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(INCLUDING ABSTRACTS)

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EFFECT OF FLOU MENT ON THE SUITABILITY OF BRE FOR DOGS

By S. G. IMPEY E and I. M. SHARMAN

Dogs given bread-crumbs ma . heavily treated with agene promptly developed hysteria. No hysteria, o detectable ill effects, resulted from the prolonged inclusion in the diet of b nbs made from untreated flour, from flour ' improved ' by prolonged aeration o avy treatment with chlorine dioxide, potassium bromate or ascorbic acid.

Introduction

In 1946 Mellanby[1] found that the treatment of flour with the ' improver ' agene, NCl_3, renders it highly toxic to dogs, producing the nervous disorders known as ' hysteria ' or ' running fits '. Information was required, therefore, on the possible toxic effects of other methods of improvement. As Moran and his colleagues[2] have identified the toxic substance as methionine sulphoximine, which requires the nitrogen atom of agene for its formation, it seemed unlikely that this poison could be produced by other methods of improvement where the active agents contained no nitrogen. Confirmation of this theoretical conclusion by practical tests, which might reveal the presence of different toxic products produced by other treatments, nevertheless seemed desirable.

Experimental

Supplies of bread-crumb

The work was started in 1953, and the flour studied was of the 80% extraction rate then extant. The improving agents, or treatments, selected for investigation were :

Agene	600 p.p.m.	Chlorine dioxide	300 p.p.m.
Aeration	Duration 70 min.	Ascorbic acid	200 p.p.m.
Potassium bromate	200 p.p.m.		

All these treatments were at 10 times the normal levels in commercial practice at the time of the investigation.

The flour was kindly supplied by the Cereals Research Station, St. Albans. In view of the duration of the experiments several batches were necessary, but the quality was kept as uniform as possible. Treatment of the flour with the gaseous improvers, agene and chlorine dioxide, was carried out weekly at St. Albans. The flour was then sent to the Baking Industries Research Station, Chorleywood, for baking. Untreated flour 2–10 weeks old was also sent, and was baked either without further treatment, with the addition of the solid improving agents, or after the aeration procedure.

The baked bread was sliced, dried under gentle heat, and ground into fine crumbs. The reason for this comminution was to allow for the mixture of the bread with other dietary components (see below).

Feeding trials

Weekly supplies of bread-crumbs were sent to the Canine Research Station (C.R.S.) at Kennett. The crumbs were mixed into a diet, modelled on that of Mellanby, which had the following composition :

Bread-crumbs	900 g.	Yeast (not dried)	45 g.
Skimmed milk powder	60 g.	Arachis soil	30 g.
Whale meat	45 g.	Supplements of vitamins A and D (as Radiostoleum, B.D.H.) and NaCl	

The above quantities sufficed for a dog of 23 kg. body weight, and were adjusted according to the size of the animal. Enough water was stirred in to give an appropriate consistency. Dogs used as controls received the usual diet of the Canine Research Station, namely, whale meat, which made up about 40% of the ration, and meal. The composition of the meal varied from time to time, but it consisted mainly of ground cereals with smaller amounts of bran and of lucerne meal or bean meal.

The dogs available for the trials were pur d Irish setters, and spaniels,
of both sexes and various ages. Most of th up of four dogs each, with two
dogs under one year old and two older dogs i The dogs in the same group shared
the same kennels, usually with two dogs per d was given once daily, following the
usual routine of the Canine Research Stati dogs were separated during feeding.
Trials on crumbs made with agene, chlorine dio ration were started in February 1953.
Crumbs made with the other treatments, potassiu ate and ascorbic acid, did not commence
until May, and only three dogs were available ach group.

Results

Hysteria in the dogs given agenised bread-crumbs

All four dogs in this group reacted very quickly. The two older, and larger dogs, both red Irish setter bitches, ate all their food on the first two days of the experiment. On the morning of the third day they were found to be suffering from severe hysteria. Both animals alternated between leaping wildly up the walls of their enclosures, and relapsing into fits. There was no response to persons moving outside their enclosures, the eyes reacted only feebly to light, and food was refused. One bitch had periods of howling, and seemed completely oblivious to her surroundings. The two young animals, one setter bitch and one greyhound dog, ate only small quantities of food. Their hysteria was delayed until the morning of the fourth day and the afternoon of the third day respectively. The disorders were no less severe than in the older dogs and were followed by exhaustion. Particulars of the amount of food eaten, and the symptoms of hysteria, are given in Table I. The total dose of methionine sulphoximine received per kg. of body weight has been calculated very approximately, assuming that the diet contained 20 mg. per kg.

Table I

Consumption of agenised food and appearance of symptoms in dogs

Dog breed	Sex	Weight, kg.	Age, years and months	Food eaten (g.) and toxic symptoms Feb. 18	19	20	21	Total intake of methionine sulphoximine
1st Test								
Red Irish Setter	♀	23	4.6	990	990	Hysteria (a.m.)		1·7
,,	♀	20	4.6	860	860	Hysteria (a.m.)		1·7
,,	♀	18	0.6	0	234	351	Hysteria (a.m.)	0·7
Greyhound	♂	16	0.6	207	172	Hysteria (p.m.)		0·5
				March 3	March 4 (a.m.)	(p.m.)	March 5 (a.m.)	
2nd Test								
Red Irish Setter	♀	23	4.6	990	990	Inco-ordination, abdominal pain		1·7
,,	♀	20	4.6	780	780		Inco-ordination	1·6
,,	♀	17	0.6	780	780	Inco-ordination, abdominal pain		1·8
Greyhound	♂	17	0.6	690	690	Inco-ordination, abdominal pain		1·6

After the appearance of hysteria the agenised diet was temporarily discontinued. When the dogs were sufficiently recovered they were given the C.R.S. diet and also milk, both of which were readily accepted within 48 h. of the onset of fits. The older dogs soon appeared to be normal. The younger dogs remained in a nervous state for several days, but had no more fits.

Within 2 weeks of the start of the first test all the dogs had recovered sufficiently to allow a second test of the agenised crumbs. In spite of their having suffered so recently from poisoning by the same food all the dogs ate their rations freely during the first 2 days. Fresh signs of injury appeared during the afte g of the second day, but in forms somewhat different from those previously seen. ler animals had no fits, but developed severe inco-ordination, reminiscent of the r se from anaesthesia by nembutal. The two younger dogs also became inco-ordinat rtheless each had several fits. In 3 out of 4 of the animals the back was arched, ir dominal pain.

All the dogs recovered on revertin C.R.S. diet, with the addition of milk for a time. During a period of observation of 13 mon er the second test all the animals remained in good general health, with no recurrence of fit or inco-ordination.

Freedom from hysteria in other groups

No signs of hysteria or inco-ordination were observed in any of the dogs except in those given agenised crumbs. All the animals remained well, with two exceptions, for periods of observation as follows :

Untreated crumbs	15–20 months
Chlorine dioxide × 10	15–20 ,,
Aeration × 10	20 ,,
Potassium bromate × 10	17 ,,
Ascorbic acid × 10	12 ,,

Appetites were good, coats were sleek, and the dogs were in no way inferior to the controls given the usual diet of the Canine Research Station. The older dogs maintained their initial weights, and the young grew at normal rates.

Of the two exceptions, the first was a member of the chlorine dioxide group. It was a greyhound dog, weighing 38 kg. and aged 3 years 9 months when the experiment started. It died suddenly, possibly from heat stroke, after being on experiment for about 4 months. The second casualty was in the bromate group. It was a spaniel dog, aged 5 years and with an initial weight of 21 kg. After being on experiment for about 6 months it developed pneumonia, from which it recovered after treatment, including temporary dietary changes. There was no evidence in either of these dogs to suggest that the illness was due to the diet.

In view of the obvious good health of all the other dogs at the end of the tests it seemed unlikely that autopsies would have added materially to our knowledge of the dietary effects of the various improving treatments. The animals were therefore kept alive for use in other work at the Canine Research Station.

Discussion

The results confirm previous conclusions that the production of methionine sulphoximine is a specific effect of the improver agene. The injuries caused appear to be of an acute and biochemical character, since they may be both induced and cured in a short time. The difference between the abnormalities caused by the first and second periods of dosing, however, suggests that an initial experience of methionine sulphoximine may modify the reactions to the ingestion of further quantities.

The healthy conditions of all except two of the dogs given bread-crumbs made from flour with various other improving treatments indicates that no acutely toxic substances were formed by the treatments. It is also obvious that if any deterioration in nutritive value was incurred it was either unimportant to dogs or could be made good by the minor components of the diet. There was no evidence that the destruction of vitamin E by the improver chlorine dioxide[3–6] had any ill effect on the general health of the dogs.

The findings that flour treated with chlorine dioxide does not cause hysteria in dogs agree with the experience of previous workers.[7–9] As far as we are aware bread made with improvement by aeration, potassium bromate or ascorbic acid has not hitherto been tested for the production of hysteria.

Conclusions

Inclusion in the diet of dogs of bread-crumbs made from flour treated with 600 p.p.m. of agene caused the prompt development of hysteria.

Bread-crumbs made from untreated flour, or flour improved by chlorine dioxide 300 p.p.m., prolonged aeration, potassium bromate 200 p. ic acid 200 p.p.m., did not cause hysteria or adversely affect general health of t . when included in the diet for periods of 12–20 months.

Acknowledgments

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CITRUS ESSENTIAL OILS. II.*—Composition of Distilled Oil of Limes

By C. A. SLATER

The composition of West Indian distilled oil of limes has been investigated and compared with that of Mexican distilled oil. Seven substances not previously reported as being present in lime oil have been isolated together with several unidentified compounds.

Introduction

A preliminary examination[1] of distilled oil of limes from Dominica (West Indies) has shown this to be very complex, containing at least 40 volatile components of which 13 have been tentatively identified. In the present work a quantity of oil has been fractionated *in vacuo* and the major components of the oil trapped out from a preparative gas chromatography column. The materials thus obtained were examined by infra-red spectroscopy. In this way a number of components reported by Guenther & Langenau[2] as present in Mexican distilled oil of limes have been shown to be present in Dominican distilled oil. In addition seven other compounds have been identified and three unidentified hydrocarbons, three unidentified alcohols and an unidentified oxide have been separated.

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* Part I: *J. Sci. Fd Agric.*, 1961, **12**, 257

Experimental

Fractionation of distilled oil of limes

Dominican distilled oil of limes (300 ml.) was carefully fractionated through a column 24 in. long, packed with Pyrex glass helices. Pressures varying from 74 to $9{\cdot}74 \times 10^{-2}$ mm. Hg were used and at no time was the bath temperature allowed to rise above 132°. In this way 51 fractions were collected.

Examination of fractions

Each fraction was chromatographed on a column (12 × 0·5 in.) of silica gel (Hopkins & Williams M.F.C.) and the hydrocarbon and oxygenated fractions thus obtained analysed by gas chromatography and examined as thin films by infra-red spectroscopy.[3] Fractions 4, 6, 10, 20–22, 29, 35, 36–40, 41, 43, 46, 47 and 50 were selected for further examination on the basis of this preliminary investigation (see Table I).

Table I

Compounds isolated from Dominican distilled oil of limes

Fraction No.	Boiling point, ° c at mm. Hg	Vol. of distillate, ml.	% oxygenated compounds	Composition (by preparative gas chromatography and infra-red spectroscopy)
4	95–97/74	5·2	26·7	α-Pinene, camphene, β-pinene, *d*-limonene, γ-terpinene, 1,8-cineole, 1,4-cineole (?), and another oxide
6	89·5/38	7·4	32·6	*d*-Limonene, *p*-cymene, and an unidentified terpene hydrocarbon (A)
10	87·5/37	7·2	20·8	*d*-Limonene, unidentified terpene hydrocarbon (A), 1,8-cineole and 1,4-cineole (?)
20–22	83·5/30	28·8	2·2	*d*-Limonene, *p*-cymene, and γ-terpinene
29	90/30	10·2	1·8	*d*-Limonene, *p*-cymene, γ-terpinene and terpinolene
35	82–86/16	4·8	13·5	Terpinolene and an unidentified terpene hydrocarbon (B)
36–40	86–89/5–9	7·5	95	Unidentified terpene alcohol (C)
41	89–91/5	4·8	98·8	α-Terpineol and two unidentified terpene alcohols (C and D)
43	77·5–78·5/1·35	10·4	98·0	α-Terpineol, β-terpineol (?) and an unidentified terpene alcohol (D)
46	$67{\cdot}5–69{\cdot}5/9{\cdot}74 \times 10^{-2}$	3·2	89·1	α-Terpineol and an unidentified terpene alcohol (D)
47	$69{\cdot}5–74/9{\cdot}74 \times 10^{-2}$	1·8	81·0	Unidentified sesquiterpene hydrocarbons (E)
50	$80–100/9{\cdot}74 \times 10^{-2}$	9·0	23·5	Bisabolene and unidentified sesquiterpene hydrocarbons (E)

Notes (*a*) The material believed to be 1,4-cineole analysed for $C_{10}H_{18}O$ and had n^{20} 1·4441. The infra-red spectrum was consistent with its being 1,4-cineole.

(*b*) The unidentified alcohol (C) gave, on dehydration over potassium hydrogen sulphate, a mixture of hydrocarbons of which the major component was *p*-cymene. There was no terpinolene formed, so this alcohol is unlikely to be terpinen-4-ol.

Preparative gas chromatography

The fractions named were separated into hydrocarbon and oxygenated fractions[3] and these were then chromatographed separately on a 6-ft. column (18–22 mm. dia.) of polypropylene sebacate (25% on 60–80 mesh ' G-Cel ') at 135°. The carrier gas was nitrogen at 2 p.s.i. inlet pressure and the individual components were detected with a preparative catharometer unit (Gas Chromatography Ltd.) at 150°. The sample (0·5 ml.) was applied to the column by means of a serum cap and hypodermic syringe and individual components were trapped out in U-tubes cooled in acetone–dry ice.

Analysis of components

The purity of components isolated by preparative gas chromatography was checked using a 12-ft. gas chromatography column (3–4 mm. dia.) of polypropylene sebacate (25% on 60–80 mesh ' G-Cel ') at 135°. The carrier gas was argon at 20 p.s.i. inlet pressure (flow-rate 75 ml. per min.) and the detector was a Lovelock ionisation detector.

Characterisation of components

Components having 95–100% purity as judged by gas chromatography were characterised through their infra-red spectra which were recorded using thin films between rock-salt plates in a Hilger & Watts Ltd. H-800 spectrophotometer. The results of this examination are given in Table I.

Results and discussion

In Table II the substances isolated from Dominican distilled oil of limes are listed together with those isolated from a Mexican oil.[2] It is clear from this Table that the five hydrocarbons present in the latter oil are all to be found in the former together with camphene, *p*-cymene, γ-terpinene, terpinolene, two unidentified terpenes, and at least one unidentified sesquiterpene.

Table II

Comparison of Mexican and Dominican distilled oil of limes

Constituents of Mexican oil[2]	Constituents of Dominican oil*	Constituents of Mexican oil[2]	Constituents of Dominican oil*
α-Pinene	α-Pinene	Geraniol	
β-Pinene	β-Pinene	Linalol	Linalol
d-Limonene	*d*-Limonene (+ dipentene)	α-Terpineol	α-Terpineol
Dipentene		Lauryl aldehyde	
Bisabolene	Bisabolene	Lauryl alcohol	
	Camphene	Acetic acid	
	p-Cymene	n-Octanoic acid	
	γ-Terpinene	n-Decanoic acid	
	Terpinolene	Unidentified aldehyde	
	Two unidentified terpenes (A and B)	Unidentified azulene	
	Unidentified sesquiterpene (E)	Unidentified phenol	
Furfural			1,4-Cineole
n-Octanal			1,8-Cineole
n-Nonanal			β-Terpineol
n-Decanal			Unidentified oxide
Citral	Citral		Two unidentified alcohols (C and D)
Borneol			

* This paper & reference 1

No trace of furfural was found in Dominican distilled oil of limes, neither was the presence of minor components conclusively demonstrated, although the presence of citral and linalol have previously been inferred from gas chromatography data.[1] α-Terpineol was easily isolated and is clearly one of the major components of distilled oil of limes making up, together with *d*-limonene, some 80–85% of the oil. In addition to these compounds, 1,8-cineole was conclusively demonstrated to be present together with two similar oxides one of which appears to be 1,4-cineole [see footnote (*a*), Table I]. There were also isolated three alcohols of slightly lower boiling points than α-terpineol. One of these is almost undoubtedly β-terpineol and the identification of the other two together with the examination of the minor components is in hand.

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DETERMINATION OF INORGANIC NITROGEN IN SOIL

By D. G. LEWIS

Investigations were conducted to find the most suitable conditions for extraction and subsequent determination of nitrate in soils by the xylenol method. Errors were caused by two main factors :

(1) When using methods of nitrate analysis based on the formation of highly coloured nitro-compounds, low recoveries of nitrate were obtained if even small amounts of soil organic matter were present at the time of nitration. The nature and extent of this loss were investigated and a satisfactory method devised for eliminating this interference.

(2) With aqueous soil suspensions, biological loss of nitrate occurred after a few hours and progressed rapidly unless sterile or bacteriostatic conditions were maintained.

Extraction of soil with 0·02N-copper sulphate solution, precipitation of copper from the decanted supernatant as hydroxide and subsequent filtration gave solutions on which nitrate could be determined, and these interferences were obviated. Use of a solution containing sodium sulphate (N) and copper sulphate (0·02N) as extractant permits determination of both ammonium and nitrate in the same extract.

Introduction

The inorganic nitrogen ions formed during nitrification in soil (NH_4^+, NO_2^- and NO_3^-) can be readily determined once they have been brought into solution, but it is necessary to ensure that the ions are brought quantitatively into the extracting liquid and that subsequent changes in concentration with time are prevented. Further, substances which interfere with the analytical techniques must be avoided or removed before the analyses are attempted.

The form of inorganic nitrogen most commonly determined in soils is NO_3^- as this is the final product of biological decomposition of organic nitrogen compounds through the accepted inorganic stages of NH_4^+ and NO_2^-.

The nitrate ion is not held by the soil colloids but is readily and completely extractable when the soil is shaken with water or aqueous solutions. The rapidity of extraction was indicated by Kelly & Brown[1] who extracted identical amounts of nitrate after 5 min. or 8 h. shaking. Riehm[2] showed that extraction for 5 min. was as efficient as 45 min., while Harper[3] and Noyes[4] claimed good recoveries from soil by extracting for 10 min.

With the xylenol method of analysis for nitrate, there was lack of reproducibility of duplicate analyses carried out on the same day or different days. This led to an investigation into the proper conditions for extracting nitrate from soils. Results obtained showed that there were two main factors leading to erroneous values of nitrate unless proper control was exercised ; viz., (1) very small amounts of soil organic matter, if present at the time of nitration, gave rise to low values ; (2) in aqueous soil suspensions which were set aside for more than a few hours, biological loss of nitrate occurred and progressed rapidly on further keeping unless bacteriostatic conditions were maintained.

Experimental

Recommended procedure for preparation of the soil extracts

(a) *When nitrate only is required.*—Shake the desired amount of soil, usually 50 or 100 g., with a measured volume of copper sulphate solution (0·02N or sufficient to ensure a slight excess of soluble copper) for a convenient period, usually between 15 and 30 min. (The longer the period of shaking, the greater is the amount of organic material brought into solution.) Set aside for 5–10 min. to allow the larger soil particles and the flocculated colloids to sediment, and decant a volume of supernatant liquid more than sufficient for the analyses. Add 0·1–0·2 g. of finely powdered calcium or magnesium oxide and shake occasionally for a few minutes to ensure complete precipitation of the copper hydroxide. Filter through a dry folded paper or under suction, rejecting the first runnings. The resultant filtrate should be clear and colourless and samples can be taken safely for nitrate determination.

(b) *When both ammonium and nitrate are required.*—The recommended extractant is sodium sulphate (1·0N) containing 0·02N-copper sulphate. Shake the desired amount of soil with a measured volume of extractant in the ratio of 1 g. of soil to 5 ml. of liquid for 1 h. Filter the extract through a folded filter paper (18·5 cm. Whatman No. 30) in a conical funnel or through a Buchner funnel, discarding the first runnings. This filtrate can be sampled directly for determination of ammonium.

Decant some of the filtrate, precipitate the copper by adding excess of magnesium oxide and filter through a dry folded paper or under suction, rejecting the first runnings. This colourless filtrate can be sampled for determination of nitrate.

Xylenol method of nitrate analysis

Although *m*-xylenol (2,4-dimethylphenol) was first suggested by Blom & Treschow[5] as an ideal reagent for nitrate determination, Holler & Huch[6] investigated all six xylenol isomers and concluded that 3,4-xylenol was a better reagent because of a greater reliability in colour development. The procedure as outlined,[6] however, gave erratic results, and so, after a thorough investigation[7] to determine the optimum conditions, the method was modified to enable accurate analysis of low concentrations of nitrate.

Reagents and method

Xylenol reagent. Dissolve 1 g. of 3,4-xylenol (B.D.H.) in 100 ml. of acetone.

Sulphuric acid (5 + 1). Cautiously add 5 volumes of pure sulphuric acid (98%) to 1 volume of distilled water and cool before using.

Silver sulphate solution. Dissolve 5 g. of pure nitrate-free silver sulphate in 60 ml. of conc. aqueous ammonia and boil off excess ammonia. Dilute to 100 ml. and store away from the light.

Standard potassium nitrate solution. Dissolve 0·7218 g. of A.R. grade potassium nitrate in distilled water and dilute to 1 l. This solution contains 100 µg. of nitrate-nitrogen per ml. from which working standards of lower concentrations can be prepared as required.

Procedure.—Pipette a sample of the nitrate solution containing not more than 150 µg. of nitrogen into a 500-ml. boiling flask. If the sample volume is less than 15 ml., adjust the volume with the required amount of distilled water. (Since chlorides and nitrites interfere with the method, these ions should be eliminated by adding a few drops of 5% silver sulphate solution or 1% sulphamic acid respectively before adjusting the total volume of sample plus reagents to 15 ml.) Add 50 ml. of (5 + 1) sulphuric acid, swirl gently but thoroughly and allow to return to room temperature. Add 1 ml. of the xylenol reagent, swirl thoroughly and set aside for at least 20 min. to allow nitration to proceed. Stop the reaction by adding approx. 150 ml. of distilled water and connect the flask to the distillation apparatus (Fig. 1). Distil the volatile nitroxylenols into 10 ml. of sodium hydroxide solution (0·2N) contained in a 100-ml. volumetric flask, continuing the distillation until the volume in the flask is approx. 98 ml. Cool to room temperature, dilute to the mark and mix thoroughly. Determine the transmittance of this solution against distilled water in a spectrophotometer at a wavelength of 430 mµ or in a filter photometer with any suitable blue filter, e.g., Ilford spectral filter No. 601.

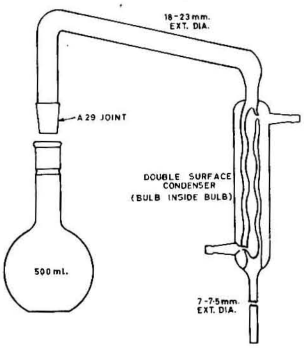

FIG. 1.—*Nitroxylenol distillation unit*

Results

(1) *Errors due to soil organic matter and their prevention*

When nitrate determinations were carried out on samples pipetted from aqueous soil suspensions, which had been kept for at least 30 min. to allow the greater part of the soil to sediment, there was frequent lack of agreement between replicate determinations. This could arise from some soil substituent remaining in suspension to a variable degree and so causing variable interference. To test this, 10-mg. portions of two different soils were added in 500-ml. flasks to solutions containing 25, 50 or 100 μg. of nitrate-nitrogen before analyses were carried out. One soil was a black organic fen soil from Rendelsham (S.A.) which had been previously leached free from nitrate and dried rapidly. The other soil was a heavy clay subsoil (sample 33–54″) from Rocky Gully, W.A., which was very low in organic matter and free from nitrate. The amounts of nitrate found in each set of determinations are shown in Table I.

Table I

Effect of added soil on the recovery of nitrate

(10 mg. of soil was added in each case: results are means of duplicate experiments)

Soil added	Nitrate added, μg. N	Nitrate found, μg. N	Difference	
			μg. N	%
Rendelsham	25	17·8	−7·2	−29
Rendelsham	50	37·6	−12·4	−25
Rendelsham	100	77·0	−23·0	−23
Rocky Gully	25	25·1	+0·1	0
Rocky Gully	50	51·5	+1·5	+3
Rocky Gully	100	101·3	+1·3	+1

In all cases where 10 mg. of organic soil had been added, the amount of nitrate recovered was considerably less than that added, while the loss became progressively greater as the amount of nitrate was increased, although the % loss remained almost constant. On the other hand the addition of 10 mg. of the mineral subsoil had no effect on the determination, and the amounts of nitrate found were, within experimental limits, equal to those added.

Addition of peptised organic matter.—A solution of organic matter was prepared by shaking 10 g. of leached Rendelsham soil for 5 min. with 200 ml. of 0·1N-sodium hydroxide and then filtering. Various amounts of this extract were added to 100-μg. samples of nitrate-nitrogen and nitrate determination carried out 2 h. after addition of sulphuric acid (5 + 1). The amounts of nitrate recovered are shown in Table II.

Table II

Effect of added soluble organic matter on nitrate recovery

(Sulphuric acid added 2 h. before xylenol reagent: results are means of duplicate analyses)

Organic extract added,	Nitrate added, μg. N	Nitrate found, μg. N	Loss, μg. N
1 ml., 1%	100	74·5	25·5
1 ml., 5%	100	35·0	65·0
2 ml., 5%	100	15·9	84·1

Table III

Effect of time of contact of organic matter and sulphuric acid on nitrate recovery

(1 ml. of 1% organic extract and 100 μg. nitrate-N added in each case)

Time of contact, h.	Nitrate found, μg. N	Nitrate lost, μg. N
1	80·6	19·4
3½	73·7	26·3
20	56·1	43·9

These values show that very considerable losses occurred, the loss being greater when larger additions of organic matter were made. The loss of nitrate was also dependent on the time during which the organic extract was in contact with the sulphuric acid and nitrate prior to the addition of the xylenol reagent. A constant amount (1 ml.) of organic extract was added to 100-μg. samples of nitrate-nitrogen and the xylenol reagent added at various times after the sulphuric acid addition. The amount of nitrate recovered decreased as the time of contact with the sulphuric acid and nitrate increased, as shown in Table III, indicating clearly that organic matter should be avoided as it interferes in the determination.

Effect of filtration.—Filtration of the soil extract to eliminate any suspended organic matter led to greater reproducibility in the replicate determinations. For the organic soil, the values for nitrate as determined on the filtered extract were slightly but significantly higher than those on the suspension, but the recovery of nitrate added to an identical sample of filtrate was still low. Corresponding determinations on the mineral subsoil showed no significant difference between determinations on the suspension and filtrate, and recoveries of nitrate added to the soil before shaking were excellent, as shown by Table IV.

Table IV

Nitrate determination on equivalent volumes (15 ml.) of soil suspension, filtrate and clarified filtrate

(10 g. of soil shaken with 250 ml. of extractant in all cases; results in μg. of N are means of duplicate analyses)

Soil	Nitrate added	Clarified filtrate* Nitrate found	Suspension†		Filtrate‡	
			Nitrate found	Loss	Nitrate found	Loss
Rendelsham	0	26·4	22·5	3·9	24·4	2·0
Rendelsham	50	76·4	67·2	9·2	71·8	4·6
Rocky Gully	0	0	0	0	0·2	−0·2
Rocky Gully	30	30·0	30·6	−0·6	30·8	−0·8
Rocky Gully	75	75·0	75·0	0	74·0	1·0

* As determined by the recommended procedure, eliminating all interferences
† Suspensions were allowed to sediment for 30 min. before sampling
‡ Filtrate obtained by filtering through a Whatman No. 2 paper under gentle suction

The low recovery of nitrate when added to the filtered Rendelsham extract indicated the presence of some interfering constituent in the filtrate. It is suggested that the organic matter undergoes a slow nitration reaction, while in contact with the acid nitrate mixture, leaving a reduced amount of nitrate available to form the desired volatile nitroxylenol compounds. Thus accurate results obviously cannot be obtained if the extract contains organic matter in solution or suspension. The phenoldisulphonic acid method also suffers interference from organic matter, but this is usually regarded as only a direct effect of coloured or colloidal solutions interfering with the photometric comparisons. This method, however, can also be affected indirectly as is the xylenol method. In both methods the reagent and the organic matter compete for the nitrate with a likely decrease in the yield of the desired component. Hence for any method involving a nitration reaction, it is important to prepare extracts which are perfectly clear and colourless.

Removal of organic matter.—Various flocculating agents have been suggested to remove organic substances from solution. Harper,[3] after a critical study of methods of clarifying solutions for nitrate analysis, finally recommended extracting the soil with an aqueous solution containing a slight excess of a copper salt and subsequently precipitating the copper as the hydroxide by addition of calcium oxide followed by magnesium carbonate to remove any excess calcium oxide. The copper hydroxide precipitate evidently absorbed or coated the organic matter and after filtration a colourless extract was obtained. As copper is known to form very insoluble complexes with some constituents of soil organic matter, the efficacy of this treatment for removal of the interfering substances in the aqueous soil extracts was examined.

Suspensions were prepared by shaking, for 30 min., 25 g. of soil (with or without the addition of known amounts of nitrate) with 250 ml. of 0·02N-copper sulphate. The copper hydroxide was then precipitated by adding 0·5 g. of calcium oxide and 1·0 g. of magnesium carbonate, and the mixture shaken for a short time. The suspension was filtered through a dry filter paper, the first runnings being rejected. This technique gave clear and colourless filtrates for all soils examined, covering a wide range of organic content. The amounts of nitrate found in four different soils and the recoveries of known amounts of added nitrate are shown in Table V. For comparison, determinations were also carried out on some aqueous suspensions prepared at the same time (without copper treatment) to assess the errors introduced by the soil organic matter. These extracts were allowed to sediment for 30 min. before the samples were pipetted from the supernatant liquid.

The right-hand column of Table V indicates that, where copper pre-treatment was included, excellent agreement was obtained between the amounts of nitrate added and the amounts

Table V

Efficiency of copper treatment in removing interferences due to organic matter

10 g. of soil shaken with 250 ml. of extractant (water or KNO_3 solutions): results are µg. of nitrate-N in 10-ml. samples

Method A: Determination on aqueous suspensions after 30 min. sedimentation
Method B: Determination on filtrate after removal of organic matter by precipitation with copper hydroxide)

Soil used	Nitrate added	Method A		Method B		Recovery, %
		Nitrate found	Loss	Nitrate found	Loss	
Rendelsham	0	22·5	3·9	26·4	—	
Rendelsham	20			46·3	0·1	99·5
Rendelsham	40			66·7	−0·3	100·7
Rendelsham	50	67·2	9·2	76·1	0·3	99·4
Urrbrae	0			14·4	—	
Urrbrae	20			34·3	0·1	99·7
Urrbrae	40			53·3	1·1	97·4
Urrbrae	60			72·4	2·0	96·7
Montague Swamp	0	2·1	1·3	3·4	—	
Montague Swamp	20			22·7	0·7	96·5
Montague Swamp	40	36·4	7·0	42·7	0·7	98·3
Montague Swamp	60			61·3	2·1	96·5
Seddon	0	4·9	0·7	5·6	—	
Seddon	20			25·5	0·1	99·5
Seddon	40	43·3	2·3	45·7	−0·1	100·2
Seddon	60			64·3	1·3	97·9

subsequently recovered. In most cases the recovery was between 98 and 101% of that added, which is regarded as quite satisfactory, being approximately the limits of reproducibility of the xylenol method. Further, the determinations, carried out without clarification of the soil suspension by preliminary copper treatment, yielded low results. The absolute losses are not great in some cases, although they frequently attain 7–15% of the amount determined, particularly if much organic matter is present. Because clarification by means of copper hydroxide readily eliminates the error due to soil organic matter or other reducing substances in the soil, its use is recommended in the standard preparation of the soil extracts for nitrate analysis.

(2) *Errors due to biological loss of nitrate*

In the course of some experiments in which the aqueous soil suspensions were kept for varying periods of up to four days before analysis, it was found that there was a very considerable decrease in the amount of nitrate found. Samples of Rendelsham soil each of 20 g. were shaken for 30 min. with 500 ml. of (*a*) distilled water, (*b*) KNO_3 solution containing 5 µg. of nitrate-nitrogen per ml., (*c*) 0·02N-copper sulphate, or (*d*) as (*c*) containing 5 µg. of nitrate-nitrogen per ml. After various periods, the nitrate contents of the supernatant solutions were determined. In the extracts without copper, the soluble organic matter was removed just before nitrate analysis by dissolving 0·1 g. of copper sulphate in 100 ml. of the decanted extract and subsequently precipitating the copper by addition of excess calcium oxide. The extracts containing copper initially were also treated with calcium oxide. All extracts were then filtered and nitrate analyses carried out on the colourless solutions. The nitrate contents of the solutions after various periods are shown in Table VI.

Table VI

Changes in nitrate content in aqueous extracts on storage

10 g. samples of Rendelsham soil A shaken with 250 ml. of extractant: organic matter removed from solution before analysis by precipitation with copper hydroxide; results are nitrate-N in µg. in 10-ml. samples

Extractant	Nitrate added	Nitrate-nitrogen found after storage for			
		30 min.	24 h.	48 h.	96 h.
Water	0	26·4	16·4	10·0	4·3
KNO_3 solution (5 µg. N/ml.)	50	76·1	54·8	38·7	20·2
0·02N-$CuSO_4$	0	26·4	25·6		27·1
0·02N-$CuSO_4$ + KNO_3 (5 µg. N/ml.)	50	76·0	75·6		76·2

Where the extracts were prepared without copper addition, considerable losses occurred on keeping even during the first 24 h., the loss becoming greater with time. In the continued presence of soluble copper, however, the amounts of nitrate-nitrogen after 1 or 4 days were essentially the same as at the beginning of the experiment. If the disappearance of the nitrate in the aqueous extracts was due to biological action, then a concentration of copper of 0·02N in the suspension effectively prevented it.

At the author's suggestion, Martin & Cox[8] incorporated 0·02N-copper sulphate in the extracting solution used in their investigations, but when fungal growth was noted in some extracts they also included chloroform as a fungicide. In the author's experience, when there is an *excess of soluble copper*, no further bacteriostatic agent is necessary or desirable. The soils used by Martin & Cox[8] were heavy black clays and it is very likely that all of the copper was removed from solution by exchange or complex formation with the clay or organic matter, leaving no free copper ions. The addition of chloroform is questionable since quite serious interference has been noted when this reagent is present. Although pure washed chloroform does not interfere, commercial samples all contain stabilising additives which are extractable by water and can interfere strongly with the analyses.

Effect of autoclaving.—To confirm the biological nature of this loss of nitrogen on keeping, suspensions of 10 g. of another Rendelsham soil were prepared in 250 ml. of (*a*) distilled water, (*b*) potassium nitrate solution containing 5 μg. of N per ml., or (*c*) 0·02N-copper sulphate solution, and after being shaken for 5 min. the suspensions were autoclaved for 2½ h. at 1½ atmospheres pressure. Separate suspensions were prepared for each sampling period and these were kept under sterile conditions until required. Samples of 100 ml. of decanted supernatant liquid were treated with 0·1 g. of copper sulphate where necessary and the copper precipitated as usual. The clear filtrates were then analysed for nitrate.

Table VII

Nitrate content of autoclaved soil suspensions

10 g. of Rendelsham soil B, shaken and autoclaved with 250 ml. of extractant: suspensions stored under sterile conditions until analysed: 10-ml. samples taken from filtrates after removal of organic matter

Extractant	Nitrate-N (μg.) after storage for	
	16 h.	96 h.
Water	90·8	93·7
KNO_3 solution (5 μg. N/ml.)	143	142
0·02N-$CuSO_4$ solution	91·0	90·9

Table VII shows that when the suspensions were prepared and maintained under sterile conditions, the amount of nitrate-nitrogen after 4 days is, within the limits of experimental errors, identical with that found initially. There was no loss of the nitrate originally present in the soil or of the added nitrate.

The quantitative recovery of the added nitrate from all autoclaved samples gives further evidence of the efficiency of the copper treatment in removing the interfering effects of organic matter, since autoclaving the soil suspensions increased very considerably the amount of organic matter in solution. A direct determination on the aqueous filtrate without removing organic matter showed only 4·7 μg. of nitrate-nitrogen per ml., whereas 9·1 μg. per ml. were actually present. Thus the organic matter brought into solution by autoclaving could cause a loss during analysis of almost 50% of the nitrate unless the organic matter were removed by prior copper treatment.

Effect of aeration of suspension.—The change in nitrate content of a soil suspension maintained under aerobic conditions was also examined. A 10-g. sample of Rendelsham soil was suspended in 250 ml. of distilled water and air was led through a sintered glass plate at the bottom of the container so that the whole of the suspension was continuously aerated. After 4 days, a sample was taken and after removal of the organic matter by copper treatment 11·6 μg. of nitrate-nitrogen were found per ml. of filtrate. Determinations carried out on the decolorised filtrate from a freshly prepared suspension showed 10·8 μg. of nitrate-nitrogen per ml. These values indicate that under aerobic conditions there was no biological loss of nitrate and in fact nitrification probably occurred.

The results in these tables show some of the precautions necessary in soil studies of nitrate production. The success of the perfusion technique of Lees & Quastel[9,10] depends upon thorough aeration both of the percolating liquid and the soil column, as otherwise a decrease in the amount of nitrate present may occur quite rapidly. Similarly, losses may be expected if anaerobic conditions develop during storage or transport of soil collected in the field under wet conditions.

Conclusion

From these experiments it is evident that the recommended extraction procedure gives colourless filtrates in which nitrate can be determined quite readily and satisfactorily with no interference from organic matter or changes due to biological activity. The precipitation of the copper hydroxide by calcium oxide and magnesium carbonate in the presence of the soil, as suggested by Harper,[3] was satisfactory, but in the case of some acid soils relatively large amounts of these reagents were necessary. In such cases, the procedure found to be more convenient and economical is as follows. When the extracts are set aside for 5–10 min., most soils will sediment fairly rapidly due to the electrolyte content. Then a sample of the supernatant liquid, usually about 100 ml., can be decanted and the copper precipitated in this slightly turbid solution with a small amount of calcium oxide (excess of oxide has no effect on the nitrate determination).

Estimation of both nitrate and ammonium nitrogen

Piper[11] has shown that ammonia can be extracted from soils as readily by using a buffer of sodium sulphate and sulphuric acid as when the more usual buffer of potassium chloride and hydrochloric acid is used.[12] This sodium sulphate–sulphuric acid buffer was investigated for ammonia determination because of the possibility of determining nitrates in the same extract, since nitrate cannot be determined readily in the Olsen extract by the xylenol method owing to interference by chloride ions. To this end, copper sulphate was also incorporated into the extracting buffer so that a small excess of soluble copper was present. The recommended buffer contained 1·0N-sodium sulphate, 0·1N-sulphuric acid and 0·02N-copper sulphate, although a similar solution without the acid was later found quite satisfactory for most soils encountered.

When both ammonium- and nitrate-nitrogen are to be determined on the sample, the extraction procedure needs to be modified slightly, as outlined in the Experimental Section. The recommended shaking period of 1 h. is usually sufficient to establish equilibrium, although for heavy clay soils a complete displacement of the total ammonium may not be accomplished. The degree of ammonium ion replacement at equilibrium is dependent on both the extent and the nature of the clay content of the sample, as well as the ratio of solid to liquid. For comparative measurements on the same or essentially similar soils the equilibrium value determined by the suggested technique is quite satisfactory. If, however, absolute values of the amount of ammonium are required, a continuous leaching technique would be preferable.

The initial filtrate can be sampled directly for determination of ammonium-nitrogen by distillation from alkaline solution and subsequent titration or Nesslerisation.

For the determination of nitrate-nitrogen a sample of the initial extract should be clarified by the addition of excess magnesium oxide and the precipitate filtered off. Magnesium oxide is preferred to calcium oxide in this case as, with the latter, the high sulphate concentration gives a copious precipitate of calcium sulphate as well as the copper hydroxide, making filtering very tedious. Magnesium sulphate does not interfere in this way and the small volume of copper precipitate can be filtered much more readily.

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TREATMENT OF MEATS WITH IONISING RADIATIONS. VIII.*—pH, Water-binding Capacity and Proteolysis of Irradiated Raw Beef and Pork during Storage, and the ATP-ase Activity of Irradiated Rabbit Muscle

By R. A. LAWRIE, J. G. SHARP, J. R. BENDALL and B. COLEBY†

The immediate effects of 5-Mrad ionising radiation on beef and pork *longissimus dorsi* muscles were an increase in pH, a decrease in water-holding capacity, in increment in gel-volume for a given pH rise, and in soluble protein, and increased resistance to low- and high-speed homogenisation. The indications of cross-binding induced by irradiation were supported by studies of isolated myofibrils from rabbit *psoas* muscle. Irradiation markedly reduced the syneresis (18°, $\mu = 0{\cdot}04$) and the swelling (0°, $\mu = 0{\cdot}25$) induced by ATP and, to a lesser extent, over-all fibrillar ATP-ase activity (the initial fast phase being depressed more than the slower second phase of the reaction). On storage (at $-20°$ to $+37°$), pH and water-binding capacity increased generally with increase of temperature. Changes in pH occurred earlier with pork and to a greater extent than with beef. In sterile beef *longissimus dorsi* (irradiated or unirradiated) there was a decrease in soluble protein during storage for 60–90 days at 37° (indicating denaturation) and increases in TCA-soluble nitrogen and tyrosine (indicating proteolysis, which was more marked in unirradiated samples). The absence of soluble hydroxyproline and the presence of clearly marked cross-striations indicated that the autolysis must have involved sarcoplasmic and not fibrillar or connective tissue protein.

Introduction

As part of a general investigation into the post-mortem behaviour of muscle, changes in pH, in water-binding capacity and in certain protein fractions were followed during prolonged storage of *longissimus dorsi* muscle from beef and pork. Microbial spoilage was prevented by irradiating the muscle samples with 2-MeV electrons, giving doses of 5 Mrads :[1] some sterile samples obtained by aseptic dissection were also studied. Although some incidental observations on the rise of pH during storage of irradiated meat have previously been made,[2] there has been little attempt hitherto to relate this phenomenon to water-binding capacity or to concomitant alterations in nitrogen distribution.

Another way in which the effects of irradiation can be elucidated is to test the ATP-ase activity and the ATP-induced syneresis of washed myofibrils prepared from minced muscle. Studies of this nature have already proved useful in assessing changes in water-binding capacity during simple freeze-drying.[3] In the present work, rabbit *psoas* muscle was used instead of beef or pork, because of the ease with which fibrils can be prepared from it.[4]

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Experimental

Sampling

The *longissimus dorsi* muscle was dissected from a loin of beef and a loin of pork on the day following slaughter. Uniform slices (about 1 cm. thick) were cut sequentially from the fore end of each muscle, with a bacon slicer having a disc cutter. Samples (30 g.) of these slices, freed from adhering connective tissue and fat, were placed in shallow aluminium cans (1·2 cm. deep); they were then gas-packed in nitrogen and kept at 0° overnight. On the following day all cans other than controls were irradiated at room temperature from above and below, with electrons from a 2-MeV Van de Graaff generator, to give a dose of 5 Mrads. Samples were taken for immediate examination (fresh) and the remainder stored in batches at −20°, 0°, 15°, 25° and 37°. Since irradiation itself is known to affect the water-binding capacity of proteins,[5, 6] both irradiated and unirradiated samples were held at −20° as controls.

Samples of both pork and beef were examined after storage for 1, 4, 26 and 52 weeks and also of pork at 2 and 12 weeks. The pH and water-binding capacity of all samples were determined. Histological sections were prepared from the beef samples for following the changes in certain protein fractions.

Analysis

pH was measured by a glass electrode on a homogenate of 1 g. of muscle in 10 ml. of distilled water.

Water-binding capacity

Two g. of muscle were homogenised with 6 ml. of phosphate buffer (0·02M, pH 7·0 and containing 0·1M-potassium chloride) for 2 min. in a high-speed homogeniser. The homogenate was poured into a 10-ml. graduated centrifuge tube and a further 3 ml. of buffer used to wash out the cup: the washings were added to the tube, and the whole centrifuged for 15 min. at 3500 r.p.m. The gel volume at the foot of the centrifuge tube was regarded as a direct measure of water-binding capacity.[7]

ATP-ase activity

For the experiments on ATP-ase activity, the *psoas* and *longissimus dorsi* muscles were removed from a rabbit immediately after death, allowed to pass into rigor, and then minced and mixed, one half being irradiated as described for beef and pork above, and the other half kept as control. Portions of each were then homogenised at high speed in KCl/imidazole buffer at pH 7·2, $\mu = 0{\cdot}10$, washed thoroughly in buffer after removal of the coarser particles by passage through fine muslin, and finally stored at −10° in a mixture of 70 parts of buffer and 30 parts of glycerol. Fibrils so prepared preserve their ATP-ase activity indefinitely. The conditions for determination of ATP-ase activity were: μ 0·04, imidazole 40 mM, ATP 4 mM, magnesium chloride 4 mM, calcium chloride 0·2 mM, pH 7·20 and T 18·5°. The activity is due to the so-called Mg-activated ATP-ase of actomyosin. For tests of swelling or syneresis, the washed fibrils (≡ 0·6 g. of fresh muscle) were suspended in 6 ml. of the appropriate buffer, and the volume of the fibril layer measured before and after addition of ATP (4 mM) and magnesium chloride (4 mM) at pH 7·2 by centrifuging for 5 min. at 2800 r.p.m. As seen in Table III the ionic strength at 18° was 0·04, which is best for testing syneresis, whereas in the experiments at 0° it was 0·25, which is best for testing swelling in the presence of ATP.

Nitrogen fractions

With the object primarily of detecting changes in the collagen fraction, the procedure for the extraction of soluble collagen was mainly followed.[8, 9]. In point of fact the complete absence of hydroxyproline in any of the soluble fractions showed that no breakdown of collagen into a soluble form occurred. Estimation of the total nitrogen in the separated fractions served however to show changes which occurred in the sarcoplasmic and myofibrillar proteins.

The samples were homogenised in 0·1M-KCl and aliquots removed for estimation of total nitrogen, trichloroacetic acid-soluble nitrogen (TCA sol. N) and total tyrosine. The remaining homogenate was centrifuged and the residue extracted successively at low temperature (0–7°) with (1) 0·1M-potassium chloride, (2) 0·2M-disodium phosphate buffer, pH 8·9, and finally (3) 0·1M-sodium citrate buffer, pH 3·7. The three soluble fractions were dialysed against water to remove non-protein material, and the extracts, including any precipitated protein, hydrolysed by boiling in 8N-HCl for 8 h. The main insoluble residue was hydrolysed without previous dialysis.

TCA-soluble N

One volume of 0·1M-potassium chloride homogenate was treated with 1 volume of 20% TCA and the mixture centrifuged. The insoluble residue was washed with 10% TCA and the extract and washings filtered. The process was carried out quickly to ensure that no breakdown of protein (as found by Bendall[10]) took place during extraction. TCA was extracted with ether, and the concentrated aqueous extract hydrolysed in 8N-HCl.

Tyrosine

The tyrosine value of all hydrolysates was estimated by measuring the absorption at 293mμ in 0·1N-NaOH and expressing the result in tyrosine equivalents. It is realised that the absorption need not be entirely due to tyrosine but partly to other substances present, such as adenylic acid, for example, but the value was regarded as a satisfactory index for this preliminary study. The tyrosine values presented do not distinguish between free and bound tyrosine but give only the total tyrosine after acid hydrolysis.

Hydroxyproline

Hydrolysates were analysed for hydroxyproline by the Neuman & Logan method.[11]

Results

Changes in pH

pH changes in the *longissimus dorsi* muscles of beef and pork are presented in Figs. 1 and 2 respectively. In both species irradiation caused an increment in pH which persisted at all temperatures and for all times of storage studied, and a further increase was brought about by freezing (the data at zero storage time in Figs. 1 and 2 are those for ' fresh ' muscle). It will be noted that irradiated pork, after one month at −20°, had a higher pH than had samples stored at 0°, 15°, 25° or 37°, although the value subsequently fell, after a further 11 months, to a level similar to that for corresponding beef samples. It is also obvious from Figs. 1 and 2

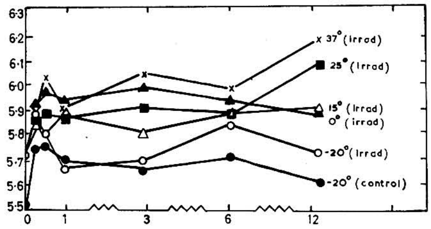

FIG. 1.—*Changes with time and temperature of storage in the pH of beef* longissimus dorsi *muscle*

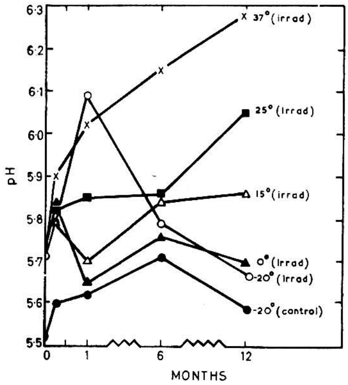

FIG. 2 (*right*).—*Changes with time and temperature of storage in the pH of pork* longissimus dorsi *muscle* that, apart from irradiation and freezing, time and temperature of storage are associated with an elevation of pH in both beef and pork *longissimus dorsi* muscles ; and that, as judged by the values after one year's storage, this effect was a direct function of temperature. An anomalous but consistent feature of the data was that, at storage times less than 12 months, pH increments in beef were greater at 0° than at 15° or 25°. The figures also show that the effect on pork of different storage temperatures was more marked (and earlier) than that on beef, since, after one month, the difference between extreme values in the irradiated samples was 0·45 and 0·27 pH units in pork and beef respectively. Although during the ensuing 11 months there was a proportionately greater rise of pH in beef than in pork, the final pH difference over the various storage temperatures was 0·60 units in pork compared with 0·45 units in beef. It should be noted that sterile, unirradiated beef held at 37° for 6 months showed a relatively small pH change (Table III, animal C).

Water-binding capacity

Changes in water-binding capacity (gel volume) after storage for 1, 4 and 52 weeks are given in Table I. From the results for the fresh samples and for those stored at —20°, irradiation diminished the water-binding capacity of both pork and beef muscle, despite the concomitant increase of pH (Figs. 1 and 2). In all cases the effect was greater in beef than in pork.

Table I

Effect of irradiation (5 *Mrads) and storage on centrifuge volume of* 1 *g. of homogenised* longissimus dorsi *muscle from beef and pork*

Storage time, weeks	Storage temp., ° C	Volume, ml.			
		Beef		Pork	
		Control	Irradiated	Control	Irradiated
Nil (' Fresh ')	—	1·50	1·15	1·55	1·25
1	—20	1·90	1·30	1·50	1·25
	0	—	1·25	—	1·25
	15	—	1·25	—	1·20
	25	—	1·25	—	1·20
	37	—	1·20	—	1·30
4	—20	1·80	1·20	1·50	1·05
	0	—	1·35	—	1·15
	15	—	1·40	—	1·30
	25	—	1·45	—	1·30
	37	—	1·45	—	1·40
52	—20	1·65	1·30	1·45	1·20
	0	—	1·40	—	1·30
	15	—	1·75	—	1·40
	25	—	1·60	—	1·60
	37	—	1·55	—	1·40

The looser structure of the irradiated samples observed in the histological sections (Fig. 4) is no doubt related to the large quantities of ' weep ' fluid observed on opening the tins. Notwithstanding this superficial feature of the intact meat samples, however, the water-binding capacity of the fibrils produced by subsequent homogenising increased on storage. Thus, although storage of pork and beef at 0°, 15°, 25° and 37° for one week had no effect on water-binding capacity in relation to irradiated control muscle at —20° (and again despite a concomitant rise of pH), there was a relative increase, at all temperatures, after storage for 4 weeks and this was even more marked after 52 weeks. It is noteworthy that the increment after one year's storage was greatest at 15° with beef and at 25° with pork. Fig. 3 shows that water-binding capacity tended to increase with increase of pH, as would be expected, in both irradiated and unirradiated samples. The increase brought about by given pH increment was, however, considerably less in irradiated than in unirradiated samples.

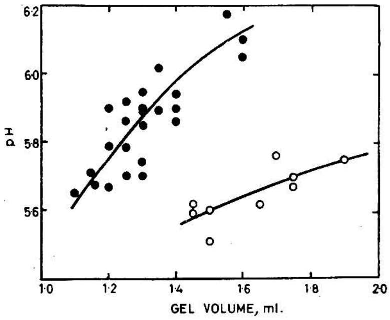

FIG. 3.—*Relationship between gel volume and pH in irradiated* (●) *and unirradiated* (○) longissimus dorsi *muscles of beef and pork*

Microscopical observations

As observed microscopically in sections of fixed tissue, irradiation at a level of 5 Mrads appeared to cause some disorientation of the muscle fibres (Fig. 4A and B). Storage at 37° for one month (Fig. 4C) brought about a general close packing of the fibres, but, after storage for one year at 37°, there was considerable separation of individual fibres (Fig. 4D).

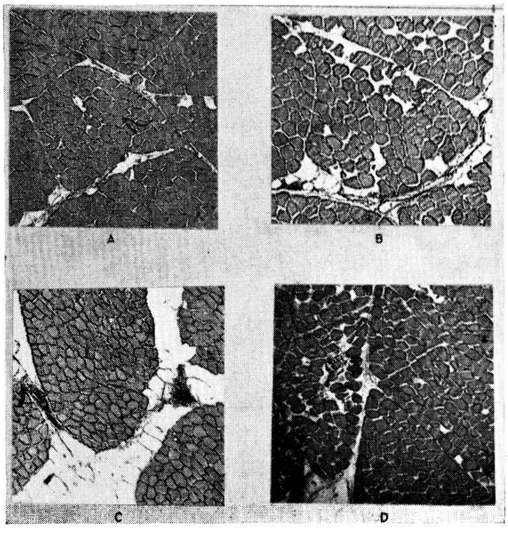

FIG. 4.—*Transverse sections of beef* longissimus dorsi *muscle*

A. Control: no storage
B. Irradiated (5 Mrads): no storage
C. Irradiated (5 Mrads): stored 4 weeks at 37°
D. Irradiated (5 Mrads): stored 52 weeks at 37°

Fixed in formalin: Celloidin embedded
Stained: haematoxylin, van Giessen (× 30)

Microscopical examination of low-speed homogenates in buffer at pH 7 showed (1) in unirradiated control samples, complete disintegration to sections of single fibrils (dia. 1 μ × length 10–25 μ), and (2) in samples immediately after irradiation, only partial disintegration to sections of fibres (dia. 100–150 μ × length 200–300 μ) in a background of single fibril sections.

High-speed homogenisation produced a greater degree of breakdown, but the effect of irradiation was still apparent. Control samples consisted entirely of small sections of fibrils (dia. 1 μ × length 3–5 μ), whereas irradiated samples consisted mostly of sections of fibres (dia. 3–7 μ × length 12–15 μ). The effect of irradiation in this respect was the same whether irradiation was carried out at 10–15° or at −75°.

Low-speed homogenates of irradiated samples held for one year at 37° and at −20° gave, in both cases, long, smooth undegenerated fibres. A similar homogenate of the control unirradiated sample held at −20° for one year consisted almost entirely of single fibrils. After high-speed homogenisation, the two irradiated samples still retained an appreciable proportion of sections of fibres in a background of fibrils.

Corresponding low-speed homogenisation of unirradiated sterile samples of beef held at 37° for 6 months, also gave mostly long fibres but, in this case, the fibres showed zones of longitudinal and transverse degeneration and cleavage which gave them a tattered and torn appearance.

ATP-ase activity

In line with the above changes in pH and water-holding capacity brought about by irradiation of beef and pork muscle, considerable changes were found in the ATP-ase activity and the ATP-induced swelling or syneresis of rabbit myofibrils. Splitting of ATP by the Mg-activated ATP-ase of the fibrils in general has a rapid initial phase, followed by a slower phase. In the present experiments, the calculated velocities of these phases in the control fibrils were respectively $9 \cdot 8 \times 10^{-2}$ and $4 \cdot 5 \times 10^{-2}$ μmoles of inorganic phosphate formed per sec. per mg. of fibrillar N. Irradiation reduced the initial velocity to 40% and the velocity of the second phase to 80% of the control. A similar effect can be produced in control fibres, by first treating them with ATP to bring about shortening, and then washing out the ATP and replacing it by fresh. It is again the first, fast phase of splitting which is the more inhibited. These inhibitory effects of irradiation are, however, less marked than the effects on the ATP-induced syneresis at 18°, $\mu = 0 \cdot 04$, or on the ATP-induced swelling at 0°, $\mu = 0 \cdot 25$, as shown in Table II. This Table indicates not only that the fibril volume before addition of ATP is much reduced by irradiation, as shown by the observations on beef and pork muscle, but also that these shrunken fibrils cannot be induced, by addition of ATP, either to shrink further, as they should at 18°, or to swell, as they should at 0°. As we have seen, this change in physical state does not reduce the ATP-ase activity proportionately, except perhaps in the first, fast phase.

Table II

Changes in gel-volume on addition of ATP

Conditions: ATP 4 mM, $MgCl_2$ 4 mM, 0·6 g. of fibrils in 6 ml. of imidazole buffer, pH 7·2

Fibril-volume (ml./g. fibrils) after centrifuging for 5 min. at 2800 r.p.m.

	0° $\mu = 0 \cdot 25$		18° $\mu = 0 \cdot 04$	
	Before ATP	After ATP	Before ATP	After ATP
Control	2·40	3·60	2·25	1·20
Irradiated	1·50	1·80	1·30	1·20
Differences	−0·90	−1·80	−0·95	0

Changes in nitrogen fractions

In Table III, values are given for nitrogen distribution in samples from three beef animals of the same prime quality grade. Those for animal F demonstrate the reproducibility of the extraction results for unirradiated sterile beef. Values for animal B correspond with those for samples taken for pH and water-retention (Table I).

Table III

Changes in the N fractions over long periods of storage of sterile unirradiated and irradiated samples of longissimus dorsi *muscles of beef animals*

N values are % of total N ; PN = Protein N ; NPN = Non-protein N ;
PN sol. in 0·1M-KCl = TN sol. in 0·1M-KCl − TN sol. in TCA

Storage temp. and days	pH (1) Initial (2) After irradiation (3) After storage	N sol. in TCA (NPN)	PN sol. in			(*a*+*b*+*c*) Total sol. PN	Insoluble residue, PN	Total tyrosine, mg./g. of muscle	% Total tyrosine sol. in TCA
			0·1M-KCl (*a*)	0·2M-Na-HPO$_4$ (*b*)	0·1M-citrate (*c*)				
Animal F									
Unirradiated									
−20° (7)	(1) 5·61 (3) 5·61	10·9	20·0	7·4	1·3	28·7	58·0	15·7	10·9
−20° (16)	(3) 5·61	10·9	18·8	7·4	1·7	27·9	57·5	—	—
Animal B									
Unirradiated									
	(1) 5·5								
−20° (90)	(3) 5·7	11·8	—	—	—	—	—	17·6	—
−20° (180)	(3) 5·7	11·3	20·1	—	—	—	—	17·6	—
−20° (365)	(3) 5·7	12·7	19·3	9·8	1·7	30·8	54·5	17·6	13·1
Irradiated									
−20° (90)	(2) 5·7 (3) 5·7	12·6	12·3	5·8	1·0	19·1	63·5	17·6	—
−20° (365)	(3) 5·8	11·8	13·4	4·6	0·7	18·7	67·2	17·6	11·7
Irradiated									
37° (90)	(3) 6·05	25·0	3·2	1·4	0·1	4·7	65·8	17·6	—
37° (180)	(3) 6·05	27·0	5·2	0·8	1·2	7·2	62·5	17·6	31·8
37° (365)	(3) 6·20	34·8	3·5	0·7	0·1	4·3	59·2	17·6	39·6
Animal C									
Unirradiated									
37° (63)	(1) 5·6 (3) 5·85	25·6	4·8	—	—	—	—	20·6	21·4
37° (172)	(3) 5·80	31·4	4·4	1·6	0·1	6·1	60·8	20·6	34·8

Since the values for the unirradiated control samples of animal B are similar to those for animal F, it would seem that no significant changes had taken place in these N fractions over a period of one year at −20°. There were no changes even in the irradiated samples between 90 and 365 days at −20°. In the absence of values immediately after irradiation, it can be assumed that the difference between the unirradiated and irradiated samples at −20° is due to the irradiation process which reduces the percentage of soluble protein from approximately 31 to 19% of the total N without any change in the TCA sol. N.

During storage at 37°, the changes in both the unirradiated and irradiated samples are very similar. There is a further large fall within 60–90 days in the solubility of the protein. A similar fall of the same magnitude takes place in unirradiated meat within a few days at 37°.[12] In both cases the fall is accompanied by proteolysis and results in a steady increase in TCA sol. N with concomitant increase in the total tyrosine content of this fraction. (Tyrosine crystals are evident in both pork and beef after storage for one year both at 25° and at 37°.)

After one year at 37°, 34·8% of the total N of irradiated beef is soluble in TCA, representing autolysis of protein equivalent to 22·6% of the total N or 25·8% of the original total N. Since the fibres still retain their striations after storage (Fig. 5) this would suggest that it is the sarcoplasmic proteins which are autolysed, leaving the structural proteins intact.

By extrapolation, the production of TCA sol. N in 90 days was found to be 0·065 mg. per g. of muscle per day in the unirradiated sample, compared with 0·052 mg. per g. of muscle per day for the irradiated sample. This difference is less than might be expected from published data on the inactivation of proteolytic enzymes by irradiation.[13, 14] It must be noted that the conditions in the two experiments were different. The irradiated samples were held in nitrogen, whereas unirradiated samples were held in sealed containers in air. The importance of this difference requires investigation before any final comparison can be made. Even so, it is clear

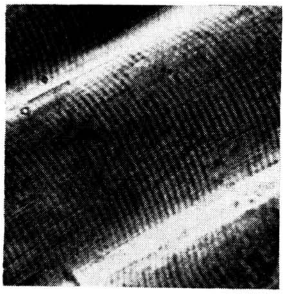

FIG. 5.—*Longitudinal section of beef* longissimus dorsi *muscle after storage for 52 weeks at 37° (irradiated)*

Fixed in formalin: Celloidin embedded
Stained: iron-haematoxylin (× 500)

that any reduction in proteolytic activity in meat by irradiation does not lead to any significant difference in this respect after long periods of storage at 37°.

In none of the samples was there any evidence of the breakdown of collagen or elastin leading to the presence of hydroxyproline in the hydrolysed soluble fractions. This corresponded with the absence of visible signs of significant changes in the main connective tissue structure of the tissues, even after 12 months at 37°. However, despite the similarity in the N fractions of the unirradiated and irradiated samples after 6 and 12 months respectively at 37°, there was, as noted above, a significant difference in the texture of the two samples when they were homogenised at low speed, indicating a greater degree of degeneration in the unirradiated sample. Apart from accumulation of 'weep' fluid, the main difference between control samples, irradiated and unirradiated, held at −20° and irradiated samples held at 37°, was that the latter had a semi-coagulated, crumbly texture, similar to the texture of rare cooked meat.

Discussion

The increase of muscle pH caused by a sterilising dose of ionising radiation aligns with previously published data,[15] as does that caused by freezing.[10, 16, 17] In both cases it appears to be due to denaturation of the muscle proteins, which is known to result in an increase in pH[10, 16, 18] and which is shown by a decrease in solubility of the proteins (Table III). That some structural modifications occur is shown firstly by the observed initial decrease in gel-volume of the treated muscles of beef, pig and rabbit (Tables I and II) and secondly by the feeble response of the irradiated myofibrils of the rabbit *psoas* to ATP, whether under optimal conditions for syneresis (i.e., at low ionic strength) or those for swelling (i.e., at high ionic strength). It is of some interest that the changes caused by irradiation reduce the ATP-ase activity of the irradiated myofibrils only to about 40% of the control in the first phase of splitting, and only to about 80% in the second, slower phase. These results, together with the increased resistance of the tissue to disintegration to single fibrils by high-speed homogenisation, are consistent with the supposition that irradiation causes cross-linking between protein molecules, resulting in some contraction of the fibrils and a more rigid structure. As a result, the fibrils will neither swell nor contract so readily as unirradiated fibrils when treated with ATP under appropriate conditions. The fact that fibrils from irradiated muscle continue to react with ATP indicates that the linkages involved in the cross-linking may be different from those involved in reaction with ATP, although the slower rate of reaction shows that the cross-links have a hindering effect. Hunt & Matheson[3] showed that freeze-drying has a similar effect on meat: water-binding, i.e., gel-volume, is more drastically affected than ATP-ase activity. Despite this evidence of denaturation, however, it is clear that the pH changes which can be attributable to it are insufficient to account for the whole increase of pH observed during storage of the irradiated samples. Thus the pH of beef muscle rises after complete heat-denaturation from about pH 5·6 to about 5·92,[10] whereas that of pork muscle rises under the same conditions by about 0·25 units. Taking the different buffer capacities of the two species into account, these increases in pH correspond in each case to the absorption of 7·5 g.-equiv. of H^+ per 10^5 g. of dry protein. On the other hand, in the extreme case of irradiation followed by storage for

12 months at 37°, the pH increase amounts to about 0·65 pH units for beef and about 0·75 units for pork (see Fig. 2), representing absorption of 14·6 g.-equiv. and 21·7 g.-equiv. of H^+ per 10^5 g. of protein, respectively. These discrepancies may be explained by the large degree of proteolysis which occurs during storage at 37°, as shown by the results in Table III and by the presence of crystalline tyrosine in the samples.

Another feature of the results, which suggests that the structural proteins are denatured but not proteolysed during irradiation and storage, is the persistence of the cross striations on the fibrils (Fig. 5), and the tendency for the gel-volume to increase with time of storage. As shown in Fig. 3, this increase can be attributed to the concomitant rise in the pH of the homogenate rather than to a breakdown of the proteins. It may be compared with a diminution of about 40% in the degree of drip (after freezing and thawing) in the *longissimus dorsi* muscle of beef between pH 5·6 and 5·8.[19]

The high pH of pork *longissimus dorsi* muscle stored for one month at −20° may be an artefact, since it can scarcely be due to either autolytic or simple denaturation changes; alternatively, it could represent some persistent effect of irradiation which is particularly emphasised in the limited aqueous phase still remaining at this temperature. That it should be found only in pork and not in beef is somewhat anomalous, but this accords with other differences between the *longissimus dorsi* muscles of these two species noted above. Thus, pork responds more markedly than beef to temperature- and time-dependent changes represented by an elevation of pH on storage: beef is more susceptible to loss of water-binding capacity induced by irradiation and to the gain induced by freezing; and the two species differ in the particular storage temperature which is associated with maximum gel volume. Such species differences have frequently been observed at the organoleptic level: the variation in taste panel scores attributable to the irradiation products hydrogen sulphide, mercaptans and carbonyls is twice as great in beef as in pork.[20, 21]

It would have been expected that changes due to denaturation or autolysis would be greatest at the highest temperature of storage (37°). The pH changes conform to this view. Yet the greatest increment in gel volume, after the 12-months storage period, was at 15° with beef and at 25° with pork. It is not possible to explain this phenomenon on present knowledge.

Owing to the different experimental conditions, the present results on irradiated beef cannot be compared with the observations of Radouco-Thomas and his colleagues on beef and pork.[22] In their work, the surface layer only was sterilised by irradiation (to a depth of a few mm.). Comparison of changes in the surface layer and in the interior showed that, although on storage the increases in TCA sol. N were approximately the same, the effect on the muscle fibres was quite different. In the unirradiated interior appreciable breakdown of fibres had taken place, whereas in the irradiated surface layer this breakdown had been largely inhibited.

The present results give a certain amount of support to these findings, since the fibres present in low-speed homogenates of beef muscle showed a significant effect of irradiation. Whereas irradiated fibres were smooth, with little sign of degeneration after one year at 37°, unirradiated fibres showed longitudinal and transverse zones of degeneration and cleavage after 6 months at 37°. There was, however, no evidence in beef of the advanced degree of degradation of the tissue structure observed by Swiss workers in rabbit muscle.[23] Both irradiated and unirradiated beef samples after 6–12 months at 37° had a texture similar to half-cooked meat but they were both still tough. Coleby, Ingram & Shepherd, although mainly concerned with changes in appearance, odour and taste, noted a softening of texture in beef and pork samples immediately after irradiation and further breakdown during storage.[24]

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ANTIFUNGAL ACTIVITY OF SUBSTITUTED NITROANILINES AND RELATED COMPOUNDS

By **N. G. CLARK** and **A. F. HAMS**

The preparation of a group of nitroanilines and some related compounds is described, together with two of the biological methods adopted for the assessment of their antifungal activity. Certain of these compounds show high activity against *Botrytis cinerea*, and one of them, 2,6-dichloro-4-nitroaniline, has been developed as a commercial fungicide (' Allisan ').

Introduction

While a number of Botrytis dusts are commercially available, none is entirely satisfactory and a search for an improved material has been pursued in these laboratories for some years. In the course of routine testing it soon became clear that a group of nitroanilines was highly active in certain tests.

This paper contains a description of the biological methods employed for initial screening and a discussion of the structure/activity relationships which emerged from the testing results. Since this work was completed, one of the compounds has been formulated and marketed under the name ' Allisan '.[1] This is a dust containing 2,6-dichloro-4-nitroaniline, used for the control of *Botrytis* in lettuce grown under glass during the winter. Its practical applications and overall efficacy form the subject of another paper.

Experimental

Preparation of materials

All the chemicals used in this investigation are known in the literature and were prepared by standard methods. The preparation of some of them, however, raised points of interest, and these are now reported briefly.

2,4,6-*Trichloro*-3-*nitroaniline*.—A poor yield of the desired material, highly contaminated with tar, was obtained by the action of chlorine on a solution of *m*-nitroaniline in hydrochloric acid, but nitration of acet-2,4,6-trichloroanilide[2] and then hydrolysis afforded a satisfactory yield.

6-*Bromo*-4-*chloro*-2-*nitroaniline*.—The only previous references to this compound appear to be by Körner,[3] who gives a m.p. of 106·4°, and by Orton[4] who quotes a value of 114–115°. The present work yielded a compound melting at 107–107·5° (Found: C, 28·9; H, 1·5. Calc. for $C_6H_4BrClN_2O_2$: C, 28·6; H, 1·6%).

4-*Bromo*-6-*chloro*-2-*nitroaniline*.—This compound is best prepared by direct chlorination of 4-bromo-2-nitroaniline.[5] A solution of 4-bromo-2-nitroaniline (25 g.) in benzene (750 ml.) was maintained below 30° while a slow stream of chlorine was bubbled in for about 1 h. The resulting solid was collected, washed with a little benzene, stirred with cold water for 10 min., and again collected. Two crystallisations from ethanol gave 9·1 g. of material m.p. 117–118°. Orton[4] gives m.p. 114°.

4,6-*Dichloro*-N-*methyl*-2-*nitroaniline*.—A more convenient method of preparing this compound is as follows. A solution of 4-chloro-*N*-methyl-2-nitroaniline[6] (10 g.) in glacial acetic acid (100 ml.) was treated with conc. hydrochloric acid (50 ml.); an aqueous solution of potassium chlorate (3·1 g. in 50 ml.) was then dropped in during 1 h. After being stirred for a further 2 h. during which time nearly all the solid dissolved, the liquid was filtered, diluted with water, and the resulting solid collected. Thorough washing with water, followed by two crystallisations from ethanol, gave the product (4·2 g.) as orange-red crystals m.p. 78–79°. Blanksma[7] quotes m.p. 80°.

2,3,5,6-*Tetrachloro*-4-*nitroanisole*.—The only previous references to this compound are by Berckmans & Holleman,[2] who give a m.p. of 105–106°, and by Peters *et al.*,[8] who quote a value of 112–113°. The present work yielded a compound melting at 104–105° (Found: C, 29·1; H, 1·2. Calc. for $C_7H_3Cl_4NO_3$: C, 28·9; H, 1·0%).

4-*Chloro*-NN-*dimethyl*-2-*nitroaniline*.—A more convenient method of preparation appears to be the following. A mixture of 2,5-dichloro-nitrobenzene (90 g.), methanolic dimethylamine solution (120 g., 30% w/w) and ethanol (120 ml.) was heated in an autoclave for 4 h. at 160°. After being cooled, the solid product was removed, washed with a little methanol, and recrystallised from the same solvent. Yield, 75 g., m.p. 54–55°. Clemo & Smith[9] give m.p. 56°.

2,6-*Dichloro*-N-*methyl*-4-*nitroaniline*.—This material is conveniently obtained as follows. A solution of 2-chloro-*N*-methyl-4-nitroaniline[10] (10 g.) in a mixture of glacial acetic acid (100 ml.) and conc. hydrochloric acid (50 ml.) was stirred during 30 min. while an aqueous solution of potassium chlorate (3·1 g. in 50 ml.) was added dropwise. After being stirred for a further 2 h., the reaction mixture was diluted with water, the precipitate collected, washed thoroughly with water, and crystallised twice from ethanol. Yield, 3·4 g., m.p. 83–84°. Qvist & Nermes[11] report a m.p. of 82·5–83°.

2,3,5,6-*Tetrachloro*-4-*nitrophenol*.—A mixture of 2,3,5,6-tetrachloro-1,4-dinitrobenzene (20 g.), aqueous sodium hydroxide solution (140 ml. of 1N) and water (140 ml.) was refluxed for 3 days, cooled and filtered. The deep red filtrate was acidified with hydrochloric acid, the precipitate collected, washed with a little water, and recrystallised from aqueous acetic acid. The yield of colourless needles was 5·4 g., m.p. 147–148° (decomp.). This compound has been obtained previously only as a by-product, m.p. 148–149° (decomp.).[8]

Methods of testing

(a) *Impregnation test*

This test is designed to assess the effect of chemicals upon the mycelial growth of fungi by physical contact and also, where the compounds are volatile, by vapour-phase action.

Agar containing 2% malt extract is prepared, melted and divided into 20-ml. portions each in a separate tube; these are then sterilised for 20 min. at 20 p.s.i. steam pressure. Calculated quantities of stock acetone solutions of the test chemicals are added to the tubes of hot agar to give an initial concentration of 10 p.p.m. of chemical; most of the acetone evaporates. The contents of the tubes are poured into sterilised Petri dishes, which are agitated to remove the last traces of acetone and to mix the chemical thoroughly with the agar. When set, these plates are inoculated with a loopful of a spore suspension of *Botrytis cinerea*. This is done with the plates inverted so that the agar surface is facing downwards at the time of inoculation. The plates are incubated in this position for 6–7 days at 23°, by which time the diameter of the fungus colony in the control plates (without chemical) is approximately 80 mm. Assessment is based on the mean colony diameter, measured in two directions at right-angles, for each of three replicates.

The percentage degree of inhibition of mycelial growth is given by the formula $100(C-T)/C$, where C and T are the diameters of the untreated (control) and treated colony, respectively. Where any concentration of chemical produces a control of greater than 50%, the compound is re-tested at lower concentrations down to 1 p.p.m.

(b) *Spore germination test*

A detailed description of this technique has already been given.[12] The test requires drops of an aqueous spore suspension of the test fungus to be placed on glass slides previously coated with a thin layer of the chemical under investigation. After incubation for 18 h., the effect of the chemical upon spore germination is assessed microscopically. The antifungal activity is expressed as percentage inhibition of germination, and is related to the strength of the acetone solution of the chemical used to coat the glass slide (not, directly, to the density of the deposit).

Since the previous publication, the range of fungi has been altered, and now consists of *Botrytis cinerea*, *Cladosporium fulvum* and *Venturia inaequalis*. Also, the highest concentration of test chemical in acetone has been reduced to 80 p.p.m., giving a dilution sequence of 80, 16, 8, 4, 2.

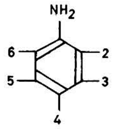
Results

Table I contains a list of the 48 compounds examined for antifungal activity, and includes

Table I

Impregnation test

Nitroanilines

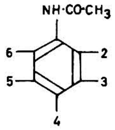


	Substituents						% Control at a concentration of		
	N	2	3	4	5	6	5 p.p.m.	2·5 p.p.m.	1 p.p.m.
1		Cl	CH_3	Cl		NO_2	73	86	10
2		Cl		Cl		NO_2	98	83	44
3		Cl		NO_2		Cl	86	74	42
4		Br		Cl		NO_2	84	72	52
5		Cl		Br		NO_2	80	63	40
6		Br		NO_2		Cl	98	77	47
7		Br		NO_2		Br	78	49	—
8		I		NO_2		I	52	39	—
9	CH_3	Cl		Cl		NO_2	82	58	37
10	CH_3	Cl		NO_2		Cl	81	58	40
11	$2CH_3$	Cl		NO_2		Cl	68	17	—
12		Cl		NO_2	Cl		61	43	—
13		Cl	NO_2	Cl		Cl	82	68	29
14		Cl	Cl	NO_2	Cl	Cl	37*		
15		Cl		NO_2		NO_2	79	42	—
16		NO_2		NO_2		NO_2	55	24	—
17		NO_2		Cl			52	4	—
18		Cl		NO_2			16	—	—
19		NO_2		Br			24	—	—
20		Br		NO_2			47	—	—
21	CH_3	NO_2		Cl			33	—	—
22	CH_3	Cl		NO_2			37	—	—
23	$2CH_3$	NO_2		Cl			21	—	—
24	$2CH_3$	Cl		NO_2			16	—	—
25		Cl		NO_2		CH_3	45	—	—

Table I (*continued*)

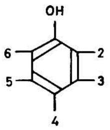
N	Substituents 2	3	4	5	6	% Control at a concentration of 5 p.p.m.	2·5 p.p.m.	1 p.p.m.
26	Cl		NO_2		CH_3O	23	—	—
27	NO_2		CH_3			5	—	—
28	NO_2		CH_3O			23*	—	—
29	CH_3		NO_2			38*	—	—
30	CH_3O		NO_2			13*	—	—
Standard	(T.C.N.B.)					80	58	45

Acetanilides



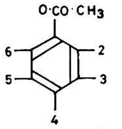
N	2	3	4	5	6	5 p.p.m.	2·5 p.p.m.	1 p.p.m.
31	Cl		Cl		NO_2	33*	—	—
32	Cl		NO_2		Cl	41*	—	—
33	Cl		NO_2		NO_2	24*	—	—
34	NO_2		Cl			14	—	—
35	NO_2		CH_3			50	—	—
36	NO_2		CH_3O			12*	—	—
37	CH_3O		NO_2			0	—	—

Phenols



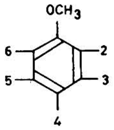
N	2	3	4	5	6	5 p.p.m.	2·5 p.p.m.	1 p.p.m.
38	Cl		NO_2		Cl	27	—	—
39	Cl	Cl	NO_2	Cl	Cl	56	34	—
40	Cl		NO_2			61	9	—

Phenyl acetates



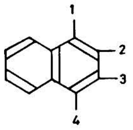
N	2	3	4	5	6	5 p.p.m.	2·5 p.p.m.	1 p.p.m.
41	Cl		NO_2		Cl	23	—	—
42	Cl	Cl	NO_2	Cl	Cl	51	14	—
43	Cl		NO_2			32	—	—

Anisoles



N	2	3	4	5	6	5 p.p.m.	2·5 p.p.m.	1 p.p.m.
44	Cl		NO_2		Cl	83	38	—
45	Cl	Cl	NO_2	Cl	Cl	76	27	—

Naphthalenes



N	Compound	5 p.p.m.	2·5 p.p.m.	1 p.p.m.
46	1-Amino-4-chloro-2-nitro-	28	—	—
47	1-Acetamido-4-chloro-2-nitro-	0	—	—
48	1-Amino-2,4-dinitro-	25	—	—

* At a concentration of 10 p.p.m.

their chemical structure and biological activity in the impregnation test. The results obtained with 2,3,5,6-tetrachloronitrobenzene (T.C.N.B.), used as a standard, are also included. Because of the generally high activity displayed in this test, as distinct from the poor response in the other test, the compounds are arranged in Table I in a manner best designed to illustrate the relationship between chemical structure and activity against *Botrytis cinerea*. The same numerical arrangement is retained for the other test.

Table II shows the results of the spore germination test. These are presented as the approximate strength of an acetone solution of the chemical which would effect a 50% inhibition of spore germination (i.e., the approx. LD_{50}).

Table II

Spore germination test

(results are approx. LD_{50} in p.p.m.)

Compound no.	*Botrytis cinerea*	*Cladosporium fulvum*	*Venturia inaequalis*
1	>80	>80	>80
2	>80	>80	>80
3	>80	>80	>80
4	50	>80	>80
5	>80	>80	>80
6	>80	>80	>80
7	>80	>80	>80
8	>80	>80	>80
9	>80	>80	>80
10	50	>80	>80
11	>80	60	>80
12	>80	50	>80
13	50	60	50
14	>80	>80	>80
15	50	>80	50
16	60	12	50
17	>80	>80	>80
18	>80	>80	>80
19	50	>80	70
20	70	70	70
21	>80	>80	>80
22	>80	>80	>80
23	>80	>80	>80
24	>80	>80	>80
25	>80	>80	>80
26	>80	>80	>80
27	>80	>80	>80
28	>80	>80	>80
29	>80	>80	>80
30	>80	>80	>80
31	80	>80	>80
32	80	>80	>80
33	>80	>80	>80
34	50	>80	>80
35	>80	>80	>80
36	>80	>80	>80
37	>80	>80	>80
38	70	>80	70
39	50	60	2
40	50	60	5
41	50	>80	14
42	>80	16	14
43	50	50	50
44	>80	>80	>80
45	>80	70	>80
46	>80	>80	>80
47	>80	>80	>80
48	>80	>80	>80

Table III presents a typical selection of results from greenhouse trials. Five of the compounds giving the best control of *Botrytis cinerea* in the impregnation test were formulated at 4% dusts and used for treating commercially grown lettuce. Results on untreated lettuces are included.

Table III

Greenhouse trials

Compound no.	No. of survivors after 15 weeks (max. 140)	% marketable	Average weight per plant, oz.
2	140	46	1·3
3	130	94	1·8
4	137	97	1·1
5	125	89	1·2
6	133	90	2·1
Untreated	74	40	1·5

Discussion

(a) *Impregnation test*

The present series of compounds was prepared and tested as a result of the high activity exhibited in the impregnation test by compound 1, an intermediate submitted for routine screening. The methyl group makes this compound relatively inaccessible, but the lower homologue (without the methyl group) is readily available. It was not surprising to find, therefore, that omission of the inert methyl group (compound 2) caused no loss of activity. A comparable level of activity was also exhibited by an isomer, 2,6-dichloro-4-nitroaniline (compound 3), which was ultimately developed as a commercial product.[13] Taking compounds 2 and 3 as models, a group of analogues was prepared in which one or both of the chlorine atoms has been replaced by bromine or iodine (compounds 4–8). High activity was again evident, although there were signs of this diminishing in the high-molecular-weight di-bromo and di-iodo derivatives.

Mono-methylation of compounds 2 and 3 in the amino-group (compounds 9 and 10) had no effect on the activity, but dimethylation caused a diminution in antifungal power (compound 11).

Another isomer of compounds 2 and 3 had good activity (compound 12) ; this was also demonstrated by compound 13 containing three chlorine atoms. Compound 14, however, containing four chlorine atoms, was almost devoid of activity. The presence of halogen appeared to be essential for high activity, since partial deactivation occurred when one or both of the chlorine atoms in compound 3 were replaced by nitro-groups (compounds 15 and 16).

Few other structural modifications could be effected without greatly impairing the biological efficacy of the compounds. Thus, omission of one of the halogen atoms, leaving one halogen and one nitro-group, reduced activity to a low level (compounds 17–24), while replacement of one of the two chlorine atoms of compound 3 by a methyl or methoxyl group had a similar effect (compounds 25 and 26). Some other mono-substituted nitroanilines were almost devoid of activity (compounds 27–30).

The essential nature of the amino-group, if activity is to be retained, is revealed by the poor biological activity of the remaining compounds in Table I. Acetylation of compounds 2, 3 and 15 almost eliminated the activity (compounds 31–33), and did not improve a poor activity in other cases (compounds 34–37). Replacement of the amino-group by hydroxyl gave a group of phenols (compounds 38–40), but neither they nor their acetates (compounds 41–43) showed the same general activity that the related anilines exhibited. Two anisole derivatives, in which the original amino-group has been replaced by methoxyl, showed moderate activity (compounds 44–45). There appeared to be no compound of promise in the naphthalene series (compounds 46–48).

It is unwise to suggest a possible mode of action of these compounds based on the present limited evidence, but the presence of a free amino-group bearing at least one hydrogen atom seems essential for high activity. If this feature is destroyed (by acetylation or dimethylation) or masked (by two massive iodine atoms or nitro-groups in the *ortho* positions), then biological activity is markedly diminished.

(b) *Spore germination test*

In this test, the chemicals presented an almost uniform picture of inactivity, although compounds 39 and 40 showed high activity against one of the fungi.

(c) *Greenhouse trials*

Results from the impregnation test showed that nine compounds (1–6, 9, 10 and 13) exhibited high activity against *Botrytis cinerea*, and warranted further testing. Because of the relatively difficult syntheses involved (which would have resulted in too expensive a final product), four of these were omitted (compounds 1, 9, 10, 13). The remainder, in the form of 4% dusts, were assessed for their control of *Botrytis cinerea* on winter lettuce grown on a commercial scale. Some typical results from randomised block trials are given in Table III.

Only compounds 3 and 6 produced a satisfactory crop of lettuce, considering both numbers and size. The former, 2,6-dichloro-4-nitroaniline, was preferred to the latter, the closely related 2-bromo-6-chloro-4-nitroaniline, because it was more economical to manufacture. 2,6-Dichloro-4-nitroaniline has now been developed commercially.

Acknowledgments

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EFFECT OF SUPPLEMENTING WITH METHIONINE, CYSTEINE AND DERIVATIVES OF THIAZOLIDINE-4-CARBOXYLIC ACID ON THE NUTRITIVE VALUE OF HERRING-MEAL PROTEIN

By L. R. NJAA

The nutritive value of herring-meal protein was improved by supplementation with methionine, cysteine and derivatives of thiazolidine-4-carboxylic acid.

The effects with graded amounts of methionine were greater than would be expected from the contents of methionine and cystine in herring-meal and from the requirement of the young rat for these amino-acids.

Methionine supplementation also improved the nutritive value of an acetone-dried meal prepared from herring fillets.

The results are explained primarily by the fact that methionine + cystine is the first limiting factor in herring protein. Destruction of amino-acids during meal production seems to be small, although some destruction of the *S*-amino-acids cannot be completely excluded.

Introduction

The utilisation of fish-meal protein by the young rat is improved by supplementing it with methionine. Miller[1] explained this by assuming that protein damage during fish-meal production was due to reactions of the Maillard[2] type.

The present work was undertaken to test whether, and to what extent, methionine was the limiting amino-acid in herring-meal. Supplementation with cysteine, and with some reaction products of cysteine and some aldehydes, was also tested. The latter products, which have been referred to as colourless Maillard compounds, are probably derivatives of thiazolidine-4-carboxylic acid.[3-5]

Experimental

Twelve nitrogen-balance experiments were performed, eleven with herring-meal (H-m) as the sole source of protein and one with extracted soya-bean meal (S-m), by procedures described previously,[6] except that the collection periods were 5 instead of 6 days. The daily food intake was kept constant at 10 g./rat/day in all experiments except in Expt. 13 in which it was 8 g. The protein content of the diets was 8% except in Expts. 1 and 5 in which it was 10%. The term 100 U/I[6b] was used as a measure of utilisation, high values corresponding to low utilisation and *vice versa* (U = urinary nitrogen ; I = nitrogen intake).

In two experiments protein efficiency ratios (P.E.R.)[7] were determined for 28-day periods ; the food was given *ad lib.* Herring-meal and an acetone-dried meal prepared from herring fillets were used as protein sources.

Table I

Protein contents of products used

Product no.	Protein content (% N × 6·25)	Description	Product used in
H-m* 6	72·9	Vacuum-dried whole meal	Expts. 8, 9
H-m 7	73·9	Flame-dried press-cake meal	Expts. 6, 7
H-m 11	75·5	Flame-dried whole meal	Expts. 1, 5, 6, 7
H-m 52	73·8	Flame-dried whole meal	Expts. 2, 3
H-m 80	76·3	Flame-dried whole meal	Expts. 12
H-m 107	72·3	Flame-dried whole meal	Expts. 13, 14
H-m 110	71·4	Flame-dried whole meal	Expts. 10, 11
S-m† 111	47·8	Heated and solvent-extracted	Expt. 4
F-m‡ 112	83·8	Acetone-dried	Expt. 11

* H-m = herring-meal ; † S-m = soya-bean meal ; ‡ F-m = meal from herring fillets

Descriptions of the products and their protein contents are given in Table I. Each experiment comprised four treatment groups made up from six groups of four litter-mate rats 4–5 weeks old. Within litters the rats were of the same sex. The amino-acid supplements were made isonitrogenous by use of glycine. The nitrogen in food and urine was determined by a micro-Kjeldahl technique.[8]

The derivatives of thiazolidine-4-carboxylic acid were synthesised by methods given in the literature.[9] The nomenclature for these compounds followed here is that of Weitzel *et al.*,[9b] who combined the name of the aldehyde with cysteine. Thus, thiazolidine-4-carboxylic acid, which is the reaction product obtained from cysteine and formaldehyde, becomes cysteine-formaldehyde.

Results

Experiments with graded amounts of methionine

The results obtained by supplementing herring-meal (Expts. 1–3) and extracted soya-bean meal (Expt. 4) with graded amounts of methionine are summarised in Fig. 1.

The herring-meals were flame-dried whole meals, H-m 11 being used in Expt. 1 and H-m 52 in Expts. 2 and 3. Both meals gave improved results with methionine supplements, thus confirming the work of Miller[1] with fish-meals. It is seen that each increment in the methionine supplement improved the utilisation of herring-meal protein. The effect of equal increments as measured by the reduction in the value of 100 U/I was greater at the lower supplementation levels than at the higher. Statistical analyses indicated significant treatment effects in all experiments. In Expt. 1 the supplement was increased on a geometric, in Expts. 2 and 3 on a linear, scale. On these scales the linear components of the sum of squares,[10] but not the quadratic and the cubic, were significant in all cases.

In the experiment with soya-bean meal, methionine supplementation improved the protein utilisation up to the 2% level, with no further improvement at the 3% level. The supplement was increased on a linear scale on which the linear and the quadratic components of the treatment sum of squares were significant.

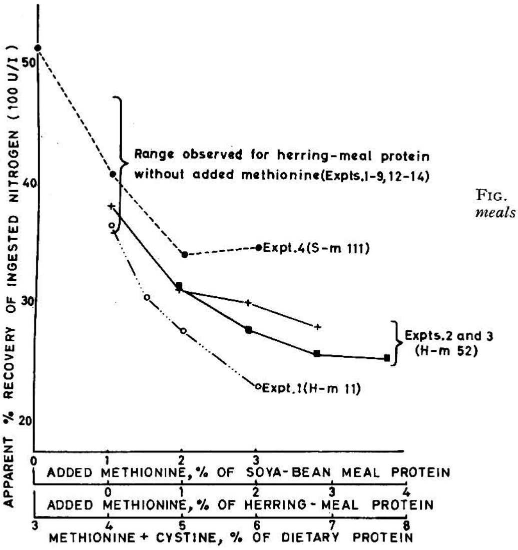

FIG. 1.—*Supplementation of two whole herring-meals and of an extracted soya-bean meal with graded amounts of methionine*

Further supplementation experiments

Results from experiments involving supplementation with methionine are summarised in Table II, those with derivatives of thiazolidine-4-carboxylic acid in Table III. The types of meal used are described in Table I.

Table II

Supplementation of herring-meals (H-m) and of a fillet-meal (F-m) with methionine, alone and in combinations

Expt. no.	Protein source	Supplement (% of protein)	100 U/I	P.E.R.
5	H-m 11	Gly (0·5)	41·3	
		Lys·HCl* (0·6)	40·3	
		CySH·HCl (1·1)	33·9	
		Met (1·0)	33·9	
		S.E. (15df)	±1·61	
	H-m 7	Gly (0·6)	35·9	
		Met (1·25)	22·8	
	H-m 11	Gly (0·6)	37·0	
		Met (1·25)	24·8	
		(H-m 11)-(H-m 7)±S.E. (5df)	1·6±0·83	
		Gly-Met±S.E. (5df)	12·6±1·01	
7	H-m 7	Gly (1·2)	47·4	
		Met (2·50)	31·1	
	H-m 11	Gly (1·2)	44·9	
		Met (2·50)	29·9	
		(H-m 11)-(H-m 7)±S.E. (5df)	−1·9±1·12	
		Gly-Met±S.E. (5df)	15·6±0·98	
8	H-m 6	Gly (1·9)	41·2	
		Gly (0·6) Lys·HCl (1·5)	45·1	
		Gly (1·25) Met (1·25)	32·4	
		Lys·HCl (1·5) Met (1·25)	30·9	
		S.E. (15df)	±1·14	
9	H-m 6	Gly (1·9)	45·9	
		Gly (0·6) Val (2·0)	44·5	
		Gly (1·25) Met (1·25)	32·3	
		Val (2·0) Met (1·25)	32·8	
		S.E. (15df)	±1·23	
10	H-m 107	Gly (2·44)		2·39
		Gly (1·89) Try (1·5)		2·41
		Gly (0·55) Met (3·75)		3.18
		Try (1·50) Met (3·75)		3·09
		S.E. (15df)		±0·11
11	H-m	Gly (1·0)		2·73
		Met (2·0)		3·11
	Fm	Gly (1·0)		3·02
		Met (2·0)		3·29
		(H-m)-(F-m)±S.E. (5df)		0·23±0·11
		Gly-Met±S.E. (5df)		0·33±0·048

* HCl = hydrochloride

In all experiments, supplementation with methionine (Expts. 5–11) or cysteine (Expts. 5, 12–14) enhanced the utilisation of herring-meal protein. A flame-dried press-cake meal (H-m 7) and a flame-dried whole meal (H-m 11) were improved by methionine to the same extent at the two levels tested (Expts. 6 and 7). A vacuum-dried whole meal (H-m 6) was also improved to about the same extent (Expts. 8 and 9). At the 1% supplementation level equivalent amounts of methionine and cysteine hydrochloride were equally effective (Expt. 5).

Lysine, DL-valine and DL-tryptophan were without any effect when tested alone or in combination with methionine (Expts. 5, 8–10). The supplements used represent increases in the amounts of lysine present in whole meal[11] of about 7% (Expt. 5) and 15% (Expt. 8), about 19% for valine (Expt. 9), and about 100% for tryptophan (Expt. 10), when only the L-forms in the supplements are considered. Lysine was tested because of its known tendency to be affected by the Maillard reaction,[12] while calculations indicated that valine and tryptophan might be the limiting amino-acid in herring-meal protein (see under Discussion).

The meal from acetone-dried herring fillets was improved by methionine supplementation but to a lesser extent than was the whole meal with which it was compared (Expt. 11). With methionine herring-meal was as good as fillet-meal without methionine. The overall difference in P.E.R. between fillet-meal and herring-meal was not significant. Miller[1] did not test supplementation of the carefully dried laboratory products.

Table III

Supplementation of herring-meals (H-m) with cysteine hydrochloride, and with derivatives of thiazolidine-4-carboxylic acid

Expt. no.	Protein source	Supplement (% of protein)	100 U/I
12	H-m 80	Glycine (0·40)	41·1
		Cysteine hydrochloride (0·84)	36·7
		Cysteine-glucose (1·50)	37·8
		Cysteine-arabinose (1·35)	40·5
		S.E. (15 df)	±2·09
13	H-m 107	Glycine (0·80)	42·0
		Cysteine hydrochloride (1·68)	32·9
		Cysteine-glucose (3·00)	34·0
		Cysteine-arabinose (2·70)	37·7
		S.E. (15df)	±2·48
14	H-m 107	Glycine (0·43)	43·6
		Cysteine hydrochloride (0·90)	32·8
		Cysteine-formaldehyde (0·76)	33·6
		Cysteine-butyraldehyde (1·00)	34·1
		S.E. (15df)	±1·28

In the experiments with derivatives of thiazolidine-4-carboxylic acid (Table III), cysteine-arabinose seemed to be less well utilised than cysteine hydrochloride and cysteine-glucose (Expts. 12, 13), but the treatment effect was not significant. However, the treatment effect was significant when the results in Expts. 12 and 13 were pooled in the analysis of variance. In Expt. 14, in which cysteine-formaldehyde and cysteine-butyraldehyde were tested, the treatment effect was significant. Subdivision of the treatment sum of squares in Expts. 12 and 13 combined and in Expt. 14 indicated significant differences only between the negative control group on the one hand and the mean of the other three groups on the other. Cysteine-glucose, cysteine-arabinose, cysteine-formaldehyde and cysteine-butyraldehyde were then apparently equally good sources of cysteine as was cysteine hydrochloride. It is possible, however, that, if further experiments were performed, cysteine-arabinose might prove a poorer source than the other.

Discussion

The first limiting amino-acid in herring-meal

The results obtained are in agreement with those of Miller[1] in so far as they show that herring-meal protein is improved by supplementation with methionine.

It is of interest to discuss whether the results could have been predicted from the amino-acid content of herring-meal seen in relation either to a standard protein[13] or to the amino-acid requirements of the growing rat.[14] Two sets of amino-acid analyses of Norwegian herring-meal are available.[11, 15] Chemical scores[13] were calculated with two sets of standard values based on whole egg-protein[13, 16] and, with one set of so-called 'target' values,[17] per cent deficits of the individual amino-acid were calculated on the basis of two sets of amino-acid requirements determined for the young rat.[18, 19] One set of analyses[15] indicated that valine[13, 16, 17, 19] and tryptophan[18] were the first limiting amino-acids, the other set[11] indicated tryptophan[13, 16, 18, 19] and methionine + cystine.[17] The combination of 'target' values[17] with the analyses of Boge[11] predicts correctly that methionine + cystine is the first limiting factor in both press-cake meal and whole meal. Similarly, combination of 'target' values with the analyses of fibrin used by Kumta & Harper[20] correctly predicts that methionine is not the first limiting factor in this protein source. This contrasts with the predictions based on rat requirements[18, 19] made by Kumta & Harper.[20]

The few experiments bearing on this point (Expts. 8–10) did not disclose the second limiting amino-acid in herring-meal protein. Similarly, Miller[1] did not establish the second limiting amino-acid in fish-meal. In both instances the failure may have been due either to the use of too low levels of methionine supplementation or to failure to test the proper amino-acid in combination with methionine. The problem of the sequence in which the amino-acids become limiting in fish protein including herring and herring-meal is to be investigated.

The quantitative aspects of methionine and cysteine supplementation are discussed for whole meal because this type of meal was used when graded amounts of methionine and when cysteine hydrochloride were tested. Whole meal contains about 4% of its protein as methionine + cystine,[11, 15] the cystine/methionine ratio being about 0·5. The 'target' value for the sulphur-containing amino-acids is 4·7 with a cystine/methionine ratio of about 0·75.[17] Ratios in the range 0·5–0·7 seem to allow effective utilisation of the cystine.[21] Therefore, effective supplementation of whole-meal protein with methionine would be expected up to the 0·7% level and with equivalent amounts of cysteine or cystine. The observed effects of cysteine supplementation may therefore probably be accounted for (Expts. 5, 12–14). For methionine, on the other hand, the effects of supplementation were observed above this level (Fig. 1). Three explanations may be suggested for this: (1) the 'target' value[17] is too low; (2) methionine and/or cystine in herring-meal are not fully available to the rat; (3) the analyses of these amino-acids are not correct.

The results obtained with soya-bean meal (Expt. 4, Fig. 1) may throw some light on the first two of these points. Methionine is the limiting amino-acid in soya-bean protein, which contains about 3% methionine + cystine.[22–26] The cystine/methionine ratio is higher than unity and methionine supplementation should be effective up to about the 2% level, with no further improvement above this. This is actually what was observed in Expt. 4. The results may, therefore, be interpreted to indicate that the 'target' value[17] for the sulphur-containing amino-acids is approximately correct. According to the analyses, soya-bean protein with 1% methionine should be equivalent to herring-meal protein without methionine. This is shown in Fig. 1. As the 100 U/I values for the former were within the range observed with the latter (Fig. 1), it is concluded that the availabilities of the sulphur-containing amino-acids in the two protein sources were not significantly different. Rosenberg *et al.*[27] obtained with chicks a greater effect of methionine supplementation in fish-meal diets than in a soya-bean meal diet, which agrees with the present results with rats.

The question whether the analyses of sulphur-containing amino-acids in herring-meal are incorrect cannot be answered definitely. The values reported for Norwegian press-cake meal[11, 15] are in agreement with literature values for herring-meal which probably refer to press-cake meals.[25, 26, 28, 29] The values for whole meal were, as expected, a little lower.[11] Any errors in the values may be attributed to losses during the preparation of the samples for analysis, but this would not explain the results obtained in the supplementation experiments.

Experiments with derivatives of thiazolidine-4-carboxylic acid

These substances were included in the study to test whether the postulated damage of fish-meal protein during the production procedure[1] arose from reactions between cysteine and aldehyde groups leading to the formation of compounds containing the thiazolidine ring.

Unlike the Maillard reaction, this reaction does not require free amino-groups and the thiazolidine ring may be formed on the peptide chain[30] (Fig. 2). Lea *et al.*[31] showed that propylaldehyde reacts with cod muscle protein and that more aldehyde reacts than can be accounted for by the disappearance of free amino-groups. Propylaldehyde and malondialdehyde were mentioned as types of volatile and non-volatile aldehydes in herring-meal capable of reaction.[31]

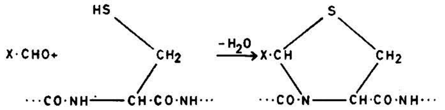

FIG. 2.—*Assumed reaction between cysteine-containing peptides and aldehydes*

The results obtained with the thiazolidine-4-carboxylic acid derivatives showed that they were available to the rat, as are cysteine-formaldehyde and cysteine-acetaldehyde.[32] The present results indicate that, even though the differences in utilisation between the compounds and cysteine hydrochloride are not significant, the latter tends to be utilised slightly better than the former. This may indicate that compounds containing the thiazolidine ring are utilised more slowly than is cysteine hydrochloride. Utilisation of peptide-bound compounds may be even slower if the presence of the thiazolidine ring affects the enzymic breakdown of the peptide containing it.

The unexpected extra effect at high methionine levels of supplementation (Expts. 1–3, Fig. 1) may be explained by assuming the presence in herring-meal protein of pools of sulphur-amino-acids with different rates of utilisation. Those utilised at the slower rates would be decreasingly drawn upon at increasing supplementation levels, so that simple summation of supplement and sulphur-amino-acids present in the meal would not apply. Pools of cystine with different chemical reactivity have been demonstrated in wool,[33] in some food proteins[34] and in insulin.[35] Such pools in herring-protein might be utilised differently or they may be differently affected during manufacture of herring-meal, with possible effects on biological utilisation. It has been demonstrated that some derivatives of thiazolidine-4-carboxylic acid are utilised better than others as cysteine sources for *Leuconostoc mesenteroides.*[36]

Methionine and methionine-sulphoxide are equivalent for slow-growing rats.[37] It is possible, however, that, at higher rates of gain, methionine and its sulphoxide may constitute pools with different rates of utilisation. Lea *et al.*[31] pointed out that formation of the sulphoxide was very probable during the production of herring-meal.

Experiment with fillet-meal

Miller[1] surmised that part of the methionine in fish-meal was ' rendered unavailable during processing ', but supplementation of carefully dried laboratory products was not tested.

In Expt. 11 methionine supplementation improved the utilisation of both fillet-meal and herring-meal. This is explained by assuming that the *S*-amino-acids are the limiting factor in herring-protein. Available analyses,[28, 38] compared with ' target values ',[17] confirm this.

It was expected that herring-meal would be less well utilised than fillet-meal because of the presence in the former of proteins from bones and viscera.[39] The difference observed was in the expected direction, but was statistically insignificant.

The results taken together seem to indicate that the extent of destruction of amino-acids during production of these herring-meals is of little importance. Some destruction of *S*-amino-acids cannot, however, be excluded.

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CARBON DIOXIDE PRODUCTION DURING INCUBATION OF SOILS TREATED WITH CELLULOSE AS A POSSIBLE INDEX OF THE NITROGEN STATUS OF SOILS

By **A. H. CORNFIELD**

The amount of carbon dioxide produced during incubation (3 weeks at 28°) of 18 soils (of varying texture and pH) treated with 1% cellulose was highly correlated with the total mineral nitrogen content of unamended soils after 3 weeks' incubation. This 'indirect incubation technique' appears a convenient method for assessing the nitrogen status of soils, as there is no need to extract and determine mineral nitrogen.

Introduction

The technique of assessing the potential nitrogen-supplying power (nitrogen status) of soils by incubation under standard conditions of temperature and moisture, followed by the determination of the amount of nitrogen mineralised, has been much used and has in many cases shown to be suitable for indicating the nitrogen fertiliser requirements of soils. This subject has been well summarised by Harmsen & van Schreven.[1] The main drawback of the method is the time and labour required in extracting and determining the nitrogen mineralised during incubation. An alternative procedure which avoids this would obviously be desirable.

If a soil is mixed with an excess of a nitrogen-free easily decomposable material, e.g., cellulose, and then incubated, the amount of carbon dioxide produced during incubation will be dependent, providing no other factor is limiting, on the amount of mineral nitrogen present initially, as well as on that mineralised during incubation. No mineral nitrogen will accumulate since it will be used as it is formed by the organisms decomposing the cellulose. Harmsen & van Schreven[1] have discussed the possibilities of this ' indirect incubation technique ' for assessing the nitrogen status of soils and have pointed out that little work appears to have been done on it. Bould[2] has applied the method for assessing the availability of nitrogen in composts.

The work reported in this paper was carried out to see whether there was, in fact, a significant correlation between the accumulation of mineral nitrogen (initial mineral nitrogen plus that formed during incubation) during ordinary incubation of a variety of soils and carbon mineralisation when the same soils were incubated after addition of 1% cellulose.

Experimental

Eighteen soils (air-dried, 2 mm. sieved) of widely different texture and pH, selected on the basis of a screening test[3] to give a wide range of mineralisable nitrogen values during incubation, were used for the study. The soils had pH ranging from 4·9 to 8·2 and total nitrogen ranging from 0·04 to 0·39% ; 6 soils contained free carbonate.

Incubation of unamended soils and determination of total mineral nitrogen accumulation

The method used, which has been described in detail elsewhere,[4] consisted briefly in incubating in test-tubes for 21 days at 28°, 10-g. portions of soil with water added to 50% of the water-holding capacity. The mineral nitrogen accumulated was extracted with 0·5N-sodium acetate and determined by the method of Bremner & Shaw.[5]

Incubation of soils containing 1% added cellulose and determination of carbon dioxide released

The method, described in detail elsewhere,[6] was briefly as follows : 10 g. of soil was mixed with 0·1 g. of cellulose powder (Whatman), the mixture placed in a 6 in. × $\frac{3}{4}$ in. (i.d.) test-tube and water added to 50% of the water-holding capacity. Resting on the soil was a 2 in. × $\frac{1}{2}$ in. (external dia.) vial containing 0·2 g. of barium peroxide plus 1 ml. of water. The test-tube was closed with a rubber bung and incubated at 28° for 21 days. The contents of the vial gave an alkaline system which absorbs carbon dioxide and releases oxygen in approximately equivalent amounts. The vial was removed periodically, replaced with a freshly charged vial, and its carbonate content determined in a calcimeter of the Collins type.[7] The test-tubes were shaken gently for a few seconds each day to break the scum of barium carbonate which forms in the vial.

Results

The soils studied contained initially from 9 to 28 p.p.m. mineral nitrogen (ammonia + nitrate) and yielded, after incubation without added cellulose, further amounts of mineral nitrogen ranging from 8 to 82 p.p.m. The cellulose-treated soils yielded, during incubation, amounts of carbon dioxide ranging from 1030 to 8450 p.p.m.

Fig. 1 shows the relationship between total mineral nitrogen accumulation after incubation of unamended soils, and carbon mineralisation during incubation of soils treated with 1% cellulose. The linear correlation coefficient is 0·925 and is significant at $P < 0·0001$. In the diagram the soils are indicated in three groups, viz., calcareous soils, those with pH > 6 and those with pH < 6. The pattern of the relationship appears to be independent of pH and the presence of free carbonates.

A further incubation test with the soils showed that the addition of extra major elements (except nitrogen) as well as trace elements did not affect the rate of carbon mineralisation.

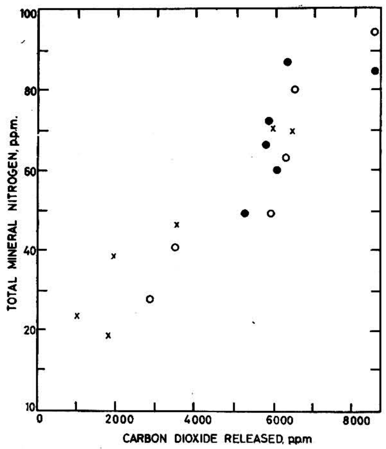

FIG. 1.—*Relations between total mineral nitrogen after incubation of unamended soils and carbon dioxide released during incubation of cellulose-treated soils*

● Soils with free $CaCO_3$ ○ soils pH > 6 × soils pH < 6
$r = 0{\cdot}925$

This indicates that all the soils already contained sufficient of these elements for securing maximum carbon mineralisation and that nitrogen was apparently the only nutrient limiting the process.

Discussion

In this study an average of 86 units of carbon dioxide was produced per unit of nitrogen used by the micro-organisms. This corresponds to the decomposition of 52 units of cellulose per unit of nitrogen. This value lies within the range of 25 to 54 units cellulose decomposed per unit of nitrogen reported by Jensen[8] for bacteria and fungi in pure culture.

The indirect incubation method would obviously have to be tested with a large variety of soils to see whether it is correlated with nitrogen mineralisation determined by the usual incubation technique, before it could safely be recommended to replace the latter. The possibility cannot be excluded that there may be some soils with some nutrient other than nitrogen which limits carbon mineralisation and it may be necessary to correct this. It may be argued that such treatment may alter the rate of nitrogen mineralisation and hence that of carbon mineralisation. However, correction of this deficiency is justified since, if any nutrient is likely to be limiting carbon mineralisation, it is also likely to be limiting for plant growth, and the soil in question would receive the nutrient in any case.

The indirect incubation method for advisory work where many samples are handled can only replace the ordinary incubation method if its use results in a saving of labour and/or materials. This could be the case if the method for determining carbon dioxide release as used in this study were modified by using a smaller sample of soil and more barium peroxide in the vial so that only a single analysis would need be made at the end of the incubation period. Also, the test-tubes could be held in a rack so that they could all be agitated together, or, as a final refinement, the rack could be agitated mechanically.

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COLORIMETRIC DETERMINATION OF 2,6-DICHLORO-4-NITROANILINE IN PLANTS AND SOIL

By J. ROBURN*

Colour reactions of the fungicide 2,6-dichloro-4-nitroaniline are discussed. An analytical method for the determination of its residues on the surface and within the tissues of lettuce and fruit and in soil is described in detail. Work carried out to demonstrate the validity of the method is summarised and discussed.

The analysis is based on reduction to 2,6-dichloro-*p*-phenylenediamine, followed by oxidation, in the presence of aniline, to a blue indamine dye.

Introduction

2,6-Dichloro-4-nitroaniline (dicloran, ' Allisan ') has recently been introduced as a fungicide for the control of *Botrytis cinerea* (Grey Mould) on lettuce.[1] Its performance against plant diseases caused by other species of *Botrytis* has also been assessed. In view of its promise as a fungicide for use on edible crops, an analytical method was required for the determination of residues of this substance in plant material.

In the preliminary work, a number of colour reactions of 2,6-dichloro-4-nitroaniline were investigated. The substance is bright yellow becoming more intense on treatment with alkali, particularly alcoholic potash. This colour is, however, too pale for use in analysis of residues. Other quite stable and strong colours are obtained by diazotising 2,6-dichloro-4-nitroaniline in very dilute aqueous solution with dilute hydrochloric acid and sodium nitrite, followed by coupling to form azo-compounds.

Alternatively, 2,6-dichloro-4-nitroaniline may be quantitatively reduced to 2,6-dichloro-*p*-phenylenediamine, the latter being determined by a colour reaction. Attempts to employ the azo-dye formed by coupling the diazotised diamine with 1-naphthol resulted in a colour which was strong and stable, but variable in intensity. Another fairly strong and stable colour can be obtained by oxidising the diamine in the presence of phenol or cresols to a violet-blue indophenol compound.[2]

The most promising approach was the formation of a dark blue indamine dye. In the presence of aniline, *p*-phenylenediamine can be oxidised to the unstable ' phenylene blue '. This reaction has been used as a spot test for *p*-phenylenediamine,[3] and was subsequently adapted for detecting this compound in hair dyes.[4] Aniline has been determined in a similar way by use of *p*-phenylenediamine.[5] 2,6-Dichloro-*p*-phenylenediamine also undergoes a reaction of this type, producing a very intense blue colour. This reaction, carried out on the diamine obtained by reducing 2,6-dichloro-4-nitroaniline, forms the basis of the analytical method described in this paper.

Experimental

Extraction of plant material

Surface deposits on lettuce.—The lettuce is weighed and its separated leaves are put into a flask. Four ml. of toluene per g. are added and the stoppered flask is placed on a shaker for 15 min. The toluene extract is filtered and an aliquot (3–40 ml.), preferably containing 30–150 μg. of 2,6-dichloro-4-nitroaniline, is pipetted into a 100-ml. round-bottomed flask.

Total residues in lettuce.—Lettuce (100 g.) is cut into strips 0·5–1 in. wide, macerated with 200 ml. of acetone for 3 min. and, after transference to a wide-neck 500-ml. flask, put on a shaker for 30 min. The plant debris is filtered off on a Buchner funnel and washed with about 50 ml. of acetone and water mixture (3 : 1). The volume of the filtrate is measured and a 20-ml. aliquot pipetted into a small separating funnel. After addition of 20 ml. of toluene, the top layer is extracted with 2 ml. of 4%, 10 ml. of 0·8% and 10 ml. of 0·8% NaOH and finally with 10 ml. of 0·5N-HCl, all the aqueous layers being rejected. The toluene layer is passed through a dry

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5 × 2 cm. alumina column (aluminium oxide for chromatographic absorption analysis as supplied by B.D.H., is satisfactory), eluted with 40 ml. of 1 : 9 acetone in toluene, and all the eluate collected in a 100-ml. flask.

Surface deposits on fruit.—The fruit is shaken for 15 min. with 1 ml. of toluene per g. The extract is filtered and an aliquot taken for analysis. If the blank with untreated fruit is higher than a reading corresponding to approximately 1 μg. of 2,6-dichloro-4-nitroaniline, the aliquot is passed through a 3 × 2 cm. dry alumina column, eluted with 40 ml. of acetone in toluene and collected in a 100-ml. flask.

Total residues in fruit.—If the fruit is larger than 0·5 in. in diameter, it is cut into small cubes before maceration. The rest of the procedure is exactly as for total residues in lettuce.

Extraction from soil

A weighed amount of soil, containing not more than 0·5 mg. of 2,6-dichloro-4-nitroaniline, is put in a filter paper thimble and extracted with acetone for 1 h. in a Soxhlet apparatus. The acetone extract is made up to 50 ml. and 50 ml. of toluene are added. After being washed with 3 × 100 ml. of 1% aqueous crystalline sodium sulphate, 20 ml. of the extract are mixed with 2 ml. of acetone and passed through a 2 × 2 cm. alumina column, which is eluted with 30 ml. of acetone in toluene (1 : 7).

Determination of 2,6-dichloro-4-nitroaniline

To the toluene extract are added 0·5 g. of zinc (zinc shot AnalaR, Hopkin & Williams). 15 ml. of distilled water and 0·5 ml. of N-HCl. The flask is fitted with a solvent trap (Fig. 1) with its tap closed and a condenser (an air condenser 50–70 cm. in length is suitable) and put on a small electric heater. The flask is shielded from strong light by placing the apparatus in a dark corner of the laboratory and behind an opaque screen. When toluene starts steam-distilling into the trap, the lower layer is let down periodically into the flask, so that the contents of the latter are always above 7 ml. After all the toluene has been driven off, the refluxing is continued for 15 min., and when boiling stops the water in the trap is allowed to run into the flask. The flask is then placed in a dark cupboard until its contents have cooled to 25° or below.

Ten ml. of this solution, after filtering where necessary, are pipetted into a boiling tube or directly into a cell, followed by 2 ml. of 0·2% aniline in glacial acetic acid, and 1 ml. of 0·05% aqueous potassium dichromate. Light absorption at approximately 650 mμ is measured 12 min. after addition of the dichromate.

Calibration.—A calibration curve is obtained by carrying out the analysis with 25 ml. of toluene containing 10–200 μg. of 2,6-dichloro-4-nitroaniline. When macerated plant material or soil is analysed, the results read off the calibration curve are multiplied by 1·4 to allow for 71% recovery.

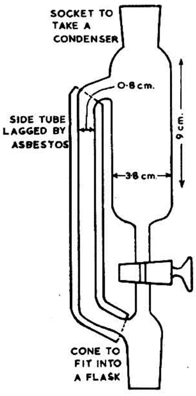


FIG. 1.—*Solvent trap*

Examination of conditions for the reactions

A summary is given below of the results of work carried out to investigate the variables involved in the analytical procedure.

Reduction.—Heating 0–200 μg. of 2,6-dichloro-4-nitroaniline with 0·5 of zinc shot in 15 ml. of 0·03N-HCl reduced it completely as soon as all the toluene had been driven off. The amount of zinc was not critical, but other forms, such as dust, filings, etc., were less satisfactory than shot. When the reduction was carried out in strong light or the reduced solution was allowed to cool on the bench, irregular low readings were frequently obtained. Carrying out the reduction in subdued light and cooling the reduced solution in the dark eliminated this difficulty.

Oxidation.—Amounts of 50 or 100 μg. of 2,6-dichloro-*p*-phenylenediamine in a final volume of 13 ml. were used to study the effect of the following variables on the intensity and stability of the blue dye.

(*a*) *Concentration of hydrochloric acid.* Intensity and stability of the colour were unaffected in 0·01N or weaker acid, both being at their maximum. At higher concentrations a progressive adverse effect was observed, e.g., in 0·5N acid the intensity was only about one-quarter of the maximum and it decreased rapidly with time.

(*b*) *Amount of acetic acid.* Increasing the concentration of acetic acid increased both the intensity and stability of the colour but it decreased its rate of development. Maximum colour intensity was reached at 40% of acetic acid, above which it again decreased slowly.

(*c*) *Amount of aniline.* Below 0·01% the reaction was incomplete but an intense stable colour was obtained at 0·03–0·05%. At much higher concentrations a dark blue colour was formed, which masked the indamine colour.

(*d*) *Concentration of potassium dichromate.* Below approximately 0·002% the reaction was incomplete. Between 0·003 and 0·010% the colour was uniform and stable.

(*e*) *Effect of temperature.* The intensity of the colour tended to decrease as the oxidation temperature increased. At 25° or less the results were not affected.

(*f*) *Time.* The colour changed slowly from a blue-green to a more purple shade, but when the solutions were not more than 0·05N with respect to HCl the light absorbance at 650 mμ remained constant for over 20 min. after addition of the oxidising agent.

Recoveries in the presence of plant material

The following tests were carried out in order to establish the efficiency of the extraction and purification stages. Lettuce was used in the work described but similar results were obtained with strawberry fruit and tomatoes. The analytical procedure was exactly as given in the preceding section, and all readings were taken on the Hilger Biochem Absorptiometer with the OY2 filter and Biochem tubes.

Calibration for surface deposits.—(*a*) 2,6-Dichloro-4-nitroaniline in toluene solution. Varying amounts of the material were dissolved in toluene and analysed. The average results of four analyses for each concentration are given in Fig. 2.

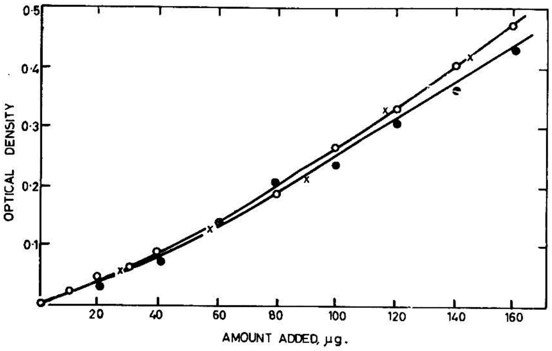


FIG. 2.—*Recovery from surface deposits*

●——● 2,6-dichloro-4-nitroaniline added to toluene
○——○ 2,6-dichloro-4-nitroaniline added to lettuce extract
×——× 2,6-dichloro-*p* phenylenediamine added to toluene, plotted as the equivalent amount of 2,6-dichloro-4-nitroaniline

(*b*) 2,6-Dichloro-4-nitroaniline in lettuce extract. Varying quantities of the chemical were added to 25 ml. of filtered surface extract (4 ml. of toluene per g. of lettuce) and analysed. The average readings of ten analyses for each concentration are plotted on Fig. 2 and the range of readings and standard deviations are given in Table I.

Table I

Calibration for surface deposits of 2,6-dichloro-4-nitroaniline

μg. added	Average reading	Range	Standard deviation μg.	Standard deviation %
0	0·004	0·002–0·006	0·6	—
10	0·023	0·016–0·026	1·4	14
20	0·045	0·040–0·048	1·2	5·8
30	0·067	0·062–0·072	1·4	4·7
40	0·089	0·076–0·089	4·0	10·1
60	0·135	0·116–0·135	3·7	6·2
80	0·193	0·168–0·218	6·6	8·3
100	0·263	0·224–0·316	7·1	7·1
120	0·333	0·308–0·358	5·8	4·8
140	0·409	0·326–0·444	13·0	9·3
160	0·472	0·444–0·486	4·3	2·7
180	0·518	0·426–0·588	18·0	10·0

(*c*) 2,6-Dichloro-*p*-phenylenediamine was added to 25 ml. of toluene and analysed, including the reduction stage. The average results are plotted on Fig. 2.

(*d*) Storage of extract. 0, 1 and 5 μg. of 2,6-dichloro-4-nitroaniline were added per ml. of filtered toluene extract of lettuce (in duplicate), left on the bench and analysed periodically. No significant loss was recorded after 2 or 4 days, but after 8 and 14 days a loss of nearly 20% occurred and this increased to approximately 40% after 3 and 4 weeks. The blank remained unaltered.

Calibration for total residues.—(*a*) Recovery from acetone extract of macerated lettuce. Varying amounts of 2,6-dichloro-4-nitroaniline were added to 20 ml. of the extract, which was then analysed. Results are plotted on Fig. 3.

(*b*) Recovery from macerated lettuce. Lettuce was macerated in acetone and, after addition of various quantities of 2,6-dichloro-4-nitroaniline, was shaken for 30 min. and analysed. Percentage recoveries are given in Table II, and the average readings on Fig. 3.

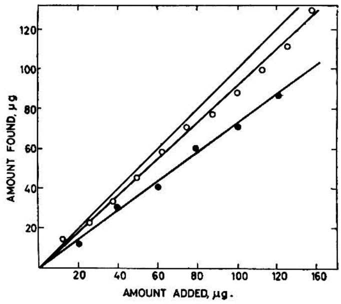


FIG. 3.—*Recovery of 2,6-dichloro-4-nitroaniline from macerated lettuce*

—— 100% recovery
○——○ when added to filtered extract
●——● when added to macerated tissue

Table II

Recovery from macerated lettuce

μg. of 2,6-dichloro-4-nitroaniline added per g. of lettuce

2·5	5·0	7·5	10·0	12·5	15·0
Recovery, %					
70	97	75	85	71	81
60	68	67	71	75	67
60	65	62	66	69	68

(*c*) Recovery from macerated lettuce after storage. Lettuce was macerated in acetone, shaken for 1 h. after addition of 12·5 μg. of 2,6-dichloro-4-nitroaniline per g., stored in the dark and analysed periodically. Storage times of 1, 4 and 6 days (duplicate samples) all gave 67% recovery.

Specificity of the colour test

Since crops are often treated with other pesticides, tests were carried out to establish whether any common spray chemicals interfere with the analysis. The effect of impurities found in technical batches of 2,6-dichloro-4-nitroaniline and of related compounds was also examined. All the readings were taken on the Hilger Biochem Absorptiometer using the OY2 filter.

Pesticides.—Solutions of 0·4 mg. of different pesticides in 25 ml. of toluene were analysed for apparent 2,6-dichloro-4-nitroaniline content (undissolved residues were filtered off). All the substances tested, namely DDT, γ-BHC, derris, pyrethrum, malathion, Chlorparacide, colloidal sulphur, copper oxychloride, phenylmercuric nitrate, Melprex, captan, TCNB and PCNB, gave the same reading as the blank, i.e., 0·002 or 0·004.

Technical impurities and related compounds.—Approximately 25-ml. aliquots of toluene containing 120 μg. of the various substances were tested by the procedure described. *p*-Nitroaniline, 2-chloro-4-nitroaniline and 2,5-dichloro-4-nitroaniline gave strong blue colours with readings of 0·162, 0·324 and 0·278 respectively (the corresponding reading for 2,6-dichloro-4-nitroaniline is 0·333). *o*-Nitroaniline, 4-chloro-2-nitroaniline, 2,4-dichloro-6-nitroaniline, 2,4-dinitroaniline and 6-chloro-2,4-dinitroaniline gave an orange or brown colour with readings of 0·052, 0·048, 0·028, 0·014 and 0·026, respectively. (Higher readings were obtained with other light filters.) No colour was obtained with *m*-nitroaniline, 2,4,6-trichloro-3-nitroaniline, 2,6-dichloro-4-nitroacetanilide, trichloroaniline and chloranil.

Discussion

2,6-Dichloro-4-nitroaniline is a relatively stable chemical. It is unaffected by boiling dilute acid or alkali, and is not, therefore, expected to hydrolyse in or on the plant. When exposed to strong sunlight for a long time it darkens slightly, but this change appears to occur much more slowly than with most aromatic amines. 2,6-Dichloro-4-nitroaniline is easily reduced to 2,6-dichloro-*p*-phenylenediamine in the laboratory and the possibility of enzymic reduction in the plant was therefore considered. The problem is complicated by the fact that added traces of the diamine reacted in the lettuce tissue and only 15–20% could be recovered. No evidence has yet been obtained, however, to suggest that 2,6-dichloro-4-nitroaniline is reduced in plants or soil or that the roots or leaves absorb either this substance or the diamine. It was, therefore, not considered necessary to employ a test for the diamine as a routine measure in the analysis of treated plants.

Theory of the analytical procedure

2,6-Dichloro-4-nitroaniline (**I**) is reduced to 2,6-dichloro-*p*-phenylenediamine (**II**) with zinc and dilute HCl. The diamine is then oxidised by very dilute dichromate in the presence of aniline, to a blue compound with maximum light absorption at 655 mμ. This blue compound is slowly converted to a paler red one which has maximum absorption at 540 mμ. Neither of these substances has been definitely identified but they are probably salts of dichloroindamine (**III**) and dichlorophenosafranine (**IV**), respectively. Since an excess of aniline and dichromate is always present in the aqueous acid solution, the conversion to **IV** cannot be prevented. Under the recommended conditions, it is slowed down so that readings at a suitable wavelength are constant between 5 and 20 min. after adding the oxidising agent. The reaction can be stopped at the indamine stage by making the solution alkaline and extracting with chloroform, so that the dye is transferred into the solvent as a more stable red form with an absorption maximum at 520 mμ. The spectrophotometric curves are given in Fig. 4.

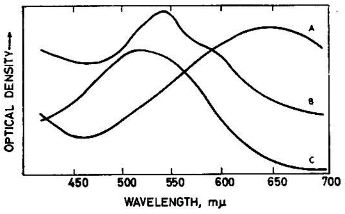
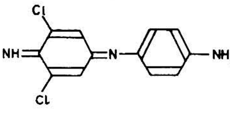


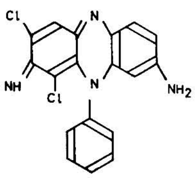
FIG. 4.—*Spectrophotometric curves*

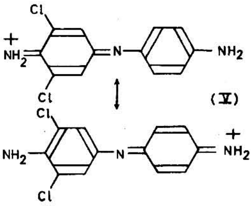
Curve A blue solution 30 min. after oxidation
Curve B pink solution 24 h. after oxidation
Curve C red solution in chloroform, obtained by making the freshly oxidised solution alkaline and extracting with chloroform


(I)


(II)


(III)


(IV)


(V)

(VI)

The blue colour in aqueous solution is reversibly suppressed by increased concentration of HCl but not of acetic acid. This suggests that the intense colour at pH 2 is due to a monovalent cation stabilised by resonance, whose two main contributing forms are **V** and **VI**.

When similarly treated, *p*-nitroaniline and its other halogenated derivatives also form a blue dye. *m*-Nitroanilines give no colour, while *o*-nitroaniline and its halogen-substituted derivatives react to give an orange to brown colour. 2,4-Dinitroanilines give a weaker orange colour, and anilines do not react.

The analytical method described above is more specific than the formation of azo-dyes from 2,6-dichloro-4-nitroaniline, either directly or after reduction, since the blue colour is only formed from aromatic compounds which are easily reduced to *p*-diamines.

Reaction conditions

Reduction appears to be complete under the conditions described, since similar readings were obtained when equivalent amounts of 2,6-dichloro-*p*-phenylenediamine were used instead of 2,6-dichloro-4-nitroaniline (Fig. 2). The occasional lower readings are probably due to slight oxidation of the diamine after it is formed.

The oxidation stage can be affected by many variables. The temperature of the solution should not be above 25°, and the concentration of the HCl must be very low (since an excess of zinc is present, only an extremely small amount of the acid remains after reduction). Acetic acid is required to stabilise the indamine dye, and it also intensifies the colour. It acts as a buffer pH 2·0, but its effect cannot be explained by buffering action alone. The amount of aniline must be high enough to give a complete reaction but not sufficient to form any emeraldine. The concentration of dichromate must be adequate for complete reaction yet not high enough to affect the blank appreciably.

Extraction with cold toluene appears to extract all the surface deposits, since further extraction did not yield any significant amounts of 2,6-dichloro-4-nitroaniline. In the case of macerated lettuce to which this substance has been added, only another 5% could be recovered by a second acetone extraction. Washing the toluene–acetone mixture three or four times reduces the acetone content and enables the extract to be purified on a short alumina column, so that the first 40 ml. of the eluate contain all the 2,6-dichloro-4-nitroaniline with no interfering substances.

The results of storage tests indicate that both filtered extracts and macerated lettuce can be kept for a few days without any appreciable loss of 2,6-dichloro-4-nitroaniline.

Validity of the method

The standard deviation for determination of surface residues is below 10% (Table I) except with samples containing less than 15 μg., when accuracy tends to diminish with the amount of 2,6-dichloro-4-nitroaniline present. Recovery from plant surfaces may be considered to be 100%.*

Results obtained with macerated lettuce (Table II) and with strawberries indicate that the reproducibility is comparable to that above, but recovery is only approximately 71%. A correction factor of 1·4 must, therefore, be applied when determining total residues.

Less than 2 μg. of 2,6-dichloro-4-nitroaniline can be detected. With 40 ml. of surface extract, this figure corresponds to 0·2 p.p.m. for lettuce and 0·05 p.p.m. for fruit. With macerated plant material and 20 ml. of the extract, this value corresponds to 0·3 p.p.m., although in practice the limit may often be somewhat higher owing to slight interference. The sensitivity of the method could probably be increased, if necessary, by taking larger aliquots and possibly using a more involved clean-up procedure.

None of the pesticides tested interfered with the analysis. Technical batches of 2,6-dichloro-4-nitroaniline contain a little 2-chloro-4-nitroaniline. The latter substance gives a similar colour reaction, but it is both more volatile and more water-soluble than the dichloro compound and its presence in residues on plants can therefore be ignored. No other impurities are present in sufficient quantities to interfere with the analysis.

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* Two theoretical sources of error, acting in opposite directions, exist: (1) extraction of some of the chemical from damaged tissue; (2) solvent penetrating surface layers may carry with it some of the dissolved pesticide.

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SOME RELATIONSHIPS BETWEEN ISOTOPICALLY EXCHANGEABLE PHOSPHATE, SOIL ANALYSIS AND CROP GROWTH IN THE GREENHOUSE

By G. E. G. MATTINGLY and A. PINKERTON*

An experiment on perennial ryegrass in small pots compared yields of grass and responses to added superphosphate with the analyses of 15 soils and with their total isotopically exchangeable phosphate (' A ' value). The highest positive correlations between yields, and negative correlations between responses to added superphosphate, were obtained with ' A ' values. The phosphate extracted by 0·5M-$NaHCO_3$, 0·5N-acetic acid–sodium acetate, 0·3N-HCl and 0·002N-H_2SO_4 (reagents 1–4, respectively) also correlated well with yield. Yield and phosphate soluble in 0·5N-acetic acid (reagent 5) were not significantly correlated.

The soils were mixed with ^{32}P-labelled superphosphate, stored for 30 days at field moisture capacity, air-dried and extracted with the five reagents listed above. The amounts of $^{32}PO_4^{3-}$ that exchanged with soil phosphate during extraction were compared with the amounts that exchanged in soils similarly treated and cropped with ryegrass in the greenhouse. More soil phosphate exchanged during extraction with reagents 3–5 in the laboratory than in the greenhouse experiment. These reagents dissolved soil phosphate, or exposed new surfaces on the soil, from which phosphate is not taken up by ryegrass. The soil phosphate that exchanged in reagents 1 or 2 was more closely correlated with, and nearly equal to, the ' A ' value of the soils enriched with superphosphate. Reagent 1 did not increase soil phosphate surfaces in most soils, but reagent 2 dissolved some phosphate, which did not exchange in the greenhouse, from soils containing more than 4% $CaCO_3$.

Introduction

Orthophosphates, labelled with the isotope ^{32}P, exchange with only part of the total soil P. This fraction is called the ' labile pool ' of phosphate in soil[1, 2] and is largely inorganic because soil organic P does not readily exchange isotopically. Greenhouse and laboratory methods for estimating labile P have been discussed elsewhere.[3] The most satisfactory methods use water or neutral salts[4, 5, 6] which dissolve much less P from soil than acid or alkaline reagents.

The present investigation was planned to see which reagents, in the concentrations used in soil analysis, dissolved soil P, or exposed new phosphate surfaces on the soil that were not part of the ' labile pool ' sampled by ryegrass. The phosphate exchanging during the extractions was compared with the ' A ' values of the soils in a greenhouse experiment. The results are discussed with reference to crop responses on the soils.

Experimental

Soils used in the experiments

Fifteen samples of surface soil (approximately 0–6 in.) were taken in spring, 1956, from sites in Central England and Wales. All the fields were under commercial arable cropping

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except soil A 10081 which was a shallow rendzina developed under grass on chalk. The sites represent varied and typical arable soils derived from different parent materials, and include acid, neutral and calcareous soils with clay contents varying from 8% to 53% clay. Table I gives descriptions, chemical analyses and clay contents.

Table I

Description, chemical analyses and clay contents of soils

Reference No.	Location	Geology	pH[a]		% of air-dried soil			
			in water	in 0·01M $CaCl_2$	Clay (<2μ)	$CaCO_3$	Total P	Inorganic P
A 10068	Dilhorne, Staffs.	Coal Measures	6·8	6·5	39·0	0·2	0·064	0·028
A 10069	Much Wenlock, Salop	Silurian limestone	8·0	7·6	19·5	9·4	0·052	0·019
A 10070	Shipston-on-Stour, Warwicks.	Lower Lias	8·0	7·6	48·3	16·0	0·083	0·037
A 10071	Whichford, Warwicks.	Jurassic oolitic limestone	7·9	7·5	33·7	5·8	0·106	0·050
A 10072	Harpenden, Herts.	Clay-with-flints	6·9	6·6	19·7	0·1	0·064	0·033
A 10073	Milton, Derby	Bunter sandstone	6·6	6·5	8·5	nil	0·065	0·028
A 10074	St. Neots, Hunts.	Chalky-Jurassic Boulder Clay	7·8	7·5	33·0	4·4	0·080	0·047
A 10075	Little Dalby, Leics.	Lower Lias	7·2	7·0	53·0	0·2	0·081	0·033
A 10076	Hathern, Leics.	Triassic drift	6·3	5·8	14·5	nil	0·056	0·032
A 10077	Sutton Bonington, Notts.	Keuper marl	6·8	6·5	19·7	1·8	0·072	0·039
A 10078	Tickencote, Rutland	Jurassic oolitic limestone	7·9	7·6	32·5	10·7	0·081	0·037
A 10079	Alconbury Weston, Hunts.	Oxford Clay	7·3	7·1	44·3	0·3	0·063	0·032
A 10080	Trawscoed, Cards.	Silurian shales	5·6	5·2	28·7	nil	0·105	0·036
A 10081	Ivinghoe, Bucks.	Chalk	7·5	7·4	25·0	36·4	0·132	0·031
A 10082	Northaw, Middx.	London Clay	6·6	6·2	29·7	nil	0·076	0·036

[a] 10 g. of soil : 25 ml. of solution ; measured after 2 h.

Analytical methods

Total P in soils was determined by digesting a 2-g. sample (<40 mesh) with 60% perchloric acid. Inorganic P was estimated as the difference between total and organic P. Organic P was measured by an ignition method[7] and $CaCO_3$ with a Schrötter tube.[8]

Table II describes the five methods used to extract P from soils ; in several the amounts of soil taken, but not the ratios of soil/solution, are smaller than in the original methods.

Table II

Methods of soil extraction used in the experiments

Method	Reagent	Wt. of soil, g.	Vol. of solution, ml.	Time of shaking
Rapid test (used at Rothamsted)	0·3N-HCl	4	10	1 min.
Truog[9]	0·002N-H_2SO_4 (buffered with ammonium sulphate at pH 3)	1	200	30 min.
Williams & Stewart[10]	0·5N-acetic acid	1·25	50	6 h.
Morgan (modified by Tinsley & Pizer[11])	0·5N-acetic acid in 0·75N-sodium acetate	5	25	15 min.
Olsen *et al.*[12]	0·5M-$NaHCO_3$ (adjusted to pH 8·5 with NaOH)	5	100	30 min.

Greenhouse experiment

For this experiment 135 pots were arranged in three randomised blocks of 45 treatments comprising the following factorial combinations :

$$[15\ \text{soils}] \times \begin{bmatrix} P_0 \\ P_{10} \\ PR_{10} \end{bmatrix}$$

P_0 No superphosphate
P_{10} 10 mg. of P per pot as superphosphate applied at sowing
PR_{10} 10 mg. of P ,, ,, ,, ,, ,, 30 days before sowing

In treatment PR_{10}, unlabelled superphosphate was mixed with the soils and the pots were kept at field moisture capacity for 30 days. The soils were then removed, air-dried and sieved before sowing.

The following nutrients were applied in solution to all pots in the experiment: 12·5 mg. N as NH_4NO_3; 12·5 mg. K as K_2SO_4; 2·5 mg. Mg as $MgSO_4,7H_2O$ and 0·5 mg. P as $Ca(H_2PO_4)_2,H_2O$ containing 5 μc of ^{32}P. After being kept overnight the soils (100 g.) were mixed mechanically with 150 g. of quartz (2–3 mm.). Perennial ryegrass seed, S.23 strain (0·1 g. per pot), was sown on 24 June 1958 and covered with 20 g. of acid-washed flint. A second nutrient solution containing minor elements was applied 6 days after sowing and immediately after germination, to give the following concentrations in the soil (p.p.m.):

Mn and Fe 50; B 25; Cu and Zn 5; Mo 1.

Further additions of 25 mg. of N and 25 mg. of K were made every 3 weeks during the experiment. The pots were watered daily to maintain the soil at, or near, field capacity. The grass was cut after 51, 91 and 134 days but only the first cut was analysed. The 'A' values given in Tables III and IV were calculated from the ratio of $^{32}P/^{31}P$ in the crop 51 days after sowing. Total P in the crop was determined by the phosphovanado-molybdate method,[13] on samples ashed for 1 h. at 500°; ^{32}P was measured by counting in an M12 liquid counting tube.

Table III

Yields of ryegrass in the greenhouse, yield responses to 10 mg. of P per pot as superphosphate, 'A' values phosphate extracted by five reagents and corresponding correlation coefficients

(The soils are arranged in three groups in order of increasing yields)

Soil No.	g. of dry matter per pot		mg. of P per 100 g. of soil					
	Yield[a] (no added phosphate)	Yield response from 10 mg. of P per pot before sowing	'A' value	0·3N-HCl	0·002N-H_2SO_4	0·5N-acetic acid	0·5N-acetic acid –sodium acetate	0·5M-$NaHCO_3$
Group 1								
A 10070	1·72	2·51	5·6	0·1	0·7	5·2	0·07	0·7
A 10068	1·82	2·33	5·7	1·6	4·2	1·1	0·06	0·5
A 10080	1·96	2·11	8·6	0·3	0·7	0·1	0·03	0·6
A 10069	1·98	2·43	4·9	0·1	0·9	2·1	0·36	0·8
A 10071	2·86	1·80	8·1	1·7	3·9	4·9	0·22	1·1
Means	2·07	+2·24	6·6	0·8	2·1	2·7	0·15	0·7
Group 2								
A 10081	3·20	2·08	12·7	0·1	0·5	3·4	0·22	0·8
A 10074	3·26	1·27	8·2	2·6	4·1	12·1	0·65	1·5
A 10078	3·65	1·06	11·0	0·1	1·7	2·5	0·34	2·2
A 10072	4·07	0·68	11·8	3·1	4·6	4·0	0·53	1·5
A 10075	4·47	0·93	16·1	1·4	4·0	1·2	0·20	1·7
Means	3·73	+1·20	12·0	1·5	3·0	4·6	0·39	1·5
Group 3								
A 10082	4·56	0·55	16·6	2·5	4·4	1·4	0·32	1·4
A 10079	4·88	0·48	18·1	3·8	6·8	2·7	1·14	4·0
A 10073	4·96	0·51	17·7	9·8	10·6	8·7	2·12	4·6
A 10077	4·97	0·83	11·8	6·9	8·7	6·6	0·84	2·4
A 10076	5·13	0·17	16·1	9·9	12·4	10·1	1·89	3·4
Means	4·90	+0·51	16·1	6·6	8·6	5·9	1·26	3·2
Correlation coefficients for yield without phosphate			+0·902***	+0·732**	+0·774***	+0·349	+0·704**	+0·806***
Correlation coefficients for yield response			−0·858***	−0·717**	−0·770***	−0·361	−0·701**	−0·795***

** $0·01 > P > 0·001$ *** $P > 0·001$

[a] All pots received 0·5 mg. P as ^{32}P-labelled $Ca(H_2PO_4)_2,H_2O$

Laboratory experiments

Powdered ^{32}P-labelled superphosphate, ground to pass a 60-mesh sieve, was mixed mechanically with 500 g. of each soil. (The amount of phosphate added, 10 mg. of P per 100 g. of soil, is approximately equivalent to a dressing of 15 cwt. of superphosphate per acre mixed intimately with the top 6 in. of soil.) The soils were stored in glass pots at approximately field moisture capacity; after 30 days they were again air-dried, mixed thoroughly, ground to pass a 2 mm. sieve and extracted by the methods in Table II.

Results

Table III gives total yields of three cuts of grass from soils receiving ^{32}P-labelled monocalcium phosphate (treatment P_0) and increases in yield from 10 mg. P per pot as superphosphate (treatment P_{10}). These results are given in three groups of five soils in order of increasing yields. In Group 1 the mean yield response was about 100%, in Group 2 30% and in Group 3 10% of the yield without phosphate. Table III also gives phosphate extracted from the soils by five methods (Table II), total isotopically exchangeable phosphate (' A ' value) in the unmanured soils, and mean values of these quantities for each group of soils. Some relationships between these mean values and those in the literature for phosphate-responsive soils are discussed below.

Table IV

' A ' values (x) and isotopically exchangeable phosphate (y_1 to y_5) in 15 soils previously enriched with ^{32}P-labelled superphosphate (10 mg. of P per 100 g.) and extracted with five reagents

Soil No.	mg. of P per 100 g. soil					
	' A ' values (x)	0·3N-HCl (y_1)	0·002N-H_2SO_4 (y_2)	0·5N-acetic acid (y_3)	0·5N-acetic acid–sodium acetate (y_4)	0·5M-$NaHCO_3$ (y_5)
A 10068	13·1	29·3	28·2	31·4	14·9	11·7
A 10069	12·5	25·4	17·4	21·8	17·5	11·8
A 10070	12·8	30·5	20·3	35·3	17·2	12·1
A 10071	15·9	48·2	35·7	36·0	19·2	14·2
A 10072	18·5	23·0	22·8	27·7	17·2	14·5
A 10073	24·7	28·3	25·0	32·7	24·1	24·7
A 10074	15·7	43·4	36·6	41·5	18·5	15·4
A 10075	23·3	33·6	30·2	32·6	21·4	21·4
A 10076	23·4	37·2	29·9	32·2	22·7	21·0
A 10077	19·6	37·6	29·7	33·5	19·5	19·1
A 10078	18·9	20·2	23·3	33·0	21·6	17·0
A 10079	25·0	35·4	30·6	28·2	24·2	24·5
A 10080	14·5	16·7	18·9	16·6	10·1	12·9
A 10081	19·5	12·1	18·6	21·6	17·1	12·5
A 10082	23·4	37·4	32·4	31·4	20·6	17·6
Means	18·7	30·6	26·6	30·4	19·1	16·7
Correlation coefficients between y and x		0·152	0·341	0·120	0·778***	0·896***

*** $P > 0·001$

' A ' values gave the highest positive correlations with yield, and negative correlations with yield responses. Yields were also highly correlated with the amounts of phosphate soluble in 0·3N-HCl, 0·002N-H_2SO_4, 0·5M-$NaHCO_3$ and 0·5N-acetic acid sodium–acetate. There were no significant correlations between yields, or yield responses, and the phosphate dissolved by 0·5N-acetic acid.

The total amounts of soil P that exchanged with $^{32}PO_4^{3-}$ during extraction by the methods in Table II were calculated from :

$$\begin{array}{c}\text{Total isotopically exchangeable P} \\ \text{(mg. P per 100 g.)}\end{array} = 10·0 \times \frac{\text{(specific activity of superphosphate)}}{\text{(specific activity of soil extract)}}$$

All the values given in Table IV refer to soils with which superphosphate (10 mg. of P per 100 g. of soil) was mixed 30 days before sowing (Treatment PR_{10}).

The total amounts of phosphate exchanging, shown in columns y_1, y_2 and y_3 in Table IV, were all much greater than and not correlated with the ' A ' values (x) obtained in the greenhouse. The amounts of soil phosphate exchanging with ^{32}P-labelled superphosphate shown in Table IV, columns y_4 and y_5 were, however, highly correlated with, and nearly equal to, ' A ' values estimated from the specific activity of the ryegrass (x). Fig. 1 shows differences between values ($y_4 - y_5$) in these two reagents as a function of the calcium carbonate content of the soils. More soil P exchanged isotopically during extraction with 0·5N-acetic acid–sodium acetate than with 0·5M-$NaHCO_3$, particularly in soils containing more than 4% of $CaCO_3$.

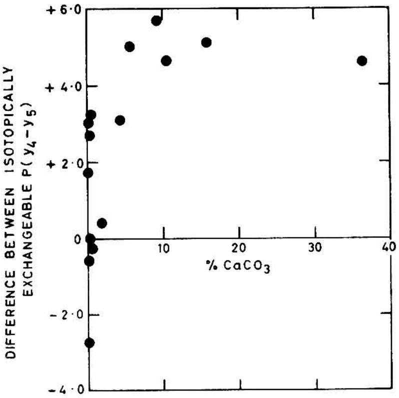

FIG. 1.—*Differences between soil phosphate (mg. P/100 g. of soil) exchanging in 0·5N-acetic acid–sodium acetate (y_4) and 0·5M-NaHCO$_3$ (y_5) as function of CaCO$_3$ content of 15 soils*

Discussion

The figures below give the mean values for ' soluble P ' for soils from Group 1 (Table III) and some published values for levels of soil P below which large responses to superphosphate would be expected by crops in the field.

Reagent	Values from greenhouse experiment	Published values from field experiments	References
	(mg. P/100 g. soil)		
0·3N-HCl	0·8	<1·0	*
0·002N-H_2SO_4	2·1	0·5–1·5	14
0·5N-acetic acid	2·7	1·0–3·0	15
0·5N-acetic acid–sodium acetate	0·15	<0·15	16
0·5M-$NaHCO_3$	0·7	0·5–1·0	12

* Unpublished data, Chemistry Dept., Rothamsted

The two figures agree well for all reagents except 0·002N-H_2SO_4, which gave high values in the greenhouse experiment because four of the soils in Group 1 (Table III) were calcareous. The published values from field experiments, for 0·5N-acetic acid-soluble P, were obtained[15] after shaking for 2 h. The longer period of shaking used here (Table II) may account for the higher values given by this reagent, particularly on the highly calcareous P-responsive soils A 10070 and A 10071.

No laboratory measurement infallibly predicts yield or response to phosphate even in the greenhouse. ' A ' values or the phosphate soluble in 0·5M-$NaHCO_3$ gave the most consistent relationships with yield (Table III). The acid reagents, except 0·5N-acetic acid, picked out soils on which responses to phosphate were very small (A 10073, A 10076, A 10077, A 10079), but they did not detect as clearly as ' A ' values or 0·5M-$NaHCO_3$-soluble P those soils which gave small yields and large responses to phosphate in the greenhouse. Nevertheless, Table III shows that 0·002N-H_2SO_4 and the simple and rapid test with 0·3N-HCl were useful, and results by these methods correlated well with yields in the greenhouse.

The limitation of acid reagents in the analysis of soils derived from different parent materials can be explained largely by the soil P they dissolve, or by the new surfaces on the soil they expose, from which phosphate ions are not taken up by ryegrass. Williams[17] showed in pot tests that soils extracted with dilute acids and then adjusted to their original pH, gave higher yields than untreated soils, and he attributed his results to the redistribution of acid-soluble P in an adsorbed form. The acetate buffer (pH 4·8) gave results intermediate between those with the acid reagents and 0·5M-$NaHCO_3$. With most acid and neutral soils (A 10073, A 10075, A 10076, A 10077, A 10079, A 10082) the total soil P exchanging during extraction with the buffer solution was almost the same as in the greenhouse (Table IV). More phosphate exchanged in calcareous soils (Fig. 1), probably because chalk and limestone fragments, which contain phosphate soluble in acids,[18] were dissolved by 0·5N-acetic acid–sodium acetate.

Several of the conclusions from this work, where a much wider range of soils was used, agree closely with those of Nethsinghe.[19] He showed that the $^{32}P/^{31}P$ ratio in extracts of an Upper Greensand soil, previously equilibrated with ^{32}P, was smaller in acid reagents, and that isotopically exchangeable P was greater, than in 0·01M-$CaCl_2$ or 0·5M-$NaHCO_3$. The $^{32}P/^{31}P$ ratio was the same in his experiments in 0·01M-$CaCl_2$ as in 0·5M-$NaHCO_3$, although bicarbonate removed ten times as much phosphate. Both ratios were equal to the specific activity of the crops he grew. With a basaltic soil from Northern Ireland, 0·5M-$NaHCO_3$ extracts had lower specific activities than 0·01M-$CaCl_2$.

The correlation coefficient ($r = +0{\cdot}896$) between ' A ' and isotopically exchangeable P during extraction with 0·5M-$NaHCO_3$ (Table IV) is smaller than the value ($r = +0{\cdot}985$) recently established in this laboratory between ' A ' and isotopically exchangeable P in 0·02M-KCl for a group of 37 arable soils.[20] Phosphate concentrations in neutral electrolytes (0·01M-$CaCl_2$; 0·02M-KCl), however, are often low and difficult to measure. 0·5M-$NaHCO_3$, first used by Olsen *et al.*,[12] has the advantage over all acid reagents for advisory work in that it removes measurable amounts of phosphate from the ' labile pool ' without dissolving much phosphate that is not isotopically exchangeable in the greenhouse.

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EFFECTS OF AIR-DRYING AND AIR-DRY STORAGE ON THE MINERALISABLE-NITROGEN OF SOILS

By J. K. R. GASSER

Samples of eight soils were air-dried and stored in brown-paper or in sealed polythene bags for periods up to 98 weeks. Portions of the fresh and re-wetted air-dry soils were incubated at 25° for 10–84 days. The soils were extracted before and after incubation and the increase in mineral-N (NH_4^+-N + NO_3^--N) content (Δ Mineral-N) was measured. Air-drying increased Δ Mineral-N. Air-dry storage further increased Δ Mineral-N, the value increasing with the period stored, but the mineralisable-N of soils kept in sealed polythene bags increased less than that of soils in paper bags, the increases occurring only in the easily mineralisable-N ; most of the extra mineral-N was formed during the first 10 days' incubation and all by 42 days, thereafter the air-dried soils had the same rate of mineralisation as the fresh soils. The mineral-N content of the air-dry soils increased with storage.

In a further similar test with 14 soils, air-dried and stored in brown-paper bags for periods up to 32 weeks, the mean value of Δ Mineral-N varied irregularly with time of storage up to 18 weeks but thereafter increased with increasing period of storage before incubation. The mineral-N content of the air-dry soils varied, the maximum value being found in the samples stored for 12 weeks.

Introduction

Buddin[1] noted in 1913 that more mineral-N was produced when soil was air-dried and re-wetted before incubation than when it was incubated fresh. Waksman & Starkey[2] confirmed this, and increases in mineralisable-N after air-drying have since been noted in studies of the mineralisation of soil organic nitrogen[3, 4] and of carbon–nitrogen relationships in soils.[5, 6] Birch[6b, c] showed that the amount of easily mineralisable-N increased with the period the soils were stored air-dry before being re-wetted and incubated, but Harpstead & Brage[7] found soils air-dry for less than 9 weeks produced less nitrate-nitrogen (NO_3^--N) on incubation than the fresh soils, although the amount of NO_3^--N mineralised increased with time of storage. Soils produced more NO_3^--N when incubated after storage in sealed glass bottles than in bottles with porous tops.[8]

There is no information on the duration of the period for which previously air-dried soils produce more mineral-N or to show whether re-wetted soil maintains increased values indefinitely, returns to the same rate, or ultimately produces less than does fresh soil. This paper describes the effects of air-drying and prolonged air-dry storage on the mineral-N produced when soils were incubated for periods up to 12 weeks. Changes in the mineral-N content of air-dry soils during storage were also followed. These results suggested that the changes occurring during the initial period of storage differed from those obtained with soils stored air-dry for prolonged periods. A second group of soils was therefore stored for shorter periods and the mineral-N content and mineralisable-N determined at intervals, after one period of incubation only.

Experimental

Analytical methods

The method of Bremner & Shaw[9] was used to extract the soils with acidified potassium sulphate solution and to determine the NH_4^+-N and NO_3^--N contents of the filtered extracts with their modified microdiffusion units. Total-N was determined by the Kjeldahl method and organic carbon by wet oxidation with dichromate.[10] pH was measured in a suspension of 1 part of soil with 2·5 parts of water. The water-holding capacity (W.H.C.) of the fresh soils was determined by saturating two 50-g. portions of each soil with water, allowing to drain overnight and weighing. When required, water was added to each incubation to bring the soil to 50% W.H.C. The moisture content of all soils was determined by drying 10 g. overnight at 105°.

For the fresh soils, the residue remaining in the shaking bottle after filtration was transferred to a 2-mm. sieve, and the portion retained was washed, dried and weighed. This weight was subtracted from 50 g. to give weight of fresh sample less than 2 mm.

All results (NH_4^+-N, NO_3^+-N and Mineral-N) are expressed as p.p.m. of N in soil below 2 mm. dried at 105°.

Samples, air-drying and storage

The first group of 8 soil samples (Nos. 1–8), taken in March 1958, was selected to provide a range of textures, organic matter and calcium carbonate contents. Soils 4 and 8 had received fertiliser nitrogen, their mineral-N contents being comparable with that of a fallow soil in the summer; these samples were included to test whether the presence of larger amounts of mineral-N affected the subsequent behaviour of the soils. The second group of 14 normal arable soils (Nos. 9–22) were taken in March 1959 when cultivations were complete but before seed was drilled or fertiliser applied.

The fresh soils were incubated as described below. The remainder of each sample was dried in a layer 2–3 cm. deep in an oven at 25–30° for 4–5 days without forced air-circulation, ground by hand in an iron pestle and mortar to pass a 2-mm. circular aperture brass sieve, returned to the trays and dried for a further day. Soils 1–8 were divided into 450-g. sub-samples, and soils 9–22 into 180-g. ones. Sub-samples of soils 1–8 were stored both in single-ply brown-paper bags and also in 500 gauge (0·005 in. thick) polythene bags (sealed at each end) at room temperature (15–18°) for 0, 16, 32, 64 or 98 weeks before incubation. Sub-samples of soils 9–22 were stored in brown-paper bags at room temperature for 1, 4, 8, 12, 18, 24 or 32 weeks before incubation.

Sub-sampling procedure

Fresh soils.—Ten 50-g. portions of soils 1–8 and four 50-g. portions of soils 9–22 were weighed. Two portions were extracted immediately with acidified potassium sulphate solution and the NH_4^+-N and NO_3^--N contents of the extracts determined. The other portions were placed in an incubator at 25° and aerated twice weekly. Two portions of soils 1–8, removed after 10, 21, 42 and 84 days, were extracted as above. The other two portions of soils 9–22 were removed after 21 days.

Air-dry soils.—Ten 40-g. portions of soils 1–8 and four 40-g. portions of soils 9–22 were weighed. Two were extracted immediately. After addition of water to the others to bring the soil to 50% W.H.C., they were incubated as above.

Results

Table I describes some of the characteristics of the soils used. The soils had organic carbon contents of 0·76–4·15% C and total-N contents of 0·086–0·494% N, and were slightly acid or alkaline. Some soils were calcareous, with free calcium carbonate contents up to 27%. Moisture contents of the air-dry soils varied from 0·9 to 4·3%.

Changes in the mineral-N content of stored air-dry soils

Table II gives the NH_4^+-N and NO_3^--N contents of soils 1–8 and 9–22 when stored in the air-dry state. Both NH_4^+-N and NO_3^--N contents of the soils increased when stored air-dry, the NH_4^--N content changing the more. The mineral-N contents of sub-samples of soils 1–8 kept in sealed polythene bags changed less than those of samples kept in paper bags, but both contained most mineral-N after 64 weeks. Mineral-N content of soils 9–22 also varied and they contained most mineral-N after being stored air-dry for 12 weeks.

Mineral-N produced on incubation for 21 days

Table III gives the mineral-N produced when soils 1–8 were incubated for 21 days, either fresh or air-dry, and stored for 0, 16, 32, 64 or 98 weeks in paper bags before being re-wetted and incubated.* Soil 8 produced slightly less mineral-N when the unstored air-dry sample (re-wetted) was incubated than when the fresh soil was incubated, air-dry samples of all other soils produced more. Soils stored for 16 or 32 weeks progressively produced more mineral-N when incubated, but the relative changes were not the same for all soils, so that the order was altered. From 32 to 98 weeks the changes varied more, but the order was maintained, except that soils 4 and 5 (with very similar values) reversed positions between 32 and 64 weeks.

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* For convenience the following terminology is used: Increase in mineral-N content of fresh soil when incubated = Δ Mineral-N_{fresh}. Increase in mineral-N content of air-dry soils when re-wetted and incubated = Δ Mineral-$N_{air-dry}$.

Table IV gives the values of Δ Mineral-N for soils 9–22 when fresh or stored for 1, 4, 8, 12, 24 and 32 weeks before incubation. All soils produced more mineral-N after being air-dried than in the fresh state. The values of Δ Mineral-$N_{\text{air-dry}}$ varied irregularly for the individual soils but those mineralising the least and most nitrogen maintained their relative positions, i.e., soil 9 always produced least and soils 21 and 22 most.

Table I

Some characteristics of soils used to test effects of air-dry storage on mineralisable nitrogen

Soil*	Rothamsted soil sample No.	Type	pH	Total-N, % N	Organic C, % C	Moisture in air-dry soil, %
1		Sand	6·8	0·086	0·76	1·0
2		Clay loam	7·9	0·108	0·98	1·7
3		Sand	6·8	0·176	1·76	1·2
4	F77	Calcareous clay	8·0	0·229	1·96	3·2
5	F78	Very stony loam	7·8	0·164	1·65	1·6
6		Clay loam	7·6	0·235	2·60	1·9
7	F76	Silty-clay loam	7·3	0·226	2·18	2·5
8	F75	Chalky loam	7·8	0·320	2·88	2·7
9	F135	Sandy loam	7·9	0·139	1·98	1·0
10		Clay loam	8·0	0·191	1·80	2·2
11	F148	Clay loam	6·9	0·176	1·65	3·1
12	F134	Chalky loam	8·0	0·177	1·90	1·7
13	F142	Loamy sand	7·0	0·162	1·80	0·9
14	F141	Silt loam	7·6	0·177	1·95	1·7
15	F137	Calcareous clay loam	8·1	0·173	1·55	2·7
16	F133	Calcareous loam	8·0	0·229	1·91	2·1
17	F143	Silty loam	7·6	0·205	2·10	1·9
18	F138	Silty loam	7·4	0·157	1·62	1·9
19	F131	Calcareous silty-clay loam	8·0	0·210	2·01	2·4
20	F140	Clay loam	7·8	0·264	2·36	3·8
21	F132	Silt loam	7·6	0·199	2·27	1·6
22	F139	Loam	6·3	0·494	4·15	4·3

* Soil 1 Unfertilised plots } Market Garden Experiment, Woburn Experimental Station, Husborne
3 Farmyard manure plots } Crawley, Beds.

2 Plot 3 Section V } Broadbalk Field, Rothamsted
6 Plot 2b Section V }

10 Deacon's Field, Rothamsted

Table II

Mean NH_4^+-N and NO_3^--N contents of two groups of soils when fresh and stored air-dry for varying periods

Weeks stored	Soils 1–8			
	Paper bags		Polythene bags	
	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N
Fresh	9	7		
0	6	9		
16	6	10	6	10
32	7	11	5	11
64	12	11	8	11
98	9	10	6	10

Weeks stored	Soils 9–22 Paper bags	
	NH_4^+-N	NO_3^--N
Fresh	4	10
1	5	13
4	7	13
8	5	13
12	8	15
18	7	14
24	6	14
32	8	14

Table III

Increases in mineral-N content (Δ Mineral-N) of soils (p.p.m. of N) when incubated for 21 days at 25° either fresh or after being stored air-dry before re-wetting and incubation

Soil	Fresh soil	Air-dry soil Time of storage, weeks					
		0	16	32	64	98	Mean
1	8	9	10	12	12	21	13
2	8	14	19	23	19	26	20
3	19	23	26	31	31	38	30
4	11	17	33	44	39	46	36
5	16	24	32	43	40	52	38
6	14	30	44	46	47	62	46
7	17	18	44	52	55	67	47
8	21	20	47	63	76	73	56

Table IV

Increases in mineral-N content (Δ Mineral-N) of soils (p.p.m.-N) when incubated for 21 days at 25° either fresh or after being stored air-dry before re-wetting and incubation

Soil	Fresh soil	Air-dry soil Time of storage, weeks							
		1	4	8	12	18	24	32	Mean
9	14	23	18	24	18	26	24	28	23
10	11	27	31	28	26	27	37	33	30
11	11	27	28	30	27	30	35	34	30
12	17	25	26	32	30	28	34	37	30
13	18	27	27	30	29	29	36	34	30
14	16	34	35	36	34	34	36	40	36
15	18	38	29	36	34	34	39	42	36
16	19	40	35	40	44	40	39	48	41
17	16	38	37	40	38	43	45	48	41
18	20	42	42	42	41	42	50	50	44
19	23	45	44	47	45	49	54	59	49
20	17	48	46	50	47	53	56	58	51
21	25	55	53	55	56	58	66	71	59
22	35	87	75	93	93	97	111	103	96

Fig. 1 shows the mean values of Δ Mineral-N and Δ NO_3^--N for soils 1–8 and 9–22. For soils 1–8 the amount of mineralisable-N increased with time of storage. Δ NO_3^--N was greater than Δ Mineral-N, because the extra NH_4^+-N present in the air-dry soil above the equilibrium value is nitrified during incubation, and in addition because the NH_4^+-N formed during air-dry storage was nitrified during subsequent incubation and included in the value for Δ NO_3^--N. Soils 1–8 were also incubated for 21 days after being stored air-dry for 2, 4 and 8 weeks. Mineral-N in the air-dry soils was not determined so the results are not given in Table I and Fig. 1, but the mean values for Δ Mineral-N using interpolated values for the initial mineral-N content were 26, 26 and 22 p.p.m. of N, respectively. Δ Mineral-N for soils 9–22 varied irregularly when stored up to 12–18 weeks, but then increased with further storage. Δ NO_3^--N increased with time of storage and was greater than Δ Mineral-N, except for 1 and 8 weeks when they were the same.

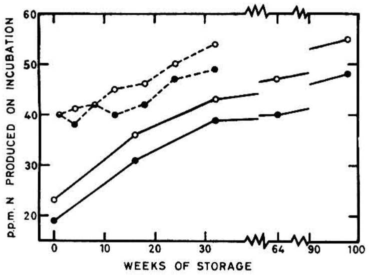

FIG. 1.—*Mineral-N or NO_3^--N produced on incubation for 21 days at 25° of soils stored air-dry and then re-wetted*

● NH_4^+-N + NO_3^--N ——— Soils 1–8
○ NO_3^--N — — — Soils 9–22

Effects of air-dry storage on mineralisation during prolonged incubation

Fig. 2 shows the average mineral-N and NO_3^--N produced when soils 1–8 were incubated for 10, 21, 42 and 84 days in the following conditions: (*a*) fresh, (*b*) re-wetted air-dry, but unstored, (*c*) re-wetted air-dry, after being stored for periods up to 98 weeks, in paper or sealed polythene bags. (The results for soils stored for 64 weeks have been omitted from Fig. 2 because they were concordant with those for 32 and 98 weeks and were intermediate in value.)

The term ' easily mineralisable-N ' is used to describe the difference between the mineral-N produced by the treated soils and that by the fresh soil at the start of incubation. Fig. 2A shows that this quantity was increased when the soils were stored air-dry. This increase was greater when the soils were kept in paper bags than in sealed polythene bags. Most of the easily mineralisable-N was produced in the first ten days and all had been produced by 42 days. The

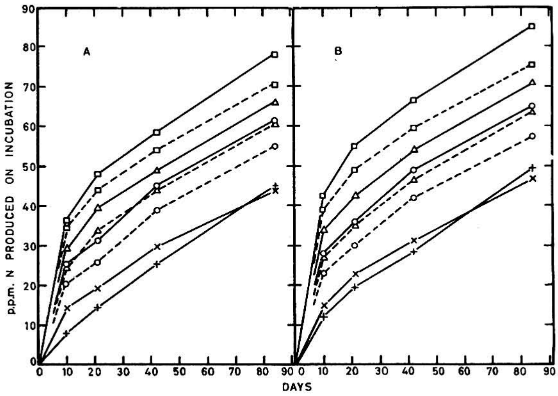

FIG. 2.—*Mineral-N (Curves A) or NO_3^--N (Curves B) produced on incubation of fresh soils or re-wetted air-dry soils stored either in brown paper or in sealed polythene bags*

(Results are averages for soils 1–8)

+	Fresh soils		———	Fresh, unstored and soils stored in paper bags
×	Air-dry soils.	Unstored		
○	Air-dry soils.	Stored for 16 weeks		
△	Air-dry soils.	Stored for 32 weeks	— — —	Soils stored in sealed polythene bags
□	Air-dry soils.	Stored for 98 weeks		

stored soils produced mineral-N at the same rate (p.p.m. of N per day) as the fresh soils between 42 and 84 days (although air-dry soils not stored produced slightly less). The rate at which mineral-N is produced decreases with time of incubating fresh soils, and also for soils stored for 32 or 98 weeks, but for air-dry soils incubated immediately after being dried, and for those stored for 16 weeks, mineralisation is slower between 10 and 21 days than between 21 and 42 days. The corresponding results for the NO_3^--N produced (Fig. 2B) show a decreasing rate with increasing time of incubation for all samples (except for the fresh soils between 42 and 84 days compared with 21–42 days). As previously noted, Δ NO_3^--N is larger than the corresponding Δ Mineral-N because some of the NH_4^+-N present in the soil at the start of the incubations was nitrified.

Discussion

Effects of method of storage on mineralisable-N

Samples stored in sealed polythene bags produced less mineral-N when incubated than those in paper bags. The numerical difference between the two sets for incubation times of 21 days or longer was the same. The method of storage altered the easily mineralisable-N formed in the stored soils, but did not affect the course of the incubation once this excess had been mineralised. The main difference between the methods of storage was the amount of gaseous exchange from within the soils to the atmosphere. This exchange occurred easily for soils stored in paper bags and the moisture content of these samples also varied. Soils stored in sealed polythene bags exchanged less with the atmosphere, although data show this plastic is permeable to oxygen and carbon dioxide (permeability of 1 mm. thick polythene sheet in c.c./sq. m./24 h. cm. Hg at 20°: N_2 0·95; O_2 2·75; CO_2 8·64).[11] The moisture content of these soils did not vary. Acharya & Jain[8] found greater values for Δ NO_3^--N with soils in closed glass bottles than for those in bottles with a porous seal. This is the reverse of the present results. All the soils used by Acharya & Jain[8] behaved similarly, and all those used in the present work were consistent. This problem requires further investigation.

Relationships between moisture content, organic-C and mineralisable-N

The moisture contents of the sub-samples stored in paper bags varied, some being greater and some less than those kept in sealed polythene bags, which had on average 0·17% less moisture than sub-samples in paper bags. Birch[6b] found, however, that soils dried more thoroughly produced more mineral-N on subsequent incubation. As the average moisture contents differed little between the two methods of storage in this experiment, this does not explain the different results.

Table I shows that in general the soils with heavier texture retained more moisture than those with light texture and that soils with higher organic carbon contents retained more moisture than those with lower.

The effects of moisture and organic carbon contents of air-dry soils on the easily mineralisable-N formed during air-drying and air-dry storage were investigated by correlating the values (mean Δ Mineral-$N_{\text{air-dry}}$ — Δ Mineral-N_{fresh}) with air-dry moisture and organic carbon contents of soils 9–22. These values were correlated with both moisture and organic-C content ($r = 0{\cdot}75^{**}$ and $r = 0{\cdot}89^{**}$ respectively) but the last two quantities were also correlated ($r = 0{\cdot}60^{*}$). The corresponding partial correlations between easily mineralisable-N and air-dry moisture or organic carbon had coefficients $r' = 0{\cdot}59^{*}$ and $0{\cdot}83^{**}$; and the multiple correlation had its coefficient $R = 0{\cdot}93^{**}$. These results show that easily mineralisable-N was correlated with either air-dry moisture or organic carbon contents of the soil, organic carbon having more influence; but the best correlation was with both together.

Effects of air-dry storage on mineralisable-N

Incubation for 21 *days.*—Air-dry soils mineralised more nitrogen when incubated than fresh soils. After prolonged storage the amount mineralised increased with the time the soils were kept air-dry before incubation, but the trend was established only after the first 12–16 weeks, before this time Δ Mineral-N varied. This may be compared with the results of Harpstead & Brage[7] who found air-drying decreased Δ NO_3^--N, but its value increased with the period of storage and regained the fresh value after nine weeks. Birch[6c] stored air-dry soils for up to 15 weeks and found both air-drying and time of storage increased Δ Mineral-N on subsequent incubation. The results of different workers probably differ because they used various methods of drying, storage and incubation. The findings of Harpstead & Brage[7] that air-drying initially decreased Δ NO_3^--N differ from those of other workers,[1–6] but otherwise all results show the same trends.

Incubation for 84 *days.*—From 42 to 84 days all samples had the same rate of mineralisation (p.p.m. of N per day) as the fresh soil (except for those incubated immediately after air-drying): therefore air-drying affects only the easily mineralisable-N and once this has been formed the soils return to the fresh condition. Fig. 2A shows that mineralisation was slower from 10 to 21 days than from 21 to 42 days for the samples incubated immediately after being air-dried and for those stored for 16 weeks before incubation. This effect is attributed in this work to the interactions of organic matter and the increased microbiological activity after re-wetting,[12] evidenced as a flush of carbon dioxide and mineral-N produced.[6b, c] The amount of readily decomposable organic matter increases with increasing time of air-dry storage,[6b, c] which affects the relative contributions from this and 'normal' decomposition until the soil has returned to the fresh state. The effect was most pronounced immediately after air-drying, was less after 16 weeks' storage and had disappeared after 32 weeks' storage.

Comparison of Δ NO_3^--N with Δ Mineral-N

The values of Δ NO_3^--N (Fig. 2B) did not show the effect noted in the preceding paragraph and the rate of mineralisation decreased with time for all samples except for fresh soils from 42 to 84 days. The values are all higher than the corresponding ones for Δ Mineral-N because the equilibrium value of NH_4^+-N is lower for the incubated than for the field soils, and NH_4^+-N formed during air-dry storage is also nitrified and included. NH_4^+-N similarly altered the relative performance of Δ Mineral-N and Δ NO_3^--N for soils 9–22 when incubated for 21 days. The mean value of Δ Mineral-N fluctuated for periods of storage up to 8 weeks and then increased with further storage, whereas Δ NO_3^--N increased regularly with the period of storage. Because the NH_4^+-N content of stored soils and the equilibrium value of NH_4^+-N both vary, Δ Mineral-N is considered to measure the mineralising power of soils better than Δ NO_3^--N. Also, soils are known in which mineralisation but not nitrification occurs, and then NH_4^+-N accumulates. With these Δ NO_3^--N is grossly misleading as a measure of mineralisable-N.

Cause of air-drying effect

Birch discussed[6b, c] the possible mechanisms which lead to the flush of mineralisation on re-wetting a dry soil, and concluded that the increased surface area of organic matter exposed by drying the colloids was responsible. Harada[13] found that the readily soluble humus in paddy soils was most easily mineralised by drying and considered this humus fraction to be held on the surface of the colloidal particles. Other factors may be involved, however, because heat caused a similar flush of mineralisation;[6b] alternate freezing and thawing soils also increased the mineral-N formed on subsequently incubating the fresh soil.[14] The microbiological equilibrium is disturbed by air-drying, which leads to a partial sterilisation,[3] and a higher metabolic rate on re-wetting.[12] The changes occurring in the mineral-N content of the air-dry soils during storage indicate that microbiological activity continues slowly, and that sealing the soils in polythene bags with less gas exchange decreases both the changes in mineral-N content and the magnitude of the flush of mineral-N produced on subsequent incubation. The relative contributions of the different processes to the increased mineralisation cannot be assessed from present results.

The application of these results to agriculture is beyond the scope of the present work, but it is relevant that, whenever soil which has become dry is re-wetted, mineralisation of nitrogen is temporarily increased.

Conclusions

1. The results confirm that air-drying soil leads to increased mineralisation of nitrogen on re-wetting (Δ Mineral-$N_{\text{air-dry}}$).

2. Δ Mineral-$N_{\text{air-dry}}$ increases with time of air-dry storage, but the trend is not clearly established for 12–16 weeks, before which values fluctuate.

3. After the early flush of mineralisation has finished, air-dry soil has the same rate of mineralisation as fresh soil.

4. Δ Mineral-N measures the mineralising power of soils better than Δ NO_3^--N, because the equilibrium content of NH_4^+-N is lower in incubated than in field soils and NH_4^+-N accumulates in air-dry stored soils.

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SOME OBSERVATIONS ON THE OXIDATIVE DETERIORATION OF THE LIPIDS OF CRUDE LEAF PROTEIN

By C. H. LEA and L. J. PARR

Measurements of oxygen absorption have been carried out on a sample of crude leaf protein, containing about 20% lipid, at temperatures between 37° and −12° and pH 2·6–6·0. An enzyme system was active in the moist but not in the freeze-dried product. Attempts to protect the lipid of the freeze-dried protein by the addition of antioxidants were unsuccessful.

Introduction

Morrison & Pirie[1] have described a process for the extraction of edible protein from leaves, in which the juice from fresh, pulped leaves is freed from most of the starch grains, fibre and detritus, and coagulated quickly by steam. The crude protein coagulum is then filtered off, acidified to about pH 4 and pressed into blocks containing 30–40% of dry matter. In this form it is said to keep under refrigeration. Alternatively, the protein coagulum can be dried to a product described as 'fairly stable' at room temperature, but the lipids present have to be removed, e.g., by extraction with acetone, before the protein will keep permanently. No quantitative data on keeping properties or on deteriorative changes are reported.

Since oxidation of the lipid seems to be a key factor in limiting the storage life of these crude vegetable protein products, at least in the dried state, it was thought desirable to measure the rate of this oxidation and to ascertain whether it would be readily amenable to control by the addition of antioxidants.

Experimental

Most of the present work was carried out on a single large sample of crude pea haulm protein supplied by Dr. Pirie as a frozen block. On arrival the block was broken up, packed in gas-tight cans under oxygen-free nitrogen and stored at −20°. Under these conditions it was completely stable, samples after storage for 15 months giving oxygen absorption curves identical with those obtained on the fresh material. The pH of the wet cake as received was unusually low (2·6), apparently the result of over-acidification, a more normal figure being 4·5. Some stability determinations were therefore carried out on the material as received and some after careful adjustment with sodium hydroxide solution to pH 4·5 and 6·0.

Lipid content

The proportion and approximate composition of the lipid present in the crude protein is indicated in Table I. Diethyl ether extracted practically none of the phospholipids, which were presumably bound as lipoprotein, and only part of the chlorophyll. In view of the recent observations of Shorland[2] and of Weenink[3] that the acetone-soluble lipids of grass, clover and rape leaves consist largely of galactolipids, rather than of triglycerides, it is probable that the fatty acids in the pea haulm are also present mainly as esters of galactosyl-1- and digalactosyl-1-glycerol, rather than of glycerol itself.

Oxygen absorption experiments

The rates of absorption of oxygen by the wet, crude protein cake were measured at various temperatures in conical flasks fitted with mercury manometers and filled with oxygen. A tube of NaOH solution inside the flask absorbed carbon dioxide. Heating pre-treatments under oxygen-free nitrogen were carried out in a number of cases to destroy enzymes. Pre-adjustment to required pH values was facilitated by a curve prepared by titrating, with NaOH solution and a glass electrode, a portion of the thawed-out press cake diluted with water to a slurry. After adjustment to the required pH the excess water was removed in a rotary vacuum evaporator at <40°.

Oxygen absorption curves on freeze-dried materials were obtained in a similar manner, after pre-equilibrium *in vacuo* at 0° over H_2SO_4 solution calculated to give an equilibrium relative humidity (R.H.) of 30% or 60%. NaOH absorption tubes of appropriate concentration maintained the required R.H. in the flasks. When required, antioxidants were mixed into the proteins as described below. Results in all cases have been expressed as ml. of O_2 (S.T.P.) absorbed/g. of original total solids.

Results

Effects of heating and of drying

The protein cake as received, despite its preparation by steam coagulation, still contained active enzyme (Fig. 1). Heating the wet material at ~90° for 30 min. reduced its initial rate of absorption of oxygen to about one-third (Table II).* Stronger heating at 110° or 130° produced no further retardation, but rather increased the rate again slightly over the 90° figure. Cyanide (4 mg. of KCN/100 g. of wet cake) inhibited oxidation to about the same extent as heating at 90° (Fig. 1).

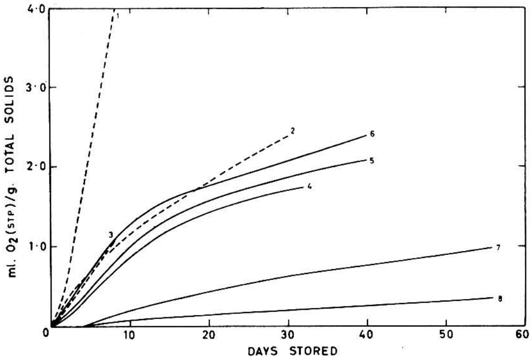

FIG. 1.—*Oxygen absorption by crude pea protein at pH 2·6 and 20°*

Wet material - - - - - -

Curve 1 original
,, 2 preheated at 90°
,, 3 cyanide added

Dry material ———

Curve 4 original (60% R.H.)
,, 5 preheated before drying (60% R.H.)
,, 6 ,, ,, ,, (30% R.H.)
,, 7 ether extracted (60% R.H.)
,, 8 chloroform-methanol extracted (60% R.H.)

Table I

The lipids of pea haulm crude protein

Extraction of the lipid	% of the total solids**
1 Hot acetone extract of the wet cake	21·2
2 Ether-soluble fraction of 1	18·7
3 Hot ether extract of the freeze-dried cake	15·8
4 Hot chloroform–methanol (2 : 1, v/v) extract of the freeze-dried cake	22·4
5 Chloroform-soluble fraction of 4	20·4
Composition of the lipid extracts	**% of the lipid**
N-content of extract 3	0·84
N-content of extract 4	1·47
N-content of extract 5	1·05
P-content of extract 3	<0·01
P-content of extract 4	0·27
P-content of extract 5	0·27
Chlorophyll content of extract 3	3·3
Chlorophyll content of extract 5	5·3
Carotenoid content of extract 5	0·6
Iodine value of extract 5	~106†

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* The effect of heating appeared to be due to inactivation of an enzyme rather than to destruction of micro-organisms, because during the rapid oxidation there was very little production of CO_2.

** 40·5% of the wet cake.

† Approximate value only, owing to the dark green colour of the crude lipid. If this material is mainly galacto-lipid (see text) the iodine value of the corresponding mixed fatty acids would be about 145.[2]

The freeze-dried crude protein absorbed oxygen at about the same rate as the heated wet material, the rate being slightly greater at 30% than at 60% R.H. (Fig. 1). Preheating the wet material at 90° before drying slightly accelerated rather than retarded oxidation after drying (60% R.H.). Apparently the heat-labile enzyme system was unable to function in the ' dry ' products.

Since extraction with ether, and more particularly with chloroform-methanol (which removed more of the lipid, Table I), greatly reduced the rate of oxidation (Fig. 1), it would appear that the constituents of the crude protein undergoing oxidation were mainly lipids. Even after extraction with chloroform-methanol small quantities of firmly bound lipids probably remain to contribute to the slow residual absorption of oxygen observed (Fig. 1).

Effect of temperature of storage and of pH

The influence of storage temperature over the range 37° to −12° on the rate of oxidation of both the wet (unheated) and dry (60% R.H.) materials is shown in Fig. 2 and Table II. The more rapid absorption of oxygen by the wet, as compared with the dry, samples was probably due largely to the action of the enzyme.

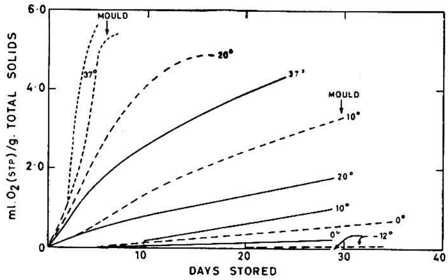

FIG. 2.—*Effect of temperature on the absorption of oxygen by crude pea protein at pH 2·6*

- - - - - - wet ———— dry (60% R.H.)

Even at the lowest pH (2·6), mould soon appeared at 37° and the sharp and erratic rise in the oxygen absorption curve after 2 days was probably due to this cause. At 20° mould appeared variably after 8–30 days and at 10° after 12–30 days.

The rate of uptake of oxygen by the (preheated) wet material seemed to increase with increasing pH (Table II), but signs of microbial growth also appeared much earlier at the higher pH. At 20°, for example, oxygen absorption at all three pH values proceeded approximately linearly for the first 3 days but thereafter the rate at both of the higher pH values increased sharply and there was visible mould after 4 days. At 10° oxygen absorption proceeded normally in the material at pH 6 for about 7 days but then increased rapidly and visible mould had appeared

Table II

Effects of temperature and of pH on the absorption of oxygen by the crude protein

Material	pH	O_2 absorption (ml. O_2/g. dry wt./day) at				
		37°	20°	10°	0°	−12°
Wet[1]	2·6	0·6	0·45	0·12	0·02	0·003
	2·6*	—	0·15	—	—	—
	4·5*	—	0·22	—	—	—
	6·0*	—	0·30	0·09	0·025	0·006
' Dry '[2]	2·6	0·183	0·069	0·033	0·008	—

[1] Measured over 2 days at 37°, 3–4 days at 20°, 7 days at 10°, 35 days at 0° and −12°. After 124 days the pH 6 samples had absorbed 1·83 and 0·64 ml., respectively, at 0° and −12°.
[2] Measured over 24 days. 60% R.H.
* Pre-heated to destroy enzyme

by the 10th day. At 0° no obvious mould was present after 6 weeks. The approximate rates of oxygen absorption quoted in Table II for the wet samples are for the period preceding rapid microbial growth.

The pH (before drying) appeared to have little effect on the rate of absorption of oxygen by the freeze-dried material. At 37° and 30% R.H. samples at pH 2·6, 4·5 and 6·0 had all absorbed 1·40–1·45 ml. of O_2/g. dry wt. after 4 days and 1·75–1·85 ml. after 6 days (Table III). These products had not been preheated before drying.

Table III

Effect of solvents and of antioxidants on the absorption of oxygen by freeze-dried pea haulm crude protein
(Oxygen atmosphere, 37°, 30% R.H.)

Expt.	pH	Solvent	Antioxidant (% dry wt.)	ml. O_2 (S.T.P.) absorbed/g. dry wt. after days 4	6	8	12	18
3	2·6	—	—	1·40	1·85	2·25	2·90	3·60
6	2·6	—	—	1·40	1·80	—	—	—
7B	4·5	—	—	1·45	1·80	—	—	—
7B	6·0	—	—	1·41	1·75	—	—	—
4	2·6	1% PG[1]	—	1·22	1·53	1·78	2·15	2·67
,,	,,	,,	EMQ[2] 0·005	1·17	1·48	1·75	2·12	2·60
,,	,,	,,	,, 0·015	1·10	1·45	1·70	2·05	2·55
,,	,,	,,	,, 0·045	1·02	—	—	—	—
6	2·6	—	EDTA[3] 0·2	1·15	1·60	—	—	—
8A	4·5	1% PG[1]	—	1·16	1·43	1·70	—	—
,,	,,	,,	EMQ[2] 0·015	1·10	1·37	1·55	—	—
,,	,,	,,	BPHF[4] 0·015	1·20	1·47	1·70	—	—
8B	6·0	1% PG[1]	—	1·30	1·52	1·70	—	—
,,	,,	,,	EMQ[2] 0·015	1·12	1·35	—	—	—
,,	,,	,,	EMQ[2] 0·015 + TOA[5] 0·1	1·12	1·38	—	—	—
,,	,,	,,	BPHF[4] 0·015	1·17	1·45	—	—	—
,,	,,	,,	BPHF[4] 0·015 + TOA[5] 0·1	1·17	1·42	—	—	—
10*	6·0	5% GO[6]	—	0·93	1·35	1·55	1·88	2·26
,,	,,	,,	EMQ[2] 0·015 + TOA[5] 0·1	0·85	1·27	1·50	1·80	2·20
,,	,,	,,	EMQ[2] 0·015 + CA[7] 0·1	0·78	1·22	1·40	1·70	2·12
11*	6·0	5% GO[6]	—	0·95	1·27	1·50	1·85	2·45
,,	,,	,,	T[8] 0·015 + CA[7] 0·1	1·00	1·30	1·55	1·90	2·50
12*	6·0	5% GO[6]	—	0·90	1·28	—	—	—
,,	,,	,,	T[8] 0·015 + CA[7] 0·1 + AA[9] 0·1	0·90	1·27	—	—	—

* Preheated to destroy enzyme
[1] Propylene glycol
[2] 6-ethoxy-2,2,4-trimethyl-, 1,2-dihydroquinoline (' Santoquin ')
[3] Ethylenediaminetetra-acetic acid disodium salt
[4] 6-t-butyl-3,7,8,2′,5′-pentahydroxyflavone
[5] Tri-isoctylamine
[6] Groundnut oil
[7] Citric acid
[8] D-α-Tocopherol
[9] L-Ascorbic acid

Addition of antioxidants

The water-soluble metal deactivator EDTA was added in aqueous solution to the wet protein before drying. In most of the other experiments the antioxidant, in solution in propylene glycol (1% of the dry material), was sprayed from a scent-spray type of atomiser on to the dried and equilibrated (30% R.H.) crude protein as it was being ' tumbled ' in a tilted rotating vessel. The solvent itself showed an appreciable inhibitory effect on the rate of oxidation at all three pH, but the further protective effects of the antioxidants added in it were very small (Table III).

In several experiments with refined groundnut oil as vehicle (5% of the dry material), the oil, with or without an antioxidant in solution, was ' blended ' with the wet protein before freeze drying. The rate of oxidation of the dried product was found to be slowed down a little further by the vegetable oil, but the antioxidants added in this way were no more effective than when added in propylene glycol.

Because the oxygen absorption curves for the ' stabilised ' samples were closely parallel to those of the controls the results have been presented in summary form only in Table III.

Discussion

Oxidation by atmospheric oxygen of lipids such as those present in crude leaf proteins, can produce a variety of flavour defects. The polyunsaturated fatty acids of the galactoglycerides and phospholipids can develop ' fishy ', ' grassy ' and ' painty ' odours and flavours, and oxidative deterioration of the carotenoids and chlorophylls of dried green vegetable matter can give rise to ' violet ' and ' hay-like ' off-odours. Carotenoids and chlorophyll can be destroyed by a coupled oxidation as the unsaturated fatty acids autoxidise and probably also by direct oxidation : Friend & Mayer[4] have recently reported the presence of an enzyme system in the chloroplasts of green leaves which appears to oxidise carotenoids directly.

The rapid enzymic oxidation observed in the present experiments in the wet (but not in the dried) crude protein could, no doubt, be avoided by somewhat stronger heating during preparation of the wet protein curd. The residual non-enzymic oxidation was still quite rapid, however, and appears to be difficult to control except by low temperature or exclusion of oxygen.

Considerable quantities of heavy metal catalyst, particularly of iron, no doubt entered the product by action of the acidic juice and mineral acid on the non-stainless plant used, but the protein itself will tend to limit the activity of the metal and quite a large addition of EDTA conferred only a small degree of further protection.

The appreciable protective effects observed with additions of the solvents propylene glycol and, in more marked degree, groundnut oil, are in line with the improved stability of carotene which obtains when fat or oil is added as a dust controller to dried green crops such as lucerne or grass. The protective effect may be due to the oxidisable lipids being brought into closer contact with natural autoxidation inhibitors already present.

The action of added antioxidants was disappointing. Although high protection factors can be obtained for extracted fats or for carotenoids in mineral or glyceride oil solution, it is well known that the degree of protection obtainable by the use of antioxidants in dried green crop or in mixed animal feed containing it is much less. Nevertheless, a useful measure of protection *can* be obtained and, for this purpose, the aromatic amine inhibitors EMQ (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline, ' Santoquin ') and DPPD (*NN'*-diphenyl-*p*-phenylenediamine) have been the most successful.[5] (EMQ, at concentrations up to 0·015%, is a permitted additive for dried green crop in the U.S.A.)

The flavonoid compound tested (6-t-butyl-3,7,8,2',5'-pentahydroxyflavone) is a synthetic substance of the type prepared and tested by Simpson & Uri.[6] It is reputed to have an extremely high primary antioxidant activity in model systems and particularly in the presence of free fatty acids,[7] which reduce the activity of many inhibitors and which might well be present or develop in the lipid of the crude leaf protein. As a flavonoid it is related to a class of natural oxidation inhibitors which might be expected to be protective in a plant lipoprotein system.

d-α-Tocopherol (vitamin E) and L-ascorbic acid are natural oxidation inhibitors commonly present in vegetable materials. Tri-iso-octylamine is one of a group of aliphatic amino substances which have recently been shown to exert a powerful synergism with certain primary (chain-breaking) antioxidants, including those under test here.[8, 9] Citric acid is a well-known, non-toxic synergist and metal deactivator. Since the effectiveness of an antioxidant can be influenced considerably by the pH of the system, tests on the dried pea protein were run on material adjusted to three pH before drying.

As can be seen from Table III, EMQ showed a detectable antioxidant activity but, at reasonable concentrations, it was too small to be of value. The amine synergist failed to improve the protective action of EMQ ; and citric acid improved it only slightly. The pentahydroxyflavone, either alone or with the amine synergist, was even less effective than EMQ, and neither tocopherol nor tocopherol plus ascorbic acid gave any protection at all.

While specific oxidation inhibitors or synergistic mixtures may still be found to stabilise these plant products, the results reported here are not encouraging, and it would seem that protection against oxidative deterioration during storage will most easily be achieved by refrigeration and/or oxygen-excluding wrappers or packing in inert gas. Even under refrigeration, oxidation at the exposed surface of a frozen block may still be fast enough to impose some limitation on storage life (Table II) unless an oxygen-excluding wrapper is also used.

Acknowledgments

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DETERMINATION OF DRY MATTER IN SILAGE BY DISTILLATION WITH TOLUENE

By W. A. DEWAR and P. McDONALD

The determination of dry matter in silages by distillation with toluene gives satisfactory results when an allowance is made for volatiles present in the aqueous distillate. A simple correction procedure, involving a single titration of the distillate, has been developed, and the method is considered suitable for routine purposes.

Introduction

In an earlier paper,[1] an apparatus was described which enabled the volatile constituents, produced during the oven-drying of silage, to be collected and determined quantitatively. An examination of 28 silages, ranging in pH value from 3·7 to 5·2, showed that appreciable losses of lower fatty acids, lactic acid and ammonia occurred during oven drying. The apparatus used in these studies could be used for the accurate determination of dry matter in silage, but for routine purposes the method was considered tedious, since it required a detailed analysis of the distillate for volatile components. Since there is a need for a rapid and accurate method of determining dry matter in silage, an alternative method was sought and the present paper summarises the results from a study of the toluene distillation technique which has already been suggested as a possible alternative to the normal oven-drying method.[2–4]

The apparatus used in the toluene distillation technique has been described by Bidwell & Sterling.[5] The sample under test is heated in a distilling flask with an excess of toluene. The distillate containing water and toluene is condensed and collected in a graduated receiver or trap, so designed that the excess toluene flows back into the boiling flask. Distillation is continued until all the water has been driven off from the sample and the volume of water in the trap is then measured.

The accuracy of the method depends largely upon the precision with which the volume of water may be measured in relation to the sample size. With very wet materials, such as fresh herbage or silage, the accuracy in measuring the volume of aqueous distillate can be increased by using a trap provided with an ovoid-shaped bulb at its lower end and graduated stem above, similar to that described by Evans & Fetzer.[6] This type of trap allows a larger sample size to be used.

Where the toluene method has been used previously in silage studies, the volatiles present in the distillate have either been ignored or considered to be of little importance; but it is desirable to know what the possible errors are. The purpose of the present investigation was to study the magnitude of these errors and to decide if the toluene distillation technique would be suitable as an accurate routine method for determining dry matter in silage.

Experimental

Apparatus

The distillation apparatus used in these studies is shown in Fig. 1. The trap was made by cutting the stem from a conventional Bidwell & Sterling trap[5] of capacity 8 ml. and fusing it to an ovoid-shaped bulb of about 50 ml. capacity.

The trap was recalibrated and was found to be suitable for measuring volumes of water between 51·70 and 58·20 ml. to the nearest 0·05 ml. It was connected by a reduction adapter, with ground joints, to a 1000-ml. wide-mouthed, round-bottomed flask which was supported in an Electro-thermal heating mantle fitted with a sensitive temperature control. A single-surface water condenser stoppered with a silica gel absorption tube, to exclude atmospheric moisture, was attached to the top of the trap.

Recovery test with volatiles

Known volumes of water and aqueous solutions of acetic, propionic, butyric and lactic acids, ammonia and ethanol were separately distilled in the apparatus, with about 400 ml. of toluene, until the volume of aqueous distillate collected in the trap was constant. Details of the procedure are given below.

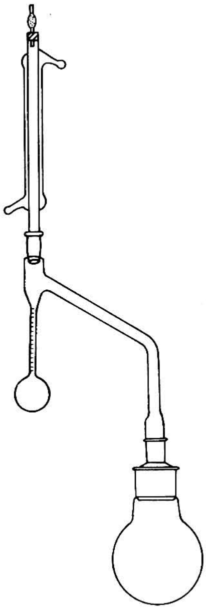

FIG. 1.—*Toluene distillation apparatus*

Volumes occupied by volatile materials in aqueous solution

Before applying the distillation technique to the determination of dry matter in silage, it was necessary to know the volume occupied by individual volatiles in aqueous solution. These volumes were found by determining the densities at 20° of known concentrations of aqueous solutions of the volatile substances mentioned in the previous paragraph. Since the density of water at 20° is 0·9982 g./ml., the volume of water, and hence the volume occupied by a known weight of solute, could be calculated.

The volume occupied by ammonia in 1% v/v acetic acid was determined by a different method in which the contraction in volume was measured in a graduated flask after the addition of ammonia solution to dilute acetic acid.

Application of the toluene distillation method to silage

Determinations of dry matter were carried out on 16 different silage samples ranging in pH value from 3·7 to 5·4. These silages had all been made in farm silos from grass or grass/clover mixtures without additives. The procedure was as follows:

Samples of 65 g. of chopped silage were accurately weighed into the distillation flask and heated with about 400 ml. of toluene in the apparatus. The distillation was continued for 7–8 h. at a rate of 2–3 drops per second. At the end of this period, a fine jet of toluene was directed down the condenser to remove the last traces of water and the distillation continued for a further 15 min.

Before the volume of water was read the graduated trap was immersed in a water bath at 20° for 20 min. The apparatus was disconnected and the aqueous layer was transferred into a 100-ml. graduated flask and made up to the mark with CO_2-free distilled water.

The total acid content of each distillate was determined on an aliquot of the diluted distillate by the method of Foreman,[7] which depends on the fact that in 80% alcoholic solution ammonia is neutral to phenolphthalein. Samples of 20 ml. of the diluted distillates were therefore diluted with 80 ml. of neutral ethanol and titrated with 0·1N-NaOH to the phenolphthalein end point.

In addition to the Foreman titration, samples of the distillate were analysed for individual volatile fatty acids, lactic acid, ammonia and alcohol by the methods described in an earlier paper.[1]

Oven-dry matter determinations

In order to compare the toluene distillation technique with the oven-drying method, the dry matter values of the 16 silages were determined in the apparatus as described in an earlier paper.[1] This method enables values for volatile matter, and hence for corrected dry matter, to be determined for the silages.

Results

Recoveries of water and aqueous solutions of lower fatty acids, lactic acid, ammonia and ethanol in the distillates after toluene distillation using the apparatus described earlier, are shown in Table I. Almost complete recoveries of water were obtained in all tests.

Table I

Recoveries of volatiles in aqueous distillates from toluene distillation of pure solutions

	Concentration, g./100 ml.			% recovered in distillate			Mean % recovered in distillate
	a	b	c	a	b	c	
Acetic acid	0·185	0·370	0·740	96·8	97·0	97·1	96·9
Propionic acid	0·137	0·274	0·548	65·3	65·9	65·0	65·4
Butyric acid	0·181	0·362	0·724	20·2	18·5	19·0	19·2
Lactic acid	0·301	0·903	1·505	6·5	7·9	7·7	7·1
Ammonia	0·038	0·076	0·114	91·4	90·8	90·0	90·5
Ethanol	0·117	0·234	0·351	85·4	82·1	90·0	85·8
Water	—	—	—	99·96 99·96	99·92 99·92	99·94 99·94	99·94

The volumes occupied by the various volatile compounds in aqueous solution at three concentration levels were studied, viz., at 0·2, 0·6 and 1·0 g. of solute per 100 ml. of solution. Within this range, good agreement was obtained for individual compounds. The volumes occupied by 1 g. of acetic, propionic and butyric acids were 0·864, 0·916 and 0·958 ml. respectively. The corresponding value for 1 g. of lactic acid was 0·778 ml. and for alcohol 1·219 ml. The value for ammonia in acetic acid solution was 0·247 ml.

The 16 silages were examined by both the oven distillation method and the toluene distillation technique. The composition of these silages and the volatiles produced during oven distillation are shown in Table II. Alcohol and propionic acid were absent from most of the silage samples.

The weights of volatiles in the aqueous distillates obtained from the 16 silages examined by toluene distillation are shown in Table III. In addition to the weights of volatiles, the volumes have also been calculated using the appropriate factors described earlier. The percentage volatilities of the various acids have been calculated and the mean values for the

16 silages were as follows: acetic, 87·7; propionic, 55·7; butyric, 7·3; and lactic acid, 3·6. These are lower than the corresponding figures obtained on pure solutions. The values in the last column of Table III (F) have been calculated in each case by dividing the total volume due to volatiles (acid + ammonia) in the distillate by the volume of 0·1N-NaOH, equivalent to the total acids present. The total acid titre can be determined by means of the Foreman titration described earlier. 'F' is therefore a factor used to calculate the volume of volatiles in the distillate from the titratable acidity. The mean value of F for the 16 silages was 0·00555.

Table II

Composition of silages and volatiles produced during oven distillation method

No.	pH	mg./100 g. of fresh silage									
		Nitrogen		Acetic acid		Butyric acid		Lactic acid		Dry matter, %	
		Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile	Apparent	Corrected
1	3·7	397	nil	298	291	nil	nil	1928	187	15·60	16·08
2	3·8	488	nil	261	255	161	152	1818	145	20·65	21·20
3	3·8	422	nil	303	285	nil	nil	2046	256	18·75	19·37[a]
4	3·8	294	10	302	281	20	15	1922	213	19·65	20·27[b]
5	3·9	378	nil	401	375	nil	nil	2021	130	20·81	21·32
6	4·0	330	10	341	320	nil	nil	1321	148	18·38	18·86
7	4·1	310	33	358	328	63	60	2020	199	18·57	19·28[c]
8	4·2	295	15	361	337	nil	nil	1311	65	16·78	17·20
9	4·5	455	36	505	490	317	300	1427	110	21·77	22·71
10	4·6	471	55	419	400	266	243	33	nil	13·81	14·51
11	4·7	405	98	390	364	nil	nil	605	55	21·75	22·27
12	4·9	268	73	491	460	335	315	299	20	15·62	16·49
13	5·0	555	91	789	741	610	576	96	10	13·61	15·08[d]
14	5·0	458	74	576	489	515	498	13	nil	12·80	13·86
15	5·2	635	120	434	389	615	602	19	nil	16·67	17·79
16	5·4	647	162	649	641	633	607	13	nil	15·82	17·43[e]

[a] including 0·08% volatile alcohol
[b] including 0·11% ,, ,,
[c] including 0·09% volatile propionic acid
[d] including 0·05% ,, ,, ,,
[e] including 0·20% ,, ,, ,,

Table III

Volatiles in aqueous distillates obtained by the toluene distillation method from 65-g. samples of fresh silage

No.	Ammonia		Acetic acid		Butyric acid		Lactic acid		Total volume of acids and ammonia in distillate, ml. (x)	ml. 0·1N-alkali equivalent to acids in distillate (y)	$\frac{x}{y}$(F)
	wt., g.	volume, ml.	wt., g.	volume, ml.	wt., g.	volume, ml.	wt., g.	volume, ml.			
1	nil	—	0·177	0·153	nil	—	0·038	0·029	0·182	33·7	0·00540
2	nil	—	0·153	0·132	0·010	0·010	0·038	0·030	0·172	30·8	0·00558
3	nil	—	0·179	0·155	nil	—	0·031	0·024	0·179	33·3	0·00538
4	0·005	0·001	0·171	0·148	nil	—	0·028	0·022	0·171	31·6	0·00541
5	nil	—	0·234	0·202	nil	—	0·034	0·026	0·228	42·7	0·00540
6	0·006	0·002	0·188	0·163	nil	—	0·040	0·031	0·194	35·7	0·00585
7[a]	0·018	0·004	0·204	0·176	0·003	0·003	0·027	0·021	0·239	42·3	0·00565
8	0·008	0·002	0·208	0·180	nil	—	0·029	0·023	0·205	37·9	0·00544
9	0·018	0·004	0·299	0·258	0·012	0·012	0·025	0·019	0·293	54·0	0·00543
10	0·031	0·008	0·251	0·217	0·013	0·013	nil	—	0·238	43·3	0·00550
11	0·054	0·013	0·227	0·196	nil	—	0·005	0·004	0·213	38·4	0·00555
12	0·040	0·010	0·279	0·241	0·012	0·012	0·004	0·003	0·266	48·3	0·00549
13[b]	0·051	0·013	0·435	0·376	0·027	0·026	0·004	0·003	0·435	78·6	0·00554
14	0·038	0·009	0·282	0·244	0·025	0·024	nil	—	0·277	49·8	0·00556
15	0·072	0·018	0·229	0·198	0·035	0·034	nil	—	0·250	42·2	0·00569
16[c]	0·104	0·025	0·345	0·317	0·028	0·027	nil	—	0·431	70·0	0·00615

[a] Propionic acid, total 0·038 g., volume 0·035 ml.
[b] Propionic acid, total 0·019 g., volume 0·017 ml.
[c] Propionic acid, total 0·069 g., volume 0·062 ml.

The values for dry matter determined by the oven distillation method and the toluene distillation technique are summarised in Table IV. The uncorrected dry matter values are the values obtained when the volatiles are not taken into account. The 'corrected' values for dry matter given under the 'toluene distillation' column in Table IV have been obtained by multiplying the total titre in ml. of 0·1N-alkali, obtained from a Foreman titration of the distillate, by the mean factor (F) 0·00555. This volume is subtracted from the observed volume of distillate to give the true volume of water. The latter is converted into g. by multiplying the number of ml. by 0·998.

Table IV

Comparison of percentage dry matter values obtained in oven distillation method and in toluene distillation method

No.	Oven distillation apparatus at 100°			Toluene distillation procedure			
	Uncorrected (a)	Corrected (b)	$\frac{b-a}{b} \times 100$	Uncorrected (c)	$\frac{b-c}{b} \times 100$	Corrected using 'F' (d)	$\frac{b-d}{b} \times 100$
1	15·60	16·08	2·99	15·88	1·24	16·21	−0·81
2	20·65	21·20	2·59	20·84	1·70	21·07	0·61
3	18·75	19·37	3·20	18·92	2·32	19·20	0·87
4	19·65	20·27	3·06	19·93	1·68	20·20	0·35
5	20·81	21·32	2·39	21·00	1·50	21·37	−0·24
6	18·38	18·86	2·55	18·53	1·75	18·84	0·16
7	18·57	19·28	3·68	19·07	1·09	19·42	−0·73
8	16·78	17·20	2·44	16·92	1·63	17·23	−0·17
9	21·77	22·71	4·14	22·31	1·76	22·77	−0·26
10	13·81	14·51	4·82	14·16	2·25	14·51	0·00
11	21·75	22·27	2·33	21·84	1·93	22·16	0·49
12	15·62	16·49	5·28	15·84	3·94	16·27	1·34
13	13·61	15·08	9·75	14·47	4·04	15·13	−0·33
14	12·80	13·86	7·65	13·46	2·89	13·96	−0·72
15	16·67	17·79	6·30	17·31	2·70	17·81	−0·10
16	15·82	17·43	9·24	16·88	3·15	17·46	−0·20

The calculation is summarised in the following equation:

$$D = 100 - 99{\cdot}8(V - 0{\cdot}0055T)/W$$

where D = % dry matter
V = observed volume of distillate
T = total titre of distillate (ml. of 0·1N-NaOH)
W = weight of sample in g.

Discussion

The volatility of the lower volatile fatty acids determined by toluene distillation decreased with increasing molecular weight. This is probably because the solubilities of the volatile acids in water decrease with increasing molecular weight and consequently more of the higher acids tend to pass into the toluene during distillation. This means that the toluene method has an advantage over the oven-drying method in that the error due to volatiles is reduced. The toluene distillation apparatus was designed to accommodate 65 g. of silage. This size of sample allowed distillate volumes to be measured in the trap when the silage dry matter was within the range 11–21%. With dry matter contents higher than 21% it was necessary at the end of the determination to add a known volume of water to the trap in order to read the level in the graduated stem. It was found that a minimum time of 7 h. was necessary in order to ensure complete recovery of water from silages. The distillation, however, could be stopped overnight if necessary and completed the following day without error, provided the apparatus was not disconnected.

The volatility of lactic acid during toluene distillation was relatively low. The lactic acid recovered in the aqueous distillate was 7·1% for the pure solution and only 3·6% for the 16 silages studied. The former value is not dissimilar to that reported by Smith[8] during steam distillation.

It is clear from the results shown in Table IV that the volatiles present in the aqueous distillate obtained during toluene distillation can affect significantly the dry matter value of the silages. These volatiles range from 1·24 to 4·04% of the corrected dry matter value. Hence, in order to obtain an accurate value for silage by toluene distillation, it is necessary to determine individual volatile substances. Such determination is, however, unsuitable for routine purposes and a simplified procedure for correcting for volatiles in the distillate is desirable. A study of Table IV shows that the volatiles are mainly acidic in nature and an attempt was therefore made to relate the titratable acidity of these volatiles (determined by the Foreman method) to the volume occupied by them in the aqueous distillate. The factor for converting titratable acidity into volume of volatiles was designated 'F' and ranged from 0·00538 to 0·00615 with a mean value of 0·00555 for the 16 silages studied. The value of 'F' will vary according to the relative amounts of acids and ammonia present in the distillate. For silages with low pH, the main volatile component of the aqueous distillate will be acetic acid and the correction factor for acetic acid, namely 0·00518, may be used instead of the calculated mean value of 0·00555. The corresponding factors for propionic, butyric and lactic acids are 0·00678, 0·00843 and 0·00700, respectively. The greatest error likely to result from the use of 0·00555 as the correction factor would be an underestimate of 34% if butyric acid is the only acid present in the distillate. This is very unlikely to occur, but even if it were, the actual error in the dry matter determination would be small because of the relatively low volatility of butyric acid under these conditions of distillation.

Ammonia would cause serious interference in the calculation only when present in large amounts and if necessary this could easily be determined in the distillate. Ammonia, however, is not likely to be present in any quantity in well-preserved silages.[9] Alcohol was detected in only two of the silages. Failure to take the volume due to alcohol into account in the calculation of dry matter value resulted in the values for dry matter being underestimated by about 0·5 and 0·75% of the corrected value.

Dry matter values obtained by the toluene method using the correction factor 0·00555 in the calculation agree well with the corrected results obtained by the oven distillation method. With one exception (sample 12) the differences between the two sets of results are well below 1%.

Conclusions

The most accurate method of determining the dry matter content of silages is by the oven distillation procedure in which the volatiles are determined individually. This method is laborious for routine use, however, and the toluene distillation procedure, coupled with the Foreman titration technique, provides a simple and reliable method for determining the dry matter content of silage.

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FIELD OBSERVATIONS ON THE 'EXHAUSTION' OF SUSPENSION AND EMULSION SHEEP DIP FORMULATIONS

By N. P. H. GRAHAM

The 'exhaustion', or decrease in insecticide concentration, of sheep dip washes comprising emulsions, suspensions or colloidal suspensions of BHC, dieldrin or DDT was investigated under field conditions. All showed some degree of exhaustion during dipping, but the emulsions exhausted more rapidly than the suspensions. The 'exhaustion rate' was constant for each formulation, and the percentage drop in concentration was closely related to the percentage of the wash volume removed on the sheep.

If dip washes are replenished with a certain concentration, sheep carry out the same *mean* deposit of insecticide irrespective of the rate of exhaustion of the formulation, the frequency of replenishments or the volume of wash through which they pass. These three factors, however, affect the extent of the variation about the mean value.

If the wash is replenished continuously at approximately the same rate as it is being removed by the sheep, the deposit of insecticide per sheep will be constant for all sheep, and, with exhausting formulations, this system will give the highest minimum deposit for any concentration of replenishments.

Introduction

When sheep are dipped for the control of ectoparasites, the fleece is saturated with an insecticidal wash. The surplus wash is allowed to drain out of the fleece and is returned to the wash reservoir. The physical state and chemical composition of the wash change, as dipping proceeds, because of contamination with dirt, urine, faeces, wool wax and suint. The ingredients of the original wash may also be removed by filtration through the fleece, by absorption on to the fleece, or by precipitation on to the bottom of the bath or floor of the draining pens or sediment sumps.

For many years the changes in the concentration of insecticide in the dip wash were disregarded. However Imes[1] recorded that, when sheep were passed through a cresylic wash, the concentration of cresylic acid fell. Freney *et al.*[2] found that when sheep were dipped in a suspension of arsenic trisulphide the drainings from the sheep contained only half as much arsenic as did the original wash. Graham & Scott[3] demonstrated that, when sheep were dipped in a suspension of tembo root or in a derris phenol emulsion, the insecticidal efficiency fell during dipping.

When DDT and BHC came into wider use, depletion of insecticide during dipping was commonly observed and came to be known as 'exhaustion' or 'stripping'. This decline in insecticidal concentration has been recorded,[3-9] but no reason has been suggested for it. Addison & Furmidge[10] studied the effect of suint on the stability of emulsions, and Machin[11] investigated the relationship between the solubility of various insecticides in wool fat and their 'exhaustion' from emulsion dips, under laboratory conditions.

Experimental

Methods

Two methods of dipping sheep are commonly used in Australia. One is the plunge or swim dip and the other is the power spray or shower dip. For the swim dip, the initial volume of wash is comparatively large; but not more than 30–50% can be used before the bath becomes too shallow and must be replenished. In the shower dip the sheep are held in a pen, while the dip is pumped from a sump through a series of nozzles over the sheep and the drainings are returned to the sump. In the shower dip the initial volume of wash is small, but it is possible to use 75–80% of the initial volume before replenishment is necessary.

With the increasing use of emulsion and suspension sheep dips, exhaustion has become more important and a number of field trials were carried out, over a period of years, to determine whether exhaustion could be reduced by altering the usual dipping procedures, or whether it would be possible to devise means whereby the concentration of insecticide in the wash could be maintained above predetermined levels. The initial work involved the use of DDT and BHC formulations under field conditions. The later trials were designed to observe the exhaustion rate of a number of proprietary formulations.

The procedure adopted was the same in all tests. The dipping vat or shower sump was thoroughly cleaned and charged with freshly prepared wash. With swim dips the wash was stirred manually, and with shower dips it was circulated through the sprays for several minutes. The wash was then sampled and the volume measured to determine the starting concentration and volume for all subsequent calculations. Further samples were collected during dipping, dipping being halted on each occasion, until all the drainings from the dipped sheep had returned to the vat or sump. The wash was then thoroughly mixed before being sampled. The volume of wash remaining and the number of sheep dipped at each sampling interval were recorded. As experience was gained, it was found that samples of 800–4000 ml. of wash were required for analysis, particularly when low concentrations were expected.

The samples were analysed by a dehydrohalogenation method, but, because of the large number involved, considerable time often elapsed between collection and analysis.

The concentration shown by analysis of the first sample was frequently lower than the theoretical initial concentration of the bath and, in particular, the case when suspension formulations were used in shower dips. One obvious source of loss was deposition on the floor of the spraying pen after the preliminary mixing.

As will be shown, there was a fairly constant relationship between the drop in concentration, expressed as a percentage of the starting concentration, and the volume of wash removed, expressed as a percentage of the original volume. By plotting the percentage drop in concentration against the percentage of the wash volume removed, it was possible to show the exhaustion rate graphically. If no additions of wash or insecticide were made during dipping, the cumulative effect of exhaustion was recorded. If, however, additions of water or insecticide were made, it was more convenient to plot separately the percentage drop in concentration and volume for each stage between sampling.

Results

A preliminary series of tests was carried out under different conditions to see if the exhaustion rate exhibited a fixed pattern which was governed by (*a*) the number of sheep dipped, (*b*) the volume of wash removed by the sheep or (*c*) the volume of wash in the vat.

A second series of tests was carried out to examine the effect on the exhaustion rate in shower dips of (*a*) varying the period of contact between the wash and the fleece by prolonged showering, and (*b*) varying the concentration of the wash.

In the third series of tests, the rates of exhaustion of a number of different formulations were compared.

(a) *Effect of different types of dip vats on exhaustion*

Trial 1. *The rate of exhaustion in small swim dips.*—Four groups of sheep were dipped in small-volume swim dips. The first group was dipped in 33 gallons of wash in a drum, and the other three groups in a small vat of 100 gal. capacity. The wool length varied from group to group. 'Rucide', a solubilised DDT product which gives a micro-crystalline suspension on dilution with water, was used. The theoretical starting concentration was 0·1% p.p.i. DDT. Table I shows the actual starting and finishing concentrations of the wash, the volume removed, the number of sheep dipped and their wool length (Tests 1/1–1/4).

Table I

Rate of exhaustion of 'Rucide' in four small plunge dips

Test no.	Volume, gal.		Conc. of p.p.i. DDT, %		No. of sheep dipped	Wool length, in.	% Vol. removed	% Concn. drop	Vol. removed per sheep, gal.
	Start	Removed	Start	End					
1/1	33	30	0·081	0·039	30	2	91	52	1
1/2	100	53	0·098	0·022	50	1–1¼	53	22	1
1/3	100	63	0·09	0·025	102	¾–1	63	28	0·6
1/4	100	36	0·1	0·08	102	¼–½	36	17	0·35

There was no relationship between the drop in concentration and the size of the dipping vat or the number of sheep dipped, but there was fairly close agreement between the percentage drop in concentration and the percentage volume of wash removed. Increased wool length required a larger volume of wash per sheep, but, as is shown in Fig. 1 (Trials 1 and 2), there is no evidence in this test that the longer wool filtered out a higher proportion of insecticide.

Trial 2. The rate of exhaustion in shower dips.—Four tests (Tests 2/1–2/4) were carried out in a 'Buzacott R60' shower dip, with the DDT formulation 'Rucide' at a theoretical starting concentration of 0·1% p.p.i. DDT. All the tests were made on the same property. In each test 700–900 fine-wool Merino sheep were dipped about 2 weeks after shearing. Each pen of sheep was sprayed for 2 min. with the overhead sprays, which were then shut off and the bottom sprays used for a further 2 min. Each pen of sheep was allowed to drain for 3 min.

Table II shows the percentage drop in concentration for each test at two stages during dipping. At stage 1, 34–39% of the wash had been used and, at stage 2, at the end of dipping, 65–84%.

Table II

Exhaustion rate of 'Rucide' in a shower dip

Test no.	Volume, gal.	Start Concentration p.p.i. DDT, %	Stage 1		Stage 2		No. of sheep dipped	Vol. removed per sheep, gal.
			% Wash removed	% drop in concn.	% Wash removed	% drop in concn.		
2/1	385	0·103	37	9	74	36	940	0·3
2/2	390	0·1	36	10	84	35	944	0·35
2/3	394	0·1	39	13	75	39	893	0·34
2/4	394	0·106	34	12	65	27	766	0·33

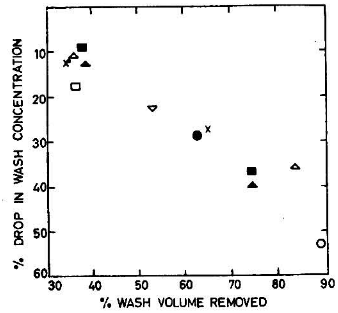

FIG. 1.—*Exhaustion rate of 'Rucide' in swim and shower dips*

Symbol	Test	Bath type	Bath capacity, gal.	Wool length, in.	Symbol	Test	Bath type	Bath capacity, gal.	Wool length, in.
○	1/1	Swim	80	Long wool	■	2/1	Shower	385	¼–½
▽	1/2	,,	100	1¼–1¾	△	2/2	,,	390	,,
●	1/3	,,	100	¾–1	▲	2/3	,,	394	,,
□	1/4	,,	100	¼–½	×	2/4	,,	394	,,

These results confirmed those of Trial 1 in that the drop in concentration was not related to the number of sheep dipped or to the number of gallons of wash removed, but to the volume removed expressed as a percentage of the starting volume. This is more clearly seen in Fig. 1, where the percentage drop in concentration has been plotted against the percentage of the volume removed for all the tests in these two trials.

Trial 3. The rate of exhaustion of BHC suspensions in swim and shower dips.—The product Rucide' used in Trials 1 and 2 did not become established as a sheep dip and, in Trial 3, two commercial BHC suspension formulations were used, one a paste concentrate containing 50% commercial BHC with 12% of γ-isomer, and the other a wettable powder containing 50% of a similar grade of commercial BHC. Both these formulations had a similar particle size

The swim dips used in Trial 1 were unusually small, and in Trial 3 the observations were made with swim dips of the normal size. Altogether five tests were made with each formulation—three in swim dips and two in shower dips. One further trial was carried out in which the concentration of the wash was maintained above 0·03% BHC by addition of additional concentrate. In these tests all dips were charged at a theoretical concentration of 0·06% BHC. Merino and Corriedale sheep were used and at least four samples were collected during each dipping. In Fig. 2, the cumulative percentage drop in concentration has been plotted against the cumulative percentage of the volume of wash used for these 11 tests (Tests 3/1–3/11).

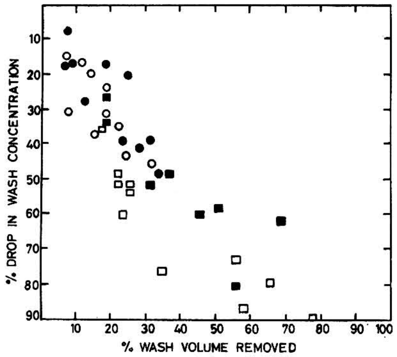

FIG. 2.—*Exhaustion rate of BHC suspensions in swim and shower dips*

Test	Type of dip	Capacity, gal.	Formulation	Test	Type of dip	Capacity, gal.	Formulation
3/1	Swim	85	BHC dispersible powder	3/7	Shower	453	BHC dispersible powder
3/2	,,	1000	,, ,, ,,	3/8	,,	413	,, ,, ,,
3/3	,,	1200	,, ,, ,,	3/11	,,	490	,, ,, ,,
3/4	,,	83	BHC paste	3/9	,,	292	BHC paste
3/5	,,	1000	,, ,,	3/10	,,	413	,, ,,
3/6	,,	1400	,, ,,				

○ BHC dispersible powder tests 3/1–3/3
□ ,, ,, ,, ,, 3/7, 3/8, 3/11
● BHC paste tests 3/4–3/6
■ ,, ,, ,, 3/9, 3/10

There was again reasonably close agreement between the exhaustion rates in the various tests whether they were carried out in a swim dip or in a shower dip. The exhaustion rate of these two BHC formulations was more rapid than that of 'Rucide'. There was a slight tendency for the exhaustion rate to be slower in the swim dips and for the paste to exhaust more slowly than the wettable powder, but it is doubtful if either of these trends is of importance.

(b) *Effect of varying dip management on exhaustion*

Trial 4. *The effect of increased contact time on the exhaustion rate.*—In Trial 4, the effect of prolonging the period of contact between the dip wash and the sheep was examined by increasing the spraying time in a shower dip. Two flocks of fine wool Merino sheep were dipped in a shower dip, 2 weeks after shearing, with 'Rucide' at a concentration of 0·1% p.p.i. DDT. In Test 4/1 each pen of sheep was sprayed for a total period of 4 min., and in Test 4/2 for 8 min. Table III shows the volume of dip removed, the drop in concentration and the number of sheep dipped in these two trials.

Table III

Effect of prolonging the period of spraying

Test	Spraying time, min.	Volume at start, gal.	Concn. at start, p.p.i. DDT, %	Volume removed, gal. (A)	Drop in concn., % (B)	B/A	No. of sheep dipped	Gal. per sheep
4/1	4	394	0·106	256	0·029	0·00011	766	0·33
4/2	8	397	0·092	287	0·032	0·00011	570	0·49

The sheep which were sprayed longer carried out more wash per head, indicating that complete saturation was not obtained with the shorter period. The percentage drop in concentration for the percentage of the wash volume removed was, however, the same in each test, so that prolonged contact did not increase the exhaustion rate.

On another occasion, a pen of sheep was sprayed for successive 4-min. periods. They were allowed to drain thoroughly and samples of wash were taken for analysis between each spraying period. There was a normal rate of exhaustion until the sheep became fully saturated, after which there was no apparent drop in concentration, nor was any more wash removed, although the sheep were sprayed for two additional 4-min. periods.

Trial 5. *The effect of increased concentration on the rate of exhaustion.*—At one time some sheep dip formulations were used at a relatively low concentration for the control of keds and lice, and at a considerably higher concentration for the control of fly strike. A trial was therefore carried out in a shower dip on two flocks of fine-woolled Merino sheep, which were showered within 24 h. of being shorn, one at a concentration of 0·06% BHC and the other at 0·48% BHC, two tests being made at each concentration. At the lower concentration the wash was reinforced as required with additions of concentrate to maintain a minimum concentration above 0·03% BHC. The amount of concentrate to be added was calculated from the exhaustion rate exhibited by this formulation in Trial 3. At the higher concentration no further insecticide was added after the sump had been charged.

The results of these tests are plotted in Fig. 3. As additions of insecticidal concentrate and water were made to the wash in Tests 5/1 and 5/2, it was necessary to calculate the exhaustion rate for each stage between samples separately and a similar procedure was adopted for Tests 5/3 and 5/4.

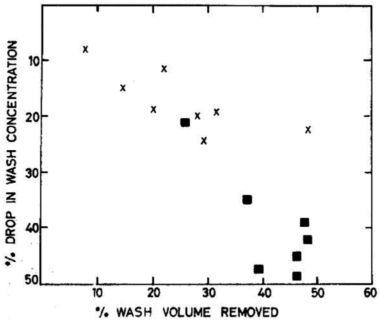

FIG. 3.—*Effect of wash concentration on the exhaustion rate of a BHC paste formulation*

■ 0·06% BHC, tests 5/1, 5/2
× 0·48% BHC, tests 5/3, 5/4

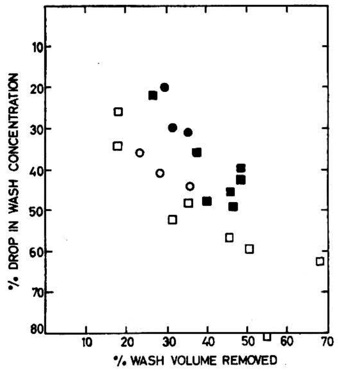

FIG. 4.—*Effect of wool length on exhaustion rate*

Symbol	Test	Formulation	Wool length
□	3/9	BHC paste	4–6 weeks off shears
□	3/10	,, ,,	
○	6/1	Dieldrin wettable powder	

Symbol	Test	Formulation	Wool length
■	5/1	BHC paste	off shears
■	5/2	,, ,,	,,
●	7/2	Dieldrin wettable powder	,,

There was not much difference between the exhaustion rates for the high and the low concentrations, that for the former tending to be slightly slower, but it is doubtful if the difference is of any practical importance.

Trial 6. *The effect of dipping sheep ' off shears ' on the rate of exhaustion.*—It was noticed in Trial 5 that the exhaustion rate of a BHC suspension was slower when it was used on sheep within 24 h. of shearing (Tests 5/1 and 5/2) than it was on sheep carrying 4–6 weeks' wool in Trial 3 (Tests 3/9 and 3/10). A further test (6/1) was therefore made with the same type of formulation in a shower dip using a 50% dieldrin wettable powder on Merino sheep carrying 4–6 weeks' wool. The formulation was the same as that used in Test 7/2 on sheep ' off shears '. In Fig. 4 the exhaustion rates are shown for the BHC and dieldrin formulations used in a shower dip ' off shears ' (wool length about ¼ in.) and 4–6 weeks after shearing (wool length 1–1¼ in.). The exhaustion rate is slower when sheep are dipped ' off shears '.

(c) *Effect of formulation on the exhaustion rate*

Formulations for sheep dips may be of three types: solutions, suspensions or emulsions. Exhaustion does not occur in washes of sodium arsenite, the only water-soluble insecticide in common use. In the following tests, the exhaustion rate of a number of suspension and emulsion formulations containing BHC or dieldrin were compared.

Trial 7. The exhaustion rate of BHC and dieldrin suspensions.—Manson (personal communication, 1951) found that the exhaustion rate of sulphur is more rapid in suspensions of large particle size. In Trial 7 the particle size of the suspensions tested was fairly uniform but the kind of insecticide, wetting agent and filler or powder vehicle varied. The tests were carried out in a shower dip with fine-woolled Merino sheep straight ' off shears ', so that these tests with dieldrin suspensions are comparable with those in Trial 5 in which BHC suspensions were used.

Two dieldrin wettable powders were used, one containing 10% dieldrin in a china clay base (Test 7/1), and the other 50% dieldrin in a bentonite base (Test 7/2). They were used at a theoretical starting concentration of 0·01% dieldrin in the wash and no further concentrate was added during the test.

The intermittent exhaustion rate was calculated for each sampling interval and is shown graphically in Fig. 5, together with those for the BHC paste used in Trial 5 (Tests 5/1 and 5/2)

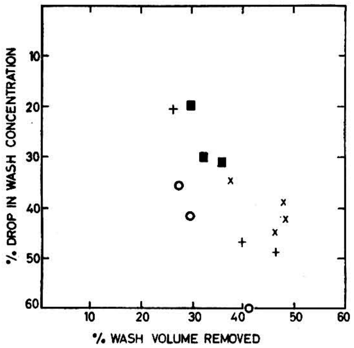

FIG. 5.—*Exhaustion rate of a BHC paste and dieldrin wettable powders*

Symbol	Test	Formulation	Symbol	Test	Formulation
×	5/1	50% BHC paste	○	7/1	Dieldrin wettable powder
+	5/2	,, ,, ,,	■	7/2	,, ,, ,,

There was little difference between the exhaustion rate of these two dieldrin suspensions and the BHC suspension used in the earlier trial.

Trial 8. The exhaustion rate of BHC and dieldrin emulsions.—In another series of tests, Merino sheep were shower-dipped ' off shears ' with three different BHC emulsions and two dieldrin emulsions. As these were all commercial preparations their exact formulae were not known. It was known, however, that different emulsifying agents were used, none of which were cationic, and that the solvents included bone oil and aliphatic and aromatic petroleum products. The cumulative exhaustion rate for each formulation is shown in Fig. 6.

Although there was a considerable variation in the composition of these emulsions, their exhaustion rates were similar and were characterised by a very rapid initial fall in concentration. The rate of exhaustion decreased in very dilute washes at the end of the tests. It is not known if this change was due to increased errors in the analysis of the very dilute wash or to the emulsion breaking. These tests with emulsions were carried out under the same conditions as those in Trial 7 with suspensions and are therefore comparable. When 30–40% of the wash had been removed, the concentration of the suspensions dropped by 30–40% (Fig. 5) and by 60–70% in the case of the emulsions (Fig. 6).

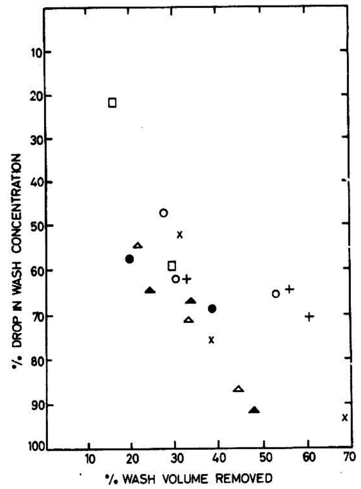

FIG. 6.—*Exhaustion rate of BHC and dieldrin emulsions*

Symbol	Test	Formulation	Symbol	Test	Formulation
○	8/1	BHC emulsion, miscible oil, 14% total BHC, 4% γ-BHC	△	8/5	15% Dieldrin emulsion
	8/2	,, ,,	▲	8/6	15% ,, ,,
●	8/3	BHC emulsion, miscible oil, 11% total BHC, 3·2% γ-BHC	+	8/7	15% ,, ,,
□	8/4	BHC emulsion, animal oil solvent, 10% total BHC, 5·3% γ-BHC	×	8/8	Dieldrin emulsion, (solubilised product, 10% dieldrin in alcoholic solvent)

Note. The wetting agent in test 8/5 was different from that in tests 8/6 and 8/7

(d) *Miscellaneous observations*

During these trials, a constant watch was kept to see if any sources of insecticidal loss could be detected. With suspension formulations, deposit was rarely found on the bottom of the shower dip sumps, but a small amount was generally present on the bottom at the entrance of large swim dips. There was always some deposition of material from suspension dips on the concrete floor of shower pens and draining yards, but the amount seen could account for only a small proportion of the insecticide lost during dipping. In shower and, to a lesser extent, in swim dips, a greasy froth or scum collects on the surface of the wash. On a few occasions, as much scum as possible was collected from shower sumps and analysed. The insecticidal content was always high, but the amount present accounted for only a small proportion of the total insecticide lost by exhaustion. It must be concluded therefore that, although small amounts are lost in various ways, the bulk of the insecticide lost by exhaustion is carried out on the sheep.

Discussion

The series of dippings recorded in this paper showed that the exhaustion rate for each formulation was fairly constant. Within the range tested, variations in wash concentration and the period of contact between the wash and the fleece had no appreciable effect on the exhaustion rate. The rate was slightly slower however with sheep dipped ' off shears ' than with sheep carrying more wool.

There was a considerable difference between the exhaustion rates of different types of formulation, the suspensions showing a slower rate of exhaustion than the emulsions. Within each type of formulation, differences in composition had little effect on the exhaustion rate. BHC and dieldrin showed similar rates when used in comparable types of formulation, thus supporting Machin's[11] observations on *in vitro* tests. The only DDT formulation used in these tests showed a slower exhaustion rate than BHC or dieldrin, but whether this was due to the insecticide itself or to the type of formulation is not known.

Large amounts of insecticide were not left as residues in the dipping equipment, so it must be assumed that the insecticide lost from the wash by exhaustion was carried out on the sheep.

Field trials of this type have their limitations. The reliability of analytical results varies considerably, due in part to errors in the sampling technique, such as the inclusion of different amounts of the greasy surface scum, or to variations in the dipping procedure, such as variation in the proportion of drainings actually returned to the wash reservoir. It is difficult to analyse accurately a fouled dip wash, especially when the insecticidal content is very low. Moreover, the number of sheep available for experimental dippings is generally limited, so that changes in concentration of the wash can be followed only for a short time. As the rate of exhaustion is constant over a wide range of wash concentrations, however, it was possible to calculate the changes in wash concentration when a number of different dipping procedures were employed.

In the field the procedure for using a dip formulation may be varied by (*a*) altering the initial concentration at which the bath is charged, (*b*) altering the concentration at which the bath is replenished with water and concentrate, (*c*) adding concentrate only from time to time to ' reinforce ' the wash, or (*d*) altering the frequency with which replenishments are added. The effect of these variations is discussed below.

With a non-exhausting formulation, the wash concentration is determined partly by the charging concentration and partly by the concentration of replenishments, and eventually the bath concentration is determined by the concentration of the replenishments. With exhausting formulations, the wash becomes stabilised over a range of concentrations which have a maximum just after replenishment and a minimum just before replenishment. The concentration of the replenishment determines the mean value of this range, and the mean is unaffected by the volume of the bath or the frequency with which replenishments are made. When the wash concentrations are in this stable condition, the amount of insecticide added to the bath and removed by the sheep is equal.

The frequency with which additions of insecticide are made determines the range of variation about the mean wash concentration. Infrequent replenishments give a wide range of concentration, but an equivalent amount of material added as a number of small portions at frequent intervals gives a narrower range and a higher minimum concentration.

The use of a system of ' reinforcement ', i.e., the addition of insecticide without an appropriate amount of water, will produce a wider range of concentrations and a higher maximum and a lower minimum than if the same quantity of insecticide had been added with an appropriate amount of water as a normal replenishment.

The minimum wash concentration is therefore determined by the concentration and frequency of the replenishments. The charging concentration has only a transitory effect on the wash concentration and the period of its effect is determined mainly by the volume of the initial charge and the frequency of the replenishments. The initial charging concentration need not be higher than the maximum that can be sustained by the replenishments subsequently used. With intermittent replenishments, a given minimum wash concentration can be maintained either by using infrequent replenishments of a high concentration or frequent replenishments of a lower concentration. A system of continuous replenishment where fresh wash is added at the same rate as it is removed by the sheep will give the highest minimum wash concentration for a given amount of material and the wash concentration will remain uniform and will be equivalent to the mean value given by the replenishment concentration.

The mean wash concentration sustained by any given concentration of replenishments is determined by the exhaustion rate of the formulation. The more rapid the exhaustion rate, the lower will be the mean wash concentration. This can be expressed numerically as the amount of insecticide removed with a given volume of wash, divided by the amount of insecticide in an equivalent volume of wash at the replenishment concentration. This 'exhaustion factor' for non-exhausting formulations would be 1, and for exhausting formulations some value greater than 1.

The actual deposit of insecticide on the sheep cannot be measured accurately, and it is usually assumed to be equivalent to the insecticide removed from the bath during a period of dipping. This amount would be calculated as the product of the volume of wash removed by the mean concentration of the wash and the exhaustion factor, so that, although the more rapidly a wash exhausts, the lower is the concentration required to give a fixed deposit per sheep, the *mean* deposit is, in fact, directly proportional to the concentration of replenishments, irrespective of the exhaustion rate of the formulation. The exhaustion rate of different formulations will, however, determine the frequency of replenishment required to maintain a fixed minimum deposit per sheep.

In practice, therefore, the problem of using exhausting formulations in sheep dips is that of controlling the variation of the deposit per sheep around its mean value. This can be reduced only by greater frequency of replenishment and, to control the minimum deposit accurately, this frequency would have to be specified in addition to the concentration.

A system of continuous replenishment has many advantages once the mechanics of its implementation are overcome. For a given concentration of any type of formulation there is the same deposit on all sheep, and this deposit is higher than the minimum that can be achieved by any other system using an equivalent amount of material.

Acknowledgments

This work was initially instituted as a research programme of the Division of Animal Health & Production, C.S.I.R.O., and later continued on behalf of William Cooper & Nephews (Aust.) Ltd. The author thanks those graziers who kindly made their flocks available, particularly Mr. G. Gill of Emu Creek, Walcha, N.S.W., on whose property most of the trials were carried out. Thanks are also due to Miss M. T. Scott, who was closely associated with much of the programme and who undertook the DDT analyses, and to Mr. W. J. Bliss who carried out the dieldrin and BHC analyses. Thanks are also due to the Directors of William Cooper & Nephews (Aust.) Ltd. for permission to use data collected whilst employed by that Company.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

NOVEMBER, 1961

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

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

NOVEMBER, 1961

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Modification of climate near the ground by irrigation for pastures on the Riverine Plain. D. A. de Vries and J. W. Birch (*Aust. J. agric. Res.*, 1961, **12**, 260—272).—Irrigation gives rise to a decrease in air and soil temp. and an increase in atm. humidity near the ground. The effects, varying with irrigation rate and the distance down wind from the boundary of the irrigated area, were approximately 1—2° and 10° for air and soil temp. and 5—10% for R.H.
E. C. APLING.

Influence of soil moisture content on plant transpiration and application of the cobalt chloride paper method to the measurement of transpiration of the coffee bush. J. Vieira da Silva, J. Esteves Baptista and M. Antonieta Nunes (*Estud. agron., Lisboa.*, 1960, **1**, 271—282).—Transpiration (measured gravimetrically) of bean plants, pearl millet and sorghum in glasshouses, depended on the moisture content of the soil. The $CoCl_2$ paper method was used to measure transpiration in coffee plants in a field test. The transpiration was inversely proportional to the hydration of the leaves, estimated by their relative turgidity, and was possibly related to the strains used. (17 references.) L. A. O'NEILL.

Transpiration and evapo-transpiration of maize as related to meteorological factors. L. J. Fritschen and R. H. Shaw (*Agron. J.*, 1961, **53**, 71—74).—Measurements of soil temp., net radiation and physiological maturity indicated that different microclimates existed in plastic covered plots and natural plots and that the difference between evapo-transpiration (measured in natural plots) and transpiration (measured in plastic-covered plots) does not give an accurate measure of evaporation. When measured in this way transpiration was 0·73 × evapo-transpiration, but when adjusted, using the ratios of energy intercepted by the plant canopies, transpiration was 0·89 × evapo-transpiration. A. H. CORNFIELD.

Theoretical p*F* curve and its importance in soil water economy. M. Pereira Gomes and A. Antunes da Silva (*Estud. agron., Lisboa*, 1960, **1**, 67—77).—A method is described for deriving the p*F* curves using the expression $Y = A \exp[-(pF^2/B^2]$ where Y is the % moisture at a given p*F*, and A and B are specific parameters calculated from two experimental points. Results for 15 samples of soil from Portugal and overseas are examined statistically.
L. A. O'NEILL.

Interpretation of the upper plastic limit of clays. B. P. Warkentin (*Nature, Lond.*, 1961, **190**, 287—288).—The upper plastic limit or liquid limit of clays is that water content at which enough free water is present to allow clay particles to slip past one another under a certain applied force. An " interaction vol." for a unit mass of clay particles is that vol. within which individual particles or units of particles interfere with the movement of adjacent particles or units. Interaction vol. decreases as the applied force increases. The influence on the liquid limit of such factors as exchangeable ion, salt concn. and pH of the clay paste, e.g., montmorillonite, kaolinite and other clays is discussed. At a high electrolyte concn. and in presence of dissolving salt, clay pastes may become stiff or show increase in liquid limit. E. M. J.

Growth rates of barley and maize as affected by changes in soil moisture stress. W. D. Kemper, C. W. Robinson and H. M. Golus (*Soil Sci.*, 1961, **91**, 332—338).—Restricted growth of the plants under conditions of moisture stress was followed by temporarily very rapid growth when the stress was relieved. A possible mechanism of these changes is indicated. The more severe stresses introduced by high salinity in the nutrient delayed the maturation of maize. A. G. POLLARD.

Glassbead-glycerol model for non-steady-state tile drainage. B. L. Grover and D. Kirkham (*Proc. Soil Sci. Soc. Amer.*, 1961, **25**, 91—94).—The construction and use of a reduced scale model for studying the falling water table in non-steady-state drainage problems are described. A capillary fringe is accounted for in the model. Stratified soils may be simulated. The model is useful for obtaining information on drainage theory and design.
A. H. CORNFIELD.

Accumulation of moisture in soil under an impervious surface. J. Le R. Mickle (*Dissert. Abstr.*, 1961, **21**, 2654).—The terminal equilibrium moisture contents in a soil column under an impervious surface can be predicted from desorption curves on undisturbed samples of soils providing water-table and temp. data are available. Only extreme temp. affect terminal moisture contents. At a given moisture tension, the equilibrium moisture content of a soil exceeds that in one which has greater density. O. M. WHITTON.

Soil management in fruit culture. J. Butijn (*Versl. landbouwk. Onderz.*, 1961, 66.7 i, ii, and iii, 403 pp.).—A review of published results with special reference to conditions in Holland. The interactions between the physical properties of orchard soils, soil-water status, climatic factors, root development, and uptake of water and nutrients are considered. A further section deals with the influence of the chemical properties of soils on the effects of deficiencies and nutritional disorders observed on some Dutch soils. A final section deals with practical advice. (742 references.) P. S. ARUP.

Microbiological and chemical techniques for assay of available soil nitrogen. F. C. Boswell (*Dissert. Abstr.*, 1961, **21**, 2427).—A technique intended for the measurement of soil-N by the pigment produced by *Pseudomonas aeruginosa* showed a highly negative correlation with nitrifying capacity as determined by the incubation method, and with soil-N as determined by the Truog method. Although the results of the Truog and the Purvis methods show a high degree of positive correlation, no significant correlation exists between the results of the Purvis and the above microbiological method. Possible explanations for the above findings are considered. P. S. ARUP.

Contribution of [determination of] soil nitrogen to [assessment of] requirements of crops on Florida soils. M. N. Malik (*Dissert. Abstr.*, 1961, **21**, 2420).—Comparative experiments with oats, millet and rye, manured at varying rates with NH_4NO_3 show the soil-N and the NO_3^--N produced during incubation of the soil to be equally good as indicators of N requirements. The test for total N is preferable for use in service laboratories on account of its greater convenience. P. S. ARUP.

Biological availability of nitrogen in certain condensation products of urea and formaldehyde. F. L. Long (*Dissert. Abstr.*, 1961, **21**, 2419—2420).—In greenhouse experiments, the comparative availability of the N from the water-insol. reaction product to a first crop of oats (two clippings and the roots) is less than that of the N from an NH_4^+-N mixture, but three times as great with respect to a succeeding crop of millet; the latter effect is due to the comparatively early depletion of the NH_4^+-N. The total recovery of N from the insol. product increases with increasing ratios of urea to formaldehyde. The availability of N in the following compounds is in the (decreasing) order: methylenediurea, dimethylenetriurea and trimethylenetetraurea. P. S. ARUP.

Pedological survey of phosphorus in twelve soils derived from granite. A. Wild (*Aust. J. agric. Res.*, 1961, **12**, 286—299).—Chemical analysis of 12 soil profiles in N.S.W. and Queensland showed that all except two had lost PO_4^{3-} by leaching. Loss of P is not correlated with present rainfall, and possible reasons are differences in soil age, level of org. matter and rate of weathering of the parent rock. Up to two-thirds of retained P is chemically bound with Fe_2O_3 and Al_2O_3. (23 references.) E. C. APLING.

Application of Ostwald's dilution law for estimation of available potassium in soils. E. Fischer (*S. Afr. J. agric. Sci.*, 1960, **3**, 539—550).—In five soils of low saline content, the determined concn. of K in the aq. phase for widely differing soil/water ratios are in good agreement with the values calculated according to the approx. dilution law, applicable to salts of strong bases and weak acids. The validity of the calculations is confirmed by the responses (or non-response) of crops to applications of K. P. S. ARUP.

Sulphur acidity in South Carolina tidal marsh soils. J. F. Fleming and L. T. Alexander (*Proc. Soil Sci. Soc. Amer.*, 1961, **25**, 94—95).—Soils from the tidal marsh area of South Carolina showed pH reductions of up to 3 units when drained and dried, giving a final pH as low as 2·6 in the topsoil and 1·7 at lower depths. The increased acidity is probably due to oxidation of S^{2-}, derived by reduction of SO_4^{2-} in the seawater under waterlogged conditions, to SO_4^{2-} during drying. A. H. CORNFIELD.

Mineralisation of sulphur from soil organic matter. N. J. Barrow (*Aust. J. agric. Res.*, 1961, **12**, 306—319).—Extraction of soils with 0·15% $CaCl_2$ is advocated for the determination of available S.

Drying of soil results in increased inorg. SO_4^{2-} extracted, presumably derived by splitting from org. sulphates, and the additional S is available to plants. Pot tests with S may be affected by this increase in available S, which is probably also of importance under field conditions. (33 references.) E. C. APLING.

Loss of zirconium from twelve soils derived from granite. A. Wild (*Aust. J. agric. Res.*, 1961, **12,** 300—305).—Analysis of soil profiles in N.S.W. and Queensland show general loss (20—50%) of Zr in the lower horizons of the profiles, implying loss of Zr from grains of zircon. Further investigation is needed before zircon can be considered a dependable index material to follow changes in soil profiles. E. C. APLING.

Radioactive pollution of soils: aspects and remedies. Cohen and Gailledseau (*C. R. Acad. Agric. Fr.*, 1961, **47,** 226—229).—The decontamination of soils polluted with ^{90}Sr is discussed. Effective methods are: addition of Ca salts, KH tartrate or ethylenediaminetetra-acetate, the last being the most efficacious. Sr is also removed from soil if vegetative fermentation takes place in the vicinity. J. V. RUSSO.

Soil organic matter–metal complexes. III. Exchange reactions of model compounds. T. E. Lewis and F. E. Broadbent (*Soil Sci.*, 1961, **91,** 341—348).—Interactions between Cu, U, exchange resins and a range of model substances having phenolic and carboxylic groups are recorded. Adsorption of Cu^{2+} by the model substances was conditioned mainly by pH; it occurred at pH ranges above a limiting value with release of H^+, continuing until the limiting pH was reached. The limiting pH was largely independent of the $[Cu^{2+}]$ in the solution and of the amount of Cu already present on the exchanger. Adsorption of U and Ba followed a similar pattern although there were differences in limiting values and in the influence of other factors. A. G. POLLARD.

Liberation of [plant] nutrients from minerals by soil fungi. G. Müller and I. Förster (*Zbl. Bakt.*, 1961, II, **114,** 1—10).—The release of K from feldspar by *Aspergillus niger* is examined. Conditions under which the Niklas technique gives reliable data on the availability of nutrients in soil are discussed. A. G. POLLARD.

Ecology of Streptomycetes. III. Antibiotic activity in the rhizosphere of barley. H.-J. Rehm (*Zbl. Bakt.*, 1961, II, **114,** 147—155).—In the rhizosphere of barley *S. diastaticus*, *S. olivochromogenus*, *S. antibioticus*, *Actinomyces chromogenes* and *A. cretaceus* were present. Shortly before ear-formation the anti-fungal streptomycetes diminished; at the ripening stage their no. exceeded those in fallow soil. A. G. POLLARD.

Glycerol–peptone–agar, an improved medium for bacteriological studies of soil. W. Hirte (*Zbl. Bakt.*, 1961, II, **114,** 141—146).—For the qual. and quant. examination of soil bacteria a glycerol–peptone–agar medium to which meat broth is added, is recommended. For the qual. examination of antinomycetes the Na–asparaginate–agar medium (Conn and Staff, *ibid.*, 1952, **107,** 129) is preferable. A. G. POLLARD.

Biotic relationships between soil algae and other micro-organisms. B. C. Parker and H. C. Bold (*Amer. J. Bot.*, 1961, **48,** 185—197).—The association between a *Bracteacoccus* sp. and a heterotrophic bacterium in soil is based on the breakdown of complex N compounds by the bacterium thus providing more assimilable forms of N for the alga. A form of symbiosis between a *Chlamidomonas* sp. and a *Streptomyces* sp. is conditioned partly by a CO_2–O_2 interchange between the organisms. The latter lowered the N supply to the former by competition and also assimilated its extra-cellular polysaccharide; both effects stimulated the growth and motility of the alga while the growth and conidia production of the actinomycete also increased. A preliminary examination of the apparent antagonism between a *Phormidium* sp. and an unidentified fungus is described. A. G. POLLARD.

Plough-down application seals anhydrous ammonia in soil. C. L. W. Swanson (*Farm Chem.*, 1961, **124,** No. 3, 23, 26).—Distribution of anhyd. NH_3 applied behind the plough is examined. Losses of NH_3 were 1·04% with 3 in., 0·35% with 6 in. and 0·15% with 9 in. ploughing. Of these losses 97% occurred within 1 h. of application. The delivery hose should release NH_3 $\nless 4$ in. from the furrow wall and 3—4 ft. behind the point of the share. The slice should be completely inverted. A. G. POLLARD.

Fertilising value of guanyl-[amidino-]urea. O. T. Rotini, N. Guerrucci, P. Rotini and G. Guatteri (*Ric. sci.*, 1960, **30,** 2114—2120).—The anti-mitotic limit for bulbs of *Allium cepa* L. is 0·00625 and 0·0125% for solutions of amidino-urea sulphate and phosphate respectively. For various cultivated plants the limits for the two compounds were similarly: wheat, 0·025, 0·05; maize, 0·0125, 0·025; barley, 0·00625, 0·0125; and vetch, 0·00156, 0·00312%. Above these limits the compounds are not phytotoxic and may be of value for the nitrogenous fertilisation of crops. (14 references.) L. A. O'NEILL.

Hydrothermal treatment of natural phosphates in a cyclone furnace. S. J. Volfkovich (*Chim. et Industr.*, 1961, **85,** 588—593).—Hydrothermal treatment occurs in a cyclone furnace at 1400—1500°, the main equation used being:

$$n\mathrm{Ca_{10}(OH)_2(PO_4)_6} + m\mathrm{SiO_2} = 10n\mathrm{CaO},3n\mathrm{P_2O_5},m\mathrm{SiO_2} + n\mathrm{H_2O}$$

The heat needed for the reaction is supplied by liquid oil or natural gas. 95—99% of the phosphates are converted into a form soluble in citric acid and F content is $<0·2$%. Tables are given of the mineral contents of different phosphates treated in this way. Experiments have proved that the phosphates obtained by this process are valuable as fertilisers and animal foods. K. RAVEN.

Phosphate availability in superphosphate-treated organic manures. A. K. Dutt (*Fertil. News*, 1961, No. 6, 7—11, 26).—The application of superphosphate to Indian soils is reviewed. Reference is made to U.S.A. data on the 26-year average yields of maize, wheat, clover treated with superphosphate applied with manure. Adaptation of these findings to Indian conditions is recommended. E. M. J.

[A] **Liquid fertilisers from wet-process phosphoric acid and superphosphoric acid.** J. A. Willbanks, M. C. Nason and W. C. Scott. [B] **Liquid fertilisers from superphosphoric acid and potassium hydroxide.** J. M. Potts, H. W. Elder and W. C. Scott (*J. agric. Fd Chem.*, 1961, **9,** 174—178, 178—181).—[A] The pptn. of impurities from the wet-process acid during and after neutralisation with aq. NH_3 can be prevented by the presence of " superphosphoric acid " (or its NH_3 salt), a product containing 76% of P_2O_5, about 50% of which is present as condensed phosphoric acids; the best results are obtained by running this acid, the wet-process acid, and the aq. NH_3 simultaneously into the mixing tank, containing a " heel " of water. A pH of 6·6 is maintained during the mixing. Liquid fertilisers to which 20—40% of the P_2O_5 has been added as superphosphoric acid (according to the purity of the wet-process acid) compare favourably, as regards stability during storage, with those made from electric furnace acid.

[B] Laboratory experiments indicate that high-grade liquid fertilisers of low Cl content can be made by adding superphosphoric acid (see [A]), water, and (if required) aq. NH_3 to KOH (or possibly K_2CO_3). Neutral liquid fertilisers, stable during 1 week at 0°, can thus be produced with NPK ratios of 0 : 1 : 1 or 1 : 4 : 4; such grades cannot be produced with KCl as the source of K. P. S. ARUP.

Sphalerite for zinc additions to fertilisers and soils. W. H. MacIntire (*Farm Chem.*, 1961, **124,** No. 7, 22).—Caking resulting from incorporation of $ZnSO_4$ into mixed fertilisers is overcome by use of finely-ground sphalerite (ZnS) which is stable under acid conditions likely to be met with in fertilisers (H_3PO_4, superphosphate). The S^- is readily oxidised to SO_4^{2-} in soil. A. G. POLLARD.

Polarographic study of fertilisers, organic manures and mixtures. Z. Ferenczy and A. Almásy (*Commun. Res. Inst. heavy chem. Ind. Hung.*, 1960, **2,** 155—159).—Polarographic methods were applied to the estimation of P, N and K in fertilisers and manures. In general, accuracies of better than 2% were obtainable, but interfering elements had to be removed, and for NO_3^- estimation the concn. of NO_3^- had to be adjusted as the linear relationship of the wave and concn. held only in a narrow range. The methods of Neuberger and Novak were used for P determination, of Ruzicka and Tokuoka for NO_3^- and of Abresch for K. K. RIDGWAY.

Plant Physiology, Nutrition and Biochemistry

An estimate of a minimum quantum yield of photosynthesis based on ecologic data. J. R. Bray (*Plant Physiol.*, 1961, **36,** 371—373).—Data based on calorific content of a *Picea omorika* plantation show above ground accumulation of $740·9 \times 10^8$ cal./ha./year, total accumulation of $946·4 \times 10^8$ cal./ha./year and total production of 1893 cal./ha./year. Direct solar radiation is $57,000 \times 10^8$ cal./ha./year. Analysis of radiation measurements in conifer stands suggest a max. visible radiation of $23,864 \times 10^8$ cal./ha./year is available to the photosynthetic pigments. Efficiency in relation to visible radiation is 7·9%. Min. quantum yield estimate for the plant community over the year is 0·038, max. 0·053 cal. E. G. BRICKELL.

Pigment formation and photosynthesis in dormant lettuce seeds. A. H. Haber and W. L. Carrier (*Plant Physiol.*, 1961, **36,** 351—353).—Lettuce seeds prevented from germinating by maleic hydrazide can develop chloroplasts in an internal sector of cotyledons exposed to light. Chlorophyll and carotenoid-like pigments can be extracted from such seeds and the latter perform a light-dependent fixation of $H^{14}CO_3^-$ with radioactivity appearing in sucrose, sugar phosphates, and amino- and keto-acids. E. G. BRICKELL.

Mechanism of stimulation of respiration by purines. I. Inhibitor studies. M. K. Bach (*Plant Physiol.*, 1961, **36,** 317—322).—Two classes of compounds inhibited purine-induced respiration without affecting endogenous respiration. One group consisted primarily of sulphydryl inhibitors while the second was made up of compounds which are either substrates or inhibitors of known flavo-protein enzymes. The stimulatory purines caused a marked decrease in the specific activity of respired $^{14}CO_2$ from cells which had been allowed to fix $^{14}CO_2$ for short periods, this being interpreted as a stimulation of fatty acid oxidation by the purines.

E. G. Brickell.

Activities of acid phosphatase, peroxidase and polyphenolase in etiolated shoots from control and irradiated maize seeds. F. A. Haskins and S. C. Downs (*Agron. J.*, 1961, **53,** 90—93).—There were enzymic differences between etiolated shoots from irradiated maize seeds and shoots from control seeds. Differences in sp. activities of acid phosphatase and peroxidase were apparent only when individual portions (first internode, coleoptile, and unexpanded foliage leaves) of the shoot were considered. The contribution of the various shoot portions to total activities and the effect of irradiation on these contributions are reported. A. H. Cornfield.

Oxidation of reduced diphosphopyridine nucleotide by mitochondria from normal and crown-gall tissue cultures of tomato. T. Tamaoki, A. C. Hildebrandt, R. H. Burris and A. J. Riker (*Plant Physiol.*, 1961, **36,** 347—351).—Normal tissue particles always showed higher enzyme activities than crown-gall particles but there was no qual. difference in their catalytic properties. The mitochondria of both have virtually identical pathways for the transport of electrons from the nucleotide to O. A. G. Brickell.

Grain sorghum caryopsis development. II. Changes in chemical composition. J. F. Kersting, A. W. Pauli and F. C. Stickler (*Agron. J.*, 1961, **53,** 74—77).—The N % in grain sorghum caryopses remained constant after about 10 days after pollination. Total sugars % decreased rapidly until 20 days after pollination and declined slowly thereafter. Starch % increased rapidly after pollination to max. of 64 and 79% in two years. Acid-hydrolysable carbohydrate % was closely correlated with starch %. The total quantities of N, total sugars, starch and acid-hydrolysable carbohydrates were at a max. at or near the dates of max. dry wt. Diastatic activity was highly correlated with % germination.

A. H. Cornfield.

Relationship between moisture stress and uptake and translocation of phosphorus by plants. J. N. Corgan (*Dissert. Abstr.*, 1961, **21,** 2428).—Moisture stress reduces the translocation of P to the plant-tops of apple seedlings grown in soil, or in tomato seedlings grown in a nutrient solution in which the stress is induced by the addition of mannitol; reduction of transpiration by conditions of R.H. also reduces the transfer of P (as ^{32}P). In bean seedlings, the relationship between root-^{32}P and top-leaf-^{32}P is similarly affected provided that the concn. of P in the nutrient solution is high.

P. S. Arup.

Cation absorption by excised barley roots from soil suspensions and their equilibrium true solutions at different time intervals. A. K. Helmy and S. Oliver (*Soil Sci.*, 1961, **91,** 339—340).—The excised roots absorbed Ca and K much more extensively from a soil suspension than from an aq. solution in equilibrium with it; Na was absorbed to a greater extent from the aq. solution.

A. G. Pollard.

Uptake of magnesium and its interaction with calcium in excised barley roots. D. P. Moore, R. Overstreet and L. Jacobson (*Plant Physiol.*, 1961, **36,** 290—295).—Mg was rapidly absorbed by 6-day-old excised barley roots, the absorption being depressed by both low temp. and dinitrophenol. Excess absorption was related to org. acid production in the root. A large fraction of the Mg absorption was blocked very effectively by small amounts of Ca even at concn. where there was a net loss of Ca from the root. Ca probably acts by altering the selective permeability properties of the cell surface.

E. G. Brickell.

Iron absorption of roots as affected by plant species and concentration of chelating agent. J. C. Brown, L. O. Tiffin, A. W. Specht and J. W. Resnicky (*Agron. J.*, 1961, **53,** 81—85).—Increasing the concn. of chelating agent (diethylenetriaminepenta-acetic acid) in a nutrient medium decreased the capacity of plant roots to absorb Fe. Of 11 species tested, growth was impeded sharply in 6 when the molar concn. of chelating agent exceeded that of Fe. Other plant species absorbed Fe at higher concn. of chelating agent.

A. H. Cornfield.

Biochemical indications as means of distinguishing between iron- and manganese-deficiency symptoms in citrus plants. A. Bar-Akiva (*Nature, Lond.*, 1961, **190,** 647—648).—Several varieties of 3-month-old citrus seedlings were grown in nutrient solutions containing gradually decreasing amounts of Fe or Mn. In the leaves of Mn-deficient plants free-pentose contents increased, peroxidase activity decreased and the ratio (**I**) of *a-/b*-chlorophyll decreased. In Fe-deficient plant leaves the free-pentose content decreased, the peroxidase activity increased and **I** remained virtually constant.

S. A. Brooks.

[A] **Redox potential in iron-induced chlorosis.** [B] **Polyphenols in iron-induced chlorosis.** L. Heras (*An. Aula Dei*, 1960, **6,** 135—163, 164—169).—[A] Correction of Fe deficiency either by injecting Fe^{2+} or by lowering the light intensity involves an increase in E_h, the former process producing a permanent and the latter only a temporary change. Three states of chlorophyll are distinguished, (i) initial, (ii) light-activated and (iii) stabilised. In photosynthesis (i) is activated by light and stabilised by Fe^{3+} which acts as a protective agent prior to photosynthesis, the Fe^{3+} being reduced to Fe^{2+} in the stabilising process, probably through the agency of a quinone–polyphenol system. In chlorotic tissue there is little Fe^{3+} and very little of the (ii) is stabilised, the remainder being decomposed. Lowering the light intensity also restricts the amount of (ii) formed and enough Fe^{3+} may be present to stabilise part of this. Restoration of full light increases (ii) excessively and part of this decomposes. Photodecomposition of (ii) is unaffected by the relative proportions of chlorophyll-*a*- and -*b* present.

[B] In pear leaves the formation of polyphenols follows that of chlorophyll and is determined by the amount of chlorophyll-protector present. Reduction of quinone to polyphenol may be effected by Fe^{2+} resulting from the protection of chlorophyll by Fe^{3+}. The rôle of the quinone–polyphenol system in Fe-deficiency chlorosis is discussed. A. G. Pollard.

Stability and concentration of metal chelates, factors in iron chlorosis of plants. J. C. Brown, L. O. Tiffin, A. W. Specht and J. W. Resnicky (*Agron. J.*, 1961, **53,** 85—90).—Uptake of Fe by milo from nutrient solutions containing four different chelates was related to the stability constant of the Fe chelates. This relationship did not occur with soya-beans. More Cu and Mn were absorbed relative to Fe by milo than by two varieties of soya-bean. The root of one variety of soya-bean had a greater capacity to alter the availability of Fe from the metal chelates than had another variety of soya-bean or milo. A. H. Cornfield.

Cell wall carbohydrates in tobacco pith parenchyma as affected by boron deficiency and by growth in tissue culture. C. M. Wilson (*Plant Physiol.*, 1961, **36,** 336—341).—B deficiency caused a marked increase in the total amount of cell-wall material accompanied by a drop in the relative galactan content but little change in the relative amounts of pectic substances or cellulose, except that the amounts in young deficient tissue were increased towards the levels found in mature normal tissue. Pith tissue in sterile culture was stimulated to expand without cell division by adding IAA to the medium. Cell walls from tissues which had more than doubled in size contained a lower proportion of cellulose while the percentage of araban was increased. E. G. Brickell.

Translocation and re-use of boron in broccoli. N. R. Benson, E. S. Degman and I. C. Chmelir (*Plant Physiol.*, 1961, **36,** 296—301).—Broccoli was grown for about half its growth period with an adequate supply of B, after which a B-free nutrient was given. At 24 days B-deficiency symptoms developed and as growth continued a redistribution of the internal B occurred. There was no loss from the basal leaves, but marked changes occurred in the younger leaves, e.g. the 22nd leaf from the base changed in concn. from 33 to 2 p.p.m., and from 18 to about 6 μg. E. G. Brickell.

Influence of γ-radiation on the microflora of cucumber fruit and blossoms. R. N. Costilow, T. A. Bell and H. A. Rutherford (*Appl. Microbiol.*, 1961, **9,** 145—149).—The initial heterogeneous effects were much higher on the blossoms than on the fruit but survival curves show that distribution was not comparable for both types of material. The asporogenic bacteria (coliform and acid-forming) were the most sensitive to radiation while aerobic and anaerobic spore formers were the most resistant; filamentous fungi occupied an intermediate position. (15 references.) C. V.

Effects of ionising radiations on rice plants. M. M. Thaung (*Nature, Lond.*, 1961, **190,** 242—243).—Several varieties of Burmese rice grains were irradiated with either *X*-rays or thermal neutrons before sowing. No differences in germination-rate of rice grains were noted but mortality and retardation in height of seedlings increased with *X*-ray dosage. Delay in sowing enhanced mortality of seedlings from grains irradiated with *X*-rays but did not affect germination. Most of the tillers of treated plants were late or poorly developed and unproductive. (18 references.) S. A. Brooks.

Carbon dioxide fixation by auxin-treated tissues. A. S. Gupta and S. P. Sen (*Plant Physiol.*, 1961, **36,** 374—380).—IAA in concn. which are known to promote growth stimulates ^{14}C-incorporation into *Avena* coleoptile tissues. The contribution of CO_2 to the synthesis of cell-wall components is affected in a varied manner; IAA stimulates the fixation into polyuronide hemicelluloses, polysaccharides and α-cellulose with relatively long incubation periods; it has a distinct inhibitory effect on fixation into protopectin; the inhibition is marked after a few h. CO_2 is metabolised into a large no. of components within a few min. E. G. Brickell.

Isolation of indole-3-acetic acid from maize kernels and etiolated maize seedlings. R. H. Hamilton, R. S. Bandurski and B. H. Grigsby (*Plant Physiol.*, 1961, **36**, 354—359).—Indolylacetic acid (IAA) was detected in 80% ethanol extracts of maize kernels and shoots, as well as vegetative sugar beet roots and leaves and may occur as a labile complex in these extracts. An 80% ethanol-sol. water-insol. fraction from maize kernels released IAA on alkaline hydrolysis and this fraction may be protein in nature. E. G. Brickell.

Steady state growth of *Avena* coleoptile sections in high auxin concentrations. N. P. Kefford and J. Bonner (*Plant Physiol.*, **36**, 323—325).—Steady-state growth rates are inversely proportional to auxin concn., as was expected on the basis of the two-point interaction concept. E. G. Brickell.

Effect of photochemically produced oxidants on growth of *Avena* coleoptile sections. L. Ordin and B. Propst (*Plant Physiol.*, 1961, **36**, 326—330).—Cell elongation is inhibited by fumigating the sections with photochemically produced oxidants albeit at concn. exceeding those found in metropolitan atm. to date. E. G. Brickell.

Mediation of geotropic response by lateral transport of auxin. B. Gillespie and W. R. Briggs (*Plant Physiol.*, 1961, **36**, 364—368).—Equal amounts of auxin diffuse out from vertical and from horizontal coleptile tips, and the lower halves of partially split coleoptile tips release a larger amount than do the upper halves. When coleoptile tips are completely bisected prior to stimulation, however, the same quantity of auxin is obtained from each half indicating that auxin, or possibly an auxin precursor, is translocated from the upper to the lower side of a stimulated coleoptile. E. G. Brickell.

Influence of 2,3,5-tri-iodobenzoic acid, indole-3-acetic acid and method of sample collection on translocation of foliar applied radio-calcium. G. A. Taylor, J. N. Moore and W. O. Drinkwater (*Plant Physiol.*, 1961, **36**, 360—363).—Pretreatment of plants with tri-iodobenzoic acid and indolylacetic acid (IAA) had no appreciable effect on ^{45}Ca-translocation but samples from plants treated with tri-iodobenzoic acid exhibited somewhat less radioactivity than those from plants treated with IAA or control plants. Dehydration of intact plants in a forced draft oven resulted in a 100-fold over-all increase in radioactivity when compared with plants which were dismembered prior to drying. This was true regardless of chemical treatment. E. G. Brickell.

Effects of 2,4-dichlorophenoxyacetic acid on carbohydrate metabolism of etiolated maize seedlings. C. C. Black, jun. (*Dissert. Abstr.*, 1961, **21**, 2455).—The enzymic extracts prepared from the seedlings previously treated with a buffer solution containing 2,4-D contain (like seedlings treated with the buffer alone) enzymes of the pentose-phosphate and the glycolytic metabolic pathways; the 2,4-D treatment, however, increased the relative activity (*in vitro*) of the pentose-phosphate pathway, as shown by increases in the utilisation of ribose-5-phosphate, in the formation of heptulose and hexose from ribose-5-phosphate and in the rate of oxidation of glucose-6-phosphate and 6-phosphogluconate. Decreases in the activity of some of the enzymes of the glycolytic pathway are also demonstrated as results of the 2,4-D treatment. P. S. Arup.

Effect of certain benzazole compounds on plant growth and development. M. J. Klingensmith (*Amer. J. Bot.*, 1961, **48**, 40—45).—Benzthiazole (**I**) suppressed the elongation of the primary root in cucumber. Adenine did not counteract this effect but supplemented the (weaker) inhibitory action of benzimidazole (**II**) and benztriazole (**III**). When added to the root medium **III** restricted internodal growth and stimulated axillary development in beans, coleus, tomato and wheat; **I** initiated adventitious roots in bean and tomato and **II** injured laminar tissue in roots. A. G. Pollard.

Influence of antibiotics on germination of tomato seed and development of the seedlings. W. Krüger (*S. Afr. J. agric. Sci.*, 1960, **3**, 561—571).—When seeds are soaked in solutions of the antibiotics in high concn., washed, and then germinated on filter-paper, toxic effects and penetration of the antibiotics to the growing parts are observed in a few cases; no such effects are observed when the seeds are sown in soil. The seed treatment is unlikely to protect the young seedlings against bacterial invasion. (16 references.) P. S. Arup.

Production of inactive mutants of *Actinomyces rimosus* and formation of an antibiotic during their cultivation. S. I. Alikhanyan, S. Z. Mindlin, Z. M. Zaĭtseva and N. V. Orlova (*Dokl. Akad. Nauk SSSR*, 1961, **136**, 468—471).—Details are given for obtaining inactive mutants of *Act. rimosus* by irradiation with u.v. light. Tabulated results show the antibiotic activity of cultures grown on two different media using pairs of inactive variants. R. A. Keen.

Quantitative determination of saccharin in plants. A. M. Lasheen (*Proc. Amer. Soc. hort. Sci.*, 1961, **77**, 135—139).—The plant material was blended with a small amount of water acidified with acetic acid and oxalates and citrates were precipitated with Pb acetate and the saccharin extracted with ether and finally after elution determined polarographically. L. G. G. Warne.

Detection of trace quantities of acenaphthene by gas chromatography. W. G. H. Edwards, J. G. Clarke and A. G. Williamson (*Nature, Lond.*, 1961, **190**, 531).—From 1 to 10 μg. of acenaphthene (**I**) (used for *c*-mitotic effect) in biological extracts can be determined from the chromatogram obtained by passing a decalin solution of the sample through a 6-in. column of brick-dust impregnated with 15% silicone oil. A flame-ionisation detector (*ibid.*, 1958, **181**, 760) and H_2 as carrier are used. Tailing of decalin does not interfere; lower limit of detection is 0·2 μg. Root-tips of onions grown in **I** had 60 μg. of **I** per l. W. J. Baker.

Stabilised gibberellic acid composition. Imperial Chemical Industries Ltd. (Inventor: N. Senior) (B.P. 844,549, 7.2.58).—A solid composition containing gibberellic acid and or a salt thereof is stabilised against decomposition by addition of a dehydrating agent, e.g., Na_2SO_4, $MgSO_4$ or Na_2CO_3 (which form non-deliquescent hydrates), or a plant nutritive anhyd. salt capable of absorbing water (to form a non-deliquescent hydrate), e.g., $CuSO_4$, $CaSO_4$, partly dehydrated salts of polybasic acids, such as Na and K pyrophosphates, metaphosphates and pyrosulphates), also oxides which form innocuous hydrated products, e.g., SiO_2 gel. F. R. Basford.

Crops and Cropping

Phosphorus responses by dryland spring wheat as influenced by moisture supplies. J. F. Power, P. L. Brown, T. J. Army and M. G. Klages (*Agron. J.*, 1961, **53**, 106—108).—Application of P to fallowed northern Great Plains soils did not always result in increased spring wheat yields. Yield responses to P applications were related to available P prior to sowing, soil moisture at sowing and rainfall from tillering to heading. Soils of medium P availability in particular showed increased responses to P applications with increasing available soil moisture at sowing. A. H. Cornfield.

Effect of depth of transplanting on tillering and seed production in wheat. R. Halwagy (*Nature, Lond.*, 1961, **190**, 513—514).—Reaction of culms of *Triticum vulgare* (50 cm. long, grown from seed) when transplanted in clayey loam at depths of 10—28 cm., was studied. Optimum vegetative growth and seed production was induced by burial at a depth of 15 cm. This is ascribed to approx. constant soil-temp. and decreased liability to desiccation. That deeper burial does not induce better tillering is unexplained. W. J. Baker.

Availability of native and radioactive fertiliser phosphorus to oats on Lake Charles clay. L. E. Golden (*Soil Sci.*, 1961, **91**, 349—355).—Using ^{32}P-labelled material, 12—0—12 and 12—12—12 fertilisers were applied to oats either in bands or mixed with the topsoil. Yields of forage (3 cuttings) were greater with the banded application but grain yields were the same with both methods. During 12 weeks of growth more fertiliser-P was taken up by the crop from the banded than from the soil-mixed fertiliser; the amount of soil-P taken up was greater from the soil-mixed fertiliser. Differences in the total P contents of the two series of plants disappeared after 8 weeks growth. Movement of P from the band of application was negligible. The availability of soil-P was measured more effectively by extraction with 0·1N-HCl + 0·03N-NH_4F than with 0·025N-HCl + 0·03N-NH_4F or 0·1N-HCl. A. G. Pollard.

Nitrogen and potash on barley. F. V. Widdowson, A. Penny and R. J. B. Williams (*J. agric. Sci.*, 1961, **57**, 29—33).—Some response to 0·5 cwt. of K_2O/acre is obtained but effects at 1 cwt./acre are variable. Total applications of 0·7 cwt./acre over 2 years increases yield whilst total applications of 1·4 cwt. has a variable effect. K does not reduce lodging tendency nor does it affect grain size to any extent. N increases lodging and % of N in grain but reduces grain size. M. Long.

Zinc deficiency of maize and potatoes as related to soil and plant analysis. D. L. Grunes, L. C. Brown, C. W. Carlson and F. G. Viets, jun. (*Agron. J.*, 1961, **53**, 68—71).—Zn-deficiency symptoms appeared on maize and potato plants grown under irrigation on a Gardena loam (Chernozem) the surface foot of soil of which had been removed to aid gravity irrigation. The exposed soil was low in 0·1N-HCl-extractable Zn and leaf tissue also had a low Zn concn. The deficiency was cured by application of ZnSO$_4$ (15 lb. Zn per acre) to the soil or sprayed on the plants or by manure (20 tons per acre).

A. H. Cornfield.

Fertiliser responses of maincrop potatoes: a re-examination of experimental evidence. D. A. Boyd (*J. Sci. Fd Agric.*, 1961, **12,** 493—502).—Effects of varied soil and climatic conditions on the form of fertiliser/yield response curve, on the interaction between fertiliser nutrients and the effect of farmyard manure on fertiliser requirements of the crop are examined. In >100 experiments average responses to N 0·8, P$_2$O$_5$ 1·0 and K$_2$O 1·5cwt./acre were 1·8, 1·4 and 2·0 tons/acre respectively differing by $\not>$0·2 ton/acre from the earlier figures of Crowther and Yates. E. M. J.

Influence of the proximity of forest on the occurrence of *Hemiptera-Heteroptera* on potatoes as exemplified by materials from Wandzin. T. Ziarkiewicz (*Ann. Univ. M. Curie-Skłodowska*, 1959, **14E,** 193—209).—Of the three species discussed, *Lygus pratensis* and *L. rugulipennis*, Popp, occurred in smaller no. in the vicinity of the forest (1—15 m.) than on plots located 15—50 m. distant. *L. punctatus* (Zett) increased in plots nearer the forest. Proximity of the forest may be useful in the growing of potatoes as it controls the *Hemiptera-Heteroptera* likely to cause injury. (12 references.)

E. M. J.

Effect of nitrogen supply on the response of Majestic potato to gibberellic acid. E. C. Humphries and S. A. W. French (*Ann. appl. Biol.*, 1961, **49,** 331—339).—Application of gibberellic acid (GA,) 50 p.p.m., 2—6 sprays) to young potato plants increased the initial production of dry matter, but the effect did not persist, even when extra N was applied to the soil or leaves. GA decreased the no. of leaves on lateral branches partly because leaf production on laterals on lower nodes was inhibited. N increased the rate of leaf production on lateral branches. GA, but not N, increased the area of the main-stem and lateral-stem leaves. There were no interactions between GA and N. GA decreased the total N content per unit area of leaf.

A. H. Cornfield.

Production of early spring grass. I. Effect of autumn management and different levels of nitrogenous manuring on the production of early spring grass from a general-purpose ley. H. K. Baker (*J. Brit. Grassland Soc.*, 1960, **15,** 275—280).—Application of N in spring increased early spring yields by 9—28 lb. of dry matter per lb. of N applied. Autumn-applied N increased autumn yields by 12—22 lb. of dry matter per lb. of N. Autumn-applied N caused only small increases in yields in the following spring. Autumn grazing had inconsistent effects on spring yields and the effects were small compared with those obtained from applying N in the spring.

A. H. Cornfield.

Relation between weather and meadow-hay yields in England, 1939-56. L. P. Smith (*J. Brit. Grassland Soc.*, 1960, **15,** 203—208).—A close relationship was established between yields of meadow hay and estimated "actual" transpiration during the growth period. County data for yield and transpiration were combined, using grass acreages as weighting factors, into regional and national values. After adjusting for the steady improvement in yields after 1946, the correlation between actual and calculated yields for 1939—56 was 0·95. A. H. Cornfield.

Influence of decreased sowing standards on yield and specific composition of meadow mixture. S. Włodarczyk (*Ann. Univ. M. Curie-Skłodowska*, 1959, **14E,** 157—192).—Decrease of sowing standards to 80, 60, 50 or 40% did not decrease yields in first, scond and fourth years, but in the third year highest yields were obtained from 80% sowing standard and lowest from 40%. In general differences in yields were not significant in the four years' study. A greater % of seedlings developed with decreased sowing standards. *Dactylis glomerata*, L. and *Arrhenatherum elatius*, L. grew well. *Phleum pratense*, L. grew well only in the 100% sowing in the first, third and fourth year. *Bromus inermis*, *Festuca rubra*, L., *Medicago lupulina*, L. and *Trifolium repens*, L. were poorly developed. Infestation with quick grass was greatest in the 40 and 60% and smallest in the 80% sowings. The greatest average (14·06%) of annuals and weeds in hay was in the 50% sowing (1959). (51 references.)

E. M. J.

Root development in a grass sward on deep peat; use of radioactive tracers. R. Boggie and A. H. Knight (*J. Brit. Grassland Soc.*, 1960, **15,** 133—136).—The rooting depth of a grass–clover mixture was measured by placing ^{32}P-labelled PO$_4^{3-}$ at various depths. On a deep peat, roots were confined to the top 6 in. and there was no difference in rooting depth due to the height of the water table.

A. H. Cornfield.

Influence of dissolved gypsum on pasture establishment on irrigated sodic clays. J. L. Davidson and J. P. Quirk (*Aust. J. agric. Res.*, 1961, **12,** 100—110).—The texture of these heavy grey and brown soils can be materially improved for the establishment of clovers and grasses by the introduction of 0·86% of gypsum as a fine suspension into the irrigation water. P. S. Arup.

Magnesium in forage plants. II. Distribution in grasses and clovers. T. R. Todd (*J. agric. Sci.*, 1961, **57,** 35—38).—The acetone-sol. Mg fraction is virtually identical with chlorophyll-Mg. The water-sol. matter of the herbage is 25% of the total dry matter; the remaining insol. fraction contains only ⅓ of the total Mg. Variations in total Mg are largely reflected in the water-sol. fraction, so that at Mg levels causing hypomagnesaemic tetany in ruminants the water-sol. Mg may be only ⅓ of the total, whilst when total Mg is >0·2% the total water-sol. fraction may amount to half the total.

M. Long.

Comparison of the value of various leguminous plants grown for green fodder. F. Pawłowski and L. Malicki (*Ann. Univ. M. Curie-Skłodowska*, 1959, **14E,** 123—156).—On the basis of yield of green fodder, weed infestation and value, as preceding crops for winter wheat, *Vicia faba minor* and *Lathyrus sativa*, grown singly or mixed, are not recommended. Similarly with a mixed crop of *Vicia sativa* + *Pisum arvense* + *Avena sativa*, better yields are obtained when *Lupinus luteus* is mixed with other leguminous plants. (50 references.) E. M. J.

Yield and composition of lucerne, grass and clover under different management systems. III. Effect of nitrogen and frequency of cutting. W. E. Davies, R. O. Davies and A. Harvard (*J. Brit. Grassland Soc.*, 1960, **15,** 106—115).—Higher yields over 4 years were obtained with Du Puits lucerne alone or with grasses (ryegrass, timothy or meadow fescue) than with Grimm lucerne or red clover with grasses. Yields from lucerne + grasses were not significantly better than those from lucerne alone. Du Puits lucerne eliminated practically all the grasses under favourable conditions. Application of N encouraged growth of grasses at the expense of the legumes, less so with Du Puits lucerne than with the other legumes. K applications benefited lucerne survival in later years. Protein yields from the best lucerne plots were double those from pure grass swards with heavy N treatments. Three cuts of lucerne in the first 2 harvest years had a very bad effect on carry-over of lucerne to the third year. A. H. Cornfield.

Distribution of carbohydrates, nitrogen and lignin in soluble fractions of the stems and leaves of lucerne hay. J. F. Couchman (*J. Brit. Grassland Soc.*, 1960, **15,** 169—173).—The N-free extractives and total N contents of the stems and leaves of lucerne were separated into three fractions, sol. in (*a*) 90% EtOH, (*b*) cold water and (*c*) acid + alkali in the concn. used for crude fibre determinations. The sol. carbohydrates in the stems comprised a lower proportion of the (*a*)-sol. and a higher proportion of the (*c*)-sol. material than in the leaves. The lignin content of the stems was more than 3 times that of the leaves, and sol. lignin was low in both. In the stems protein-N constituted a lower proportion of the total N, and was possibly of a less digestible type, than that in the leaves.

A. H. Cornfield.

Productivity of lucerne fields with dry and irrigated culture in relation to the effect of sowing density. II. Results of two years of tests. I. Eynard (*Ric. sci.*, 1960, **30,** 2407—2415).—Yields of lucerne and total green matter under dry and irrigated conditions in a comparatively dry and in a rather wet year, are compared. Yields of total green matter did not vary appreciably, but the yield of lucerne increased progressively with the density of sowing.

L. A. O'Neill.

Birdsfoot trefoil, *Lotus corniculatus*, yields as affected by soil properties. C. D. Foy and S. A. Barber (*Agron. J.*, 1961, **53,** 109—110).—Yields of birdsfoot trefoil in pot tests with 20 Indiana soils of widely varying texture, limed where necessary, were significantly correlated with aggregation index and P extractable by 0·75N-HCl, but not with other soil factors. Multiple correlation involving aggregation index, available P and org. matter content accounted for 58% of the yield variation between the soils. The inclusion of other factors did not improve this correlation. A. H. Cornfield.

Tips on selecting fruits and vegetables. W. W. Morrison (*U.S. Dep. Agric.*, 1961, *Market Bull.* 13, 44 pp.).—Desirable characteristics for commonly used fresh fruits and vegetables are described.

E. G. Brickell.

Phosphorus–potassium fertilising of fruit trees in irrigated culture. H. Rebour (*C. R. Acad. Agric. Fr.*, 1961, **47,** 223—226).—For effective fertilisation it is necessary for the P-K components to penetrate to the base of the roots of the tree and for there to be sufficient moisture for them to be absorbed. Vertical furrow irrigation is advised, with repeated but not copious watering.

J. V. Russo.

Chemical thinning of fruits. M. P. Lorne (*C. R. Acad. Agric. Fr.*, 1961, **47,** 218—223).—The spraying of apple blossom with naphthyl-acetic acid (9—15 p.p.m.) is described. The exact concn. needed differs with the variety of apple. J. V. Russo.

Photoperiodic response in pineapple. D. P. Gowing (*Amer. J. Bot.*, 1961, **48**, 16—21).—Flower initiation in the " Smooth Cayenne " pineapple is conditioned jointly, by photoperiod, temp. range and time of planting slips. Daily application of indolylacetic acid at the beginning of the dark periods did not influence the effect of short-day conditions. Treatment with the anti-auxin, 4-chlorophenoxyisobutyric acid, did not alter the suppression of flowering by interruption of the dark periods. A. G. Pollard.

Potassium, calcium and magnesium in the nutrition of pineapples in Guinea. II. Influence on the saleable yield. P. Martin-Prével (*Fruits d'outre mer*, 1961, **16**, 113—123).—Pineapple shoots planted in 1956 were treated with varying amounts of Mg, Ca and K (*ibid.*, 1961, **16**, 49—56). The results of acetylene treatment to promote flowering were variable. Factors influencing the effects of different amounts of these elements are examined. For near-optimum effects on mean wt. of fruits, exportable tonnage and shoot production, the fertiliser should contain K 30—60, Mg 30—65, and Ca 0—17%. For late fruit production K 50, Mg 35 and Ca 15% should be applied; and for early production K 35, Mg 50 and Ca 15%. The costs of the recommended treatments are discussed. S. G. Ayerst.

Manuring fruit crops. VI. Papaya. S. L. Katyal and K. L. Chadha (*Fertil. News*, 1961, No. 6, 16—19).—Fertiliser recommendations for papaya trees grown in different regions are discussed. For female and hermaphrodite plants, farmyard manure (20 lb. per plant) is applied at planting, and 80—100 lb. 4—5 months later, the treatment being repeated in the following year before the rains. Bonemeal is recommended after the rains. Plants 1 year old may be treated with $(NH_4)_2SO_4$, lime and bonedust. E. M. J.

Sultana vine. VII. Comparison of crop regulation by pruning with regulation by disbunching. A. J. Antcliff, W. J. Webster and P. May (*Aust. J. agric. Res.*, 1961, **12**, 69—76; cf. *ibid.*, 1958, **9**, 328).—Alteration of the leaf/fruit ratio by disbunching has not materially improved the yields obtained from this vine during four seasons. P. S. Arup.

Effects of certain mineral nutrients on growth and nitrogen-fixation of inoculated bean plants, *Phaseolus vulgaris*, L. J. C. Burton, O. N. Allen and K. C. Berger (*J. agric. Fd Chem.*, 1961, **9**, 187—190).—The effectively inoculated plants grown in sand cultures show marked responses as regards growth, pod-yield and N-fixation to high levels of P, K, Ca and Mg in the presence of a moderate level (200 p.p.m.) of inorg. N. The fixation of N by ineffective rhizobia cannot be improved by increasing the concn. of the nutrients. (16 references.) P. S. Arup.

Leaf removal in grain sorghum. I. Effects of defoliation treatments on yield and components of yield. F. C. Stickler and A. W. Pauli. **II. Trends in dry matter, carbohydrates and nitrogen following defoliation.** A. W. Pauli and F. C. Stickler (*Agron. J.*, 1961, **53**, 99—102, 102—105).—I. Seed yields of sorghum declined curvilinearly with increasing removal of leaf area; the effect of the removal increased with the amount removed. The no. of seeds per head was affected to a greater extent by leaf area removal than were no. of heads per unit area or seed size. Greater yield reduction resulted from removing leaf area from upper than from lower portions of the plant. Removing alternate leaves was more deleterious than removing half of each leaf. Yield decreases of 23—95% resulted from removing 33—100% of the leaf area at late boot and anthesis stages.

II. Accumulation of dry matter, total sugars, acid-hydrolysable carbohydrates and N were reduced in plants and grain of sorghum partially or completely defoliated at the boot or anthesis stage. A. H. Cornfield.

Responses of seeds of *Pinus virginiana* to light. V. K. Toole, E. H. Toole, S. B. Hendricks, H. A. Borthwick and A. G. Snow, jun. (*Plant Physiol.*, 1961, **36**, 285—290).—Germination was promoted by red and inhibited by far-red radiant energy, about 1×10^6 ergs/sq. cm. of energy being needed for conversion of 50% of the pigment by red or far-red energy. Action was immediately and repeatedly reversible. E. G. Brickell.

Balanced fertiliser application gives record groundnut yields. M. R. Panikkar (*Fertil. News*, 1961, No. 6, 22—25).—In the chief regions of cultivation the responses of groundnuts to NPK are varied. Although the need for a balanced NPK fertiliser is indicated, large-scale tests are necessary before manurial schedules can be formulated to give max. yields. Effects of water, season, seed, cultivation, cropping, etc., are also considered in relation to soil requirements. E. M. J.

Indispensibility of boron for hop plants. W. Schropp (*Brauwissenschaft*, 1961, **14**, 167—168).—B is indispensable to the growth of hop plants whether they are cultured in water or in sand. J. V. Russo.

Hazardous nature of horticultural production. H. G. Kronenberg (*Versl. landbouwk. Onderz.*, 1961, 67.2, 104 pp.).—A review with special reference to the influence of climatic and other factors on the cultivation of apples, carrots and chrysanthemums in Holland. (82 references.) P. S. Arup.

Methods of combatting frost. G. Perraudin (*Promotionsarb., Zürich*, 1961, No. 3121, pp. 229).—Use of covers, screens, chemical fogs and air circulation for preventing frost damage to crops (e.g., fruit, flowers, potatoes) in Switzerland is discussed. Details of extensive experimental work with various types of water sprinklers and heaters are presented. A. G. Pollard.

Pest Control

(A) **New insecticides.** R. Schuppon (*Chim. et Industr.*, 1961, **85**, 252—264).—The chemical composition, physico-chemical properties, toxicology and insecticidal action of various new insecticides including Barthrin, Pyrolan, Isolan, Sevin, Thiodan, Kelthane, Isochlorthion, Delnav, Phenkapton, Gusathion and Ethion are summarised. (94 references.) J. M. Jacobs.

[B] **Recent attainments in the field of insecticides.** R. Schuppon (*Chim. et Industr.*, 1961, **85**, 421—436).—The continuation of the review deals with systemic organo-P insecticides for which the physical and chemical characteristics, toxicological properties, insecticidal activity and metabolism in plants and animals are given. The organo-P insecticides are classified according to their toxicological properties. The corresponding antidotes are indicated and the permanency of their residues at the times of harvest and consumption is considered. M. Sulzbacher.

Practical problems and recent trends in nematode control. F. C. Peacock (*Ann. appl. Biol.*, 1961, **49**, 381—383).—A critical review and discussion. A. H. Cornfield.

Evaluation of an experimental nematicide *OO*-diethyl *O*-2-pyrazinyl phosphorothioate (Cynem). R. E. Motsinger (*Plant Dis. Reptr*, 1961, **45**, 335—340).—Soil treatment with Cynem (10—20 lb./acre) 1—2 weeks before planting, at planting, or 10 days after planting gave excellent control of nematodes in tobacco. Initial phytotoxic symptoms had disappeared 38 days after treatment. Shoots of plants growing on treated soils were toxic to aphids feeding on them. A. H. Cornfield.

Fungitoxicity of metal ions. E. Somers (*Ann. appl. Biol.*, 1961. **49**, 246—253).—The *in vitro* fungistatic activity of 24 metal cations was determined against *Alternaria tenuis* and *Botrytis fabae.* The log. metal ion concn. at the ED_{50} value conformed to the exponential relationship with electronegativity proposed by Danielli and Davies (*Adv. Enzymol.*, 1951, **11**, 35). The results are discussed in relation to the site of action of metal cations on the fungal cell. A. H. Cornfield.

Influence of mineral salts on *Plasmodiophora brassicae*, Wor. H. Bochow (*Phytopath. Z.*, 1961, **40**, 420—421).—Spore germination of *P. brassicae* in sand with different concn. of nutrients indicates that the nutrient content of the medium affects not only the growth of the host plant but also the development of the pathogen. A. G. Pollard.

Fungicidal activity and chemical constitution. IX. Activity of 6-n-alkyl-8-hydroxyquinolines. R. J. W. Byrde, D. R. Clifford and D. Woodcock (*Ann. appl. Biol.*, 1961, **49**, 225—232).—Max. fungistatic activity in a series of 6-n-alkyloxines, tested against the mycelium of *Aspergillus niger*, occurred with a chain length of 6—7 C. 6-n-Hexyloxine was twice as active as the corresponding 5-n-hexyl compound. In the presence of Cu^{2+} max. activity occurred with oxine and 6-methyloxine. With some higher members of the series a rapid initial growth rate fell sharply after 2 days. This growth inhibition was due to the production of oxalic acid by the fungus which resulted in liberation of free oxine. A. H. Cornfield.

Fungitoxic derivatives of salicylaldehyde. II. Chlorinated and brominated derivatives of salicylideneaniline and related compounds as eradicants of cucumber powdery mildew, *Erysiphe cichoracearum*, D.C. III. *N*-Substituted derivatives of halogenated salicylideneimines as eradicants of cucumber powdery mildew. R. J. Smith and W. H. Read (*Ann. appl. Biol.*, 1961, **49**, 233—241, 242—245).—II. Of a no. of halogenated derivatives of salicylideneaniline tested for eradicative activity against cucumber powdery mildew on young plants, the 3-bromo- and 3-chloro-deriv. were the most promising. When applied at 0·08—0·10% in a commercial-scale trial both materials were more effective than Karathane [2,4-dinitro-6-(1-methylheptyl)phenyl crotonate] in controlling severe mildew infection, but showed phytotoxic effects on mature plants. Preliminary tests on the toxicity of the materials to other fungi are also reported.

III. *N*-Alkyl, *N*-aryl and *N*-heterocyclic deriv. of three halogenated salicylideneimines were tested for eradicative activity against cucumber powdery mildew. Although the activity of

several of these compounds approached that of 3-chlorosalicylidene-aniline, no compound of practical interest was discovered.
A. H. CORNFIELD.

Dimethylacrylic ester of 4,6-dinitro-2-s-butylphenol, a new acaricide. J. Lhoste and J. Lambert (*C. R. Acad. Agric. Fr.*, 1961, **47**, 48—54).—The effect of the new acaricide when tested on three varieties of mite (e.g. *Tetranychus cinnabarinus*) compared very favourably with that of other known acaricides.
J. V. RUSSO.

Phosphoro-organic insecticides. Derivatives of β-dicarbonyl compounds. M. I. Kabachnik, P. A. Rossiĭskaya, M. P. Shabanova, D. M. Paĭkin, L. F. Efimova and N. M. Gamper (*Zh. obshch. Khim.*, 1960, **30**, 2218—2223).—Deriv. of phosphorothionic acid which contained alkoxyl or dialkylamide groups were synthesised from dialkyl phosphorochloridates or appropriate dialkylamide derivatives with β-dicarbonyl compounds under Clausen acylation conditions. The deriv. obtained readily combined with S in CS_2 to form the required deriv. of phosphorothionic acid. Esters of diethylphosphoric acid and enol forms of acetoacetic methyl esters combined with Cl_2 to give diethyl (methoxycarbonyldichloroprop-2-yl) phosphate. Tests showed that the compounds which contained methoxycarbonylpropenyl groups were more toxic than those which contained pentenonyl groups.
A. L. B.

Effect of time and temperature on toxicity of insecticides to insects. III. Tests of seven poisons in the range 10—28°. M. Das and A. H. McIntosh (*Ann. appl. Biol.*, 1961, **49**, 267—289).—Results are presented with rotenone, 5,5-dimethyldihydroresorcinol dimethylcarbamate (Dimetan), 2-bromomercurithiophen, 2-isovaleryl-1, 3-indanedione (Valone), α-chlordane, toxaphene and DDT on four insect species.
A. H. CORNFIELD.

Escape of ^{35}S-labelled methyl isothiocyanate from soil into the air and its uptake by tomato plants. J. Willenbrink, E. Schulze and K. Junkmann (*Z. PflKrankh.*, 1960, **68**, 92—98).—Of the isothiocyanate (MS) injected into a compost soil, approx. one-third had evaporated in 22 days; the residual S in the soil was mainly oxidised to SO_4^{2-}. Tomato plants grown in the treated soil showed ^{35}S activity in all parts, totalling up to 1% of the amount injected. Of this 56—90% was present as SO_4^{2-}. Absorption of MS, as such, by plant roots is unlikely.
A. G. POLLARD.

Chalk from sedimentation mud as a filler in plant protectives. I. Dodig (*Kem. u. Industr., Zagreb*, 1961, **10**, 27—29).—The sedimentation mud from sugar beet refineries, in Yugoslavia, contains $CaCO_3$ 35—50, H_3PO_4 1—2, N 0·2—0·4 and K_2O 0·1—0·3%, and is suitable for prep. of valuable chalk products (cf. J. Vašátko and V. Križan, *Chemické Zvesti, Bratislava*, 1953, **7**, 299—316). The chalk used as a filler with 7% HCH prep. gave good protection against sugar beet moth (*Phtorimaea ocellatella*) and against *Calandra granaria* in rye. In field tests using HCH or DDT with fillers, the sedimentation mud chalk was found effective against *Calandra granaria*, *C. oryzae* and *Acanthoscelides obtectus* when used in a fused form without DDT or HCH.
A. L. GROCHOWSKI.

Influence of environment on the action of nematicides for the control of the potato nematode (*Heterodera rostochiensis*, Woll): use of model preparations. A. Dieter (*Z. PflKrankh*, 1961, **68**, 80—91).—Tests with tetrachlorobutane (TCB), a NaOH–$CaCO_3$ mixture (PII) and Me dimethyldithiocarbamate (DMCM) on cysts in various soils under different conditions are recorded. TCB and DMCM were most effective on light soils but had little action in humus soils. The efficiency of PII diminished with increasing clay content of the soil irrespective of its humus content.
A. G. POLLARD.

Relation of soluble manganese to the incidence of common scab in potatoes. J. J. Mortvedt, M. H. Fleischfresser, K. C. Berger and H. M. Darling (*Amer. Potato J.*, 1961, **38**, 95—100).—Addition of Mn to the tuber-setting zone of potatoes grown in sand culture reduced the incidence of scab. Increasing application of $MnSO_4$ (50—150 lb./acre) in a field test tended to reduce scab incidence, but the differences were not significant.
A. H. CORNFIELD.

New means of controlling [virus] vectors in potatoes by systemic seed dressings. K. Küthe and W. Ronnebeck (*Z. PflKrankh.*, 1961, **68**, 209—218).—The spread of leaf-roll virus by aphids was prevented by application of Disyston (granular) at the rate of 0·5—1·0 g. per tuber at planting or by spraying with Metasystox (800 ml./hectare). No harmful residues were detected in the crop.
A. G. POLLARD.

Ley deterioration and soil insects. G. W. Heath (*J. Brit. Grassland Soc.*, 1960, **15**, 209—211).—Application of dieldrin mixed with fertiliser to a 5-year-old ley in March and May, 1955 (1 lb. total dieldrin per acre) resulted in a 15% increase in dry matter yields of herbage cut in June, 1956 in comparison with the plots receiving fertiliser only. In March, 1956 wireworm no. were reduced by the treatment to 20% of the control plots. Mite and collembola no. were also reduced.
A. H. CORNFIELD.

Insect problems of lucerne seed production in South Australia. K. M. Doull (*J. Aust. Inst. agric. Sci.*, 1961, **27**, 11—15).—Improvement in seed yields is dependent largely on better control of insect pests and more efficient pollination. Pests of economic importance during 1956—9 were the pink cutworm *Agrostis munda* Walk., the climbing cutworm, *Heliothis punctigera* Wall., the lucerne pod-borer *Etiella Behrii* Zell., and the lucerne seed chalcid *Bruchophagus gibbus* Boh. Poor pollination is mainly due to a shortage of honeybees, but in some areas native solitary bees, *Megachile quinquilineata* (Cockerell) and *Nomia australica* Smith, are also of potential importance.
E. C. APLING.

Viruses of red clover in Wisconsin. E. W. Hanson and D. J. Hagedorn (*Agron. J.*, 1961, **53**, 63—67).—Viruses inciting red clover vein mosaic, pea common mosaic, bean yellow mosaic, Wisconsin pea streak, alsike clover mosaic and lucerne mosaic were isolated repeatedly from naturally infected red clover. The first three were most prevalent. The symptoms incited by each virus varied sufficiently from clone to clone so that it was frequently impossible to distinguish the different diseases on the basis of symptoms. There were clonal differences in reaction to the viruses studied.
A. H. CORNFIELD.

Concentrate spraying of apple trees. II. Dosage/volume relationships of lime–sulphur in disease control and spray damage. M. H. Moore (*Ann. appl. Biol.*, 1961, **49**, 254—266).—Control of apple scab and powdery mildew by lime–S sprays depended primarily on the dosage and not merely on the vol. of spray applied, but the vol. of spray was sometimes a critical factor in spray damage. Dosage also affected the extent of infestation by the red spider mite and sawfly. The incidence of fruit russet and poor skin finish was sometimes related to impaired foliage. Gravity flow and droplet size of spray together increased with increasing dilution of lime–S.
A. H. CORNFIELD.

Control of powdery mildew, *Microsphaera alni*, of blueberry. J. E. Huguelet, R. H. Fulton and M. A. Veenstra (*Plant Dis. Reptr*, 1961, **45**, 368—372).—Of a no. of materials tested, the best results were given by wettable S (6 lb.), Karathane (0·75 lb. per 100 gal.), actidione 2 and cycloheximide semicarbazone 5 p.p.m.
A. H. CORNFIELD.

DDT and copper residues in a vineyard soil. E. F. Taschenberg, G. L. Mack and F. L. Gambrell (*J. agric. Fd Chem.*, 1961, **9**, 207—209).—After three or four annual sprayings during 6 and 12 years, the accumulations of DDT in the 0—3-in. layer were 18 and 27 lb./acre, i.e. 50 and 33%, respectively, of the total DDT applied. Regular spraying with Cu increased the Cu content from 40 to 80 p.p.m. after 6 years, and to 120 p.p.m. after 11 years. Very little DDT or Cu was found in the 3—6-in. layer. Small proportions of DDT occurred as the decomposition product DDE. (13 references.)
P. S. ARUP.

Cherry leaf-spot and its control. H. L. Keil (*U.S. Dep. Agric.*, 1961, *Leafl.* 489, 5 pp.).—The disease caused by *Coccomyces hiemalis*, Higgs, is described. Bordeaux mixture, fixed Cu prep., liquid lime–S, captan, Dodine, ferbam, glyodin and cycloheximide give control.
E. G. BRICKELL.

Effectiveness of certain fungicides against the peach leaves curl (*Taphrina deformans*) and the curyneum blight (*Clasterosporium carpophilum*). Z. Prpić (*Kem. u Industr., Zagreb*, 1961, **10**, 23—24).—Field tests carried out in 1960 with a series of fungicides showed that org. prep. based on TMTD (0·15—0·2%) or Ziram (0·2—0·5%), applied before the growth of plants, gave reasonable protection against peach leaf curl; good protection was given by applications once before and twice during growth. A single winter application of a standard fungicide based on Cu compounds 3% or Kreozan 1·5%, followed by two applications of the above org. prep. during growth, provided good protection against both leaf curl and blight.
A. L. GROCHOWSKI.

Control of soil rot and damping-off of cucumbers with soil fungicides. J. P. Jones (*Plant Dis. Reptr*, 1961, **45**, 376—379).—The incidence of soil rot in cucumbers was reduced by soil application of Hercules 3944 or combinations of PCNB with Dexon, captan and actidione. Pythium damping-off was controlled by captan, actidione, Omadine Mn and Hercules 3944.
A. H. CORNFIELD.

Systemic control of powdery mildew, *Sphaerotheca pannosa*, of roses with the semicarbazone derivative of actidione (cycloheximide). B. M. Jones and H. G. Swartwout (*Plant Dis. Reptr.* 1961, **45**, 366—367).—Soil treatment with cycloheximide semicarbazone (20—100 p.p.m.) 3 days prior to inoculating roses with powdery mildew spores gave complete control of the disease.
A. H. CORNFIELD.

Control of bacterial scab, *Pseudomonas marginata*, and *Fusarium* corm rot, *Fusarium oxysporum* f. *gladioli* of gladiolus. L. P. Nichols

(*Plant Dis. Reptr*, 1961, **45,** 344—346).—The best control of bacterial scab of gladiolus was obtained with pre-plant treatment of the corms with Delsan A-D and Panoram D-31 dusts. The best control of *Fusarium* corm rot was obtained by treatment with the Hg materials Emmi, Ceresan and New Improved Ceresan.

A. H. CORNFIELD.

Determination of insecticide residues on green and flue-cured tobacco and in main-stream cigarette smoke. T. G. Bowery and F. E. Guthrie (*J. agric. Fd Chem.*, 1961, **9,** 193—197).—The determinations of Guthion are made by the method of Meagher *et al.* (cf. *Analyt. Abstr.*, 1961, **8,** 1758) and those of Sevin by the method of Miskus *et al.* (cf. *Analyt. Abstr.*, 1960, **7,** 2502). Destruction of the residues on tobacco amounts to 73% for Guthion and 88% for Sevin during curing, and $\nless$99% for both in the course of smoking. With 0·5 lb. of Guthion or 1 lb. of Sevin per acre, no detectable amounts of either can occur in the main stream of cigarette smoke.

P. S. ARUP.

Attempts to control dissemination of internal cork virus of sweet potatoes with insecticides. D. H. Habeck, L. W. Nielsen and C. H. Brett (*Plant Dis. Reptr*, 1961, **45,** 330—333).—Weekly applications of six insecticides to sweet potato foliage throughout the season did not reduce the incidence of internal cork virus.

A. H. CORNFIELD.

Developments in weed control. W. C. Shaw (*Farm Chem.*, 1961, **124,** No. 4, 18—20).—A short survey of recent trends.

A. G. POLLARD.

How surfactants affect herbicides. Anon. (*Farm Chem.*, 1961, **124,** No. 8, 16, 22).—Notes on interactions between surfactants, herbicides and plant species are recorded. A. G. POLLARD.

Pre-emergent herbicidal activity of certain aldehyde and chloro-substituted aldehyde addition products and related compounds. J. S. Pizey and A. Bates (*J. Sci. Fd Agric.*, 1961, **12,** 542—547).—Three series of chloro-substituted aliphatic aldehyde addition products mostly derived from chloral were studied. The chloral-nitro-paraffin and chloral-amide addition products showed selective herbicidal toxicity which might depend on their breakdown, in soil, to trichloroacetic acid. Unsaturated dehydration products of the nitro-alkanols, their acetyl deriv. and corresponding methoxy compounds showed little toxicity to plant species tested; all active compounds tested were toxic to wheat and other monocotyledons but not to dicotyledonous plants. Chloral-acetamide, monochloral-urea and 1,1,1-trichloro-3-nitrobutan-2-ol seemed worth testing under field conditions. (18 references.) E. M. J.

Diquat as arboricide. J. S. Gunn and P. B. Tatham (*Nature, Lond.*, 1961, **189,** 808—809).—Diquat (*ibid.*, 1958, **181,** 446) is consistently effective for poisoning old oil-palms before or during replanting in W. Africa. The dosage is 40—50 g. per tree, in a water-filled hole punched in the trunk ~3 ft. above ground; trees are killed in 3—6 weeks. Though expensive it is safer and more effective than Na_3AsO_3. W. J. BAKER.

Chemical control of brush and trees. R. Behrens, H. M. Elwell, W. A. Gentner, H. M. Hull, D. L. Klingman, F. A. Peevy, F. L. Timmons, F. H. Tschirley and R. E. Larson (*U.S. Dep. Agric.*, 1961, *Fmrs' Bull.*, 2158, 23 pp.).—Foliage and basal spraying, frill, notch, stump and soil treatments, with 2,4-D, 2,4,5-T, Silvex [2-(2,4,5-trichlorophenoxy)propionic acid], Amitrole (3-amino-1,2,4-triazole) and NH_4 sulphamate, are described. E. G. BRICKELL.

Witchweed, *Striga hermonthica*, on rain-grown pearl millet in nitrogen-deficient sandy soil of the central Sudan. S. A. J. Tarr (*Ann. appl. Biol.*, 1961, **49,** 347—349).—Spraying with 1 lb. per acre of 80% 2,4-D (Na salt) 2 weeks after sowing reduced the incidence of the root parasite witchweed attacking pearl millet in sandy soil. This control resulted in increased yields of millet only on land very deficient in available N and not when $(NH_4)_2SO_4$ was applied.

A. H. CORNFIELD.

Collaborative Pesticides Analytical Committee. Recommended analytical methods for pesticides. I. Mercury in formulated or technical products. (*FAO Plant Protection Bull.*, 1960, **9,** No. 2, pp. 17—28, Reprint).—Differences caused by the presence of fillers, Pb arsenate, solvents, surface-active agents, colouring matter, BHC, etc., are discussed. A study of the composition of the sample before selection of method of analysis is important. Details are given of three basic procedures: the sample (i) is decomposed by heating with CaO + sucrose and the liberated Hg is dissolved in HNO_3; (ii) refluxed with conc. H_2SO_4 and KNO_3; (iii) the Hg compounds are decomposed with glacial AcOH and Zn wool, the Hg–Zn amalgam is isolated and decomposed with HNO_3. In each case the Hg is determined volumetrically with NH_4CNS.

E. M. J.

Analysis of highly toxic organic phosphorus acid esters. Kh. Lohs and P. Franz (*Acta chim. hung.*, 1961, **26,** 451—457).—A review (89 references) is made of the literature concerning the detection and determination of trace quantities of phosphorus insecticides such as parathion, Paraoxon, DFP, Tabun, Sevin and Soman. The method of Schönemann is recommended. This depends on the formation of a colour produced by the action of a peroxide (H_2O_2, Na perborate) on the P compound to give a per-acid which then reacts with an amine (especially benzidine or *o*-tolidine) at pH 9·5—10·5. Sensitivity is 10 μg./ml. for parathion to 0·1 μg./ml. for Soman (pinacolyl methylphosphorofluoridate). PCl_3, $POCl_3$, $MePOCl_2$ and certain carboxylic acid chlorides interfere.

K. RAVEN.

Pesticidal organic dithiophosphates. Murphy Chemical Co. Ltd. (Inventors: M. Pianka and A. H. Oxtoby) (B.P. 844,741, 29.3.55).—Compounds useful as acaricides, insecticides and fungicides, comprise dithiophosphates, $OR(OR')\cdot PS_2\cdot CHR''\cdot CH_2\cdot NO_2$ (R and R′ are alkyl of $\ngtr$4 C; R″ is CCl_3, or substituted or unsubstituted Ph or furyl). As an example, the method prep. of OO-Et_2 S-2-*nitro*-1-*phenylethyl thiolothionophosphate*, a pale oil, is described. A spray composition containing 0·003% of the ester is 100% lethal to red spider. F. R. BASFORD.

Derivatives of hexachlorotoluene. Velsicol Chemical Corp. (B.P. 845,036, 3.1.58. U.S., 8.1.57).—Compounds with insecticidal properties (against Mexican bean beetle and Southern army worm) comprise hexachlorotoluene deriv., e.g., hydroxymethyl-α,α,α,2,3,4-hexachlorotoluene, which are obtained from 1,2,3,4,7,7-hexachlorobicyclo[2,2,1]hepta-2,5-dienes by heating at 190—295°.

H. S. R.

Nuclearly substituted *N*-heterocyclic compounds. Farbenfabriken Bayer A.-G. (B.P. 845,062, 27.8.58. Ger., 27.8.57).—Compounds with (*inter alia*) insecticidal properties (e.g., against *Tetranychus telarius* on bean plants) are pyridines, quinolines and isoquinolines and their methyl deriv. with F-substituent in the α- and/or γ-positions. Compounds made by interaction of the corresponding OH-compound with cyanuric fluoride include 2-*fluorolepidine*, b.p. 140°/16 mm., 2,4-*difluoroquinoline*, b.p. 91·5°/11 mm., and 1,3-*difluoroisoquinoline*, m.p. 57°, b.p. 112°/14 mm. H. S. R.

Organic phosphorus compounds having pesticidal properties· Société des Usines Chimiques Rhône-Poulenc (B.P. 845,647, 17.2.58. Fr., 4.3.57).—Compounds $[(OR)_2\cdot PS_2]_2CH_2$ are claimed (R is alkyl of 1—3 C); they are useful as pesticides and are also claimed as such. They may be obtained by interaction of $(OR)_2PS_2M$ with a dihalogenomethane (at 50—100° in an alkanol or aliphatic ketone) (M is alkali metal). The prep. is detailed of *bis*-(OO-*diethyl thiothionophosphoryl*)*methane*. F. R. BASFORD.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 845,447, 25.4.57. Ger., 27.4.56).—Thiophosphoric acid esters $(OR_3)(OR_4)PS\cdot O\cdot CH(CH_2\cdot NR_1R_2)\cdot CH_2\cdot SR$ (R is an aromatic radical, R_1—R_4 are 1—4-C alkyl radicals or R_1 and R_2 form, together with the N-atom, a heterocyclic ring which may also contain other hetero atoms), useful as insecticides, are produced by reacting appropriate *OO*-dialkyl phosphorothionyl halides with the appropriate 1-(substituted amino)-3-(arylmercapto)-2-hydroxypropanes in an inert organic solvent (e.g., benzene) in the presence of an acid binding agent (e.g., alkali metal hydroxide) at a temp. between room temp. and 100° (60—70°). OO-*diethyl* O-(1-*diethylamino-3-phenylthioprop-2-yl*) *phosphorothionate*, an oil, is prepared.

E. ENOS JONES.

Dithiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 845,373, 2.12.57. Ger., 10.12.56).—Dithiophosphoric acid esters of the general formula $R_3\cdot C_6H_4\cdot S\cdot CH_2\cdot CH_2\cdot S\cdot PS(OR_1)(OR_2)$, where R_1—R_3 are 1—4-C alkyl radicals or R_3 is halogen, especially Cl or Br, are produced when a β-chloroethyl aryl sulphide is reacted with an *OO*-dialkyl dithiophosphoric acid, or the NH_4 salt thereof, in an inert solvent having b.p. 90—150° (e.g., xylene). *OO-Diethyl* S-2-*p-chlorophenylthioethyl phosphorothiolothionate*, b.p. 144°/0·01 mm., is prepared. E. ENOS JONES.

Fungicidal compositions. Boots Pure Drug Co. Ltd. (Inventors: N. G. Clark, H. A. Stevenson, R. F. Brookes and A. F. Hams) (B.P. 845,916, 17.9.57 and 12.6.58).—Compositions for use in combating fungi, especially *Botrytis cinerea*, *B. tulipae*, *Pythium debaryanum*, *Corticium solani*, *Plasmopara viticola* and *Phytophthera infestans*, comprise dusts, dispersions, emulsions, smokes and aerosols containing compounds *p*-$NRR'(X)\cdot C_6HWYZ$ (R and R′ are H, low-mol. alkyl, or R is acyl; W and X are halogen, NO_2, alkyl or alkoxy; Y and Z are H, halogen or NO_2 provided that 1—3 of W, X, Y and Z is or are NO_2). A typical compound is 4-chloro-2-nitroaniline.

F. R. BASFORD.

Biguanide salts. Imperial Chemical Industries Ltd. (Inventor: N. Senior) (B.P. 843,676, 31.12.57).—Compounds claimed comprise bis-biguanide salts, viz., salts of bis-guanides $[CH_2]_m[NH\cdot C(:NH)\cdot NH\cdot C(:NH)\cdot NR\cdot X\cdot A]_2$ (e.g., chlorhexidine) and C_{6-26}-aliphatic or olefinic acids or monohydroxy, carboxy, or ester deriv. thereof. Suitable acids include arachidic, stearic, palmitic, lauric, oleic, linoleic, linolenic and ricinoleic acid, also glycerol diesters of such acids. Anti-microbial and antifungal compositions containing these salts are also claimed. F. R. BASFORD.

[Herbicides]. Monsanto Chemicals Ltd. (Inventor: J. P. Brown) (B.P. 844,329, 26.2.58).—2-Allylphenols with 1 or 2 halogen and 1 or 2 alkyl groups in the benzene nucleus (the *p*-position being unsubstituted if only 5 substituents are present) are active against fungi (including *Botrytis cinerea*) and useful as herbicides. Compounds prepared include 2-allyl-4-chloro- and -4,6-*dichloro*-3,5-*dimethylphenol*, m.p. 32—34°. F. R. BASFORD.

Phenoxyglycerol diesters and their use as herbicides. Badische Anilin-u. Soda-Fabrik A.-G. (Inventors: G. Steinbrunn and A. Fischer) (B.P. 845,632, 2.1.59. Ger., 4.1.58).—Compounds with herbicidal properties comprise phenyl glycerol esters, $OR{\cdot}CH_2{\cdot}CH(O{\cdot}COR'')CH_2{\cdot}O{\cdot}COR'$ and $OR{\cdot}CH(CH_2{\cdot}O{\cdot}COR'){\cdot}CH_2{\cdot}O{\cdot}COR''$ [R is Ph substituted by 2 or 3 Cl or Me groups; R and R″ are (chlorinated) alkyl groups of 1—4 C]. The prep. is detailed of 1-(2,4-*dichlorophenoxy*)-2,3-*di*-(αα-*dichlorobutyryloxy*)*propane*. F. R. BASFORD.

Animal Husbandry

Nitrogen requirements of some ruminal cellulytic bacteria. M. P. Bryant and I. M. Robinson (*Appl. Microbiol.*, 1961, **9,** 96—103).—All strains of ruminococci studied [*Ruminococcus flavaciens* (**I**), *R. albus* (**II**)] grew with NH_3 as the sole source of N other than the B-vitamins. NH_3 was essential even in presence of amino-acids (17), purines (3) and pyrimidines (2). *Bacteroides succinogenes* (**III**), previously shown to require NH_3 in presence of amino-acids, grew with asparagine and glutamine as an N-source in the place of NH_3 but at a slower rate. **I, II** and **III** fixed similar amounts of NH_3 in media containing high or low levels of amino-acids and peptides. **I, II** and **III** digest cellulose in the rumen and synthesise all but a small amount of their cellular-N compounds from exogenous NH_3 even when large amounts of org. N are present. (28 references.) C. V.

Protein-saving action of synthetic lysine and methionine. R. E. Evans (*J. agric. Sci.*, 1961, **57,** 111—121).—With high-protein barley, supplemented with lysine and methionine, protein concentrates are no longer necessary for max. growth rate and food conversion economy. Lysine and methionine in low-protein vegetable diets produce nearly as good growth and economy as a diet containing white fish meal in amounts giving max. growth. Max. N retention, however, does not necessarily occur unless min. digestible protein is present. M. LONG.

Calcium and magnesium in the alimentary tract of sheep. I. The distribution of calcium and magnesium in the contents taken from various parts of the alimentary tract. II. Effect of reducing the acidity of abomasol digesta, *in vitro*, on the distribution of calcium and magnesium. J. E. Storry (*J. agric. Sci.*, 1961, **57,** 97—102, 103—109).—I. Except in the abomasum where no bound Ca and Mg exists, Ca and Mg are found mainly in non-ultrafilterable forms. The pH of the digesta appears to decide the proportion of non-ultrafilterable (increase at low pH) Ca. The effect is less noticeable in the case of Mg. It is suggested that only in the abomasum and duodenum are Ca and Mg in sufficient concn. for absorption.

II. Increasing the pH of abomasol contents, *in vitro*, reduces ultrafilterable Ca and Mg due to binding of the ions by suspended matter in the digesta. The binding capacity for Ca is greater than that for Mg. Pptn. of both ions is prevented by the binding process. M. LONG.

Effect of mechanical treatment on the rate of drying and loss of nutrients in hay. J. C. Murdoch and D. I. Bare (*J. Brit. Grassland Soc.*, 1960, **15,** 94—99).—Cutting the crop with a forage harvester resulted in rapid drying but high nutrient losses. When the crop was tedded with the harvester the hay dried more quickly than with conventional tedding. Nutrient losses were similar for both treatments, and exceeded those sustained with less severe treatments; tedding reduces considerably the risk of hay being weathered. A. H. CORNFIELD.

Digestibility of herbage. VIII. Digestibility of S37 cocksfoot, S23 ryegrass and S24 ryegrass. D. J. Minson, W. F. Raymond and C. E. Harris (*J. Brit. Grassland Soc.*, 1960, **15,** 174—180).—For the first growths in spring digestible org. matter (*D*) remained almost constant until the ears started to emerge from the leaf sheath, when *D* decreased at about 0·5% per day. S23 ryegrass showed comparable values of *D* 3 weeks later than did S24 ryegrass, because of the later ear emergence of the former. S37 cocksfoot showed lower *D* values than did S24 ryegrass, even though their growth stages occurred at similar times. The *D* values of monthly regrowths declined with each successive cut. Application of N had little effect on *D* values. *D* values were similar over two years, even though yields were different due to weather. A. H. CORNFIELD.

Fungal processing of fodder by self-heating. I. Examination of the fodder prepared in the laboratory. G. Müller (*Zbl. Bakt.*, 1961, **II, 114,** 192—202).—A fattening ration comprising rye bran, milo, chopped soya-bean and rye with dried sugar-beet slices was moistened with hot (60°) water and packed in containers (Rohde process). The temp. of the mass rose to 48° and after 3 days was fed to pigs. The distribution of micro-organisms in the material (8 species of bacteria, 15 yeasts and 3 mould fungi) was examined. None of the organisms present decomposed cellulose. Proteolytic activity was small. Most of the bacteria were lactic-acid formers. A. G. POLLARD.

Estimates of the areas of pasture fouled by the excreta of dairy cows. D. S. Maclusky (*J. Brit. Grassland Soc.*, 1960, **15,** 181—188).—The average area covered by faeces was 7·3 sq. ft. per cow per day. Faeces dropped during grazing had a negligible effect on the utilisation of herbage at that grazing, but each dung pat probably affected the growth and palatability of an area of herbage about 6 times as great at the next grazing. Urine affected about the same area of herbage as did faeces, but unlike faeces, improved the palatability of the herbage to the cow. A. H. CORNFIELD.

Nutrient losses and efficiency of conserving herbage as silage, barn-dried hay and field-cured hay. W. R. B. Carter (*J. Brit. Grassland Soc.*, 1960, **15,** 220—230).—A review of U.S. literature. A. H. CORNFIELD.

Effect of temperature in the mass on chemical composition of silage. J. C. Murdoch and M. C. Holdsworth (*J. Brit. Grassland Soc.*, 1960, **15,** 240—245).—When conditions in the silage were favourable for the formation of lactic acid, either through treatment or because of the herbage itself, a low max. temp. in the mass had no adverse effect on silage quality. There were indications that with chopped or lacerated materials a low max. temp. was associated with better silage quality. A. H. CORNFIELD.

Digestibility and feeding value of green and ensiled maize fodder. N. D. Dijkstra and W. R. Becker (*Versl. landbouwk. Onderz.*, 1960, 66.14, 48 pp.).—The effects of variations in plant density are small. In goat feeding experiments, better results as regards content of dry matter, starch equiv. and digestibility are obtained with green fodder or silage from an early than from a late variety of maize. Increases in dry matter are observed during growth; changes in digestibility during growth are small. Digestibilities of the silages (of good quality) are generally lower than those of the green fodder. The average losses of nutrient value during ensiling are ~38% for digestible crude protein, and ~29% for starch equiv. Satisfactory correlations are obtained between chemical composition and nutrient value. (24 references.) P. S. ARUP.

Guano as source of protein for cattle. W. A. Verbeek and M. K. S. L. von la Chevallerie (*S. Afr. J. agric. Sci.*, 1960, **3,** 613—616).—In comparative feeding experiments in which the supplementary N constitutes 40% of the ration, supplementary guano shows the same protein-value as urea; its content of P and other mineral matter is, however, a point in its favour. The value of guano appears to be somewhat, but not significantly, lower than that of groundnut oil-cake. (In Afrikaans.) P. S. ARUP.

Symptoms of sodium deficiency in animals and results of too large doses of common salt. J. J. Lehr (*Tijds. Diergeneesk.*, 1961, **86**; reprint *Chilean Nitrate agric. Serv.*, 1961 [August]).—A review with 43 references concerning the symptoms of deficiency of Na in domestic (farm) animals, the effect of K/Na ratio in the fodder, and toxicity of Na (especially as chloride). The beneficial effects of $NaNO_3$ in curing the deficiency are pointed out. H. S. R.

Production of phosphate-containing feed salts [for animal husbandry]. B. Balla, O. Gyimes and A. Tancsa (*Commun. Res. Inst. heavy chem. Ind. Hung.*, 1960, **2,** 1—13).—Two methods were developed for the prep. of $CaHPO_4$, suitable for feeding stuffs. (*a*) Apatite is treated with 50% HNO_3 at 60° with agitation for 45 min. $NaNO_3$ is then added to form the Na fluosilicate, thus removing 75—80% of the F on filtration. The filtrate is diluted NH_4NO_3 is added, and the solution is blown with NH_3/air mixture, when a further ppt. forms. The filtrate from this operation is neutralised with more NH_3, when $CaHPO_4$ with low F content is precipitated. (*b*) Ordinary superphosphate is extracted with water (1·5 cu. m./ton), and washed after extraction with 0·5 cu. m./ton. $CaCl_2$ is added to precipitate F as Ca fluosilicate, or alternatively a suspension of lime is added to form a phosphate ppt. which contains all the F. K. RIDGWAY.

Grass as a food for the calf. I. Value of cut grass in the diet of the unweaned calf in comparison with hay and concentrates. II. Effects of feeding grass or concentrates-with-hay during the pre-weaning period on the intake and utilisation of grass during the post-weaning period. D. T. Chambers (*J. agric. Sci.*, 1961, **57,** 71—76, 77—82).—I. Good quality grass can replace hay and concentrates in the diet of unweaned calves, no significant differences existing between the three, although rumen development may be affected. No interaction exists between type of dry food and milk ration or housing conditions.

II. After 2 weeks, grass-reared calves make slightly better gains, although without appearing to digest food more efficiently or to consume more herbage dry matter. M. Long.

Growth of Afrikaner calf in relation to production and composition of milk of its dam. II. Milk production of dam and growth of calf. H. Heyns (*S. Afr. J. agric. Sci.*, 1960, **3**, 517—530).—Highly significant direct correlations are found between gains in wt. and body measurements by calves and production of milk (measured by weighing the calves before and after suckling) and (especially) of protein. The lower milk production by late- as compared with early-calving cows is significantly reflected in lower weaning-wt. Birth wt. is highly correlated with (*a*) milk production of the cow, (*b*) gain in wt. of the calf and (*c*) weaning-wt. Milk production and weaning-wt. reach max. values at maternal ages of 6—7 years. P. S. Arup.

Summer stall feeding [of dairy cows]. A review of the literature. H. Hovius (*Landbouwdocumentatie*, 1961, **17**, 707—713).—A description of the principles of " zero grazing " is followed by a consideration of the relative advantages and disadvantages of the system. (39 references.) P. S. Arup.

Effects of frequency of feeding on production characteristics and feed utilisation in lactating dairy cows. John Roy Campbell (*Dissert. Abstr.*, 1961, **21**, 2424).—Increases in the no. of daily feedings (2—4—7 times) cause upward trends in the production of milk, milk solids, and milk fat; significance at the 5% level is, however, approached only in the case of wt. of milk and of 4% fat-adjusted milk. Similar trends occur for feed-intake, -efficiency and -digestibility, but not for body-wt. P. S. Arup.

Dairy production from pastures: comparison of two methods of controlled grazing at two rates of stocking. A. S. Foot and C. Line (*J. Brit. Grassland Soc.*, 1960, **15**, 155—162).—Using the high rate of stocking (possibly somewhat restrictive) rotational grazing gave an average milk yield of 40 lb. and daily strip folding 38 lb. per cow per day. With a 20% lower rate of stocking (considered good practice) both methods gave 42 lb. milk per cow per day. With the high stocking rate milk yield per acre was 1007 gal. from rotational grazing and 997 gal. from daily strip-folding. With the low stocking rate yields were 881 and 874 gal. per acre respectively. A. H. Cornfield.

Blood serum-calcium and -magnesium levels in bullocks grazing simple leys and old pasture. W. M. Ashton and K. B. Sinclair (*J. Brit. Grassland Soc.*, 1960, **15**, 296—299).—Serum-Mg levels of bullocks on leys (S24 ryegrass and S170 tall fescue) were higher than those on old pasture. Serum-Mg levels were also higher on the tall fescue than on the ryegrass. Serum-Ca levels were similar on all pastures. A. H. Cornfield.

Influence of night grazing on the growth rate of Zebu cattle in East Africa. A. D. H. Joblin (*J. Brit. Grassland Soc.*, 1960, **15**, 212—215).—Restriction of night grazing resulted in 30% less live-wt. gains over 10 months as compared with unrestricted grazing. A. H. Cornfield.

Production of beef from Zebu cattle in East Africa. III. Value of feeding a phosphatic supplement. G. H. Lampkin, D. A. Howard and M. L. Burdin (*J. agric. Sci.*, 1961, **57**, 39—47).—Supplements of 0·7—1·0 oz. of $CaHPO_4$ per head daily increased the blood-inorg. P level and (slightly) improved the condition of the animals; only during drought. Fertility was improved throughout the experiment. Symptoms of apparent aphosphorosis disappeared on raising the general standard of nutrition. M. Long.

Influence of nutritional level during prenatal and early post-natal life on adult fleece and body characters. P. G. Schinkel and B. F. Short (*Aust. J. agric. Res.*, 1961, **12**, 176—202).—In addition to reducing the wt. of the lambs at birth and maturity, restriction of the prenatal diet reduces the development of wool-follicles in no. by 15%, and ultimate yields of wool by 8·5%. Restricted dieting during the first four months retards growth and reduces the mature body wt. and the productivity of (the normal no. of the) follicles by 10 and 12·5%, respectively. (26 references.) P. S. Arup.

Reproductive performance of New Zealand Romney sheep grazed on red clover (*Trifolium pratense*) pastures. T. S. Ch'ang (*J. agric. Sci.*, 1961, **57**, 123—127).—Oestrogenic red clovers cause ewe lambs to accept males earlier, but no corpus luteum is found in the ovary nor is subsequent reproductive performance affected. However, the lambing season is protracted and at 6 years lambing performance is reduced. M. Long.

Effects of nutrition on the growth and sexual development of ewe lambs. D. M. Allen and G. E. Lamming (*J. agric. Sci.*, 1961, **57**, 87—95).—Puberty occurs at a lower live-wt. with moderate than with high-plane nutrition levels. Ovulation and the no. of ovarian follicles >2 mm. dia. are unaffected by nutrition, although at high levels more follicles <2 mm. appear. Injections of pregnant mare serum increase ovulation but no interaction with nutrition exists. Ovarian wt. and dimensions of uterus, thyroid and anterior pituitary glands tend to be greater and adrenal glands smaller in lambs receiving high- than in those receiving moderate-plane rations. Nutrition has no effect on the fertilisation of ova. M. Long.

Suppression of oestrus in sheep with progesterone. D. R. Lamond and L. J. Lambourne (*Aust. J. agric. Res.*, 1961, **12**, 154—170).—Intramuscular injections of progesterone in oil of 5 mg. per day or 10 mg. per 2 days are necessary and sufficient to suppress oestrus in most ewes during the breeding season. Results obtained by injecting at 4-day intervals are less successful. Ewes inseminated during the treatment are nearly always infertile. Among ewes inseminated after the injection period, the fertility is greatest in those injected every other day. (15 references.) P. S. Arup.

Depletion of insecticides in sheep-dipping baths: further field trials. I. R. Harrison and P. G. Marshall (*J. Sci. Fd Agric.*, 1961, **12**, 548—552).—In a trial the no. of sheep dipped varied from 400 to >2000. To keep the level of insecticide (e.g., aldrin/γ-BHC) concentration more constant during the early dipping period, a lower initial concentration was used and the bath was replenished more frequently. The physical composition and behaviour of the bath after a large no. of sheep have been dipped, in relation to the depletion rate of insecticide at the beginning and towards the end of a trial, are discussed. E. M. J.

Effect of feeding different levels of protein to baconers. P. J. S. Pieterse and E. J. Penzhorn (*S. Afr. J. agric. Sci.*, 1960, **3**, 573—580).—Supplementation with white fish-meal at levels $>2·5\%$ of the ration during growth between 100 and 200 lb. live wt. gives significant increases in the thickness of the back fat and kidney wt., but has no effect on gains in live wt., feed utilisation or carcass development. P. S. Arup.

Phosphorus requirement of pigs from two to seven weeks of age. J. M. Vandepopuliere (*Dissert. Abstr.*, 1961, **21**, 2426).—Comparative experiments based on determinations of the bone-density and bone ash composition of the (surgically extracted) fibula show the P requirements to be 0·44% of a ration of fortified maize–soya-bean oil meal with a Ca/P ratio of 0·9 : 1. The direct relationship of bone ash to bone-density is confirmed. P. S. Arup.

Effects of supplementary detergents on the digestibility of poultry diets. W. Bolton (*J. agric. Sci.*, 1961, **57**, 83—86).—A non-ionic detergent (Lissapol N) aided the digestion of fat but not that of protein or carbohydrate; an anionic (detergent) slightly and a cationic detergent seriously impaired fat absorption. The latter also impaired carbohydrate digestion. M. Long.

Winter egg-laying rate. I. Coefficient of correlation between the yearly egg-laying rate and the egg-laying rate in each part of the year. B. Kraszewska-Domańska (*Ann. Univ. M. Curie Skłodowska*, 1959, **14E**, 283—295).—The highest correlation was between the laying rate of the last 4 months of the egg-laying year and the yearly rate. The coeff. of correlation between the winter and the yearly egg-laying rates depended on the mean daily min. temp. in Nov. and Feb. Selection for winter egg-laying should be made after considering conditions in that period. (24 references.) E. M. J.

Chick oedema factor. II. Isolation of a toxic substance. A. Yartzoff, D. Firestone, D. Banes, W. Horwitz, L. Friedman and S. Nesheim (*J. Amer. Oil Chem. Soc.*, 1961, **38**, 60—62).—A cryst. halogenated compound isolated from a sample of triolein was toxic when administered to Cebus monkeys. When fed to chicks at the level of 0·1 p.p.m. of feed, it produced oedema symptoms; it showed properties similar to those of a compound reported by Harman *et al.* (*J. Amer. chem. Soc.*, 1960, **82**, 2078). A solution of the compound in iso-octane exhibits an absorption max. at 245 and 300 mμ, and the substance sublimes at 239°. (11 references.) G. R. Whalley.

Increasing the effectiveness of antibiotics as chemotherapeutic agents. J. S. Kiser (*Antibiot. & Chemotherapy*, 1961, **11**, 261—266).—Chlortetracycline incorporated into broiler feed, low in Ca, produced a significantly higher blood concn. and proportionately longer survival time in chicks experimentally infected with *Salmonella gallinarum*. C. V.

Crude fibre analysis. II. Comparison of different methods for determining crude fibre. E. Mergenthaler (*Z. LebensmittUntersuch.*, 1961, **114**, 18—26; cf. J.S.F.A. Abstr., 1959, ii, 103).—Analyses are given of 52 different types of plant material by the following methods: the original standard method using 1·25% H_2SO_4 and

1·25% KOH, the methods of Fincke and Thaler, Hampel, Glimm and Hansen, and of König, and the ethylene glycol method. The results show considerable variations according to the method used. No one of the later methods shows any particular advantage as regards reproducibility or correlation with the original standard method. The Fincke and Thaler, Glimm and Hansen and the ethylene glycol methods are convenient for routine analyses.
P. S. Arup.

Composition for the control of gastro-intestinal parasites in animals. Dow Chemical Co. (B.P. 845,368, 16.8.57. U.S., 1.10.56).—A composition, for use in the control of gastro-intestinal parasites (e.g., strains of *Eimeria tenella* coccidia) in animals, especially poultry, comprises a suitable diluent (e.g., poultry feed) and 0·001—1 wt.-% of a polynitrobenzamide, viz., Z·COR [Z is 2,4,6-$(NO_2)_2C_6H_2X$, 2,4,5-$(NO_2)_2C_6H_2Y$, 3,5,4-$(NO_2)_2C_6H_2X$, 3,5,6-$(NO_2)_2C_6H_2Q$, or 2,4,6-$(NO_2)_3C_6H_2$; X is H, NH_2, low-mol. alkyl or alkoxy; Y is H, low-mol. alkyl or low-mol. alkoxy; Q is NH_2, low-mol. alkyl or low-mol. alkoxy; R is NH_2 or alkylamino]. Thus, addition of 0·1% of 3,5-dinitro-4-methylbenzamide to poultry feed affords 100% control of coccidiosis in infected poultry.
F. R. Basford.

Animal feed. Chas. Pfizer & Co. Inc. (B.P. 845,786, 19.12.57. U.S., 26.12.56).—A glucocorticoid, e.g., hydrocortisone or prednisone (0·01—0·1 mg. per kg.), is added to animal feed (for simple-stomached animals—rats, chickens, turkeys, pigs, dogs, cats, etc.), to increase the growth rate. F. R. Basford.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Purification and preparation of cereals spoiled by sprouting. W. Seibel and H. Zwingelberg (*Getreide u. Mehl*, 1961, **11**, 53—60).—The Heid density fractionater is tested on five types of flour of different moisture and " spoiled grain " content. The machine produces six fractions, one fraction of which has a considerably lower " spoiled grain " content than the original flour. Speeds of action are varied and the optimum conditions are evolved. Higher moisture contents lead to poorer fractionation. J. V. Russo.

Sterol esters in cereals and their importance in differentiating durum and *vulgare* wheat. A. Guilbot (*Getreide u. Mehl*, 1961, **11**, 49—53).—Sterols can be extracted from flour by digesting 150 g. of flour with 300 ml. of acetone at 38° for 24 h. After filtering hot, the extract is cooled to 5° for 2 h. and the ppt. is filtered off at 5—10°. The ppt. contains a mixture of sterols and sterol esters, which gives a colour reaction with the Liebermann–Burchard reagent. The sterol esters can be separated from the sterols by the digitonin pptn. method, by chromatography on Al_2O_3 or by paper chromatography. On samples of durum wheat studied, the sterol ester content varied from 20 to 62 mg.-% and on three samples of *vulgare* wheat from 18 to 21%. Further study is necessary if this method is to be used to differentiate the two types of wheat. (30 references.) J. V. Russo.

Mucilages of cereals. I. Chromatographic fractionation of water-soluble wheat pentosans on diethylaminoethylcellulose. W. Kuendig, H. Neukom and H. Deuel (*Helv. chim. Acta*, 1961, **44**, 823—829).—The water-sol. starch-free polysaccharides of a wheat flour (Manitoba II) were chromatographically adsorbed on diethylaminoethylcellulose and selectively eluted. Five separate fractions were obtained. Fraction 1 was a pure araboxylan. The other fractions contained glycoproteins, their polysaccharides being composed of xylose, arabinose and galactose in various proportions.
M. Sulzbacher.

Biochemistry of rye flour and rye bread. M. Rohrlich (*Dtsch. LebensmittRdsch.*, 1960, **57**, 83—89).—A review of modern views on methods for the quality testing of rye flour, the microbiology and biochemistry of sour dough development, the effects of souring on the starch and protein in rye doughs and the nature of the acids and flavour compounds in rye doughs and rye bread. (44 references.)
E. C. Apling.

Effect of package type on stored flour and farina. R. K. Larmour, J. H. Hulse, J. A. Anderson and C. J. Dempster (*Cereal Sci.*, 1961, **6**, 158—164).—Flour and farina commercially milled from one lot of Canadian hard red spring wheat were stored for 5 years in various packaging materials. Samples of two initial moisture contents (14·5% and dried to 8% for flour, and 15·0% and dried to 10·0% for farina) were stored outdoors at a mean annual temp. of 45 to 50°F and indoors at a constant temp. of 75°F. Once a year changes in moisture, baking strength, fat acidity and fat content, in lipoxidase activity and pigment content, and in physical dough properties were determined. All packages which retarded moisture interchange kept the dried flour and farina in good condition, but a rapid deterioration of baking strength was noted in normal moisture flour and to a lesser degree in normal moisture farina. In moisture-permeable packages, normal moisture and dried farina underwent only a slight decrease in baking strength, while normal moisture and dried flour underwent an appreciable and progressive decrease. (10 references.) I. Dickinson.

Vitamin content of wheat flour as a function of the degree of grinding. A. Schulerod (*Brot u. Gebäck*, 1961, **15**, 61—64).—Thiamine and riboflavin values for many flours of different degrees of grinding are collected and % total vitamin is plotted against degree of grinding. Average curves are drawn and it is found that these curves rise steeply above 78% degree of grinding.
J. V. Russo.

Baking quality of rye flour under the influence of acids and salt. H. Huber (*Brot u. Gebäck*, 1961, **15**, 88—95).—Baking tests were made on rye flour with and without the addition of NaCl and citric acid. The presence of NaCl has more effect on the quality of the loaf than the presence of acid. Optimum results are obtained with 20 g./kg. of NaCl and 12 g./kg. of citric acid. The α-amylase activity of the flour is not altered much by the presence of salt, but it is virtually destroyed when the pH falls to 4·8. (12 references.)
J. V. Russo.

Use of selective desorption from carbon columns for the determination of maltotriose in starch conversion products. S. J. Patterson and J. L. Buchan (*Analyst*, 1961, **86**, 160—163).—The method previously described for the determination of dextrose and maltose in starch conversion products (Patterson *et al.*, J.S.F.A. Abstr., 1958, ii, 69) has been extended to the determination of maltotriose. The carbon–kieselguhr column is lengthened to 15 in. and narrowed to 0·5 in. dia. (the wt. of material being unaltered) and elution is with water (100 ml.) for dextrose, 5% ethanol (100 ml.) for maltose and 8% ethanol (150 ml.) for maltotriose, the temp. being maintained throughout at 40°. Unless the determination of maltotriose is required the shorter column (*loc. cit.*) should be used for determining dextrose and maltose. A. O. Jones.

Fundamental studies on dough with the Brabender Extensograph. I. Determination of stress–strain curves. H. G. Muller, M. V. Williams, P. W. Russell Eggitt and J. B. M. Coppock (*J. Sci. Fd Agric.*, 1961, **12**, 513—523).—A method is described for converting the empirical load-extension diagrams obtained by stretching flour–water–salt doughs on the Brabender Extensograph, into stress–strain curves plotted in absolute units. Variation in stress during extension is a major deficiency of this type of instrument. The weaker the flour, the greater is the flow and the consequent increase in the effective dough mass. Differences between strong and weak flours tend to be misrepresented; instrumental and baking tests on individual flours may be at variance. Probable errors are defined and assessed. (20 references.) E. M. J.

Possible effect of wheat dehydrogenases on the meaning of physical tests on unyeasted doughs. R. W. Crawley (*J. Sci. Fd Agric.*, 1961, **12**, 559—561).—Pieces of fermenting dough (pH 5·6) removed at intervals, show decrease of extensibility, and quality of the bread sample improves until the optimum condition (mature) is reached for dough and loaf. The extensibility of unyeasted doughs (pH 6·1) increases with time. This is postulated to be brought about by dehydrogenases, the activity of which falls sharply below pH 6. At pH 6·4 dehydrogenase activity of a germ suspension persists for many hours; at pH 5·6 it is destroyed in 1 h. at 37°. (10 references.) E. M. J.

Buffering action of phytin in multistage and continuous acid [baking] process. M. Rohrlich, A. Schulz and H. Stephan (*Brot u. Gebäck*, 1961, **15**, 85—88).—The addition of Ca phytate to acid doughs, both multistage and continuous, increased the bacterial count in the dough and produced bread of improved crumb elasticity but of a slightly acid flavour. J. V. Russo.

Quality assessment of fine bakery products. A. Rotsch (*Brot u. Gebäck*, 1961, **15**, 85—97).—A marking scheme for the quality assessment of fine bakery products is drawn up. The total points are 20, comprising: 6 for outward appearance, 6 for inner appearance (crumb, etc.) and 8 for aroma, flavour and keeping quality. Any faults in any of these groups are underlined and corresponding marks are awarded. J. V. Russo.

Importance of rye mucins in preservation of freshness in bakery products. G. Rehfeld (*Ernährungsforschung*, 1961, **6**, 82—95).—Extracts of rye meal are prepared by shaking with water the meal which has previously been shaken with 80% EtOH. The use of these extracts in place of water in making the dough for wheaten or starch breads results in greatly improved keeping properties of

the loaves. Small amounts of arabinose, probably arising from the hydrolysis of the mucins, are found in the aq. extracts.
P. S. ARUP.

Fat content of Swedish crispbread and a newly discovered method for arriving at a better composition of the diet. D. Karp (*Brot u. Gebäck*, 1961, **15**, 97—100).—Swedish crispbread is analysed to find total lipid content and the unsaturated fatty acid content of these lipids. The relationship between these fatty acids and human physiological effects is discussed. The vitamin E (tocopherol) content of crispbreads is considered in relation to its effect on muscular effort. (17 references.) J. V. RUSSO.

Salt determination in bakery goods. M. Obrtel and J. Purš (*Prům. potravin*, 1961, **12**, 385—387).—Methods of NaCl determination in bakery goods are reviewed and the carbonising methods criticised on account of low values resulting from the reaction of NaCl with acid phosphates present in the material examined, and loss of gaseous HCl. This drawback can be obviated either in determining the Na ions with flame photometer, or eliminating the HCl losses by combustion in alkaline medium, or by binding the Cl ions with Ag ions in wet combustion in acid medium. The procedures giving exact values are described in detail. J. S. B.

Effect of freezing and frozen storage on the freshness and firmness of Danish pastry. K. Kulp and W. G. Bechtel (*Food Technol.*, 1961, **15**, 273—275).—Freezing and defrosting caused no appreciable loss of freshness. At 0°F the product was protected from staling and firming for at least 8 weeks. (10 references.) E. M. J.

Flour of improved biscuit-making properties. Research Association of British Flour-Millers, A. P. Bowman, R. G. Cousins and P. Halton (B.P. 846,262, 7.11.55).—The biscuit-making properties of flour are improved by adding thereto a modifier, viz., an extracellular proteinase derived from *B. subtilis*, cysteine, cystine, glutathione and/or a salt of the last three (to convert the protein of the flour into relatively large molecular units), so that the unyeasted dough made from the mixture has an extensibility of $\nless 18$ and a spring value of $\ngtr 330$. F. R. BASFORD.

Sugars and confectionery

Field sugar cane sampling technique for the analysis of sugar contents in the sugar cane. T. P. Pao, S. C. Yang and P. C. Sun (*Rep. Taiwan Sug. Exp. Sta.*, 1961, No. 23, 1—18).—Sampling methods using either 3, 5 or 7 stalks per plot and/or 1—3, 4—6 and 7—9 stalks per plant were compared. The methods of determining the suitable no. of replications, the size of sample per plot and the relative % of efficiency are described and discussed with reference to the relative costs of taking samples. Lodging cane was of greater variability and lower efficiency than standing cane. The sugar content of standing cane was higher. There was no significant difference in sugar content between blossom and blossomless, and stripped and non-stripped cane. (26 references.)
S. G. AYERST.

Coloured substances balance in the sugar refining process. J. Buriánek, J. Bernatová and K. Paulfranzová (*Listy cukrovar.*, 1961, **77**, 153—165).—The ratio of the quantity of coloured matter to that of inorg. substances can be used for criterion of the colouring or decolorisation of any intermediate sugar refinery product. Only with refined syrup a certain reservation is advisable, the possible inaccuracy due to retention of a portion of inorg. substances by the decolorising medium or to the alkalisation of the syrup is of no essential significance. The above ratio can be established as the relation of the colour quotient to the inorg. substances quotient. By the term colouring is meant the relative increase of colour due to coloured substances, which is shown not before the refining process. (11 references.) J. S. B.

Colour index and degree of whiteness of Taiwan plantation white sugar. W. Chen and S. H. Wu (*Rep. Taiwan Sug. Exp. Sta.*, 1961, No. 23, 117—124).—The colour indexes and degrees of whiteness of superior white crystal sugar from 14 Taiwan factories were compared. The mills could be divided into three levels and although there were slight changes throughout the season the quality level tended to remain constant. A visual grading method for estimation of whiteness has been devised and is described. S. G. AYERST.

One layer cylinder plate method for routine antibiotic assay. P. Soong, Y. Y. Jen and Y. S. Hsu (*Rep. Taiwan Sug. Exp. Sta.*, 1961, No. 23, 81—86).—The accuracy of the method is the same as the standard two-layer cylinder plate method. Advantages over the standard method are : (i) it saves 60% of the medium for assay ; (ii) it saves labour and time for prep. ; (iii) it minimises the chance of contamination ; (iv) it gives a clear and larger zone of inhibition ; (v) the formation of the zone of inhibition is earlier ; (vi) the range of the standard curve is larger. S. G. AYERST.

An examination of the occurrence of honeydew in honey. II. K. C. Kirkwood, T. J. Mitchell and I. C. Ross (*Analyst*, 1961, **86**, 164—165).—Twenty-eight samples of honey were analysed for moisture, ash, colloid, dextrin and reducing sugars, and their sp. rotation and pH value were measured, the object being to test the efficiency of the discriminant function previously reported (Kirkwood *et al.*, J.S.F.A. Abstr., 1961, i, 31) for distinguishing floral from honeydew honey and in particular to distinguish Scottish heather honey from honeydew honey. Ling heather honey can be distinguished by its high colloid content and honeydew honeys of the same colloid content are much darker. Honeydew honeys had higher dextrin and lower reducing sugar contents and were dextrorotatory whereas floral honeys were laevorotatory. The efficiency of the discriminant function (*loc. cit.*) for distinguishing honeydew honey from floral honey was confirmed. Eleven of the samples tested contained honeydew. A. O. JONES.

Hydroxymethylfurfural in honey. I. Determination and conversion of hydroxymethylfurfural [HMF]. E. Romann and M. Staub (*Mitt. Lebensm. Hyg., Bern*, 1961, **52**, 44—58).—The presence of HMF and cause of formation in native and foreign honeys were studied ; the Maillard reaction, aldol condensation and polymerisation are discussed. Oxidation of HMF to hydroxymethylpyromucic acid and rearrangement to 5-methylpyromucic acid were observed. Extraction of HMF and determination by measurement of the coeff. of extinction, having regard to interfering substances, are described. (29 references.) E. M. J.

Fermentation and Alcoholic Beverages

Flor yeast in wine production. Laboratory study. I. I. J. Saavedra and J. M. Garrido (*Rev. Cienc. apl.*, 1961, **15**, 97—107).—The production of wines with the aid of yeasts capable of causing veiling, e.g., *Saccharomyces cheresiensis*, has been studied. Trials on some white wines have been carried out and relationships between the volume of wine and the areas of veiled surface examined.
L. A. O.NEILL.

Proportion of fructose in residual sugar of wines. F. Prillinger (*Mitt. Wein u. Obstbau, Wien*, 1961, **11**, 30—35).—Fructose was determined in a wide variety of wines by means of the alkaline $CuSO_4$ oxidation method at 50°, excess Cu being determined iodimetrically. The ratio of glucose : fructose in the residual sugars varied but was always $<0\cdot35$. Ratios higher than this would therefore indicate adulteration. J. V. RUSSO.

Production of glycerol and 2,3-butylene glycol in wine fermentation. Differentiation between naturally pure and sugared wines. E. Kielhöfer and G. Würdig (*Z. LebensmittUntersuch.*, 1961, **114**, 376—397).—The factors influencing the quotients of the content of glycerol (**I**) and of 2,3-butylene glycol (**II**) in relation to the EtOH content (expressed as 100 g. per l.) are examined ; these factors include the original composition of the must and the conditions of fermentation. The results of comparative experiments show the **I** quotient (average 9·6 for 26 natural and 8·8 for 13 sugared wines) to be a more reliable guide to the detection of added sugar than the K value obtained by the Rebelein formula. The **II** quotient (incorporated in the Rebelein formula) is of no value in assessing the ripeness of the grapes or the addition of sugar. (45 references.)
P. S. ARUP.

Detection of additions of alcohol or sugar in wines. Comparing and contrasting the methods of Rebelein and Seith. A. Blumenthal (*Mitt. Lebensm. Hyg., Bern*, 1961, **52**, 59—65).—The method of Seith (extract no.) is based on determination of the original total sugar content (e.g., 182·2 g./l.) and on that of the extract without sugar added (e.g., 21·2 g./l.). The extract no. is then $21\cdot2 \times 100/182\cdot2 = 11\cdot6$. The method of Rebelein requires additionally the determination of glycerol and of 2,3-butylene glycol. Wines may be classified as (*a*) natural, (*b*) those with evident additions of alcohol or sugar and (*c*) those for which an accurate expression is not possible. An analysis according to Rebelein can give a more precise estimate of wines of the (*c*) class. E. M. J.

Determination of ethyl acetate in wines by gas chromatography. P. Ribéreau-Gayon (*Chim. anal.*, 1961, **43**, 161—164).—The ethyl acetate (**I**) content of the wine is determined on a distilled aliquot. 100 ml. of the wine are distilled and exactly 20 ml. collected. 10 to 40 μl. of the distillate are injected into the gas stream of the chromatograph. The column (2 metres) is packed with polyethylene glycol (Perkin–Elmer column, type K). The operating temp. is 95° and the carrier gas is He at an inlet pressure of 0·7 kg./cm^2. The content of **I** is obtained by comparing the peaks with a standard curve. Results compare well with those of the chemical method.
R. A. HENDEY.

Determination of chloropicrin in wine. V. Astegiano and M. Matta (*Chim. e Industr.*, 1961, **43**, 266—270).—Chloropicrin in wine is extracted by xylene, recovered by steam distillation, and determined by $PhNMe_2$ in the presence of Bz_2O_2. The colour intensity developed is measured at 380 mμ and compared with a standard absorption–concn. curve. C. A. Finch.

Distillation at a high proof figure in the continuous distillation of alcoholic beverages. H. M. Brau (*Rev. Soc. quím. Mex.*, 1961, **5,** 51—57).—A study of the separation of fusel oil from alcohol and desirable impurities under a variety of conditions of column operation shows the advantage of distillation at the highest possible proof strength. Selection of reflux ratio and point of collection from the column (take-off height) makes possible the production at high proof strength of practically pure spirit, or spirit containing any desired proportion of impurities. Present legislation in many countries regarding max. proof strength of distillates retards the application of modern distillation techniques to the production of alcoholic beverages. E. C. Apling.

Determination of fruit esters in spirits by paper chromatography. A. Osztrovszky and J. Ujszászi (*Acta chim. hung.*, 1961, **27,** 285—293).—A qual. and quant. determination of esters contained in fruit distillates is based on the conversion of the extracted esters into hydroxamic acids and their separation by paper chromatography. $FeCl_3$ serves as developer. Spirits made from cherries, morelloes, apricots and plums were examined. (In German.) M. Sulzbacher.

Determination of nitrate in brewing waters. E. Schild, L. Then and H. Diemer (*Brauwissenschaft*, 1961, **14,** 122—123).—The brucine colorimetric method for the determination of NO_3^- in brewing waters gives poor results. The method of Ulsch, which depends on the reduction of NO_3^- to NH_3 by Fe in the presence of H_2SO_4 gives excellent results in the range 25—150 mg./l. of NO_3^-. J. V. Russo.

Iron and manganese in brewing waters : importance, detection, determination and removal. II. H. Wildner (*Brauwissenschaft*, 1961, **14,** 127—130).—Open and closed plant for the removal of Fe is described. The basic processes consist in air oxidation of Fe to Fe_2O_3, conversion to gelatinous $Fe(OH)_3$ and filtration. Mn is removed similarly either by air oxidation at an alkaline pH, or by bacterial oxidation. (32 references.) J. V. Russo.

A co-precipitation and spectrophotometric determination of micro quantities of iron in Japanese brewing water and liquor. K. Otozai and K. Mizumoto (*Mikrochim. Acta*, 1961, 217—226).—Traces of iron are co-precipitated with MnO_2 from aq. solution and determined spectrophotometrically with *o*-phenanthroline. The sensitivity for water samples is 0·0002 p.p.m. of Fe and for saké samples 0·005 p.p.m. of Fe. (In English.) W. T. Carter.

Composition and anthocyanogen content of hordein of barley. G. Kloos (*Bauwissenschaft*, 1961, **14,** 223—227).—Electrophoretic analyses reveal that the extraction of the anthocyanins from barley meals by 40% EtOH is incomplete and that a part of the proteins is removed together with the anthocyanins. The diagrams for the hordein fraction extracted by 40% EtOH show a marked decrease in the rapidly-moving α- and β-fractions, and an increase in the slowly-moving δ- and ϵ-fractions in comparison with those for normally-extracted hordein. Dialysis of the hordein removes $\sim$67% of the anthocyanins after a few days, without markedly affecting the composition of the proteins. The anthocyanins (present chiefly in the δ- and ϵ-fractions) are probably linked, for the most part, with the proteins sol. in 75% EtOH. The proteins extracted by 75% EtOH from de-spelted malt contain much less anthocyanin than those extracted from ordinary malt. (11 references.) P. S. Arup.

Technology of malting. L. Weith (*Brauwissenschaft*, 1960, **13,** 214—218).—The determination of α- and β-amylase and of diastatic activity during the germination of barley and the effects of variety and grain size are described and discussed. J. V. Russo.

Use of gibberellic acid in malting. H. Stadler, H. Kipphahn and S. Gallinger (*Brasserie*, 1961, **16,** 103—109).—Gibberellic acid increases the rate of disintegration of barley in malting and leads to higher extracts and Kolbach indexes. The amount necessary for optimum results varies with the variety of barley and micro-malting tests are advised, as excess acid yields poor brewer's malt of too high Kolbach index ($>$40) and of a deep colour. In general doses of 0·01—0·02 mg./kg. yielded good results. Hetero-auxin (indol-3-ylacetic acid) yielded results analagous to those with gibberellic acid. J. V. Russo.

Studies to improve the fine–coarse extract difference figure. L. R. Bishop (*J. Inst. Brew.*, 1961, **67** [New Ser. **58**], 244—248 ; see also *Brasserie*, 1961, **16,** 110—114).—The discrepancies in results in the measurement of malt modification by the difference between fine and coarse extract yields are substantially reduced by modified grinding to give regular sized particles. Thus, mean values of the fine–coarse extract difference for the same set of malts by the current procedure were 1·8 and 4·0% in two laboratories, whereas on another set of malts using the modified grinding, values of 2·15 and 2·45% were found. The modified grinding is done in a Casella mill fitted with a sieve, 0·5 or 1·0 mm. for fine and 2·5 mm. for coarse grinding. The feature of the mill is that the action is one of cutting rather than crushing. The 1·0 mm. sieve gives the same values as the 0·5 mm. but reduces the time of grinding. The standard deviation of fine–coarse extract differences is reduced in the Casella mill to about one-fourth of that in the current standard procedure. J. I. M. Jones.

Kaffircorn malting and brewing studies. X. Susceptibility of sorghum starch to amylolysis. L. Novellie and R. J. Schütte (*J. Sci. Fd Agric.*, 1961, **12,** 552—559 ; cf. J.S.F.A. Abstr., 1961, ii, 174).—Gelatinised starch gives Kaffir beer its characteristic creamy body and keeps in suspension the particles of grain and malt, essential constituents. The effects of brewing conditions, chiefly pH and temp., on gelatinisation of the starch and subsequent breakdown by malt enzymes were studied. The temp. of gelatinisation of sorghum starch is 68—75·5°. Kaffircorn malt is not heated $>$60° in mashing, yet some breakdown of malt starch was observed. Amylases present during heating, attack the raw starch as well as that which has been gelatinised. Grain at pH 3·9—2·8 was gelatinised to the same extent as that at pH 7 ; with a buffered solution gelatinisation was decreased. The effect of buffer was similar to that of a salt. (19 references.) E. M. J.

Growth- and fermentation-limiting factors in continuous fermentation. T.-M. Enari and V. Mäkinen (*Brauwissenschaft*, 1961, **14,** 253—256).—Experiments made in a laboratory continuous fermentation apparatus show atm. O_2 to be a growth-limiting and the concn. of the wort carbohydrates a fermentation-limiting factor. An advantageous feature of the apparatus is an upward-sloping run-off tube from the base of the fermentation-cylinder which is bent back downwards at the level of the fermenting liquor in the cylinder. Comparative analyses show no difference between beer produced by continuous and ordinary fermentation. (11 references.) P. S. Arup.

Problems in connexion with determination of bitter substances in hops and beer. E. Mikschik (*Brauwissenschaft*, 1961, **14,** 272—278).—A review with 39 references. P. S. Arup.

Energy supply in yeast cells under anaerorbic metabolic conditions. W. Nordheim (*Mschr. Brauerei*, 1961, **14,** 71—80).—Various vegetable and animal fats, lecithin, serine and unsaturated fatty acids "catalyse" anaerobic yeast cell proliferation. "Energy rich" compounds (ATP, ADP, phosphocreatine, etc.) and saturated fatty acids do not have this effect. (40 references.) J. V. Russo.

Protein synthesis in yeast. G. Harris (*Brauwissenschaft*, 1961, **14,** 205—209).—A review with special reference to the probable function of the peptidyl nucleotidates as synthetic intermediates. (35 references.) P. S. Arup.

Influence of yeast strain on loss of bittering material during fermentation. J. S. Hough and J. R. Hudson (*J. Inst. Brew.*, 1961, **67** [New Ser. **58**], 241—243).—Iso-humulones in hopped beer worts are adsorbed by yeasts but the amounts adsorbed are not correlated with the amount of yeast present or its nature (degree of flocculence and ability to form yeast head). With some yeasts the amounts adsorbed increase with increasing original gravity of the wort. J. I. M. Jones.

Use of non-sugar substances from waste liquors of citric acid fermentation for cell-substance synthesis of adapted *Torulopsis utilis* and their de- and re-adaptation. H. Leopold, Z. Valtr and H. Svašková (*Brauwissenschaft*, 1961, **14,** 139—144).—De- and re-adaptation of yeast is closely examined. The phenomenon of diauxie is observed during culturing in waste liquor. Yeast is de-adapted after several 6-h. cultures made in rapid succession and will no longer assimilate non-sugar substances. It can be regenerated by passing several times through waste liquor having an increasing content of dry substance and decreasing sugar content. Regeneration can also be effected by passing through waste liquor containing 1% of org. acids (citric, succinic, fumaric or lactic). (30 references.) J. V. Russo.

Effects of different adsorbents on proteins of beer. K. Raible (*Brauwissenschaft*, 1961, **14,** 263—271).—Whilst the N compounds adsorbed by kieselgels are practically limited to the Lundin A-fraction, those adsorbed by bentonites include, in addition, considerable amounts of proteins belonging to the B- and C-fractions. Six samples of kieselgel show considerable differences in quant. adsorptive capacity which are not reflected in the differences observed as regards effects on the $(NH_4)_2SO_4$ pptn. values. The results of the adsorption experiments show no relationship to the widely different colloidal stabilities of several beers. (11 references.) P. S. Arup.

Adsorptive action of kieselgels on beer proteins. K. Raible (*Mschr. Brauerei*, 1961, **14,** 80—84).—Two types of kieselgel (no. 14 and 16) were tested in varying concn. in beer. The beer was subjected to full analysis including cold stability (turbidity) and force test (measurement of turbidity formed after two heat–cold treatments and of that formed after five heat–cold treatments). 100 g./hl. of gel No. 14 produced a very stable beer. (12 references.) J. V. Russo.

Properties of reversible and irreversible turbidities in beers. L. Chapon, B. Chollot and E. Urion (*Brasserei*, 1961, **15,** 42—55).—Reversible turbidity is defined as that which appears on cooling to 0° and disappears on warming to 20° or above, irreversible turbidity does not disappear on warming. Degrees of turbidity are measured with a nephelometer standardised with " formazine." The effects of pH and ionic force on the formation of turbidity are studied. Artificial turbidity is promoted by the addition of tannins (to precipitate protein substances) and polyvinylpyrrolidone (to precipitate polyphenols) and the protein fraction is analysed. The speed and intensity of turbidity formation at different pH with different promoters are measured. J. V. Russo.

Direct quantitative determination of polypeptides in beer. J. Hummel (*Kvasný průmysl*, 1961, **7,** 145—152).—Polarographic methods can be successfully applied for direct determination of polypeptides in beer to be delivered from breweries. The method is based on comparison of a standard sample of beer of the same strength. Using the method quant. changes in polypeptides during salting out with $(NH_4)_2SO_4$ were studied, samples of beer during stabilisation tests and the effect of the diatomite and sorption earth and filtration on the polypeptide content in 8° beer were examined. The Brdička reaction can be used for testing the intensity of colloidal stabilisation, using the reduction of the catalytic wave height expressed in mg.-% cystine, compared with non-stabliised beer of the same strength, as criterion. (22 references.) J. S. B.

Adsorption of chill haze protein from barley, malt, wort and beer by nylon. W. O. S. Meredith (*J. Inst. Brew.*, 1961, **67** [New Ser. **58**], 255—256).—Powdered nylon 66 adsorbs amino-acids, proteins, carbohydrates and anthocyanins from barley and malt extracts, wort and beer. The adsorbed materials are eluted from the nylon with 85% aq. acetone and the presence of the above substances shown by chromatography. The protein adsorbed from barley and malt extracts, wort and beer all prepared from Montcalm barley, when chromatographed after hydrolysis with 6N-HCl showed the same amino-acid pattern as a Dutch Chill Haze II similarly treated. (11 references.) J. I. M. Jones.

Measurement of beer turbidity in EBC formazin turbidity units. W. Kleber and G. Franke (*Brauwissenschaft*, 1961, **14,** 279—281).—In order to improve the accuracy of the measurement in the lower critical range, it is proposed to dilute the stock solution 1 : 100 instead of 1 : 10 for comparative purposes. The results might with advantage be stated in the well-known Helm units as whole no. P. S. Arup.

***Brettanomyces*. I. Occurrence, characteristics and effects on beer flavour.** R. B. Gilliland (*J. Inst. Brew.*, 1961, **67** [New Ser. **58**], 257—261).—The occurrence and characteristics of the yeast genus *Brettanomyces* are reviewed and their presence in beers is investigated. The isolation of four strains from each of 11 bottles of old and/or abnormally flavoured beers, based on the ability of the genus to grow in media containing actidione is described. The 44 strains so obtained were divisible into three groups : (i) 21 strains fermenting glucose, sucrose and maltose only, and identified as *Brett. bruxellensis*, (ii) 11 strains fermenting glucose and galactose only and identified as *Brett. schanderlii*, found in wine but not hitherto reported in beer, and (iii) 12 strains fermenting glucose, galactose, sucrose and lactose, possibly a hitherto unrecognised species. Tasting tests on beers to which cultures were added showed the development of objectionable flavours with ageing. (32 references.) J. I. M. Jones.

Occurrence of phen[-yl-]ethyl alcohol in beer. T. Ayrapaa (*J. Inst. Brew.*, 1961, **67** [New Ser. **58**], 262—266).—A fraction constituting 30—50% of the lipid-sol., neutral, non-volatile substances in beer was found by gas chromatographic analysis to consist of nearly pure phenylethyl alcohol (β-phenylethanol). From 8 to 10 l. of beer, 250 to 400 mg. of neutral, lipid-sol. material was obtained by extraction under specified conditions with $CHCl_3$-methanol 3 : 1. From 9 to 19 mg. of β-phenylethanol were obtained per l. of beer. The substance is not present in wort and arises from the action of the yeast on phenylalanine. Approx. quant. determination on carefully purified extracts may be made by measuring the difference in optical density at 267·5 and 266 mμ. Control experiments showed 52—68% recovery and the amounts found must be corrected on this basis. Duplicate analyses show a mean error of only ±4%. β-phenylethanol contributes to the aroma of beer. (13 references.) J. I. M. Jones.

Diethyl pyrocarbonate, a new preservative for beer? G. Mönch (*Brauwissenschaft*, 1961, **14,** 257—263).—This substance produces an unpleasant flavour in beer when used in concn. sufficient to afford effective bacteriostatic action against spoilage micro-organisms. P. S. Arup.

Non-alcoholic and low-alcoholic beer. F. Hlaváček and G. Klazar (*Kvasný průmysl*, 1961, **7,** 152—155).—Brewing processes recommended for the manufacture of non-alcoholic and low-alcoholic beer are reviewed and various brewing recipes compared and evaluated. Preference is given to the technology based on interrupting the main fermentation or after-fermentation, resulting in obtaining beer with low alcohol content. According to organoleptic tests palatable beer containing 0·35—0·40% alcohol can be produced from 4° wort. J. S. B.

Continuous production of yeast. Distillers Co. Ltd. (Inventor : H. N. Sher) (B.P. 845,315, 3.8.57).—A continuous process for the production of high quality yeast by growth under conditions of aeration in a liquid nutrient medium in at least two fermentation vessels is claimed and the apparatus illustrated. F. R. Basford.

Fruits, Vegetables, etc.

Evaluation of apple pectin of high and low ester content by the Tarr-Baker method. D. Lange, W. Bock and K. Täufel (*Ernährungsforschung*, 1961, **6,** 65—81).—The methods for determining sugar- and water-binding capacities are described and critically examined. The considerable variations in acidity and buffering capacities of different samples necessitate the carrying out of a series of test-boilings with different amounts of acid (or acid and Ca^{2+}) to determine the optimum pH for gelatinisation. The same principle applies to the determination of the optimum concn. of pectin ; these cannot always be determined by interpolation between two points. The time for test-boilings should be 8 min., additions of acid being made as late as possible. The test-gels may be firmed (during 24 h.) at room temp. (15—30°). (20 references.) P. S. Arup.

Determination of residues of 3-amino-1,2,4-triazole in cranberries. E. Kröller (*Dtsch. LebensmittRdsch.*, 1961, **57,** 107—114).—The properties and analytical chemistry of 3-amino-1,2,4-triazole (**I**) are reviewed. In the proposed method **I** is extracted from the cranberries with methanol and the extract is boiled with Na dithionate, cooled and passed through a column of strongly acidic ion-exchange resin, and the eluate rejected. **I** is eluted from the column with 2N-aq. NH_3 ; the eluate is concentrated, decolorised by passing through a column of activated alumina ; and **I** is determined colorimetrically by reaction with chromotropic acid, with β-naphthylamine or *N*-ethyl-*N*-benzylaniline-3-sulphonic acid, or with " H-acid." (14 references.) E. C. Apling.

Production of high moisture raisins. M. W. Miller and C. D. Fisher (*Food Technol.*, 1961, **15,** 276—279).—The factors studied were : medium and time of rehydration and resulting loss of sol. solids. With rehydration by immersion in hot water raisins began to show excess water or " syrup " when rehydrated to ~21—22% moisture. Syrup increased with storage temp. and moisture content. Raisins rehydrated in atm. of high R.H. retained the " bloom." E. M. J.

Potassium, calcium and magnesium in the nutrition of pineapples in Guinea. III. Influence on the quality of fruit. P. Martin-Prével, R. Huet and L. Haendler (*Fruits d'outre mer*, 1961, **16,** 161—180).—The fruits from pineapple plants treated with varying amounts of Mg, Ca and K (*Fruits d'outre mer*, 1961, **16,** 49—56 and 113—121) were harvested, and a sample of 50 fruits from each parcel was tested and assessed for size, shape, colour of the skin, dia. of the core, texture, coloration of the flesh, number of cavities in the fruit, amount of juice, dry matter, acidity, smell, taste and the relationship between taste, dry matter and acidity. The types of fruit preferred by the public for dessert and by the manufacturers for jams, juice, crush and canned slices and cubes are discussed. The effects of various fertiliser treatments on the above factors are recorded. With careful use of fertilisers it is never necessary to sacrifice quality to quantity. S. G. Ayerst.

Magnitude and origins of storage losses in Nigerian yams. D. G. Coursey (*J. Sci. Fd Agric.*, 1961, **12,** 574—580).—Very serious losses occur during storage of yam tubers (*Dioscorea* spp.) under normal Nigerian conditions, the losses being greatly increased by rotting of the tubers, while sprouting has comparatively little effect. *D. rotundata* stores somewhat better than *D. cayenensis*. Wt. loss is caused by loss of moisture in drying, and loss by respiration, probably as CO_2. Fungicidal treatments had some beneficial effects for storage periods of $\ngtr 3$ months, in the case of *D. rotundata*. E. M. J.

Biochemical changes in shelled groundnuts caused by storage fungi. I. Effects of *Aspergillus tamarii*, four species of the *A. glaucus* group and *Penicillium citrinum*. H. S. Ward, jun., and U. L Diener (*Phytopathology*, 1961, **51**, 244—250).—Deterioration of groundnuts caused by the fungi was largely due to hydrolytic rancidity of the oil, a dark reddish-coloured oil having a mouldy acid odour being formed. There was little change in peroxide value, total carbonyls, total tocopherol or protein N of the nuts. The relative activity of the fungi in causing the deterioration was, *A. tamarii* > *A. glaucus* group > *P. citrinum*. A. G. POLLARD.

Modified Gibb method for determination of 1 p.p.m. or less of o-phenylphenol in fruits. L. R. Leinbach and J. E. Brekke (*J. agric. Fd Chem.*, 1961, **9**, 205—206).—The efficiency of the recovery of the preservative by steam-distillation from homogenised fruits or from orange juice is improved by the use of the Cash apparatus (with a widened still-head joint) in which the tube containing the sample is heated by immersion in the boiling water in the steam generator Recoveries of the preservative are very considerably improved by steam-distillation in the presence of $CaCO_3$. Recoveries of 85—100% can be obtained by collecting 2 or 3 × 85 ml. of distillate from 100 g. of the sample. (10 references.) P. S. ARUP.

[A] **Effect of insecticides and fungicides on flavour quality of fruits and vegetables.** E. F. Murphy, A. M. Briant, M. L. Dodds, I. S. Fagerson, M. E. Kirkpatrick and R. C. Wiley. [B] **Influence of herbicides on flavour of processed fruits and vegetables.** F. J. McArdle, A. N. Maretzkl, R. C. Wiley and M. G. Modrey (*J. agric. Fd Chem.*, 1961, **9**, 214—223, 228—230).—[A] A reference summary is given of the results of collaborative evaluations of 23 crops treated with 47 pesticides, singly and in combination, at seven experimental stations and at the U.S. Dep. of Agric. Lindane, BHC, toxaphene, endrin, malathion and PCNB (Terrachlor) are particularly prone to the production of off flavours. Chlordane, DDT, dibrom, dilan, Dimethoate, heptachlor, phosphamidon, Sevin, Thiodan and Trithion are generally free from objectionable results. The results are graded on a five-point scale ranging from " better " down to " definite off flavour."

[B] A summary is given of collaborative tests on 28 herbicides made at the Universities of Pennsylvania and Maryland. The use of 17 of the herbicides at the suggested rates fails to reduce the scores of eight frozen or canned crops. Reductions in scores caused by the remaining 11 herbicides are generally slight or very slight, and do not fall below the grade " slightly off flavour." P. S. ARUP.

Autoxidation of potato granules. I. Changes in fatty acids. R. G. Buttery, C. E. Hendel and M. M. Boggs. **II. Formation of carbonyls and hydrocarbons.** R. G. Buttery (*J. agric. Fd Chem.*, 1961, **9**, 245—248, 248—252).—I. Gas-liquid chromatography of the Me esters of the fatty acids contained (free or as glycerides) in the dehydrated granules reveals linolenic, linoleic, palmitic and stearic acids as the main constituents ; small amounts or traces of other acids (8) are also found. The ratio linoleic + linolenic acid/stearic and palmitic acid decreases with length of storage under air ; the decrease is accelerated by storage under O_2. The decrease is correlated with the vol. of O_2 absorbed and deterioration in flavour.

II. The most volatile of the compounds formed by passing O_2 over the granules and those formed in the headspace of tins during subsequent storage under air are collected in a dry ice trap. The other volatile compounds are obtained by steam-distillation. Gas–liquid chromatography and paper-chromatography of the dinitrophenylhydrazones of the carbonyl compounds are used for the identification of the volatile compounds ; the chief of these include CH_4, C_2H_6, C_3H_8, C_4H_{10}, C_5H_{12}, acetaldehyde, propanal, 2-methylpropanal, pentanal, 2- and 3-methylbutanal and hexanal. (26 references.) P. S. ARUP.

Problems in pneumatic drying of potato starch. J. Valchář (*Prům. potravin*, 1961, **12**, 362—365). J. S. B.

Effect of residual sulphur dioxide on the quality of pre-peeled potatoes. F. J. Francis and B. L. Amla (*Amer. Potato J.*, 1961, **38**, 89—94).—Potato strips dipped in $NaHSO_3$ of varying concn. (SO_2, 625—5000 p.p.m.) showed increasing residual SO_2 with increasing concn. of solution but not with time of dip (2—10 min.). Potatoes peeled by hand showed much lower residues of SO_2 than did abrasion-peeled samples. SO_2 residues decreased with increasing pH of the dipping solution (4—10). SO_3^{2-} flavour could be detected in cooked mashed potatoes prepared from those containing SO_2, 115 p.p.m., but not from those containing 87 p.p.m. A. H. CORNFIELD.

Vitamin stability in fortified potato flakes. J. Cording, jun., R. K. Eskew, G. J. Salinard and J. F. Sullivan (*Food Technol.*, 1961, **15**, 279—282).—Retention of vitamins in the potato-flake process by pilot plant was tested during drying and subsequent storage. Retention of natural and added vitamin C during processing was 71—73%. On storage for 28 weeks at 75°F in an air pack, flakes with 5% moisture, containing antioxidant, retained 70—76% and in a N_2 pack substantially all was retained that survived processing. Based on retention, fortification with vitamins A, B_2 and niacin seems practical. (10 references.) E. M. J.

Composition and properties of dried peas in relation to their suitability for canning. Lakshminarayana Setty and G. S. Siddappa (*J. Sci. Fd Agric.*, 1961, **12**, 537—541).—Typical wrinkled-seeded varieties, e.g., W.K. Marrowfat and E.W. Marrowfat, having a high water-absorption capacity are good for canning. They have a low starch : protein ratio, low alcohol-insol. solids (A.I.S.) and a high sugar content. The starch is chiefly of the amylose type. Smooth-seeded varieties, e.g., R.O. Dutch Blue and S.O. Dutch Blue, have low water-absorption capacity, high swelling ratio on processing and give a mealy canned product. They have a high starch : protein ratio, high A.I.S. content and low % of sugar. The starch is of amylose and amylopectin types. (16 references.) E. M. J.

Effect of added sugar on consumer acceptance of canned peas. K. G. Weckel, W. D. Mathias, G. F. Garnatz and M. Lyle (*Food Technol.*, 1961, **15**, 241—242).—Within the range 3·1—8·7% of added sugar in the brine of canned peas, the level 8·7% was preferred significantly and the next-highest level 7·35% was preferred. Added sugar increased total solids and alcohol-insol. solids slightly, gave a technically significant increase in drained wt., and an apparent reduction in Hunter L values. E. M. J.

Use of Kramer shear-press in forecasting harvest dates for Fordhook lima beans. H. J. Neumann, L. R. Frame, L. Morgan and R. L. Olson (*Food Technol.*, 1961, **15**, 225—228).—With representative sampling and use under specific conditions shear-press values supply useful added information on relative growth rates of the various fields, especially in the all-green stage, a few days before harvest. (14 references.) E. M. J.

Stability of chlorophyll in vegetables as affected by pH. J. P. Sweeney and M. E. Martin (*Food Technol.*, 1961, **15**, 263—266).—Destruction of chlorophyll *a* was the principal factor responsible for loss of colour in cooked green vegetables. The pH range 6 to 7 was critical. In cooking frozen green beans, by use of citrate–phosphate buffers, pH 6·2—7·0, progressive increases in colour were obtained with increase in pH. Above pH 7·0 flavour deteriorated. (15 references.) E. M. J.

Media for enumerating enterococci on frozen vegetables. D. F. Splittstoesser, R. Wright and G. J. Hucker (*Appl. Microbiol.*, 1961, **9**, 303—308).—Azide dextrose broth yielded the highest no. of confirmable enterococci and the sensitivity was comparable with that of non-selective media. Ethyl violet azide broth resulted in a no. of false positive tests. Growth in a broth medium containing 6·5% NaCl, incubated at 45° for two days was a more specific confirmatory test for enterococci. (13 references.) C. V.

Jellies. Alginate Industries Ltd. (Inventor : R. H. McDowell) (B.P. 845,024, 10.4.57).—A hot aq. solution containing alkali metal alginate is admixed with a hot aq. solution containing tartaric ,citric or lactic acid, an alkali metal salt of such an acid (to prevent gelling while hot) and a Ca salt of the acid (to ensure gelling on cooling), then the hot mixture is cooled, to produce a jelly. More specifically the final composition contains Na alginate (0·4—2 g. per 100 c.c.), Ca citrate (0·2—1·5 times the chemical equiv. of the alginate), Na citrate (1—10 times the chemical equiv. of the Ca citrate), and citric acid (0·5—2 times the chemical equiv. of the Na citrate). F. R. BASFORD.

Improved gelatin composition. General Foods Corp. (B.P. 845,274, 27.9.56. U.S., 6.10.55).—A dry, cold-water-sol. gelatin, non-clumping composition, suitable for use in compounding of table-jelly mixes, is produced by dissolving a dispersant in a gelatin solution, then drying the solution from a sol state without allowing the gelatin to pass through a gelled state. The dispersant may be a silicone, a high-mol. polyethylene glycol, a polyoxyethylene derivative of a fatty acid ester of sorbitan, a polyoxyethylene derivative of sorbitan, or an ester of a polyhydric alcohol and a fatty acid. F. R. BASFORD.

Non-alcoholic beverages

Gelation in concentrated orange juices. E. Primo Yúfera, J. Koen Mosse and J. Royo Iranzo (*Rev. Cienc. apl.*, 1961, **15**, 126—130).—Changes in the proportions of the different types of pectin in the pulp and in the liquid fractions of orange juice, on concentration and on ageing, and the effect of pasteurisation on the changes have been examined. L. A. O'NEILL.

New results in science and technology of fruit juices. (*Int. Fed. Fruit Juice Producers. Proc. sci. tech. Comm. III. Wageningen* [*Holland*], 1961, 123 pp.). **Reactions of sulphur dioxide in juices and**

fermented products. A. Pollard pp. 7—15.—Fermented cider in the U.K. may contain 200 p.p.m. of SO_2. The amount required to kill 50% of a culture of *Saccharomyces uvarum* within 6 h. at pH 3·0 was 18 p.p.m. of free SO_2 or 1·0 p.p.m. of H_2SO_3 equiv. To keep total SO_2 as low as possible, bound SO_2 should be at a min. At a total SO_2 content of 192 p.p.m. glucose solution (6%) binds 64 p.p.m.; acetaldehyde solution (0·0132%) binds 190 p.p.m. Pyruvic acid can also bind a major part of the SO_2 present. Up to 75% of dehydroascorbic acid is converted into L-xylosone (**I**) in presence of SO_2 and **I** in the finished product, indicates the original ascorbic acid content.

Gelification in concentrated orange juices; IV. Enzymic action in pulp suspensions and its influence on the pectin content and clarification. E. Primo Yúfera, J. Koen Mosse and J. Royo Iranzo pp. 17—31.—Gelification in conc. orange juice is influenced by the degree of concentration, proportion of pulp, total pectin and pectin with low methoxyl content (**I**), **I** being the most effective. Most of the pectins and enzymic activity are in the pulp. The evolution of the pectin fractions in the isolated pulp, re-suspended in water and in synthetic juices containing sugar, citric acid and Ca, was studied. The presence of protopectinase was demonstrated.

Content of metal in fruit juices. A. Kern pp. 33—50.—The unfavourable action of Cu, Pb and Zn in fruit juices and occurrence of these metals in drinking water are discussed. In the industrial processing of fruit juices contamination by the equipment may increase metallic content. The limiting amounts allowed by U.K., Canada and Switzerland are discussed. The Commission recommends as max. limits for all fruit juices, in mg./kg., Cu 5, Fe 10, Pb 0·5, Zn 5, metals calculated as Fe precipitated by $K_4Fe(CN)_6$ 12, and that the metal content is determined after dilution to: apple and pear juices 11·0° Brix, grape juice 16·0° and citrus fruit juices 11·5°. (25 references.)

Judgement of quality of aroma concentrates and aroma distillates of fruit juices. A. Mehlitz and K. Gierschner pp. 51—62.—The aroma concentrate (0·5 l.) is extracted with isopentane (300 ml.) dried over Na_2SO_4 in presence of 1—2 ml. of N_2. The residual aroma oil is analysed by gas chromatography. Nine compounds of an aroma concentrate of apples have been tentatively identified as: acetone, n-butyraldehyde and ethyl acetate, ethyl methyl ketone, isobutyl alcohol, n-butyl alcohol, n-ethyl butyrate, amyl alcohol, amyl acetate. The value of gas chromatography as a method of analysis is discussed. (10 references.)

Determination of aroma in fruit juices and fruit juice concentrates with a quick method. M. Jensen pp. 63—68.—An apparatus is described in which steam from boiling water, bubbles through the test material (**I**) driving off the volatile substances. Dilution of **I** by condensed steam is avoided; the distillation proceeds for 20 min. and $\sim$100 ml. will have been distilled, a quantity which contains most of the volatile substances in apple juice. An aliquot is oxidised with 0·2N-$K_2Cr_2O_7$, 0·5 g. of KI is added and the mixture titrated with thiosulphate.

A simple method for measuring colour in apple juices and apple-juice concentrates. H. Luthi and H. Schroeter pp. 69—85.—The colour tints of apple juices and concentrates of increasing density derived from them are defined from the physical viewpoint and characterised according to international standards. A scale of 25 colour tints which change regularly has been established. With standardised coloured glasses arranged in three colour disks the tints are measured in the "Neo-Comparateur," Hellige's system. (10 references.)

Measure of the colour of orange juice. P. Dupaigne pp. 87—95.—Maxwell's method of spinning coloured disks and easily obtainable commercial colours of Lefranc: dark Cd yellow, Persian lemon yellow and Prussian blue, were used; the three coloured disks were varnished, the fourth black disk was unvarnished. The scale of concentration is given. The yellow disappears progressively, while browning of the product is shown by increase in the % of black. (10 references.)

Deaeration, pasteurisation and concentration of fruit juices with the "Centri-therm" heat exchanger. M. Mautner pp. 97—115.—The process and apparatus are described. Fruit juices can be treated at 100° without spoiling quality. Pulpy juices containing a large quantity of pectins can be evaporated to highest *d* without loss of quality. Vitamin content, colour, flavour and structure of conc. juices undergo only very small changes. (13 references.)

Heat transmission in evaporators. J. Veldstra pp. 117—125.—As a measure of total heat transmission (*a*) from the heating medium to the wall, (*b*) through the wall and (*c*) from the wall to the liquid under test, the coeff. k is used which rises with increase of the boiling temp. and decreases with increase of η and with an increasing surface tension. The influence of difference in temp. between the heating steam and the boiling liquid on the k value depends mainly on the type of evaporator.

Utilisation of dried fruits. P. Dupaigne pp. 127—137.—Trials are made with: (*a*) dried prunes + a little conc. grape juice and crushed to a pulp; this may be used as jam; the prep. of a prune juice. (*b*) Apricots: infusion of dried apricots gives sweet liquids. (*c*) Grapes: Greek raisins with small berries without pips give a clear light yellow juice with sweet taste. (*d*) Figs. (*e*) Bananas and (*f*) dates after drying can be used in prep. of beverages with pleasant tastes. (15 references.)

Blackcurrant, development of cultivation. Growing importance for sweet must as a health beverage. J. Baumann pp. 139—145.—The development of best quality blackcurrant bushes in Western Germany, especially in the last 10 years, is reviewed. The cultivation of blackcurrants with >190 mg.-% of vitamin C is recommended. Blackcurrant beverages should have acidity of 10 g./l. calculated as tartaric acid. Good quality blackcurrants contain at least 50 g./l. of acids and high content of vitamin C.

Preservation of "moscatel" grape juice with sodium bisulphite, sodium benzoate and potassium sorbate at their threshold concentrations. B. Lafuente, E. Hernandez, P. Cuñat and E. Primo pp. 151—166.—Organoleptic threshold concentrations of the three substances were 34 p.p.m. (as SO_2), 222 p.p.m. and 463 p.p.m. respectively. With K sorbate (463 p.p.m.) good control of micro-organisms was obtained for 9 months at 3—5°. When all three substances were added at threshold level good control was effected for 9 months at $\ngtr 10°$.

Preservation of wine and fruit juices with diethyl pyrocarbonate. H. Luthi, K. Mayer and E. Hotz pp. 167—179.—The use of diethyl pyrocarbonate for preserving unfermented fruit juices even after reduction of a large no. of micro-organisms is unsatisfactory. With regard to wines with residual sugar, a final decision cannot at present be given.
E. M. J.

Tea, coffee, cocoa

Oxidation of *l*-epicatechin gallate during the processing of Assam tea leaf. [A] I. S. Bhatia and Md. R. Ullah, [B] E. A. H. Roberts (*Chem. & Ind.*, 1961, 1169, 1170).—[A] The test material (unprocessed dried green tea leaf or black tea) was extracted with methanolic HCl and run, after concentration, on a two-way chromatogram. The spots (detected under u.v. light after exposure to NH_3 vapour) were eluted with a known amount of water and measured at 275 mμ. The results indicate that appreciable amounts of *l*-epicatechin gallate, in addition to *l*-epigallocatechin and *l*-epigallocatechin gallate, are involved in the fermentation process.
E. BRIMACOMBE

[B] The implications of the findings in [A] are discussed.
H. S. R.

Acidification of the fat in the raw coffee grain with time. A. Baião Esteves (*Estud. agron. Lisboa*, 1960, **1**, 297—317).—The acidity of the fat extracted from various types of coffee (dry and wet process) was measured over storage periods of 1 to 2 years and the results were examined statistically. (15 references.)
L. A. O'NEILL.

Chlorogenic acids in coffee beans and their alteration on roasting. III. R. Krasemann (*Arch. Pharm., Berl.*, 1961, **294**, 266—273).—Chlorogenic acids in raw and roasted coffee are extracted into 70% ethanol, separated by paper chromatography and determined by eluting the zones (detected by fluorescence under u.v. light) and measurement of the absorption at 324 mμ. On heating pure chlorogenic acid (Freudenberg, *Ber. dtsch. chem. Ges.*, 1920, **53**, 232), six chlorogenic acids were formed, with R_F characteristics identical with those of fractions present in extracts of roasted coffee. Variations in amounts of the various chlorogenic acids formed with the period of roasting are discussed.
A. G. COOPER.

Coffee extract in the form of a dry powder. E. Griffiths Hughes Ltd. (B.P. 844,514, 20.5.58. U.S., 10.4.58).—A process for the production of a dry coffee extract in powder form comprises making from ground, roasted whole beans an aq. infusion (containing flavour and aroma), adding and homogenising with a non-fatty, low-boiling org. solvent (e.g., light petroleum b.p. 36—45°, chloroform or dichloroethylene, 2—28 vol.-%); then desiccating the mixture, e.g., at 65—85° by spray-drying.
F. R. BASFORD.

Milk, Dairy Products, Eggs

Milk phosphatases. III. Influence of temperature on activity of acid milk phosphatase. F. Kiermeier and E. Meinl (*Z. Lebensmitt-Untersuch.*, 1961, **114**, 407—416; cf. J.S.F.A. Abstr., 1961, ii, 126).—The activity is reduced by $\sim$5—10% after heating the milk at 62—66° during 10—40 sec. About 10% of the activity persists after heating at 95° during 100 sec. A linear relationship between the temp. and the log of the holding time is restricted to the range showing 30—70% inactivation. No activity is found in sterilised or condensed milk, but a slight residual activity is readily detectable in uperised milk. (29 references.) P. S. Arup.

Calcium-binding capacity of peptide fractions of enzymic casein hydrolysates. J. Schormüller and R. Fresenius (*Z. Lebensmitt-Untersuch.*, 1961, **114**, 397—406).—An improved form of the murexide spectrophotometric method is used for the determination of Ca in combination with peptides and phosphoproteins. A direct relationship is found between the Ca-binding capacity and the phosphate-ester content of the tryptic hydrolysates of casein, phosphovitin (from egg-yolk) and a phosphopeptone. A reduction in the binding capacity accompanies the dephosphorylisation (by a prep. of wheat phosphatase of low proteolytic activity) of phosphovitin. In the phosphoprotein, the binding of Ca by the free carboxyl groups occurs subsequently to the saturation of the phosphate-ester groups. The binding capacity of the phosphopeptone phosphate groups is greater than that of those of the other org. phosphate groups, including also that of inorg. phosphate. (29 references.) P. S. Arup.

Chromatography of milk proteins on anion-exchange cellulose. M. Yaguchi, N. P. Tarassuk and H. G. Hunziker (*J. Dairy Sci.*, 1961, **44**, 589—606).—Fractionation of milk protein from a dialysed sample was effected by adsorption on an anion-exchange cellulose column (DEAE-SF) and elution by varying concn. of NaCl in a PO_4^{3-} buffer (pH 6·6—7·0) using either the gradient or the stepwise procedure. Whey proteins (8) were eluted by 0—2M-NaCl and casein components (10 or 11) by concn. >2M, there being a clean-cut separation of the two groups. A. G. Pollard.

High-temperature short-time sterilised evaporated milk. III. Influence of the lipid phase on heat and storage stability. A. Leviton and M. J. Pallansch (*J. Dairy Sci.*, 1961, **44**, 633—643).—In some sterilised evaporated milks stability to heat and storage was impaired by the presence of lipids. Three types of milk were distinguished on this basis. The relative heat-stabilities of a stable and a labile milk were partly reversed by exchanging the fat globules. Replacement of milk protein by an equal wt. of fat lowered the stability to both heat and storage. Milks liable to coagulation by an unstable lipid phase showed increased stability after fore-warming. The heat- and storage-stabilities of mixtures of fore-warmed and non-fore-warmed samples were intermediate between those of the two original samples. Reconstituted milks were stabilised by adding fat-sol., interfacially-active material to the butter oil. A. G. Pollard.

Autoxidation of milk lipids. II. Relationship of sensory to chemical methods for measuring the oxidised flavour of milk fats. D. A. Lillard and E. A. Day (*J. Dairy Sci.*, 1961, **44**, 623—632).—Parallel tests by chemical means and by the flavour threshold of milk samples previously oxidised to different extents are recorded. Close correlation was established between the thiobarbituric acid no., peroxide value, total saturated and unsaturated carbonyls, volatile saturated and unsaturated carbonyls. The non-volatile carbonyls comprised 97—99% of the total carbonyl-reactive matter in the fats. Significant correlation coeff. exist between the chemical tests and the reciprocal of the flavour threshold. A. G. Pollard.

Trisaturated glycerides of milk fat. C. Boatman, A. E. Decoteau and E. G. Hammond (*J. Dairy Sci.*, 1961, **44**, 644—651).—The content of trisaturated glycerides (*TG*) in milk fat was 21·5—32·0%. The relation between the amount and the m.p. of the *TG* occurring in many other fats does not apply to butter fat. A. G. Pollard.

Quantitative fatty acid analysis of milk fat by gas–liquid chromatography. L. M. Smith (*J. Dairy Sci.*, 1961, **44**, 607—622).—The method is based on the prep. of the Me esters of the fatty acids which are dissolved in EtCl and separated by gas–liquid chromatography using diethylene glycol succinate as the liquid phase. All the principal and many of the minor acids were thus determined. A. G. Pollard.

Structural components of milk triglycerides. H. J. Ast and R. J. Vander Wal (*J. Amer. Oil Chem. Soc.*, 1961, **38**, 67—69).—The glyceride composition of winter butter fat is determined after hydrolysis with pancreatic lipase by gas–liquid chromatography. The results indicate that the fatty acid groupings are not randomly distributed throughout the glycerol C-atoms. When they are considered only on the basis of saturated and unsaturated fatty acids, they appear to be randomly distributed intermolecularly, but tend to assume specific positions intramolecularly. G. R. Whalley.

Method for extracting fat from whole milk powder. S. J. Emery and D. P. Schwartz (*J. Dairy Sci.*, 1961, **44**, 721—722).—The powder is extracted first with MeOH and then (twice) with hexane. After removal of the solvents the residue is dissolved in methylene chloride and the solution dried (anhyd. Na_2SO_4) and filtered into a tared vessel. The solvent is removed and the fat dried to constant wt. A. G. Pollard.

Complexometric determination of calcium and magnesium in milk. J. Mašek (*Prům. potravin*, 1961, **12**, 384—385).—Rapid method and procedure of complexometric determination of Ca and Mg in milk, using methylthymol blue as the indicator, are described. J. S. B.

Volumetric microdetermination of potassium with tetraphenyl boron. II. Determination of potassium in milks. F. Bermejo Martínez and A. Margalet Barral (*Inf. Quím. anal.*, 1961, **15**, 36—40).—The milk is treated with aq. Na_2WO_4 and H_2SO_4 to remove proteins, which are separated by centrifuging. The supernatant liquid is treated at pH 9 with a small amount of CH_2O and a known amount of standard $NaBPh_4$ solution. The ppt. is filtered off and the excess $NaBPh_4$ measured by titration with a standard solution of a quaternary ammonium salt (cetyldimethylbenzylammonium chloride) using bromophenol blue indicator. L. A. O'Neill.

Lactoflavin in cows' milk. F. Kiermeier and K. Haisch (*Z. LebensmittUntersuch.*, 1961, **114**, 417—421).—A decrease of 0·7—0·23 mg. per 100 ml. is observed in the colostrum of one cow during the first 12 milking periods ; the main decrease occurs during the first three periods. Published data show very wide variations in the lactoflavin content of milk. Some feeding trials demonstrate the sensitivity of the content to the nature of the feed. (42 references.) P. S. Arup.

Co-vitamin studies. IV. Interrelationship between vitamin A, carotene and tocopherol contents in cow colostrum. K. M. Narayanan and C. P. Anantakrishnan (*Indian J. Dairy Sci.*, 1960, **13**, 179—185).—The colostrum of 171 crossbred, Red Sindhi and Gir cows was analysed by methods previously reported (Narayanan and Anantakrishnan, *Indian J. Dairy Sci.*, 1959, **12**, 68 ; J.S.F.A. Abstr., 1960, ii, 35). The length of the dry period had no effect on the vitamin A, carotene and tocopherol contents of colostrum. There was strong correlation between the vitamin A, carotene and tocopherol contents, but none between these substances and the fat %. There seems to be no consistency in the yield, fat %, vitamin A and carotene values from the same animals in successive calvings. S. G. Ayerst.

A pigment-producing spoilage bacterium responsible for violet discoloration of refrigerated market milk and cream. E. W. Seitz, P. R. Elliker and W. E. Sandine (*Appl. Microbiol.*, 1961, **9**, 287—290).—A psychrophilic strain of bacteria, identified as *Chromobacterium lividum*, was the causative agent ; it was rod-shaped and Gram negative. Since the organism was destroyed by exposure to 56° for 5 min. post-pasteurisation contamination had occurred. The characteristics of this organism and the other chromobacteria are discussed. C. V.

Sweet foods based on gelatinised milk (definition, regulations for, analysis). J. Pien (*Ann. Falsif., Paris*, 1960, **53**, 391—399).—Legal requirements for fermented milks (yoghurts), milk products with sucrose, flavoured, e.g., with chocolate or caramel and coagulated by rennet, and milk products containing vegetable thickeners are discussed in detail. J. V. Russo.

Stability of tocopherol in ghee during storage. K. M. Narayanan and C. P. Anantakrishnan (*Indian J. Dairy Sci.*, 1960, **13**, 186—189).—Milk samples were taken from cows and buffaloes at five stages of lactation and ghee was prepared. Peroxide value and tocopherol contents were determined initially and after 2, 4 and 6 months. The stage of lactation had no effect on the storage of the ghee with reference to stability of tocopherol and peroxide development. The initial tocopherol content of the ghee was influenced by the level of green fodder fed to the animals, but the storage properties were not affected. S. G. Ayerst.

Starter metabolism. V. Mechanism of acetoin formation as determined with ^{14}C-labelled substrates. W. G. Mizuno and J. J. Jezeski (*J. Dairy Sci.*, 1961, **44**, 579—588).—A starter culture consisting mainly of *Strep. cremoris* and *Leuconostoc* spp. transferred C from the 2-, 3-, 4-, or 6-position but not from the 1- or 5-position of citric acid to the acetoin mol. (**I**). When uniformly-labelled glucose was added to a medium containing non-labelled citrate or pyruvate or when uniformly labelled citric acid was added to a medium containing unlabelled glucose the resulting **I** was labelled. The Me- and α-keto but not the carboxyl-C of pyruvic acid were transferred to **I**. Carboxyl-C appeared as CO_2. The mechanism of **I** formation is discussed. A. G. Pollard

Standardisation of starters for butter and cheese production. E. Voss (*Milchwissenschaft*, 1961, **16**, 60—67).—Using a freeze-dried starter a culture was used for 6—7 months ; it was maintained at 6—8°. Activity was assessed by rate of souring, production of diacetyl and protein titre. C. V.

Determining sugars in cheese and milk products by electrophoresis. V. Palo (*Prům. potravin*, 1961, **12**, 385—387).—A simple rapid method for determination of sugars in cheese and milk products by paper electrophoresis is described. Complete partition of galactose, lactose, glucose and sucrose, using potential gradient 8 V/cm. and borate buffer of pH 9·2, takes 4—5 h.; partition of sucrose from lactose in sweetened products takes 30—40 min. J. S. B.

Lipolytic fat hydrolysis (rancidity) in milk and cheese. M. Bachmann (*Promotionsarb., Zürich*, 1960, No. 3043, 71 pp.).—The term rancidity is limited to the enzymic hydrolysis of the glycerides of fatty acids. Conventional methods for the determination of lipolytic activity in normal milk and in milk becoming rancid spontaneously were unsatisfactory. The titration of the free higher mol. fatty acids gave an accurate and reliable determination of the rancidity. A simple and quick chromatographic method was developed to assay the total amount of free fatty acids containing >5 C atoms. The same procedure could be used for estimation of rancidity in ripening cheese. Two kinds of spontaneous rancidity were observed, one in the milk of pregnant cows and one in the milk of animals with a disturbed hormone system. This second kind of spontaneous rancidity could be artificially caused by injection of oestrogenic hormones. ($\sim$140 references.) M. Sulzbacher.

Dichlorofluorescein and potassium chromate as indicators in a titration test for salt in cheese. W. M. Breene and W. V. Price (*J. Dairy Sci.*, 1961, **44,** 722—725).—The Volhard method as modified by the American Dairy Science Association (*J. Dairy Sci.*, 1937, **20,** 27) and the direct titration method using K_2CrO_4 or dichlorofluorescein (**I**) are compared. The end-point given by **I** is sharper than that by K_2CrO_4 and gives results more closely correlated with the A.S.D.A. than with the Volhard method. A. G. Pollard.

Penicillin in milk used for cottage cheese making. Kenneth Leroy Smith (*Dissert. Abstr.*, 1961, **21,** 2437—2438).—Concn. of penicillin tolerated by streptococci (45) isolated from commercial cultures are $<$0·1—0·3 units per ml. Individual tolerances are subject to large daily variations. Manufacturing operations are progressively impeded by concn. of 0·05—0·15 units per ml. of separated milk. Scalding temp. of 49—55° (during 2 h.) fail to destroy completely, even small concn. of penicillin. When whey is used for diluting the penicillin standard, the size of the inhibition zone increases with decreasing pH of the whey; this relationship is not observed when water is used as the diluent. P. S. Arup.

Cooling and crystallisation of plastic materials in the chemical and food industry. K. F. Leopold (*Kältetechnik*, 1961, **13,** 188—193).—A plant for the production and processing of butter and similar materials, the construction of which is the result of close co-operation between refrigeration and processing engineers, viscous liquids and plastic masses being cooled and crystallised by means of twin tubes cooled externally, a screw conveyor transporting the material through the annular space, is described. The new pressure cooler used makes the Alfa-Butter process a practical one; there is no manual contact with the material, and the fat content of the buttermilk is 0·02% max. There is also a considerable economy of space. W. H. Kemp.

Edible Oils and Fats

Refining of oils and fats. S. A. Kahn, M. K. Bhatty and Karimullah (*Pakist. J. Sci.*, 1960, **12,** 103—106).—A brief general review. (31 references.) C. V.

Heated fats. I. Effects of heating on the chemical nature of cottonseed oil. D. Firestone, W. Horwitz, L. Friedman and G. M. Shue (*J. Amer. Oil Chem. Soc.*, 1961, **38,** 253—257).—When cottonseed oil is heated in air at 225° for 7—8 h. per day for about 40 days, the non-urea adduct-forming mono- and dimers (of linoleic acid) so formed are toxic when fed to rats. Such fractions contain both carbonyl and hydroxy groups together with unsaturated moieties which are difficult to remove by hydrogenation. Some cyclic products seem to also be present in the dimer fraction. G. R. Whalley.

Application of ultra-violet spectrophotometric analysis in the oil and fat field. N. Costanzo (*Rev. argent. Grasas y Aceites*, 1960, **3,** 84—86).—The use of spectrophotometry for the control of olive and other edible oils is reviewed. (24 references.) L. A. O'Neill.

Characterisation of the methyl esters of higher saturated fatty acids by infra-red spectroscopy and gas chromatography. U. Pallotta (*Riv. ital. Sostanze grasse*, 1961, **38,** 191—197).—The normal saturated even no. 18—26 C fatty acids may be differentiated by i.r. spectra of their Me esters, determined in a paraffin oil mull, particularly from the no. of bands in the 7·5—8·5 μ region, which is equal to half the no. of C atoms in the chain. The 20—26-C acids also differ from stearic acid by having a third band in the 8·26—8·5μ region Some identification is possible of binary mixtures, but with more complex mixtures a preliminary fractional crystallisation is necessary to reduce the components to $\ngtr 2$. The use of the technique for examining adulteration of olive oils is described. The gas chromatographic separation of the Me esters of the 12—24-C acids is reported. L. A. O'Neill.

Concurrent oxidation of cholesterol and corn [maize] oil sterols in autoxidising lipid films. L. N. Norcia (*J. Amer. Oil Chem. Soc.*, 1961, **38,** 238—240).—The atm. oxidation of maize oil sterols and cholesterol films in maize oil or their fatty acids has been determined over 3 monthly periods at room temp. or over 2 months at 60° in the dark; and measuring cholesterol losses by the method of Sperry-Webb (*J. biol. Chem.*, 1950, **187,** 97). Under these conditions cholesterol is quite stable, but when the films become liquefied it is readily decomposed, the rate of which appears to be related to the stability of the film to autoxidation. G. R. Whalley.

Fractionation of compound lipids by dialyis in an organic solvent against an organic solvent. Sen-itiroh Hakomori and Koh-kiti Takeda (*Nature, Lond.*, 1961, **190,** 265—266).—The dialysability of various compound lipids in a no. of org. solvents (pyridine, $CHCl_3$, etc.) has been examined. Almost complete separation of individual compound lipids (phosphatidyl-choline, -ethanolamine, and -serine and cerebroside) from mixtures has been achieved. S. A. Brooks.

Obviating emulsification of natural and hardened oils on deacidification. S. Fiala (*Prûm. potravin*, 1961, **12,** 342—345).—On the basis of theoretical exhibition of problems involved in the formation of water–oil and oil–water emulsions, optimal conditions for the deacidification process in refining natural and hardened oils are examined. The most important cause of persistent emulsification is the negative charge of the colloidal particles forming the disperse phase that are not disposed to sediment unprompted. For their sedimentation the influence of a charge change predominates over that of sp. gr. difference. In the deacidification of oils with soda lye the undesirable emulsification is due chiefly to the formation of soaps and acid soaps giving no positive reaction with phenolphthalein and detectable only using acetone solution of bromophenol blue as the indicator. The emulsion stability is also influenced by the temp. For washing out the acid soaps from the oil 30° is optimum. J. S. B.

Analysis of glyceride structure of cocoa butter by thermal-gradient crystallisation. G. V. Jones and E. G. Hammond (*J. Amer. Oil Chem. Soc.*, 1961, **38,** 69—73).—Cocoa butter is separated into fractions in a column −29° at one end and +2° at the other. The fractions are then analysed by gas–liquid chromatography after conversion to their Me esters. Considering the formation of eutectics, the glyceride composition of cocoa butter agrees with the composition, predicted by restricted random distribution. Cocoa butter containing 10% of triolein was analysed by this method in an attempt to resolve the GU_3 and GS_2U constituents; the results show that separation is incomplete. (24 references.) G. R. Whalley.

Glyceride structure of vegetable oils by counter-current distribution. V. Comparison of natural, interesterified and synthetic cocoa butter. H. J. Dutton, C. R. Scholfield and T. L. Mounts (*J. Amer. Oil Chem. Soc.*, 1961, **38,** 96—100).—The component glyceride structure of cocoa butter is examined by gas–liquid chromatography of the Me fatty esters, counter-current distribution, determination of spectro-iodine values and isotope dilution methods using tritiated palmitic and ^{14}C-labelled stearic acids. The palmitic and stearic acids are randomly esterified at the 1- and 3-glycerol positions with oleic acid at position 2. Cocoa butter, when interesterified in the presence of NaOMe, shows a completely random structure, thereby altering its physical properties. A synthetic glyceride prepared from equal amounts of all three acids shows properties similar to those of cocoa butter. (20 references.) G. R. Whalley.

Metabolic studies of glyceride esters of adipic acid. R. L. Shull, L. A. Gayle, R. D. Coleman, R. B. Alfin-Slater, A. T. Gros and R. O. Feuge (*J. Amer. Oil Chem. Soc.*, 1961, **38,** 84—86).—The digestibility, absorption and *in vivo* oxidation of two radioactive types of glyceride adipic acid esters, a diglyceride adipate, a polymeric fatty acid, adipic acid and glycerol were tested in rats. All the compounds are readily digested, and although stearic acid is well absorbed, it is only slowly oxidised. Stearic acid oxidation is increased when it is fed as the diglyceride adipate. G. R. Whalley.

Autoxidation and conservation of unsaturated fats. O. J. Debrus (*Riv. ital. Sostanze grasse*, 1961, **38,** 229—235).—A review. (31 references.) L. A. O'Neill.

Study of unsaponifiables by chromatography on silicic acid. P. Capella, G. de Zotti, G. S. Ricca, A. F. Valentini and G. Jacini (*Riv. ital. Sostanze grasse*, 1961, **38,** 198—202).—The unsaponifiable matter of fatty oils freed from all fatty acid or soap is dissolved in hexane adsorbed on a silicic acid column and eluted with a series of solvents of increasing polarity. The components are separated into their main groups, viz., hydrocarbons, waxes, sterol esters, alcohols, sterols, etc., which are examined by colour tests and i.r. spectroscopy. The results obtained with an extracted olive oil are given; the principal sterol is β-sitosterol. (31 references.)

L. A. O'NEILL.

Decolorisation and purification of oils and fats. R. Raffaeta (B.P. 846,474, 18.2.57. It., 16.2.56).—The process comprises the steps of subjecting a mixture of the oil or fat and an adsorption agent (e.g., $ZnCl_2$) to a sudden rise in temp. (to 140—220°) by contact with a pre-heated portion of the mixture in a heat-exchanging contact zone at a temp. below that which will cause polymerisation of the oil or fat, and then heating the mixture with superheated steam in counter flow to remove colour and impurities from the oil or fat.

E. ENOS JONES.

Modified edible fat products containing essential fatty acids. Allied Laboratories Inc. (B.P. 845,147, 9.4.58. U.S., 10.4.57).—A fatty acid mixture consisting of edible vegetable oil (of $\not<$40% of linoleic acid) 75—92 and hydrogenated vegetable oil (m.p. 112—145°F) 8—15% is admixed (73—90) with glyceryl monostearate and/or lecithin emulsifier (0·1—2) and a fluid aq. milk product (10—25 pt.) above the m.p. of the fatty mixture. The resulting emulsion is then rapidly cooled, to give a solid spreadable product. The edible oil may be maize germ, soya-bean, safflower, cottonseed, sunflower and/or groundnut oil, while the hydrogenated material is preferably hydrogenated coconut oil; if desired, the composition may also contain NaCl, butter flavouring and antioxidants.

F. R. BASFORD.

Meat and Poultry

Relationship of post-mortem acidity and temperature. J. Wismer-Pedersen and E. J. Briskey (*Food Technol.*, 1961, **15**, 232—236).—When pH was decreased to ~5·4 (lactic acid ~1%) in freshly killed muscle (e.g., *longissimus dorsi* muscles) held at temp. >25°, soft exudative tissue resulted. When the tissues were cooled rapidly extreme pH variations and muscle structure alterations were prevented. The rapid chilling of carcasses (post mortem) by submersion did not give the same degree of improvement, but substantially retarded the decrease of pH. Colour intensity and water-binding capacity in fresh tissue were much improved and gelatin formation in the canned product was reduced. (21 references.)

E. M. J.

Prepackaged fresh meat characteristics as related to quality and environmental factors. E. Birmingham (*Dissert. Abstr.*, 1961, **21**, 2667—2668).—Comparisons are made between firm and soft cuts of pork and beef as regards wt.-, colour- and flavour-stability during storage at 1° or 10°. Good correlations are found between objective and subjective methods for measuring firmness. Firm cuts show better shelf-life than soft cuts. P. S. ARUP.

Heat-induced haem-catalysed lipid oxidation in animal tissues. B. G. Tarladgis (*Dissert. Abstr.*, 1961, **21**, 2668—2669).—An improved distillation method for the determination of malon aldehyde in rancid foods is described. The formation of malon aldehyde from pure unsaturated fatty acids is a function of O_2-uptake, and decreases after the point of max. uptake has been passed. Suitable antioxidants reduce the development of rancidity in frozen cooked meats. Lipid-oxidation increases in frozen meats, but decreases in irradiated meats, (probably) because the ferrihaemichromogen of the former is active as a catalyst, whilst the ferrohaemochromogen of the latter meats is inactive in the absence of preformed peroxides. This theory, and a general theory based on the dependence of catalytic activity on the magnetic properties of the haem compounds, is supported by the results of other experiments on the effects of oxyhaemoglobin and methaemoglobin on the oxidation of unsaturated fatty acids. P. S. ARUP.

Effects of smoking and smoking temperatures on the shrinkage, rancidity development, keeping quality and palatability of dry-cured hams. J. D. Kemp, W. G. Moody and J. L. Goodlett (*Food Technol.*, 1961, **15**, 267—270).—Hams (150) were dry cured; in two trials, were heated in groups during smoking to 80—140°F, and stored for 6 months at 65°F and R.H. of 55—60%. Shrinkage increased with ageing and especially above 120°F. Peroxide no. increased with ageing, faster in unsmoked hams, with smaller increase with increase in temp. Free fatty acids increased with ageing but increased less as smoking temp. increased. Hams smoked below 110°F were similar in desirable appearance and aroma and were sounder than those smoked >110°F. Palatability scores were similar but the higher smoked groups were more tender. (14 references.)

E. M. J.

Creatine and creatinine in meat and meat products. Z. Dvořák (*Prům. potravin*, 1961, **12**, 381—384).—The conversion of creatine to creatinine in meat was examined and the influence of pH and temp ascertained for evaluation of the preceding heat treatment during meat processing. The ratio of creatine to creatinine is decisive, their sum remaining practically constant. Methods elaborated for accurate determination of both components, using known reactions, are reported. The evaluation method can be applied to all products containing muscle tissue. J. S. B.

[A] **Determination of 3,5-dinitro-o-toluamide (Zoalene) in chicken tissues.** G. N. Smith, B. J. Thiegs and M. G. Swank. [B] **Determination of 3-amino-5-nitro-o-toluamide (ANOT) in chicken tissues.** B. J. Thiegs, G. N. Smith and J. L. Bevirt (*J. Agric. Fd Chem.*, 1961, **9**, 197—201, 201—204).—[A] To prevent destruction of the Zoalene (**I**) samples should be frozen immediately after slaughter. The ground frozen samples are extracted (by blending) first by $COMe_2$ and then by C_6H_6. The material contained in the combined extracts (freed from the aq. phase) is obtained in $CHCl_3$ solution; the solution is passed through an Al_2O_3 column; after washing with $CHCl_3$, the **I** is eluted by 80% EtOH; this solution is carefully evaporated to near dryness. The determination of the **I** in the residue is based on the spectrophotometric determination at 560 mμ of the colour developed with 1,3-diaminopropane in the presence of dimethyl formamide. Average recoveries from muscle tissue are 77% ± 4%, and from liver tissue 86% ± 5%. No **I** is found in the meat 12 h. after the cessation of dosing of the birds.

[B] This compound, which is bound in the tissues, is a metabolite of Zoalene (see previous abstract); it is liberated from the ground frozen tissues by acid ficin digestion at 30° during 24 h. After neutralisation with $NaHCO_3$, the extraction (in this case with $COMe_2$ and $CHCl_3$) and chromatography on Al_2O_3 follow on the same lines as described for Zoalene, after which the 80% EtOH eluate is passed through a column of Dowex 50 W-X8 (H^+ form). The column is washed with 80% EtOH followed by water; the ANOT is eluted by 4N-HCl and determined by diazotisation and coupling with *N*-1-naphthylethylenediamine dihydrochloride and spectrophotometric measurement at 540 mμ. Average recoveries are 86—87% ± 5%. Most of the ANOT disappears from the tissues within 12 h. after the cessation of the dosing of the birds.

P. S. ARUP.

Micro-organisms and their control on fresh poultry meat. L. E. Dawson and W. J. Stadelman (*Mich. State Univ. agric. Exp. Sta.*, 1960, Tech. Bull. 278, 39 pp.).—Means of restricting the no. of bacteria on eviscerated carcasses and of increasing subsequent shelf life are examined. During cooling in ice-water addition of Cl_2 was more effective than that of I_2 in lengthening shelf life. Of antibiotics examined chlortetracycline was the most effective when the bacterial count was not excessive. Initial bacterial populations were lowered by β-irradiation but subsequent re-population was rapid. With >50,000 rep carcass odour was impaired but dosages $\not>$1 m. rep did not affect flavour. An atm. enriched with CO_2 (up to 25%) destroyed some bacterial species and increased shelf life.

A. G. POLLARD.

Effects of antibiotics and irradiation on the *Pseudomonas* and *Achromobacter* flora of chilled poultry. M. J. Thornley, M. Ingram and E. M. Barnes (*J. appl. Bact.*, 1960, **23**, 487—498).—Antibiotic treatment, tetracycline (**I**) and irradiation (**II**) alter the spoilage flora; in both cases the survival of yeasts (**III**) are favoured but in **III** there are no obvious changes. *Pseudomonas* (*P*) is the most sensitive to **II** while *Achromobacter* (*A*) is more sensitive to **I**. The ultimate spoilage flora was generally dominated by *P* but there was a significant difference between the three **I**-compounds used. Chloro-**I** almost eliminated *P*, only the pigmented strains appearing at a later stage; with **I** the pigmented strains were abundant but with oxy-**I** the non-pigmented strains predominated although growth was delayed. The position was complicated by a **I**-resistant group of *A*. **I** exerted only a bacteriostatic effect while **II** destroyed; **I** + **II** treatment was effective although on eventual spoilage non-pigmented *P* predominated. These findings are examined and discussed; if processing by **I** and **II** is adopted, a new criterion of spoilage would be used. (19 references.) C. V.

Effect of duration and type of chilling and thawing on tenderness of frozen turkeys. A. A. Klose, A. A. Campbell, H. L. Hanson and H. Lineweaver (*Poultry Sci.*, 1961, **40**, 683—688).—The effects of various procedures of chilling and thawing on the tenderness of turkeys of different ages were studied. A. H. CORNFIELD.

Cooking of fowl with various salts for pre-cooked poultry products. O. J. Kahlenberg and E. M. Funk (*Poultry Sci.*, 1961, **40**, 668—673).—The cooking of old fowl in solutions of salts (0·75—1·50% KCl, 2—4% NaCl, and 2—4% Na tripolyphosphate) had no advantage over cooking in water with respect to non-fat cooking losses, tenderness of cooked breast meat and the amount of fat in cooked thigh meat. Pressure cooking resulted in increased tenderness of breast meat and lower content of fat in thigh meat over simmering and boiling. A. H. CORNFIELD.

Fish

Literature survey on the effects of ionising radiations on sea foods with respect to wholesomeness aspects. S. A. Miller, J. J. Licciardello, J. T. R. Nickerson and S. A. Goldblith (*U.S. at. Energy Comm.*, 1961, Rep. TID 11610, 35 pp.).—The effects on colour, texture and flavour of cod, haddock, halibut, sol, mackerel, salmon, tuna, " other fish," crab meat, lobster, oysters, shrimps and scallops, are discussed. (77 references.) C. V.

Bacterial flora of fish caught in the Pacific. J. Liston (*J. appl. Bact.*, 1960, **23,** 469—470).—Based on three areas with widely different temp., and hydrographic conditions, the results are remarkably similar to those found for North Sea and Arctic fish. C. V.

Spoilage of fish in vessels at sea. VII. Seasonal variations in the landed quality of gutted, trawler-caught Atlantic cod and haddock. C. H. Castell and J. G. Giles (*J. Fish. Res. Bd Can.*, 1961, **18,** 295—302 ; cf. J.S.F.A. Abstr., 1959, ii, 185).—Comparison of the landed quality of gutted cod and haddock in 1959 and part of 1960 showed the same seasonal fluctuations as those observed in 1956/57. Better quality fish are landed in the late winter, spring and early autumn than in summer and late autumn. These observations were confirmed by independent observations on trawler-caught fish at another Nova Scotian fishing port. E. M. J.

Grading fish for quality. IV. Variations in the relation between trimethylamine values and grades for gutted, trawler-caught Atlantic cod and haddock. C. H. Castell, M. F. Elson and J. G. Giles (*J. Fish. Res. Bd Can.*, 1961, **18,** 303—310).—Data from grading of several hundreds of fish and the probability curve developed by Hoogland (1958) showed the relation between organoleptic grade and trimethylamine (TMA) values for gutted, trawler-caught Atlantic cod and haddock. These values have been verified recently by tests on 3170 cod and haddock. The relation between TMA and grade is not the same for cod as for haddock. For a given TMA level haddock show greater deterioration. Fish caught in summer and late autumn show more deterioration than those caught in spring. E. M. J.

Pseudomonas and Achromobacter group of bacteria in the spoilage of white fish. J. M. Shewan, G. Hobbs and W. Hodgkiss (*J. appl. Bact.*, 1960, **23,** 463—468).—Total colony count and analysis of the bacterial flora of the skin-slime of the cod at five-day intervals during stowage of fish in ice, showed that organisms of the genera *Pseudomonas* and *Achromobacter* became predominant in the latter stages of spoilage. (25 references.) C. V.

Observations on " green " tuna. A. M. Dollar, A. M. Goldner, W. D. Brown and H. S. Olcott (*Food Technol.*, 1961, **15,** 253—255).—Physical and chemical properties of normal and off-coloured fish in a shipment of tuna were compared with a high % of fish that were not acceptable after being cooked. The " green " fish had darker kidney suspensions, less total haem pigments, more ferri- than ferro-haem pigment, and higher peroxide content in the lipids. The variation of these measurements in fish to fish were too great to be of use as a test for greening. (12 references.) E. M. J.

Deteriorative changes in frozen shrimp and their inhibition. M. B. F. Chastain (*Dissert. Abstr.*, 1961, **21,** 2688).—The oxidative fading of the red carotenoid pigment astaxanthin is associated with loss of flavour and odour. The degree of fading is a more sensitive index of deterioration (including loss of texture due to protein denaturation) than organoleptic judgement ; it can be determined by measurement at 470 mμ of a $COMe_2$ extract (made in the presence of Na_2SO_4) of the material. Storage of the material at −18° before and after cooking gives better keeping quality than storage at −4° ; cold storage before cooking gives a definite advantage. Encouraging results have been obtained with the use of ascorbic acid, butylated hydroxyanisole and liquid smoke as antioxidants. P. S. Arup.

Increasing the water-soluble portion of animal muscle protein and preparation of muscle protein extracts. J. A. Benckiser G.m.b.H. Chemische Fabrik (B.P. 844,979, 27.2.58. Ger., 27.2.57).—The amount of water-sol. material in animal muscle protein, e.g., a fish product or sausage meat, is increased (and a product of improved quality and appearance thereby obtained) by treatment with a water-sol. ionised or ionisable Mg compound (e.g., Mg salt of citric acid, H_3PO_4 or condensed phosphoric acid) in presence of an alkali metal salt of a condensed phosphoric acid (and optionally an anti-oxidant, e.g., citric acid or a salt of ester thereof). The treatment may be undertaken before or after mincing of the product, or during extraction thereof. F. R. Basford.

Spices, Flavours, etc.

Production of volatile compounds related to the flavour of foods from the Strecker degradation of DL-methionine. P. E. Ballance (*J. Sci. Fd Agric.*, 1961, **12,** 532—536).—The volatile products were condensed in cold traps and analysed by gas–liquid chromatography. The major constituent was methyl mercaptan with trace amounts of other volatile compounds, e.g., acrolein, isobutyraldehyde, dimethyl sulphide and dimethyl disulphide. The initial step in the reaction sequence was the formation of methional which subsequently gave acrolein and methyl mercaptan. The importance of methionine as a source of volatile S compounds associated with the flavours of foodstuffs is discussed, e.g., methional with the " sunlight " flavour of milk ; methyl mercaptan with flavour and dimethyl sulphide with odour of meat, etc. (20 references.) E. M. J.

Gas–liquid chromatographic analysis of *Capsicum* amides. P. H. Todd, junc., and C. Perun (*Food Technol.*, 1961, **15,** 270—272).—Mixtures of naturally occurring may be distinguished from synthetic products by gas chromatographic analysis of the methyl esters of the fatty acids in *Capsicum* pungent amides. In the natural product, besides capsaicin, a small amount of another amide occurs, in greater proportion in *C. annuum* than in *C. frutescens*. The results are discussed with reference to i.r. spectra. (10 references.) E. M. J.

Essential oils and related products. E. Guenther, K. Kulka and J. A. Rogers (*Analyt. Chem.*, 1961, **33,** No. 5, 37R—45R).—Analytical procedures reported in the 1959—1960 literature are briefly discussed. (219 references.) C. V.

Treatment of compositional data for characterisation of essential oils. Determination of geographical origins of peppermint oils by gas-chromatographic analysis. D. Morison Smith and L. Levi (*J. agric. Fd Chem.*, 1961, **9,** 230—244).—Descriptions are given of the gas–liquid partition chromatographic technique employed, and the method used for the calculation of % compositions of samples from the peak areas of the chromatograms. The possibilities of the technique are demonstrated by numerous analyses of the oils of *Mentha piperita* and *M. arvensis* of various origins. Thus, oils which have undergone deterpenisation (by rectification) or dementholisation (by freezing) can be recognised by their terpene and menthol content, respectively. The ratio (*C*) limonene/cineole is 0·2—0·7 for (natural) *M. piperita*, and >2 for *M. arvensis* oil, but may be somewhat distorted in terpene-freed oils ; graphs plotted for ratio *C* against the ratio (*D*) menthofuran/ "menthone-related constituents " affords a greatly improved method for distinction between the two oils. The ratio *D* and similar ratios for other constituents are shown to be influenced by genetic, ecological and/or climatic factors, the maturity of the plants, or the parts of the plants from which the oils are obtained. Items of chromatographic evidence for the detection of adulterants (7) in *M. piperita* oil are tabulated. (52 references.) P. S. Arup.

Detection of 2-alkoxyphenols in vanillin and ethyl-vanillin. J. G. Hald (*Dansk Tidsskr. Farm.*, 1961, **35,** 73—77).—Traces of *o*-methoxyphenol in vanillin or of *o*-ethoxyphenol in ethyl-vanillin can be detected by the intense red colorations developed 20 min. after dissolution of the sample in 74% (by wt.) H_2SO_4. The colorations developed by the pure substances should not exceed that of a mixture of the $CoCl_2$ and $FeCl_3$ (1 : 19) U.S.P. colour standards. The smallest detectable amounts of the impurities, 0·01%, correspond respectively with the smallest amount detectable by smell in vanillin, and a distinctly detectable amount in ethyl-vanillin. Additions of Vanitrope (1-propenyl-3-ethoxy-4-hydroxybenzene) can similarly be detected by the same test. P. S. Arup.

Colouring matters

Synthetic carotenoids for the colouring of foodstuffs. O. Isler, R. Rüegg and P. Schudel (*Chimia*, 1961, **15,** 208—226).—Two provitamin A compounds commercially available are synthetic β-carotene and β-apo-8-carotene, and others under test for suitability for use are lycopene, torularhodin and cantoxanthin. The biogenesis of these compounds, their synthesis, analytical control, chronic toxicity and forms of use, are reviewed. (41 references.) (In German.) J. L. Prosser.

Preservatives

Preservation of custard apple (*Anona squamosa*) pulp. B. S. Bhatia, L. V. L. Sastry, G. V. Krishnamurthy, K. G. Nair and Girdhari Lal (*J. Sci. Fd Agric.*, 1961, **12,** 529—532).—On exposure to air the pulp turns pink by peroxidase activity, heating >55° for a short time causes the development of a bitter taste. To preserve the pulp citric acid 1, Na benzoate 0·1% and SO_2 50—100 p.p.m. (to check the enzymic activity) are added. With sugar concn. of 40° Brix enzymic activity is retarded. E. M. J.

Use of sorbic acid or its salts in pickled green olives. J. M. R. de la Borbolla y Alcalá, M. J. Fernandez Díez and P. González Cancho (*Grasas y Aceites*, 1961, **12**, 10—15).—The development of yeasts and moulds in pickled green olives is prevented by the addition of sorbic acid or K sorbate. A concn. of 0·025% is effective for 5 to 6 days and 0·15 to 0·1% for 2—3 months. Taste, acidity and pH are unaffected, but there is a slight darkening in light. L. A. O'NEILL.

Determination of sorbitol in food products. A. M. Manzone and C. Jacobelli-Turi (*Ann. Chim., Roma*, 1961, **51**, 220—229).—The amount of sorbitol in a simple solution is determined by measuring the optical rotation $[\alpha]^{25}_{D}$ of the solution ($\not> 2$ g. of sorbitol) to which has been added NH_4 molybdate (4 g.) and N-H_2SO_4 (25 ml.) (which greatly enhances the rotation) and has been made up to 100 ml., by comparison with a reference standard. In the presence of sugars, e.g., glucose or sucrose, not forming complexes with NH_4 molybdate, the $[\alpha]^{25}{}_{D}$ is determined firstly in aq. solution and then in a solution containing NH_4 molydate + H_2SO_4, and the sorbitol content determined from the incremental increase in $[\alpha]^{25}_{D}$. The procedure with food products is indicated. L. A. O'NEILL.

Rapid method for the detection of sorbic acid in foods. A. Cesari, C. Jacobelli-Turi and M. G. Malagodi (*Ann. Chim., Roma*, 1961, **51**, 312—317).—Sorbic acid is extracted from the foodstuff, e.g., margarine, butter or cakes, by steam distillation after acidification with H_3PO_4. The pH of the distillate is adjusted to 8 and it is then oxidised with 0·1N-$KMnO_4$. In the presence of sorbic acid, acetaldehyde will be produced, and if the mixture is heated on a water bath in a vessel stoppered with a disk of filter paper impregnated with Simon reagent (Na nitroprusside and diethanolamine) a blue spot will be formed; 0·3 mg. of sorbic acid may be detected. With wine, the sorbic acid must first be freed from ethanol by extraction and pptn. as Ba sorbate. L. A. O'NEILL.

Food Processing, Refrigeration

Stabilised raisins for dry cereal products. G. G. Watters and J. E. Brekke (*Food Technol.*, 1961, **15**, 236—238).—Coating with a moisture barrier, e.g., beeswax diluted with cottonseed oil was not satisfactory as, by dilution, the permeability of beeswax to water vapour was increased. Dusting raisins with starch and then dipping them in molten beeswax was effective. These raisins remained edible for ~4 times as long as untreated raisins stored with bran flakes. Pilot plant tests have been made. E. M. J.

Canadian potato dehydrating plant using flake method. W. C. Christner and D. S. Gardner. **Improving communication between research and management.** E. Thor. **Economic efficiency in food plant operations.** R. G. Bressler. **Case study in economic engineering analysis.** C. Calleros. **Economic engineering research in food processing plants.** B. C. French and L. L. Sammet (*Food Technol.*, 1961, **15**, No. 6, 4, 6, 7, 10; 13, 14; 15, 16; 18, 20, 23). E. M. J.

Radiation preservation of milk and milk products. XII. Radiation sterilisation of milk in a rotary disk apparatus. G. D. Saravacos, J. E. Hoff, S. A. Goldblith and E. L. Wick (*Food Technol.*, 1961, **15**, 239—241; cf. J.S.F.A. Abstr., 1960, i, 305).—The apparatus by which sterile conc. (3 : 1) milk, essentially free of off-odour, may be produced consists basically of an irradiation chamber, an evaporator in which off-odours are removed by aseptic vac. distillation and the milk is diluted to required concentration, and an aseptic sampler. Polyphosphate-treated milk processed in this way may be stored at 30° in sterile form without gelation for at least 6 months. E. M. J.

Uncertainties in canning process calculations. E. W. Hicks (*J. Fd Sci.*, 1961, **26**, 218—226).—The nature of safety factors and uncertainties in calculating lethal values are discussed. Most of the uncertainty in thermal data (**I**) is due to real variation from can to can and these uncertainties should be considered when calculations in canning processes are made, especially in deciding the safety factor. Uncertainties in **I** obtained from heat penetration measurements and in bacteriological data (these having the greater effect) contribute significantly to the overall uncertainty. (28 references.) E. M. J.

Thermal conductivity of meats, fats, gelatin gels and ice. C. P. Lentz (*Food Technol.*, 1961, **15**, 243—247).—At temp. above freezing, the conductivities of different meats were about equal and slightly lower than that of water. At temp. below −10° the relation between temp. and conductivity of gels and meats is expressed by a simple linear equation. The conductivity of fats varied only slightly with temp. Calculated results appeared to have limited use in practice. (15 references.) E. M. J.

Method for calculating the temperature distribution and mass average temperature in conduction-heated canned foods during water cooling. S. Charm (*Food Technol.*, 1961, **15**, 248—253).—In cooling processed canned foods to a mass average temp. >90°F but <110°F, the surface of the can dries rapidly enough to prevent rusting and at temp. <110°F thermophiles, which usually are not destroyed in processing, will not grow. A method is described to calculate the temp. distribution after a given cooling time has elapsed by use of the dimensionless heating curves developed by Gurney and Laurie. Determination of mass average temp. is also discussed. E. M. J.

Frozen food handling codes in the United States, Great Britain and Germany. F. H. Reuter (*Food Technol. Aust.*, 1961, **13**, 299, 301, 303, 305, 309, 311, 313, 314, 317).—Information on the present position of Frozen Food Handling Codes in prep. in the above named countries is reviewed. Two American codes mentioned deal with (*a*) quick frozen foods (q.f.f.) the main point being that they must be kept at 0°F or below, at all times; (*b*) the economic arrangements in the industry. The Public Health aspect, legal ways of controlling the management of q.f.f. in retail trade and comparison of the Australian problem with that of the U.S. are discussed. E. M. J.

Salad dressings stable to frozen storage. H. L. Hanson and L. R. Fletcher (*Food Technol.*, 1961, **15**, 256—262).—Factors influencing oil and water separation in emulsions and starch paste conforming to the oil and egg-yolk specifications of the standards of identity were studied at temp. from +20° to −50°F. At 20°F safflower oil did not solidify and was best of tested oils. Groundnut oil solidifies to some extent, but oil separation can be delayed for >3 months at +20°F to −30°F by appropriate combined conditions. Waxy rice flour was the best thickening agent tested; increasing egg-yolk level increased stability; as storage temp. was lowered the emulsifying power of egg-yolk decreased; increase of salt level from 0·5 to 1·7% increased stability. Of the ratio of emulsion to starch paste, increase in emulsion % increased oil separation; increase in paste increased water separation. (11 references.) E. M. J.

Thermal properties of foods at low temperatures. I. Specific heat. S. W. Moline, J. A. Sawdye, A. J. Short and A. P. Rinfret (*Food Technol.*, 1961, **15**, 228—231).—Knowledge of the specific heat of the sample of food is useful for calculating cooling and thawing times and also to determine accurately the vol. of liquid N required to cool a given amount of food to a given temp. A method is described for continuous determination of the specific heat of foods over a wide range of temp. below the f.p.; the specific heat of fats, gelatin and water were measured. (18 references.) E. M. J.

Packaging

Extractability of organo-tin stabilisers from hard polyvinyl chloride foils. M. Houška (*Z. LebensmittUntersuch.*, 1961, **114**, 373—375).—Consideration of the amounts of the stabilisers which can be extracted from the foils by means of water, aq. AcOH or EtOH, aq. 5% Na_2CO_3 or edible oil raises serious doubts as to whether their use in foils for food packing should be permitted until further information is available regarding their physiological effects. P. S. ARUP.

WVTR [Water vapour transmission rate] of aluminium foil laminates. I. Laboratory test data. II. Testing of packaged products. M. A. Miller, J. E. Stillwagon and D. B. Strohm (*Pack. Engng*, 1961, **6**, No. 2, 36—40; No. 3, 70—72).—WVTR values for various materials are examined. Al-foil is an important component of a laminate when low WVTR values are required to provide max. protection but adhesives should be applied to the paper rather than to the foil and the explanation of this is discussed. C. V.

Tin-plated cans sprayed with soluble oil formulations show increased rust resistance. R. K. Cohen (*Corrosion, Houston*, 1961, **17**, No. 3, 75).—Dealing with the problem of transport by sea, it was found that corrosion could be reduced by passing the can through a spray chamber so as to apply a very fine film of this protective material just prior to labelling; e.g. an emulsion of a sol. oil (5—20%) in water containing 0·5—2·0% $NaNO_3$ and surfactant 0·25—5%. This treatment is satisfactory for light conditions of exposure; if severe, more stringent methods are required. C. V.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Strained baby foods. I. Proximate, mineral and vitamin composition of canned fruit and vegetable pulps and fruit custards. II. Drying of strained mango pulp and mango custard. III. Relative effect of canning and drum drying on the proteins, ascorbic acid and β-carotene of mango custard. IV. Shelf-life of mango custard powder. G. S. Siddappa and S. Ranganna (*Food Sci., Mysore*, 1961, **10**, 29—36, 37—40, 41—44, 45—48).—I. Canned samples of strained indigenous fruit and vegetable pulps prepared in the laboratory were compared with similar products manufactured in other countries. Results of cut-out examination and the proximate, mineral and vitamin composition are tabulated. Strained mango and papaya pulps are rich sources of β-carotene. The Nendran variety of banana contains β-carotene and is also rich in thiamine unlike the other varieties. (20 references.)

II. Drum-, spray- and freeze-drying of *Badami* mango pulp showed that the drum-dried product is of thin, papery texture, difficult to powder. Mango custard blend, however, has all characteristics of a good powder, and shows little non-enzymic browning. Proximate, mineral and vitamin composition and the effect of methods of drying on the ascorbic acid content, β-carotene, total colour and residual SO_2 in mango powder and custard are given. Retention of ascorbic acid is higher in freeze-dried products (88·2 mg.-%) than in spray-dried ones (77·05 mg.-%). (16 references.)

III. The insolubility of protein in a medium in which it is soluble, while still native, was taken as a criterion for determining denaturation. The loss of solubility in water and salt solution was determined. The solubility of the protein and the retention of ascorbic acid is higher in the drum-dried powder than in the canned product. β-carotene and total carotenoid pigments are stable in canning and drum-drying.

IV. Mango custard powder fortified with fat, vitamins A and D, thiamine, riboflavin, niacin, pyridoxine and B_{12} can serve as a complete infant food. Shelf-life tests revealed that it can be stored in tins under N_2 at 5° for over a year without signs of deterioration. Addition of fat (0·8%) does not cause rancidity, it helps to improve the flavour. I. DICKINSON.

Supplementary value of strained foods based on mango pulp to milk-cereal diet. S. Korula, K. Joseph, M. Narayana Rao, K. Indiramma and G. S. Siddappa (*Ann. Biochem.*, 1960, **20,** 327—332).—In rats fed a rice-milk diet and in those fed a similar diet to which mango custard was added, no significant increase in wt. was observed. Red blood cell counts were higher in rats fed the supplemented diet but there was no significant difference in the haemoglobin content of the animals or in the moisture, fat, protein or ash contents of the body; the fat content of the liver was significantly lower. The rats retained significantly larger amounts of N, Ca and P than those on the control rice-milk diet. E. M. J.

Chemical composition and nutritive value of bajra (*Pennisetum typhoideum*) and bajra diets. P. P. Kurien, M. Swaminathan and V. Subrahmanyan (*Food Sci., Mysore*, 1961, **10,** 3—6).—A review on bajra, a food grain, which can be cooked as rice or used as flour for unleavened bread, deals with the chemical composition of different varieties, composition of amino-acids present, distribution of protein, Ca and P between husk and endosperm, digestibility and biological value of the proteins, availability of Ca and P, nutritive value, human metabolism studies, malt prep. and enzymes. Bajra has a high nutritive value as a food grain and can help to overcome the shortage of cereals. (25 references.) I. DICKINSON.

Castor beans as food and fodder. W. D. Raymond (*Trop. Sci.*, 1961, **3,** 19—24).—The causes of toxicity of the castor bean are reviewed and methods for de-toxification described. Castor seed may be rendered non-toxic by dehusking the seed and heating, followed by fermentation for two days. G. W. DOUGLAS.

Effect of storage temperature and antioxidant treatment on the chemical and nutritive characteristics of herring meal. B. E. March, J. Biely, C. Goudie, F. Claggett and H. L. A. Tarr (*J. Amer. Oil Chem. Soc.*, 1961, **38,** 80—84).—When herring meal is stored at −20° there is a larger decrease in the ether extractable material, and in I value of the latter than with material extracted from meal stored at 25·5°. In the presence of butylated hydroxytoluene (BHT) there was no change in ether extractables and I values at both temp. Digestion tests with pepsin on BHT-treated meals showed the lowest quantity of undigestible N, which was highest in the meal stored at −20°. Both meals have similar nutritive values with respect to protein content, even after 9 months of storage, but that stored at −20° gave slowest rate of growth in chicks. (14 references.) G. R. WHALLEY.

Symposium on the microstructure of proteins. *J. Poly. Sci.*, 1961, **49,** 1—175.)—The following papers are presented: **Sequence of amino-acid residues in proteins.** F. Sanger pp. 3—29 (81 references.), **Influences of three dimensional configuration on the chemical reactivity and stability of proteins.** C. B. Anfinsen pp. 31—49 (31 references.), **Peptide synthesis and protein structure.** P. G. Katsoyannis pp. 51—74 (70 references.), **Function of neighbouring groups and side chains in the enzyme chymotrypsin.** M. L. Bender, G. R. Schonbaum and G. A. Hamilton pp. 75—103 (53 references.), **Phase changes in proteins and polypeptides.** P. J. Florey pp. 105—128 (38 references.), **Use of rotary dispersion in the determination of protein structure.** J. A. and C. G. Schnellman pp. 129—151 (35 references.), **Use of X-ray diffraction in the determination of protein structure.** B. W. Low pp. 153—175 (38 references.). C. V.

Nutritional value as protein of some of the nitrogenous constituents of marine algae, *Chondrus crispus* and *Laminaria digitata*. B. A. Larsen and W. W. Hawkins (*J. Sci. Fd Agric.*, 1961, **12,** 523—528).—Nitrogenous constituents of alkali-extracted *C. crispus* and *L. digitata* and of isopropanol-extracted *C. crispus* were incorporated into diets for young rats at levels of 7—10% crude protein. The digestibility of these constituents was lower than that of egg albumin. The biological value, as measured by retention of absorbed N, was for the *L. digitata* prep. 75% that of egg albumin and for the *C. crispus* prep. about the same as that of egg albumin. None of the prep. supported an adequate rate of growth. (22 references.) E. M. J.

Food uses and properties of soya-bean protein. I. Food uses. A. K. Smith and W. J. Wolf. **II. Physical and chemical properties of soya-bean protein.** W. J. Wolf and A. K. Smith (*Food Technol.*, 1961, **15,** No. 5, 4—6, 8, 10; 12—13, 16, 18, 21, 23, 26, 28, 31, 33).—I. A review. The U.S. grows ~55% of the world's supply of soya-beans and this potential market for protein has advantages over other commercial oil-seeds in the high nutritional value of the protein, relatively high ratio of protein to oil, low proportion of seed coat and low cost of production. (34 references.)

II. The basic physical and chemical properties were studied to provide a more scientific basis for prep. of soya-bean meal, its protein fractions and application to foods. Details are given of extraction, separation (ultracentrifuge) and separation of the whey proteins by chromatographic methods. (51 references.) E. M. J.

Changes in dry weight, protein, nucleic acid and chlorophyll contents of growing pea leaves. R. M. Smillie and G. Krotkov (*Canad. J. Bot.*, 1961, **39,** 891—900).—The leaves were examined over a 10-day growth period. Chlorophyll content increased rapidly during early leaf expansion, then remained fairly constant. As the leaves developed, small differences only were found in % of sol. protein, but the levels of ribonucleic and deoxyribonucleic acids decreased continuously. These changes are discussed in relation to similar changes found, e.g., in leaves of tobacco, sugar cane, tomato, etc. (38 references.) E. M. J.

Isolation of leaf components. I. I. H. Chayen, R. H. Smith, G. R. Tristram, D. Thirkell and T. Webb (*J. Sci. Fd Agric.*, 1961, **12,** 502—512).—The yield of sol. N from a leaf remains fairly constant at 75—85% when alkaline solution is used as extractant. Disintegration is effected by the impulse process, i.e., a mechanical rupture of the membranes of the cells by a series of high-speed impulses through the medium of the liquid. Evidence suggests that high-quality grass has a high protein content, and that the yield of protein falls when grass reaches the flower stage. (10 references.) E. M. J.

Sequence of amino-acid residues in proteins. F. Sanger (*J. Polym. Sci.*, 1961, **49,** 3—29).—A general review dealing with the approach to this problem. Attention is drawn to the similarity between proteins and peptides from the same organ. Examples are the two hormones of the posterior pituitary (oxytocin and vasopressin) which possess very similar structures but very different physiological function, and corticotropin and the melanocyte-stimulating hormone of the anterior pituitary and the proteases of the pancreas (trypsin, chymotrypsin and elastase). These possess the same tetrapeptide sequence round their reactive serine residue; other examples are given. Of three possible explanations given for these similarities, the most probable is based on the biological origin of the protein, three proteases being evolved from one protein, and that some primitive organism may have had but one protease, which has developed along three separate lines by change in its amino-acid sequence to give three enzymes with different specificities but with certain sequences in common which are survivals of the parent protein. Amongst other sequences shown that contain 158 amino-acid-residues is the protein of mosaic tobacco virus. (81 references.) C. V.

Effects of chain length on the metabolism of saturated fatty acids by the rat. S. L. Kirschner and R. S. Harris (*J. Nutr.*, 1961, **73,** 397—402).—Four randomly mixed triglycerides containing either butyric-, caprylic-, lauric- or palmitic-1-^{14}C (**I**) acids were administered to young adult rats by stomach tube and detailed examination of urine, faeces and breath analysis was carried out over 24 h. Faecal samples showed there was an 86% absorption from the intestinal tract of **I**, this being significantly lower than that obtained from the three shorter chained ones (98—100%). (12 references.) C. V.

Production of vitamin B_{12} by photosynthesising bacteria. E. N. Kondrat'eva and V. É. Uspenskaya (*Dokl. Adak. Nauk SSSR*, 1961, **136,** 718—719).—A study was made to determine whether vitamin B_{12} and its derivatives are formed by photosynthesising bacteria found in polluted ponds and sludges where vitamin B_{12} occurs. *Chlorobium thiosulphatophilum, Chloropseudomonas ethylicum,* *Chrometium minutissimus* and *Rhodopseudomonas palustris* were cultivated on media containing alkali salts and traces of B, Zn, Mn, Cu, Fe and Co with 0·1% of Na_2S, $Na_2S_2O_3$ or an org. additive. All these bacteria formed cobalamins active for *Escherichia coli* in quantities which many heterotropic micro-organisms synthesise. Co content (50 μg./l.) was found adequate. P. W. B. Harrison.

Unclassified

Symposium on *Pseudomonas* and *Achromobacter* (*J. appl. Bact.*, 1960, **23,** 373—514).—**Introductory reflections on the *Pseudomonas-Achromobacter* group.** M. Ingram and J. M. Shewan pp. 373—378 (12 references.); **Determinative scheme for the identification of certain genera of Gram-negative bacteria with special reference to the pseudomonadaceae.** J. M. Shewan, G. Hobbs and W. Hodgkiss pp. 379—390 (53 references.); **Some results of a computer analysis of strains of *Pseudomonas* and *Achromobacter* and other organisms.** J. Liston pp. 391—394; **Computation of similarities between strains of *Pseudomonas* and *Achromobacter* isolated from chicken meat.** M. J. Thornley pp. 395—397; **Identification of flagella stains.** W. Hodgkiss pp. 398—399; **Composition of carbohydrate metabolism and localisation of enzymes in *Pseudomonas* and related micro-organisms.** J. de Ley pp. 400—441 (139 references.); **Differential techniques and methods of isolation of *Pseudomonas*.** K. Klinge pp. 442—462 (110 references.); **Mechanism of multiplication of *Pseudomonas* in the hen's egg.** J. Brooks pp. 499—509 (10 references.); **Distribution and ecology of Gram-negative organisms other than the enterobacteriaceae in lakes.** V. G. Collins pp. 510—514. C. V.

Relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry and eggs. J. C. Ayres (*J. appl. Bact.*, 1960, **23,** 471—486).—In freshly ground beef >80% of the micro-organisms present may be composed of chromogenic bacteria, moulds, yeasts and spore-forming organisms; 75—80% of the colonies on chicken parts were similarly distributed; at the time of spoilage (off-odour and/or sliminess) these meats reveal primarily single, paired or short-chained motile Gram-negative, non-sporing rods; some of these produce fluorescent yellow, green or bluish pigments; most do not. These organisms are discussed at some length and a suggested grouping and classification is examined but before this can be accepted further data are required, e.g., a study of their growths at 5, 15, 25, 37 and 42°, and standardisation of procedures. (64 references.) C. V.

Preservation of micro-organisms by freeze drying. II. Destructive action of oxygen. Additional stabilisers for *Serratia marcescens* and experiments with other micro-organisms. R. G. Benedict, E. S. Sharpe, J. Corman, G. B. Meyers, E. F. Baer, H. H. Hall and R. W. Jackson (*Appl. Microbiol.*, 1961, **9,** 256—262).—Atm. O_2 killed 95% dried *Serratia marcescens* (**I**) in 10 min. but the presence of some reducing agents prevented this; humidity played no part in this reaction. Urea and several of its derivatives protected **I** during drying and glucose and isomaltose improved the protective action of the supernatant liquor; isomaltotriose, dextran and mucin did not. Attempts to freeze-dry *Saccharomyces capsularis* (**II**) and *Eremothecium ashbyii* gave very poor results, **II** being mostly destroyed possibly on account of its large size. Optimum results for organism survival were obtained using moderately low temp. and drying for a short period at an elevated platten temp. The presence of protective colloids did not contribute to the survival of **I**. *Leuconostoc mesenteroides* and *Pseudomonas aurefaciens* were also studied. (13 references.) C. V.

***Salmonella saint paul* infection in England and Wales.** N. S. Galbraith, J. F. Archer and G. H. Tee (*J. Hygiene, Camb.*, 1961, **59,** 113—141).—The incidence of infection increased from three, annually, before 1953 to 100 in 1958. Isolations of this organism from non-human sources (turkey poults, pigs, bone meal, etc.) are analysed and discussed; one-third of the 83 cases in 1959 was attributed to home-produced meat coming from two affected abattoirs. It is considered that this animal infection is due to imported feeding stuffs. (22 references.) C. V.

Determination of residual organo-phosphorus insecticides in foodstuffs. A review. E. D. Chilwell and G. S. Hartley (*Analyst*, 1961, **86,** 148—159).—After an introductory discussion of the nature of the problems involved in the determination of insecticide residues, the general procedures available for extraction of the insecticide, its separation from possible interfering substances and its subsequent determination are reviewed. Methods available for the determination of 19 individual insecticides are briefly summarised. (72 references.) A. O. Jones.

Noxious heterogenous substances in foods. J. Špinka (*Prům. potravin*, 1961, **12,** 369—370).—Warning comments appearing in the press about the presence of noxious substances in customary foods endangering public health by possible physiological accumulation are reviewed: e.g., the tar and phenolic substances formed in smoking meat and fish with special reference to carcinogenity, antibiotics in meat from their use in animal production, synthetic dyestuffs such as Butter-yellow (dimethylaminoazobenzene), Pb in canned foods, insecticide residues on vegetables, overheated oils and fats, and substances formed by action of light and irradiation. (17 references.) J. S. B.

Examination of foodstuffs for radioactivity. M. Depner (*Dtsch. LebensmittRdsch.*, 1960, **57,** 89—92).—The theoretical and practical requirements for effective estimation of radioactive contamination of foodstuffs by fall-out, or as a result of catastrophe, are reviewed. Method of distinguishing relatively small amounts of contamination (due mainly to ^{90}Sr, ^{137}Cs and ^{140}Ba) superimposed on the natural radioactivity of the material are briefly discussed, with particular reference to the examination of water, milk, cereals and fish. E. C. Apling.

Caesium-137 in air, precipitation, drinking water, milk and beef in Norway 1959—60. T. Hvinden and A. Lillegraven (*Nature, Lond.*, 1961, **190,** 402—404).—Continuous measurements at three principal stations—Bergen, Røros and Lillestrøm—over the period Jan. 1959—Dec. 1960 are reported. The main contribution to the intake of ^{137}Cs by cows is from grass, hay and fodder; the differences in the ratios of ^{137}Cs in fall-out to ^{137}Cs in the milk at the three locations are interpreted in terms of the differences in local grazing-field qualities, although other factors may also be important. G. H. Hudson.

Analytical methods for determination of iodine-131 in vegetation, milk, thyroid glands and natural waters. U.K.A.E.A., P.G. Rep. 204 [W].)—Radio-I, with added carrier, is separated by extraction as elemental I into CCl_4 and back extracted as iodide into dil. H_2SO_3. The iodide is precipitated as AgI, mounted and the β-activity is measured; the observed counting rate is corrected for background chemical yield and radioactive decay, and the activity of the original sample is calculated as $\mu\mu$c(/g./l. or per sample) by comparison with a standard ^{131}I source. E. M. J.

Hydrolysis of protein substances. Cyklus Ltd. (B.P. 846,682, 10.3.59. Switz., 14.3.58).—Protein substance, e.g., extracted meat, is hydrolysed with strong volatile mineral acid at 90—140°, and when the ratio of the total N of the hydrolysis products to the NH_2-N is <10 but >1 these products are removed (by filtration) and freed from acid (by evaporation of the latter). Acid equivalent to that thus removed is added to the hydrolysis mixture, together with fresh protein if desired, and further hydrolysis is effected. The protein hydrolysate thus obtained has a meat-like taste, and is suitable for use as seasoning. F. R. Basford.

3.—SANITATION

Evaluation of surface-active disinfectants for use in milk production. C. M. Cousins, W. A. Hoy and L. F. L. Clegg (*J. appl. Bact.*, 1960, **23,** 359—371).—A can test for evaluating detergent-sterilisers used on dairy farms is described. Such materials are compared with a standard hypochlorite detergent to determine their cleansing capacity of milk-soiled surfaces. The results are compared with those obtained with the suspension test. The latter is of value for initial screening but products may give good disinfection in suspension yet may fail to cleanse a soiled surface. (12 references.) C. V.

Assessment of the activities of surface-active agents for use in the catering industry. B. C. Hobbs, N. Emberley, H. M. Pryor and M. E. Smith (*J. appl. Bact.*, 1960, **23,** 350—358).—Cleaning and bactericidal efficiencies of detergents were examined by laboratory tests, the technique being described. Field trials in a large and small canteen gave similar results. The ability of *Salmonella paratyphi* B to survive and grow after inoculation with samples of used dishwater was demonstrated. (10 references.) C. V.

Activity of insecticides on absorbent surfaces. N. van Tiel (*Span*, 1961, **4,** 90—94).—Field work, using experimental huts with plastered mud interior walls, is reported. Dieldrin and dieldrin–Aroclor gave an equal performance during the rainy season when high humidity caused a high effectiveness of absorbed dieldrin; in the dry season dieldrin–Aroclor showed a progressive improvement. Favourable humidity conditions in most areas prevent absorption from being a major problem of mosquito content in anti-malaria campaigns. (13 references.) E. G. Brickell.

House fly populations. III. Influence of insecticides on population trends. R. L. Beard (*Conn. agric. exp. Sta.*, 1960, Bull. 631, 22 pp.).—House flies, reared to 45 generations whilst being exposed to various insecticides, showed variations in biological response to the insecticides. There were indications of behavioural or physiological changes in the flies. Resistance to DDT developed commonly and resistance to dieldrin quickly succeeded resistance to DDT. An unusual cross-resistance to γ-$C_6H_6Cl_6$ also appeared. Flies maintained at a marginally low level by severely limiting their food and breeding resources increased to full no. when these resources were supplied and were much more resilient than populations suppressed by insecticides for many generations and then reared in the absence of insecticides. A. H. CORNFIELD.

Estimation of carbon dioxide in stored grain: an indication of condition. M. Calderon and E. Shaaya (*Trop. Sci.*, 1961, **3,** 25—30). —Metabolic activity in stored grain causes a temp. rise with the production of CO_2 and may serve as an indication of the presence of mould or insect infestation. The concn. of CO_2 may be taken as a measure of this activity and measured by a simple thermal conductivity gas analyser of the type used to determine fumigant concn. by comparing the thermal conductivity of the sample with a CO_2-free sample of air. G. W. DOUGLAS.

Water analysis. M. W. Skougstad and M. J. Fishman (*Analyt. Chem.*, 1961, **33,** No. 5, 138R—164B).—A very detailed literature review of recent analytical methods. (448 references.) C. V.

Arsenic accumulation by fish in lakes treated with sodium arsenite. W. W. Ullmann, R. W. Schaefer and W. W. Sanderson (*J. Wat. Pollut. Control Fed.*, 1961, **33,** 416—418).—Data showing the arsenic accumulation in fish in lakes treated with Na arsenite (used as herbicide) are given and discussed. There was no noticeable increase in the As content of the fish during the 21 days after the herbicide had been added. As contents up to 0·38 p.p.m. were recorded in the fillets. O. M. WHITTON

Measuring coliforms in water. J. A. McCarthy, J. E. Delaney and R. J. Grasso (*Wat. & Sewage Wks*, 1961, **108,** 238—243).—Discrepancies encountered with the use of the membrane filter for measuring coliforms in water have been analysed. A two-step procedure is proposed. (15 references.) O. M. WHITTON.

Coliform population in stored sewage. R. M. Cody, R. G. Tischer and H. K. Williford (*J. Wat. Pollut. Control Fed.*,.1961, **33,** 164—166).—Changes in coliform population during storage for 24 h. at 45°F or less are not statistically significant. B. F. FULLAN.

Culture and harvest of attached algae grown on domestic sewage. I. G. W. Reid and J. R. Assenzo (*Wat. & Sewage Wks*, 1961, **108,** 248—250).—The use of attached algae to improve waste water plant effluents and their possibilities as a food source are discussed. (24 references.) O. M. WHITTON.

Distribution of carbohydrate metabolism in sewage sand filters. G. B. Morgan and F. W. Gilcreas (*Wat. & Sewage Wks*, 1960, **107,** 485—488).—Chemical and radiochemical methods have been applied to the study of distribution of carbohydrate metabolism in pilot-plant and laboratory-scale intermittent sand filters. The loss of B.O.D. removal ability at lower levels in the sand (more than 6 in. from top surface) and absence of N_2 fixation under anaerobic conditions suggest the need for ventilation. C. A. SLATER.

Use of sewage effluent for irrigation of truck crops. S. G. Dunlop and Wen-Lan Lou Wang (*J. Milk Food Tech.*, 1961, **24,** 44—47).—*Salmonella* (**I**), *Ascaris* ova (**II**) and *Endamoeba coli* cysts were recovered from >50% of samples of irrigation water contaminated with raw sewage or primary-treated chlorinated effluent. Only one in 97 samples of vegetables yielded **I** but **II** was found twice in 34 samples; in this latter case raw sewage was being discharged into the stream. (11 references.) C. V.

Nitrate as the nitrogen source in B.O.D. dilution water. P. E. Gaffney and R. S. Ingols (*Wat. & Sewage Wks*, 1961, **101,** 91—95).—Nitrate was substituted for ammonia in B.O.D. dilution water, the use of ammonia being the present standard method. Oxidation lags were shown in lower 5- and 10-day B.O.D. values. Long-term studies of the order of 20 days showed increases in B.O.D. values with decreasing sewage volume with ammonia dilution but were much less with nitrate dilution, oxidation demand for nitrification is eliminated and B.O.D. values give a better representation of ultimate B.O.D. values for any carbonaceous residue. B. F. FULLAN.

New platinum electrode system measures DO and BOD. J. D. Eye, L. H. Reuter and K. Keshavan (*Wat. & Sewage Wks*, 1961, **108,** 231—236).—The application of a plastic-covered, stationary, Pt electrode system for measuring the dissolved O_2 and BOD content of water and domestic sewage has been studied. Reproducibility and accuracy are comparable to those of standard (Winkler) tests. Operation of the cell is satisfactory with either KOH or KCl, in varying concn., as an internal electrolyte. The system should be frequently standardised or the battery replaced every 10—15 days. Polyethylene film is a satisfactory electrode membrane. Plastic components inert to biological oxidation should be used. (14 references.) O. M. WHITTON.

Substituted sulphonic acid amides. Farbenfabriken Bayer A.-G. (B.P. 845,834, 10.11.58. Ger., 11.11.57).—Compounds useful (0·00001—1 wt.-% concn.) as insecticides (effective against aphids, flies, mites and lice) comprise substituted sulphonic acid amides, viz., $SR\cdot[CH_2]_2\cdot SO_2\cdot NR'R''$ (R′ is alkyl of 1—5 C; R″ is H or alkyl; or R′ and R″ together with N form a piperidino or morpholino radical; R is substituted or unsubstituted alkyl, aryl, aralkyl, acyl, aroyl or acid radical of an alkyl phosphonic acid, etc.). They are produced by interaction of RSH with $CH_2{:}CH\cdot SO_2NR'R''$. 2-(*Phenylthio*)*ethane*-1-*sulphon*-NN-*diethylamide* is prepared. F. R. BASFORD.

4.—APPARATUS AND UNCLASSIFIED

Diazotised sulphanilic acid reagent for endrin analysis. J. E. Fahey and M. S. Schechter (*J. agric. Fd Chem.*, 1961, **9,** 192—193).—The presence of an excess of sulphanilic acid in the reagent (as used by Bann *et al.*) gives rise to a background colour due to the reaction of the free with the diazotised acid. This can be prevented by the use of a slight excess of $NaNO_2$, the remainder of which can be destroyed by the addition of NH_4 sulphamate after the complete diazotisation of the acid. Directions are given for the prep. of the reagent on these lines. P. S. ARUP.

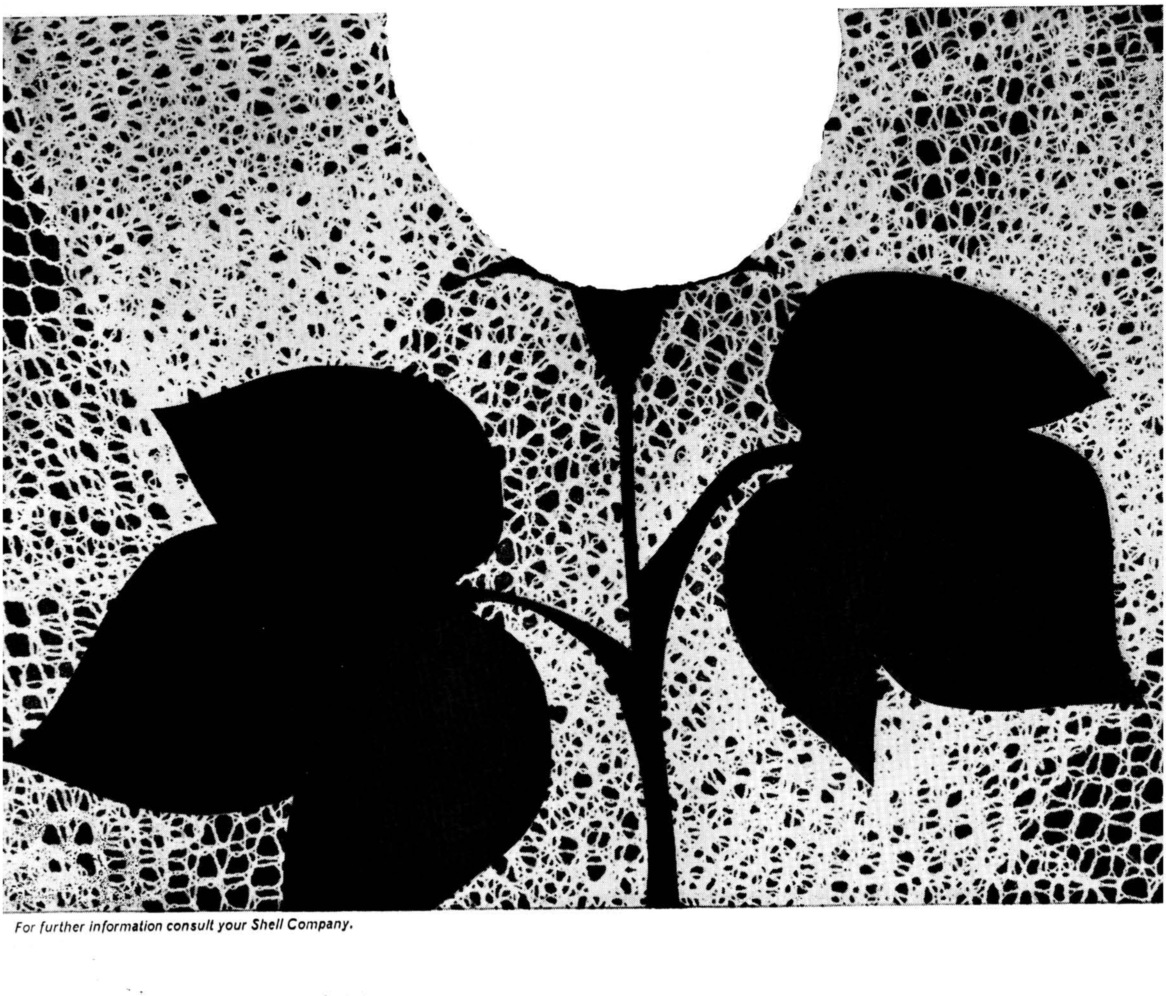
An agar-diffusion method for the estimation of organic phosphate insecticides. E. Sandi and J. Wright (*Chem. & Ind.*, 1961, 1161—1162).—An agar diffusion method similar to the cup-plate assay method for antibiotics has been developed to determine traces of insecticides which do not contain easily detectable groups and which penetrate the plant tissue. Details of the procedure adopted are given. Systox (demeton) and Metasystox can be estimated at a concentration of 5×10^{-6} moles/litre. E. BRIMACOMBE.

Estimation of phenothiazine and some of its oxidation products in biological material. W. T. Oliver, H. S. Funnell and N. Platonow (*J. agric. Fd Chem.*, 1961, **9,** 213—214).—Phenothiazine (**I**), phenothiazone (**II**), thionol (**III**) and phenothiazine-5-oxide (**IV**) are extracted from homogenised poultry tissues, intestinal contents or feeding stuffs by shaking with a mixture of $COMe_2$, MeOH and formaldehyde and separated by paper-chromatography under N_2 with $AcOH–C_6H_6–H_2O$ (1 : 2 : 3) as ascending solvent. Eluates of the sections of the paper containing **I**, **II**, **III** and **IV** are tested by reagents producing coloured deriv. suitable for spectrophotometric measurement. **I** and **II** are converted into their nitroso deriv. (**II** after reduction by $Na_2S_2O_3$), **III** by the colour developed at pH 9·7 and **IV** by the colour developed on the addition of 8N-HCl, with a little $FeCl_3$ as stabiliser. Recoveries range from 83·5—93% for **III** to 97—101·5% for **I**. P. S. ARUP.

Micro-Kjeldahl determination of nitrogen: effects of added salt and catalysts. P. R. W. Baker (*Talanta*, 1961, **8,** 57—71).—The effects of K_2SO_4 concn. and of various catalysts, on recoveries of NH_3 from $(NH_4)_2SO_4$, nicotinic acid and benzylisothiouronium chloride, have been studied. The use of HgO does not cause losses of NH_3 during the digestion of $(NH_4)_2SO_4$, and leads to good recoveries from nicotinic acid. The other catalysts examined gave low recoveries from nicotinic acid or $(NH_4)_2SO_4$, or from both. For a 5-mg. sample a digestion mixture consisting of HgO (20 mg.), K_2SO_4 (2·25 g.) and H_2SO_4 (1·5 ml.), is recommended. R. M. ROWLEY.

Controlled use of adsorbents in enzyme purification. I. T. Oliver (*Nature, Lond.*, 1961, **190,** 810—811).—The graded fractional adsorption procedure for enzyme purification with either $C\gamma$-Al_2O_3 or Ca_3PO_4 gels ensures a higher purification factor (*F*) than does preferential adsorption of contaminants or of enzymes from solution. Small vol. of adsorbent are added successively and residual enzyme is rapidly titrated by a standard method. Results are reported for prep. of (*i*) glucose-6-phosphate dehydrogenase and (*ii*) uridinediphosphoglucose dehydrogenase; in (*i*) *F* was ~5 in comparison with 1·9 for the usual method, whilst in (*ii*) differential adsorption of protein occurred. W. J. BAKER.

Paper chromatography of hexosamines and *N*-acetylhexosamines. R. Heyworth, H. R. Perkins and P. G. Walker (*Nature, Lond.*, 1961, **190,** 261—262).—The separation of amino-sugars and their *N*-acetyl deriv. by paper chromatography was improved by preliminary treatment of the paper with 0·1M-$BaCl_2$ which increased the solvent movement and the variations in R_F. Talosamine was detected in acid hydrolysates of alkaline extracts of Ba chondroitin sulphate from dried bovine nasal cartilage. S. A. BROOKS.



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