JOURNAL
OF THE
SCIENCE OF FOOD AND AGRICULTURE
(INCLUDING ABSTRACTS)

Published by the Society of Chemical Industry

Volume 16 | No. 4 | April, 1965

SOCIETY OF CHEMICAL INDUSTRY

FOUNDED IN 1881 INCORPORATED BY ROYAL CHARTER 1907

Offices of the Society: 14 Belgrave Square, S.W.1

Telephone: BELgravia 3681/5

Annual Subscription to the *Journal of the Science of Food and Agriculture*

£15 post free, single copies £1 17s. 6d. post free

AMINO-ACID COMPOSITION OF LUCERNE AND OF LUCERNE AND GRASS PROTEIN PREPARATIONS

By R. F. WILSON and J. M. A. TILLEY

The amino-acid composition of lucerne herbage cut at six stages of maturity has been determined by an ion-exchange chromatographic procedure and compared with that of protein preparations isolated from lucerne and grasses.

The protein preparations from grass and lucerne were of closely similar amino-acid composition; no appreciable difference in composition could be detected between protein preparations from young or mature lucerne.

The amino-acid composition of lucerne-herbage N differed from that of the protein preparations; this difference arose because these herbages contained about 30% of their nitrogen in a water-soluble form, only part of which was present as free amino-acids. The amino-acid composition of lucerne herbage changed little as maturity increased.

Introduction

There have been conflicting reports as to the amino-acid composition of herbages[1–9] or of protein preparations isolated from them.[10–15] To determine whether this could be due to the herbage species tested having been sampled at different stages of maturity, a study has been made of the amino-acid composition of lucerne herbage, cut at six different stages of growth. The composition of protein preparations isolated from three of these lucerne samples, and from five grasses, have also been measured.

Some of the published data may contain considerable errors;[16] certain amino-acids may have been destroyed under the conditions of hydrolysis used and some of the analytical procedures used to estimate the amino-acids in the hydrolysates are likely to have been of low accuracy. In the present work, hydrolysis of herbages and of protein preparations has been carried out under conditions where losses could be kept to a minimum.[17–19] The amino-acids were determined by the ion-exchange chromatographic technique of Moore & Stein,[20] which permits the complete separation of all the amino-acids present in an hydrolysate.

Lysine, cystine, methionine and tryptophan are probably the most important amino-acids in protein feeds intended as supplements to cereal-based diets,[21] as the other essential amino-acids are usually present in adequate quantities in the unsupplemented feed. Unfortunately, both tryptophan and cystine are destroyed during the acid hydrolysis used to liberate the other acids; alternative procedures have therefore been used for these two acids.

Experimental

Two types of sample were used in these investigations: (i) samples of whole lucerne herbage, and (ii) samples of protein concentrates prepared from juice expressed from minced lucerne or grass.

Lucerne herbage samples

Samples of lucerne were cut at six stages of maturity during first growth, dried overnight at 100° in a Unitherm oven, and ground in a Christy and Norris 8-in. laboratory mill fitted with an 0·8 mm. grid. Details of crops are given in Table I. Nitrogen contents were determined by conventional Kjeldahl analysis and soluble N by the procedure of Barnett & Miller.[22]

Protein preparations

Samples of fresh lucerne or grass were macerated in a hand-mincer and the juice expressed by hand through butter muslin. Four volumes of industrial alcohol were then added and the mixture allowed to stand overnight at 0°. After decanting the supernatant, the protein was collected by centrifugation and washed successively with 80% alcohol, hot alcohol, hot water and acetone. The product was air dried and passed through a 200 mesh sieve. During this preparation, the sample was not heated above 80°. A sample of 'cytoplasmic protein' was prepared from lucerne 4 juice; chloroplastic material was removed by centrifugation at 23,000 *g* for 1 h. and the cytoplasmic protein preparation recovered from the supernatant liquid by

Table I

Description of herbages together with their total-N content (dry-matter basis) and the % of this N which was water-soluble. The % N in protein preparations from these herbages is also given

Sample	% N of herbage	% of N which was water-soluble	% N of protein preparation	Description of herbage and date cut
Lucerne (var. Du Puits)				
1	4·8	33	—	1st cut 2 April 1957; 6–8 in.
2	4·3	31	13·1	1st cut 16 April 1957; 12 in.
3	4·5	29	—	1st cut 1 May 1957; 18 in.
4	3·1	29	12·5 13·9 (cytoplasmic protein)	1st cut 14 May 1957; 20 in.
5	2·5	34	10·3	1st cut 4 June 1957; 30–36 in., a few flowers
6	2·3	37	—	1st cut 25 June 1957; 36 in., full flower
*Grasses**				
7	2·3	17	11·2	S.23 perennial ryegrass, 1st cut 27 May 1958
8	2·8	15	11·1	S.22 Italian ryegrass } Cut 12 August 1958, re-growth after cutting on 9 July 1958
9	3·1	16	9·9	S.53 meadow fescue
10	2·9	14	7·7	S.48 timothy
11	2·1	14	7·1	S.37 cocksfoot

* Grass herbages not analysed for amino-acids

alcohol precipitation as above. Table I gives the % N contents of the protein preparations (dry-matter basis).

Acid hydrolysis

All samples were hydrolysed for 22 h. under reflux with constant-boiling hydrochloric acid. Lucerne herbage samples containing 8 mg. of N were hydrolysed with 200 ml. of acid, and protein preparations containing 10–15 mg. of N were hydrolysed with 100 ml. of acid. Humin from herbage hydrolysates was removed by filtration. The hydrolysates were evaporated to dryness at 38° on a rotary evaporator,[23] taken up in 10 ml. of 0·5N-hydrochloric acid, centrifuged to remove humin and insoluble material, and then stored in a refrigerator for analysis. Humin and insoluble materials were combined and total-N content determined by Kjeldahl analysis. Ammonia N in the hydrolysate was determined by a microdiffusion method.[24]

Chromatography

The method of Moore & Stein[20] was followed apart from minor modifications. The resin used was Zeo-Karb 225 of nominal 8% cross-linkage with a particle size of approximately 50 μ (The Permutit Co., Ltd.), from which coarse particles were removed with a 200 mesh sieve and fine particles by decantation. One ml. of hydrolysate was used for each determination. Fractions of 1 ml. were collected, and the acids were determined by a photometric ninhydrin procedure.[25] Ninhydrin (indanetrione hydrate, British Drug Houses Ltd.) was purified by the method of Hamilton & Ortiz.[26] All amino-acids separated well, with the exception of serine and threonine; the best separation of these acids was achieved with an eluting buffer pH of 3·38 (rather than pH 3·42[20]); it was then possible to make a reasonably accurate estimation of both acids. Tyrosine was readily separated from phenylalanine.

γ-Aminobutyric acid was present only in the herbage hydrolysates. As reported,[19] these hydrolysates also contained products arising from the degradation of the carbohydrate material of herbage which reacted with ninhydrin reagent to give a red colour. Another small peak, due to methionine sulphoxide, was detected; all other compounds reacting with ninhydrin were identified as amino-acids.

Losses of amino-acids

Hydrolysis with relatively large volumes of acid reduces these losses[17–19] so that, in this work, humin formation amounted only to 5·5–8·4% of the herbage nitrogen and 2·0–4·8% of the protein nitrogen. Part of the methionine was destroyed during hydrolysis and despite addition of the antioxidant thiodiglycol to the buffer, a further part was oxidised on the column

to methionine sulphoxide ;[19] the yield of the latter was small and a correction factor was not applied. The data reported for methionine therefore refer only to that recovered as amino-acid. Corrections for losses of serine and threonine[27] and for glutamic acid[20] have been suggested, but they were not applied here. The recoveries of N as amino-acids from protein preparations in these experiments were of the same order as those found by Chibnall *et al.*[15] In lucerne herbage a proportion of the total-N is not present as amino-acid-N or protein-N ; the lower recoveries and higher humin-N and ammonia-N analyses (Table II) were an indication of this. Cystine and tryptophan, destroyed completely by acid hydrolysis, were determined separately (see below). The humin-N and ammonia-N values in Tables II and III were obtained from acid hydrolysates and so probably include a contribution from the breakdown of these two acids.

Cystine (cysteine) estimation

Cystine and cysteine were oxidised to cysteic acid,[28] using 0·2–0·3 g. herbage or 0·1 g. of protein and 25 ml. of performic acid reagent at 0° for 16 h. After removing excess reagent under vacuum, the residue was hydrolysed with 100 ml. (herbage) or 50 ml. (protein) of constant-boiling hydrochloric acid for 22 h. The solution was then prepared for analysis as described for the other acid hydrolysates, the final extract being made up to 10 ml. in 0·5N-hydrochloric acid, from which 2-ml. (herbage) or 1-ml. (protein) aliquots were taken for chromatography. A 40 × 0·9 cm. column of Zeo-Karb 225 was used, buffered initially with pH 3·38, 0·1M-sodium citrate buffer. The aliquot of hydrolysate was mixed with an equal volume of 0·2M-sodium citrate buffer at pH 5, and the pH of the mixture adjusted to 2·5 with N-sodium hydroxide before applying to the column. Elution was carried out with pH 3·38 buffer ; a total of 30 ml. of effluent was collected at a rate of 5 ml./h., with the cysteic acid emerging after 15 ml. of effluent had been collected. This method gives a 10% loss of cystine ;[28] this was confirmed and the results have been corrected for this loss.

Tryptophan estimation

Acid hydrolysis destroys tryptophan completely ; even under alkaline conditions some tryptophan may be lost because of oxidation or of combination with carbohydrates or other amino-acids. Drèze[29] used partially hydrolysed starch as a reducing agent to minimise losses. The recovery of tryptophan was studied under alkaline conditions, either in air or in an atmosphere of nitrogen, and with and without the addition of hydrolysed starch. Hydrolysis in the presence of nitrogen alone gave the best recoveries (90–95%) and was used here. Either 0·5 g. herbage or 0·2 g. protein were taken for hydrolysis under reflux with 15 ml. of 6N-barium hydroxide solution for 16 h., a slow stream of nitrogen from a cylinder being passed continually through the hydrolysate. After cooling, the hydrolysate was neutralised with concentrated hydrochloric acid, and insoluble material was removed by centrifugation. The supernatant and washings were made up to 50 ml. and 5 ml. were applied to a 30 × 0·9 cm. column of purified potato starch. Tryptophan was eluted from the column with 0·1N-hydrochloric acid[30] containing 0·33% w/v BRIJ detergent (Honeywell & Stein Ltd.), and was determined by the photometric ninhydrin reaction.[25] The results in Tables II and III were not corrected for the 5–10% loss of tryptophan on hydrolysis.

Results and discussion

The amino-acid contents of the six samples of lucerne herbage, expressed as a percentage of total herbage N, are shown in Table II. The largest differences in content and the highest standard errors of individual values were found for those amino-acids present in the greatest quantity in the herbage. While there were significant variations between herbage samples in their content of most of the individual amino-acids, these differences were not marked and were usually within ±10% of the mean value. This result was despite the considerable range of herbage total-N (2·3 to 4·8% N ; Table I) and of stage of maturity. (The errors quoted in Table II are a measure mainly of the analytical error and do not permit an assessment of field sampling errors.)

Table II

Amino-acid analysis of lucerne herbage

(Results as N % of total herbage-N before hydrolysis)

	Means of duplicate hydrolysates						Standard error of an individual value	Mean	Mean as N % of amino-acid-N recovered†
	1	2	3	4	5	6			
Aspartic	7·7	8·2	7·1	6·6	7·4	8·0	±0·20	7·5	11·4
Threonine	2·9	2·9	2·9	2·8	2·9	3·0	±0·05	2·9	4·4
Serine	3·1	3·2	3·3	3·5	3·3	3·2	±0·06	3·3	5·0
Glutamic acid	5·1	5·2	5·3	5·2	4·9	4·7	±0·09	5·1	7·8
Proline	3·1	3·4	3·3	3·6	3·5	3·3	±0·06	3·4	5·2
Glycine	4·9	5·1	5·4	5·6	5·3	5·2	±0·07	5·2	7·9
Alanine	5·3	6·0	5·4	5·2	5·1	5·0	±0·07	5·3	8·1
Valine	3·6	3·7	4·1	3·8	3·9	3·6	±0·06	3·8	5·8
Methionine	0·6	0·4	0·4	0·6	0·6	0·5	±0·03	0·5	0·8
Isoleucine	2·5	2·6	2·7	2·7	2·7	2·5	±0·04	2·6	4·0
Leucine	4·4	4·4	4·5	4·7	4·6	4·1	±0·05	4·4	6·7
Tyrosine	1·5	1·6	1·6	1·5	1·6	1·6	±0·04	1·6	2·4
Phenylalanine	2·0	2·2	2·4	2·3	2·3	2·2	±0·04	2·2	3·3
γ-Aminobutyric acid	0·8	0·8	0·8	0·7	0·9	0·7	±0·04	0·8	1·2
Histidine	3·1	3·1	3·0	3·0	3·3	3·1	±0·04	3·1	4·7
Lysine	5·6	5·4	4·8	4·7	5·6	4·7	±0·06	5·1	7·8
Arginine	9·8	9·5	9·3	8·4	8·6	8·3	±0·08	9·0	13·7
N recovered as :									
Amino-acids	66·0	67·7	66·3	64·9	66·5	63·7		65·8	(100)
Ammonia	13·5	13·8	12·3	11·2	11·7	13·3	±0·32	12·6	—
Humin	6·2	5·5	7·7	8·4	6·1	7·9	±1·04	7·0	—
Total N recovered	85·7	87·0	86·3	84·5	84·3	84·9		85·4	—
*Cystine	0·8	0·8	0·8	0·8	0·8	0·9		0·8	—
*Tryptophan	1·3	1·2	1·4	1·3	1·4	1·4		1·3	—

* Single analyses, determined separately on special hydrolysates
† Excluding cystine and tryptophan

The samples of lucerne herbage contained from 29 to 37% of their N in water-soluble non-protein fractions (Table I). Only part of this water-soluble-N was present as amino-acids or peptides. In consequence after hydrolysis more of the total-N of the lucerne protein preparations was recovered as amino-acids (Table III), and the amino-acid composition of these protein preparations, as a percentage of total sample N before hydrolysis, varied considerably from those reported for the herbages in Table II. When the data were expressed as a percentage of the recovered amino-acid-N, much of the difference disappeared. γ-Aminobutyric acid is not a constituent of proteins and was therefore found only in the herbage samples. Aspartic acid and its amide, asparagine, are predominant in the water-soluble fraction of lucerne,[31] and the concentration of aspartic acid found in the herbages was much greater than that found in the lucerne protein preparations. The variation between the lucerne protein preparations in amino-acid composition seemed to be less than that of the herbages ; this is perhaps a reflection of the greater purity of the protein fractions, as shown by the lower losses on hydrolysis.

Compared on the basis of recovered amino-acid-N, the amino-acid composition of the lucerne cytoplasmic protein preparation differed little from the mean values for the lucerne proteins. The difference in lysine content reported by Yemm & Folkes[32] between barley whole protein and cytoplasmic protein was not found, a result in agreement with that of Chibnall *et al.*[15] for spinach protein preparations.

Within the individual protein preparations from different grass species, there was some variation in the proportion of the total-N recovered as amino-acids (81·8–87·3%). In particular the S.37 preparation gave rise to a relatively large amount of humin-N ; the low lysine value for this preparation may have been due to destruction of this amino-acid during hydrolysis

Table III

Amino-acid analysis of protein preparations from lucerne and grasses

(Single analyses. Results as N % of total protein N)

	Lucerne protein preparations					Lucerne 4 cytoplasmic protein		Grass protein preparations						
	2	4	5	Mean	Mean as N % of amino-acid-N recovered†	As N % of amino-acid-N recovered†		7	8	9	10	11	Mean	Mean as N % of amino-acid-N recovered†
Aspartic acid	6·5	6·6	6·8	6·6	7·8	7·2	8·0	6·6	7·1	7·5	6·9	6·3	6·9	8·1
Threonine	3·2	3·7	3·6	3·5	4·2	4·2	4·6	3·9	3·8	4·0	4·2	3·4	3·9	4·6
Serine	3·5	3·5	3·5	3·5	4·2	3·5	3·9	3·4	4·4	4·4	4·2	4·0	4·1	4·8
Glutamic acid	6·9	6·5	6·2	6·5	7·7	7·8	8·6	6·9	6·7	6·5	6·2	6·4	6·5	7·6
Proline	4·1	3·6	3·7	3·8	4·5	3·6	4·0	4·0	3·8	4·1	3·8	3·9	3·9	4·6
Glycine	6·4	6·0	6·6	6·3	7·5	6·1	6·7	6·9	6·4	6·9	6·4	6·9	6·7	7·9
Alanine	6·3	6·0	5·8	6·0	7·1	6·8	7·5	7·0	6·5	6·8	6·9	6·7	6·8	8·0
Valine	5·2	4·9	5·0	5·0	5·9	4·6	5·1	5·5	5·6	5·5	5·6	5·1	5·5	6·4
Methionine	1·1	0·9	1·0	1·0	1·2	1·2	1·3	1·2	1·0	1·1	1·0	1·1	1·1	1·3
Isoleucine	3·8	3·7	3·6	3·7	4·4	4·6	5·1	3·7	3·6	3·8	3·6	3·4	3·6	4·2
Leucine	6·1	6·4	6·4	6·3	7·5	6·8	7·5	6·3	6·2	6·2	6·2	5·7	6·1	7·2
Tyrosine	2·3	2·4	2·5	2·4	2·8	2·8	3·1	2·4	2·4	2·6	2·4	2·2	2·4	2·8
Phenylalanine	3·3	3·2	3·4	3·3	3·9	4·0	4·4	3·3	3·3	3·4	3·2	3·6	3·4	4·0
Histidine	4·4	4·2	4·2	4·3	5·1	4·5	5·0	4·1	4·0	4·0	4·3	3·6	4·0	4·7
Lysine	8·5	7·6	7·6	7·9	9·4	8·5	9·4	8·4	8·2	8·2	8·4	6·6	8·0	9·4
Arginine	14·4	13·7	14·0	14·0	16·6	14·2	15·7	13·7	11·5	12·1	11·3	12·9	12·3	14·4
N recovered as :														
Amino-acids	86·0	82·9	83·9	84·3	(100)	90·4	(100)	87·3	84·5	87·1	84·6	81·8	85·1	(100)
Ammonia	8·8	9·4	10·0	9·4	—	4·0	—	9·4	8·6	8·4	8·8	9·2	8·9	—
Humin	2·0	2·6	2·2	2·3	—	1·3	—	2·1	2·2	2·3	2·4	4·8	2·8	—
Total N recovered	96·8	94·9	96·1	95·9	—	95·7	—	98·8	95·3	97·8	95·8	95·8	96·7	—
*Cystine	0·8	0·8	0·9	0·8	—	1·1	—	0·9	0·7	‡	‡	‡		—
*Tryptophan	1·9	2·0	1·7	1·9	—	2·2	—	2·1	2·0	‡	‡	‡		—

* Determined separately on special hydrolysates
† Excluding cystine and tryptophan
‡ Not determined due to insufficient sample

rather than to a real difference in lysine content. The mean content of amino-acids in the grass protein preparations, as a percentage of the recovered amino-acid-N, was very similar to that of the lucerne protein preparations.

To summarise these results, no large compositional differences between the amino-acids of the protein preparations of the five grasses, or of the lucerne protein preparations taken from plants cut at different stages of growth, were found. They also show that, for lucerne, the amino-acid composition of the nitrogenous fraction of the whole plant was unaffected by growth stage. The distribution of amino-acids in the water-soluble-N of grass is different to that of lucerne ;[31] the proportion of soluble-N in grass is lower (Table I) so that the amino-acid composition of grass herbage would be expected to differ considerably from that of lucerne herbage.

Duckworth & Woodham[33] discussed the nutritive value to poultry and rats of crude leaf protein preparations and concluded that certain processing methods often brought about a reduction in nutritive value, either by lowering the digestibility of the protein, or by rendering particular amino-acids unavailable. Low and variable results for the nutritive value of these materials have therefore often been reported even though they had a high potential in terms of amino-acid content. The results reported here for the amino-acid content of purified protein preparations from the leaves of herbage plants also indicate that the nutritive value of such preparations, when calculated from their amino-acid composition, is high. Thus, when suitably prepared, their nutritive value should not be inferior to other high-quality plant proteins such as soya protein (see Table 2 of reference [16]).

Acknowledgments

The authors wish to thank Dr. William Davies for providing facilities, Mr. W. F. Raymond for his interest and Mr. L. C. Chapas and Mr. P. J. Radford their their valued assistance with the statistical analysis.

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Received 13 August, 1964

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RELATION OF THE FORMULATION OF CATTLE SPRAYS TO THE DEPOSITION AND LOSS OF DDT

By W. J. ROULSTON and H. J. SCHNITZERLING

The deposition and loss of DDT from various DDT formulations sprayed on to cattle were investigated as part of a general study aimed at increasing the persistence of acaricides on cattle. Exceptionally high DDT deposits resulted from several types of formulations: (*a*) a cationic emulsion, the charge of which was adjusted to take advantage of the known negative charge on cattle hair, (*b*) an emulsion which broke during spraying, possibly through the hardness of the water, (*c*) a wettable powder. The high deposition from (*a*) was presumably due to the electrostatic attraction of the emulsion droplets to the hair and that of (*b*) and (*c*) to the filtering action of the hair on the relatively large particles in the spray fluid.

The time for half the initial deposit to be lost from the hair of cattle was used as a basis for comparison. Within experiments the rate of loss of DDT varied little and the rate was unaffected by addition of resins or ' stickers '. It is apparent that the factors involved in the removal of DDT from cattle coats differ in nature from those affecting deposits on inert surfaces.

The rate of loss was less in winter than in summer.

Introduction

Hitchcock & Mackerras[1] showed that cattle dipped in a suspension of DDT for the control of cattle ticks, *Boophilus microplus* (Can.), were protected against reinfestation by larval ticks for a number of days after treatment. They advanced the reasonable suggestion that the destruction of larvae attaching to the animal during this protective period depleted the population of larval ticks in the pastures and that this could be the most important mode of action of DDT against cattle ticks. Residual effects of toxicants for stock pests have in fact come to be regarded as highly important, and there is an obvious need to increase the persistence of acaricides on cattle, particularly with the organophosphorus compounds which have assumed prominence since the use of chlorinated hydrocarbons was banned in Queensland for the control of cattle ticks.[2]

Suggestions have been made that the addition of resins or ' stickers ' may increase the effectiveness and persistence of insecticides. Matthysse[3] commented favourably on treatment of the ears of cattle for the control of Gulf Coast tick with DDT emulsions containing an unspecified adhesive or with DDT in grease. Wilkinson[4] found that coumarone resin added to a DDT emulsion increased the residual toxicity of sprayed calves to tsetse flies. Sullivan & Hornstein[5] showed that deposits of lindane on an aluminium surface continued to kill cockroaches for a longer period when the lindane was mixed with ' Aroclor ', a chlorinated polyphenol. Tapley[6] reported that 10% dieldrin in a urea–formaldehyde lacquer protected coffee trees against borer beetles for 8–9 months and claimed this result was not possible with an emulsion containing dieldrin only. Hornstein *et al.*[7] showed that the rate of loss of lindane from inanimate surfaces was reduced by the addition of ' Aroclor ' and the biological effectiveness of such deposits was enhanced. There was, therefore, a good case for testing the efficacy of resins and stickers in extending the persistency of acaricides on cattle. Moreover, a melt-type formulation was considered to be more effective against cattle ticks than a dispersible powder or paste of DDT,[8] so that it was also of interest to investigate the possibility of achieving further advantages by incorporating resins or stickers into the melt-type formulation.

Another approach to the problem was suggested by Prof. A. E. Alexander,[9] based on the belief that, when insecticidal emulsions made from anionic emulsifiers were sprayed on to cattle, the negative charge known to be on the hair would repulse the negatively charged emulsion. This electrostatic repulsion could be overcome if the emulsion carried a positive charge, but an emulsion prepared from a cationic emulsifier would be likely to give the substrate a positive charge and again make it repulsive. This problem could be solved, he suggested, by stabilising the emulsion with a non-ionic agent and adding only sufficient cationic agent to give it the necessary positive charge. Emulsions suitably formulated were supplied to check this theory.

The experiments described in this paper were made with DDT and thus do not have direct relationship to cattle tick control in Australia, but the results contribute to a wider understanding of the losses of acaricides from cattle.

Experimental

Treatment of cattle

To overcome the large inter-animal variation in the loss of DDT on cattle, a ' half-animal ' technique was used in which one side was sprayed with one formulation and the other side with a different one. This allowed the closest possible comparison of two formulations. The cattle were sprayed with suspensions of DDT prepared from concentrates just prior to use. The spraying fluids contained 1·0% *pp'*-DDT, except in Experiment ix, where the concentration was 0·5%. They were applied by means of a portable spraying unit at a pressure of 150–200 p.s.i. In Expts. i and ii, the volume sprayed on to the sampling area on the barrel of the animal was empirically fixed at 0·5 gal., which appeared to wet the hair thoroughly. However, the amount of hair on cattle varies with the season, thus, if the volume of spray were constant, the deposit could vary. In the later experiments the animals were sprayed until the area was considered to be saturated. The side of the animal on which a particular formulation was sprayed was alternated within groups and any one formulation was compared with each of the others on at least two animals. After treatment all the cattle grazed together in the same area. Hair samples were clipped from the animals, weighed after solvent extraction, and analysed for DDT as described by Roulston *et al.*[10] The first samples were taken after the coats of the animals had dried.

Formulations

The formulations tested were of three types: emulsions, wettable powders and colloidal dispersions (prepared by heating technical DDT and surface-active agent to a clear melt before addition to water). Some of the formulations were commercially available and others were prepared in the laboratory. The laboratory-prepared emulsions were based on xylene as a solvent and usually either ' Lissapol NX ' or ' Triton X-100 ' as emulsifiers. ' Brinol ' (sodium salt of sulphonated butyl oleate) was used to prepare the colloidal suspensions. The cationic surface active agent used was Amine Salt 220 (1-hydroxy-2-heptadodecenylimidazolidine).

Results

The logarithm of the mean DDT deposit (μg. DDT/g. clean dry hair) and the mean weights of hair samples taken from the cattle in all experiments, together with the rainfall, are shown in Fig. 1. A straight-line relationship was found to exist between log mean deposit and time. This is characteristic of first-order rate reactions. On this basis, the time for half the initial deposit to be lost ($t_{\frac{1}{2}}$ value) may be determined and this allows comparison of rates of loss irrespective of the magnitude of the initial deposit.

The mean values of all DDT analyses for the trials are shown in Table I.

(i) *Comparison of formulations A, B and C*

Formulation A, when diluted to a 1% *pp'*-DDT emulsion, contained 8·0% of lanolin and 2·7% of resin. It was based on LBE sheep-branding fluid[11] which persists on sheep for up to 1 year, but the pigment was replaced by DDT; B was a commercially available emulsion and C was a non-ionic emulsion provided by Prof. Alexander. Nine animals were treated on 22 October, 1952, each formulation being applied to six half-animals. The emulsion in formulation A broke during spraying, possibly as a result of pumping or because the water may have been harder at the time of spraying than when samples were tested at the laboratory (hardness known to range from 60 to 310 p.p.m.).

Hair samples were taken from the 18 spraying sites as soon as the animals had dried and again after 3 and 6 weeks.

Formulation A gave a higher initial deposit than B or C and there was little difference between the deposits from formulations B and C. After 3 weeks there was no marked difference

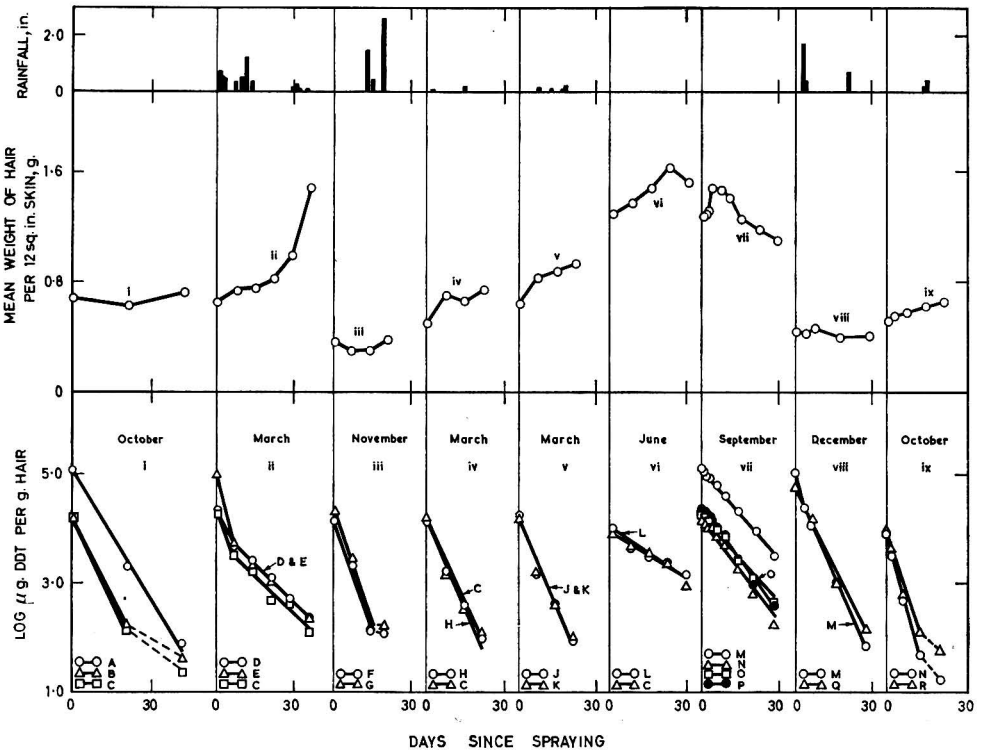

FIG. 1.—*Mean deposits of DDT on cattle hair at intervals after spraying with different formulations, the mean weight of hair from 12 sq. in. of skin on each sampling occasion, and the daily rainfall during the nine experiments which were commenced in the months indicated*

between the percentage retention from treatments A, B and C, the $t_{\frac{1}{2}}$ values of the deposits being 3·5, 3·2 and 3·3 days respectively. These values were determined over the first 21 days.

(ii) *Comparison of formulations C, D and E*

Formulations C, D and E were prepared from the same solvent and non-ionic emulsifier, but differed in the amounts of cationic agent. When diluted, C contained 0·0% ; E 0·01% and D 0·10% of Amine Salt 220, and 0·75, 0·74 and 0·65% of Lissapol NX respectively. Nine animals were sprayed on 12 March, 1953, and hair samples were taken immediately the animals had dried and again after 1, 2, 3, 4 and 5 weeks.

Formulation E gave a higher initial deposit than did formulations D or C, which differed little from one another. Over a period of 1–5 weeks the deposits from formulation E did not differ greatly from those of C or D. The greatest relative loss during the first week was shown by formulation E ; but the rate of loss was similar for all formulations over the period of 1–5 weeks as indicated by the same $t_{\frac{1}{2}}$ value of 6·3 days. Because of the rapid rate of loss during the first week, possibly caused by heavy rain, the $t_{\frac{1}{2}}$ value was calculated from an initial deposit obtained by extrapolation.

(iii) *Comparison of formulations F and G*

Formulations F and G were prepared from the same solvent and emulsifier, but when diluted G contained 1·0% of ' Aroclor 5460 '. Six animals were sprayed on 12 November, 1953, and the sprayed areas were sampled immediately after treatment and again after 1, 2 and 3 weeks. There was no marked difference in the deposits either immediately after treatment or during the subsequent three weeks. For both formulations the $t_{\frac{1}{2}}$ value was 2·6 days.

Table I

Deposits of DDT on cattle hair (expressed as mg./g. hair) taken from cattle at varying periods after treatment with different formulations containing 1% pp′-*DDT*

Experiment	Formulation*	Mean deposit of DDT (mg. per g. hair) on samples taken at intervals (days) after treatment									
		0	1	2	3	4	6	7	8	9	13
(i)	A (6)	109·3									
	B (6)	14·2									
	C (6)	14·1									
	D (6)	21·0						4·7			
(ii)	E (6)	101·4						5·3			
	C (6)	21·1						3·0			
(iii)	F (6)	14·7						2·1			0·1
	G (6)	22·2						3·0			0·2
(iv)	H (6)	15·7						1·8			
	C (6)	16·1						1·7			
(v)	J (6)	17·8						1·6			
	K (6)	17·4						0·8			
(vi)	L (4)	11·5†	10·9						5·0		
	C (4)	10·0†	9·0						5·2		
	M (9)	140·5	121·6	98·4	92·5		71·3			44·9	
(vii)	N (9)	17·4	15·7	12·8	13·4		9·0			5·7	
	O (9)	20·2	18·9	17·1	14·2		11·2			8·4	
	P (9)	26·6	23·6	20·8	19·6		12·0			8·6	
(viii)	M (9)	129·1				28·0		13·5			
	Q (9)	70·8				27·5		19·0			
(ix)	N (6)	10·2‡		4·1				0·6			
	R (6)	11·9‡		5·5				0·9			

Experiment	Formulation*	14	15	17	20	21	22	28	29	35	42
(i)	A (6)					2·0					0·10
	B (6)					0·2					0·04
	C (6)					0·1					0·02§
	D (6)	2·6				1·3		0·5		0·2	
(ii)	E (6)	2·3				1·2		0·4		0·2	
	C (6)	1·6				0·5		0·4		0·1	
(iii)	F (6)				0·1						
	G (6)				0·2						
(iv)	H (6)	0·4				0·1					
	C (6)	0·3				0·1					
(v)	J (6)	0·5				0·1					
	K (6)	0·5				0·1					
(vi)	L (4)		3·3				2·7		1·6		
	C (4)		3·9				2·7		1·0		
	M (9)	24·0				11·2		3·7			
(vii)	N (9)	2·1				0·8		0·2			
	O (9)	3·0				1·4		0·6			
	P (9)	3·2				1·2		0·5			
(viii)	M (9)			1·3				0·1			
	Q (9)			1·2				0·2			
(ix)	N (6)	0·1						0·02§			
	R (6)	0·2						0·1			

* Number of samples per formulation in brackets
† Extrapolated values
‡ Cattle sprayed with 0·5% *pp′*-DDT
§ Above limit of detection

(iv) *Comparison of formulations H and C*

Formulation H contained, when diluted, 0·1% of coumarone–indene resin (m.p. 95–124°) and was compared with formulation C. Six animals were sprayed on 8 March, 1954. The sprayed areas were sampled on the day of treatment and again after 1, 2 and 3 weeks, but there was no marked difference between the deposits. For both formulations, the $t_{\frac{1}{2}}$ value was 2·6 days.

(v) *Comparison of formulations J and K*

Formulation K, containing coumarone–indene resin, required heating to a clear melt before addition to water to form a suspension of DDT and 0·1% coumarone–indene resin. Formulation J did not contain the resin, but was treated similarly. Six animals were sprayed on 10

March, 1954, and the sprayed areas were sampled on the day of treatment and 1, 2 and 3 weeks later but no marked differences between DDT deposits were detected, the $t_{\frac{1}{2}}$ value being 2·1 days for both formulations.

(vi) *Comparison of formulations L and C*

Formulation L contained, when diluted, 0·2% of coumarone-indene resin and was similar to formulation H except for the higher concentration of resin. It was compared with formulation C, which contained no resin. Four animals were sprayed on 7 June, 1954. The sprayed areas were sampled on the day after treatment and again after 1, 2, 3 and 4 weeks, but no marked differences between deposits could be detected. The $t_{\frac{1}{2}}$ values were 9·5 days for formulation L and 9·8 days for C.

(vii) *Comparison of formulations M, N, O, P*

Formulation M was a DDT wettable powder, N was a DDT emulsion, O was a DDT melt-type formulation and P was a DDT melt-type formulation containing, when diluted, 1·0% of polyisobutylene. Twenty-four animals were sprayed on 17 September, 1957, and the sprayed areas were sampled on the day of treatment, and again after 1, 2, 3, 4, 5, 6, 9, 14, 21 and 28 days. Formulation M gave a higher initial deposit than those of the other three formulations but the rates of loss were similar for all four formulations. The $t_{\frac{1}{2}}$ values were 5·6 days for formulations M and O, and 5·0 days for N and P.

(viii) *Comparison of formulations M and Q*

Formulation Q was a wettable powder suspension containing, when diluted, an emulsified suspension of 1·0% of polyisobutylene. Nine animals were sprayed on 13 December, 1957, and the sprayed areas were sampled on the day of treatment then after 4, 7, 17 and 28 days. The initial deposit from formulation M was higher than from Q but there was no marked difference between the deposits over the period from 4 days to 4 weeks. Over this period, the $t_{\frac{1}{2}}$ values were 2·8 days for formulation M and 2·9 days for Q.

(ix) *Comparison of formulations N and R*

Formulation R was a DDT emulsion containing, when diluted, 0·5% of 'Polyox' resin. Six animals were sprayed on 22 October, 1958, and the sprayed areas were sampled on the day of treatment and after 2, 7, 14, 21 and 28 days, but there were no marked differences between the deposits at any time. The $t_{\frac{1}{2}}$ values were 1·7 days for formulation N, and 1·9 days for R.

Discussion

In relation to rates of loss of DDT from cattle hair, the results obtained are similar to those presented by Gunther & Blinn[12] for the behaviour of insecticide residues on vegetable and fruit substrates. In some experiments changes of slope are evident. The factors causing the change in slope in deposits below the level of 200 μg. of DDT per g. of hair are unknown, but deposits of this order are believed to have little effect on populations of invading larvae.

Although there was considerable variation between DDT deposits from the same formulation on different animals, there were clear-cut differences between formulations. In some trials certain formulations gave exceptionally high initial post-treatment deposits but it is considered that the reasons for this were diverse. (*a*) In the case of the lanolin emulsion (A, Expt. i) this high initial deposit could be associated with emulsion instability. Roulston *et al.*[10] believed that suspension instability, caused by different factors from those in Expt. i, largely explained the greatly increased deposits of DDT from a melt-type formulation which occurred 2 weeks after charging the dipping vat and again 2 weeks after replenishing it with freshly prepared suspension. A similar phenomenon is known to occur in a dieldrin suspension prepared from a melt-type formulation.[13] (*b*) The higher initial deposit with the higher concentrations of cationic surfactant (E, Expt. ii) is believed to be the result of the predicted electrostatic attractions between the emulsion and hair. This is in agreement with some earlier published work. Thus, Moore[14] reported that the adherence on field plants of a positively charged calcium arsenate

was 196–259% greater than the uncharged formulation. Heath & Mitchell[15] showed that, when a wool fibre was dipped in an anionic-stabilised emulsion, the droplets were randomly distributed, but when a cationic stabilised emulsion was used the droplets were attracted to the fibre. (*c*) The initial deposits of DDT on cattle sprayed with 1% DDT suspension as a wettable powder were also high (M : Expts. vii and viii), in fact approximately 10 times as high as the initial deposit reported by Roulston *et al.*[10] on cattle dipped in the same formulation containing 0·5% of DDT. When cattle are sprayed it appears likely that the hair acts as a filter when the suspension is forced through it, thus removing a much larger quantity of suspended material than would occur when dipping. Presumably the magnitude of this deposit on a given area would be a function of the amount of spray applied and the wettability of the suspension.

For various reasons, therefore, formulations A, E and M gave exceptionally high initial deposits of DDT. It is known from other experiments[16] that, in general, higher deposits of DDT produce longer protective periods. This is in agreement with Norris[17] who found that the period for which cattle were protected against buffalo flies generally increased with concentration, which presumably resulted in progressively higher deposits. A similar finding was recorded by Bruce & Blakeslee[18] for horn flies. However, for any additive to have practical value it would be necessary for the material to decrease the rate of loss and in all trials (except for the first 4 days of Expt. viii) the rate of loss from the formulations containing additives differed little from those without. Over the first 4 days of Expt. viii the rate of loss of DDT from the formulation containing polyisobutylene was less than from that without polyisobutylene, but thereafter there was no marked difference. It appears that, in respect to prolonging the residual effectiveness of DDT, the additives are of minor importance in reducing acaricide loss on cattle in comparison with the effect of the magnitude of the initial deposit.

Some of the earlier work in which ' Aroclor ' was demonstrated to enhance biological effectiveness, was based on lindane, so with the possibility in mind that the effect might be more or less specific to lindane, cattle were sprayed with emulsions of lindane with or without ' Aroclor '. No difference in rates of loss resulted, so it appears that any reduction in losses of acaricides on cattle that might be due to the use of ' stickers ', is offset by the nature and scale of operation of the processes removing the acaricide from the hair. These have been discussed by various authors. Norris[17] reported that the haired surface of a piece of fresh cattle hide, sprayed with DDT, remained toxic to buffalo flies for months, even though the hide had in the week following treatment been washed and exposed to sunshine. By contrast he found the biological effectiveness of DDT on live cattle to be short-lived and discussed the following factors in relation to the early disappearance of DDT from cattle : (*a*) rainfall, (*b*) solar radiation, (*c*) detoxification by dust or mud, (*d*) rubbing and licking by the cattle, (*e*) natural shedding of hair, (*f*) absorption into the skin and hair, (*g*) removal or destruction by skin secretions, (*h*) removal by flakes of epithelium. He concluded that cattle licking themselves and rubbing against plants and the ground caused the greatest loss of insecticide. Hackman[19] also discussed most of the above factors and concluded that licking was the major cause of removal of DDT from cattle hair. Roberts & Chamberlain[20] demonstrated that most of the loss of insecticides from cattle ' could be accounted for by the abrasive action of the tail, contact with ground, rubbing and other exercise, rain and licking '. These authors also reported that, while licking was considered an important factor in the loss of insecticide from areas within reach of the animal's tongue, the quantity removed by licking was masked by other factors.

In the experiments recorded here no attempt was made to control licking, and the effect of rain on the loss of the DDT deposits is not clear. It is likely that heavy rain soon after spraying the cattle in Expt. ii resulted in a much more rapid loss of DDT, especially from formulation E. Heavy rain in the early part of Expt. viii probably increased the rate of loss of the wettable powder formulation M, some of which would not be firmly attached to the hair. It is probable that heavy rain falling soon after treatment of cattle with insecticides would be likely to remove loosely attached particles of insecticide. The proportion of loosely attached insecticide would probably decrease with time so that rain falling later in the experiment would have less effect.

In view of the rôle of abrasive action in removing insecticides from cattle, it is somewhat surprising that the additives used in these trials did not increase the persistence of DDT.

The initial deposits of DDT appeared to be influenced by an animal factor, because on many occasions the animal with the highest deposit of one formulation would also have the highest deposit of the second formulation on the other side. Likewise another animal might also have the lowest deposits from two formulations on opposite sides. On many occasions the order of the deposits on the cattle remained approximately the same throughout the trial.

From Fig. 1 it can be seen that the slope of the lines changes between experiments. The mean weight of hair removed from 12 sq. in. also varied between experiments and there was an apparent relationship between season and rate of loss of DDT. Thus half the initial deposit of formulation C was left after 3·3, 6·3 and 9·8 days, when the animals were sprayed in November, April and June respectively. The long June persistence of DDT coincided with some of the greatest hair yields. Bracey[21] reported that the persistence of DDT crystals on the coats of cattle and goats was proportional to the length of the hair, but, although it appears that seasonal increases in hair yields on cattle are largely due to increase in hair length, the present experiments do not indicate a very clear relationship between cattle hair yield and rates of loss of DDT.

Conclusions

Although the immediate aim of employing ' stickers ' to make DDT persist on cattle for a longer period was not achieved, the experiments provided more data on the complex problem of acaricide loss from cattle hair. A relationship between DDT deposit on cattle hair and time was established and the rate of loss of DDT was also shown to vary from summer to winter. This information may assist in an understanding of what happens to other acaricides applied to cattle.

Acknowledgments

Formulation A was supplied by Dr. M. Lipson, C.S.I.R.O., and formulations C, D and E by Prof. A. E. Alexander, University of Sydney. Mr. P. G. Thompson assisted in determining the amounts of acaricide on cattle hair. The authors are indebted to Mr. K. R. Norris and Dr. R. H. Wharton for constructive criticism of the manuscript.

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Received 28 September, 1964

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INSECTICIDAL ACTIVITY OF FRESH AND DRY PYRETHRUM FLOWERS

By P. J. GODIN,* HELENE S. INGLIS* and J. H. STEVENSON†

Acetone extracts of fresh pyrethrum flowers and of flowers dried at a low temperature over silica gel were compared by gas–liquid chromatography and by biological assay. Results showed that there was no effect of drying on the amounts of the known insecticidal constituents and that activity was not diminished. Tests on extracts of pyrethrum flowers dried at various temperatures showed no significant change in composition or activity when flowers were dried at temperatures below 80°, but at 120° there was a significant loss.

Introduction

Munro[1] reported that artificial drying of pyrethrum flowers at 82° yields as much total pyrethrins as sun-drying, provided drying is not prolonged. Earlier,[2] quick drying in the sun was found not to cause loss of pyrethrins, but prolonged drying in sun or shade did ; artificial drying at 60° but not at 50° caused slight loss of pyrethrins.

The experiments now described compare the pyrethrin content of fresh and of dried flowers by biological and chemical assays, so that any loss of pyrethrin can be related directly to insect toxicity. Pyrethrin content was estimated by spectrophotometric[3] and gas–liquid chromatographic[4] techniques.

Reduced insecticidal activity could reflect a loss by decomposition of one or more of the five known insecticidal constituents ; cinerin I, pyrethrin I, cinerin II, pyrethrin II and jasmolin II,[5] or an unknown constituent could be decomposed or lost.

Experimental

Materials and methods

The flowers.—For these experiments seed of pyrethrum (*Chrysanthemum cinerariaefolium*), obtained from Kenya, was sown in the autumn and the young plants were kept in pots in a heated greenhouse until they were set out in the open or in an unheated Dutch-light house in the following spring. Plants growing in the open grew longer and produced a bigger total yield of flowers than those under glass, but they remained in flower under glass slightly longer.

Flowers were picked when two or three rows of the disk florets were fully open, when their total pyrethrins content was usually between 1·4 and 1·8% of dry weight. Moisture content varied only between 76 and 78%. Flowers were picked by cutting the stems as close to the receptacle as possible.

Drying.—To retain the maximum insecticidal activity, flowers were dried to constant weight in a desiccator over silica gel at 15–18°, which took about 10 days. To determine the effects of drying temperature, the flowers were laid out on sheets of filter paper, not more than one flower deep, in ovens at 40, 80 and 100° and in a constant-temperature room in the dark at 25° (relative humidity 48%) (Table II).

Extraction.—Two methods of extraction referred to as ' hot ' and 'cold ' were used. For hot extraction, the dried flowers were first ground to a fine powder in a sealed ball mill (Dangoumau Analytical Grinder), which took 5 min. and produced a much finer powder than that prepared for commercial extraction. Powdered flowers (approx. 1 g.) in each batch were extracted in a Soxhlet apparatus with ' n-hexane fraction (low in aromatic hydrocarbons) ' (25 ml.).

Cold extraction was used in comparing fresh and dry flowers, because the water in fresh flowers made extraction with hexane impractical. Hexane was preferred to acetone where possible because acetone interfered with spectrophotometric analysis.

Cold extractions were made by grinding the flowers in the ball mill with 10% v/v methanol in acetone for 20 min. After being ground, the mixture was centrifuged and the liquid decanted, further extractions were made until the liquid obtained was colourless.

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Estimation of pyrethrins

(1) *Gas-liquid chromatography.*—The gas–liquid chromatographic (G.L.C.) method was that previously described[4] except that 74–85 mesh ballotini were used as support instead of 85–100 mesh and a flow rate of 6 l. of nitrogen per hour instead of 4 l.

(2) *Spectrophotometry.*—The spectrophotometric method described by Ward & Newham[3] was used to measure the intensity of absorption of the extract solutions in 'aromatic-free' n-hexane at 225 mμ, and total pyrethrins concentration was calculated on a specific absorption of 1166.[3]

(3) *Biological assay.—Tribolium castaneum* (Herbst.) (the rust-red flower beetle) and *Aedes aegyptae* (L.) (the yellow fever mosquito) were used to assess the biological activity of the flowers.

(a) *Tribolium castaneum.*—Unsexed adult *Tribolium castaneum* were used between 2 and 10 days after emergence from pupation. Using a microdrop applicator developed at Rothamsted, drops of 0·5 μl. of the extracts, or of appropriate serial dilutions, were placed on the dorsal surface at the base of the elytra. The beetles, unanaesthetised, were held at the end of a suction tube during the application. Four or five concentrations of the test insecticides were applied to two or three replicates each of 20 insects, which were then kept at 25°. Response to the treatment was assessed after 24 or 48 h. as failure to behave normally (i.e., unable to walk with co-ordinated movement) when transferred to a warm brass plate.[6]

(b) *Aedes aegypti.—Aedes aegypti* larvae, 3 days old, were treated in a slightly modified form of the Burchfield–Harris photomigration assay.[7] Larvae were put in tubes containing 15 ml. of water, and 1 ml. of a series of diluted solutions of each extract was added to separate tubes. Two lots of 20 larvae were treated at each of five concentrations. After 3 h. in the test solution, the larvae and solutions were transferred to the trough of the Burchfield–Harris apparatus containing 80 ml. of water. The larval response to strong, unidirectional light was then assessed in the usual way. This procedure avoids the necessity of keeping larvae during exposure in large volumes of water as in the original Burchfield technique and increases the sensitivity of the method.[8]

(4) *Direct biological assay of flowers.*—To check whether the extraction procedure, particularly the use of heat, affected the pyrethrins, a direct and sensitive biological method of assaying the flowers was needed. Assays were attempted by placing various species of beetle, which attack store products, in the ground flowers but were unsatisfactory. A technique using three-days-old *Aedes aegypti* larvae succeeded.

The quantity of flowers required to produce dosage response curves was too small to handle conveniently so the flowers were diluted with an inert powder. Several powders were tried and the method adopted was to dilute one part of the flowers in 5000 parts of chromatographic cellulose powder ; dilution and thorough mixing was obtained by grinding two or three times in the ball mill ; there were at least 50,000 particles in each mg. of powder. Weighed amounts (10–100 mg.) of this mixture were then put into tubes and 15 or 20 *A. aegypti* larvae in water (15 ml.) were added. After a suitable time (usually 3 h.) at 20° the response was assessed using the Burchfield–Harris method[7] already described.

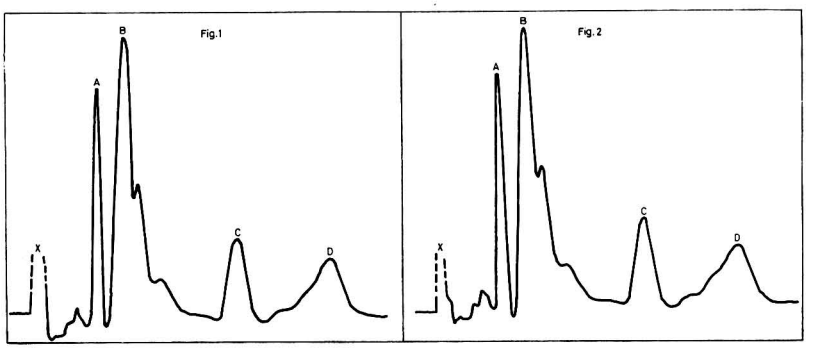
Biological assays were made solely to compare effects of different treatments of the flowers ; thus there was no need to relate results directly to absolute concentrations of pyrethrins and the tests were independent of the chemical assays. Equal volumes of the solutions used represented equal weights of flowers extracted. It was convenient to assign to these solutions a concentration value of unity. LD_{50} values therefore represent relative differences in the toxicities of equal weights of flowers.

(5) *Analysis of biological results.*—Biological assay values were obtained as dosage/response regression curves, which were analysed statistically using the probit transformation method of Finney.[9] Results are presented as median response dose and slopes of the curves together with standard errors. The lines did not depart significantly from parallelism (at 5% level) and results of χ^2 test for heterogeneity of each curve were not significant except for the figure in Table III, experiment II at 80°.

Results

(1) *Comparison of extracts of fresh and of dried flowers prepared at room temperature*

To make comparisons reliable with only 12–25 flowers, each flower was carefully bisected with a sharp scalpel along a diameter and the halves were adjusted to equal weight to give two equivalent batches of flowers. Batch I was then dried over silica gel before extraction by the cold method, and batch II was extracted immediately. Equal aliquots of extract from fresh and dried flowers were passed through the G.L.C. column alternately and no significant quantitative difference was detected between the two extracts (Figs. 1 and 2).



Gas–liquid chromatography trace of extract of fresh (FIG. 1, left) *and dried* (FIG. 2, right) *flowers prepared by 'cold' method*

Peak A = Cinerin I; B = pyrethrin I; C = cinerin II; D = pyrethrin II; X = solvent peak

Biological assays made with *T. castaneum* and *A. aegypti* larvae (Table I) also showed no significant difference in toxicity between the two solutions. For comparison, equivalent solutions obtained by extraction of fresh and of dried flowers were each assigned an arbitrary concentration value of unity.

Table I

Comparison of biological activity of extracts of fresh and dried flowers using Tribolium castaneum *adults and* Aedes aegypti *larvae*

		Log LD_{50} × 10	±Standard error	LD_{50}	Slope	±Standard error
I.	*A. aegypti*					
	Fresh	0·595	0·023	0·391	4·191	0·448
	Dry	0·627	0·024	0·424	4·135	0·444
II.	*T. castaneum*					
	Fresh	2·122	0·121	1·13	1·646	0·328
	Dry	1·932	0·066	0·85	2·353	0·390
III.	*T. castaneum*					
	Fresh	1·007	0·045	1·02	3·164	0·572
	Dry	0·995	0·029	0·99	4·973	0·768

LD_{50} values in arbitrary units as described in text

(2) *Comparison of extracts of flowers dried at different temperatures under controlled conditions*

Random samples of flowers of equal weight, obtained from bulk by a riffling procedure, were dried on trays to a constant weight at 25, 40, 80, 100 and 120° as described above. 'Hot' extracts in n-hexane were prepared and analysed spectrophotometrically for total pyrethrins and by G.L.C. (Table II) for pyrethrin I. The quantity of pyrethrin I was calculated by calibration of the detector of the G.L.C. instrument against pure pyrethrin I isolated from commercial extract.[10] When these determinations were made, no other pure constituent of the extract was available, but comparison of their peak areas showed that all constituents had approximately the same stability up to 80°.

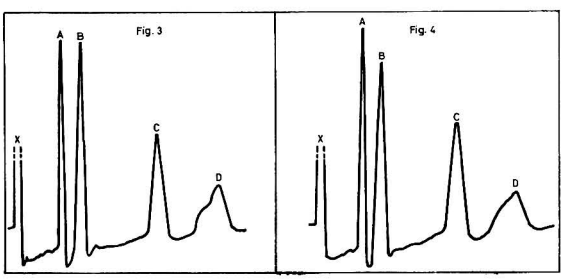
Table II

Concentration of pyrethrins and pyrethrin I in flowers dried at different temperatures

Drying temperature	Drying time	Moisture content, %	Concentration* total pyrethrins, % dry wt.	Concentration† pyrethrin I, % dry wt.
A. 100°	6 h.	77·2	1·99	0·45
80	13 h.	77·1	2·25	0·56
40	52 h.	78·1	2·28	0·56
25	7 days	78·0	2·18	0·53
B. 100°	5 h.	77·1	1·60	0 44
80	11½ h.	77·0	1·93	0·50
40	52 h.	77·8	2·16	0·55
25	7 days	77·5	2·08	0·49

* Estimated spectrophotometrically
† Estimated by gas–liquid chromatography

In another experiment gas–liquid chromatograms of extracts of flowers dried at 25°, 40° and 80° were similar, but after drying at 120° some pyrethrin I was lost; the peak for this constituent was smaller relative to cinerin I than it was after drying at the lower temperatures (Figs. 3–4). The trace for 40° is given as typical of those obtained at 25°, 40° and 80°.



Gas–liquid chromatography trace of extracts of flowers dried at 40° (FIG. 3, left) *and* 120° (FIG. 4, right) *prepared by the 'hot' method*
Peaks as in Figs. 1 and 2

Biological assays, with *T. castaneum* adults, showed very little difference between the potency of solution derived from flowers dried at different temperatures up to 80° (Table III). There is satisfactory agreement between these figures and those obtained chemically (Table II).

Table III

Comparison of biological activity of extracts of flowers dried at 100, 80, 40 *and* 25° *using* Tribolium castaneum

	Log LD_{50} × 10	±Standard error	LD_{50}	Relative potency	Slope	±Standard error
I. 100°	1·29	0·155	1·197	100	2·727	0·879
80	0·98	0·042	0·95	127	3·972	0·730
40	0·86	0·026	0·73	164	4·943	0·727
25	0·95	0·032	0·89	134	4·894	0·842
II. 100°	0·78	0·053	0·61	100	4·267	1·205
80	0·69	0·022	0·49	123	5·526	0·715
40	0·67	0·024	0·47	128	4·869	0·658
25	0·71	0·024	0·51	119	4·749	0·642
III. 100°	0·84	0·039	0·70	100	3·594	0·557
80	0·64	0·051	0·43	161	4·799	1·104
40	0·65	0·029	0·45	155	4·978	0·667
25	0·66	0·027	0·46	153	5·611	0·783

LD_{50} in arbitrary units derived as described in text

(3) *'Direct' biological assay of flowers*

Because *T. castaneum* was not sufficiently sensitive, ground flowers were assayed as already described with *A. aegypti* larvae to compare the biological activity of fresh flowers with those dried at 80°, because previous results suggested this temperature does not harm the pyrethrins, and at 120°. Twelve flowers were each cut on a diameter to produce equal weights and thus two equivalent batches. Batch I was immediately ground and diluted with cellulose and batch II was dried in the oven. Table IV shows no significant loss of activity in the flowers dried at 80° but some loss at 120°.

Table IV

Comparison of biological activity of fresh flowers and of flowers dried at 80° and 120° using Aedes aegypti *larvae*

		Log LD_{50}	±Standard error	LD_{50}, mg.*	Relative potency	Slope	±Standard error
I.	Fresh flowers	1·760	0·044	57·4	100	1·831	0·290
	Dried at 80°	1·735	0·038	54·3	95	2·043	0·292
II.	Fresh flowers	1·234	0·061	17·1	100	2·295	0·395
	Dried at 120°	1·454	0·040	28·4	60	2·946	0·403

* Expressed as mg. of cellulose/flowers mixture

Conclusion

The G.L.C. traces (Figs. 1 and 2) show that the amounts of the known biologically active chemicals in extracts from fresh and dry flowers are very similar, both qualitatively and quantitatively in so far as they can be compared by this technique. However, it must be clearly recognised that active constituents of high molecular weight would be retained on the column and also that insecticidal constituents which do not give peaks using an electron capture detector may also affect the activities of the extracts under comparison. The activity of the two extracts was therefore compared by bioassay (Table I) and again no significant difference was found. It can, therefore, be concluded that drying at laboratory temperature (approximately 15–18°) does not change the chemical constitution of the extract in ways that affect its insecticidal activity, and that there would be no commercial advantage in extracting the fresh flowers.

The second series of experiments (Tables II and III) show that drying temperatures up to 80° have little or no effect on the pyrethroid content and insecticidal activity of extracts from dried flowers. It might therefore be advantageous to shorten the drying time by applying heat, but out of direct sunlight so that free-radical oxidation reactions are less likely to decompose the insecticidal constituents. Figs. 3 and 4 show that, although some of all the pyrethrins was lost at 120°, the principal loss is of pyrethrin I and is not pyrethrin II.

Acknowledgments

This work forms part of a collaborative research programme between Rothamsted Experimental Station and the Tropical Products Institute. One author (J. H. S.) acknowledges the receipt of a grant from the Pyrethrum Boards of Kenya and Tanganyika. The authors thank our colleagues in the Insecticide departments of both Institutes for their help and suggestions.

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Received 24 August, 1964

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CARBONYL COMPOUNDS AND THE NON-ENZYMIC BROWNING OF LEMON JUICE

By K. MARY CLEGG and A. D. MORTON

During the non-enzymic browning of lemon juice the build-up of carbonyl compounds is believed to be associated with the breakdown of ascorbic acid. This has been studied by separation of their 2,4-dinitrophenylhydrazine derivatives on thin-layer chromatograms, and by spectroscopic examinations. The results have been compared with those from model systems. Twelve carbonyls were found in browned samples, half of these have been tentatively identified and the remainder have been classified as aldehydes or ketones. Work with model systems confirmed that the α,β-unsaturated carbonyls are potent browning agents and also that dicarbonyls of the glyoxal type make a contribution to browning in the early stages. The rôle of sugars in these systems has been considered.

Introduction

In a previous paper[1] the non-enzymic browning of lemon juice has been related to conditions such as pH, ascorbic acid and amino-acid contents. Sugar–amino-acid reactions, leading to a build-up of reactive carbonyl compounds, seemed unlikely to be the main contributors to the formation of melanoidin pigments because of the high acidity of the system. Under such conditions a more probable explanation was that the carbonyl compounds, which subsequently react with amino-groups and polymerise to give brown pigments, originated from the oxidation of ascorbic acid.[2, 3]

Studies on the mechanism of non-enzymic browning in natural products and model systems, whether by sugar–amino-acid condensation[4] or ascorbic acid decomposition,[5] have shown the development of furfural. This observation led to the idea that furfural and related compounds were the active carbonyls responsible for the formation of melanoidin pigments. More recent work of McWeeny & Burton[6] supports the hypothesis that furfural compounds are relatively inactive and tend to accumulate in the system while other more reactive carbonyls, which have formed as a result of oxidation and fragmentation of larger molecules, are mainly responsible for the development of browning.

An investigation of the build-up of all carbonyl compounds during the browning of lemon juice and simulated model systems has therefore been undertaken. An attempt has been made to classify some of these breakdown products with a view to following the reactions occurring during the non-enzymic browning of an acidic product with a high ascorbic acid content such as lemon juice (pH 2·5). Carbonyl compounds were extracted from the systems, both before and after reaction with various specific reagents for terminal groups, as their 2,4-dinitrophenylhydrazine derivatives. These complexes were then separated by thin-layer chromatography. The browning potential of different classes of carbonyls when added to these model systems has also been investigated.

Experimental

Materials

Commercial samples of pasteurised lemon juice, preserved with sulphur dioxide at a level of 350 p.p.m., of the following approximate composition per 100 ml. were used: citric acid 5·8 g., ascorbic acid (fortified) 100 mg., nitrogen 70 mg. and sugars 3 g. Samples at various stages of browning under aerobic conditions were obtained by incubating the juice, at 37° for different lengths of time, in filled 1-oz. screw-top bottles with two 1-mm. dia. holes in the caps. Freshly-squeezed lemon juice was also examined.

Preparation of 2,4-dinitrophenylhydrazine derivatives (DNPH's)

Preliminary extraction of the carbonyls with ethyl acetate was found to give no advantage over the simpler technique of precipitation by the direct addition of saturated 2,4-dinitrophenylhydrazine, in acidified carbonyl-free methanol, to the test solution. The washed and dried precipitate was usually dissolved in carbonyl-free dioxan for application to thin-layer chromatographic plates. No heat treatment was required by this method thus affording minimal loss of carbonyl compounds.

Thin-layer chromatographic technique (TLC)

In agreement with other workers[7, 8] the neutral adsorbent Kieselgel G in the particle size range 5–25 mμ was found to be successful in the chromatography of DNPH's. In the preliminary investigations the solvent system benzene/light petroleum (60–80°) 3 : 1 recommended by Dhont & de Rooy[9] was used but unresolved material remained at the origin indicating more polar solvents were required. A multiple development technique was then evolved in which the plates were first run in benzene/ethyl acetate 1 : 1, of intermediate polarity, for 6 cm., secondly in the non-polar solvent benzene/light petroleum 3 : 1 for 10 cm., and finally in the highly polar solvent ethyl alcohol/ethyl acetate 3 : 2 for 3 cm. This technique was found to give reasonably good separation of all the DNPH's obtained from a browned sample of lemon juice. Unfortunately it was impossible to separate the DNPH's of glucose and ascorbic acid which ran as one spot.

Results

Development of carbonyl compounds in lemon juice

DNPH's were prepared from fresh juice immediately after squeezing the lemons, from untreated commercial lemon juice, and from commercial lemon juice which had been incubated for 2, 3, 6, 9, 12, 15, 19 and 26 days, and then separated by TLC (Fig. 1). Usually, a standard mixture of DNPH's of ascorbic acid, citral, furfural, hydroxymethylfurfural and diacetyl was run for comparison since R_F values alone cannot be relied upon with TLC.

The fresh lemon juice showed only three bands which, by comparison with an ascorbic acid standard, were thought to be glucose/ascorbic acid, dehydroascorbic acid and 2,3-diketogulonic acid (Fig. 2); ascorbic acid dissolved in de-ionised water is known to be unstable.[10] More than three bands were found in the commercial lemon juice, indicating that the first stages of non-enzymic browning had already begun. After 3 days' incubation, nine bands were detectable and after 26 days' incubation at least twelve carbonyl bands could be seen. An interesting observation was the development of a very pink band, compared with the orange colour of all the other bands, in the samples incubated for 2 and 3 days, which had disappeared by 6 days incubation. From the position of this spot on the chromatogram and its colour, it seemed likely to be α-ketogulonic acid, a breakdown product of ascorbic acid (Fig. 2) which is known to form a red DNPH; a standard sample of α-ketogulonic acid was not available for confirmation. Another feature was the late appearance of a band corresponding to hydroxymethylfurfural which was not seen until after 9 days' incubation, although a trace of furfural could be detected in the original commercial juice and increased in amount with incubation. A standard sample of methylfurfural was prepared from rhamnose[11] and converted to the DNPH which moved fractionally ahead of furfural, but it was not found in any of the lemon juice samples.

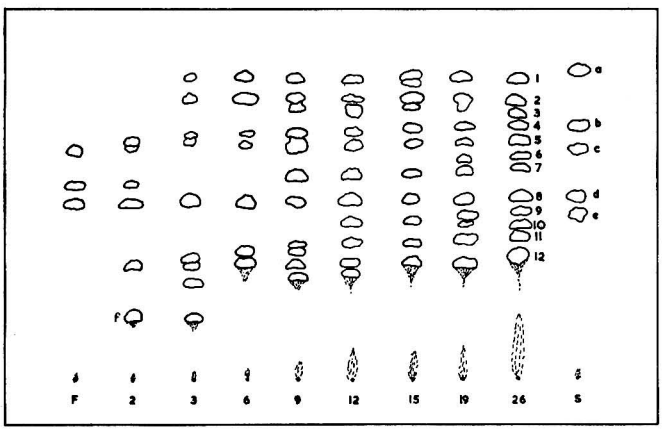

FIG. 1.—*Thin-layer chromatogram of DNPH's of fresh lemon juice (F), commercial lemon juice incubated 2–26 days, and a standard (S)*

a = citral; b = furfural; c = diacetyl; d = ascorbic acid; e = hydroxymethylfurfural; f = pink spot, α-ketogulonic acid?

Classification of carbonyl compounds found in browned lemon juice

The lemon juice which had been incubated for 26 days was taken as the standard material from which to classify tentatively the carbonyl compounds. The twelve bands on the chromatogram were numbered starting furthest from the origin.

(i) *Colour of DNPH's in alcoholic sodium hydroxide.*—Stadtman[12] noted a connexion between adsorption and the colour of DNPH's in alkaline alcoholic solution. The twelve bands were scraped individually off the plate and transferred to a small volume of ethyl alcohol which was made alkaline with 40% sodium hydroxide. The findings are summarised in Table I and show

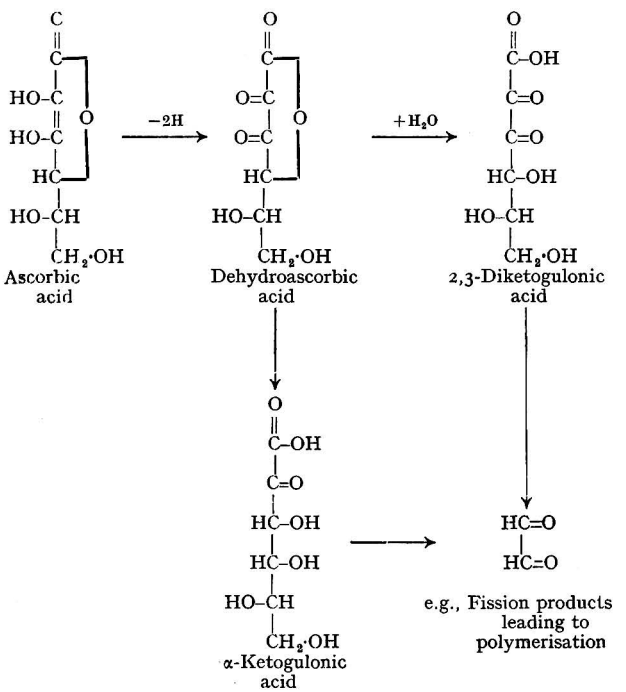

FIG. 2.—*Breakdown of ascorbic acid*

Table I

Classification of DNPH's of browned lemon juice separated by TLC

Band no. (see Fig. 1)	Colour of DNPH in alkaline alcoholic solution*	Formation of bisulphite complex†	Reaction with Tollens reagent‡	Comparison with standard DNPH's	Tentative carbonyl classification
1	pink	yes	positive	—	aldehyde
2	lilac	no	none	methylglyoxal	—
3	,,	no	none	glyoxal	—
4	pink	yes	positive	furfural	—
5	deep pink	no	none	—	ketone
6	lilac	yes	positive	—	aldehyde
7	blue	yes	,,	—	aldehyde-dicarbonyl ?
8	,,	no	none	glucose/ascorbic acid	—
9	violet	yes	positive	hydroxymethylfurfural	—
10	blue	yes	none	acetylacetone	—
11	,,	yes	,,	—	methylketone-dicarbonyl ?
12	,,	yes	positive	—	aldehyde-dicarbonyl ?

* Blue colour indicates carbonyls capable of forming bis-hydrazones

† No complex formation shows that these carbonyls are ketones other than methyl ketones; glucose is an exception as it also does not complex with bisulphite

‡ No reaction indicates that these carbonyls are ketones

that the colour was blue for the bands near the origin and pink for those less strongly adsorbed; the exceptions were bands 2 and 3 which were bluish. It is known that compounds such as glucose, glyoxals and dicarbonyls which are capable of forming bis-hydrazones give blue colour changes in alcoholic sodium hydroxide.

(ii) *Specific reagents.*—The browned lemon juice was treated with the following reagents which react characteristically towards certain types of carbonyls. By this means it should be possible to classify the DNPH bands; for example, after preliminary reaction of the lemon juice with a reagent which specifically combines with the aldehyde group, any DNPH bands which are subsequently developed must be of ketonic origin. However, it was realised that the presence of unsaturated ketones, for example, might confuse some of the observations.

(*a*) *Saturated sodium bisulphite solution.* This reagent combines with all aldehydes and methyl ketones; glucose is an exception as it fails to form a bisulphite complex. Bands 2, 3, 5 and 8 for DNPH's were obtained which indicated that these carbonyls are probably ketones, other than methyl ketones.

(*b*) *Tollens reagent* (ammoniacal silver nitrate). This reagent is specific for aldehydes. Bands, 2, 3, 5, 8, 10 and 11 for DNPH's were found from the ketones in the lemon juice which had not reacted with the silver nitrate. These results for reagents (*a*) and (*b*) indicate that bands 10 and 11 may be methyl ketones.

(*c*) *Fehling's solution.* This reagent is also specific for aldehydes but proved unsuccessful because heat is necessary for the reaction and there was loss of volatile carbonyls.

(*d*) *Thiobarbituric acid.* This reagent has been used to identify aromatic and α,β-unsaturated aldehydes from other carbonyls;[13] for example, substances of the furfural type give precipitates. However, side reactions with glyoxals can occur[14] and no conclusive evidence was obtained with thiobarbituric acid.

(*e*) *Isonicotinyl hydrazide.* This reagent combines with Δ^4-3- and Δ^{1-4}-3-ketones.[15] The results for this trial were disappointing, but bands 2 and 3 were missing after this treatment, indicating that they may be ketones of this nature.

(iii) *Comparison with standard DNPH's.*—A large number of pure recrystallised DNPH's were prepared from commercial carbonyl compounds, subjected to TLC, and compared with lemon juice incubated for 26 days.

Carvone, which has been isolated from grapefruit concentrates,[8] was not present in lemon juice, nor was α-ketoglutaric acid which has been postulated as an oxidation product of reductic acid.[16]

A series of homologous methyl ketones, acetone, β-ionone, diacetyl and acetylacetone showed that acetylacetone moved in a similar manner to band 10 in lemon juice. The reactions

with bisulphite and Tollens reagent had indicated that band 10 probably was a methyl ketone, also a dicarbonyl had been expected from the blue colour in alcoholic sodium hydroxide.

A series of straight-chain aldehydes from acetaldehyde to heptaldehyde, crotonaldehyde, citral, methylglyoxal, glyoxal, furfural and hydroxymethylfurfural indicated that the last four were present in browned lemon juice corresponding to bands 2, 3, 4 and 9, respectively. From the previous tests the aldehyde nature of bands 4 and 9 was expected; bands 2 and 3 were thought to be ketones, but their α,β-dicarbonyl structure could have interfered with the usual chemical reactions. Citral was found to be the major component of lemon essence but its concentration in the juice was too low to be detected by TLC and it did not constitute one of the twelve bands.

Glucose and ascorbic acid gave DNPH's which moved together equivalent to band 8. As ascorbic acid is known to disappear after 3 days under these standardised conditions, band 8 in the juice which had been incubated for 26 days must be due to glucose. This is in agreement with the bisulphite finding but not with the DNPH derivative for glucose which was observed after treatment with Tollens reagent but would be expected to be missing. Ascorbic acid yielded two subsidiary DNPH bands which were taken to be dehydroascorbic acid and 2,3-diketogulonic acid, and corresponded to the three bands found in freshly squeezed lemon juice. However, these bands have also disappeared after prolonged incubation and are not included in the 12 bands under observation. Similarly, the transient pink band found after a short incubation, and believed to be α-ketogulonic acid, cannot be numbered in the 12 carbonyls of 26-day-incubated juice.

The results of this attempted classification are summarised in Table I which shows that six of the 12 carbonyls separated from browned lemon juice have been tentatively identified. The remaining six have been classified as aldehydes or ketones.

Browning of model systems

The evidence, so far, indicated that during the non-enzymic browning of lemon juice both aldehydes and ketones were found. The ultra-violet spectrophotometric analysis of the separated DNPH bands[17–19] showed that the absorption maximum in the near visible region gradually broadened and shifted from 370 mμ to 420 mμ, with concurrent decrease in relative absorption, from band 1 to band 12. These characteristics indicated increasing complexity of the DNPH'S due to conjugated unsaturation of the parent carbonyl.[19, 20] The browning potential of different classes of carbonyl compounds therefore was investigated using model systems.

In the first instance the browning of ascorbic acid alone, and in the presence of other components of the model system (Table II), was determined before investigating the effect of added carbonyls. All solutions were adjusted to pH 2·5 and withdrawn from the incubator at 37° after varying lengths of time. The degree of browning in the visible range was measured at 400 mμ[1] and the comparative build-up of conjugated unsaturated carbonyl compounds was assessed by absorption in the ultra-violet range at 285 mμ.[19]

Visually, ascorbic acid alone gave little browning even after 30 days' incubation; addition of citric acid and citric acid + amino-acids gave increased browning. The complete model system D gave considerable browning, but with a plateau between 15–22 days' incubation followed by a further increase in pigmentation with longer incubation. Browning of the lemon juice control was linear throughout and therefore appeared greater than model system D.

In the ultra-violet range, readings for ascorbic acid alone increased up to 10 days' incubation and then showed a slight falling off in carbonyl content. Model systems B and C followed a similar pattern but the maximum was not reached until 17 days after and the presence of amino-acids had a depressing effect. Model system D behaved like ascorbic acid alone but the falling off ceased by 17 days when another increase in carbonyls began, perhaps due to the presence of glucose. With the lemon juice control a carbonyl content approximately three times that ever reached during 30 days' incubation of any of the model systems was obtained after 3 days; between the 3rd and 10th days there was a slight increase after which a linear increase took place for 8 days when the readings remained constant for the rest of the incubation period. These findings with model systems suggested that amino-acids were implicated in ' mopping up '

Table II

Composition of model systems

Model system	Ascorbic acid, %	Citric acid,%	Amino-acids,* %	Glucose, %
A	0·1	—	—	—
B	0·1	5·0	—	—
C	0·1	5·0	0·44	—
D	0·1	5·0	0·44	3·0

* Hydrolysed casein

carbonylic fragments; also, since ascorbic acid/citric acid systems can brown, some of the pigments must arise from polymerisation of the primary fragments.

Development of carbonyl compounds in model systems

DNPH's of carbonyl compounds which had developed during 13 days' incubation of model systems B, C and D were investigated by TLC. In model system B, containing only ascorbic acid and citric acid, roughly the same bands for 12 DNPH's could be seen as were found in browned lemon juice. In model system C which contained additional amino-acids, the only bands which were detectable were the two or three moving near the solvent front which indicated that the other free carbonyl groups had been blocked by the amino-acids at this stage of browning. The presence of glucose in model system D almost restored the picture to that of ascorbic acid/citric acid only, except that the faster-moving bands which remained in model system C were relatively faint.

Browning of model system + carbonyl compounds

Model system D of definable composition, rather than lemon juice, was taken as the basal medium for the investigation of the browning capacity of various carbonyl compounds, even though this system had failed to produce the same intensity of browning, and had developed less than a third of the carbonyl material found when lemon juice was incubated under identical conditions. The following carbonyls, obtained commercially and without further purification, were chosen as representative of different classes and were incubated at 1 millimolar concentrations in model system D: acetaldehyde (aliphatic aldehyde), glyoxal (bis-aldehyde), furfural, crotonaldehyde (α,β-unsaturated aldehyde), methyl isobutyl ketone, diacetyl (diketone), hydroxymethylfurfural, methyl vinyl ketone (α,β-unsaturated ketone).

The results over a 21-day incubation period showed that acetaldehyde appeared to inhibit the browning of model system D, and methyl isobutyl ketone had no effect. Carbonyls of the type which would arise from fragmentation of larger molecules, such as glyoxal and diacetyl, accelerated browning in the early stages and then levelled off. The furfurals were potent additives giving a marked linear increase in browning over the incubation period; browning of the model system + furfural was approximately equal to that of lemon juice and slightly greater with hydroxymethylfurfural as the additive. The straight-chain α,β-unsaturated carbonyls, crotonaldehyde and methyl vinyl ketone, gave the most marked increases in browning, especially the latter; by the 15th and 10th days, respectively, the absorption at 400 mμ was double that of the model system alone after 25 days' incubation. The rate of browning of model system D + methyl vinyl ketone was even greater than the lemon juice control. No latent period was observed in the presence of the additives except for the plateau reached with the 'fragmentary' carbonyls.

Discussion

Compared with freshly squeezed lemon juice the commercial sample was in the first stages of non-enzymic browning. The one or two extra DNPH bands in the commercial juices were near the origin of the TLC plates and the blue colour in alcoholic sodium hydroxide of these more strongly adsorbed compounds would indicate dicarbonyls. Also, bands 2 and 3, which were tentatively identified as the dicarbonyls methylglyoxal and glyoxal and gave a bluish/pink colour in alcoholic sodium hydroxide, were clearly visible after 2 days' incubation. This early

appearance of dicarbonyls, and especially those of a 'fragmentary' nature, indicates their important association with the initiation of non-enzymic browning. Supporting evidence comes from the browning capacity of carbonyls added to a model system in which glyoxal gave the greatest initial boost to the development of brown pigments, even though the potential was not maintained compared with other carbonyls.

In agreement with Burton *et al.*[21a] the α,β-unsaturated carbonyls were found to be the most potent browning agents and maintained a linear increase over the 21-day incubation period. These workers have also suggested that furfural and hydroxymethylfurfural are relatively inert in the browning of sugar–amino-acid model systems but may play a more important part in the browning of fruit juices.[21b] The present investigation has shown that in ascorbic acid/citric acid/amino-acids/glucose model systems these cyclic carbonyls do in fact have a high browning capacity, but in the natural product although furfural was detected in the early stages hydroxymethylfurfural was late in forming.

The fluctuations in the carbonyl content during browning were indicated by the readings at 285 mμ of model systems. In solutions of ascorbic acid alone there was no linear increase; instead, a maximum carbonyl development was recorded after 10 days' incubation, after which this level must have been reduced by polymerisation. In the presence of citric acid and citric acid + amino-acids, the maxima were delayed another 7 days. Although the amino-acids depressed the carbonyl level as measured by absorption at 285 mμ, absorption was increased in the visible range at 400 mμ. This indicated that carbonyl/amino interaction, in addition to polymerisation, had taken place and eliminated some of the free carbonyl groups.

Carbonyl development in model systems containing glucose was similar, but at a reduced level, to that of lemon juice except that in the latter the initial maximum was earlier, probably due to the lemon juice having undergone the first stages of browning before the incubation experiments began. The presence of glucose as a contributor to the build-up of carbonyls appeared not to take effect until after 18 days' incubation. Under acid conditions hydroxymethylfurfural can be formed from sugars.[22, 23] Possibly in non-enzymic browning mainly due to ascorbic acid breakdown, glucose makes a separate delayed contribution to α,β-unsaturated carbonyl development. Burton *et al.*[21a] have found that straight-chain saturated carbonyls retard browning in glucose/glycine systems at pH 6·5; a similar result has now been shown with acetaldehyde in a model system at pH 2·5.

Considering that the test carbonyls were added to a model system at a low concentration and that the α,β-unsaturated carbonyls produced such striking increases in browning, it is not surprising that the 'complete' model system failed to reproduce the browning capacity of lemon juice. The natural product need only contain a trace of carbonyl to give it this lead. Citral could be a factor contributing to the difference between lemon juice and the model system, although its concentration was so low as to be undetectable by TLC; however, addition of 0·05% citral to lemon juice was not found to augment the rate of browning. Other trace substances must account for the discrepancy; the rôle of trace elements has not been included in this study.

After 12 days' incubation under aerobic conditions at 37°, 12 DNPH's could be separated by TLC and the pattern remained constant with further incubation up to 26 days. Stadtman[12] also isolated twelve carbonyls from browned apricot concentrates, which is of interest since apricots are less acidic (pH 3·5) and browning of this product is generally attributed to sugar–amino-acid reactions. Only furfural and hydroxymethylfurfural were identified and it may be coincidental that the number of carbonyls was the same as found in the present investigation, rather than proof of similar chemical reactions in apricots. In this study an attempt has been made to identify more of the carbonyls as their DNPH's which were precipitated from lemon juice (Table I). The blocking of specific carbonyl groups with various reagents has proved to be a useful technique. The inconclusive results with isonicotinyl hydrazide were not unexpected since it has been primarily used with more complex ketosteroid compounds;[15] the two carbonyls which reacted with this reagent could have been an artefact as they were subsequently thought to be methyl glyoxal and glyoxal. The general classification of the 12 DNPH's with sodium bisulphite and Tollens reagent was shown to be generally correct for some of the carbonyls identified by comparison with standard DNPH's. All of the DNPH's

which have been identified and gave a bluish colour in alcoholic sodium hydroxide are carbonyls which form bis-hydrazones, and it is likely that unidentified bands 7, 11 and 12 are also dicarbonyls. Fractionation of the carbonyls in browned lemon juice and incubated model systems into volatile and non-volatile groups could possibly assist their identification.

The evidence from this investigation shows that carbonyls are the reactive compounds in the non-enzymic browning of an acidic product such as lemon juice. Hodge[24] and Burton & McWeeny[23] also stress the rôle of carbonyls in sugar–amine systems under non-acidic conditions. The melanoidin end-products of the two different types of system may therefore be similar.

Acknowledgment

The authors wish to thank Beecham Food & Drink Division Ltd. for their practical interest in this investigation.

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Received 5 October, 1964

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THE EFFECTS OF CERTAIN SALTS ON DOUGHS

By RUTH BENNETT and J. A. D. EWART

The effect of sodium lactate and some other salts on the extensometer curves of wheaten flour doughs is usually to increase the breaking stress and reduce the extensibility. Anions which can crosslink by means of secondary forces appear to have more effect than the chloride ion. The effects of salts on dough become more marked as the protein content decreases. This is probably because low-protein flours contain a higher proportion of salt-soluble proteins which adsorb ions strongly and take up an extended configuration in salt solutions, thus increasing the intermolecular cohesion of the network.

Introduction

The studies of the effects of acids on dough described earlier[1] have been extended to observe the action of certain salts.

Experimental

The flours listed in Table I were examined.

Table I

Characteristics of flours used

Flour	Protein content of moist flour, %	% of protein extractable by 0·8% NaCl	Moisture content, %	Water added to 280 g. flour
English biscuit wheat	7·9	(13·2)*	14·7	132
English weak	8·7	12·0	14·8	138
English medium	9·1	11·2	14·4	138
Scottish winters	9·3	11·4	14·1	148
Bread	11·7	8·8	15·3	158
Strong	13·3	7·8	14·8	163

* This figure was not determined on the actual batch of flour used for the experiments but on a sample of similar specification from the same source with a protein content of 8·0%.

Doughs were mixed in a Majorpin mixer for 4 min. with 280 g. of flour and an amount of water previously determined by the Simon Water Absorption Meter. Doughs were also made with the same volume of a solution of the desired additive after the pH had been adjusted to 6·5 with 5N-sodium hydroxide or conc. hydrochloric acid. The doughs were tested on the Simon Extensometer by the 1 h. method. The following additives were used on the 'bread' and the English biscuit wheat flours: sodium chloride, sodium glycollate, sodium lactate, glycine, sarcosine (*N*-methylglycine), sodium mandelate and sodium β-hydroxybutyrate. Only sodium lactate and sodium chloride were used on the other four flours.

Results

Considerable scatter is inevitable with the rheological techniques normally used in testing doughs. In the figures smooth curves and, if possible, straight lines are drawn through the points so that the general trend can be followed. This practice tends to eliminate kinks which seem to have no significance. The units on the graphs for resistance (R) and extensibility (E) are the arbitrary ones given by the Extensometer. Figs. 1 and 2 show that sodium mandelate reduces E for the bread and the English biscuit flours but the other additives only reduce E for the low-protein flour, the hydroxy-aliphatic anions having a more pronounced effect than the chloride. The R curves have not been plotted because, as shown previously,[1] a plot of the product R $\times$ E is more meaningful, giving a rough measure of breaking stress, whereas R takes no account of the thickness of the test-piece. It appears from Figs. 3 and 4 that, while sodium mandelate had a slight toughening effect on the doughs, the other additives increased the breaking stress, the effect being much greater in the case of the biscuit flour. The hydroxy-aliphatic anions had a particularly marked effect in the case of the biscuit flour.

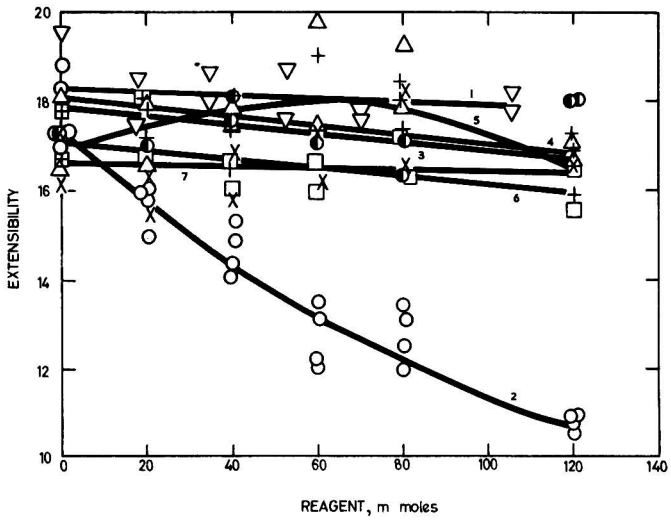

FIG. 1.—*Extensibility of bread flour dough vs. mmoles of reagent per* 280 *g. of flour*
1, ▽ Na β-hydroxybutyrate; 2, ○ Na mandelate; 3, ◐ NaCl; 4, △ Na glycollate; 5, + Na lactate; 6, □ glycine; 7, × sarcosine

In order to confirm whether the difference between the two flours was significant, four more were tested using only sodium lactate and sodium chloride. These tests (Figs. 5 and 6) confirmed that both salts reduced E for the low-protein flours, lactate having more effect than chloride, and caused a small increase in E in the high-protein flour. The breaking stress of the four flours was increased by the salts, lactate again being more effective than chloride, and the weaker flours showing the biggest change.

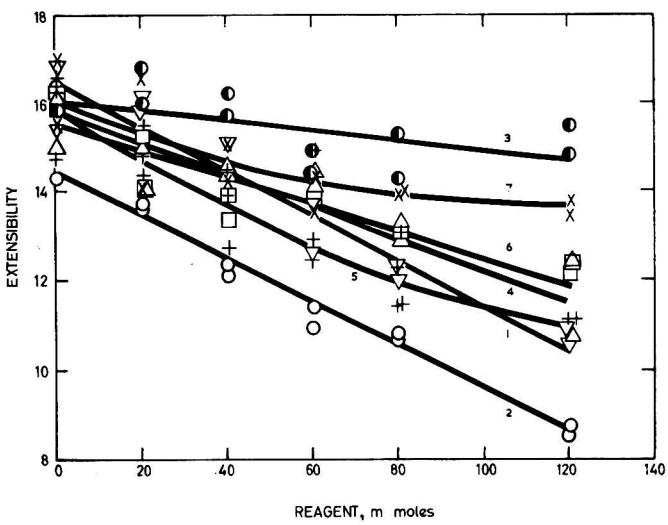

FIG. 2.—*Extensibility of English biscuit dough vs. mmoles of reagent per* 280 *g. of flour*
(legend as Fig. 1)

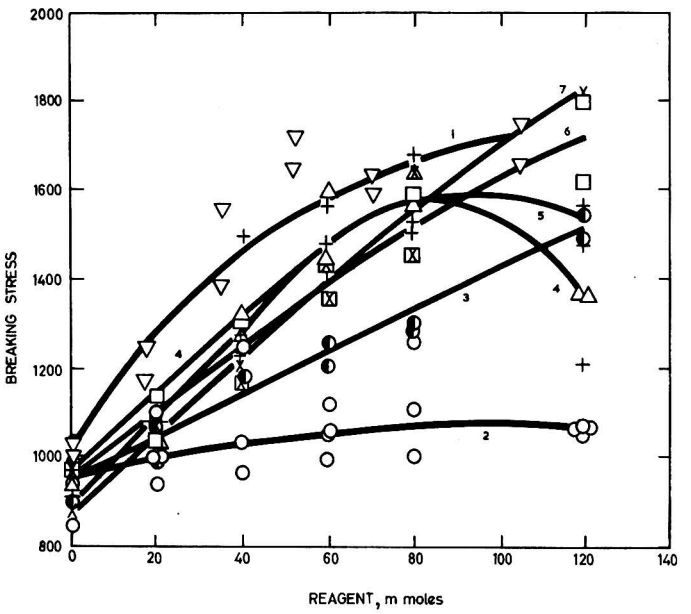

FIG. 3.—*Breaking stress of bread flour dough vs. mmoles of reagent per* 280 *g. of flour*
(legend as Fig. 1)

Discussion

Preliminary remarks

In a dough sample prepared from 280 g. of flour the quantities of substances that would be associated with 1000 g. of water are set out in Table II.

Calculations based on the data of Greer & Stewart[2] assuming a damaged starch content of 3% suggest that starch will absorb 61% of the water in the case of the English biscuit flour and 43% in the case of the strong flour. On this basis some 20–30 moles of water will be associated with the protein and the additives, which means that though the system is concentrated it can still be considered as a solution. The ratio of protein to associated water is approximately the same in the case of the ' strong ' and English biscuit flours, these two representing the extremes of protein content and water absorption.

The additive concentration will vary by ~20% owing to the range of water absorptions, but the effect on the breaking stress varies over 10 times this range. The action of the anions is probably more important than that of the sodium ions since the latter are a common factor in every case save those of the amino-acids. Anions tend to be preferentially adsorbed compared with cations, and hydroxy-aliphatic anions can form hydrogen bonds at numerous sites on proteins. Moreover it is conceivable that in addition to the normal screen of water molecules surrounding the ion due to ion–dipole interaction, many polarisable sites on the proteins could interact with the anions by penetrating into the secondary solvating layer if not into the first.

Table II

Type	Starch (as moles of anhydro-glucose)	Protein (as moles of anhydro-amino-acid)	Additive (moles)	Water calc. to be associated with protein (moles)
English biscuit	7·7	1·1	0–0·69	22
Strong	5·6	1·6	0–0·59	32

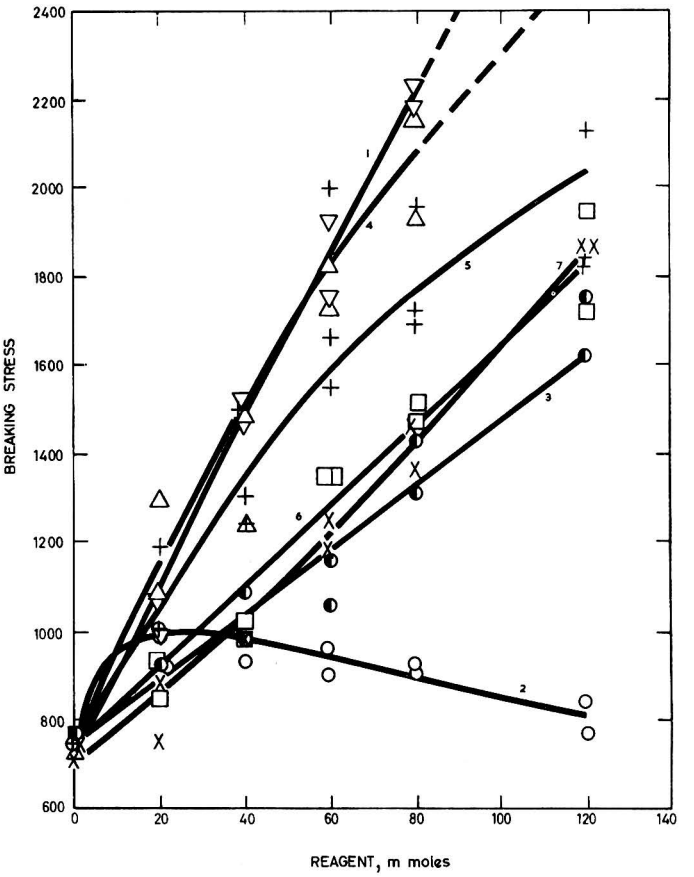

FIG. 4.—*Breaking stress of English biscuit dough vs. mmoles of reagent per* 280 *g. of flour*
(legend as Fig. 1)

Effect on breaking stress

As described earlier,[1] when a dough is stretched to the limit of breaking the protein molecules have been uncoiled to a considerable extent and tend to be arranged with their peptide chains in the direction of stress. The stress is determined by the intermolecular adhesion, made up of salt links, ion–dipole bonds, polar interactions, hydrogen bonds and van der Waals forces, which is just unable to prevent molecular slippage taking place on a large scale. Cross-linking agents such as the hydroxy-aliphatic anions and zwitterions will therefore increase the value of the breaking stress (cf. Figs. 3, 4 and 6). The bulky mandelate ion will probably interfere with the hydrogen-bonding of its own hydroxyl group, although its aromatic group could form hydrophobic bonds with non-polar side chains. The free energy of the changes from native to denatured forms of proteins are chiefly determined by the hydrophobic bonds according to Tanford.[3] Any strengthening due to hydrophobic crosslinks with mandelate ions, however, will be countered by interference with the close alignment of peptide chains as they extend under stress, hence the net effect will be small (cf. Figs. 3 and 4).

Although the chloride ion could act as a difunctional reagent accommodating two dipolar groups in its outer solvation shells this is less likely because, owing to the short radius, the two sites to be crosslinked would have to be present in a smaller volume than in the case of the organic ions.

Influence of protein content

The lower-protein flours contain a higher proportion of protein soluble in 0·8% sodium chloride solution (equivalent to the addition of ~25 mmoles of sodium chloride in the dough tests). Gluten proteins precipitate above an ionic strength of 0·04[4, 5] corresponding to a level of addition of 7–8 mmoles of sodium chloride. Since the gluten proteins are insoluble in the presence of the salts they will favour compact forms minimising protein-solvent contacts. The salt-soluble proteins on the other hand will favour elongated configurations giving a maximum number of protein-solvent contacts. Upon mixing the dough the shearing stresses will tend to uncoil the proteins but the insoluble ones will usually resume compact forms on resting, giving a network which is interlaced by the extended soluble proteins. The latter contain more basic amino-acids than the gluten proteins[6] and will more strongly adsorb anions thereby participating more fully in inter-chain bonding. Hence doughs will have a higher breaking stress in the presence of salts as the proportion of salt-soluble protein increases, i.e., as the total protein content of the flour decreases (Table I). The properties of such a network are very sensitive to the number of crosslinks, and so a small change in the proportion of protein capable of reacting more intensely with ions has such a marked effect (Figs. 3, 4 and 6).

Effect of extensibility

It seems reasonable to conclude that the extensibility will be determined chiefly by the reserve of coiled and folded molecules which give the material capacity to yield, but that it will also be influenced by interchain forces. If the latter increase to a marked extent then there will be an increase in the number of sites where especially favourable orientation enables portions of coiled or folded peptide chains to withstand shearing stresses.

The low-protein doughs contain the highest proportion of salt-soluble proteins, which will have adopted extended configurations in the presence of salts thereby reducing the capacity of the network to yield. Furthermore these flours are those where, as the network unfolds under stress, there are likely to be the greatest number of favourably oriented sites ; this pronounced toughening effect will cause E to fall to an even greater extent as the protein content decreases (Figs. 1, 2 and 5).

Mandelate will also reduce extensibility but by a different mechanism. It is likely that this ion will also attach itself to proteins by means of hydrophobic bonds with its aromatic

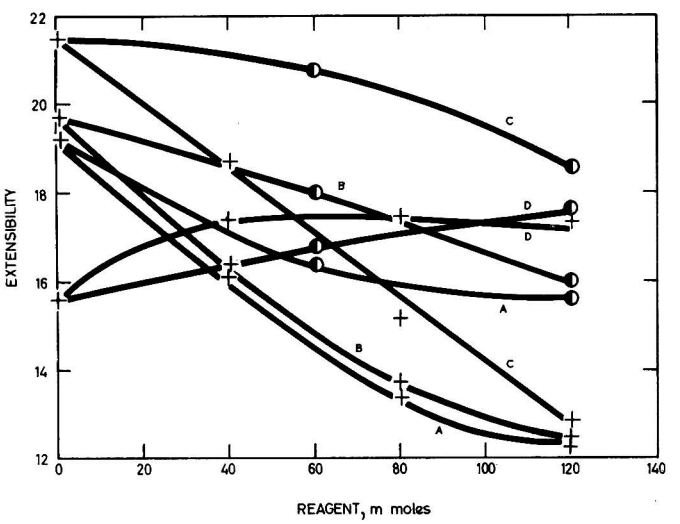

FIG. 5.—*Extensibility of doughs vs. mmoles of additive per* 280 *g. of flour*

● NaCl ; + Na lactate
A Scottish Winters ; B English medium ; C English weak ; D Strong

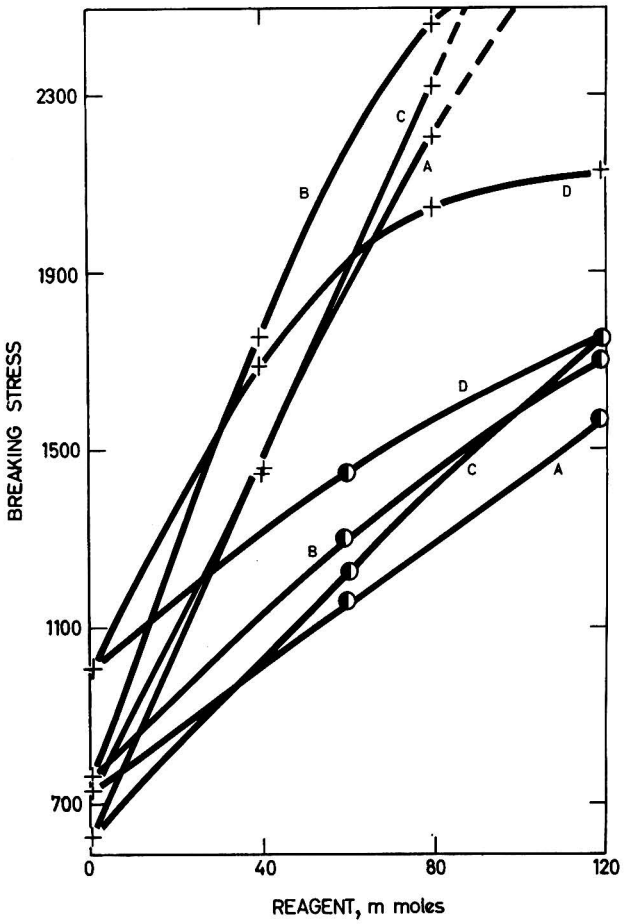

FIG. 6.—*Breaking stress of dough vs. mmoles of additive per* 280 *g. of flour*
(legend as Fig. 5)

group, particularly to the gluten proteins which have more hydrophobic residues than the salt-soluble proteins. Steric hindrance coupled with the repulsive effect of the anions on one another will present the resumption of compact forms for the insoluble proteins during resting, thus greatly reducing its capacity for further extension. Since the percentages of protein insoluble in 0·8% sodium chloride for the bread and English biscuit flours are respectively 91 and 87% it will be seen why the effect on the two flours is similar (Figs. 1 and 2).

The effect of the salts is generally to reveal differences between high- and low-protein flours, whereas no such divergence was found when acids were used.[1] The acids destroyed salt linkages thereby removing one of the main causes of divergence in behaviour between the two flours.

Acknowledgments

The authors thank Messrs. Bowmans Chemicals Ltd. for a grant towards the work, Drs. G. A. H. Elton and D. W. E. Axford for helpful advice, Miss M. D. Moody for the Extensometer tests, and Miss W. A. Keddie for experimental assistance.

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Received 1 October, 1964

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MEASUREMENT OF COLOUR IN TEA INFUSIONS. I.—Effects of Tea Composition on the Colour of Infusions

By R. F. SMITH and G. W. WHITE

A spectrophotometric method was used for determining theaflavin and thearubigin contents, and colour and brightness of infusions of samples of garden tea selected according to tea tasters' descriptions of the colours of their infusions. The results indicated differences in composition according to the country of origin and type of manufacture. After the addition of milk to the infusions, the colours were measured by reflection spectrophotometry, and correlations were found between % Reflectance at 700 mμ, % Brightness calculated from absorbance at 460 mμ, and tea tasters' assessment of 'brightness'. Further confirmation was thus obtained of the importance of theaflavins in determining the 'brightness' of tea infusions, and hence in providing an indication of the quality of black teas.

Introduction

Previous investigations on the phenolic substances of manufactured tea have shown the importance of colour in judging the quality of tea infusions, and as a result a spectrophotometric method was proposed for the evaluation of tea liquors.[1, 2] The present paper is an account of an extension of the earlier work to a more detailed examination of the individual effects of the phenolic constituents of manufactured tea on the colour of tea infusions.

When a tea taster examines a sample of tea, an infusion of the sample is prepared by a recognised method in a special tasting mug, the infusion decanted into a shallow tasting bowl, and then tasted. The taster's judgment on flavour is influenced to a certain extent by the appearance and colour of the infusion by transmitted light, as seen in the tasting bowl and in the tasting spoon. It is generally considered that a bright orange-red colour is most desirable. The infusion is however more usually tasted after the addition of a measured volume of milk, and the colour of the infusion again judged, this time by reflected light. The colour may be described by the taster in terms of hue (e.g., 'grey', 'yellow', 'golden', 'brown', 'red' or 'rosy-pink'), and in terms of brightness (e.g., 'bright', 'average' or 'dull'). The colour characteristics are determined by the country of origin of the tea, the method of manufacture and 'specie' (or grade) of the sample.

The principal coloured constituents of black tea are the orange-red theaflavins and the brownish-red thearubigins, which are two groups of phenolic oxidation products formed during fermentation of the tea leaf mainly by oxidation of (−)-epigallocatechin and (−)-epigallocatechin gallate.[3] A spectrophotometric method for determining these two groups of coloured substances has been published, and the colours of tea infusions expressed in terms of two index figures Total Colour and % Brightness, obtained from the spectrophotometric measurements.[1, 2] Total Colour is related to the sum of the theaflavin and thearubigin contents, and hence to 'strength', whereas % Brightness is an expression of the proportion of the colour that is due to the theaflavins.

Colour descriptions are apparently closely connected with flavour characteristics, and hence must influence the taster when forming his judgment. Colour is certainly an important factor, because it is found that daylight (or illumination from colour matching lamps), as opposed to tungsten filament light, is essential when tasting tea infusions. The ultimate consumer is certainly influenced by its appearance in the cup, which may add or detract from its acceptability.

Experimental

Tea samples

A series of garden samples of tea that had been selected by tea tasters and classified according to the colours of their infusions were used for most of this investigation. These teas had been placed in five groups according to their colours (or hues) which were described as ' grey ', ' golden ', ' coloury-brown ', ' coloury-reddish ' or ' rosy-pink ' respectively. Each group contained three samples each with a different brightness, described as ' dull ', ' average ' or ' bright ' respectively, so making a total of 15 samples (Table I). A strong ' coloury ' Assam C.T.C. tea (Kharjan fannings) was used for the preparation of solutions of theaflavins and thearubigins in the examination of the individual effects of these tea constituents on colour.

Preparation of the tea infusions

All tea infusions were prepared by infusing 9 g. of the tea sample with 375 ml. of distilled water by the method of Roberts & Smith.[1]

Milk

Pasteurised (non-homogenised) milk was used throughout this part of the investigation.

Transmission spectrophotometry

The infusions were examined by the spectrophotometric method of Roberts & Smith.[1, 2] Theaflavin and thearubigin contents were calculated from absorbance readings obtained at a wavelength of 380 mμ, and Total Colour and % Brightness values were calculated from readings obtained at a wavelength of 460 mμ. A Unicam SP 600 spectrophotometer was used for measurements of absorbance.

Reflection spectrophotometry

This system was used for the examination of tea infusions to which milk has been added. Except where otherwise stated, 3·6% v/v of pasteurised milk was added to the hot tea infusion, a proportion normally used by tea tasters. The % Reflectance was measured at wavelengths between 400 and 800 mμ with a Unicam SP 500 spectrophotometer fitted with the reflectance

Table I

Description of samples of garden teas

Sample no.	Country of origin	Type of manufacture	Colour grading
1	Nyasaland	Orthodox	Grey—dull
2	50% Nyasaland/50% Assam	,,	,, —average
3	Assam	,,	,, —bright
4	Ceylon	Orthodox	Golden—dull
5	,,	,,	,, —average
6	,,	,,	,, —bright
7	Assam	C.T.C.	Coloury brown—dull
8	,,	,,	,, ,, —average
9	,,	,,	,, ,, —bright
10	Assam	C.T.C.	Coloury reddish—dull
11	50% Assam/50% Dooars	C.T.C./Legg-cut	,, ,, —average
12	Dooars	Legg-cut	,, ,, —bright
13	Dooars	Legg-cut	Rosy-pink—dull
14	,,	,,	,, —average
15	,,	,,	,, —bright

attachment. The milked tea infusion was contained in a glass cell 2 cm. deep, and with a 1-in. dia. glass cover-slip placed on the surface of the liquid, to provide a flat surface and to ensure that it was in the same plane as the magnesium carbonate block used as the reference standard.

Visual colorimetry

In this system, the colours of the infusions to which milk had been added (see above) were measured under Illuminant C (equivalent to mean daylight of colour temperature 6660° K) with a Lovibond-Schofield Tintometer. The red and yellow glasses were used to match hue and saturation, and the obturator vane to match brightness, employing two observers. The average readings obtained were converted to colour attributes by means of the conversion graph provided, so obtaining values for luminance (lightness), dominant wavelength (hue) and excitation purity (saturation).

It was decided however that this method of visual colorimetry, although it provided more information on colour attributes, was not sufficiently sensitive for our purpose, which required the detection of very small variations in the colour of tea infusions. The results obtained are therefore not included here, and the method of reflection spectrophotometry was consequently adopted. The visual colorimetry method is basically similar to that used by tea tasters, except that colour matching is made under controlled conditions using internal standards, but the field of view is restricted to 2°, which involves some loss of discrimination.

Results

Compositions of garden samples of tea

A systematic examination of the variations in composition of samples of tea was first made by examining the distilled water infusions of the 15 classified garden samples of tea by transmission spectrophotometry. The results (Table II) show that these samples cover a wide range of values, and they are characteristic of the types and origins of the teas in each of the five groups,[1, 2] although they are not necessarily representative of the tea produced in these countries. The ' grey ' group had low contents of both theaflavins and thearubigins, and hence low total colours. The ' golden ' group (Ceylon teas) had average theaflavin and thearubigin contents; also high E_{380}/E_{460} ratios of the thearubigins, indicating low colouring powers of the thearubigins. The ' coloury-brown ' group (Assam C.T.C. teas) had high theaflavin and average thearubigin contents. The ' rosy-pink ' group (Dooars teas) had very high theaflavin contents, but thearubigin contents somewhat below average, so giving very bright infusions. The thearubigins had high E_{380}/E_{460} ratios, and hence were lightly coloured.

Table II

Spectrophotometric analyses of distilled water infusions of tea samples

Sample no.	TF, %	TR, %	E_{380}/E_{460} for TR	Total Colour	% Brightness
1	0·38	8·57	5·69	2·05	16·4
2	0·50	9·34	5·14	2·40	17·7
3	0·67	9·83	4·66	3·03	19·8
4	0·62	13·90	5·85	3·19	17·8
5	0·75	14·85	6·53	3·38	19·8
6	0·89	15·20	7·05	3·36	25·0
7	0·79	13·30	4·49	4·12	17·8
8	1·03	12·93	4·34	4·34	22·7
9	1·17	13·02	4·65	4·17	25·5
10	0·91	15·10	4·48	4·40	19·1
11	1·02	13·30	4·91	3·89	25·4
12	1·11	12·30	5·66	3·41	30·5
13	1·24	11·20	5·42	3·48	34·9
14	1·33	12·03	5·62	3·90	32·7
15	1·56	13·04	5·82	4·10	38·4

TF = Theaflavins TR = Thearubigins

The ' coloury-reddish ' group was intermediate in composition to the ' coloury-brown ' and ' rosy-pink ' groups, having high theaflavin and average thearubigin contents.

Comparison of the average values for each of the five groups of samples shows that (1) theaflavin contents increased from group to group, (2) thearubigins were highest in the three middle groups, (3) Total Colours were highest in the middle group, and (4) % Brightness increased from group to group, and within each group increased in the order ' dull '–' average '–' bright '. The classification of the samples according to the determined composition of the distilled-water infusions of the samples was therefore also in accord with the tea tasters' classification.

Reflection spectrophotometry of distilled-water tea infusions

The colours of the milked tea infusions were compared by reflection spectrophotometry. The reflectance curves obtained for the infusions of the 15 garden teas showed that reflection was mainly in the red part of the spectrum with maxima at or above 700 mμ, and varied according to the taster's description of ' hue ' and of ' brightness '. Typical reflectance spectra are shown in Figs. 1*a* and 1*b*. Variations according to colour group are illustrated by Fig. 1*a*, in which the curves for the ' average ' samples in the ' grey ', ' coloury-brown ' and ' rosy-pink ' groups show that the reflectance maxima increased with increasing redness of ' hue '. Within each colour group the reflectance maxima increased with increasing ' brightness '. As an example Fig. 1*b* illustrates variations in reflection according to ' brightness ' within the ' golden ' group.

In Table III are tabulated the values for reflectance at 700 mμ, showing that they increased in each group of samples in the order ' dull '–' average '–' bright '; and also that the mean reflectance for each group increased from the ' grey ' group to the ' rosy-pink ' group. Similar variations in % Brightness had been found by transmission spectrophotometry, and Fig. 2 shows that there is a correlation between % Reflectance at 700 mμ and % Brightness.

Individual effects of theaflavins and thearubigins on the colour of tea infusions

The important effects of theaflavins on colour have been demonstrated, but the effects of thearubigins were not fully understood previously. The following experiment was therefore designed to demonstrate the individual effects of theaflavins and thearubigins on colour. Solutions of theaflavins and thearubigins were prepared by separating these components from a tea infusion. For this purpose a strong infusion of a coloury Assam C.T.C. tea (containing

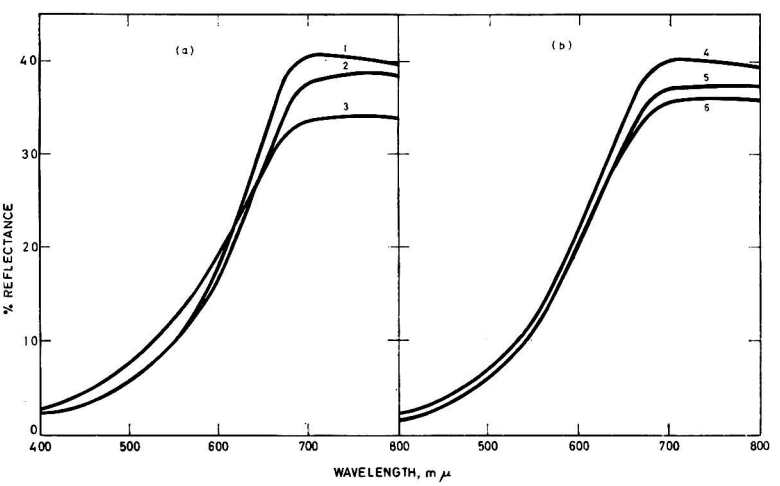

FIG. 1.—*Reflectance spectra of milked tea infusions*

(a) *Variations in reflectance according to colour group of samples of ' average ' brightness*

curve 1 rosy-pink curve 2 coloury-brown curve 3 grey

(b) *Variations in reflectance according to ' brightness ' of samples in ' golden ' colour group*

curve 4 bright curve 5 average curve 6 dull

Table III

% Reflectance at 700 mμ of distilled-water tea infusions

Colour group	Grey	Golden	Coloury-brown	Coloury-reddish	Rosy-pink
Dull	32·4	36·3	36·4	37·1	39·6
Average	33·7	37·3	37·7	38·0	41·0
Bright	34·7	40·3	40·0	39·1	42·2
Mean	33·6	37·9	38·0	38·1	40·9

1·58% of theaflavins and 12·4% of thearubigins) was prepared. The cooled infusion was shaken with an equal volume of isobutyl methyl ketone (IBMK) and the two layers separated. This extraction was repeated twice more, the IBMK extracts combined, and the IBMK removed by distillation, leaving a crystalline residue consisting mainly of theaflavin gallate, together with some theaflavin, some thearubigins (free acids) and caffeine. This residue was dissolved in an ethanol–water mixture (approx. 20% v/v ethanol) and the theaflavin and thearubigin contents of an aliquot portion determined spectrophotometrically. The aqueous solution remaining after extraction of the infusion with IBMK, containing the greater part of the original thearubigins and a trace of unextracted theaflavins, was boiled to expel residual IBMK, then cooled and diluted to a known volume. The theaflavin and thearubigin contents of an aliquot portion of this solution were determined spectrophotometrically.

The two prepared solutions of theaflavins and thearubigins were mixed in various proportions and diluted with water to produce nine reconstituted solutions. The compositions of these solutions were calculated to correspond to infusions of tea containing minimum, intermediate and maximum theaflavin contents (1, 2 and 3 respectively) each combined with either minimum, intermediate or maximum thearubigin contents (A, B and C respectively) in a 3 × 3 array. These nine artificial infusions covered the extreme range of theaflavin and thearubigin contents that have been found in teas. The actual compositions of the solutions were then checked spectrophotometrically, and Total Colour and % Brightness values also calculated (Table IV*a–d*). These results show that, with increasing theaflavin content, both Total Colour and % Brightness increased ; but that with increasing thearubigin content, Total Colour increased and % Brightness decreased.

The normal proportion of milk was added to each artificial infusion, and the colours measured by reflection spectrophotometry. Figs. 3*a–c* show the effects of increasing theaflavin content at minimum, intermediate and maximum thearubigin contents respectively. At each thearubigin level, the % Reflectance at 700 mμ increased with increasing theaflavin content (associated with increasing % Brightness) as in genuine tea infusions. Increasing thearubigin content (associated with decreasing % Brightness) had the effect of decreasing the % Reflectance

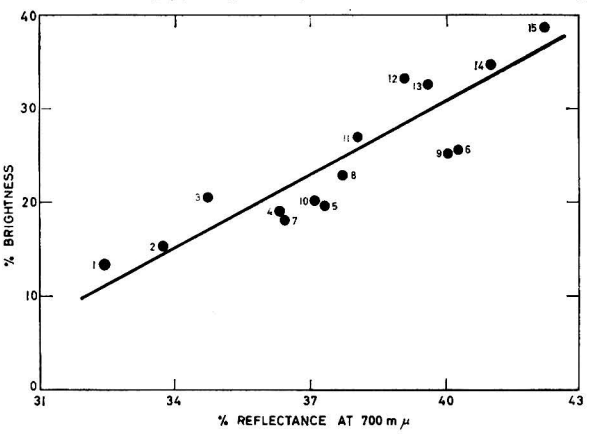

FIG. 2.—*Correlation between % Brightness of unmilked distilled water infusions of tea and % reflectance at 700 mμ of the milked infusions*

(numerals refer to samples described in Table I)

Table IV

Determined compositions of artificial tea infusions

(a) % *Theaflavins*

	1	2	3
A	0·32	0·94	1·44
B	0·38	0·96	1·52
C	0·37	1·05	1·67

(b) % *Thearubigins*

	1	2	3
A	8·6	8·7	8·1
B	11·5	12·1	12·3
C	15·9	16·1	16·4

(c) *Total Colour*

	1	2	3
A	2·80	3·34	3·43
B	3·68	4·34	4·60
C	5·06	5·96	6·55

(d) % *Brightness*

	1	2	3
A	10·3	24·5	38·8
B	9·0	20·6	29·2
C	6·5	17·0	26·1

1 → 2 → 3 Increasing theaflavin content

A → B → C Increasing thearubigin content

at 700 mμ (Table V), and of moving the wavelength of maximum Reflectance up to 800 mμ, which is the limit of normal vision, thus increasing the slope of that part of the curve between 700 and 800 mμ. There was also a good correlation between % Reflectance at 700 mμ and % Brightness.

The Reflectance curves of theaflavins and thearubigins (Fig. 4) were obtained by the use of prepared solutions of these constituents to which milk had been added. The theaflavin solution (0·036%) showed a Reflectance maximum at about 700 mμ (red) which is complementary to the wavelength of maximum absorbance of theaflavins (460 mμ in the blue). The Reflectance curve of the thearubigin solution (0·345%), which has been extended beyond the limit of normal vision (800 mμ), showed a maximum just on this limit. The shapes of the Reflectance spectra of the artificial tea infusions, and those of genuine tea infusions, are therefore the results of the combined effects of theaflavins and thearubigins on Reflectance. Fig. 4 shows that the theaflavins have a much greater effect on Reflectance at 700 mμ relative to the thearubigins, so that in a tea infusion, although there may be a considerable difference between the concentrations of theaflavins and of thearubigins, their individual contributions to the

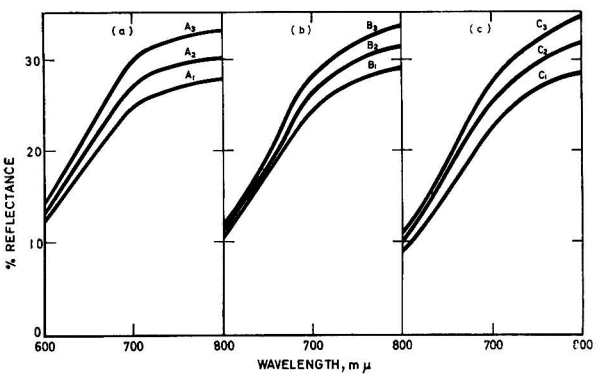

FIG. 3.—(a) *Reflectance spectra of milked artificial tea infusions*

Min. thearubigin content equiv. to 8·5% in tea
A1. Min. theaflavin content " " 0·32% " "
A2. Intermediate theaflavin content " " 0·94% " "
A3. Max. theaflavin content " " 1·44% " "

(b) *Reflectance spectra of milked artificial tea infusions*

Intermediate thearubigin content equiv. to 12·0% in tea
B1. Min. theaflavin content " " 0·38% " "
B2. Intermediate theaflavin content " " 0·96% " "
B3. Max. theaflavin content " " 1·52% " "

(c) *Reflectance spectra of milked artificial tea infusions*

Max. thearubigin content equiv. to 16·1% in tea
C1. Min. theaflavin content " " 0·37% " "
C2. Intermediate theaflavin content " " 0·05% " "
C3. Max. theaflavin content " " 1·67% " "

Table V

% *Reflectance at 700 mμ of milked artificial tea infusions*

		Increasing theaflavins →		
		1	2	3
Increasing thearubigins ↓	A	25·0	27·2	30·2
	B	24·8	26·8	28·4
	C	22·8	25·0	27·3

Reflectance at 700 mμ can be of the same order. In a similar manner the relatively high contribution of the theaflavins to the absorbance at 460 mμ (viz., % Brightness) of unmilked tea infusions has been explained by the greater absorbance of the theaflavins relative to that of the thearubigins.

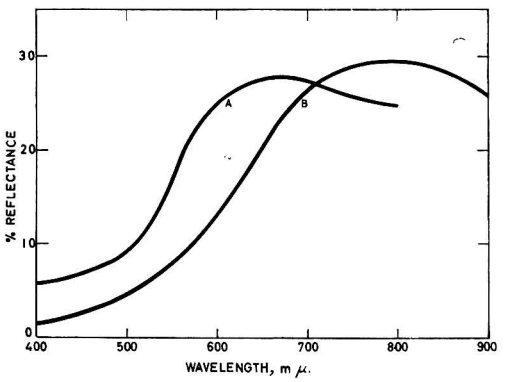

FIG. 4.—*Reflectance spectra of milked solutions of theaflavins and thearubigins*
curve A Solution containing 0·036% theaflavins (equivalent to 1·60% in tea)
curve B Solution containing 0·345% thearubigins (equivalent to 15·2% in tea)

Summary and conclusions

Spectrophotometric examination of distilled-water infusions of a series of tea samples classified by tea tasters into five groups according to hue and into three grades in each group according to brightness showed that there were differences in composition in each group which were characteristic of the sources and type of the samples. Values obtained for ' brightness ', calculated from the spectrophotometric measurements, confirmed that the tasters had arranged the samples in the groups, and within each group, in orders of increasing ' brightness '.

Infusions of these same samples of tea after the addition of milk had characteristic reflectance spectra with maxima at wavelengths at or near 700 mμ. The significant variations in reflectance at this wavelength correlated well with variations in ' brightness ' measured by transmission spectrophotometry. The reflectance maxima also showed good correlation with the tasters' assessments of ' brightness ', which also correlated markedly with theaflavin contents; the tasters therefore unconsciously made assessments of relative theaflavin contents when they compared the colours of milked tea infusions.

Theaflavins and thearubigins have different characteristic reflectance spectra in solutions with added milk, theaflavins having a maximum at about 700 mμ and thearubigins at about 800 mμ. Variations in theaflavin content are therefore mainly responsible for the variations in reflectance at 700 mμ of the tea infusions.

Evidence was obtained that theaflavins increase and thearubigins decrease the ' brightness ' measured by reflection, just as they affect the ' brightness ' measured by transmission.

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Received 6 August, 1964

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MEASUREMENT OF COLOUR IN TEA INFUSIONS. II.*—Effects of Methods of Preparation on the Colour of Tea Infusions

By R. F. SMITH and G. W. WHITE

A spectrophotometric method was used for determining the compositions and colours of tap-water infusions of selected garden samples of tea, and to demonstrate the effects of temporary hardness in the water on colour. Other factors affecting colour, such as continued boiling of the water, variations in the infusion time, the processing of the milk used, and proportion of milk added, were also investigated. After the addition of milk, the % Reflectance at 700 mμ was used as a measure of 'brightness' of the infusions.

Introduction

The preceding paper[1] described the use of two spectrophotometric methods for investigating the effects of the composition of tea on the colour of distilled water infusions. The importance of the two principal coloured constituents of black tea, the theaflavins and thearubigins, was demonstrated. The present paper describes the results of the application of these methods to the examination of the effects of factors other than the composition of the tea on the colour of infusions. These factors were (*a*) the composition of the water used for preparing the infusion, (*b*) the time for which the water is boiled, (*c*) the time of infusion with the water, (*d*) the strength of the infusion, (*e*) the proportion of milk added and (*f*) the kind of processing which the milk had received.

Experimental

Tea samples

The series of 15 garden teas obtained for Part I of this investigation was used again, but a popular blend of tea of good average quality was used for the investigation of the effects of variations in the method of preparation of the infusions, and of the effects of added milk on the colours of the infusions.

Milk samples

Pasteurised (non-homogenised) milk was used in all cases. In the examination of the effects of processing of different types of milk on the colour of tea infusions, homogenised, sterilised, evaporated full-cream, and condensed full-cream sweetened milks were also used. The evaporated and condensed milks were first diluted with water before use, to a strength equivalent to that of whole milk.

Preparation of the tea infusions

Tea infusions were prepared by the method of Roberts & Smith,[2, 3] excepting that the 9 g. of tea sample was infused with London (Metropolitan Water Board) tap water, a moderately hard water (~260 p.p.m. total hardness and 58 p.p.m. non-carbonate hardness, both as $CaCO_3$), in place of distilled water. Except where otherwise stated, the tap water used was brought

* Part I: preceding paper

just to the boil, thereby ensuring that there was no significant reduction in temporary hardness. As an example, the total alkalinity (as p.p.m. $CaCO_3$) determined by titration to methyl orange with N/50-hydrochloric acid, was in one case 220 before boiling and 213 after bringing just to the boil. The strength of the infusions prepared in this way corresponded to a strength normally used by tea tasters, but other infusions that were examined (using 5·5 g. of tea in 375 ml. of water) had a lower strength, more in accord with average domestic practice.

Colorimetric analysis of tap-water tea infusions

In order to examine the effects of the mineral constituents of water on the composition of tea infusions, the 15 garden samples of tea were infused with a tap water with a composition similar to that used by the tea tasters to classify the samples, and the infusions were examined by transmission spectrophotometry (Table I). Comparison of these results with those previously obtained on distilled-water infusions[1] shows that tap water had a different effect on each group of samples. The changes in composition were similar in nature to those previously observed,[1] but varied in amount according to the types of tea in each group of samples. The net result of these determinations was to show that the spectrophotometric method again confirmed the tasters' classification of these samples according to colour, the teas having been arranged by the tasters both in groups and within the groups in order of increasing ' brightness ', which they apparently also associate with increasing redness and decreasing brownness of the infusions.

The mechanism of the changes taking place in the composition of the polyphenolic constituents of tea and the action of the mineral constituents of the water used for infusing are not yet fully understood. It obviously does not consist simply of a conversion of theaflavins to thearubigins. Increases in the colour of the thearubigins are indicated in the decreases in the value of the E_{380}/E_{460} ratio. Increases in total colour, resulting in decreases in brightness, can result from increases in the ratio of thearubigin salts to free acids. Other more deep-seated changes may take place in the thearubigins, but cannot be fully explained until the exact structure of the thearubigins is known.

Reflection spectrophotometry of tap-water tea infusions

The colours of the milked tea infusions were also measured by reflection spectrophotometry. The reflectance spectra between the wavelengths 400 and 800 mμ for the 15 tea infusions were

Table I

Spectrophotometric analyses of tap-water infusions of tea samples

Sample no. (see Part I[1])	Total alkalinity* of water after boiling	% TF	% TR	E_{380}/E_{460} (TR)	Total colour	% Brightness
1	189	0·26	9·40	4·64	2·48	8·5
2	189	0·43	9·95	4·26	3·03	12·6
3	188	0·44	10·30	3·70	3·27	12·0
4	188	0·52	15·45	4·32	4·03	11·8
5	190	0·66	16·25	4·94	4·12	14·7
6	190	0·77	17·48	4·77	4·04	19·0
7	185	0·65	14·75	3·85	4·88	12·5
8	185	0·95	15·90	3·76	5·61	16·5
9	185	1·10	16·45	3·86	5·79	18·6
10	196	0·77	16·67	4·97	4·87	15·0
11	196	0·86	14·80	4·99	4·61	18·0
12	199	0·94	13·52	5·59	3·97	22·0
13	194	0·97	12·82	5·28	4·30	22·7
14	196	1·09	12·95	5·18	4·14	24·2
15	196	1·30	14·05	5·53	4·65	26·6

* as p.p.m. $CaCO_3$. Total alkalinity before boiling = 210 (London tap water)

TF = Theaflavins TR = Thearubigins

similar to those previously obtained with distilled-water infusions, showing again that reflection was mainly in the red part of the spectrum, with maxima in the region of 700 mμ. Table II shows however that the average % Reflectance at 700 mμ for each group of teas was less than that obtained for distilled water infusions of the same teas (Table III of the preceding paper[1]), indicating decreases in brightness. Again, the average Reflectance for each group (with the exception of the 'golden' group) increased from the 'grey' group to the 'rosy-pink' group and (with one exception) increased within the groups in the order 'dull'–'average'–'bright'. Fig. 1 shows that there is a correlation between % Reflectance at 700 mμ and % Brightness determined by transmission spectrophotometry. Both factors are therefore related to the tasters' classification of the samples according to 'hue' and 'brightness', using water with a composition similar to that used in these experiments to prepare their infusions.

Effects of the time of boiling of the water used on the colour of tea infusions

Evidence has been obtained of the effects on colour of the hardness of the water used for preparing tea infusions, by comparing the compositions of infusions of the same samples of tea prepared with soft water (distilled) and hard tap water. Temporary (bicarbonate) hardness increased the Total Colour and decreased the % Brightness of the infusions, but permanent hardness had little or no effect.[3] For these reasons infusions made with soft natural water supplies are much brighter than those made with hard water supplies. It is generally considered that freshly-boiled water should be used for tea making, but Schurer[4] has demonstrated that boiling hard water for a short time (5–10 min.) improved the appearance of tea infusions and reduced film formation, but that there was a tendency for the flavour to deteriorate after prolonged boiling of the water.

The effects of time of boiling of a hard water on the colours of tea infusions were examined by preparing a series of infusions (9 g. of tea in 375 ml. of water) of a tea blend of average quality, using London (M.W.B.) water that had been boiled for various times up to 30 min.,

Table II

% Reflectance at 700 mμ of tap-water tea infusions

Colour group	Grey	Golden	Coloury-brown	Coloury-reddish	Rosy-pink
Dull	28·7	32·0	29·5	31·8	37·3
Average	31·8	34·2	32·0	32·8	38·3
Bright	29·6	38·4	35·3	33·9	39·5
Mean	30·0	34·9	32·3	32·8	38·4

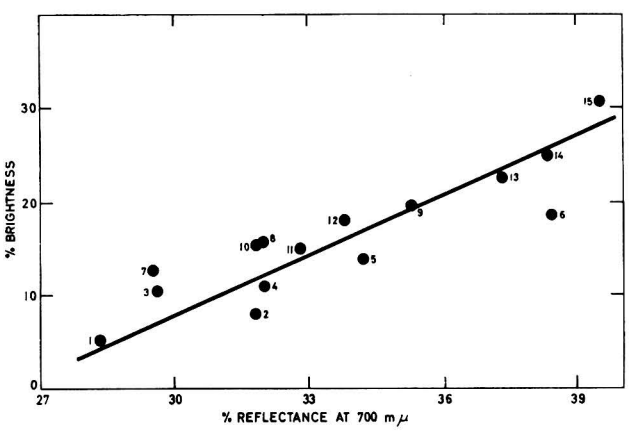

FIG. 1.—*Correlation between % Brightness of unmilked tap water infusions of tea and % Reflectance at 700 mμ of the milked infusions*

(numerals are tea sample numbers—see Part I[1])

and infusing the tea for 10 min. The compositions and colours of these infusions, also of a distilled-water infusion, were determined by the spectrophotometric method, and the percentage reflectances at 700 mμ measured after the addition of milk (16·6 ml. per 100 ml. of infusion). The temporary (bicarbonate) hardness of the water used for preparing each infusion was determined by titrating the alkalinity to methyl orange with N/50-hydrochloric acid, and the pH values of the boiled water samples and tea infusions were measured (Table III).

Temporary hardness was only slowly removed from the water by boiling, and was still not completely removed within 30 min. boiling. Reductions in hardness and alkalinity resulted in appreciable increases in theaflavin content and appreciable apparent decreases in thearubigin content, resulting in decreases in Total Colour and increases in % Brightness. Reflectance at 700 mμ also increased with increasing 'brightness'. Boiling for 30 min. resulted in values which differed only slightly from those obtained with the distilled-water infusion. It therefore follows that, as far as it concerns colour and appearance, tea infusions are improved if hard tap water is boiled for 5–10 min. before preparation of the infusion, confirming the observations made by Schurer; but it does not follow that flavour is also improved. It is generally considered that the water used for tea making should be freshly boiled, otherwise a deterioration in flavour described as a 'flatness' results, which is attributed to loss of gases from the water. Air is rapidly eliminated from water before it reaches the boiling point, but carbon dioxide may persist for some time in the form of bicarbonates. The evolution of gases from tea made with freshly-boiled water is therefore apparently a result of a production of carbon dioxide gas by reaction of the residual bicarbonates with acidic constituents of the tea. Natarajan *et al.*[5] have observed a slight improvement in taste of tea when the water used contained 10–25 p.p.m. of bicarbonate ions, but the colour of the infusion became darker when the concentration of bicarbonate ions was more than 50 p.p.m.

Effect of time of infusion on the colour of tea infusions

The time of infusion is another important factor in determining the composition of a tea infusion. This also was investigated by examining a series of infusions prepared by infusing a similar blend of tea (9 g. of tea in 375 ml. of water) with freshly-boiled water for various times up to 20 min. The compositions and colours of the infusions were determined by the spectrophotometric method, and the reflectances of the infusions measured at 700 mμ after the addition of milk in two different proportions (i.e., 3·6 and 16·6 ml. of milk per 100 ml. of infusion). The results (Table IV) show that the extraction of coloured constituents (viz., Total Colour) was almost complete in 10 min. (the time for which tea is normally infused by tea tasters). Natarajan *et al.*[5] obtained similar results on the rate of extraction of soluble constituents. Table IV also shows that the rate of extraction of theaflavins, and hence the rate of increase in brightness, was slower than the rate of extraction of thearubigins, due presumably to the lower solubility of the former in water.

At the lower milk concentration the reflectance at 700 mμ was less than at the higher milk concentration, but the variation in reflectance between infusions was greater at the lower concentration. Differences in the colour of tea infusions are therefore accentuated at a low milk concentration, such as that used by the tea tasters.

Table III

Effects of variations in time of boiling tap water on the compositions of tea infusions

Time of boiling of tap water, min.	0†	5	10	20	30	Distilled water
Bicarbonate alkalinity* of water (p.p.m.)	213	112	60	33	31	nil
pH of water	8·60	8·65	8·40	8·35	—	—
pH of infusion	6·30	5·70	5·20	5·10	5·10	4·95
% Theaflavins	0·74	0·77	0·89	0·85	0·85	0·86
% Thearubigins	12·4	12·1	12·1	10·8	11·7	11·3
Total Colour	4·04	3·86	3·75	3·45	3·50	3·44
% Brightness	16·7	19·1	23·7	23·5	22·3	23·6
% Reflectance at 700 mμ	50·0	52·0	54·5	54·8	55·3	55·2

* Original tap water: 220 p.p.m. bicarbonate alkalinity
† Water brought just to boil

Table IV

Effects of variations in time of infusion with tap water on the compositions of tea infusions

Time of infusion with tap water, min.	2	4	10	20
% Theaflavins	0·55	0·69	0·84	0·96
% Thearubigins	9·1	10·8	13·4	13·8
Total Colour	3·14	3·62	4·34	4·33
% Brightness	14·2	17·6	18·0	19·9
% Reflectance at 700 mμ (3·6% v/v milk added)	28·8	29·4	31·3	31·3
% Reflectance at 700 mμ (16·6% v/v milk added)	52·2	51·7	52·3	51·7

Effects of the type and proportion of milk on the colour of milked tea infusions

A preliminary account has been given[6] of an investigation made on the effects of homogenisation and heat treatment during processing of different types of milk on the appearance of tea infusions. To demonstrate this, a series of tea infusions was prepared by adding 375 ml. of boiling tap water to 5·5 g. of tea, and straining the infusion after 5 min. To 100-ml. portions of infusion were added 16·6 ml. of each type of milk examined (i.e., 14·3% v/v of milk in the mixture). The strength of the infusion and proportion of milk added corresponded to average domestic practice.

The reflectance curves of the milked infusions (Fig. 2) show that the % Reflectance at 700 mμ (or brightness) of the milked infusions increased in the order pasteurised–condensed–evaporated–homogenised–sterilised. The average diameters of the fat globules in these milks were 1·4, 1·0, 0·6, $0{\cdot}5_5$ and 0·5 μ, respectively, confirming that the last three milks, with the smallest fat globules, had been homogenised during processing. The whitening powers of these different types of milk, when added to tea infusion, therefore increased with decreasing size of fat globule, i.e., with increasing numbers of fat globules.

Similar variations are shown by the reflectance curves of the milks themselves (Fig. 3). The condensed, pasteurised and homogenised milks had reflectance curves similar in form, but differing only in the value of maximum reflectance, and were similar to that obtained by Timmermans[7] for evaporated non-sterilised milk. The reflectance spectra of the sterilised and evaporated milks show that, as a result of the heat treatment received during processing, reflectance was considerably reduced at the blue end of the spectrum. The reflectance spectrum of the tea infusion containing evaporated milk also showed considerable reduction in reflectance in the blue, which accounted for the colour of the infusion being visibly different in hue from that of the remainder of the infusions, appearing brighter but yellowish.

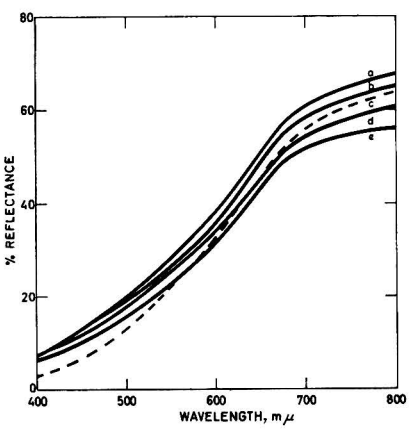

FIG. 2.—*Reflectance spectra of milked tea infusions effects of the processing of the milk on colour*

curve a sterilised curve b homogenised
c evaporated ,, d condensed
curve e pasteurised

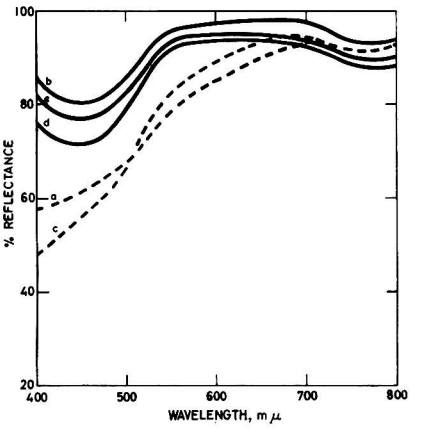

FIG. 3.—*Reflectance spectra of various types of milk—effects of processing on colour*
(legend as for Fig. 2)

The proportion of milk added to a tea infusion also has an important effect on the appearance of the tea. Increasing the proportion of milk increased the whiteness of the infusion, and as a result the % Reflectance at 700 mμ was increased, but the variation in reflectance between different teas was reduced. This can be seen in the results obtained with infusions of the three samples of tea in the ' coloury-brown ' group shown in Table V, which includes reflectance values obtained with two different proportions of milk, corresponding to those used in tea tasting and in normal domestic usage. The tea tasters, by using a low concentration of milk, as well as a high tea concentration, are thus able to accentuate differences in colour as well as differences in taste, so increasing the discrimination of their tasting test.

One point about which there is some disagreement amongst tea consumers is whether it is preferable to add the tea to the milk or to add the milk to the tea in the cup. It is a matter of personal preference, but tea tasters have reported that they are able to detect a difference in flavour according to the procedure. A flavour with a characteristic like that of cooked milk can be detected if the milk is added to the hot tea, due presumably to the fact that the temperature of the milk, which comes into immediate contact with hot tea, rises rapidly then falls to that of the final mixture. In the reverse process, the temperature of the milk is slowly raised by the addition of the hot tea until it reaches that of the final mixture.

The effects of the two methods of dilution upon the colour of milked tea infusions were compared by preparing tea infusions (9 g. tea in 375 ml. boiling tap water) and (*a*) adding tea infusion at 85° to cold milk, and (*b*) adding cold milk to tea infusion at 85°. Milk was added at the minimum and maximum levels (namely 3·6 and 16·6 ml. per 100 ml. of infusion respectively), the final temperatures of the mixtures being about 85° and 75° respectively. The colours of the milked infusions were measured by reflection spectrophotometry, and the results obtained with the three samples of ' coloury-brown ' tea show that in each case the % Reflectance at 700 mμ was greater when the tea was added to the milk than when the milk was added to the tea (Table V). The reflectance readings were highest with the greater proportion of milk,

Table V

Effect of method of addition of milk on the % Reflectance at 700 mμ of milked tea infusions

Proportion of milk used	3·6 ml. of milk to 100 ml. of infusion			16·6 ml. of milk to 100 ml. of infusion		
Tea sample no.	7	8	9	7	8	9
Tea added to milk	31·3	33·6	36·8	45·3	46·3	48·0
Milk added to tea	30·1	31·3	34·9	44·5	45·6	47·0

but differences due to the method of mixing the tea and milk were most marked at the lower milk concentration. These differences in colour were perceptible to the eye, the colours being ' brightest ' in tea tasters' terms when tea was added to milk. It can therefore be concluded that, so far as colour is concerned, the addition of tea to milk is to be recommended.

Variations in appearance indicate differences in the polyphenolic tea constituents that may account for the flavour differences detected by tea tasters. These might be explained by variations in the reactions between tea constituents and milk proteins. Brown & Wright[8] have found that when a tea infusion is mixed with milk the coloured tea polyphenols interact with the α-casein complex and β-casein of the milk to form soluble casein–polyphenol complexes, and they concluded that this interaction is, at least at first, due to the formation of hydrogen bonds. If there is insufficient casein to form soluble complexes with the polyphenols, the excess polyphenols could combine with the milk serum proteins β-lactoglobulin and α-lactalbumin to form insoluble precipitates. This might occur when milk is added to the tea infusion, and the polyphenol molecules are initially in excess, but when tea is added to milk the casein molecules are initially in excess. It is highly probable that the theaflavins are more reactive than the thearubigins, so that variations in brightness of the colour could result from differences between the interactions of the theaflavins and thearubigins with the milk proteins.

Discussion

Comparisons of the composition and colour, as determined by a spectrophotometric method, of the infusions made with a moderately hard water (London, Metropolitan Water Board) and with distilled water shows differences which were similar in nature to those previously observed.[3] namely, decreases in theaflavin content and in % Brightness, and increases in thearubigin content and in Total Colour. The amount of change varied from sample to sample and, because the decreases in theaflavin content were not equal to the apparent increases in thearubigin content, it was obvious that the changes did not consist of a simple conversion of theaflavins to thearubigins. The changes in composition, which were brought about by the temporary hardness of the water, resulted in an increase in the range of Total Colour values, but a decrease in the range of % Brightness values in this series of samples. The results still however corresponded with the tasters' classification of the samples according to colour and brightness. Although these teas were not examined in a naturally soft water supply, such a water (e.g., upland surface water) would be expected to produce results similar to those obtained with distilled water, or intermediate between those obtained with distilled water and with hard water.

In addition to examining the effects of the compositions of the tea and of the water used for preparing the infusion on the colour of tea infusions, other factors concerning the method of preparing the infusion were examined. It is generally considered that freshly-boiled water should be used for infusing tea, in order to obtain the best flavour. Evidence was however obtained that continued boiling slowly reduced the temporary hardness and alkalinity of water, and resulted in decreased total colour and increased brightness of the infusion. Without considering the effect on flavour, continued boiling of hard water for up to 10 min. produced improvements in the colour and appearance of tea infusions. Associated improvements in flavour would be expected, but the flatness of the taste of such infusions observed by tea tasters must result from other factors, such as loss of carbon dioxide. Dissolved gases, such as air, contrary to popular belief, are normally eliminated from water before it reaches the boiling point, but the water can however still contain carbon dioxide combined as bicarbonates, and this would be liberated by reaction with acidic constituents of the tea.

The time for which tea is infused is also important, as it determines the strength and composition of the infusion. Extraction of soluble constituents was almost complete after 10 min., the time for which tea is infused by tea tasters, and a further 10-min. infusion produced only small increases in soluble extractives. The rate of extraction of theaflavins was less than that of thearubigins, owing to the lower solubility of the former in water. Infusion for less than the optimum time (5 min.) produced infusions that were lacking in brightness.

The type and proportion of milk added to a tea infusion had important effects on the beverage. Homogenisation of the milk, by increasing the number of fat globules, increased the whitening power of the milk, and so increased the brightness of the tea infusion. Heat

treatment, as applied to sterilised milk and evaporated milk, which develops pinkish-brown colours, produced a further increase in brightness of tea infusions. Increasing the proportion of milk added to tea infusions increased whiteness, but also reduced the variation in colour in different tea infusions. Tea tasters, by deliberately using a lower concentration of milk, as well as a higher strength of infusion, thus increase the discrimination of their tasting test.

Evidence was also obtained that the addition of hot tea to milk resulted in a brighter colour than the addition of the milk to the hot tea, so that from the point of view of colour alone the former practice is preferable. Differences in flavour that can be detected by tea tasters might be explained by variations in order and nature of the reactions between milk proteins and tea polyphenol compounds.

Acknowledgments

The authors wish to thank Dr. E. B. Hughes in the first place for suggesting this investigation, and Dr. J. H. Bushill for his interest and helpful advice in the preparation of the manuscripts for these two papers.

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Received 6 August, 1964

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EFFECTS OF PROTEIN INTAKE ON THE STORAGE OF COPPER IN THE LIVER OF SHEEP*

By A. MacPHERSON and R. G. HEMINGWAY

Death from copper poisoning resulting from administration of 1·0 g. of $CuSO_4,5H_2O$ per day occurred after periods ranging from 10 to 34 weeks in two groups of housed sheep on concentrate diets containing 10 and 20% of protein, respectively. The additional protein was dried blood meal. Those on the higher protein intake appeared to be the more resistant, although the concentration of copper in the liver of both groups were in the dangerously high range. Rather less than 3% of the supplementary copper was stored in the liver. Additional protein did not affect copper storage when sheep on basal diets providing 10% of protein and containing about 6 p.p.m. of Cu were supplemented with 10 mg. of Cu per day.

Introduction

It is known that copper can accumulate in various tissues, particularly the liver, of sheep and that the feeding of supplementary copper can, under certain circumstances, lead to death from copper poisoning. Most of the cases seem to result from the grazing of orchards where copper sulphate has been used extensively. Housed sheep fed diets either naturally rich in copper or supplemented with copper sulphate may also accumulate copper in potentially toxic amounts.

However, when attempts have been made to produce copper poisoning experimentally, surprisingly large amounts of copper have generally been required. For example, Barden & Robertson[1] fed housed sheep on hay and oats with a daily supplement of 1 g. of crystalline copper sulphate, $CuSO_4,5H_2O$, which would have given an overall copper concentration in the diet of about 250 p.p.m. The first death occurred after 15 weeks (104 g. of $CuSO_4,5H_2O$). Todd *et al.*[2] fed sheep at grass with 1 g. of $CuSO_4,5H_2O$ daily, the first death (of six sheep) occurring after 28 weeks. Hemingway[3] has also fed 1 g. of $CuSO_4,5H_2O$ daily to pregnant ewes at grass for periods of 15 weeks with no obvious sign of distress to the sheep, although liver-copper concentrations increased to very high levels.

Many diverse factors affect the storage of copper in the liver. The dietary protein concentration has recently been shown to influence copper storage in both pigs and rats. Wallace *et al.*[4] found that the toxicity of 750 p.p.m. of copper (as the sulphate) fed to growing pigs decreased as the protein level was increased from 15 to 25%. McCall & Davis[5] found for rats that, when the diet contained 25% of protein, a supplement of 1000 p.p.m. of copper produced no significant increase in liver-copper concentration. When the diet contained only 10% of protein there was a highly significant increase in liver copper storage resulting from the additional copper.

This paper is concerned with the possible rôle of protein intake on liver-copper storage by sheep.

Experimental

Blackface hoggs (54) aged about 6 months were divided at random into five groups of eight, and two groups of seven sheep, in such a way that the separate groups had a similar liveweight distribution. One group of eight sheep was slaughtered at the commencement of the experiment, in order to determine the approximate mean initial liver-copper concentration for the whole group of 54.

The remaining six groups of sheep were housed and fed a basal diet of 0·75 lb. of hay per day (5·8 p.p.m. of Cu, 9·8% crude protein). Three of the groups received a daily supplement of 0·5 lb. of crushed oats (6·0 p.p.m. of Cu, 8·5% crude protein). The other three groups were fed 0·5 lb. per day of a mixture of seven parts of crushed oats and one part of blood meal (6·2 p.p.m. of Cu, 18·9% crude protein). Blood meal was chosen as the source of supplementary protein as it had a similar and low copper concentration to that of the oats, and because only

* Paper read at Meeting of Agriculture Group, 18 February, 1964

a small proportion needed to be added to the oats to materially increase the protein content of the concentrate supplement. [It should be noted that protein-rich concentrates other than blood meal contain rather more copper (i.e., 15–25 p.p.m.).] These two concentrate diets are subsequently referred to as the low-protein and the high-protein groups. These rations were chosen to provide comparable amounts of energy to those diets which are commonly fed to Blackface ewe hoggs overwintered indoors and where the expected liveweight gain over a 6-month period would be 5–20 lb.

There were three levels of copper supplementation given as $CuSO_4,5H_2O$; nil, 40 mg. (≡10 mg. Cu), and 1000 mg. (≡250 mg. Cu) per day. One of each of these supplements was given to both a low- and a high-protein group. Table I gives the total daily copper intake of the separate groups.

Table I

Total daily copper intake (as mg. Cu)

Copper supplement, mg. Cu	Low protein	High protein
Nil	3·33	3·39
10	13·33	13·39
250	253·33	253·39

Low protein = hay 12 oz., oats 8 oz.
High ,, = ,, 12 oz., ,, 7 oz., blood meal 1 oz.

The sheep were introduced to the indoor diets on 20 November, 1962. Administration of copper sulphate (given in solution by mouth) commenced on 10 December. The supplement of 10 mg. per day was given on 5 days of each week for the period 10 December to 5 April. Dosing was discontinued between 5 and 22 April and resumed during the period 23 April to 2 May. On 2 May these two groups of sheep and the two groups of control sheep receiving no copper supplement were slaughtered. By this date the sheep dosed with the low copper supplement had received a total of 960 mg. of copper given in 96 doses over a period of 163 days.

Administration of 250 mg. of Cu per day also commenced on 10 December and continued on 5 days of each week until 5 April when 88 doses (≡22 g. of Cu) had been given. The first death from copper poisoning occurred on 30 January after 49 doses (≡12·25 g. of Cu), and several deaths occurred during the period 30 January–5 April. Dosing was resumed on 22 April and continued until 12 August for those sheep which did not die earlier, when 183 doses (≡45·75 g. of Cu) had been given. Eleven of the fourteen sheep in these two groups died of copper poisoning and the other three were slaughtered at various stages of the experiment when they were still in good health.

The sheep were weighed monthly. Blood samples were obtained on 6 occasions from the groups which received either no copper or the low copper supplement. Blood sampling was much more frequent for those sheep which received the high copper supplement and which died of copper poisoning. The whole livers of all the sheep were recovered at slaughter or death for determination of total fresh weight, dry-matter content and copper concentration. Copper was determined by the method of Brown & Hemingway.[6]

Results

(1) *Control sheep and sheep receiving a daily supplement of 10 mg. of Cu*

Liveweight changes

The mean liveweights of the group of eight sheep slaughtered at the start of the experiment on 20 November was 48·1 lb. and the mean values for the other four groups ranged from 46·7 to 47·3 lb. The progressive changes in mean liveweight of these four groups are shown in Fig. 1.

The two groups which were fed the high-protein concentrate had greater increases in liveweight than those fed oats alone. Their final mean liveweight (66·95 lb.) was significantly ($P < 0·05$) greater than that for those fed the low-protein diet (57·70 lb.).

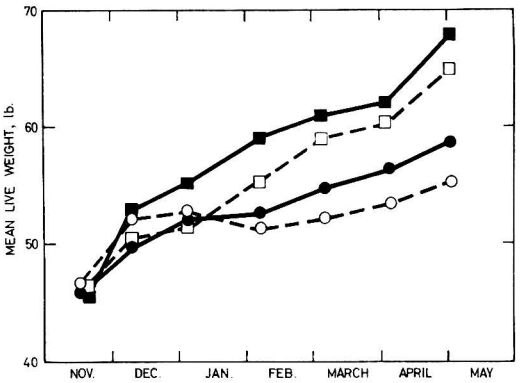

FIG. 1.—*Changes in mean live weights*

Low-protein diet
○ no Cu
● +10 mg. Cu/day

High-protein diet
□ no Cu
■ +10 mg. Cu/day

The supplement of 10 mg. of Cu per day effected some increase in liveweight for both the low- and high-protein groups. Although the increases were not significant, there was a constant trend of $2\frac{1}{2}$–$3\frac{1}{2}$ lb. mean liveweight in favour of the copper-supplemented groups. Significant regressions ($P < 0.01$) for mean liveweight increases were obtained for all groups except that fed the low-protein diet unsupplemented with copper.

Changes in blood-copper concentration

Mean blood-copper concentrations for the four groups of sheep are given in Fig. 2. At the start of the experiment the mean values of the separate groups ranged from 1·02 to 1·12 p.p.m. of Cu and the mean value of the slaughtered group was 1·15 p.p.m. Blood-copper concentrations of both groups which did not receive supplementary copper decreased steadily over the experimental period, but a significant regression ($P < 0.02$) was obtained only for the low-protein group. Blood-copper concentrations remained constant for both the low- and high-protein groups which received 10 mg. of additional copper per day.

Liver dry weight, liver-copper concentration and total liver-copper content

Table II presents the mean dry weights and copper analyses of the livers of these four groups of sheep and of the group killed initially. The daily supplement of 10 mg. of copper did not significantly increase the mean liver dry weights. For the low-protein group the increase

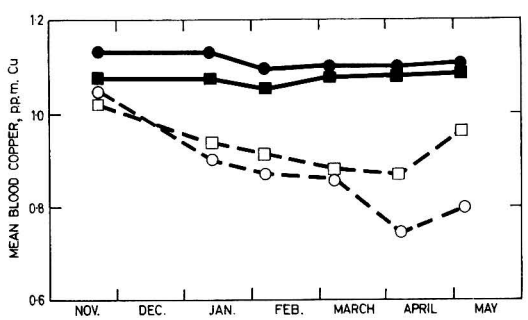

FIG. 2.—*Changes in mean blood-copper concentration*

(legend as Fig. 1)
The fall in April/May on the low-protein no-Cu diet is significant

Table II

Liver dry weight, liver-copper concentrations and liver total copper contents

(Mean and range of values for groups of 8 sheep)

Experimental diet	Liver dry wt., g.	Liver-copper concentration, p.p.m.	Liver total copper content, mg.
		Killed initially	
	90·6 (71–101)	119·5 (49–327)	10·1 (4·6–23·1)
		Killed after experimental period of 163 days	
No supplementary copper			
Low protein	93·5 (81–103)	70·1 (17–204)	6·9 (1·6–22·7)
High protein	113·2 (91–139)	106·6 (21–220)	11·9 (2·7–23·8)
Supplementary copper 10 mg./day			
Low protein	107·6 (78–134)	347·7 (116–746)	35·3 (12·2–70·1)
High protein	125·8 (97–161)	328·4 (138–505)	40·8 (17·6–69·3)
Least significant difference between means			
$P < 0.05$	15·2	127·4	11·5
$P < 0.01$	20·1	167·8	15·1

from 93·5 to 107·6 g. (13·9 g.) and the increase from 113·2 to 125·8 g. (12·6 g.) for the high-protein group should be compared with the least significant difference ($P < 0.05$) of 15·2 g. Both high-protein groups had significantly greater mean liver dry weights than the corresponding low-protein groups. Only the mean liver dry weight of the low-protein group fed no supplementary copper failed to increase significantly relative to the group slaughtered initially. These changes in mean liver weight correspond with the changes in mean liveweight of the separate groups.

The mean liver-copper concentration of the unsupplemented low-protein group (70·1 p.p.m.) was less than for the group killed initially (119·5 p.p.m.) and for those fed the high-protein concentrate (106·6 p.p.m.), but these differences were not significant. The addition of 10 mg. of Cu per day markedly (and to a similar degree) raised liver-copper concentrations, irrespective of the high- or low-protein content of the diet.

The total liver-copper content of the group killed initially was 10·1 mg. and that for the high-protein group was 11·9 mg., the mean total liver-copper content of the low-protein group being 6·9 mg. Although this reduction was not significant, it shows a similar trend to the significant fall in blood-copper concentration for this group from 1·03 to 0·81 p.p.m. over the period of the experiment (Fig. 2). In the two copper-supplemented groups, the mean total liver-copper contents of the low- and high-protein groups were 35·3 and 40·8 mg. Cu respectively; these amounts were significantly ($P < 0.01$) greater than for the groups which did not receive additional copper, but the level of dietary protein did not have a significant effect on the total amount of Cu stored in the liver. Thus, these two groups were given a total of 960 mg. of Cu (almost 4 g. of $CuSO_4,5H_2O$) more than the other groups. The proportion of this supplementary copper stored in the liver was 2·96% and 3·01% for the low- and high-protein groups respectively and at the level of copper supplementation used, the difference in protein intake did not affect the proportion of orally dosed copper stored in the liver.

(2) *Sheep receiving the high copper supplement*

Liveweight changes

The liveweight gains of both the groups of seven sheep receiving the 250-mg. Cu supplement each day were similar to those of the control sheep and those given the small copper supplement when fed the equivalent diets until 6 March when the mean weight of the low-protein group was 54·5 lb. and that for the high-protein group was 57·2 lb. After this the low-protein group started to lose weight. The high-protein group was not affected at this stage by the additional copper and continued to gain weight at a rate equivalent to both the control group and the low-Cu supplement groups: on 1 May the four surviving sheep in this group had a mean liveweight of 65·3 lb.

In the final stages of copper poisoning loss of weight occurred in most of the sheep as a result of an inappetence which developed a few days before death, and was generally the first sign of distress. One sheep on the low-protein diet lost 16 lb. in the 2 weeks before death, but a loss of about 8 lb. was more general. Loss of weight shortly before death was much more evident for the sheep on the low-protein diet, and only rarely and to a lesser extent (about 3 lb.) did a weight loss occur in sheep on the high-protein ration which died of copper poisoning.

Changes in blood-copper concentrations

The mean blood-copper concentrations at the start of the experiment were 0·98 and 1·10 p.p.m. respectively for the high- and low-protein groups. At the beginning of February when the first deaths occurred, the mean concentration of both groups was 1·10 p.p.m. After this date the mean values were greater and varied widely. This resulted from the abnormally high values found at or near the time of death of individual sheep. In four cases, values as high as 9·3, 10·6, 12·0 and 12·7 were recorded and as the inclusion of such values would greatly distort the mean for each group, such mean values are not presented here but have been discussed in detail elsewhere.[7] It is generally considered that concentrations above 1·2–1·3 p.p.m. of Cu in whole blood are rarely found in sheep of normal copper status.[7] Blood-copper concentrations of individuals during the 2 weeks prior to death commonly fell within the range 1·3–3·0 p.p.m. (with occasional much higher values) for those sheep fed hay and oats alone (low-protein). A range of 1·3–1·7 p.p.m. was more usual for the group fed supplementary protein. Only four of the eleven sheep which died from copper poisoning had blood-copper concentrations which were consistently above 1·40 p.p.m. during the 2 weeks prior to death.

In some cases, as the illness progressed, the blood packed-cell volumes fell as low as 8 compared with values of about 35 for normal sheep. In individuals in which a partial recovery occurred this value rose again to about 20.

Liver dry weight. Liver-copper concentration and total liver-copper content

Table III shows the concentrations and total amounts of copper in the livers. All the seven sheep on the low-protein diet, but only four of the seven on the high-protein diet, died of copper poisoning. The other three were slaughtered at various stages of the experiment when they were quite healthy.

The first sheep which died was one fed on the high-protein diet. Death occurred after 49 doses of 1 g. of $CuSO_4,5H_2O$. The first death in the low-protein group occurred after 51 doses. By 5 April (after 88 doses over 144 days) all seven of the sheep fed the low-protein diet had died compared with only three from the high-protein group. The two sheep slaughtered after 88 doses over 163 days and the other after 183 doses over 265 days from the high-protein group appeared to be in normal health and were not in immediate danger of death from copper poisoning.

If copper sulphate had continued to be administered until a fatal amount of copper had accumulated in these three sheep fed high-protein diet the mean amount given (103 g.) and the mean time to death (164 days) would have been increased. Although there was no significant difference in the amounts of copper needed to cause death in these two groups, this largely results from the high standard errors associated with these small groups. The sheep in the high-protein group were however noticeably better in vigour and condition throughout the experiment. One was given as much as 158 g. of $CuSO_4,5H_2O$ over 240 days before death and a further one was slaughtered after 183 g. had been given over 265 days. In spite of the lack of statistical evidence it seems reasonable to assume that an increased protein intake delayed the time of death of those sheep which were given regular doses of 1 g. of $CuSO_4,5H_2O$ over a long period.

The sheep on the high-protein diet had greater liver dry weights than those fed oats alone and this was a reflection of their increased live weight. The low-protein group had a mean liver-copper concentration at death of 4295 p.p.m. This was significantly ($P < 0{\cdot}01$) higher than both the mean level of 2446 p.p.m. for those four sheep fed the high-protein diet which died and for the mean value of 2565 p.p.m. for all the sheep in that group.

The mean total liver-copper content of 337 mg. for the low-protein group was significantly greater ($P < 0.05$) than the mean of 277 mg. for all seven sheep in the high-protein group. The difference was not quite significant at this level when the mean of 266 mg. for the four sheep which actually died of copper poisoning was considered.

There was a marked tendency for the proportion of dosed copper found in the liver at death to be greater for those sheep which died early than for those which died after a much longer period. Those sheep fed supplementary protein stored a smaller ($P < 0.1$) proportion of the dosed copper than those fed oats alone. The proportion of dosed copper stored in the liver was in the range 1·5–3·0% for the sheep fed the low-protein diet. Only the first sheep to die in the high-protein group stored more than 1·5% of the administered copper in its liver and three of the sheep in this group stored as little as 0·6–0·7% of the copper given.

Discussion

Copper requirement of housed sheep

Although the differences were not significant there was a tendency for the sheep receiving 10 mg. of Cu per day to gain rather more weight than those without copper supplement which received a total of only 3·3 mg. of Cu/day (Fig. 1 and Table I). In the absence of supplementary copper both the low- and high-protein groups showed steadily falling blood-copper concentrations compared with the steady values of those given an additional 10 mg. of Cu/day (Fig. 2). The fall was however only significant for the low-protein group. The concentration of copper and the total amount of copper in the liver were appreciably lower for the low-protein group than for those killed initially (Table II). Although the differences were not significant, reductions of the order of 30–40% were obtained. The high-protein group however had a similar mean liver-copper concentration to that of the sheep killed initially.

It is generally considered that housed sheep accumulate copper. There is no indication that this occurred in the two groups on diets containing only about 6 p.p.m. of Cu with no Cu supplement, and depletion of liver-Cu may have occurred in some cases. The results in Table II suggest that a supplement of 10 mg. of Cu allowed some accumulation of copper in the liver, but this was not associated with an increase in blood-copper concentration over the 5-month period.

Proportion of administered copper stored in the liver

The mean percentages of the total of 960 mg. of Cu administered in 10-mg. doses stored in the liver were 2·96 and 3·01 for the groups fed the low- and high-protein diets respectively. These figures have been calculated from the total liver-copper contents of these sheep compared with those fed the unsupplemented diets which were slaughtered at the same time.

These results are in good agreement with figures quoted by previous workers where similar amounts of copper have been given. Edgar[8] gave 25 mg. of Cu per day (as the sulphate) to 16 sheep at grass for 228 days and found that the mean amount of copper stored in the liver was 2·3% (range 1·0–3·9%) of the total given. Dick[9] found in a large number of experiments with both housed sheep and sheep at grass that when 30 mg. of supplementary copper (as the sulphate) was given each day, the proportion stored in the liver ranged from 2·7 to 4·1%. Hemingway *et al.*[10] dosed eight sheep at grass with 0·5 g. of $CuSO_4,5H_2O$ at fortnightly intervals, the dose averaging 9 mg. of Cu/day, and found that 2·4% of this copper was stored in the liver as measured by comparison with untreated sheep.

Of the sheep receiving a supplement of 250 mg. of Cu (= 1 g. of $CuSO_4,5H_2O$) the first two which died stored in the liver 3·15 and 3·00% of the total copper administered (Table III). The mean percentage of dosed copper found in the liver for the eleven sheep which died was 1·77. There was a marked tendency for the proportion of copper stored to be less for sheep which survived for a longer period and for those fed supplementary protein.

Amounts of copper required to cause death from copper poisoning

Table IV summarises the results of this present investigation and similar experiments conducted by Barden & Robertson[1] and Todd *et al.*[2] where regular daily doses of 1·0–2·0 g.

Table III

Quantities of copper sulphate administered and the amounts of copper stored in the liver of sheep which died of copper poisoning or were slaughtered after long-term dosing

Sheep no.	$CuSO_4,5H_2O$ given, g.	No. of days to death	Liver dry wt., g.	Liver Cu concentration, p.p.m.	Liver Cu content, mg.	% of dosed copper in liver
*Low protein**						
11	51	74	60	6530	392	3·00
42	54	79	55	5300	294	2·17
29	65	101	80	3750	300	1·85
12	77	121	103	3674	379	1·97
48	78	123	98	4718	460	2·36
18	88	136	87	4259	370	1·68
47	88	144	72	4089	337	1·53
Mean	84	129	84	4295	337	1·87
*High protein**						
40	49	71	98	3930	386	3·15
41	69	109	109	2370	259	1·50
39	88	136	84	1558	131	0·60
55	158	240	148	1924	287	0·73
5†	88	163	101	3313	334	1·52
38†	88	163	106	2435	257	1·17
33†	183	265	117	2425	284	0·62
Mean	103	164	109	2565	277	1·33
Significant difference between means	n.s.	<0·1	<0·05	<0·01	<0·05	<0·1

* See Table II † Slaughtered when in good health

of $CuSO_4,5H_2O$ were administered. The first deaths to occur in the present experiment were recorded after a shorter period of copper supplementation and after the administration of a smaller total amount of copper than those found by these previous investigators. In this case however the sheep were much smaller (60 lb. compared with about 100 lb.) and in consequence the daily supplement in terms of unit body weight was proportionately larger in the current experiment.

Table IV

Amounts of copper sulphate required to cause death in sheep when administered at 1 g. per day

	Weeks dosed	$CuSO_4,5H_2O$ consumed, g.
Barden & Robertson[1]	15–17	104
Todd *et al.*[2]		
(*a*)	17–28	80–148
(*b*)	17–29	144–154
Present experiment		
Low-protein diet	11–21	52–88
High-protein diet	10–34	48–156

The amount of copper administered at a rate of about 1 g. of $CuSO_4,5H_2O$ per day required to kill a sheep ranges from 48 to 156 g. as $CuSO_4,5H_2O$ and this wide range may depend on live weight, liver size and the composition of the diet. It would appear that death of sheep from copper poisoning might occur after 3–6 months if the diet contains about 250 p.p.m. of Cu. The present investigation suggests that a high protein intake may make sheep less susceptible to copper poisoning resulting from this order of copper supplementation. Where only small amounts of supplementary copper were given (10 mg./day) additional protein had no such effect.

The consistent findings that under 3% of orally administered copper sulphate is stored in the liver of otherwise healthy sheep, allows an estimate to be made of the total copper consumption in a suspected case of poisoning. For this the total liver-copper content is required rather than a simple expression of the copper concentration.

The sheep receiving 10 mg. of supplementary copper per day stored copper in the liver at the same rate (3%) as those which died most rapidly when given the 250 mg. of Cu/day supplement. The minimum quantity of copper required to kill a sheep under the conditions of the present experiment was 12·5 g. of Cu (Table IV).

Acknowledgments

One of the authors (A. M.) is indebted to the Horserace Betting Levy Board for a Veterinary Research Training Scholarship. A proportion of the experimental costs was met by a general grant given by Imperial Chemical Industries (Pharmaceuticals) Ltd. to the Glasgow University Veterinary School, for which the authors express their appreciation.

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THE REFRACTIVE INDEX OF THE MUSCLE OF FISH AND SHELLFISH. I.—A Possible Method for Measuring Protein Deterioration in Fresh and Frozen Cod

By M. K. ELERIAN

The refractive index of cod muscle was measured during stowage of the whole fish in ice and during cold-storage of fillets at −14° and −29°. These treatments caused, respectively, a decrease and an increase in refractive index, the increase at −14° being faster than that at −29°. The possibility of using refractive index to measure the deterioration of fish is discussed.

Introduction

The acceptability of fish muscle as an article of food diminishes as it is stored in melting ice or, more slowly, in the frozen state at low temperatures. Routine inspection for quality control and research designed to delay the deterioration depend upon methods for measuring the changes that occur in fish muscle that can become obvious to the senses. Although several methods are in use, and many possibilities have been tried, few fulfil the requirements of accuracy, speed and simplicity.[1, 2]

The purpose of the present work was to measure the refractive index of muscle juice or, if possible, whole muscle tissue, during the progress of various treatments, to see whether the readings could be used to measure deterioration.

Experimental

Material and methods

North Sea cod (*Gadus morhua* L.), 22–28 in. long, caught by trawl net, were used throughout the work. Except where stated otherwise, they were caught within 40 miles of Aberdeen. They were gutted at sea and packed in crushed ice, generally reaching the laboratory within 24 h. of catching.

Most of the determinations of refractive index were carried out with an Abbé refractometer which was maintained at 20° by means of circulated water. The light source was a sodium vapour lamp.

A small pocket refractometer (Bellingham & Stanley Ltd., London) was also used for some of the determinations, being found to give essentially the same results as the larger instrument. It was kept, between determinations, with the prisms in a bath of water at 20°, and quickly dried before use.

Expressible fluid was obtained by placing about 2 g. of muscle in a steel cylinder of about 9 mm. dia., closed at one end with fine-mesh brass gauze. A plunger pressing on the sample with a pressure of 0·7–0·8 kg. per sq. cm. liberated within a few seconds sufficient fluid for refractive index determination.

Results

Unfrozen material

Fig. 1 shows the effect of keeping whole gutted cod in crushed ice (at an ambient temperature of about 2°) on the refractive index of muscle and of press juice. A steady decline is shown, and the values are almost always lower in tissue or fluid from the posterior than the anterior end of the fillet.

The refractive index of a solution declines linearly as the solution is diluted (w/v basis),[3] and it was felt that the effect seen in Fig. 1 might be a simple dilution effect from the melt-water of the ice. Accordingly, two groups of similar whole gutted cod were stowed in crushed ice, the first as before, and the individual fish of the second being wrapped in aluminium foil to prevent contact with the melt-water.

The results (Fig. 2) show that although leaching or dilution do have a small effect, the decline in refractive index is largely independent of it.

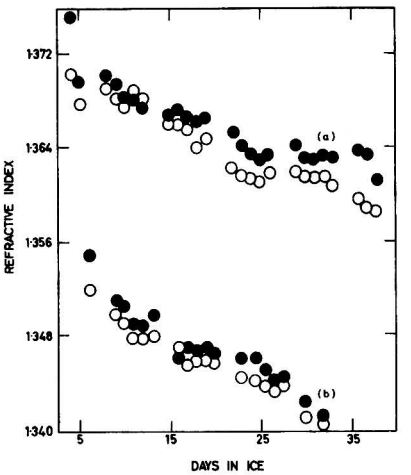

FIG. 1.—*Refractive index of cod muscle* (curves a) *and expressible fluid* (curves b) *during stowage in ice*

● From anterior half of fillet
○ From posterior half

Frozen material

Bundles of 10 anterior pieces of fillet, each of about 50 g. and each from a different fish, were wrapped in aluminium foil and frozen in an air-blast freezer at −30°. The refractive index was measured in a bundle after thawing at about 18° for 1–2 h. after various times at −14° and −29°. The results (Fig. 3) show that the refractive index rises during cold storage, somewhat more quickly at −14° than at −29°.

It was found that if cod were kept at −1·5° in a supercooled condition, i.e. without ice present in the tissue, the refractive index of the muscle did not change over a 12-day period, although it rose rapidly over the same period at the same temperature when ice was present. This accords with observations on protein denaturation measured by the cell-fragility method.[4]

The refractive index of expressible fluid from frozen and thawed fish rose, as in Fig. 3, but the results were much more variable than in whole muscle. The reason appears to be because denaturation causes a considerable amount of fluid to be released from the structural proteins, and this dilutes and contaminates the expressible fluid to a variable extent. It was therefore considered unprofitable to carry out refractive index measurements under these conditions.

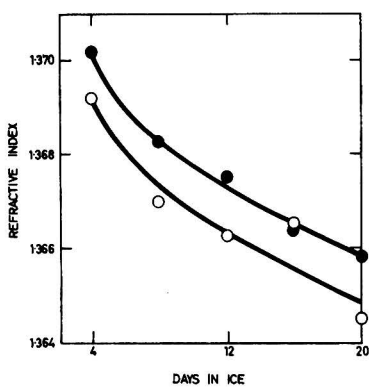

FIG. 2.—*Effect of wrapping in aluminium foil on the decline in the refractive index of cod muscle during stowage in ice*

● Wrapped ○ Unwrapped

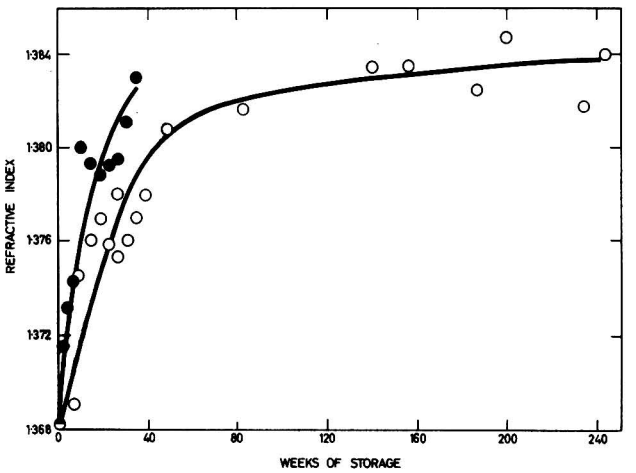

FIG. 3.—*Effect of freezing and cold-storage on the refractive index of cod muscle after thawing out*
●——● Stored at −14°
○——○ Stored at −29°

Establishment of a base line

Much has been written[5–7] about the natural variations that occur in the body constituents of cod, and of their influence on the accuracy of determinations of cold-storage changes in the tissue.[8, 9]

In order to obtain a rough idea of the extent of the variations that might be encountered in fresh fish, a few batches were obtained from various grounds. The refractive index after 3 days in ice was found (Table I) to range from 1·3700 to 1·3744.

Table I

Refractive index of the muscle of cod caught in different localities

Date of catching	Fishing ground	Refractive index	Standard error[4]
17.9.1961	50 miles E. of Aberdeen	1·3708	3·1
5.1.1962	Tod Head (near Aberdeen)	1·3700	2·4
9.5.1963	Near Hebrides (W. Coast of Scotland)	1·3708	2·3
15.7.1963	90 miles N. of Orkney Is.	1·3710	4·1
19.9.1963	15 miles S.E. of Faroe	1·3744	1·8
21.9.1963	20 miles E. of Iceland	1·3734	3·1
25.9.1963	15 miles N.E. of Iceland	1·3734	2·0

Each reading is the average of 10 each from a different fish

The lowest value was in January, which is near the time when spawning and food scarcity bring about the greatest depletion in the muscle. If samples had been obtained in February or March the results would probably[7] have been lower still.

Discussion

The complex nature of cod muscle precludes any simple comprehensive theory to account for these observations. However, Netter[10] has stated that it is reasonable to expect a change in the refractive index in either direction—a drop as a result of the extension of the polypeptide chains, or a rise because of a loss of hydration (water-holding capacity of the muscle). This conjecture is in line with the present findings with cod muscle undergoing iced storage (decrease in refractive index) or frozen storage (increase). While it is not possible to state much more than that as an indication of the mechanism, there are two features of the observations which deserve mention.

When the tissue was stored in melting ice (Fig. 1) the refractive index of the expressible fluid dropped in a manner very similar to that of the whole muscle. It is therefore possible that the changes in refractive index during chill storage, presumably the result of bacterial and/or tissue enzyme action, take place in the extracellular fluid and perhaps the sarcoplasm as well. Expressible fluid is largely extracellular in origin[11] and does not contain the 'structural' proteins actin and myosin, which are the proteins usually studied in connexion with changes during frozen storage.

If this were true, so that we could regard the observations as relating to the albumin (water-soluble) fraction of the muscle, it would account for the unusual appearance of the cold-storage curves shown in Fig. 3. Studies of changes in cod protein during frozen storage have been confined for the last 30 years to the actomyosin fraction. It was recently shown[12] that the alterations to actomyosin were virtually complete in 15 weeks at $-14°$, but required over 9 years at $-29°$. In Fig. 3 it is true that the rate of increase of refractive index is greater at $-14°$ than at $-29°$, but the changes here continued at $-14°$ for at least 34 weeks, while those at $-29°$ appeared to be completed in only 3 years. Such figures are inconsistent with the behaviour of actomyosin established after many experiments, both from solubility studies[12] and from the 'cell fragility' method.[9] Confirmation of the present data would be a lengthy undertaking; meanwhile one may cautiously suggest that a protein fraction other than actomyosin is involved.

It can be definitely stated as the outcome of this work, that the refractive index of cod muscle declines when the whole fish are kept in melting ice, and that the decline is only slightly influenced by leaching or dilution effects; further, that storage in the frozen state results in an increase in the refractive index, the rate of increase being influenced by the storage temperature in a manner somewhat reminiscent of that of the salt-solubility of actomyosin and 'cell fragility'. The changes in refractive index, up or down, are greater than those attributable to the biological 'condition' of the fish on the evidence obtained so far, but such changes do occur and might reduce the usefulness of the technique for examining fish of unknown history.

From the practical viewpoint, the technique may be suitable for assessing the deterioration of cod kept in ice. The possibility depends entirely on the long-term establishment of a 'base line' of small variability for the geographical area from which the fish were taken, i.e. further work along the lines of Table I. The technique seems to be rather more promising than measuring the refractive index of the centrifuged[1, 13] or uncentrifuged[14] eye fluid, since the eyes of the fish are often damaged during handling, and since earlier studies on the diffusion of water into fish eyes[15] suggest that the rate of melting of the ice probably influences the result.

The picture is more encouraging when one considers frozen cod, since refractive index is altered to a greater degree. The technique can be used to distinguish between fish that have been cold-stored for a while and those newly frozen or stored at very low temperatures. The degree of discrimination of the method, and the influence of time after death before freezing, require investigation.

Should the method prove suitable for the assessment of deterioration in frozen fish, it would have some advantages over existing methods. It is very quick and inexpensive, and simple to carry out. It also requires only a minute piece of the fillet, which therefore remains saleable.

Acknowledgment

The work described in this paper was carried out as part of the programme of the Department of Scientific and Industrial Research, during the tenure of a grant from the United Arab Republic.

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Received 30 July, 1964; amended manuscript 14 October, 1964

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ERRATA

In the article by Daphne J. Osborne (*J. Sci. Fd Agric.*, 1965, **16,** 1), the following amendments should be made:

Page 7 Table I Omit 40% from heading of col. 2

,, (c.p.m. in protein) 5×10^3 from heading of col. 3

Line 6 For 'entire RNA synthesis of the cell' read 'entire messenger-RNA synthesis of the cell'

Page 8 Line 20 For 'formation' read 'information'

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

APRIL, 1965

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Pedogenesis in New South Wales. VIII. Alternative hypothesis for the formation of the solodised-solonetz of the Pilliga district. E. G. Hallsworth and H. D. Waring (*J. Soil Sci.*, 1964, **15**, 158—177).—The conditions under which a solodised-solonetz can occur without ever having been through the stages of solontchak and solonetz are presented and discussed. A. H. CORNFIELD.

Soil catena on granite in Southern Rhodesia. I. Field observations. II. Analytical data. J. P. Watson (*J. Soil Sci.*, 1964, **15**, 238—250, 251—257).—Characteristics of the catena are presented and discussed. A. H CORNFIELD.

Planosolic Piedmont soils of North Carolina. I. Morphology and composition. R. J. McCracken, S. B. Weed and E. F. Goldston (*Soil Sci.*, 1964, **98**, 22—32).—The soil profiles are described and the chemical and physical composition given. Micro-morphology is also discussed and the soils are classified. T. G. MORRIS.

Soil investigations on Banks Island. J. C. F. Tedrow and L. A. Douglas (*Soil Sci.*, 1964, **98**, 53—65).—A report of the chemical, physical and mineralogical investigations on the soils. T. G. MORRIS.

Trace element contents of some Queensland soils. A. C. Oertel and J. B. Giles (*Aust. J. Soil Res.*, 1963 [1964]. **1**, 215—230).—The proportions of Co, Cu, Ga, Mn, Mo, Ni, V, Zn and Zr in 28 soil profiles are shown. Soils of similar taxonomic groups show similar patterns of trace element distribution with depth. The relative concn. of trace elements in surface soils may afford evidence of the manner of formation of the profiles. A. G. POLLARD.

Effect of temperature and trapped air on the energy status of water in porous media. R. S. Chahal (*Soil Sci*, 1964, **98**, 107—112).—The theoretical equation developed by Peck (*ibid.*, 1960, **89**, 303) is discussed and a correction applied. Published results of other workers are compared in the light of the new equation. T. G. MORRIS.

Determination of the total specific surface areas of soils by adsorption of cetylpyridinium bromide. D. J. Greenland and J. P. Quirk (*J. Soil Sci.*, 1964, **15**, 178—191).—The method is described. A. H. CORNFIELD.

Surface area measurements in soils. III. Sorption of stearic acid and iodine as measures of external surface. B. R. Puri and K. Murari (*Soil Sci.*. 1964, **97**, 417—420), cf. *ibid.*, 1964, **97**, 341).—In about 80 soils and clays, dried at 110°, total external surface area was measured by (*a*) glycol retention, (*b*) absorption of stearic acid from solution in benzene and (*c*) absorption of I_2 from $CHCl_3$,both before and after heating the soils and clays at 600° for 6 h. Methods (*b*) and (*c*) yielded substantially the same values both before and after heating; (*a*) gave values of the same order after heating the samples but much greater values for the unheated materials. Heating at 600° is assumed to cause loss of internal surface which is not recorded by (*b*) or (*c*). A. G. POLLARD.

Influence of organic materials on the determination of the specific surface areas of soils. J. R. Burford, T. L. Deshpande, D. J. Greenland and J. P. Quirk (*J. Soil Sci.*, 1964, **15**, 192—201).—The large differences that occurred in surface and total area of soil clays, as determined by adsorption of N_2 at low temp. and adsorption of cetylpyridinium bromide, before and after treatment of the soil with H_2O_2, showed the importance of removing org. matter before determining the surface areas of soil clays. A. H. CORNFIELD.

Determination of soil density. R. Sunkel (*Z. PflErnähr. Düng.*, 1964, **106**, 228—238).—Results of density measurements on an oven-dry soil using a liquid pycnometer and org. solvents are different from those obtained with an air pressure pycnometer. Both methods give different results if moist soil is used. The most accurate results are obtained using oven-dry soil and a liquid pycnometer with org. solvent. Oven drying has no adverse effects although results are between 1·5 and 4·5% lower than those from moist soil, due to compression experienced by strongly adsorbed water. M. LONG.

Effect of tillage operations on bulk density and other physical properties of the soil. D. H. Luttrell (*Dissert. Abstr.*, 1964, **24**, 4103).—The influence of certain tillage implements [plough, harrow (disk or spiked tooth section)] and operations on the bulk density, clod size distribution and surface roughness during five treatments is described. A surface roughness coeff. (**I**) was developed and soil moisture and temp. fluctuations were recorded. **I** was a sensitive index for describing the degree of surface roughness of tilled soil. Ploughing created the lowest bulk density, largest mean wt. dia. and roughest surface. F. C. SUTTON.

Neutron soil moisture monitor. E. M. Speen, W. L. Bunch and M. R. Wood (*U.S. atomic Energy Commission*, 1964, *Rep.* H.W. 82009, 19 pp.).—The method is described. C. V.

[Soil] perfusion apparatus with variable aeration. J. I. Sperber and B. J. Sykes (*Plant & Soil*, 1964, **20**, 127—130).—The Audus soil perfusion apparatus (*Nature, Lond.*, 1946, **158**, 419) was modified to allow varying periods of intermittent perfusion and aeration. A. H. CORNFIELD.

Theory of unsaturated flow into a non-uniform soil profile. D. Zaslowsky (*Soil Sci.*, 1964, **97**, 400—410).—The hydraulic flow of water in soil is discussed mathematically, with particular reference to one-dimensional, steady-water infiltration into a soil profile of non-uniform hydraulic conductivity. Two types of profile are considered, (*a*) one with well-defined layers each of which has its own conductivity differing from those of the other layers, and (*b*) one in which the conductivity changes gradually with increasing depth. A low water-head at the soil surface combined with high flow-resistance near the point of entry of water leads to unsaturated flow; conversely high water-head and adjacent low flow-resistance produces a saturated flow. The shape of the pressure distribution with depth, an analysis of vertical and diagonal flow and the formation of a 'perched' water horizon are also subjected to mathematical examination. A. G. POLLARD.

Mass-flow and salt accumulations by plants on water vs. soil cultures. F. M. Eaton and J. E. Bernardin (*Soil Sci.*, 1964, **97**, 411—416).—Barley, cotton and tomato plants were grown in soil and in water cultures with a view to establishing whether the salt tolerance of plants grown in water-culture could serve to predict the decrease in yields of these plants grown on saline field soils. With nutrient solutions containing Cl^- (120 mequiv./l.) as the Na, Ca and Mg salts the three crops accumulated in aerial organs 31, 25 and 39% respectively more Cl^- than when grown in soil culture. Transpiration rates of plants although affecting mass-flow probably do not influence the Cl^- accumulation within the plants. A. G. POLLARD.

Possible rôle of methane in affecting the hydraulic conductivity of fine quartz sand. D. Swartzendruber and R. P. Gupta (*Soil Sci.*, 1964, **98**, 73—77).—Sand was treated in a permeameter with either water or a 1% v/v solution of methane in water, and the hydraulic conductivity was measured. At the end of the flow period (70 l.) the conductivity of the sand to water only had decreased by 30% while that to methane solution was unchanged. Ethylene oxide sterilisation of the sand with 'Millipore' filtration of the water did not eliminate bacterial growth but it did keep the reduction in hydraulic conductivity to $\gtrsim$35%. T. G. MORRIS.

Physical properties of soil mixes. S. R. Richards, J. E. Warneke, A. W. Marsh and F. K. Aljibury (*Soil Sci.*, 1964, **98**, 129—132).—Privet was grown in three alluvial soils mixed with plaster sand, peat or wood shavings. The hydraulic conductivity of small 5-cm. dia. by 5-cm.-long cylinders packed under standard conditions was determined. Results indicated that the values obtained for bulk density and hydraulic conductivity on the small compact samples appeared to be of potential use in predicting these properties for the mixes for commercial use. T. G. MORRIS.

Effect of hexadecanol on water loss from soil and plants. A. A. Abdalla and W. J. Flocker (*Proc. Amer. Soc. hort. Sci.*, 1963, **83**, 849—854).—High rates of application (600 lb. or more per acre) of hexadecanol reduced evaporation from bare soils, but seriously reduced percolation of water into soil. 10—50 lb./acre enhanced the growth of sweet maize and tomato plants, but 100 lb./acre reduced growth. A. H. CORNFIELD.

Water losses from soil and rubidium uptake by adventitious roots of sunflower as related to the water content of the soil. D. S. Stevenson and L. Boersma (*Agron. J.*, 1964, **56**, 512—514).—Water absorption by adventitious roots of sunflower was closely related to the amount of root growth, which in turn increased with soil moisture content. Uptake of Rb was not consistently related to initial soil water content or to amount of root growth. Uptake of Rb may be related to the active transport of water from the roots to the plant. A. H. CORNFIELD.

Relationship between behaviour of soils (crumbling and cracking) and their texture. S. Hénin and —. Bosquet (*C. R. Acad. Agric. Fr.*, 1964, **50**, 842—846).—Observation of the behaviour of 20-g. samples moistened with water, spread evenly on glass balls (dia. 27 mm.) and allowed to dry at room temp. affords a means of classifying the soils into four groups, viz. crumbling and non-crumbling, and cracking and non-cracking. An examination of the relationship of the results with those of granulometric analyses led to the development of two equations by which the tendency to crack or crumble can be predicted from the granulometric analyses. P. S. ARUP.

Maintenance of permanent irrigation agriculture. W. P. Kelley (*Soil Sci.*, 1964, **98**, 113—117).—A review. T. G. MORRIS.

Solubility of variscite. A. W. Taylor and E. I. Gurney (*Soil Sci.*, 1964, **98**, 9—13).—The solubility product of variscite has been determined by equilibration at 25° with 0·003M-HCl or with 0·03, 0·01 or 0·003M-H_3PO_4. The mean value in HCl was 22·52 and in H_3PO_4 21·50. The difference is ascribed to errors in the calculation of the ionic activities resulting from the presence of unknown complex Al–PO_4 ions. T. G. MORRIS.

Properties of protein–bentonite complexes as influenced by equilibration conditions. D. E. Armstrong and G. Chesters (*Soil Sci.*, 1964, **98**, 39—52).—Mg–bentonite was used to form complexes with pepsin (representing proteins with an acid isoelectric pH) and lysozyme (basic isoelectric pH). Suspensions of the clay were shaken with the desired amount of protein in a buffer, the bentonite removed centrifugally and the amount of protein adsorbed was determined. Adsorption of the protein was rapid, 90% of the max. occurring in 3 min., the remainder required up to 12 h. For a given protein concn. max. adsorption occurred near the isoelectric pH of the protein. The amount of protein adsorbed by bentonite was influenced by the protein concn. and the pH of the system. For a given protein concn. the max. adsorption occurred near the isoelectric pH of the protein. Adsorption of protein below the isoelectric pH of the protein was greatly increased by the addition of electrolytes. *X*-ray diffraction showed that adsorption of protein resulted in an expansion of the clay lattice. Lattice expansions of up to 64 Å were observed. T. G. MORRIS.

Swelling of sodium montmorillonite due to water absorption. W. W. Emerson (*Aust. J. Soil Res.*, 1963 [1964], **1**, 129—143).—The swelling of orientated flakes of dry Na montmorillonite exposed to water vapour or immersed in solutions of Na or Li salts of varied concn. and pH, or of cetyltrimethylammonium bromide was measured microscopically. Montmorillonite crystals are probably joined edge-to-edge in a tactoid. Bands appearing in thin sections of expanded gels in polarised light may result from a periodicity of stacking of the silicate sheets forming the crystals. The increase in swelling induced in the previously untreated clay by rise in pH may be explained by removal of Al from the external surfaces of the crystals. The mechanism of these effects is discussed. A. G. POLLARD.

Cation-exchange reactions. R. C. Salmon (*J. Soil Sci.*, 1964, **15**, 273—283).—Bentonite, illite and fen peat were saturated with different proportions of Ca, Mg and K, and exchange between these cations was studied by measuring their activity ratios in dil. equilibrium solutions. With both clays the activity ratio a_{Mg}/a_{Ca+Mg} in solution was linearly related to the ratio of adsorbed Mg/Ca, the former being 1·22 times larger than the latter ratio. Peat held Mg much less strongly than Ca, the difference increasing with Mg saturation. With all three materials the activity ratio a_{Mg}/a_{Ca+Mg} in solution was curvilinearly related to % Mg saturation. Peat adsorbed K less strongly relative to the divalent cations than did the clays, and bentonite adsorbed K less strongly than did illite. Decreasing the Ca : Mg ratio increased the strength with which peat adsorbed K, but had no effect on K adsorption by clays. The relation between the concn. ratio [Mg]/[Ca + Mg] of 40 soils and the ratio of exchangeable Mg/(Ca + Mg) varied within the range covered by peat and bentonite or illite, suggesting that differences between soils may be due to different org. matter contents. The extent to which [Mg]/[Ca + Mg] in solution was altered by changes in the exchangeable Mg content differed considerably between soils. These differences were not all explained by variations in exchange capacity, showing that different soils adsorb Mg with differing strengths relative to Ca. A. H. CORNFIELD.

Cation-exchange equilibria with vermiculite. A. Wild and J. Keay (*J. Soil Sci.*, 1964, **15**, 135—144).—The exchange of Na^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} was studied over the temp. range 25—70°. Vermiculite showed a preference for divalent cations over Na^+ at 25° and the preference increased greatly with temp. The preference was largely determined by the increase in entropy which accompanied the replacement of monovalent ions by divalent ions in the vermiculite. Preference was less marked between divalent ions, which are similarly hydrated in the mineral. The greater affinity of Mg^{2+} compared with other divalent ions is explained by their closer approach to the silicate surfaces. A. H. CORNFIELD.

Precipitation of strontium by calcium carbonate in calcareous soils and measurement of cation-exchange capacity. E. Halevy and Y. Tzur (*Soil Sci.*, 1964, **98**, 66—67).—Tests described show that in addition to cation-exchange there exists in calcareous soils a mechanism of pptn. of Sr by $CaCO_3$. A small % of lime in a soil will cause the retention of a much larger amount of Sr than would be expected. T. G. MORRIS.

Rapid method for the determination of cation-exchange capacity of calcareous and non-calcareous soils. C. L. Bascomb (*J. Sci. Fd Agric.*, 1964, **15**, 821—823).—A convenient routine method based on the removal of Ba^{2+} ions of a Ba-soil by aq. $MgSO_4$ as $BaSO_4$ ppt. is described. The amount of Mg exchanged from a standard solution by a Ba-soil is measured by EDTA titration, and the cation-exchange capacity is calculated. Advantages are: pH remains almost constant, elimination of exhaustive washing prevents risks of hydrolysis, complete replacement of the saturating cation (Ba^{2+}) is achieved in one operation by pptn., measurement of anion is unnecessary. Results agree favourably with those given by the standard A.O.A.C. procedure. E. M. J.

Soluble silica in soils. I. Sorption of silicic acid by soils and minerals. R. S. Beckwith and R. Reeve (*Aust. J. Soil Res.*, 1963 [1964], **1**, 157—168).—When soils of varied types were shaken with solutions of monosilicic acid, (**I**) (≯135 p.p.m.) the residual concn. of **I** in near-equilibrium conditions depended on the level of added **I**, on the ratio, solution : soil and on the pH of the soil suspension. This residual concn. was controlled by an adsorption equilibrium which depended on pH; decrease in pH below 8—9 increased the residual concn. progressively. Some oxides and hydroxides of Fe and Al also sorb **I** from solutions in a manner similar to that by soil and which was also dependent on pH, notably in the range 4—9. Sesquioxides probably account for much of the sorption of **I** by soils. Bentonite, kaolinite and some natural and synthetic carbonates also absorbed **I**. The possible occurrence of native SiO_2 in the sorbed condition in soils is discussed. A. G. POLLARD.

Form and frequency of appearance of various particle size fractions. K. Hartge (*Z. PflErnähr. Düng.*, 1964, **107**, 1—10).—No soils are found with <20% silt and >30% clay nor with <5% clay and >30% silt and/or <65% sand. Log particle size distribution is nearly normal. Every particle size range has a frequency distribution with only one peak. Sand-clay mixtures, not containing silt, seldom occur as they have a twin-peaked distribution. The clay in sands and silts probably originates only from films and silt–sand aggregates, brought into suspension during laboratory handling. M. LONG.

Lime potential of soils L. E. Lisanti (*Z. PflErnähr. Düng.*, 1964, **107**, 11—18).—The lime potential (**I**) of red earths diminishes with increasing CO_2 pressure, irrespective of the % saturation of the soils. **I** is independent of the salt concn. of the extractant and also of the soil/extractant ratio. Peat behaves quite differently, **I** being variable. M. LONG.

Mineralisation of nitrogen in soil during spring. J. Chabannes, G. Barbier and J. Driard (*C. R. Acad. Agric. Fr.*, 1964, **50**, 874—881).—Previous observations are confirmed (cf. *ibid.*, 1963, **50**, 550). P. S. ARUP.

Factors in low-temperature storage influencing the mineralisable-nitrogen of soils. D. E. Harding and D. J. Ross (*J. Sci. Fd Agric.*, 1964, **15**, 829—834).—Of four soils stored at −20°, on subsequent incubation three produced significantly more NH_4^+- and NO_3^--N, these increases being produced by previous freezing and thawing and probably associated with moisture and org. matter contents. Nitrifying organisms decrease after storage for 6 months. (15 references.) E. M. J.

Mineralisation of plant nitrogen in soil following alternate wet and dry conditions. H. F. Birch (*Plant & Soil*, 1964, **20**, 43—49).—When soil was incubated with ground plant material of high N content mineral N accumulation was less when the soil was maintained moist throughout incubation than when it was subjected to a no. of dryings, followed by re-moistening, during incubation. Where plant materia of low-N content was added the extent of N immobilisation during incubation was less under alternating wet and dry conditions than under permanently moist conditions. A. H. CORNFIELD.

Comparison between the effect of fresh and dried organic material added to soil on carbon and nitrogen mineralisation. D. A. van Schreven (*Plant & Soil*, 1964, **20**, 149—165).—The rate of mineralisation of C and N in soil incubated with addition of org. materials was usually less if the org. material was dried than when it was applied in undried condition. A. H. CORNFIELD.

Non-enzymic gaseous loss of nitrite from clay and soil systems. L. H. Wullstein and C. M. Gilmour (*Soil Sci.*, 1964, **97**, 428—430).—Experimental evidence presented suggests that loss of NO_2^- from acidic field soils under certain conditions may result from interaction with transition metals, e.g., Mn, whereby Mn^{2+} is oxidised to Mn^{3+} and NO_2^- is reduced to NO. A. G. POLLARD.

Nitrogen fixation by *Azotobacter* as influenced by soil extracts. V. Iswaran (*Indian J. appl. Chem.*, 1964, **27**, 98—100).—The pH, % of C and N for 21 soils and the N fixation by the soil in Jensens medium (**I**) and by *Azotobacter* in the soil solutions were determined. N fixation is greatest in (**I**) and the soils are classified as satisfactory, fair and poor. E. C. DOLTON.

Measurement of nitrogen-supplying power of soils by extraction with sodium bicarbonate. A. A. MacLean (*Nature, Lond.*, 1964, **203**, 1307—1308).—A method developed for measuring the N-supplying power of soils is thought to be more reliable than all others tested. The soil is extracted with $NaHCO_3$ and digested with H_2SO_4/K_2SO_4 followed by direct Nesslerisation. S. A. BROOKS.

Phosphorus fractions in selected soil profiles of El Salvador as related to their development. W. C. Dahnke, J. L. Malcolm and M. E. Menéndez (*Soil Sci.*, 1964, **98**, 33—38).—The various forms in which P occurs in selected soil profiles are examined. Available P, org. P, total P and inorg. fractions were determined in 17 profiles derived from volcanic materials. Org. C varied widely in the soils and in all cases diminished with depth but was relatively greater in the lower levels of the older soils. In general, the older soils contained much less total P and less of each P fraction except occluded Fe-P than did the younger soils. All the younger soils contained considerable amounts of Al-P in the surface layers. Org. P was not clearly related to the other soil P fractions nor to org. C. T. G. MORRIS.

Phosphorus movement in a calcareous soil. II. Soil microbial activity and organic phosphorus movement. R. J. Hannapel, W. H. Fuller and R. H. Fox (*Soil Sci.*, 1964, **97**, 421—427; cf. *ibid.*, p. 350).—Addition of sucrose to a soil increased its microbiological activity as measured by CO_2 production and also increased (38-fold) the movement of P in a column of soil. 95% of the P which had moved during the experimental period was in org. forms. Addition of formaldehyde with sucrose diminished CO_2 production and also the movement of P. Much of the P was associated with microbial cells and cell débris. Probably the P which moves through soil in the soil solution is present therein as colloidal org. complexes. A. G. POLLARD.

Determination of labile soil phosphate as influenced by the time of application of labelled phosphate. S. Larsen and D. Gunary (*Plant & Soil*, 1964, **20**, 135—142).—The effect on L-values of pre-equilibrating ^{32}P with three soil types for 2, 1 and 0 months before sowing ryegrass was studied. Resin and H_3PO_4 were used as carriers. Equilibrium was established some 12 weeks after sowing and this time was virtually unaffected by the pre-equilibration treatments. The P source affected both P uptake and L-value, the resin source showing higher uptake and lower L-value. A. H. CORNFIELD.

Availability of residual phosphorus as measured by lucerne yields, phosphorus uptake and soil analysis. J. R. Thomas (*Soil Sci.*, 1964, **98**, 78—84).—Barley and lucerne were grown in the field in a clay soil which had received various amounts of P as superphosphate and horse manure previously. The crops were grown continuously for 5 years. Residual P significantly increased the yield and P content of the barley grain. The % recovery of the added P was low. Where manure had been applied with P yields of grain were reduced but the N and P content of the grain were increased. Residual P significantly increased the lucerne hay yields and also the P content over the 5 years. Recovery of residual P was influenced by the initial rates of P supplied, the total recovery for the 5-year period being 27·7% for the lowest rate (26·2 lb.) to 21·3% for the highest rate (209·6 lb./acre) cropping reduced the amount of indigenous soil P extractable with $NaHCO_3$ or dilute acid-NH_4F by 76·4 and 36·9% respectively. The uptake of P by the plants was significantly related to the extractable P. T. G. MORRIS.

Radioactive-tracer method for measuring the stability of sparingly soluble phosphates in soil. F. M. Abdou and S. Larsen (*Soil Sci.*, 1964, **98**, 94—99).—Three P sources, $CaHPO_4.2H_2O$ (**I**), hydroxyapatite and $MgNH_4PO_4.6H_2O$ (**II**), labelled with ^{32}P, were added to a soil at different moisture levels and left for different periods. The soil was then shaken with a conc. solution of phosphate saturated with the particular phosphate under test but without ^{32}P labelling. This extracted the ^{32}P released by breakdown by the soil of the added labelled phosphate with min. disturbance of the equilibrium. The extracted ^{32}P was counted and the breakdown of the material under test could be calculated. In soil having 100% of its water-holding capacity saturated, at 25° and pH 6·5, **I** was completely degraded in 1 day; **II** was broken down in 2 h., while hydroxyapatite persisted for 8 weeks unchanged. T. G. MORRIS.

Phosphate potentials of soils. I. Measurement of phosphate potential. R. E. White and P. H. T. Beckett (*Plant & Soil*, 1964, 20, 1—16).—Measurements of Schofield's phosphate potential are described. The equilibrium phosphate potential SP (**I**) was determined in a solution of 0·01M-$CaCl_2$, which underwent no net exchange of PO_4^{3-} with the soil. The results suggest that labile $PO^3{}_4^-$ in a field soil was not at equilibrium, so that **I** depended on the duration of shaking the soil in solution, and on the soil/solution ratio. In contrast after long storage (2—3 months) of a field soil at constant temp. and with careful aeration, equilibrium was achieved within the pool of labile PO_4^{3-}; **I** was then independent of the duration of shaking and the soil/solution ratio until the onset of microbial interference. Such an equilibrium may be disturbed by drying, changes in temp. and temporary anaerobic conditions. However, with the exception of the anaerobic effect the changes induced in this value were considerably smaller than the range of potentials **I** of the field soils examined. The assumptions implicit in the use of **I** as a measure of the availability of soil-PO_4^{3-} are discussed. **I** is a valid measure of the chemical potential of labile PO_4^{3-} in a soil only when soils of comparable Ca status are compared. A. H. CORNFIELD.

Measurement of chemical potential of phosphate in soil suspensions. A. W. Fordham (*Aust. J. Soil Res.*, 1963 [1964], **1**, 144—156).—Effects of the ratio of soil to solution and of the time of shaking on values obtained for the PO_4^{3-}-potential (Schofield) are examined. No evidence was obtained of the presence of complex phosphate ions which might dissociate and thus influence the PO_4^{3-}-potential. Change of potential during shaking was not influenced by dissociation of HCO_3^- from the soil surface. Enzymic activity possibly takes place in the soil with use of toluene and also after irradiation. The capacity of soil to absorb PO_4^{3-} increased during shaking; variations of PO_4^{3-}-potential then taking place were in a direction opposite to that observed in moist soil. A. G. POLLARD.

Effect of drying on the availability of phosphorus and potassium in the submerged rice soils of Kuttanad. P. K. Zachariah (*J. Instn Chem. India*, 1964, **36**, 211—214).—The available K and P increased markedly on drying and there was no apparent relationship between the available K and P under field conditions and as determined in the dried samples. (13 references.) E. C. DOLTON.

Effect of cation size and pH on potassium exchange in Nason soil. C. I. Rich (*Soil Sci.*, 1964, **98**, 100—106; cf. *ibid.*, 1963, **97**, 384).—In this very acid soil K was released to NH_4·OAc, and $MgCl_2$ but not to $Mg(OAc)_2$. The release of K by $MgCl_2$ is explained by an increase in $[H^+]$, when unbuffered $MgCl_2$ was added to the acid soil. The exchange of K depended largely on the dia. of the solvated cation, except in the case of H^+. The clay minerals of the soil include a proportion of dioctahedral vermiculite in which the expanded layers probably do not all extend to both edges of the particle and thus leave wedge-shaped inter-layer zones. T. G. MORRIS.

Correlation between soil test values for potassium and crop responses to potassic fertilisers by paddy in Indian soils. V. Iswaran and P. K. Oommen (*Indian J. appl. Chem.*, 1964, **27**, 62—66).—One-pot culture experiments with paddy grown on 20 different soils were used to evaluate K as a plant nutrient. No statistically significant relation was found between soil test values and % crop responses but there was a significant correlation between exchangeable K % and crop responses for clay-loam soils. (12 references.) E. C. DOLTON.

Potassium status of West Indian soils. P. Moss and J. K. Coulter (*J. Soil Sci.*, 1964, **15**, 284—298).—The soils had 5—72% of clay and contained principally pyroxenes, soda-lime feldspars and variable quantities of glass and amphiboles. Total K content, but not the clay content, was highly correlated with K extracted in the first as well as in further extractions with boiling N-HNO_3. K intensity, $pK - 0{\cdot}5p(Ca + Mg)$ values, determined by equilibrating soils with dil. $CaCl_2$ solutions, were similar to those in soil solutions displaced by alcohol. The K-intensity status was highest in allophane soils and lowest in montmorillonite soils and was highly correlated with exchangeable, fixed and highly-sol. HNO_3-sol. K. The major factors controlling K intensity were exchangeable K relative to exchangeable Ca and Mg and the K-fixing ability. A. H. CORNFIELD.

Mechanisms of formation of sodium carbonate in soils. II. Laboratory study of biogenesis. P. Janitzky and L. D. Whittig

(*J. Soil Sci.*, 1964, **15**, 145—157).—In incubation tests an anaerobic environment and a supply of Na_2SO_4 and readily decomposable org. matter were essential for development of HCO_3^- and CO_3^{2-}. Ultimate development of high alkalinity depends on inactivation of sol. and exchangeable divalent bases as pptd. CO_3^{2-}. This favours a high exchangeable Na content as well as increases in sol. $NaHCO_3$ and Na_2CO_3. The extent of reduction of SO_4^{2-} was inversely related to the amount of HCO_3^- formed. Development of alkalinity was favoured where org. matter and Na_2SO_4 were both at high levels, but was restricted when one of these was limiting.

A. H. CORNFIELD.

Aluminium in rice soils. R. B. Cate, jun., and A. P. Sukhai (*Soil Sci.*, 1964, **98**, 85—93).—Rice seedlings grown for 4 weeks in nutrient solution were transferred to vessels for further growth by a 'split root' technique. One half of the roots were dipping into a nutrient solution and the other half into aq. $Al_2(SO_4)_3$ containing Al up to 1000 p.p.m. Plants exposed (half-root) to 1000 p.p.m. of Al died after 2 days. With [Al] 300 p.p.m. leaf mottling developed in 4—5 days and plants in [Al] 25 p.p.m. did so after 3 weeks. Plants in which roots had been cut back to 1 in. developed new roots 6—8 in. long but the corresponding Al-treated plants developed roots decreasing in length with increasing Al concn. Other cations and anions alleviate the sensitivity to Al but did not eliminate it completely. There was no correlation between the % germination of seeds and the Al concn. Under the reducing conditions normally present in rice culture, exchangeable Al is replaced by exchangeable Fe^{2+}. When the soil is re-oxidised Al re-appears in the exchangeable form. Sea water is beneficial for leaching these soils.

T. G. MORRIS.

Mobilisation of iron by aqueous extracts of plants. I. Composition of the amino-acid and organic-acid fractions of an aqueous extract of pine needles. J. W. Muir, R. I. Morrison, C. J. Brown and J. Logan. **II. Capacities of the amino-acid and organic-acid fractions of a pine-needle extract to maintain iron in solution.** J. W. Muir, J. Logan and C. J. Brown (*J. Soil Sci.*, 1964, **15**, 220—225, 226—237).—I. Seventeen amino-acids were identified in the cation-exchange-extractable portion of the aq. extract of Scots Pine needles. α-Alanine, γ-aminobutyric acid and arginine were present in the largest amounts. Shikimic, citric, quinic and malic acids, in decreasing order of amounts, as well as H_3PO_4 were present in fraction extracted by an anion-exchange resin.

II. The org.-acid fraction maintained Fe, in solution from pH 4—9·5, with max. effect at pH 8. The amino-acid fraction was ineffective above pH 4·5. A synthetic acid solution, containing the acids identified in the org.-acid fraction, behaved similarly to the latter with respect to ability in retaining Fe in solution. The water-sol. complexes formed between the org. acids and Fe occurred over a wide range of pH and in the presence of Ca^{2+}. The stabilisation of $Fe(OH)_3$ sols by the org. acids was greatly influenced by pH and the presence of Ca^{2+}.

A. H. CORNFIELD.

Rapid method for [determination of] assimilable manganese in soil. R. H. Molfino (*Rev. Fac. Agron., La Plata*, 1963, **39**, No. 1a, 95—98).—A semi-quant. method, developed from a Fiegl spot test (*Chemiker-ztg*, 1920, **44**, 689) is described. The method depends on visual assessment of the blue colour developed in acetate solution with benzidine and NaOH with or without sensitisation with KIO_4.

E. C. APLING.

Evolution of organic matter in soils. Ph. Duchaufour and F. Jacquin (*C. R. Acad. Agric. Fr.*, 1964, **50**, 376—387).—Two types of humus: grey and brown, can be separated by paper electrophoresis. The brown is less polymerised than the grey. The chemical structure of humus consists essentially of a spherical aromatic portion formed from phenols or quinones attached to chains formed by saccharides and amino-acids. Humification is encouraged by high availability of N and Ca. In very acid soils humification goes no further than the 'brown' stage. (38 references.)

J. V. RUSSO.

Physico-chemical study of the properties of humic acid and their changes during humification. S. A. Visser (*J. Soil Sci.*, 1964, **15**, 202—219).—The properties of humic acids extracted from plant material held in nylon gauze in a swamp over 2 years and from peat samples of various ages were studied. With increasing age there were increases in humic-acid C %, aromatic character, alcohol and ether groups and aliphatic side chains and double bonds, and decreases in % H, mol. and equiv. wt., and hydrophilic character. The presence of C and, to a minor extent also, of clays in decomposing plant material promoted the formation of humic acids which were very similar to those found in older peat deposits. Humic acids found in different peat materials (papyrus and sphagnum) were very similar in structure and composition. A. H. CORNFIELD.

Comparison of reagents for soil organic matter extraction and effect of pH on subsequent separation of humic and fulvic acids. T. L. Yuan (*Soil Sci.*, 1964, **98**, 133—141).—The fine sandy soils used were poorly drained and had prominent B horizons (org. pan). Samples of the surface layer and the org. pan were treated with four reagents, 0·5M-NaOH (pH >12), 0·1M-$Na_4P_2O_7$ (pH 10·2), 0·5M-NaF (pH 7·1) and Dowex A.1 resin, to extract org. matter. After a week the supernatant solutions were separated and analysed for C and N, and the buffer action of the reagents and soil extracts was examined. The org. matter extracted by the reagents had little effect on the buffer curves of the extractants. 0·5M-NaOH was the most effective extractant for C and N followed by pyrophosphate which removed 49·5 and 40·7 % of these amounts from the surface soil and 67·5 and 72·1 from the org. pan. The effectiveness of the other two reagents varied with the soil layer. The Dowex resin extracted more C and N from the surface than either pyrophosphate or NaF but from the org. pan it extracted less. For NaF the reverse was true. The C/N ratios, the C distribution in humic and fulvic acid fractions and the optical properties of the extracts suggested that the nature of the org. constituents extracted from the two soil layers by the different reagents was different. Pptn. of org. matter from the extracts varied with the extractant and with the pH, in general, increasing as pH was lowered.

T. G. MORRIS.

Chemical nature of soil organic phosphorus. I. Inositol phosphates. D. J. Cosgrove (*Aust. J. Soil Res.*, 1963 [1964], **1**, 203—214).—Constituents of the 'phytin' fraction of soil org. matter were separated by gradient elution chromatography on a column of the anion exchange resin, Dowex AG. 1—X8. In addition to myoinositol hexaphosphate corresponding deriv. of DL-inositol and scylloinositol were detected. Pentaphosphates formed the major components among the lower inositol phosphates. (26 references.)

A. G. POLLARD.

Polyphenols in plants, humus and soil. III. Stabilisation of gelatin by polyphenol tanning. IV. Factors leading to increase in biosynthesis of polyphenols in leaves and their relationship to mull and mor formation. R. I. Davies, C. B. Coulson and D. A. Lewis (*J. Soil Sci.*, 1964, **15**, 299—309, 310—318).—III. The rôle of polyphenols in the stabilisation of protein in superficial humus and the relationship of this rôle to mull and mor formation is discussed. Polyphenols extracted from a variety of plant materials were used to tan gelatin over a side range of pH. The stability of gelatin, tanned with polyphenols extracted from green beech leaves (mor site), against microbial degradation was then estimated. Over the range of pH found in soils, stability was greatest when the pH of the tanning was low. Mor-forming plant species probably contain more efficient tannins and the acid condition of the associated soil is better suited to the tanning process.

IV. Green leaves of species growing under mor conditions were usually higher in leuco-anthocyanins and tannin than were the leaves of the same species growing under mull conditions. Sand culture tests with seedlings of a no. of species showed a high content of polyphenolic substances in the leaves when N or P were not supplied to the plants.

A. H. CORNFIELD.

Decomposition of diethylstilboestrol in soil. B. Gregers-Hansen (*Plant & Soil*, 1964, **20**, 50—58).—Since practically all the diethylstilboestrol administered to animals to increase their growth rate is excreted in unchanged form there is a possibility that the material may be absorbed by plants and thus constitute a health hazard to humans and animals. The extent of decomposition of the hormone was studied in pot tests by adding labelled hormone to the soil and following the extent of release of labelled CO_2. Depending on soil type and whether or not org. matter was added the hormone decomposed to the extent of 1·6—16% after 3 months and 12—28% after 6 months.

A. H. CORNFIELD.

Mineralisation of carbon and nitrogen in some New Zealand allophanic soils. F. E. Broadbent, R. H. Jackman and J. McNicholl (*Soil Sci.*, 1964, **98**, 118—128).—Soils derived from allophane and andesilic ash were incubated at 30°, the evolved CO_2 being measured; inorganic N in soil extracts was also estimated. The ratio of C lost as CO_2 to N mineralised was fairly constant for most of the soils used; soils of high allophane content were slow in the mineralisation of both C and N. If ground clover was added to an allophane soil and a non-allophanic soil the contribution of the clover to C loss on incubation, was the same for both soils. Also in mixtures of allophanic and non-allophanic soil the C loss indicated no interaction between the two soils, i.e., that the protective effect of allophanic soils on C and N mineralisation is not conferred on fresh plant materials or on the org. matter of non-allophanic soils. Allophanic soils probably contain a stable clay–inorg. matter complex, which forms slowly.

T. G. MORRIS.

Effect of organic matter on the efficiency of urea fertiliser. V. Iswaran, S. N. Datta and A. K. Rishi (*Indian J. appl. Chem.*, 1964, **27**, 21—23).—When urea is mixed with org. matter, e.g., sawdust, leafy matter and dung etc., total nitrification is increased because of the increase in C content. Data given indicate that the rate of

nitrification is slowed down but that the % nitrification is higher in the urea-org. matter products than if urea is used alone. Crop growth experiments indicate better yields from urea-org. matter products; the efficiency of urea fertiliser is increased by mixing it with org. material in the proportion of 1 : 4. I. DICKINSON.

Factors affecting losses of ammonia from urea and ammonium sulphate applied to soils. J. K. R. Gasser (*J. Soil Sci.*, 1964, **15**, 258—272).—Urea (100 lb. N per acre) was either broadcast on the surface or mixed with two clay loams and two sandy loams and losses of NH_3 determined at moisture levels of 40% and 60% of the water-holding capacity during incubation at 5° or 25°. Urea and $(NH_4)_2SO_4$ were similarly applied to two calcareous soils which were incubated at 40% water-holding capacity at both temp. Losses of NH_3 ranged from 2% of the N applied from an unmanured clay loam to 13% from an unmanured sandy loam. Varying temp. of incubation and moisture level had little effect on losses, although the effects varied with soil type. Losses of NH_3 were similar from urea and $(NH_4)_2SO_4$ applied to the calcareous soils. NO_3^- tended to accumulate in the sandy and calcareous soils, especially at the lower temp. and highest moisture level. NH_3 was lost at 25° from calcareous soils until all the NH_3-N had been nitrified, from the slightly alkaline clay soils until 90% had been nitrified, and from the neutral sandy soils until 50% had been nitrified. A. H. CORNFIELD.

Effects of solutions of urea and of ammonium and potassium salts on the germination of kale, barley and wheat. J. K. R. Gasser (*Chem. & Ind.*, 1964, 1687—1689).—Urea appears to decrease the germination of kale (**I**), barley (**II**) and wheat (**III**) on account of its osmotic effect; although it may be hydrolysed in the seed, urea does not appear in itself to be toxic. Increasing salt concn. decreases the germination of **I** more than that of **II** and of **II** more than that of **III**. When germination was decreased, NH_4 salts were more damaging than those of K; this suggests a toxic effect of NH_4^+. SO_4^{2-} was less damaging than NO_3^- and Cl^- probably because SO_4^{2-}-solutions possess lower osmotic pressures at equal NH_4^+ and K^+ concn. The possibility that the Cl^- and NO_3^--ions may be toxic has not been excluded. C. V.

Manurial value of seaweeds. II. Effect of *Pachymenia himantophora* on manganese release and physical properties of soils. R. I. B. Francki (*Plant & Soil*, 1964, **20**, 65—73).—Application of 1·25% of seaweed meal to soils resulted in increased growth of tomato seedlings and greater leaf-Mn in soils of pH $<$6·1. In soils of pH $>$5·6 the treatment resulted in much reduced water permeability, and increased uptake of Mn by the plant to such an extent that it was toxic and reduced growth. A. H. CORNFIELD.

Effect of 30 years of manuring with inorganic and farmyard manure on some of the properties of a 'Filder' loam soil. G. Michael and M. Djurabi (*Z. PflErnähr. Düng.*, 1964, **107**, 40—50).—Compared with control (no farmyard manure plots), soil org. C increased from 0·89% to 1·12% over the 30-year period with a yearly application of 300 dz/ha. Inorganic fertilisers also increase soil C due to greater return of crop residues. No effect is observed on the subsoil and with a fertile soil no lasting effect is to be expected from farmyard manure (**I**) applications. Exchange capacity and water-holding capacity are only slightly raised by **I**, although the pore vol., especially of coarse pores, is increased. M. LONG.

Release of trace elements from FTE (fritted trace elements). M. Oosting (*Z. PflErnähr. Düng.*, 1964, **106**, 206—218).—The theory of FTE behaviour is discussed and procedure to predict release of trace elements is given. Release of trace elements is initially proportional to time but soon becomes proportional to log time. Methods used on percolation and use of buffer solutions for control are discarded in afavour of maintenance of constant pH by the addition of H_2SO_4 in a titrator. M. LONG.

Plant Physiology, Nutrition and Biochemistry

Effect of light intensity on assimilation characteristics of detached tea leaves. D. N. Barna (*J. agric. Sci.*, 1964, **63**, 265—271).—Mature tea leaves from four different sources showed considerable differences in photosynthetic rates in both strong (32 klux) or poor (4 klux) light intensities. Differences could not be attributed to the thickness of the leaf lamina nor to the chlorophyll concn. of the leaf tissue. In shade-adapted leaves rates of photosynthesis were greater at lower light intensities. Optimal light intensity varied between leaves of different sources. A. G. POLLARD.

Influence of spectrum composition and intensity of radiation on the chlorophyll content with respect to length of photoperiod. I. A. Shul'gin and L. A. Khodorenko (*Dokl. Akad. Nauk SSSR*, 1964, **156**, 712—714).—The chlorophyll content of radishes diminishes with reduced intensity of illumination, independently of period or spectrum composition. For a given intensity it increases with increase of period. For high intensity of solar radiation, blue supplementary irradiation accelerates development and increases the quantity of chlorophyll; if the intensity is diminished, stopping the growth of the plant, then irradiation with red light favours chlorophyll formation. G. F. PENNY.

Respiratory and carbohydrate metabolism of plant tissues. XIV. Determination of certain phosphate compounds in plant extracts. J. Barker, R. Jakes, T. Solomos, M. E. Younis and F. A. Isherwood (*J. exp. Bot.*, 1964, **15**, No. 44, 284—296).—Glucose-6-, fructose-6, glucose-1, fructose-1,6-di-, dihydroxyacetone-phosphates, 2- and 3-phosphoglycerate and phosphoenolpyruvate were assayed by enzymic methods with photometric or fluorimetric measurements. The substances were extracted by trichloroacetic acid, extracts being cleared with activated C (cf. Barker *et al.*, *Nature, Lond.*, 1962, **196**, 1115). Values thus obtained for potatoes, bananas, strawberry leaves, apples and tomatoes are presented. (35 references.) A. G. POLLARD.

Inducible formation and stability of nitrate reductase in higher plants. I. Effects of nitrate and molybdenum on enzyme activity in cauliflower (*Brassica oleracea* var. Botrytis). M. M. R. K. Afridi and E. J. Hewitt (*J. exp. Bot.*, 1964, **15**, No. 44, 251—271).—Leaf tissues of cauliflowers grown in sterile culture with glutamic acid or $(NH_4)_2SO_4$ (**I**) as N source in absence of NO_3^- contained no NO_3^--reductase. The enzyme was formed steadily in excised leaves on infiltration of NO_3^-. Mo-deficient plants grown with NO_3^- lacked enzyme activity which was restored by infiltration with Mo. Both Mo and NO_3^- were essential for max. production of the enzyme. (45 references.) A. G. POLLARD.

Mobilisation of fats in germinating seeds: the *in vivo* 'quantum' hydrolysis of triglycerides. A. R. S. Kartha and J. M. S. Mathur (*J. Sci. Fd Agric.*, 1964, **15**, 869—872).—Of four different seeds studied, no acetyl values of residual fats in 19 germination stages were found. The fats were composed of triglycerides as in that from the original seeds. A triglyceride mol. is split up into fatty acids and glycerol without intermediate liberation of mono- and di-glycerides. (14 references.) E. M. J.

Ascorbic oxidase activity and the ascorbic acid/dehydroascorbic acid system during germination. A. Amberger and M. El-Fouly (*Z. PflErnähr. Düng.*, 1964, **106**, 218—227).—Bush beans (**I**) and air-dried maize (**II**) contain no vitamin C, barley has only a little and rape seed (**III**) contains 44 mg./100 g. dry matter. Ascorbic acid oxidase activity on a unit seed basis is highest in **I** and lowest in **II** and **III**. After swelling of the seed, ascorbic acid activity rises linearly at first. Vitamin C and ascorbic acid formation is slower, e.g., 10-fold for **III** and 40-fold for **I** during a period of 6 to 16 days. The dehydroascorbic acid concn. rises only slightly. The green portions of the plant contain the highest concentration of vitamin C, whilst the seed residue contains little or no ascorbic acid. The roots generally contain a high ascorbic acid oxidase activity. M. LONG.

Influence of aqueous plant extracts on germination and growth of eight forage species. E. A. Grant and W. G. Sallans (*J. Brit. Grassland Soc.*, 1964, **19**, 191—197).—The effects of aq. extracts of the tops and roots of four legumes and four grasses on germination and root and shoot growth of the same species were studied, using distilled water as a check. Based on the no. of significant reactions to the extracts the species could be classified in the following order of decreasing inhibition: lucerne, birdsfoot trefoil, ladino clover, red clover, reed canary grass, bromegrass, timothy and orchardgrass. Except for lucerne, extracts of tops of the plants had greater inhibitory effects than did root extracts. A. H. CORNFIELD.

Effect of inositol on growth and nodulation of diploid and tetraploid white clover in pot culture. J. B. Weir (*Plant & Soil*, 1964, **20**, 175—183).—Treatment of white clover in pots with 0·01—0·02% aq. inositol increased no. and size of nodules and dry wt. of plants in comparison with water treatment. The treatment was more effective when applied to the soil than when sprayed on the foliage. The diploid plants produced more nodules than did the tetraploid plants. A. H. CORNFIELD.

Foliar absorption—an active uptake process. W. H. Jyung and S. H. Witler (*Amer. J. Bot.*, 1964, **51**, 437—444).—Experimental data on the absorption of P and Rb by leaves of bean seedlings indicates that the over-all process of foliar absorption over a 24-h. period is metabolic in character. Protein carriers are probably concerned in the process. A. G. POLLARD.

Uptake and release in the assimilation of potassium by young barley roots. K. Mengel (*Z. PflErnähr. Düng.*, 1964, **106**, 193—206).—K is released by both intact and excised roots and is unaffected by either NaCl or $CaCl_2$. Where uptake is depressed by metabolic inhibition or ion competition, release is relatively high. The net release is mainly dependent on the [K] of the medium and eventually

an equilibrium is set between uptake and release. Plant membranes are not completely impermeable to K ions, and release is due to a passive back diffusion independent of the carrier mechanism. An active transport mechanism here is counteracting the passive ion movement caused by an electropotential gradient. M. LONG.

Tolerance of plants to lithium. F. T. Bingham, A. L. Page and G. R. Bradford (*Soil Sci.*, 1964, **98**, 4—8).—Various plants were grown in soil treated with moss and/or fertiliser and also in sand culture with varied additions of Li_2SO_4. The harvested plants were analysed for Li, Ca, Mg, Na and K. Plants found to be least tolerant were avocado, soya-beans and sour orange ; the grasses and maize were most tolerant. There was a parallelism between Li tolerance and Na tolerance. In sand cultures toxic concn. of Li was 75 p.p.m. Levels of Li found in irrigation waters were unlikely to be toxic. T. G. MORRIS.

Effect of calcium on the uptake of sodium and the release of potassium by excised barley roots. H. Marschner (*Z. PflErnähr. Düng.*, 1964, **107**, 19—32).—K is released from barley roots to K-free media, the amount depending on the K content of the roots and the vol. of the medium. Ca reduces the release of K from roots whilst N increases it. Ca reduces Na uptake initially, but later increases it. K also reduces Na uptake, factors reducing the K content of the root, also increasing Na uptake. Thus the high endosperm K content of young roots maintained by high Ca concn. reduces Na uptake, whilst with older roots where the K content is less the effect of Ca is only slight. The effect of Ca on Na uptake is explained by a reduction of the permeability of the cell membrane or by the maintenance of membrane stability in the presence of Ca. M. LONG.

Uptake of calcium and strontium by plants from some Australian soils. C. H. Williams and D. J. David (*Aust. J. Soil Res.*, 1963 [1964], **1**, 185—202).—In pot cultures and field trials the ratio Ca/Sr in plants was closely related to the ratio, exchangeable Ca/exchangeable Sr, in soil. In roots of oats and cocksfoot the Ca/Sr ratio was similar to that in tops ; in subterranean clover and in *Erodium botrys* the proportion of Sr in the roots exceeded that in the tops. Diminution in exchangeable Ca and Sr in soil due to the growth of the clover accounted for about 80% of the total uptake of both the cations by the plant ; the smaller uptake of Ca by oats was more closely related to the water-sol. Ca than to the exchangeable Ca of the soil. Following addition of sol. Ca and Sr to the soil the ratio Ca/Sr in the top growth of oats was closely correlated with the Ca/Sr ratio of the initially exchangeable + added cations. In field soils the uptake of Ca and Sr by subterranean clover was related to the exchangeable forms in the top 4 in. of soil whereas the uptake by wheat was influenced by the Ca and Sr in soil depths >4 in. (26 references.) A. G. POLLARD.

Effect of iron ethylenediaminetetra-acetic acid on the growth and metabolism of tomato plants in water culture. W. van Driel (*Plant & Soil*, 1964, **20**, 85—104).—Tomato plants were grown in nutrient culture with continuous or intermittent treatment with $FeSO_4$ (**I**) or Fe-EDTA (**II**). Although a ppt. of hydrated oxide was rapidly formed when **I** was used, the Fe was available to the plant. Plant growth was better when **I** than when **II** was the source of Fe. Catalase and peroxidase activites were higher where Fe was supplied as **II** than when as **I**. Chlorotic plants treated for a few days with **I** exhibited enzyme activities equal to or higher than those treated with **II**. A. H. CORNFIELD.

Effects of boron on the growth and lignification of sunflower tissue in agar cultures. T. R. Dutta and W. J. McIlrath (*Bot. Gaz.*, 1964, **125**, 89—96).—The growth rate and lignification of stem callus and of root cultures of sunflower were depressed by absence of B from the nutrient. The effect of B on lignin formation is associated with lowered peroxidase activity in the tissues. A. G. POLLARD.

Pollen germination and pollen tube growth. I. Effects of boric acid and hydrogen-ion concentration on pollen of petunia and antirrhinum. P. Fähnrich. **II. Actions of trivalent metal ions and growth-substances on the pollen of petunia and antirrhinum.** P. Fähnrich and H. Ullrich (*Planta*, 1964, **61**, 187—195 ; **62**, 39—50).—I. Germination of pollen *in vitro* was stimulated by sucrose and H_3BO_3, the latter being an essential factor. Optimum pH for germination was approx. 5 for both species ; growth of pollen tubes was max. at pH 5 for petunia and pH 7 for antirrhinum.

II. Germination of the pollen and development of pollen tubes was stimulated by B, which was not replaceable by Al, In, Sc, Y or La. Al^{3+} and NO_3^- depressed germination rates and inhibited growth of pollen tubes. IAA did not induce germination and in relatively high concn. inhibited the growth of pollen tubes, the effect being partly counteracted by H_3BO_3. Gibberellic acid in presence of H_3BO_3 accelerated pollen tube growth in petunia. A. G. POLLARD.

Constitutive studies of some seed fats of the Indian arid zone with particular reference to the influence of environmental and genetic factors. A. Sen Gupta and M. M. Chakrabarty (*Indian J. appl. Chem.*, 1964, **27**, 49—61).—The seed fat composition of seven different plant families growing under wild and cultivated conditions was studied. The fatty acid composition of the seed fats is affected by the genetic factor. Seed fats of two species *Copparis aphylla* and *Gynandropsis pentaphylla* belonging to the same family show divergent composition. A low temp. during ripening increases the yield of unsaturated acids. (70 references.) E. C. DOLTON.

Anthocyanidin content of petals of a pink and a red rose. K. G. Ahuja, H. L. Mitchell and W. J. Carpenter (*Proc. Amer. Soc. hort. Sci.*, 1963, **83**, 829—832).—The cyanidin (the only anthocyanidin present in rose petals) content of petals of the red variety was much higher than that of the pink variety. Cyanidin concn. was highest at the bud and lowest at the fully opened stage. Cyanidin content increased from outer to inner petals in the buds and partly opened flower, but the reverse was true in fully opened flowers. A. H. CORNFIELD.

Comparison of some processes in homogenates and sections of healthy bean leaves resistant and susceptible to the bacterial haloblight disease. B. J. Deverall and J. M. Daly (*J. exp. Bot.*, 1964, **15**, 308—313).—Unifoliates of *Phaseolus vulgaris* were inoculated with *Pseudomonas phaseolicola* ; symptoms of resistance or susceptibility developed quickly. Homogenates of healthy unifoliates of resistant lines showed O_2 uptakes 30—75% higher than did those of susceptible lines. Rates of respiration of leaf sections and the extent of dark fixation of CO_2 were the same in susceptible and resistant lines. The O_2 uptake of the homogenates offers a promising approach to problems of susceptibility. A. G. POLLARD.

Isolation and determination of the ferric iron chelate of ethylenediaminedi-(o-hydroxyphenylacetic acid) in plant tissues. P. P. Batra and R. H. Maier (*Plant & Soil*, 1964, **20**, 105—115).—The method is described. Recovery of the chelate from tomato tissue ranged from 94 to 97%. A. H. CORNFIELD.

Leaf analysis as a guide to the nutrition of fruit crops. VI. Determination of magnesium, zinc and copper by atomic absorption spectroscopy. E. G. Bradfield and D. Spincer (*J. Sci. Fd Agric.*, 1965, **16**, 33—38 ; cf. J.S.F.A. Abstr., 1964, ii, 237).—The technique has been employed at Long Ashton over the past 3 years. Mg may be determined in solutions containing 0·01—2·0 μg. of Mg/ml., Zn in solutions containing 0·02—4·0 μg. Zn/ml. and Cu in solutions containing 0·1—15·0 μg. of Cu/ml. The effect of PO_4^{3-} and Ca^{2+} on the absorption of Mg in the coal gas/air flame was studied in relation to the position in the flame at which measurements are made ; and the effect of combinations of Mg and SO_4^{2-} on the absorption of Zn was studied. (17 references.) E. M. J.

Foliar analysis as a diagnostic technique in cocoa nutrition. I. Sampling procedure and analytical methods. D. K. Acquaye (*J. Sci. Fd Agric.*, 1964, **15**, 855—863).—Analytical methods are outlined and factors affecting sampling procedures are studied. Variability found, arising from factors other than the supply of the nutrients in the soil, does not invalidate the usefulness of foliar analysis in cocoa nutrition studies, but these factors should be considered in sampling and interpretation of results. Recommendations are : sampling should be done between 8 and 10.30 a.m. on a clear sunny day, taking 10 leaves of average size (the most recently mature leaf from the tip of the shoot)/tree from under the canopy in the shade, use of whole leaf for analysis, etc. Accuracy and precision are tested by statistical analysis. (34 references.) E. M. J.

Spectrophotometric determination of iron and aluminium in leaves of the rubber tree (*Hevea brasiliensis*). K. R. Middleton (*Analyst*, 1964, **89**, 421—427).—The ground sample is digested with HNO_3, $HClO_4$ and H_2SO_4 and the digest diluted with water and transferred to a separating funnel. Hydroxyquinoline–AcOH reagent is added and the solution neutralised with 5N-aq. NH_3. $AcONH_4$–AcOH buffer is then added followed by hydroxyquinoline–$CHCl_3$ reagent (**I**). After shaking, the optical density of the $CHCl_3$ layer at 4700 Å is measured against a reagent blank to determine Fe. After further addition of 5N-aq. NH_3 and **I** to the remaining aq. layer, the optical density of the new $CHCl_3$ layer at 3850 Å is read against a reagent blank to determine Al. The main source of error is the digestion process. The method is sensitive to 3 p.p.m. of Fe and 1·5 p.p.m. of Al, with a precision of 1% and 1·4% respectively. (14 references.) I. C.

Simple and inexpensive integrating photometer. M. C. Powell and O. V. S. Heath (*J. exp. Bot.*, 1964, **15**, 187—191).—Details of construction of the apparatus and of its calibration are presented. A. G. POLLARD.

Interactions of hormonal substances in the growth and development of plants. D. J. Osborne (*J. Sci. Fd Agric.*, 1965, **16**, 1—13).—Patterns of growth and development in plants under the control of endogeneously produced chemical regulators (plant hormones—the

auxins, kinins and gibberellins) are discussed. Current knowledge on the nature of plant hormones, on the kinds of responses they produce in plant cells and in whole plants and on ways hormones might act as regulators of the biochemical processes of the cell which lead to growth and differentiation is reviewed. (47 references.)
E. M. J.

East Malling coleoptile straight growth test method. (The late) C. R. Hancock, H. W. B. Barlow and H. J. Lacey (*J. exp. Bot.*, 1964, **15**, 166—176).—Details of the method for testing growth-promoting substances are recorded together with experimental data substantiating particular items in the technique. A. G. POLLARD.

Carbon dioxide effects on auxin responses of coleoptile sections K. E. Cockshull and O. V. S. Heath (*J. exp. Bot.*, 1964, **15**, 331—346).—In the wheat cylinder bioassay of auxins, accumulation of CO_2 in the assay tubes, depress the growth of the cylinder in presence of IAA, from 8 h. onward at 25°. Ultimately the cylinders shrink and release their IAA content into the solution. Aeration of the solutions in this method is stressed. A. G. POLLARD.

Growth-regulator interactions in the growth of the shoot system of *Avena sativa* seedlings. I. Growth of the first internode. E. K. Ng and L. J. Audus (*J. exp. Bot.*, 1964, **15**, 67—95).—Segments from the first internode of the seedlings, grown in darkness, responded to both gibberellic acid (**I**) and auxins, by increased extension. When the node between the internode and the coleoptile was included in the cut segment the action of **I** was increased and that of IAA diminished. The presence in the node of an endogenous auxin necessary for the action of **I** is postulated. No evidence of interaction of **I** and IAA or synergism between **I** and synthetic auxins was obtained. Weak anti-auxins (*p*-chlorophenoxyisobutyric [**II**] and 2,4,6-trichlorophenoxyacetic acids [**III**]) antagonised competitively the promotive action of strong auxins (IAA) and that of **I**; α-(methyl 1-naphthyl sulphide)-propionic acid (**IV**) acted similarly as an anti-auxin. The inhibitory action of high concn. of **II** and **III** was synergised by concn. of IAA above optimal whereas that of **IV** was synergised by supra-optimal concn. of IAA or of **I**. (52 references.) A. G. POLLARD.

Rôle of β-indolylacetic acid in seed dormancy. T. V. Daletskaya (*Dokl. Akad. Nauk SSSR*, 1964, **156**, 708—711).—Experiment suggests that the physiological dwarfing shown by germs from deeply-dormant seeds is connected with a high content of β-indolylacetic acid. Those factors which disturb dormancy at the same time lower the content of this substance. Growth in the germ is retarded by the action of heteroauxin, but this retardation is perceptibly weakened in conditions which disturb physiological dwarfing. (11 references.) G. F. PENNY.

Determination of auxin, separated by thin-layer chromatography, by the Söding *Avena* daylight test (*Avena* silica-gel curvature test). H. Kaldewey and E. Stahl (*Planta*, 1964, **62**, 22—38).—Details of the modified method are given and experimental data supporting the modifications are recorded. A. G. POLLARD.

Effect of applied growth substances on development of strawberry fruit. I. Induction of parthenocarpy. P. A. Thompson (*J. exp. Bot.*, 1964, **15**, 347—358).—Growth-regulating substances in lanoline were applied to flowers of pistillate varieties of strawberry. Parthenocarpic fruit developed from most of the flowers treated with 4-(indol-3-yl)butyric (**I**), 2-naphthoxyacetic and 1-naphthylacetic acids at concn. 500—6000 p.p.m. Gibberellic acid promoted growth in the 'neck' region of one variety, increased the response to **I** and reduced the period elapsing between anthesis and ripening. A. G. POLLARD.

Effect of gibberellic acid on curvature reactions; lateral transport of gibberellic acid in plants. Is gibberellic acid concerned in tropic curvatures. E. Libbert and I. Gerdes (*Planta*, 1964, **61**, 245—258).—In the *Avena* test gibberellic acid (**I**) caused no curvature of the coleoptile but, when used in conjunction with IAA, it increased the curvature by about 40%. This results from stimulation of growth on both sides of the coleoptile. The phototropic response of repeatedly decapitated coleoptiles was small and **I** had no effect. Addition of IAA to the cut surface increased the response and further addition of **I** increased the response by an additional 40%. Lateral movement of **I** in the coleoptile was rapid. The mechanism of the experimental effects is discussed. A. G. POLLARD.

Action and interaction of gibberellic acid and B995 on *Datura innoxia*. R. P. James and L. A. Sciuchetti (*J. pharm. Sci.*, 1964, **53**, 1093—1097).—Four successive weekly treatments of 33-day-old seedlings of *D. innoxia* with 50 μg. of gibberellic acid resulted in greatly increased height, stem dry-wt. and decreased alkaloid concn. (*A*), whilst those treated similarly with 100—1000 p.p.m. of aq. *N*-dimethylaminosuccinamic acid (B995) were unaffected except for slight growth retardation and decreased *A* in roots at the last harvest. When the plants were treated with both chemicals together the gibberellin effect predominated, suggesting that the concn. of B995, although not high enough to induce growth retardation, was low enough to demonstrate a slight stimulatory effect. *D. innoxia* responded to gibberellic acid like other solanaceous plants; results of selective solvent extraction of the leaf-tops by the modified Dragendorff method are reported. W. J. BAKER.

Rate and amount of absorption of maleic hydrazide by potato tubers. E. W. Franklin and E. C. Lougheed (*Amer. Potato J.*, 1964, **41**, 191—195).—When potato foliage was sprayed with maleic hydrazideamine (2500 p.p.m.) 3 weeks past full bloom sufficient absorption (6 p.p.m.) of maleic hydrazide (**I**) by the tubers occurred within 24 h. (vines were clipped at ground level) satisfactorily to inhibit sprouting of tubers stored for 6 months at 10°. Sprouting during storage was stimulated when (**I**) (4 p.p.m.) (2—4 h. absorption) was present in the tubers. Residues of **I** in the tubers increased with time up to a max. of 36 p.p.m. after 7 days.
A. H. CORNFIELD.

Effects of Etamycin on seedling growth and chlorophyll production. A. P. Cercos (*Phytopathology*, 1964, **54**, 741).—Treatment of seeds with Etamycin (**I**) (an antibiotic produced by *Streptococcus lavendulae*) affected the rate of growth of the seedlings, monocotyledons being affected more than were dicotyledons. **I** suppressed the formation of chloroplasts and greatly restricted synthesis of chlorophyll but had no further effect on established chloroplasts. Chlorosis caused by **I** was not reversible, unlike that of streptomycin; it was largely restricted to young or meristematic tissues and was ameliorated by Cu but not by Ca or Mn. A. G. POLLARD.

Responses of pear seedlings to *N*-dimethylaminosuccinamic acid, a growth retardant. H. J. Brooks (*Nature, Lond.*, 1964, **203**, 1303).—Foliar sprays of *N*-dimethylaminosuccinamic acid (**I**) applied to 2-year-old pear seedlings in July caused retardation in terminal growth and stimulation of spur-type growth the following autumn and spring. This suggests that **I** may have a potential as a regulator of growth and fruiting in commercial pear plantings.
S. A. BROOKS.

Plant growth retardant, CCC, as inhibitor of gibberellin biosynthesis in *Fusarium moniliforme*. H. Ninnemann, J. A. D. Zeevaart, H. Kende and A. Lang (*Planta*, 1964, **61**, 229—235).—Photosynthetic synthesis of gibberellin by *F. moniliforme* was suppressed by the plant growth retardant (2-chloroethyl)trimethylammonium chloride (CCC). A. G. POLLARD.

Crops and Cropping

Growth of cereals under soil conditions characteristic of an unstable tilth. C. R. Clement (*Plant & Soil*, 1964, **20**, 265—270).—Mechanical compaction of moist soil over wheat seed severely inhibited germination in a sandy loam. The destruction of surface tilth by falling water drops had no effect on the rate of emergence of either wheat or oats. Tillered wheat plants growing at winter temp. survived prolonged conditions of adverse soil aeration. A. H. CORNFIELD.

Biochemical investigations into the susceptibility of barley varieties to DDT. D. G. Upshall and T. W. Goodwin (*J. Sci. Fd Agric.*, 1964, **15**, 846—855).—The strains used were Rika (susceptible) and Proctor (resistant); internal deposits of DDT accumulated in the leaves were the same in both strains. No significant metabolism of DDT by either strain could be observed even when [^{14}C]-DDT was used. Examination of 19 analogues of DDT showed three structural requirements for toxicity to barley. The lethal effect in the susceptible strain is concerned with its effect on the structural organisation of the chloroplast, the DDT can penetrate to a structural lipoprotein and put it out of action. (22 references.)
E. M. J.

Fertiliser experiments on maincrop potatoes 1955—61. D. A. Boyd and W. Dermott (*J. agric. Sci.*, 1964, **63**, 249—263).—At low levels of P and K the influence of soil is substantial. Light-textured soils show a higher response to K and soils with impeded drainage responding more to P. Seasonal effects are more important with N than are soil influences. For heavy textured soils and those deficient in P the optimal dressing is probably 1·0 to 1·5 cwt. of P_2O_5, whilst for soils high in P the appropriate dressing is 0·5 cwt./acre. Heavy soils show little response to K. Most sands and sandy loams require 2·0 cwt. of K_2O/acre. An adequate application is generally 0·8 cwt. M. LONG.

Effect of application of manganese sulphate on tuber yields and incidence of scab in potatoes in a neutral soil. A. J. McGregor and G. C. S. Wilson (*Plant & Soil*, 1964, **20**, 59—64).—Potatoes grown on a neutral soil low in water-sol. Mn showed high scab incidence but no signs of Mn deficiency. Application of $MnSO_4$ (0·5 cwt./acre) mixed with a compound fertiliser, applied in the drill at planting

time, did not affect yields, but increased the Mn % in leaves and tubers, and markedly reduced the incidence of scab.
A. H. CORNFIELD.

Potato quality. XXV. Specific gravity and after-cooking darkening of Katahdin potatoes as influenced by fertilisers. L. Lujan and O. Smith (*Amer. Potato J.*, 1964, **41**, 274—278).—Tuber sp. gr. decreased with increasing rate of application of K (100—500 lb./acre) KCl being more effective than K_2SO_4 in this respect. Increasing P level (150 or 300 lb. of P_2O_5/acre) reduced tuber sp. gr. at the low but not at the higher rates of K. Although after-cooking darkening was not significantly influenced by source or rate of K or rate of P, there was a trend for increasing darkening with increasing rate of KCl. A. H. CORNFIELD.

Grasses and grassland cultivation of Britain. I. Before 1700. II. 1700—1900. G. E. Fussell (*J. Brit. Grassland Soc.*, 1964, **19**, 49—54, 212—217).—A review. A. H. CORNFIELD.

Influence of level of nitrogenous fertiliser on the long-term productivity of leys. H. K. Baker, J. R. A. Chard and G. Gwyne (*J. Brit. Grassland Soc.*, 1964, **19**, 42—48).—The higher N level (180 lb./acre per annum) yielded 28% more dry matter over 4 years than did the lower N level (70 lb.) and the yield was also more evenly spread throughout the season. Clover was virtually eliminated from the high-N swards, whilst with low-N the proportion of clover varied, but tended to increase with advancing season. Dry-matter yields were highly correlated with utilised-starch-equiv. output of the swards. A. H. CORNFIELD.

Mineral nutrition of several grass species. IV. Nitrogen level. A. D. Bradshaw, M. .J Chadwick, D. Jowett and R. W. Snaydon (*J. Ecol.*, 1964, **52**, 665—676).—Effects of various NO_3^- concn. on different grass species in sand cultures are shown. Responses, in terms of yields of forage varied widely with the species, *Lolium perenne* and *Agrostis stolonifera* yielding most heavily with the highest [N] applied, viz., 243 p.p.m. Variations in N levels in soil represent an important factor controlling the distribution of plant species under natural conditions. A. G. POLLARD.

Effects of fertilisers on herbage production. II. Effect of nitrogen, phosphorus and potassium on botanical and chemical composition. J. W. S. Reith, R. H. E. Inkson, W. Holmes, D. S. MacLusky, D. Reid, R. G. Heddle and G. J. F. Copeman (*J. agric. Sci.*, 1964, **63**, 209—219).—Clover almost disappeared when N was applied at 87 lb./acre, none surviving with 174 and 348 lb. of N/acre. N increased the rye-grass and cocksfoot content of the sward. P had no effect on botanical composition, whilst K in the absence of N increased clover. Crude protein in the herbage was higher in the autumn than in the summer and, where K was adequate, N at 348 lb./acre doubled the production of crude protein, although at lower levels of N the % of crude protein was lower than with no N. P in the herbage showed little seasonal variation, but with high N applications (348 lb./acre) its uptake was doubled. Applications of K increased the K content of herbage at all levels of N. The Na content showed a seasonal increase, K applications reducing Na uptake and N increasing it. Ca and Mg showed seasonal increases. N and K together depressed the Ca content of herbage. N tended to increase the Mg content, whilst K reduced it, especially in the presence of N. Only high levels of N affected trace elements, reducing Co, Mn and Sr. At moderately high levels of N, 1 lb. of K_2O per lb. of N was adequate to maintain K supplies in the soil.
M. LONG.

Pot trial investigation into the influence of nitrogenous fertilisers on the yield, botanical composition and quality of a clover-grass mixture. S. Barbier (*Z. PflErnähr. Düng.*, 1964, **107**, 32—40).—Yield, clover content, protein and mineral contents of clover and grass mixture depends on the frequency of cutting and level of N fertiliser. High N fertiliser increases yield due almost entirely to an increase in the grass fraction. Very frequent cutting reduces yield but counteracts the fall in the clover fraction at high N fertiliser levels.P, K, Ca, Mg and crop-protein fall most notably at low N levels. Yields of protein and minerals parallel total yield.
M. LONG.

Nutrition of forage legumes. III. Effect of copper on nodulation of *Trifolium subterranean* L. and *T. repens*. E. G. Hullsworth, E. A. N. Greenwood and M. G. Yates (*Plant & Soil*, 1964, **20**, 17—33).—The no. of nodules developing on the roots of *Trifolium subterraneum* increased with level of NO_3^--N in the nutrient up to 1000 μM per litre. With nutrient Cu at 0·01 μM per litre the wt. of excisable nodules decreased continuously with increasing NO_3^- supply. With nutrient Cu at 0·1—5·0 μM per litre the wt. of excisable nodules increased continuously both with increase in supply of Cu and of NO_3^--N. Increasing Cu supply also increased the growth of the plant and the quantity of N fixed. A. H. CORNFIELD.

Isotopic studies of the uptake of nitrogen by pasture grasses. I. Recovery of fertiliser nitrogen from the soil-plant system using Rhodes grass in pots. A. E. Martin, E. F. Henzell, P. J. Ross and K. P. Haydock (*Aust. J. Soil Res.*, 1963 [1964], **1**, 169—184).—Rhodes grass (*Chloris gayana*) was grown under glass in a light-textured soil pretreated with 0 or 200 lb. of N/acre as NH_4NO_3. Later the grass was cut 3 in. above soil level and aq. NH_4NO_3 (labelled $^{15}NH_4^+$) up to 800 lb. N/acre was added to the soil. Amounts of total N and of labelled N in the soil at the end of the experimental period were each linearly related to the amount of NH_4NO_3 added. 93·6 and 94% of the total and labelled N respectively were recovered in soil and plant. The mechanism of the loss of N is discussed. (48 references.) A. G. POLLARD.

Differential survival of plant types in swards. A. H. Charles (*J. Brit. Grassland Soc.*, 1964, **19**, 198—204).—Populations of *Lolium* spp., *Dactylis* spp. and *Phleum*, each based on two or more cultivars, were subjected to three managements (frequent grazing, infrequent grazing and hay aftermath) and two N levels. Rapid changes in population occurred in response to the treatments and there were differences in extent of survival within cultivars.
A. H. CORNFIELD.

Influence of date of origin of the shoot and level of nitrogen on ear size in three perennial grasses. G. J. A. Ryle (*Ann. appl. Biol.*, 1964, **53**, 311—323).—In plants of S.24 ryegrass, S.215 meadow fescue and S.37 cocksfoot grown in the field, or with controlled nutrition in the glasshouse, ear size decreased the later the date of origin of the shoot between Oct. and March, before flowering in the late spring. The smaller ear size in shoots arising at successively later dates resulted from decreased numbers of primary branches in the ear, and also from the development of fewer florets on each branch. Application of N ('Nitrochalk') to plants growing one yard apart in the field had no effect on ear size. Plants receiving smaller rates of N in the glasshouse generally developed ears with fewer florets on each lateral branch. Lack of N also decreased the no. of primary branches in ears of ryegrass and prevented many shoots of all three varieties from flowering. A. H. CORNFIELD.

Growth and nutrient uptake of Newport bluegrass as affected by soil oxygen level. J. Letey, L. H. Stolzy, O. R. Lunt and V. B. Youngner (*Plant & Soil*, 1964, **20**, 143—148).—An O_2 diffusion rate of at least 20×10^{-8} cm.$^{-2}$ min.$^{-1}$ was required for root growth of Newport bluegrass, although for optimum growth higher O_2 diffusion rates were required. Growth of tips before the first clipping was similar with 2—21% O_2 in soil atm., but was less with 1% O_2. After clipping growth increased with O_2 level up to 10% and decreased to 21% O_2 in the soil atm. Foliar N, P and K % increased with O_2 supply. Foliar Na % was high with very low O_2 levels. A. H. CORNFIELD.

Comparative productivity of herbage varieties on upland peat. I. V. Hunt (*J. Brit. Grassland Soc.*, 1964, **19**, 55—61).—The productivity and persistence of 27 varieties of herbage plants were compared following sowing on a blanket peat after ploughing, liming and measuring. Over 5 years there were significant differences in dry matter production due to variety. S59 red fescue and smooth-stalked meadow grass gave the highest yields, although the other red fescues, S170 tall fescue, S143 cocksfoot and crested dog-tail produced yields which were not significantly lower. These varieties were also the most persistent. A. H. CORNFIELD.

Competition between perennial ryegrass and meadow fescue under field-plot conditions. R. L. Crocker and P. M. Martin (*J. Brit. Grassland Soc.*, 1964, **19**, 27—29).—When grown in mixed stands over 10 weeks and 9 months the contribution of fescue to overall dry-matter yields was severely restricted by perennial ryegrass, although there were no deaths of fescue plants even with an over-wintering period. The reduced dry-matter yield of fescue was caused by decrease in size and wt. of individual plants due to competition for light and nutrients from the faster-growing ryegrass.
A. H. CORNFIELD.

Anhydrous ammonia versus ammonium nitrate as sources of nitrogen for rye crops cover on the potato soils of Long Island. S. L. Dallyn and R. L. Sawyer (*Amer. Potato J.*, 1964, **41**, 201—207).—Anhydrous NH_3 (40 lb. N/acre) was not as effective as NH_4NO_3 as a source of N for rye cover crops on a loam. Losses of N by leaching and other mechanisms were similar from both sources applied in the autumn. Although the N treatments increased cover crop yields, the yields of potatoes following the ploughing-in of the cover crops were not significantly affected. A. H. CORNFIELD.

Effect of a cover crop on the seed production of leafy cocksfoot S26. H. M. Roberts (*J. Brit. Grassland Soc.*, 1964, **19**, 62—64).—Seed yield of S26 cocksfoot was reduced in the first harvest year when it was undersown with oats. Yields were markedly improved by application of extra Nitro-chalk in spring, by using a partial cover crop and by cutting the oats for silage. Over 3 years financial returns were very similar for all treatments. A. H. CORNFIELD.

Fertiliser-seed placement with birdsfoot trefoil and lucerne. R. W. Duell (*Agron. J.*, 1965, **56**, 503—505).—When fertiliser was placed 1·25 in. from the seed lucerne was superior to birdsfoot trefoil in germination, seedling emergence, height and wt. Both species showed reduced germination when soil moisture was low. Lucerne seedlings absorbed more P than did birdsfoot trefoil, but the reverse was true for K. A. H. CORNFIELD.

Seed and forage production of irrigated Russian wildrye, *Elymus junceus*, as influenced by time and rate of nitrogen fertilisation. G. A. Rogler and R. J. Lorenz (*Agron. J.*, 1964, **56**, 501—503).—Both seed and forage aftermath yields of Russian wildrye increased with rate of application of N (50—200 lb./acre/year) during the 4th to 7th years. There were no differences in seed and forage yields between single or split application of N. Application of P (44 lb./acre) had no effect on yields in the absence or presence of applied N. A. H. CORNFIELD.

Acid, ammonium and nitrate distribution in grasses. H. W. Dougall and H. F. Birch (*Nature, Lond.*, 1964, **203**, 1308—1309).—The acid, NH_4^+ and NO_3^- distributions in *Setaria sphacelata* (**I**) and *Bracharia ruziziensis* (**II**) were compared. NH_3-N and acidity were high in **I** and low in **II** while NO_3^- concn. were high in both grasses, particularly **II**. The NH_3-N acid concn. of **I** declined with maturity. S. A. BROOKS.

Improved electronic instrument for estimation of pasture yield. M. B. Alcock (*Nature, Lond.*, 1964, **203**, 1309—1310).—The use and accuracy of an improved electronic instrument for estimation of pasture yield is described. To obtain more accurate estimations of dry-matter yield it was necessary to sample for the total water/dry wt. ratio. S. A. BROOKS.

Effect of phosphorus and potassium nutrition of sour cherry on soil population levels of five parasitic nematodes. J. D. Kirkpatrick, W. F. Mai, K. G. Parker and E. G. Fisher (*Phytopathology*, 1964, **54**, 706—712).—Application of P and K fertilisers in varied proportions had differential effects on populations of *Pratylenchus* sp., *Helicotylenchus dihystra*, *Tylenchorhynchus* sp., *Xiphinema americanum* and *Pratylenchus penetrans*. Relationships between leaf-K and -P and the no. of the various nematodes in the soil, are established. A. G. POLLARD.

Inorganic manuring of pineapple plantations in Guiana. Results of first tests and first suggestions. C. Py and A. Fouqué (*Fruits, Paris*, 1964, **19**, 262—264).—The application of fertiliser to two common but different soils in Guiana for the cultivation of pineapples is discussed. The problem of rapid leaching of fertiliser from the non-retentive soils is of extreme economic importance. W. ELSTOW.

Influence of copper gradients on various apple leaf and twig constituents as related to fire blight incidence. J. W. Bushong, D. Powell and P. D. Shaw (*Phytopathology*, 1964, **54**, 713—717).—Twig infection of apple by *Erwinia amylovora* was lowered by trunk injections of $CuSO_4$ and spray applications of Bordeaux mixture. In succulent shoots Cu concn. and fire blight incidence were inversely related. Cu treatments did not affect the protein or N levels in leaves or twigs but affected the amino-acid distribution, especially after trunk injections. Peroxidase activity in shoots was increased by low and diminished by high [Cu^{2+}]. A. G. POLLARD.

Yields of tomatoes as influenced by application of sugarcane filter-press cake and starter solutions. H. Azzam and G. Samuels (*J. Agric. Puerto Rico*, 1964, **48**, 55—59).—The highest yield of tomatoes on a clay (pH 5·6) were obtained when sugarcane filter-press cake (10 tons/acre) was used in conjunction with a starter solution and application of a 9—10—5 (N-P_2O_5-K_2O) fertiliser (1200 lb./acre). Yields were low when the filter-press cake was not applied, and there were responses to starter solutions only when the cake was applied. A starter solution made from the 9—10—5 fertiliser (12 lb./100 gal.) was as effective as two commercial starter solutions. A. H. CORNFIELD.

'Lyamungu dieback' of arabica coffee in Tanganyika. I. Symptoms, distribution and experimental treatments. D. A. Burdekin (*Ann. appl. Biol.*, 1964, **53**, 281—289).—Symptoms and distribution of the disease are described. The disorder is associated with heavy cropping and is influenced by environment and cultural management. In experimental plots its effects were diminished by artificial shading (which reduced the crop) and by heavy nitrogenous manuring. A. H. CORNFIELD.

Effects of rates of supply of nitrogen, phosphorus, potassium and magnesium on leaf and stem growth, flowering and 'topple' of Golden Harvest and Elmus tulips. W. F. Cheal and E. J. Hewitt (*Ann. appl. Biol.*, 1964, **53**, 477—484).—In sand culture factorial experiments with N, P, K and Mg at various rates, N had the greatest effect. Increasing N decreased the length of the first internode of Elmus. There were minor differences due to variety in mineral deficiency symptoms in leaf and stem. Elmus tulips receiving amounts of Ca normally considered adequate developed 'topple' when given no N, especially when combined with high K; these treatments also induced stem contortion in Golden Harvest this disorder appeared to be analogous to 'topple'. A. H. CORNFIELD.

Influence of nitrogen and potassium on susceptibility of *Chrysanthemum morifolium* to *Botrytis cinerea*. E. L. Hobbs and W. E. Waters (*Phytopathology*, 1964, **54**, 674—676).—On a sandy soil the susceptibility of *C. morifolium* to *B. cinerea* was greater where larger applications of N were made. The severity of the disease was not significantly affected by K. Where K + N fertilisers were used, the development of the disease was greater where the larger amounts of fertiliser of the same K : N ratio were applied. A. G. POLLARD.

Aluminium and phosphorus requirements of *Pinus radiata*. F. Humphreys and R. Truman (*Plant & Soil*, 1964, **20**, 131—134).—When *Pinus radiata* seedlings were grown in solutions containing adequate P for max. growth and varying amounts of Al, P uptake in roots and tops was positively correlated with Al uptake. Uptake of both nutrients increased with the Al content of the nutrient. Increased uptake of P was not correlated with increased growth rate. A. H. CORNFIELD.

Effect of iron chelate treatment of poplar upon carbon dioxide uptake, leaf size and content of leaf pigments and iron. T. Keller and W. Koch (*Plant & Soil*, 1964, **20**, 116—126).—Soil treatment of Fe-deficient poplars in pots with Fe-chelate ('Sequestren 138 Fe') increased foliar Fe, chlorophyll, β-carotene, lutein and violaxanthin contents. The treatment also increased CO_2 uptake by the leaves, more so at high than at low light intensities. The treatment did not affect the size of leaves already formed, but increased the size of leaves developing after treatment. A. H. CORNFIELD.

Manganese toxicity in soya-beans. H. J. Walters and G. W. Hardy (*Phytopathology*, 1964, **54**, 627).—Soya-beans grown on sandy soils of Arkansas showed symptoms (described) of Mn toxicity. Affected plants produced only a few distorted pods and root systems had few secondary rootlets. Soils on which severe symptoms developed had pH 4·6—4·9. Liming counteracted the toxicity. A. G. POLLARD.

Effect of irrigation and row spacing on grain sorghum in the Piedmont (U.S.A.). A. R. Brown, C. Cobb and E. H. Wood (*Agron. J.*, 1964, **56**, 506—509).—Irrigating when 10% available moisture remained in the top 2 ft. of soil was as effective in increasing sorghum grain yields as was irrigation when 50% available soil moisture remained in the top 2 ft. of soil. 20-in. row spacing gave higher yields of grain than did 40-in. spacing in 2 or 3 years over all irrigation treatments. Forage yields were greater with 20-in. than with 40-in. row spacing. Irrigation increased forage yields in 1 of 3 years. Lodging was greater in 20- than in 40-in. spacing, particularly under drought conditions. When charcoal rot was present irrigation reduced lodging induced by the disease. A. H. CORNFIELD.

Composition for preventing or inhibiting the germination of seeds. O. R. Hansen (B.P. 941,114, 23.12.59).—The composition, the effects of which may last for 4—5 months, contain as active agent (herbicide) a water-sol. salt of 2,5,1-NH_2·C_6H_3X·SO_3·NH_2 (X is halogen). Thus, in a comparative test on the germination of *Poa trivialis* in a Petri dish of 90 mm.-dia., 0·008 mg. of 2-amino-5-chlorobenzenesulphonamide (as triethanolamine salt) causes 50% inhibition whereas 0·2 mg. of 2,4-D is needed for the same result. F. R. BASFORD.

Pest Control

Applied entomology in Britain. I. Veterinary entomology. W. N. Beesley. **II. Forest entomology.** D. Bevan. **III. Forest-products entomology.** J. D. Bletchly. **IV. Medical entomology.** J. R. Busvine. **V. Stored-products entomology, 1964.** J. A. Freeman, **VI. Agricultural and horticultural entomoloy.** F. H. Jacob (*Ann. appl. Biol.*, 1964, **53**, 175—180, 180—184, 184—190, 190—199. 200—215, 215—228). A. H. CORNFIELD.

Fungicides. IX. Fungitoxicity, phytotoxicity and systemic fungicidal activity of inorganic salts. G. A. Carter and R. L. Wain (*Ann. appl. Biol.*, 1964, **53**, 291—309).—The systemic therapeutic activity of 88 inorg. salts, representing most of the known elements, was assessed by determining their ability to protect broad bean seedlings from *Botrytis fabae*, tomato plants from *Alternaria solani*, and wheat seedlings from *Erysiphe graminis* following root application to the host plant. Li_2SO_4, when applied to wheat seedlings, showed marked systemic activity against *E. graminis*. The toxicity of the salts towards *B. fabae* was determined using the standard spore germination test; their toxicity towards broad beans and

wheat seedlings was also assessed. The effect of bean sap upon the fungistatic activity of the salts was also investigated. The degree of systemic activity shown by the salts is discussed in relation to such factors as their fungitoxicity, mode of action, fungitoxicity/phytotoxicity ratios, uptake by the plant and the degree to which plant sap inactivates them. A. H. CORNFIELD.

Fungicides. X. Antifungal activity of 2-deoxy-D-glucose. R. K. Atkin, D. M. Spencer and R. L. Wain (*Ann. appl. Biol.*, 1964, **53**, 437—443).—The fungitoxicity of 2-deoxy-D-glucose was examined in spore germination tests using *Botrytis cinerea, B. fabae, Alternaria brassicicola, Aspergillus niger, Cladosporium cucumerinum, C. fulvum, Sclerotinia fructigena, Verticillium albo-atrum* and *Glomerella cingulata*. It was toxic at concn. below 10 μg./ml. to all of these fungi except *G. cingulata*, which was resistant to concn. up to 2000 μg./ml. Mycelial growth of *G. cingulata* and *A. niger* was much more resistant than that of the other species to high concn. of the compound. Results are discussed in relation to the known inhibitory nature of 2-deoxy-D-glucose in other systems. A. H. CORNFIELD.

Physical and chemical properties of pesticides. III. Physical characteristics of emulsifiable products. A. Velniceriu, I. Simulescu and C. Ciocan (*Rev. Chim. Bucharest*, 1964, **15**, 257—260).—A new method is presented, for calculation of the composition of some emulsifiable products, under conditions of under- or over-proportioning of components. A graphical method based on the Gibbs' ternary diagram, served to confirm the results of the calculation. An illustration of the method was presented for the system technical aldrin–toluene–Emulsogen I 40. M. L.

Emulsifiable pesticide concentrates. K. L. Johnson (*Soap, N.Y.*, 1964, **40**, No. 9, 93—96, 129).—The general approach to the problem is discussed with special reference to stability in every type of water. Tests are outlined to evaluate the degree of dispersion and stability. Most formulations should be neutral or midly acidic since all toxicants release acidic fragments; emulsifiers containing active N should be avoided when working with chlorinated toxicants and the concentrate should have a low moisture content since this hastens hydrolysis. Trace impurities of many metals should be avoided as these can act catalytically. C. V.

Sulphur-depletion of cells by captan. R. J. Lukens (*Phytopathology*, 1964, **54**, 881—882).—Evidence is presented showing that the CS_2 produced by the action of captan on cells of *Saccharomyces pastorianus* derives part of its S from thiols in the cells (which become depleted of S) and part from captan (probably by decomposition of thiophosgene in water). The loss of S from cellular contents may be a significant factor in the toxicity of captan. A. G. POLLARD.

Symmetrical dichlorotetrafluoroacetone: a synthetic organic rust chemotherapeutant. T. C. Allen, jun., and A. H. Freiberg (*Phytopathology*, 1964, **54**, 580—583).—Rust infections on various host plants were controlled by *S*-dichlorotetrafluoroacetone (**I**), applied to soil, foliage, seed or by root immersion. Treatment with **I** prior to inoculation of pinto beans with *Uromyces phaseoli* var. *typica* and of wheat with *Puccinia recondita* prevented infection. Foliar applications of **I** eliminated rust already established on the beans and wheat. A. G. POLLARD.

***NN'*-Dimethylphosphorodiamidate (Nellite), a new nematocide, systemic insecticide and growth-promoting substance on cotton seedlings.** J. A. P. Pinckard (*Phytopathology*, 1964, **54**, 626).—Nellite broadcast by air (1 lb./acre) over cotton seedlings or ploughed land increased the growth and improved the vigour of the plants and markedly reduced no. of *Rotylenchulus reniformis*. About two weeks after emergence a new infestation of thrips damaged more than half the seedlings on control plots and only 8% on treated plots. A. G. POLLARD.

Mode of action of 6-azauracil against powdery mildew. J. Dekker and A. J. P. Oort (*Phytopathology*, 1964, **54**, 815—818).—6-Azauracil (**I**), which acts systemically against powdery mildews, was tested against *Erysiphe cichoracearum* on cucumber and *E. graminis* on wheat. Neither spore formation nor penetration of plant tissue by the fungus was prevented by **I**, which, however, affected the formation of the first haustorium and completely inhibited the mildew. 6-Azauridine (**II**) and 6-azauridine-5'-phosphate (**III**) were similarly active. The action of **I** but not that of **II** or **III** was reversed by uracil. This reversal occurred *in vivo* or *in vitro* in tests with other bacterial or fungal pathogens, but reversal by the precursor orotic acid took place only within the plant system. A. G. POLLARD.

The free amino-acid pool of the cockroach (*Periplaneta americana*) central nervous system and the effect of insecticides. J. W. Ray, (*J. Insect Physiol.*, 1964, **10**, 587—597).—The action of DDT, dieldrin, DFP (di-isopropyl phosphorofluoridate) (**I**) and *o*-IMPC (*o*-isopropoxyphenyl *N*- methylcarbamate) (**II**) on the cockroach is associated with considerable reduction in the free proline concn. in the nerves. In the case of **I** and **II** there was a compensatory increase in α-alanine and glutamine concn. Treatment with rotenone or n-valone caused accumulation of α-alanine in the nerve chord. A. G. POLLARD.

Partial characterization of *in vivo* metabolites of DDT-^{14}C in *Triatoma infestans*. M. Agosin, A. Morello and N. Scaramelli (*J. econ. Ent.*, 1964, **57**, 974—977).—Third-instar larvae were topically treated with DDT-^{14}C and Kelthane-^{14}C. After 72 h. two DDT metabolites were found (metabolites 2 and 3). Kelthane is absorbed more slowly and after 10 days one metabolite was found. Paper chromatography, chemical reactions and u.v. spectrum indicate that metabolite 3 corresponds to Kelthane while metabolite 2 is probably similar to a Kelthane metabolite. C. M. HARDWICK.

Aqueous formations of pyrethrum for controlling phytophagous Arthropoda—an evaluation using bioassay techniques. W. N. Yule (*Ann. appl. Biol.*, 1964, **53**, 15—28).—The LC_{50} of pyrethrins in unsynergised formulations were for adult aphid, 150 p.p.m.; adult beetle, 50,910 p.p.m.; third instar caterpillar, 325 p.p.m.; and adult mite, 1168 p.p.m. Increasing the wetting power increased the toxicity to aphid and caterpillar. Addition of synergists (piperonyl) butoxide or Sulphoxide) at concn. up to 8 times that of pyrethrins increased their potencies for aphid, beetle and mite 7—10-fold, but did not affect the potency for caterpillar. A. H. CORNFIELD.

Tests of soil fungicides under uniform conditions for control of damping-off caused by *Rhizoctonia solani*. J. H. Owen (*Phytopathology*, 1964, **54**, 625—626).—In greenhouse and small field-plot soils uniformly infested with *Rh. solani* and maintained at similar temp. and water content, comparison was made of several fungicides in dust and granular (greenhouse) or liquid (field) formulations, all being applied in the furrows. In the greenhouse best control of post-emergence damping-off was obtained with pentachloronitrobenzene (**I**) + captan (or thiram, or 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole, **II**). In field plots **I** alone or with HgMe dicyanodiamide or **II** gave the best results. A. G. POLLARD.

Disappearance of dimethoate from soil. W. R. Bohn (*J. econ. Ent.*, 1964, **57**, 798—799).—Dimethoate spray was applied to a sandy loam at 1 lb./acre. Under drought conditions 50% disappeared in 4 days. When two more applications were made at 7-day intervals they did not penetrate more than 3 in. C. M. HARDWICK.

Influence of soil moisture on toxicity of insecticides in mineral soil to insects. C. R. Harris (*J. econ. Ent.*, 1964, **57**, 946—950).—Diazinon was 134·6-, parathion 28·3-, trichlorphon 20·1- dieldrin 16·4-, Zectran 16·1-, DDT 15·9-, heptachlor 12·7- and mevinphos 1·4-fold more toxic to *Gryllus pensylvanicus* in wet than in dry soil. Regression lines are given for toxicity to crickets at six moisture levels. Using last-instar *Euxesta notata* a similar effect was produced whether the insect was in or on soil. Zectran, diazinon and parathion became more toxic and mevinophos became less toxic with increased soil moisture content. An explanation is suggested. (20 references.) C. M. HARDWICK.

Influence of in-covering soil fungicides on the covering soil microflora in relation to cotton seedling disease. L. S. Bird (*Phytopathology*, 1964, **54**, 621).—Two soils were treated with (*a*) captan + folpet or (*b*) pentachloronitrobenzene + thiram and used as covering soils in the planting of cotton seedlings. Plate counts showed that (*a*) lowered the no. of actinomycetes (*A*) and increased those of bacteria (*B*), whereas (*b*) decreased both types of organisms. The incidence of post-emergence damping off among seedlings was associated with the ratio A/B; a critical level being approx. 1 although the nature of the soil had some influence. A. G. POLLARD.

Bioassay for volatile toxicants from fungicides in soil. L. T. Richardson and D. E. Munnecke (*Phytopathology*, 1964, **54**, 836—839).—A simple technique is described and data obtained with a no. of fungicides is recorded. A. G. POLLARD.

Vaporised dichlorvos for control of arthropod pests in greenhouses. B. C. Pass and R. Thurston (*J. econ. Ent.*, 1964, **57**, 832—834).—Dichlorvos (20%) impregnated in resin strands at 1 linear ft./100 ft.^{2} gave excellent control of various aphids, mites and whitefly. Application of dichlorvos liquid to steam pipes at 0·5—1·5 oz./10,000 ft.^{3} gave 100% control but could cause corrosion. There was no phytotoxicity. C. M. HARDWICK.

Comparative toxicity of some phosphoramidothioates and phosphoramidates to susceptible and acaricide-resistant strains of *Tetranychus pacificus* and *Panonychus citri*. L. R. Jeppson, M. J. Jesser and J. O. Complin (*J. econ. Ent.*, 1964, **57**, 878—881).—*p*-Chlorophenyl, some 2,4-dichlorophenyl and about 55 homologous 2,4,5-trichlorophenyl phosphoramidothioates and phosphoramidates were investigated. Of these the 2,4,5-trichlorophenyl series was the only one of significant toxicity to resistant strains. Amongst alkoxy-homologues, the isopropyl substitutions were most toxic and

propyl and butyl substitutions least. Phosphoramidates were frequently more toxic than their phosphoramidothioate analogues. The relation of structure and toxicity in mites, houseflies and mosquitoes is compared. C. M. HARDWICK.

Mechanism of malathion and parathion resistance in two-spotted spider mite, *Tetranychus urticae*. F. Matsumura and G. Voss (*J. econ. Ent.*, 1964, **57**, 911—917).—*In vitro* and *in vivo* degradation studies on four strains of mature female mites having different degrees of resistance to malathion were compared by paper chromatography. No differences were found in rate of penetration. Some differences occurred in the amounts of carboxyesterase products and in phosphatase activity. Homogenates of a strain highly resistant to malathion hydrolysed β-naphthyl benzoate at a relatively higher rate. (18 references.) C. M. HARDWICK.

Resistance to acaricides in European red mite. D. Asquith (*J. econ. Ent.*, 1964, **57**, 905—907).—The level of resistance of *Panonychus ulmi* subjected to five different series of treatments using dimethoate, Tetradifon and Kelthane is described. Evidence is presented to show that mites exposed to consecutive applications of some individual insecticides developed resistance more quickly than when the insecticides were used in mixtures or in alternate applications with others. C. M. HARDWICK.

Effect of PCNB [pentachloronitrobenzene] on isolates of *Rhizoctonia solani* under field conditions. M. N. Shatla and J. B. Sinclair (*Phytopathology*, 1964, **54**, 626).—Isolates of *Rh. solani* from diseased cotton seedlings growing on plots previously treated with PCNB were more tolerant of subsequent applications of PCNB than were isolates from plants grown on previously untreated plots. A. G. POLLARD.

Relation of chlorogenic acid and free phenols in potato roots to infection by *Verticillium albo-atrum*. S. S. Patil, R. L. Powelson and R. A. Young (*Phytopathology*, 1964, **54**, 531—535).—Three weeks after planting sprouted single-eye seed-pieces of several varieties of potato, chlorogenic acid (**I**) and free phenols in the roots diminished considerably. All varieties were resistant to *V. albo-atrum* until 4—5 weeks after planting when infection of susceptible varieties began. **I** (up to 1000 p.p.m.) was not fungistatic but its oxidation products inhibited germination of the spores of the pathogen. Root-polyphenol oxidase was much more active in a susceptible than in a resistant variety. A. G. POLLARD.

Relation between toxicity of malathion analogues and organophosphate resistance in the two-spotted spider mite. G. Voss, W. C. Dauterman and F. Matsumura (*J. econ. Ent.*, 1964, **57**, 808—811).—The effects on susceptible and resistant strains of the mite, of substituting the alkyl group by Me, Et, Pr^n, Pr^i or Bu^n group at the phosphorester or carboxyester of malathion or malaoxon are examined. LC_{50} values showed that changes in the resistance factor were directly related to toxicity of the compound to the susceptible strain. The reason for this is discussed. C. M. HARDWICK.

Adapted tolerance to organic fungicides by isolates of *Rhizoctonia solani* from seedling cotton. H. M. Elsaid and J. B. Sinclair (*Phytopathology*, 1964, **54**, 518—522).—Tolerance of *Rh. solani* to captan (**I**), dichlone (**II**), maneb (**III**), pentachloronitrobenzene (**IV**) and thiram was increased by serial transfers. The adaptation was generally temporary except that to **II** which was notably more persistent ; it was specific for **III** and **IV** but non-specific for **I** and **II**. **IV** was fungistatic but **II** and **III** were fungitoxic. A. G. POLLARD.

Toxicity to fish of mixtures of poisons. II. Copper-ammonia and zinc-phenol mixtures. D. W. M. Herbert and J. M. Vandyke (*Ann. appl. Biol.*, 1964, **53**, 415—421).—Tests with rainbow trout in mixtures of NH_4Cl with $CuSO_4$ and of PhOH with $ZnSO_4$ showed that the threshold of toxic concn. for 50% mortality occurred in solutions for which a value of 1 was obtained by summing the concn. of the individual poisons expressed as fractions of their individual threshold concn. With NH_3–Cu mixtures this method of predicting the threshold concn. became professively less adequate as lower % mortalities occurred. A. H. CORNFIELD.

Disappearance of dimethoate and S.D.-7438 from lucerne. F. R. Shaw and W. H. Ziener (*J. econ. Ent.*, 1964, **57**, 997—998).—Dimethoate spray at 0·25 lb./acre was below sensitivity level after the tenth day and at 0·5 lb./acre after the thirty-fourth day. Residues of S.D.-7438 (toluene-α,α-dithiol bis-[*OO*-dimethyl phosphorodithioate]) at 1·0 and 2·0 lb./acre decreased to 10 and 20 p.p.m. by the twenty-first day. C. M. HARDWICK.

Toxicity of insecticides to a coccinellid predator of cereal leaf beetle. Y. M. Yun and R. F. Ruppel (*J. econ. Ent.*, 1964, **57**, 835—837).—In laboratory tests Carbaryl and Guthion gave a rapid kill of *Coleomegilla maculata lengi*. Malathion was effective but slower acting. Bayer 39007 (*o*-isopropoxyphenyl methylcarbamate) gave 41% mortality. Dieldrin, lindane and endrin were only slightly toxic. Sprays and granular formulations gave similar results except that granules were slower acting. Carbaryl and dieldrin also caused high mortality of *Oulema melanopa*. C. M. HARDWICK.

Aerial applications of insecticides to control spring infestations of cereal leaf beetle on small grains. R. F. Ruppel and M. C. Wilson (*J. econ. Ent.*, 1964, **57**, 899—903).—Experiments were carried out in May and June to control *Oulema melanopa* on wheat and oats in Michigan and Indiana. Carbaryl (**I**) was still effective after 8 days while malathion had a high initial kill but was ineffective after 72 h. If applied without a sticker **I** had less residual effect ; it was as effective when applied at 50 ft. as at 5 ft. but malathion was effective only from low altitudes. Guthion was also promising. (22 references.) C. M. HARDWICK.

Control of southwestern maize borer with an experimental systemic insecticide. S. D. Hensley, W. Machado and D. R. Melville (*J. econ. Ent.*, 1964, **57**, 1011).—Granules of American Cyanamid 47470 [cyclic propylene (diethoxyphosphinyl) dithioimidocarbonate] at 4 lb./acre gave highly effective control of larvae of *Zeadiatraea grandiosella* for 62 days and at 2 lb./acre for a shorter time. This gave early season control and so increased yields. Girdling by diapausing larvae was not decreased. C. M. HARDWICK.

Effects of infection by *Puccinia graminis tritici* on the vitamin B_6, niacin and pantothenic acid contents of wheat plants. M. Husain, C. D. Hobbs and M. C. Futrell (*Phytopathology*, 1964, **54**, 502—505).—In leaves and stems of infected wheat plants the pantothenic acid (**I**) and niacin (**II**) contents increased as pustules and uredospores of the fungus developed. The spores contained much more **I** and **II** than did the plant tissues. The vitamin B_6 contents of the plants were unaffected by the pathogen. A. G. POLLARD.

Laboratory device for observing insects and mites involved in heating of stored grain. L. E. Eighme (*J. econ. Ent.*, 1964, **57**, 998—999).—The box consisted of a plywood frame with glass sides in which thermocouples and relative humidity sensing elements were installed. Moisture was added to initiate the hot spot. C. M. HARDWICK.

Use of systemic insecticides for control of potato leafhopper, *Empoasca fabae* and effect on potato yield. H. B. Wressell and G. R. Driscoll (*J. econ. Ent.*, 1964, **57**, 992—993).—Granular formulations of phorate and Disyston applied as plants were breaking through the soil gave increased yields. Four foliar sprays, at 7-day intervals, of DDT, Bayer 25141 (*OO*-diethyl *O-p*-(methylsulphinyl) phenyl phosphorothioate) and Thiocron (*OO*-dimethyl *S*-(2-methoxyethylcarbamoylmethyl) phosphorothiolothionate gave significantly greater yields. Fewer leafhoppers were found in sprayed plots. Residue levels are given. C. M. HARDWICK.

Terraclor (pentachloronitrobenzene) for control of *Rhizoctonia* in potato soils. C. H. Livingston, N. Oshima and M. D. Harrison (*Amer. Potato J.*, 1964, **41**, 239—243).—*Rhizoctonia* levels in soil treated with Terraclor (25—100 lb./acre) were not significantly affected at the beginning of the season, but were reduced by the end of the season. Tuber infection was reduced by the treatments but yields were not significantly increased. A. H. CORNFIELD.

Influence of green manures and crop rotation on common scab of potato. A. R. Weinhold, J. W. Oswald, T. Bowman, J. Bishop and D. Wright (*Amer. Potato J.*, 1964, **41**, 265—273).—Incidence of scab in potatoes was no different whether barley, cotton or sugar beet was grown in rotation with potatoes. Where potatoes were grown every year and a cover crop, grown each year, was incorporated in the soil an intermediate barley crop increased scab incidence while a soya-bean crop reduced incidence of scab compared with that when no cover crop was grown. A. H. CORNFIELD.

Phorate and Di-syston for potato insect control. R. S. Patterson and W. A. Rawlins (*Amer. Potato J.*, 1964, **41**, 196—200).—The systemic insecticides phorate (**I**) and Di-syston (**II**) were applied at varying rates in bands at planting time. **I** gave good flea beetle control throughout the season in 2 of 3 years, whilst **II** was less effective. **II** was more effective than **I** for aphid control and both were effective for potato leafhopper control. Split application (planting time and mid-season) of the materials was no more effective than the full dosage at planting time. A. H. CORNFIELD.

Effectiveness of insecticides against white grubs in bluegrass lawns. B. C. Pass (*J. econ. Ent.*, 1964, **57**, 1002—1003).—Granular formulations of trichlorphon, dieldrin and maize meal-impregnated Kepone were comparable with recommended rates of heptachlor and aldrin for control of *Phyllophaga* and *Cyclocephala*. C. M. HARDWICK.

Effect of winter burning on some pests of lucerne. H. H. Tippins (*J. econ. Ent.*, 1964, **57**, 1003—1004).—Higher insect counts were obtained in unburnt areas and finally the lucerne became completely denuded. Insecticide treatments reduced infestations. The advantages of burning extended also to disease and weed control. C. M. HARDWICK.

Pad method of recovering fruit flies from infested fruit. M. McPhail (*J. econ. Ent.*, 1964, **57**, 1012—1013).—The apparatus using Celotex pads to soak up juices, is described. In two experiments fly recovery was similar to that in the holding bag method. The use of a Celotex hood on recovery unit was also found to be advantageous. C. M. HARDWICK.

Field evaluation of insecticides for woolly apple aphid control. S. C. Hoyt (*J. econ. Ent.*, 1964, **57**, 1009).—Sprays of diazinon, endosulfan, dimethoate, Menazon and Bayer 39007 (*o*-isopropoxyphenyl methylcarbamate) gave good control of *Eriosoma lanigerum*. Bayer 39007 had some phytotoxic effects. C. M. HARDWICK.

Control of walnut aphid and codling moth on walnuts in northern California. H. F. Madsen, L. A. Falcon and T. T. Y. Wong (*J. econ.* Ent., 1964, **57**, 950—952). In experiments over 2 years, phosphamidon gave good control of *Chromaphis juglandicola* and *Carpocapsa pomonella*. Bidrin was effective in 1-year tests. Both cause some phytotoxicity. Morestan (6-methylquinoxaline-2,3-dithol cyclic carbonate) controlled the aphid and spider mites. Endosulfan was effective against the walnut aphid but had little residual action. Morestan, Bidrin and Imidan allowed survival of the predators. C. M. HARDWICK.

Control of strawberry leaf roller, *Ancylis comptana fragariae* (Lepidoptera : Tortriudae). G. A. Schaefers (*J. econ. Ent.*, 1964, **57**, 983—986).—About 22 compounds were evaluated on commercial strawberry plantings in western New York. The most effective time of application was early in season before the larvae protect themselves within folded leaves. TDE, parathion, Guthion, diazinon and Zectran gave good control of hatching larvae. Imidan, Stauffer N2404 (*O*-2-chloro-4-nitrophenyl *O*-isopropyl ethylphosphonothioate) and Bayer 44646 (4-dimethylamino-*m*-tolyl methylcarbamate) were also promising. Parathion and Guthion gave best control of established larvae. (13 references.) C. M. HARDWICK.

Control of *Drosophila* in vineyards with insecticidal dusts. E. M. Stafford and A. P. Yerington (*J. econ. Ent.*, 1964, **57**, 958—960).—*Drosophila* caused the spread of bunch rot as maturity approached and its incidence gave the best indication of *Drosophila* control. The level of bunch rot in mid-Aug. was significant in calculating the effectiveness of *Drosophila control*. Dimethoate dust was the most effective. An early application of endrin followed by two applications of Naled gave better control than did two applications of Naled alone. C. M. HARDWICK.

Some effects of γ-radiation and a chemosterilant on Mexican bean beetle. T. J. Herneberry, F. F. Smith and W. L. McGovern (*J. econ. Ent.*, 1964, **57**, 813—815).—Larvae were more sensitive than pupae to direct radiation. Untreated females mated with males irradiated as pupae or adults produced eggs which did not hatch. Pupal-irradiated females produced few or no eggs. Irradiation produced high adult mortality. Adult *Epilachna varivestis* dipped in or fed Apholate-treated foliage produced no eggs which hatched. Longevity was also affected. Untreated females produced viable eggs in subsequent matings with untreated males. C. M. HARDWICK.

Effect of insecticides, rates, intervals between, and number of applications and insecticide-oil and surfactant combinations for insect control on southernpeas. D. A. Wolfenbarger (*J. econ. Ent.*, 1964, **57**, 966—969).—Bidrin, Monsanto 40294 (*O*-*p*-nitrophenyl *O*-phenyl methylphosphonothioate) and Bayer 25141 [*OO*-diethyl *O*-*p*-(methylsulphinyl)phenyl phosphorothioate] gave good control of *Chalodermus aeneus* and *Liriomyza munda*. Guthion and carbaryl were also effective against *C. aeneus*. Comparison was made of the effectiveness of up to seven applications against *Heliothis zea*. The effect of the addition of oil to toxaphene and endosulfan was indeterminate. Penetrant NS-139 increased the effectiveness of Bidrin against *L. munda*. C. M. HARDWICK.

Red spider mite control on roses in Florida. D. O. Wolfenbarger (*J. econ. Ent.*, 1964, **57**, 1000—1002).—Sprays of Morestan gave outstanding control of *Tetranychus urlicae* while binapacryl, Aramite, chlorbenzilate and tetradiphon were promising. Tetradiphon as a thermal aerosol was more effective than Kelthane or or chlorbenzilate. C. M. HARDWICK.

Nematocidal activity of phorate and Disyston in woody ornamentals. P. Sivapalan (*Phytopathology*, 1964, **54**, 748—749).—Application of the systemic insecticides to woody plants, e.g., azaleas, produced positive growth responses which were correlated with nematocidal activity, e.g., on *Tylenchorhyncus claytoni*. In pot tests the efficiency of these substances equalled or exceeded that due to 1,2-dibromo-3-chloropropane and Zinophos (*OO*-diethyl *O*-2-pyrazinyl phosphorothioate. A. G. POLLARD.

Imported fire ant toxic bait studies : evaluation of toxicants. C. E. Stringer, jun., C. S. Lofgren and F. J. Bartlett (*J. econ. Ent.*, 1964, **57**, 941—945).—In laboratory tests using continuous feeding, limited feedings and bait transfer, the order of efficiency Mirex (**I**) > Kepone > HRS-1243 (1,1a,3,3a,4,5,5,5a,5b,6-decachloro-2-(2,3-dihydroxypropoxy) octahydro-1,3,4-metheno-2H-cyclobuta[cd] pentalen-2-one) > Bayer 30911 (*O*-2,4-dichlorophenyl *O*-methyl methylphosphonothioate). All baits were repellent to some degree but Mirex was lest repellent. In three field tests Mirex had the longest residual effect. C. M. HARDWICK.

Control of spider mites on cotton. W. J. Mistric, jun. (*J. econ. Ent.*, 1964, **57**, 855—857).—Field experiments in N. Carolina demonstrated varying degrees of resistance of *Tetranychus telarius* to sprays of demeton, ethion, Carbophenothion, Bidrin, Aramite, chlorbenzilate and Kelthane. Some failure was due to lack of utilisation of cultural and natural controls. C. M. HARDWICK.

Use of fungicides in the control of *Cercospora coffeicola* on coffee. C. A. Soto (*Phytopathology*, 1964, **54**, 501).—Comparative trials with a no. of fungicides are described. Best results were obtained with ferbam and with monthly application of Cu salts ('tribasic' Cu sulphate). Sn compounds showed promise. A. G. POLLARD.

Mistblower spray tests for control of birch leaf miner. D. P. Connola and R. C. Sweet (*J. econ. Ent.*, 1964, **57**, 1015).—Four evenly spaced spray applications of 2% Carbaryl prepared from 4-flowable formula were superior to various concn. of chlordane, lindane or malathion. C. M. HARDWICK.

Herbicidal control of broad-leaves and grass weeds in established grassland. S. Evans (*J. Brit. Grassland Soc.*, 1964, **19**, 205—211).—A review. A. H. CORNFIELD.

Trials for utilisation of o-nitroethylbenzene. II. Preparation of new substances from nitroethylbenzene, with possible herbicidal activity. P. Buchwald and A. Kövendi (*Rev. chim., Bucharest*, 1964, **15**, 261—264).—The *o*-nitroethylbenzene was obtained as waste product from chloroamphenicol production. It was used in the synthesis of a series of new aryloxyacetic acids, and of *N*-substituted- and *NN'*-disubstituted corresponding urea deriv. The raw material which contained in addition *p*- and *m*-isomers (20%) was purified at the stage of aminoethylbenzene, so that the isomers could be separated, and used individually in the syntheses. Details were presented on the reaction conditions and products. M. L.

Effect of dichlorophenoxyacetic acid on the growth nodule formation and nitrogen content of *Vicia faba*. R. Geranmayeh (*Planta*, 1964, **62**, 66—87).—Sand cultures of beans and peas were grown with N-containing and N-free nutrients and the plants were treated with 2,4-D, as Na or NH_4 salt, applied either to leaves or to roots. Spraying bean plants with the 0·001% salts diminished the dry-matter content of the aerial parts but increased the wt. of roots and stimulated nodulation. A 0·002% solution of the salts produced a smaller effect on the aerial parts and a greater effect on roots and nodules. In peas the solutions increased the dry wt. of both tops and roots and slightly stimulated nodulation. With both plants and both methods of application leaf areas were decreased and leaf form was modified. Leaf fasciation also occurred in treated beans. A. G. POLLARD.

Physiological action of 2,2-dichloropropionic acid. I. Mechanisms controlling the inhibition of root elongation. R. Prasad and G. E. Blackman (*J. exp. Bot.*, 1964, **15**, No. 43, 48—66).—The inhibitory action of 2,2-dichloropropionic acid (**I**), labelled with ^{36}Cl, is examined in relation to concn. and time using, *Sorghum vulgare*, *Zea mays*, *Helianthus annuus* and *Pisum sativum*. The concn. of **I** needed to halve the rate of root growth showed a ten-fold difference between the most sensitive (*S. vulgare*) and the least sensitive (*P. sativum*) species. Experimental evidence indicated that the action of **I** was primarily to disturb meristematic activity in the root tips. The uptake of **I** by roots was cumulative with highest concn. at the tip. The herbicidal action of **I** probably depends more on the level of accumulation in the root than on its reaction with individual cells. A. G. POLLARD.

Chromatographic detection and determination of organochlorine herbicides in soil and water. D. C. Abbott, H. Egan, E. W. Hammond and J. Thomson (*Analyst*, 1964, **89**, 480—488).—Herbicides in water were extracted with ether from aq. AcOH and in soil with ether from a slurry in dil. H_2SO_4; the solvent used to obtain paper-chromatographic resolution was a mixture of liquid paraffin, benzene, AcOH and cyclohexane as mobile phase with Whatman No. 1 paper but MCPB and 2,4-DB and MCPA and 2,4,5-T were not resolved. For complete separation and identification, thin-layer plates of kieselguhr and silica gel were spotted with **I** and developed with **II**. Spots appeared when dried plates were sprayed with alcoholic $NaNO_3$ and irradiated with u.v. light. Chromatograms and experimental data are given. C. A. P.

Gas-chromatographic separation and determination of closely related active herbicides of the phenoxyalkanecarboxylic ester type. W. Ebing and H. G. Henkel (*J. Gas Chromatogr.*, 1964, **2**, 207—214).—A method is described in which columns packed with Versanid

900 are used to separate mixtures of esters so similar structurally that identification by i.r. spectrometry is impossible. With these columns herbicide mixtures are analysed directly with katharometer and flame ionisation detection, and soil residues with flame ionisation detection. The time required for three determinations is 35—50 min. C. PEARCE.

Gas chromatography of triazine herbicides. H. G. Henkel and W. Ebing (*J. Gas Chromatogr.*, 1964, **2**, 215—218).—A method for the analysis of triazine herbicide residues in soil samples is described. A 2-m. column packed with 2·5% Versamid 900 on Diatoport S was used in conjunction with a flame ionisation detector and the total analysis time was <2 h. C. PEARCE.

Determination of triazine herbicides by gas-liquid chromatography with particular reference to atraton in soil. C. A. Benfield and E. D. Chilwell (*Analyst*, 1964, **89**, 475—479).—Triazine residues in crops or soils were determined using a Pye Argon chromatograph and a low loading of ethylene glycol adipate polyester (as liquid phase) on glass beads. A general extraction procedure with a clean-up process, applicable to crop and soil extracts is described. The procedure for determining atraton and similar compounds is simplified by the use of a second related triazine as an internal standard, the final determination involving only the ratio between the two triazines present. C. A. P.

Methods for the detection of insecticidal phosphoric acid esters and the products of their hydrolytic cleavage. H.-W. Rahn and G. Urban (*Pharmazie*, 1964, **19**, 597—602).—Paper-chromatographic procedures are described for the separation of chlorothion, parathion, parathionmethyl, ronnel and Lebaycid, and for the separation of *OO*-dimethyl- and *OO*-diethylphosphoric acids and the corresponding thio and dithio acids. The esters are best detected with the 2,6-dichloro-*p*-quinone-*N*-chloroimide reagent. A. R. ROGERS.

Microbial insecticide and production thereof. Institut Pasteur (B.P. 942,462, 14.3.60. Fr., 20.3.59).—An insecticide, suitable for the biological destruction of caterpillars and other insects which cause havoc in cultures and forests, is produced by growing, in fluid medium, up to sporulation, a stock of bacteria having a pathogenic action on the insects (*Bacillus thuringensis*)—the medium containing glucide (1—2), assimilable N (0·06—0·1%) and trace minerals (Ca, Zn, Mn and/or Mg), and the glucide being exhausted before the assimilable N. F. R. BASFORD.

4-Chloronaphth-1-yl methylcarbamate and insecticidal compositions containing the same. Union Carbide Corp., Assee of J. R. Kilsheimer and H. H. Moorefield (B.P. 942,515, 29.11.61. U.S., 30.12.60).—Directions are given for the prep. of the title compound from 4-chloro-α-naphthol and Me isocyanate. It is useful as an insecticide especially against Mexican bean beetle. F. R. BASFORD.

Insecticides. Union Carbide Corp., Assee of J. R. Kilsheimer and H. H. Moorefield (B.P. 940,699, 27.11.61. U.S., 30.12.60).—Compounds claimed comprise 5,6-(**I**) and 5,8-*dihydronaphth-1-yl methylcarbamate*. The method of prep. of **I** is detailed. Its insecticidal activity against bean aphids and houseflies is described. F. R. BASFORD.

Dithiophosphonic acid esters. Farbenfabriken Bayer A.-G. (Inventor: G. Schrader) (B.P. 939,946, 10.2.61. Ger., 13.2.60).—Compounds claimed (35 new products specifically claimed) have the general formula R·PS(OR′)·SR″, wherein R is alkenyl of ⩽3 C, alkyl, chloroalkyl, cycloalkyl or phenylalkenyl; R′ is alkyl of 1—4 C; and R″ is aryl optionally substituted, or alkyl or cycloalkyl of up to 6 C. They have insecticidal properties (especially effective against pests resistant to org. dithiophosphates) and are obtained in good yield by interaction of R·P(OR′)SX (X is halogen) with R″SM (M is alkali metal) or R″SH in presence of acid-binding agent. Directions are given for the prep. of O-*Me* S-*Ph methylphosphonothiolothionate*, b.p. 92°/0·01 mm. F. R. BASFORD.

Unsaturated phosphorus-containing esters and pesticidal preparations containing them. CIBA Ltd. (B.P. 940,033, 6.10.60. Switz. 12.10.59).—The compounds claimed have the general formula $RX_{n-1}(Y_{m-1}R^{I})\cdot PZ\cdot O\cdot CR^{IV}$: $CR^{III}\cdot CO_2R^{II}$ wherein R and R^{I} are hydrocarbyl or heterocyclyl, or together may form part of a ring system; R^{II} is residue of a heterocyclic alcohol containing at least 1 *O* as hetero atom; R^{III} is H, alkyl or halogen; R^{IV} is aliphatic, residue; X and Y are O, S, NH or NR; Z is O or S; *m* and *n* are 1 or 2. One example is *Me*$_2$ 3-[(2-*oxo*-4,5-*dihydrodioxol*-5-*yl*)-*methoxycarbonyl*]*prop*-2-*en-yl phosphate*. Its activity against bean aphids is tabulated. F. R. BASFORD.

***p*-Acid amido- or imido-phenylmethyl carbamates and parasiticides containing them.** Dow Chemical Co. (B.P. 941,049, 13.6.61. U.S., 17.6.60).—Compounds with parasiticidal properties have the general formula *p*-NYZ·C_6H_{3-m}(O·CO·NHMe)RR'_m (*m* is 1 or 2; R is H or alkyl or 1—4 C; R′ is H, Me or Et; and NYZ is acid amido group of 1—8 C or acid imido group of 4—8 C). One example (prep. described) is 4-*formamido*-3,5-*xylyl methylcarbamate*, m.p. 202—205°. R. BASFORD.

Basically substituted anthradipyrazoles. Farbenfabriken Bayer A.-G. (Inventors: S. Schutz, E. Schraufstätter and M. Bock) (B.P. 940,532, 24.2.62. Ger., 20.2.61).—Preparative details are given for the title compounds claimed as being useful in the treatment of amoebic dysentry in warm-blooded animals. One example is 2,7-*bis*-(2-*dimethylaminoethyl*)-2,7-*dihydroanthra*[1,9 : 5 : 10]*dipyrazole*, m.p. 211—213°. H. S. R.

Phosphorus-containing derivatives of compounds having a nitrogen heterocyclic nucleus of aromatic character. N.V. Philips Gloeilampenfabrieken (B.P. 940,921, 21.10.59. Neth., 23.10.58).—Compounds claimed have the general formula R·PX($NR^{I}R^{II}$)·$NR^{III}R^{IV}$, wherein R is residue of a substituted or non-substituted, condensed or non-condensed cyclic-N-containing heterocyclic compound of aromatic character [which nucleus contains an NH group in which the H is replaced by PX($NR^{I}R^{II}$)·$NR^{III}R^{IV}$]; X is O or S; R^{I}—R^{IV} are alkyl radicals, or R^{I} and/or R^{II} are H. They may be obtained by the usual methods and are useful in combating noxious organisms (49 claims). Details are given for the prep. of NNN^{I}N^{I}-*tetramethyl*-4,5-*diphenylimidazolylphosphonamide* (25%), m.p. 157°. F. R. BASFORD.

Thiophosphoric and thiophosphinic acid esters. Farbenfabriken Bayer A.-G. (Inventors: A. Dörken and G. Schrader) (B.P. 941,631, 27.2.61. Ger., 29.2.60).—Insecticidal compounds of the general formula R′R″PS·O·C_6H-RR‴·NO_2 — 2,4,5 are claimed, wherein R′ and R″ are alkoxy or hydrocarbon radicals; R and R‴ are halogen and/or alkyl of 1—4 C. The prep. is detailed of *Me*$_2$ 3,5-*dimethyl*-4-*nitrophenyl phosphorothionate*, m.p. 24—25°. F. R. BASFORD.

New phosphate ester and insecticidal compositions containing it. VEB Farbenfabrik Wolfen (Inventors: Z. El-Hewehi and M. Born) (B.P. 941,271, 23.11.61).—The insecticidal compound described is *Me*$_2$ 1,2,2-*trichloro*-2-*nitro-ethyl phosphate*, a greenish-yellow oil, b.p. 110—120°/2 mm. The method of prep. is detailed. F. R. BASFORD.

[A] Pentafluorosulphur-substituted aldehydes and [B] carboxylic acids. Imperial Chemical Industries Ltd. (Inventor: N. H. Ray) (B.P. 941,392—3, [A] 16., [B] 19.5.62).—[A] *Pentafluorosulphuracetaldehyde* (**I**), b.p. 78—80°, is made by dehydrochlorinating $SF_5\cdot CH_2\cdot CHCl_2$ under alkaline conditions (e.g., with baryta); reacting the resulting $SF_5\cdot CH$: CCl with NaOMe under anhyd. conditions to give 2-*methoxyvinyl sulphurpentafluoride* (**II**), b.p. 110—115°, and hydrolysing this with strong mineral acid (H_3PO_4). [B] **I** is oxidised to the corresponding acid by treatment with $KMnO_4$ or H_2O_2 under acid conditions, to afford *pentafluorosulphur-acetic acid*, m.p. 64°. It is a soil fumigant. F. R. BASFORD.

Synthesis of the metabolite isomer of heptachlor expoxide. Shell Internationale Research Mij N.V. (B.P. 941,130, 6.11.61. U.S., 7.11.60).—The metabolite isomer (probably stereoisomer) of heptachlor epoxide is formed by treating heptachlor with at least the stoicheiometric amount of CrO_3 in AcOH–H_2SO_4 at ⩾100° (80°). The resulting heptachlor epoxide (4.3 g.), m.p. 164·5°, is an insecticide. F. R. BASFORD.

1-(Acylaminoaryl)-3,3-di-substituted triazenes. Amer. Cyanamid Co. (B.P. 941,489, 20.9.60. U.S., 24.9.59).—The title compounds, which deter or prevent attack of plants, wool, cellulose, etc. by insects, mammals, birds and allied pests, are obtained by coupling $NHR^{III}R^{IV}$ with $N_2R\cdot C_6H_2R^{V}R^{VI}\cdot NR^{II}\cdot COR^{I}$ in aq. solution (R is acid group; R^{I} is H, Ph or alkyl of 1—6 C; R^{II} is H or alkyl of 1—6 C; R^{III} and R^{IV} are H, alkyl, Ph, aralkyl, cycloalkyl or alkenyl, at least one of R^{III} and R^{IV} being hydrocarbon radical; or $NR^{III}R^{IV}$ is heterocyclyl; R^{V} and R^{VI} are H, Cl or alkyl of 1—6 C). Thus, a diazotised solution of *p*-$NH_2\cdot C_6H_4\cdot$NHAc is treated at 0° with a solution of NaOAc and 25% $NHMe_2$ in water. When the reaction is complete Na_2CO_3 is charged, and 30 min. later the cryst. ppt. is filtered off to afford 1-(p-*acetamidophenyl*)-3,3-*dimethyltriazene*, m.p. 158°. The biological activity of this and a further 15 compounds against Southern Army Worm and Mexican bean beetle is tabulated. F. R. BASFORD.

Fluoroacetic acid derivatives and pesticidal preparations containing them. Farbwerke Hoechst A.-G. (B.P. 941,587, 4.5.61. Ger., 4.5.60).—Compounds R·CCl_2·CH(OH)·NH·CO·CH_2F and R·CCl_2·CH(OH)·N : CCl·CH_2F, useful as insecticides and acaricides, are claimed (R is Cl or α-chloroalkyl of 1—4 C optionally containing more Cl) and are obtained by reacting fluoroacetamide with R·CCl_2·CHO, then where desired, further treating the product with an acid chloride. One compound prepared from chloral is N-(2,2,2-*trichloro*-1-*hydroxyethyl*)*fluoroacetamide*, m.p. 101—102° (82% yield).

A solution of this containing 0·012% of active agent, gives 100% destruction of aphids on plants (e.g., cineraria infested with *Myzodes persicae* or sugar beet infested with *Dorsalis fabae*).
F. R. BASFORD.

Fungicides. Imperial Chemical Industries Ltd. (Inventors: H. M. Fox and M. J. A. Geoghegan) (B.P. 941,616, 10.10.60).—There is claimed a fungicidal composition (especially for use as soil fungicide in the protection of cotton plants against *Rhizoctania solani*) containing pentachloronitrobenzene and a synergist therefore, viz., an azo compound *p*-NH_2·R·N:N·R′·X-*p*′ wherein R is benzene residue optionally substituted by one or more alkyl or alkoxy of 1—4 C, or halogen; R′ is benzene residue further substituted by alkyl or alkoxy of 1—4 C or by OH; X is OH. A typical synergist is 2,3′,5′-trimethyl-4-amino-4′-hydroxyazobenzene.
F. R. BASFORD.

Fungicides. Österreichische Stutestoffwerke A.-G. (B.P. 941,046, 2.3.61. Aus., 21.3.60).—Compounds useful as active ingredients of fungicidal compositions (for killing fungi on plants, etc.) comprise 4-R-2-R′-2,3,5,10-tetrahydro-3,5,10-trioxonaphtho[2,3-*b*]-1,4-thiazines (R and R′ are H or alkyl, or R is cycloalkyl). Details are given of the method of prep. of 4-*methyl*-2,3,5,10-*tetrahydro*-3,5,10-*trioxonaphtho*[2,3-*b*]-1,4-*thiazine*, m.p. 180—181°.
F. R. BASFORD.

Alkylenebis-dithiocarbamoyl derivatives and fungicidal compositions containing them. Fabriek von Chemische Producten Vordelingenplaat N.V. (B.P. 939,246, 6.4.60. Neth., 13.4.59).—Fungicidal agents especially active against *Podosphaera leucotricha* (apple mildew) and *Venturia inaequalis* (apple scab) comprise alkylene bis-dithiocarbamoyl deriv. of the general formula $[CH_2]_n(NH·CS_2·CX·NRR')_2$, wherein *n* is 2—3; R and R′ are alkyl of 1—10 C (and may all be different); and X is O or S. Details are given of the method of prep. of *bis(dimethylthiocarbamoyl)ethylene-bis-dithiocarbamate*.
F. R. BASFORD.

Isourea ethers. Farbenfabriken Bayer A.-G. (Inventors: E. Kühle, L. Eue and O. Bayer) (B.P. 940,663, 9.2.62. Ger., 10.2.61).—Compounds claimed have the general formula NR:C(OR′)·NR″R‴, wherein R and R′ are aromatic radicals optionally substituted; R″ and R‴ are H, alkyl or alkenyl of 1—4 C, at least one of them being alkyl or alkenyl. They have herbicidal properties, and are obtained by interaction of NHR″R‴ with an aryl arylimino-halogenoformate. One example is NN-*dimethyl*-N′-(p-*chlorophenyl*)-O-(2,4-*dichlorophenyl*) *isourea*.
F. R. BASFORD.

Triphenylmethane derivatives. F. Hoffmann, La Roche & Co. A.-G. (B.P. 937,157, 8.3.62. Switz., 10.3.61).—Compounds with formula $(C_6H_3R_2)_2CX·C_6H_4R·O·[CH_2]_2·R'$ [X is H, R is alkyl (1—4 C), alkoxy, etc.; R′ is dialkylamino or *N*-heterocyclic radical containing optimally other hetero-atoms] are claimed for one *inter alia* as molluscicides and vermicides. A representative compound is (m-*chlorophenyl*)-(p-*tolyl*)]p-(2-*diethylaminoethoxy*)*phenyl*]*methane*, b.p., 193—194°/0·02 mm. (prep. described).
H. S. R.

Carbanil-hydroxamic acid esters and herbicidal preparations containing them. CIBA Ltd. (B.P. 940,321, Swtiz., 19.1.61).—There are claimed compounds of the general formula 4,2,5,1-$CF_3·C_6H_2YY'·NH·CX·NR(OR')$, also compositions containing them for use as selective herbicides (R and R′ are alkyl of 1—3 C; X is O or S; and Y and Y′ are H or halogen). The method of prep. is detailed of 1-*methoxy*-1-*methyl*-3-m-*trifluoromethylphenylurea*, m.p. 86—91°. This (10) is ground with sulphite cellulose waste liquor (2) in presence of water (100 g.), to form a stable dispersion which is highly effective in the destruction ot *Setaria italica, Dactylis glomerata, Sinapis alba, Medicago sativa, Lepidium sativum* and *Calendula chrysantha*.
F. R. BASFORD.

Animal Husbandry

Utilisation of grass by ruminants. (A symposium.) (*J. Brit. Grassland Soc.*, 1964, **19**, 81—138).—**Efficient use of grass.** W. F. Raymond (pp. 81—89). **Utilisation of the metabolisable energy of grass.** K. L. Blaxter (pp. 90—99). **Ruminal volatile fatty acid production in relation to animal production from grass.** J. A. F. Rook (pp. 100—109). **Factors affecting the voluntary intake of grass.** R. C. Campling (pp. 110—118). **Efficiency of utilisation of fresh grass.** W. Holmes and J. G. W. Jones (pp. 119—129). **Factors affecting the efficient utilisation of conserved grass.** J. C. Murdoch (pp. 130—138).
A. H. CORNFIELD.

Nutritive value and agronomic aspects of fodders in Northern Nigeria. III. Hays and dried crop residues. T. B. Miller, A. B. Rains and R. J. Thorpe (*J. Brit. Grassland Soc.*, 1964, **19**, 77—80).—Digestibility data are presented for *Andropogon gayanus* hay and bush foggage, *Arachis hypogoea* (groundnut) haulms, *Glycine max* (soya-bean) hay, *Sorghum vulgare* (sorghum) leaves and hay, *Stizolobium* sp. (velvet bean) hay, *Vigna sinensis* (cowpea) hay and haulms, and *S. vulgare–Stizologbium* mixture.
A. H. CORNFIELD.

Grassland recording. I. Investigation into grassland recording on commercial dairy farms, sponsored by the British Grassland Society. H. K. Baker, R. D. Baker, R. M. Deakins, J. L. Gould, J. Hodges and R. A. Powell. **II. Assessment of two grassland recording systems by herbage sampling and *in vitro* digestibility determinations.** R. D. Baker. **III. A reappraisal of the use of livestock and starch equivalent standards in assessing the utilised production from grassland.** R. D. Baker. **IV. Comparison of the traditional method of calculating the utilised-starch-equivalent output of fields with a recommended quicker method.** R. D. Baker. **V. Recommendations for recording the utilised output of grassland on dairy farms.** H. K. Baker, R. D. Baker, R. M. Deakins, J. L. Gould, J. Hodges and R. A. Powell (*J. Brit. Grassland Soc.*, 1964, **19**, 139—143, 144—148, 149—155, 156—159, 160—168).
A. H. CORNFIELD.

Composition, intake, digestibility and prediction of digestibility of Coastal Bermudagrass hays. G. E. Hawkins, G. E. Paar and J. A. Little (*J. Dairy Sci.*, 1964, **47**, 865—870).—Analysis revealed the following ranges in composition of 15 Coastal Bermudagrass hays: crude protein, 6·1—14·7%; crude fibre, 28·5—36·9%; lignin 9·3—11·4%; ash 3·7—6·2%; Ca 0·29—0·99%; P 0·15—0·41%; and Mg 0·08—0·22%. Digestible protein content ranged from 2·5 to 9·4% and could be estimated with a high degree of accuracy from the crude protein by the prediction equation: % estimated digestible protein = 0·821 × % crude protein − 2·57. Total digestible nutrients ranged from 52·3 to 62·2%. TDN contents of the hays were correlated negatively with those of crude fibre, cellulose and lignin and positively with those of total and invert sugars and both cold- and hot-water extractables of the hays. Intake of the hays by cows was correlated positively with crude protein and ash and negatively with lignin contents of the forages. (25 references.)
M. O'LEARY.

Interrelationships and conversion factors between expressions of the digestible energy value of forages. D. P. Heaney and W. J. Pigden (*J. Anim. Sci.*, 1963, **22**, 956—961).—Regression equations shown express relationships between digestible energy (*DE*), total digestible nutrients (*TDN*), digestible org. matter and digestible dry matter in forages. Digestible protein has a marked positive effect on the ratio, kcal. of digestible energy/g. of total digestible nutrients. Hence commonly used conversion factors do not give good estimates of *DE* from *TDN* over wide ranges of forage quality.
A. G. POLLARD.

Nutrient evaluation of summer range forage with cattle. J. M. O'Connor, V. R. Bohman, A. L. Lesperance and F. E. Kinsinger (*J. Anim. Sci.*, 1963, **22**, 961—968).—The nature and digestibility of forage consumed by cattle on two types of range (desert shrub and sage-bush–grass) were examined by rumen-fistulated animals. Grass was selected most readily although the chemical composition of the diet was substantially constant over the experimental period. The apparent digestibility of the summer range was determined, using chromogens or lignins as indicators; the former gave the more reliable results. Urinary losses of energy were small. With some nutrients digestion by the fistulated animals was less efficient than that by intact animals. Data calculated from 'grab' samples of faeces were substantially the same as those from total collections when Cr_2O_3 was used as indicator.
A. G. POLLARD.

Control of hypomagnesaemic tetany by foliar application of calcined magnesite to pasture. J. R. Todd and N. E. Morrison (*J. Brit. Grassland Soc.*, 1964, **19**, 179—182).—Dusting pasture with calcined magnesite (28 lb./acre) just before grazing prevented hypomagnesaemia and tetany in a dairy herd. Where the treatment was not given cases of tetany (including one death) and hypomagnesaemia occurred.
A. H. CORNFIELD.

Effect of 'winter burn' on the chemical composition and *in vitro* dry matter digestibility of eight grasses. D. G. Miles, G. ap Griffiths and R. J. K. Walters (*J. Brit. Grassland Soc.*, 1964, **19**, 75—76).—The 'winter-burnt' fraction of 8 grasses cut in late Nov. was lower in water-sol. carbohydrates, particularly, and in *in vitro* dry-matter digestibility than was the green fraction.
A. H. CORNFIELD.

Plant juices in relation to silage fermentation. I. Rôle of the juice. W. L. Greenhill (*J. Brit. Grassland Soc.*, 1964, **19**, 30—37).—Using ryegrass and lucerne, the relationship between plant-cell breakdown, as indicated by collapse of the silage mass and increase in its electrical conductivity, and the initiation of lactic acid (**I**) production was studied. It was found that cell breakdown and the resultant release of the plant juices was a necessary prerequisite for the production of **I** during ensiling. The complete exclusion of air resulted in much earlier cell breakdown and formation of **I**.
A. H. CORNFIELD.

Grass–legume forage fed fresh and as silage for market hogs. D. M. Bowden and M. F. Clarke (*J. Anim. Sci.*, 1963, **22**, 934—939).—

Fresh-cut grass–legume and the same converted into silage were compared in rations which included a concentrate (self-fed), the protein content of which was raised from 12 to 15% during growth from 110 to 200 lb. live-wt. Growth rates increased with the dietary protein level. The amount of forage consumed was greater with the 12%-protein ration than with those of higher protein content. Pigs receiving silage in dry lot grew faster than those fed on pasture. The latter had larger hearts and spleens but lighter livers and intestines (large and small). Consumption of the silage was low especially when self-fed concentrate was available. Reduction of intake of concentrate increased silage consumption by 200%.
A. G. POLLARD.

Silage additives. J. M. Oades, W. O. Brown and J. A. M. Kerr (*J. Brit. Grassland Soc.*, 1964, **19**, 38—41).—The use of molasses, (13 lb.), dried sugarcane juice (14 lb.) and Silotracin (Zn bacitracin 3·85 g.) added to 28 cu. ft. of grass before ensiling improved the quality of the silage compared with no addition. The silage produced with molasses was slightly higher in dry matter than the others. A. H. CORNFIELD.

Rumen metabolism. III. Effects of lipids *in vitro* and *in vivo* on microbial activity. J. A. Robertson and J. C. Hawke (*J. Sci. Fd Agric.*, 1964, **15**, 890—897, cf. J.S.F.A. Abstr., 1964, ii, 90) —Addition of lipids (linseed oil) increased the formation of NH_3 when rumen liquors were incubated anaerobically at 39° with ryegrass juice extract high in protein-N (20 ml ≡ 350 mg. of crude protein), but decreased the formation of NH_3 when ryegrass fibre low in protein-N (1 g. ≡ 200 mg. of crude protein) was used in place of the juice extract. Under this latter condition digestion by micro-organisms was decreased *in vitro* and *in vivo*. In presence of lipid, formation of AcOH decreased, that of propionic acid increased. Reductions in the digestion of cellulose by rumen micro-organisms can be brought about by lipid under a variety of dietary conditions. Increased formation of propionate in presence of lipid is related to digestion of fibrous rather than sol. constituent of pasture species. (16 references.) E. M. J.

Use of pelleted roughage in the feeding regime for feeding sheep. I. L. Lindahl and C. E. Terrill (*J. Anim. Sci.*, 1963, **22**, 953—955).—Long lucerne hay was compared with the pelleted material in feeding trials with lambs and pregnant ewes. Considerable improvements in feeding value resulted from pelleting, e.g., rates of gain in wt. of lambs were sometimes doubled. For pregnant ewes 2·5 lb. of pellets satisfactorily replaced 3 lb. of long hay. Part of the improvement may have been due to refusal or wastage of long hay; there was no wastage of pellets. A. G. POLLARD.

Utilisation and digestion of long, ground and pelleted lucerne and mixed hay. R. R. Johnson (*J. Anim. Sci.*, 1964, **23**, 94—99).—Lambs fed a 1 : 1 mixture of cracked maize and pelleted hay showed somewhat higher gains in wt. than when the hay was ground and/or pellets. As the sole ration for steers, ground or pelleted hay was more effective than long hay. When the ration was changed to a full feed of ground maize together with free choice of the three forms of hay, animals using long hay gained wt. as rapidly as did those given ground hay. The digestibility of the 1 : 1, hay–maize ration was substantially the same regardless of the form of the hay. When the hays were fed alone the digestibility of the dry matter, org. matter, cellulose, crude fibre and energy of chopped and long hay exceeded those for ground or pelleted hay. Given choice, consumption was greater for pelleted and ground hays. Retention in the digestive tract was greater for chopped or pelleted hay. *In vitro* cellulose digestibility was similar for pelleted and chopped hay and somewhat greater than that for ground hay. A. G. POLLARD.

Effect of varying milo–barley levels, ration preparation and intra-ruminal injections of vitamin A on feedlot performance of steers. A. T. Ralston, D. C. Church, W. H. Kennick and N. O. Taylor (*J. Anim. Sci.*, 1963, **22**, 943—945).—Steers were fed rations containing milo and barley prepared by (*a*) coarse grinding, (*b*) steam-rolling and (*c*) pelleting after fine grinding, together with beet pulp. The average daily gain was greatest with *a* and least with *c*. No significant differences were apparent in feed efficiency, dressing %, marbling, back-fat or grade. Intraruminal injection of vitamin A showed considerable differences in the daily gain in wt. between the three methods of preparation. A. G. POLLARD.

Effect of processing and of feeding hay on the digestibility of soya-bean hulls. H. F. Hintz, M. M. Mathias, H. F. Ley, jun. and J. K. Loosli (*J. Anim. Sci.*, 1964, **23**, 43—46).—In digestion trials with steers and sheep, soya-bran flakes showed higher digestibility than did soya-bean hulls (whether ground or not) when used as sole feed. Addition of hay increased the digestibilities of the bran flakes and the hulls and tended to eliminate differences between them. The rate of passage was faster when raw soya-bean hulls were fed alone than when fed with hay or when soya-bran flakes were fed with or without hay. A. G. POLLARD.

Digestion. III. Faecal analyses and digestibility. N. Hellström and M. Aamisepp (*J. Sci. Fd Agric.*, 1965, **16**, 27—33).—The faeces of sheep fed ryegrass and cocksfoot cut at different periods of growth were examined. The faeces were separated into five fractions, by ultrasonic irradiation and stepwise sedimentation. The correlation between the feed, the protein contents of the faeces fractions and the metabolic product is discussed. E. M. J.

Natural herbage of the sub-tropics.. Concentrations of volatile fatty acids and ammonia in the rumen of sheep fed fresh herbage. J. H. Topps and R. C. Elliott (*J. agric. Sci.*, 1964, **63**, 245—248).—The NH_3-N concn. in rumen liquor ranged from 4·06 to 13·65 mg., whilst volatile fatty acids ranged from 7·50 to 12·20 mg./100 ml. Mean molar proportions of acids were acetic 65·8%, propionic 19·5%, n-butyric 9·0% and higher acids 5·7%, the differences at different sampling times being non-significant. On the basis of volatile fatty acid concn. and proportion in young sub-tropical herbage, the net energy value is less than that of high-quality temperate herbage. M. LONG.

Ruminal ammonia formation in relation to the utilisation of groundnut and herring meal as protein sources for milk production. M. I. Chalmers and S. B. M. Marshall (*J. agric. Sci.*, 1964, **63**, 277—282).—Herring meal causes less NH_3 formation in the rumen, is used more efficiently by lactating goats and is superior to groundnut meal for milk production by dairy cows. M. LONG.

Influence of urea on the vitamin A nutrition of ruminants. G. S. Smith, S. B. Love, W. M. Durdle, E. E. Hatfield, U. S. Garrigus and A. L. Neumann (*J. Anim. Sci.*, 1964, **23**, 47—53).—Sheep fed a purified diet containing urea as the only appreciable source of N showed unexpectedly low liver-vitamin A levels. This condition persisted subsequently when a semi-purified diet containing 12% of soya-bean protein was fed. Addition of urea (5%) to a 12%-soya-bean protein diet lowered liver A concn. by enlargement of the liver without affecting its total A content. When a ration having approx. 12% of a natural protein was supplemented with urea (5%) neither liver size, liver-A storage nor carotene utilisation was affected. Steers depleted of liver-A and fed vitamin A palmitate (**I**) during a 56-day fattening period showed as good a performance without effect on vitamin A levels when soya-bean meal was replaced by urea. A single intramuscular injection of **I** in polysorbate resulted in higher liver-A storage than when the same total amount was fed in daily portions over 56 days. A. G. POLLARD.

Influence of sodium nitrate, vitamin A and protein levels on feedlot performance and vitamin A status of fattening cattle. A. Weichenthal, L. B. Embry, R. J. Emerick and F. W. Whetzal (*J. Anim. Sci.*, 1963, **22**, 979—984).—Steers receiving a ground maize–lucerne (4 : 1) ration did not respond to an increase in dietary protein (10·6—11·8%) or to supplements of vitamin A palmitate (12,000 i.u./head daily). Plasma-vitamin A of animals fed the unsupplemented ration for 153—160 days fell from the initial value of 30 to 16—18 μg./100 ml., but to a smaller extent in those receiving the A supplement. Addition of 1% of $NaNO_3$ to the ration lowered the performance of the animals without affecting the levels of vitamin A or carotene in plasma or liver. No clinical symptoms of NO_3^- toxicity were apparent. A. G. POLLARD.

Effect of sodium nitrate on the vitamin-A nutrition of sheep. R. D. Goodrich, R. J. Emerick and L. B. Embry (*J. Anim. Sci.*, 1964, **23**, 100—104).—A control ration for lambs was supplemented with (*a*) 2·5 or 3·0% of $NaNO_3$, (*b*) 3000 i.u. of vitamin A per head/day or (*c*) *a* + *b*. Methaemoglobin were very low when (*a*) was fed. Vitamin A did not protect the animals from NO_3^- poisoning. Rations (*a*) and (*c*) did not affect plasma-vitamin A but lowered liver-vitamin-A storage. A. G. POLLARD.

***In vitro* degradation of vitamin A and carotene by rumen liquor.** E. K. Keating, W. H. Hale and F. Hubert, jun. (*J. Anim. Sci.*, 1964, **23**, 111—117).—Rumen liquor was obtained from steers undergoing feeding trials involving the use of ethoxyquin (**I**) and NO_3^-. Liquor from animals receiving a 70% roughage ration with added **I** partly counteracted the destructive action of NO_3^- on vitamin A. *In vitro* additions of high proportions of NO_3^- adversely affected vitamin A retention from high-concentrate rations only. Addition of NO_3^- lowered A retention from high- and low-roughage rations. β-Carotene levels were not greatly affected by additions (other than NO_3^-) to rumen liquor after feeding a low-roughage ration. NO_2^- reduced carotene retention. Destruction of A by rumen liquor from animals on a high-roughage ration exceeded that caused by liquor from those receiving a high-grain ration. A. G. POLLARD.

Effect of heat treatment in the processing of groundnut meal on the value of the protein for ruminants, with some additional experiments on copra. M. I. Chalmers, J. B. Jayasinghe and S. B. M. Marshall (*J. agric. Sci.*, 1964, **63**, 283—288).—Toasted groundnut

meal is more effective than undenatured or air-dried meals in promoting N retention, the digestibility of protein being unaffected. Air-dried meals produce the highest NH_3 concn. in the rumen, meals of lower solubility causing less NH_3 formation. The N retention from copra meal is similar to that from groundnut meal, although NH_3 formed in the rumen is low. M. LONG.

Energy nutrition and milk secretion in the dairy cow. J. A. F. Rook and J. E. Storry (*Chem. & Ind.*, 1964, 1778—1787).—The many facets of this problem are examined and are discussed in some detail. (79 references.) C. V.

Oral administration of molybdenum and cobalt to Brahman-Angus heifers. H. L. Chapman, jun., and R. W. Kidder (*J. Anim. Sci.*, 1963, **22**, 985—988).—Yearling heifers at pasture received, twice weekly, treatments of Co (8 mg./head/day) or Mo (250 mg./head/day) or both. No supplementary Cu was given; NaCl and $CaHPO_4$ were available *ad lib.* Pasture contained, P, 0·24%; Cu, 13·5 and Mo, 0·50 p.p.m. (dry basis). Co increased blood-haemoglobin (**I**), packed-cell vol. (**II**) and liver-Cu, but lowered liver-Fe. Mo lowered **I**, **II** and liver-Cu but increased liver-Fe. Plasma-inorg. P and body wt. were unaffected. Co did not correct, entirely the diminution in liver-Cu caused by Mo. A. G. POLLARD.

Absorption, retention and tissue deposition of labelled inorganic phosphates by cattle. L. R. Arrington, J. C. Outler, C. B. Ammerman and G. K. Davis (*J. Anim. Sci.*, 1963, **22**, 940—945).—A single dose of ^{32}P-labelled $CaHPO_4$, defluorinated rock P or 'soft' phosphate (**I**) was fed to calves and steers in metabolism cages for 6 days prior to slaughter. Absorption and retention of P were greatest from $CaHPO_4$ and least from **I**. Intravenously administered ^{32}P was excreted mainly in the faeces. A. G. POLLARD.

Effects of temperature and feed intake on thyroxine-^{131}I disappearance rates in cattle. R. G. Lundgren and H. D. Johnson (*J. Anim. Sci.*, 1964, **23**, 28—31).—The rate of loss of blood-thyroxine-^{131}I by lactating cows was depressed by environmental temp. of 88°F, whether the animals were fed *ad lib* or under controlled conditions. The depression is a direct effect of high environmental and body temp. rather than of diminished feed intakes. A. G. POLLARD.

Selenum and vitamin E and K additions to a no-hay finishing cattle ration. W. Burroughs, R. Kohlmeier, R. Barringer, R. Kawashima and A. Trenkle (*J. Anim. Sci.*, 1963, **22**, 929—933).—A finishing ration for 1—2-year steers, contained no hay and inadequate vitamin E and K. It was supplemented with vitamin E (100 or 200 i.u.) and vitamin K (47 mg./head/day). The supplements increased rates of growth (average 9%), the effect being more marked in the later part of the experimental period, and increased feed utilisation Supplementary Se (0·05 or 0·10 p.p.m. as Na_2SeO_3) produced a rate of gain ≮ that given by the vitamin supplements and had a vitamin E-sparing action, as also did vitamin A. A. G. POLLARD.

Effect of cortisone acetate on steers. F. D. Carroll, S. B. Powers and M. T. Clegg (*J. Anim. Sci.*, 1963, **22**, 1009—1011).—Yearling steers received subcutaneous injections of cortisone acetate (1 g.) three times weekly during the last 9 weeks of a 12-week feeding trial. The treatment increased the energy value of the carcasses. Control animals produced higher live and carcass wt. per unit of feed consumed and the carcasses contained more protein and less fat than did those of treated animals although the total energy gains were the same in both cases. Treated animals produced hides of lighter wt. A. G. POLLARD.

Grazing control for intensive fat-lamb production. II. Effect of stocking rates and grazing systems with a fixed severity of grazing on the output of fat lamb per acre. P. J. Broadbent (*J. Brit. Grassland Soc.*, 1964, **19**, 15—19).—The effects of three stocking rates (6, 8 or 10 ewes, each with twin lambs, per acre) and four systems of grazing management (ewes set stocked or grazed in 3-, 6- and 12-paddock rotation) were studied. Managment system had no significant effect on wt. gain of lambs or total live-wt. per acre or on live-wt. of ewes. Live-wt. gains of lambs was negatively, and total live-wt. per acre was positively, related to stocking rate. Live-wt. of ewes also decreased with increasing stocking rate. A. H. CORNFIELD.

Nitrogen metabolism of the young pig. II. Effect of heat treatment on the 'available' lysine content of fish meal and the performance of pigs. A. S. Jones and A. Cadenhead (*J. Sci. Fd Agric.*, 1965, **16**, 38—51).—The diets fed to young male pigs contained barley and fish meal, viz., Peruvian or white fish meal of similar 'available' lysine content but different total lysine content, heated white fish meal with the same total but reduced 'available' lysine content, or heated fish meal with supplementary lysine, with added minerals and vitamins. N retention, growth rate and feed conversion were reduced when heated meal was given, but no significant differences were observed when Peruvian or white fish meal was fed. Depression in live-wt. gain and feed conversion was not entirely due to the lowering of the 'available' lysine content of the fish meal; there were changes in the digestibility of dry matter. (17 references.) E. M. J.

Response of pigs to graded levels of soya-bean meal and added lysine in 10%-protein rations. A. J. Clawson, E. R. Barrick and W. W. G. Smart, jun. (*J. Anim. Sci.*, 1963, **22**, 1027—1032).—Pig rations based on maize and soya-bean meal were formulated to contain 10% of total protein, of which that provided by de-hulled soya-bean meal was 12·5, 25·0 or 50·0%. A supplement of lysine was added. With increase in the proportion of soya-bean protein feed consumption and live wt. increased and leanness of carcasses was improved. A. G. POLLARD.

Effects of addition of lysine and virginiamycin to maize-soya-bean meal rations on performance of weanling pigs. J. R. Jones and W. G. Pond (*J. Anim. Sci.*, 1963, **22**, 1033—1037).—The basal ration was supplemented with L-lysine (0·16%) and/or virginiamycin (**I**) (20 mg./lb. of ration). In two of four trials, lysine significantly increased the daily rate of gain in live-wt. and lowered the feed consumption per unit gain. **I** produced similar effects. No interaction between lysine and **I** was apparent. Trials with rats showed similar trends. A. G. POLLARD.

Protein and energy nutrition of the bacon pig. II. Effect of varying the protein and energy levels in the diets of 'finishing pigs'. D. W. Robinson and D. Lewis (*J. agric. Sci.*, 1964, **63**, 185—190).—Differences in live-wt. gain and food conversion efficiency between pigs fed on eight diets at four energy levels and two levels of crude protein were not significant, although diets containing 16% of crude protein and yielding 2950 kcal. per kg. gave the best performance. High energy levels adversely affected carcass quality while high-protein improved it. No sex differences existed with regard to live-wt. gain and efficiency, but gilts produced a superior carcass compared with hogs. M. LONG.

Effect of level of dietary zinc and source and level of maize on performance and incidence of parakeratosis in weanling pigs. W. G. Pond, J. R. Jones and G. H. Kraening (*J. Anim. Sci.*, 1964, **23**, 16—20).—Pigs were fed high Ca rations based on maize and soya-bean, in which the source of the maize and the Zn content of the ration were varied. The proportion of Zn needed to prevent parakeratosis in pigs fed a ration containing 1·3% of Ca was 34 p.p.m. A. G. POLLARD.

Sheep bot fly control tests. R. E. Pfadt (*J. econ. Ent.*, 1964, **57**, 928—931).—As a nasal spray, 1·2—4·2 mg./kg. dichlorvos reduced bot flies by up to 99%. Higher doses were toxic to sheep. Bayer 9017 [*OO* diethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate] and trichlorphon–coumaphos prep. were also good nasal sprays. Nasal ointments were less effective than sprays. Bayer 9017 and 9018 [*OO*-dimethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate] gave >99% control as oral drenches or feed additives. A pour-on treatment of trichlorphon reduced bots by 56% while Famophos was ineffective. C. M. HARDWICK.

Evaluation of some chemicals as feed additives to control face fly larvae. R. E. Treece (*J. econ. Ent.*, 1964, **57**, 962—963).—Thirteen compounds were tested using coumaphos as a standard. Shell SD-8447 [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate], Shell SD-8448 [2-chloro-1-(2,4,5- trichlorophenyl)vinyl diethyl phosphate] and G.C. 4072 [2-chloro-1-(2,4-dichlorophenyl)-vinyl diethyl phosphate] gave complete control at lowest dosages tested. Most of the compounds were ineffective. C. M. HARDWICK.

Toxicological studies on dichlorvos feed-additive formulations to control houseflies and face flies in cattle faeces. C. W. Pitts and T. L. Hopkins (*J. econ. Ent.*, 1964, **57**, 881—884).—Dichlorvos was administered in two polyvinyl resin formulations at 0·5—4·0 mg./kg. One was a powder designed for rapid release and the other a resin extrusion pellet for slow release. Bioassay of manure showed excellent housefly and face fly control. Blood-cholinesterase levels in treated animals dropped rapidly in the first 2 weeks and then levelled off. There was no adverse effect on feed palatability and consumption or wt. gains. C. M. HARDWICK.

Absorption, excretion and metabolism of ^{32}P-labelled Metepa by screw-worm and stable flies. W. F. Chamberlain and E. W. Hamilton (*J. econ. Ent.*, 1964, **57**, 800—803).—Topically applied Metepa (**I**) was absorbed fastest in the first 6 h. By 24 h. 50% of the applied dose had been absorbed by the screw-worm fly and 75% by the stable fly. The metabolism of the stable fly was about twice as rapid as that of the screw-worm fly. The much larger dose of **I** required to sterilise the latter than the former insect is explained by differences in rates of absorption, metabolism and detoxication. The principal metabolite was H_3PO_4. C. M. HARDWICK.

Effectiveness and residual activity of new compounds in soil against the eye gnat, *Hippelates collusor*. M. S. Mulla (*J. econ. Ent.*, 1964, **57**,

873—878).—Dosage response lines are given for 39 compounds sprayed on to and incorporated into soil. Bayer 29492 (*OO*-diethyl *O*-[4-methylthio-*m*-tolyl] phosphorothioate) and Bayer 37289 (*O*-ethyl *O*-2,4,5-trichlorophenyl ethylphosphonothioate) gave 90% reduction in emergence of adult gnats at rates of <0·1 lb./6 in.-acre. Residual activity was determined over 4 months. Seven compounds were promising. C. M. HARDWICK.

Cattle tick control in Northern Nigeria; a field study of BHC, Sevin, toxaphene and ronnel. R. J. Thorpe and P. Walker (*Bull. ent. Res.*, 1964, **54**, 633—641).—In trials against *Boophilus annulatus, B. decoloratus, Ambylomma variegatum, Hyalomma truncatum* and *Rhipicephalus simus*, toxaphene and BHC gave the most effective control when applied at concn. 0·37 and 0·05%, respectively, at 21-day intervals in the dry season and at 7—14-day intervals in the wet seasons. A. G. POLLARD.

Foaming properties of lucerne and their relation to bloat. R. Pressy, S. H. Synhorst, J. Bertram, R. S. Allen and N. L. Jacobson (*J. Anim. Sci.*, 1963, **22**, 970—978).—The foaming properties and foam stability of extracts of lucerne collected by hand or by rumen fistula are determined (apparatus described). The foaming property was associated mainly with leaf tissue; it is preserved in plant extracts by freezing or lyophilisation but diminishes on drying or boiling (25° ar 95°) the extract. Boiling in presence of chloroplasts accelerates the loss of foaming capacity. The foaming property was dependent on pH and temp.; foam stability was max. when the material was macerated with the extractant at pH 5·5 and 20°. At 35° maceration at pH 6·5 gave max. stability. Lucerne also contains a foam inhibitor. Significant correlation is shown between foam stability and bloat. A. G. POLLARD.

Relationship of potassium and sodium content to the composition of pigs. A. H. Kirton, R. H. Cynaedinger and A. M. Pearson (*J. Anim. Sci.*, 1963, **22**, 904—910).—Carcasses of 24 pigs were used to establish possible relationships between their K content and carcass composition with the object of testing the validity of predictions of 'meatiness' of carcasses from the proportion of naturally occurring ^{40}K in the living animals. All correlations between gross chemical composition (water, protein, ether extract, ash) and the K content of the carcass were significant. Use of K values for predicting carcass composition is of doubtful value unless sources of error in measurements can be reduced. A. G. POLLARD.

Effects of marginal vitamin-A intake during gestation in swine. D. P. Heaney, J. A. Hoefer, D. E. Ullrey and E. R. Miller (*J. Anim. Sci.*, 1963, **22**, 925—928).—A ration complete in all other essentials but devoid of carotene or vitamin A was supplemented with varied amounts of stabilised vitamin A palmitate (to provide 16, 5 or 2·5 μg. of vitamin A/kg. live-wt.) and fed to gilts from age 4 months and through two complete gestation periods. A depletion period preceded the experimental feeding in the case of the two smaller dosages of vitamin A. The low level of vitamin A intake was adequate for the dams but probably on the borderline for reproduction. The vitamin A intake during gestation was reflected in the vitamin A reserves in blood-plasma and liver, the latter being the more sensitive index. Dietary levels of vitamin A were reflected in those of the milk, particularly of the colostrum, these levels being of greater importance to the new-born pigs than were their liver stores at birth. The different levels of vitamin A fed to the dams had no effects on litter size, birth wt., survival rates and rates of growth after birth. A. G. POLLARD.

Ground carobs in chicken diets. P. Vohra and F. H. Kratzer (*Poultry Sci.*, 1964, **43**, 790—792).—Diets containing 20—49% of ground carob were deficient in energy for broilers. Growth with diets containing 5—10% soya-bean oil and 20% carob was as good as control diets containing ground maize. A. H. CORNFIELD.

Raw and heated unextracted soya-beans for layers. J. C. Rogler and C. W. Carrick (*Poultry Sci.*, 1964, **43**, 605—612).—Raw unextracted ground soya-beans reduced egg production and feed conversion and produced pancreatic hypertrophy in comparison with soya-bean meal when both were supplied on an equal N basis. Heated unextracted ground soya-beans gave as good egg production and better feed efficiency than did soya-bean meal, and increased the concn. of linoleic and linolenic acids in the thigh and egg yolk lipids at the expense of palmitic, palmitoleic and oleic acids. A. H. CORNFIELD.

Acidulated cottonseed-oil soapstock. II. Attempts to reduce its gossypol content. B. Lipstein and S. Bornstein (*Poultry Sci.*, 1964, **43**, 694—701).—Treatment of cottonseed oil soapstocks (containing approx. 1% gossypol) with hot concn. alkali before acidulation produced soapstocks containing less than 0·1% gossypol, thus allowing high levels of the material to be used in broiler rations. Acidulated cottonseed oil soapstock produced in this way did not differ chemically or nutritively (for broilers) than did materials of low gossypol content produced by regular manufacturing processes. A. H. CORNFIELD.

Strain response to dietary protein level. R. E. Moreng, H. L. Enos, W. A. Whittet and B. F. Miller (*Poultry Sci.*, 1964, **43**, 630—638).—There were significant differences among four strains of birds receiving diets containing 13—17% of protein with respect to egg production and Haugh unit values. Strain differences were often also found in egg wt. and shell thickness and these were due to a specific strain and dietary protein level. A. H. CORNFIELD.

Amines as synergists for ethoxyquin in dehydrated lucerne. J. W. van der Veen and H. S. Olcott (*Poultry Sci.*, 1964, **43**, 616—617).—Of a no. of antioxidants used for protecting β-carotene in dehydrated lucerne, ethoxyquin was by far the most effective, diphenylphenylene diamine gave limited protection, whilst the other materials were ineffective. Addition of a no. of alkylamines did not increase the effectiveness of any of the materials. A. H. CORNFIELD.

Effect of a lysine deficiency on body weight and age at sexual maturity of meat-type pullets. E. P. Singsen, J. Nagel, G. Patrick and L. D. Matterson (*Poultry Sci.*, 1964, **43**, 786—787).—Birds fed a lysine-deficient (59% of normal requirement) diet for the first 4—21 weeks of age reached 25% egg production an average of 18·4 days later than did birds receiving a normal diet. Chicks fed the normal ration for 4—21 weeks and then the deficient ration over part or all of the rest of the 21 weeks showed no delay in time to 25% or 50% egg production or body wt. at 21 weeks in comparison with birds on the normal ration all the time. Feeding the deficient ration from day-old for increasing lengths of time successively reduced body wt. at 21 weeks of age, had a slight effect on age at 25% egg production, and a definite effect on age at 50% egg production. A. H. CORNFIELD.

Level of molasses in growing chick rations. M. S. Qureshi, I. A. Khan and B. H. Schneider (*W. Pakistan J. agric. Res.*, 1963, **1**, No. 2, 42—51).—In feeding trials over the first 12 weeks after hatching molasses replaced, satisfactorily, part of the cereal ration. Use of >10% of molasses in the ration is probably undesirable. A. G. POLLARD.

Feeding trial conducted at six different locations. J. R. Aitken, J. Biely, D. C. Hill, J. B. O'Neill, A. R. Robblee and J. T. Sell (*Poultry Sci.*, 1964, **43**, 744—751).—There were significant differences due to diet (varying protein and energy source) and location in wt. gains of broiler to 4 and 6 weeks of age. The interaction between the two factors was also usually significant. The use of results obtained in growth trials conducted under a given set of conditions to predict results under other conditions is discussed. A. H. CORNFIELD.

Thyroxine secretion rate in chicks and poults. W. J. Mellen (*Poultry Sci.*, 1964, **43**, 776—777).—The thyroxine secretion rate of chicks over 5—7 weeks of age averaged 1·39 μg. per 100 g. body wt. per day whilst that of poults of similar wt. averaged 1·88 μg. A. H. CORNFIELD.

Proteases, amylase and lipase of the intestinal contents of germ-free and conventional chickens. S. Lepkovsky, M. Wagner, F. Furuta, K. Ozone and T. Koike (*Poultry Sci.*, 1964, **43**, 722—726).—There were no significant differences in proteases, amylase and lipase of the intestinal contents between germ-free and conventional chicks after 70 days except in one experiment where proteases of the caecal contents were lower in conventional than in germ-free chicks. The most pronounced effect with respect to N compounds was the almost complete absence of protein-N in the caeca of germ-free birds and relatively high amounts in the caeca of conventional birds. A. H. CORNFIELD.

Voluntary intake of calcium supplements by the laying hen. P. Griminger and H. Lutz (*Poultry Sci.*, 1964, **43**, 710—716).—When housed in individual cages there was appreciable variation among hens in consumption of Ca supplements. Consumption per hen was highest in individual cages, intermediate in community cages, and lowest in floor pens with litter. In floor pens hens preferred crushed oyster shell to calcite grit when both were available together, but consumed similar amounts of the two materials when they were available one at a time. The level of dietary Ca profoundly influenced the intake of supplementary Ca of hens in floor pens, but not of those in individual cages. In floor experiments 15—32% of the Ca supplement removed by the hens from the hoppers was wasted. A. H. CORNFIELD.

Efficiency in prediction of egg weight from early measurements. N. S. Cowen, D. B. Bohren and H. E. McKean (*Poultry Sci.*, 1964, **43**, 567—573).—When all four variables (age at maturity, wt. of first five eggs, Nov. egg wt., and max. egg wt.) were included in the regression analyses and if max. egg wt. was fitted first, the other three variables did not contribute significantly to the accuracy of predicting spring egg wt. Of the first-named three variables studied, Nov.

egg wt. had virtually the same predictive power as did the three variables jointly. A. H. CORNFIELD.

Influence of breed and/or strain on the fatty acid composition of egg lipids. H. M. Edwards, jun. (*Poultry Sci.*, 1964, **43**, 751—754).—There were significant differences in the contents of palmitoleic, stearic, linoleic and arachidonic acids of egg lipids between five strains of hens. There were significant differences in palmitic and oleic acids between hens within a strain, even though there were no significant differences between strains. A. H. CORNFIELD.

Effect of dicumarol on the incidence of blood spot of eggs. E. J. Day, B. C. Dilworth and P. N. Dua (*Poultry Sci.*, 1964, **43**, 796—798).—Addition of dicumarol (0·1 g./lb. of feed) to the hen's diet reduced the incidence of blood spot in eggs and increased the prothrombin time. Addition of menadione Na bisulphite complex (0·002 g./lb. of diet) or sulphaquinoxaline (0·057 g./lb.) had no significant effect on egg blood spot incidence or prothrombin time. A. H. CORNFIELD.

Relationship of vitamin K to the incidence of blood spots in eggs and blood prothrombin time of layers. E. J. Day and R. C. Woody (*Poultry Sci.*, 1964, **43**, 794—796).—In two of four tests, addition of menadione Na bisulphite complex (vitamin K) (4 g./ton of feed) to the diet of layers significantly increased the incidence of blood spots in the eggs. Addition of 2·5% of lucerne had no significant effect. Blood prothrombin time was decreased by both vitamin K and lucerne meal additions. A. H. CORNFIELD.

Effect of sodium ascorbate on egg-shell thickness during hot weather. B. W. Heywang, B. L. Reid and A. R. Kemmerer (*Poultry Sci.*, 1964, **43**, 625—629).—Addition of Na ascorbate (0·010—0·454 g./lb. of feed) to hen diets containing 2·25% or 4·25% of Ca for 35—70 days had no appreciable effect on egg wt., shell thickness or ratio of dried shell wt. to whole egg wt. A. H. CORNFIELD.

Additives for animal feeds. A.-G. Fuu (B.P. 939,956, 21.12.61. Ger., 21.12.60).—The additive (suitable for pig, cattle, poultry or other animal feed) consists of a mixture of rennet, papain and/or pepsin, and $CaCl_2$, Ca acetate and/or Ca lactate, the rennet/Ca salt ratio being 1 : 5. F. R. BASFORD.

Penicillins. Beecham Research Laboratories Ltd. (Inventors: B. O. H. Sjoberg and B. A. Ekistrom) (B.P. 940,488—9, 21.7.61).—Methods of prep. are detailed for [A] α-azidobenzyl- and [B] α-aminobenzyl-penicillin useful as antibacterial agents, nutritional supplements in animal feeds, treatment of mastitis and as therapeutic agents for cattle and poultry. H. S. R.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Niacin content of regional varieties, hybrids and commercial varieties of maize. Wilfredo Ruben Cortes Avila (*Rev. Fac. Agron., La Plata*, 1963, **39** (No. 1a), 79—83).—Niacin contents, determined by the microbiological method (cf. Methods of Vitamin Assay, Ass. of Vitamin Chemists, 1947), are reported for 18 varieties of maize. Niacin contents found varied from 41·25 to 14·14 μg. per g. E. C. APLING.

New methods in cereal microscopy. A. Th. Czaja (*Getreide u. Mehl*, 1964, **14**, 97—101).—Many new applications of polarisation microscopy and the use of staining reagents to the identification and distinction of cereals and their adulterants are briefly described. Polarisation microscopy in non-aq. mountants (clove oil, methyl benzoate or iso-amyl phthalate) with the use of the first-order red gypsum compensator simplifies the detection of rice flour in wheat flour, detection of maize flour in other flours, identification of gelatinised flours (from the appearance of hairs and other branny tissues), distinction between wheat and rye flours (from differences in the transverse cell layers), identification of soya meal and of sweet lupin meal (crystals in the aleurone cells), identification of 80—85% amylose bean starch (centres of starch grains isotropic), identification of darnel in spelt, and the identification of the fruit of *Cephalaria syriaca*. Staining with 0·1% fuchsin solution is useful for the identification of rye, maize and barley flours and for the detection of yeast and albumin in wheat bread. Staining with I 'water (0·018% I at 10°) can be used for the identification of gelatinised rice flour, distinction between root and rhizome starches and seed and fruit starches and for the identification of gelatinised starches. The detection of ergot in rye flour by treatment with H_2SO_4 and of the fungal layer of darnel in flour by careful treatment with chloral hydrate is described. Finally brief directions are given for the identification of wool, cotton, hemp and jute fibres, copra and palm kernel meal in cereal flours. (18 references.) E. C. APLING.

Falling number, a rapid method for the determination of sprout-damage. H. Perten (*Brot. u. Gebäck*, 1964, **18**, 181—186).—The Hagberg falling no. method for the assessment of flour diastatic activity (*Cereal Chem.*, 1961, **38**, 202) and examples of its application are described. Results obtained with various wheat and rye flours are compared with those of the maltose test, dextrin figure, Amylograph test and determination of α-amylase activity by the modified Wohlgemuth procedure of Hagberg (*Cereal Chem.*, 1961, **38**, 241). The relationship of falling no. to pH value is compared with similar relationships for maltose value, and α- and β-amylose activities. Applications of the method to the comparison of the amylolytic activities of cereal enzymes on various starches, and to the formulation of flour mixtures of given falling no. are described. (Cf. *Cereal Chem.*, 1964, **41**, 127; J.S.F.A. Abstr., 1961, ii, 273.) (35 references.) E. C. APLING.

Experiences with the Maturograph and oven forcing apparatus. IV. Fermentation response of various wheat flours. W. Seibel and A. Crommentuyn (*Brot u. Gebäck*, 1964, **18**, 153—161).—Results showing the effects of pre-treatment of the wheat (steeping or conditioning), and of additions of malt flour, $KBrO_3$ and ascorbic acid on the fermentation response of flours milled from Manitoba and dark Hard Winter wheats and of mixtures of flour from Manitoba wheat and Dutch flour are presented and discussed. E. C. APLING.

Fate of sulphuryl fluoride in wheat flour. R. W. Meikle (*J. agric. Fd Chem.*, 1964, **12**, 464—467).—Experiments on the fumigation of Graham flour with sulphuryl-^{35}S fluoride indicate that the fumigant reacts with the protein of the flour, in particular with free amino-groups. This results in the liberation of fluoride which may have a potential health hazard. (15 references.) W. ELSTOW.

Laboratory studies of flour for continuous mix bread production. G. W. Schiller and J. A. Gillis (*Cereal Sci. Today*, 1964, **9**, 256, 259—263).—The Pillsbury Laboratory Continuous Mixer is described. Tests for reliability and reproducibility of results, studies of absorption, oxidation, malt, dough development vs. absorption and starch damage were made. The following were observed: as absorption is increased, mixing requirements also increase; as oxidation is increased, mixing requirements are increased; as maltose levels are increased, mixing requirements decrease; as starch damage is increased, mixing requirements increase, but tolerance to mixing and the quality of the bread are significantly affected adversely. The tests demonstrated reproducibility of results and the usefulness of the device in certain uniform and specific applications. I. DICKINSON.

Rate of energy consumption in mixing air classified flour fractions into dough. R. Gracza (*Cereal Sci. Today*, 1964, **9**, 274, 276—278, 281—282).—The review covers: the importance of characterising contributory factors to the hydration rate, which involve water, physical and chemical and procedural changes. Farinograph data are given which focus attention on divergent flour properties in certain flour fractions which constitute a single parent flour. Analytical indices characterise these divergent flour properties which affect the rheological behaviour and the mixing energy requirements of flours to produce optimum bread. (26 references.) I. DICKINSON.

Influence of moisture content on the storage stability of maize flour in respect of flour colour. H. M. B. Ballschmieter (*Getreide u. Mehl*, 1964, **14**, 101—103).—Granular maize meal was stored for from 6 to 43 weeks at 30° and 40—50% R.H. at initial moisture contents from 5·8 to 12·7% and for 2 to 11 weeks at 65—70° at initial moisture contents from 5·0 to 14·9% and evaluated organoleptically and by paste colour. Storage stabilities at 30° were 33 weeks with moisture <7·5%, 19 weeks with 9—10% moisture and 12 weeks with >11% moisture. At the higher temp. storage stability varied similarly with moisture content in the range from 11 to <2 weeks. E. C. APLING.

New methods in the production of dextrins. H. D. Schmidt (*Stärke*, 1964, **16**, 264—268).—The processes involved in dextrin production include acidification, drying, rasting, cooling, re-moistening, sifting, mixing and transportation. Improvements suggested include pneumatic drying to <1% moisture; use of an electric roaster; cooling within a few min. with pneumatic coolers; re-moistening to a 10—12% water content in a specially designed rotating bin in which water is sprayed in and the dried dextrin introduced tangentially via a special nozzle; and improved transportation involving a fluid-air pump. These measures should assist in the design of advanced continuously operating plant. A. T. CARPENTER.

Importance of starches for the detection of flours mixed with rice flour. A. Th. Czaja (*Stärke*, 1964, **16**, 276—279).—A distinct differentiation of starch-filled endosperm cells, combined and simple starch granules can be made when cereal flours are examined under the polarisation microscope using cedar wood oil as immersion agent. Use of a gypsum compensator (red type I.O.) improves the differentiation. The addition of, e.g., wheat, oat or maize flour to rice flour can thus be readily detected. (26 references.)
A. T. CARPENTER.

Rheology of bread doughs under slow deformations. A. H. Bloksma (*Brot u. Gebäck*, 1964, **18**, 173—181).—Fundamental rheological concepts are briefly reviewed and the construction and use of a special pin-disk viscometer, the Rheometer (cf. *Rheol. Acta*, 1962, **2**, 217—230), which makes possible the measurement of deformation resulting from a small constant stress, is described. Examples of measurements of dough deformation against time under stress and subsequent recovery are figured and discussed. With stresses of the same order as those occurring in fermentation (~500 dynes per cm.^{2}) applied for up to 15 min. the resulting deformations were largely irreversible or viscous (reversible or elastic component only from 10 to 20%). The deformation/stress curves for dough are non-linear, with much greater deviation from linearity for the viscous than for the elastic component. Variation in water content of the dough also changes the proportions of elastic and viscous deformations produced by a given stress: increasing the water content of an untreated flour dough by 1 ml. per 100 g. (14% moisture basis) increased total deformation by 1·26 but elastic deformation by only 1·15. The significance of these results is discussed in relation to the effects of small errors in moisture determination on the results of dough testing with the Farinograph and Extensograph. (38 references.) E. C. APLING.

Buffering and souring of doughs. A. Angermann and G. Spicher (*Brot u. Gebäck*, 1964, **18**, 162—169).—The effects of water hardness and whey additions on the pH and acidity of doughs and bread from wheat, wheat–rye mixture and rye flours are reported and discussed. A strong buffering action was observed with hard water and with whey when compared with soft water, which appeared to be due entirely to their mineral content. Removal of coagulable protein from whey produced no significant alteration in its buffering effect. In the development of sour dough (natural mixed culture), the relationship between rate of acidity development and rate of drop in pH (souring quotient, S_Q) is strongly dependent on the buffering capacity of the liquid used in dough make-up. In the period from 16 to 24 h. after doughmaking, S_Q was 9 for soft water, 11 for hard water and 17 for whey. E. C. APLING.

Use of vitamin C in breadmaking. L. Milatovic (*Brot u. Gebäck*, 1964, **18**, 186—189).—A literature review and brief summaries of rheological, baking and staling test results for flours available in Jugoslavia. (14 references.) E. C. APLING.

Protein complex of dough. C. D. Stone and M. K. Hamdy (*Bakers' Dig.*, 1964, **38**, No. 4, 36, 38, 39, 40, 43, 44, 46—48, 50, 52).—The more recent literature on the protein constituents of dough is reviewed. (93 references.) I. DICKINSON.

Physicochemical properties of gluten from three types of wheat. R. R. Matsuo and A. G. McCalla (*Canad. J. Biochem.*, 1964, **42**, 1487—1498).—The intrinsic η values of gluten from hard (**I**), soft (**II**) and durum wheat (**III**) samples, dispersed in 8% Na salicylate and in aq. lactic acid–NaCl decreased in the order **I** > **II** > **III**; under N_2 values were higher than those observed in air. Values were reduced when the gluten was treated with performic acid or large excess of Na_2SO_3, the order remaining the same. Sedimentation coeff. were significantly reduced by the treatments, the greatest reduction being shown by gluten of **III** with performic acid. Sulphydryl group analysis gave the highest value for gluten from **III** and lowest for that from **I**. (22 references.) E. M. J.

Shortenings. Processing for specialised uses. J. P. Mock (*Bakers' Dig.*, 1964, **38**, No. 4, 53—57).—New methods for 'tailoring' fats for specific foods and for the retention of their flavour for an extended period of time, are examined. Refining, bleaching, hydrogenation, interesterification, deodorisation, additives and plasticising are discussed. I. DICKINSON.

Flour brew studies. VI. Varying the proportions of total flour, salt or 'starter' dextrose in the brew. E. G. Bayfield and W. E. Young (*Bakers' Dig.*, 1964, **38**, No. 4, 58—60, 62, 63, 64—65).—Varying the % of flour in brews made without any sugar showed gassing was stimulated by increasing the amount of flour used. Acidulating the flour brews with lactic acid gave further increase in fermentation, although 50% flour plus acid did not equal 8·0% dextrose without acid. Very high gassing was detrimental and produced loaves which were blown up and open, and light in wt. Studies dealing with the amount of brew salt were less conclusive. In most cases optimum results were obtained with 1·0% salt in the brew and the remainder added at the dough mixing stage. When using 10% or low % flour brews, it is desirable to add about 0·5% of dextrose to the brew as 'starter'. The use of 5·0% dextrose in the brew produced poor bread because so much of the sugar was fermented away in the brew and not enough remained to carry the dough through the baking stage. I. DICKINSON.

Production of amylase on vermiculite by *Aspergillus oryzae*. J. Meyrath (*J. Sci. Fd Agric.*, 1965, **16**, 14—18).—Microbial amylase and/or protease produced by solid cultures, especially with one substrate, wheat bran, known to give high yields has a disadvantage that the enzyme extracts are very difficult to purify because of the great no. of undefined compounds that have been solubilised by the mould. A method is described in which synthetic substrates (allowing easy purification of the amylase) in presence of an inorg. absorbing material (vermiculite) are used. The substrate is solid and has a strong stimulating action on the rate of amylase production. (13 references.) E. M. J.

Heat-stable bacterial α-amylase in baking. Application to white bread. O. Silvestain (*Bakers' Dig.*, 1964, **38**, No. 4, 66—70, 72).—It was found that appropriate application of heat-stable bacterial α-amylase (BAA) counters ageing in bread associated with staling. The level of BAA application depends on the type of flour, baking process and degree of anti-staling effect desired. The use of BAA in other baked products has led to improvement. (24 references.)
I. DICKINSON.

Research with a pilot-scale continuous breadmaking unit. I. Replicability and experimental design. S. Redfern, B. A. Brachfeld and R. L. Bell (*Cereal Sci. Today*, 1964, **9**, 242—244, 247).—Modifications of an Amflow Unit are described on which an experimental design for the study of variables was worked out. On each of two days two different formula variations were run in duplicate. Loaf vol., data were subjected to analysis of variance. There is a large and significant variation between days, a non-significant variation between the two treatments, and a significant interaction between days and treatments. Therefore it is necessary to run experiments on different days rather than duplicating them on one day. It is suggested that the factorial-design approach is more efficient and will permit the evaluation of three factors (yeast food level, oxidant level and mixing speed—all at two levels) and their interaction. The technique of factorial experimentation with the Amflow Unit is illustrated and a description of the use of the Yates method for the analysis of the data is given. I. DICKINSON.

AMF pilot plant in continuous bread experimentation. G. W. Trum (*Cereal Sci. Today*, 1964, **9**, 248, 250, 252, 254).—The plant and the procedure used is described in detail. Experiments covered the effects of all ingredients that go into the making of white pan bread. The following were evaluated: amounts and types of non-fat dry milk, types of lard and different % of added flake and emulsifiers, differences in various available sugars, yeast foods, acid salts and buffer salts, cultures and flavour additives and types and strengths of flour grades. Results to be reported later.
I. DICKINSON.

Progress report: effects of milk components on continuous mix bread. R. R. Baldwin, R. G. Johansen, W. Keogh, S. T. Titcomb and R. H. Cotton (*Cereal Sci. Today*, 1964, **9**, 284, 287—288, 290, 308).—The classic milk fractionation procedure is not adequate for separation and determination of specific entities in milk which may cause difficulties in baking, e.g., changes in the protein fractions during the manufacture of dry milk. The casein fraction behaves as an inert diluent material which may be used within limits depending on the flour. It results in increased η and a reduced mixing requirement for dough development. Whey proteins and particularly albumin fractions tend to weaken or slacken dough, they interfere with the development of an optimum gluten film network. Heat-treatment of the globulin and albumin fractions results in some improvement in baking characteristics. The use of dry milk products in continuous-mix bread demands heat treatment and proper attention to dough mixing speed as well as to pH-buffering and water-absorption properties of the dry milk.
I. DICKINSON.

Effects of heat treatments given to skim milk and skim milk concentrate before drying. A. M. Swanson, W. B. Sanderson and J. Grindrod (*Cereal Sci. Today*, 1964, **9**, 292—295, 298).—The 1·5 mg./g. limit of undenatured whey-protein N required for a high heat product was obtained with all forewarming temp. except at 165°F for 30 min. At this temp. 190°F for 10 min. was required to reach the limit. The η of 40% reconstituted non-fat dry milk (NFDM) and the protein reducing values may indicate the amount of heat treatment the milk has received. The higher forewarming treatments and the heating of the concentrates normally resulted in higher η and protein reducing values. Two flours used to calculate the water absorption of NFDM gave different curves which indicates

that wheat proteins exert some effect on the NFDM. The max. water absorption of the NFDM manufactured with low-temp., long-time forewarming treatments is reached at 185°F for 30 min. This corresponds to the max. loaf vol. In almost all instances the increase in heat on the concentrate and the increase in total solids increased the water absorption of the resulting NFDM. (14 references.) I. DICKINSON.

Carrageenan and hydroxylated lecithin applied to continuous mix bread. E. F. Glabe, P. W. Anderson and E. C. Jertson (*Cereal Sci. Today*, 1964, **9**, 300—302, 344).—Hydroxylated lecithin increases dough stability and loaf vol., both alone with higher levels of $KBrO_3$ and KIO_3 and at lower levels of these two oxidisers when carrageenan is present. Carrageenan (an extract of Gigartinacea) significantly affects loaf vol., shape and texture when milk is present. The combination of both materials has a complementary effect to the extent that the best bread characteristics are produced in the presence of milk and at substantially reduced levels of $KBrO_3$ and KIO_3. I. DICKINSON.

Sterols in egg-dough products and their determination. III. Examination of photometric determination of sterols. L. Acker and H. Greve (*Z. LebensmittUntersuch.*, 1964, **125**, 356—363).—In continuation of previous work (cf. *ibid.*, 179), photometric determinations based on the Liebermann–Burchard reaction are shown to be unreliable owing to the development at different rates, of different extinction values by cholesterol and its esters, the phytosterols, their esters and their glycosides. The sterols in egg-dough products are unaffected by prolonged storage in the dark, but decompose on exposure to light. (13 references.) P. S. ARUP.

Staling of wheat breads containing maize flour. H. M. B. Ballschmieter and H. Vlietstra (*Brot u. Gebäck*, 1964, **18**, 189—191).—Studies on the effects of additions of vitamin C or $KBrO_3$ (at optimal, ~50 mg. per kg. of flour) or egg powder (1%) on the staling rate of bread made from a mixture of wheat and maize flours (3 : 1) are reported. The keeping quality of wheat/maize bread prepared with addition of vitamin C compared well with that of normal wheat bread, and staled less rapidly than wheat/maize bread prepared with additions of either $KBrO_3$ or egg powder. (11 references.) E. C. APLING.

Convenient apparatus for the determination of yeast activity. D. G. Weeden (*Chem. & Ind.*, 1964, 1839—1840).—The standard procedures for determining the 'strength' of bakers' yeast are briefly summarised but generally these are either bulky in dimension, or complex in construction and costly. The yeasted dough is inserted into the flask and a 3-way cock adjusted to give access to the atm. A levelling bulb filled with saturated NaCl and the apparatus (or flask only) is immersed in a water bath at 30°. When the temp. remains constant, the brine level is adjusted to zero and the air supply is cut off. Fermentation is allowed to proceed for a predetermined time and the vol. at atm. pressure and the temp. noted; the yeast activity can be calculated from the increase in vol. The apparatus is illustrated; the same procedure can be adopted for micro-estimations. (10 references.) C. V.

Disintegration of yeast by high intensity ultrasound. E. A. Neppiras and D. E. Hughes (*Biotechnol. Bioengng*, 1964, **6**, 247—270).—Tests were made using an ultrasonic transducer-probe system which is fully described and the cell breakage estimated in several ways. The disintegration was studied as a function of ambient pressure and ultrasonic power. At a fixed intensity disintegration reaches a peak with increasing ambient pressure and then falls rapidly. Increasing intensity increases the ambient pressure at which the peak is found. The results obtained support the view that all breakage is primarily dependent on the production of gaseous cavitation in the medium. (17 references.) E. C. DOLTON.

Flour. J. Nara (B.P. 940,624, 30.5.60).—A process and apparatus for milling grain (wheat, etc.) and producing flours of various grades (e.g., flour containing only embryos, refined flour containing no embryos, or bran and flour consisting of bran) are claimed. F. R. BASFORD.

Sugars and confectionery

Pilot plant work on dextrose production. V. Hansen (*Stärke*, 1964, **16**, 258—263).—The historical development of the dextrose industry is briefly reviewed. A pilot plant for the production of α-dextrose and 'total sugar' by enzymic saccharification uses a continuous Krøyer converter of adjustable capacity (50—100 l. starch slurry per h.) and variable reaction time (2—30 min.) and a number of 50-l. crystallisers. Acid conversion of a 10° Bé slurry in a test run gave DE and DX values of 92 and 87 respectively. After saccharification of the low DE hydrolysate with amyloglucosidase, DE = 95 and DX = 93. The homogeneous product from the liquefaction of starch by α-amylase was saccharified to a DE = 98 and DX = 96 product. The calculation for the operation of crystallisation of α-dextrose monohydrate is explained as an example of the use of the method. (13 references.) A. T. CARPENTER.

Glucose syrups and the confectionary industry. F.-W. Conti (*Stärke*, 1964, **16**, 254—257).—A large no. of confectionary producers obtain glucose syrups from a small no. of sources and would benefit from standardisation of this raw material. The following factors could be measured and rated as an indication of quality (acceptable values for a 'standard syrup' in parentheses): i. Concentration, usually expressed as refractometer values (81·3° Brix); ii. DE value (38); iii. pH (5), acidity (<1·6) or candy test; iv. SO_2 content (60—100 mg./kg.); v. ash content (<0·75%); vi. appearance and colour stability. Protein, Fe and Cu content should be <0·08, <0·008 and <0·0008% respectively. A. T. CARPENTER.

Saccharification of cellulose-containing material. Shin Nippon Chisso Hiryo K.K. (inventor: J. Kusama) [1](BP. 940, 314. 4.1.61.)—In the saccharification of particulate cellulose-containing materials (**I**) by the HCl gas-suspension method, an inert non-tacky substance having particle size less than that of **I** and a vapour pressure lower than that of the ambient atm. (lignin or diatomaceous earth) is added to the initial **I** in order to reduce the stickiness of the particles of **I** during the process, and to facilitate hydrolysis of **I**, and reduce stickiness of the product. H. L. WHITEHEAD.

Purification of solutions containing sugar. Farbenfabriken Bayer A.-G. (B.P. 941,011, 11.2.60. Ger., 16.2.59).—Colour is removed from sugar solutions by treatment with an acid-activated spongy cation-exchange resin (containing CO_2H or SO_3H groups). Exhausted resin may be regenerated with alkaline-earth metal hydroxide solution and/or alkaline-earth metal salt, followed by treatment with mineral acid. F. R. BASFORD.

Apparatus for drying sugar-containing material. American Sugar Refining Co. (B.P. 942,727, 19.7.62. U.S., 2.8.61).—Sugar-containing material, e.g., wet granular sugar, is dried by dispersing it through a gas and generating therein acoustic vibrations (500—30,000 cycles per sec. at 0·001 W per cm.^{2}). Apparatus is figured and claimed. F. R. BASFORD.

Fermentation and Alcoholic Beverages

Distillation of cognac. R. Lafon, J. Lafon and P. Couillaud (*C. R. Acad. Agric., Fr.*, 1964, **50**, 972—973).—A brief mention of a report from the Station Viticole de Cognac dealing with the clarification of empirical observations by gas-chromatographic analyses. P. S. ARUP.

Formation of succinic acid during fermentation of wine. K. Mayer, I. Busch and G. Pause (*Z. LebensmittUntersuch.*, 1964, **125**, 375—381).—Fermentation experiments made with wine and with synthetic media demonstrated that the disappearance of malic acid observed during the fermentation of wine is largely due to its conversion into succinic acid, and partly to its conversion into lactic acid. These conversions were confirmed by experiments with ^{14}C-labelled malic acid; they were more pronounced under aerobic than under anaerobic conditions. The conversion of glutamic acid into succinic acid (but not into lactic acid) was confirmed. (35 references.) P. S. ARUP.

Determination of sulphur dioxide in beers and wines. G. T. Jones (*Analyst*, 1964, **89**, 678—679).—Modifications to the Lloyd and Cowle method for soft drinks are described making it suitable for determining SO_2 in beer, wines, etc. A large heated desorption vessel with a condenser between the desorption vessel and the absorption tubes is used. A large sample is used to increase sensitivity. A 25-min. N_2 flow is necessary for desorption and the titration end-point is determined with wide-range indicator paper. E. C. DOLTON.

Hydrogen sulphide production by yeasts. B. C. Rankine (*J. Sci. Fd Agric.*, 1964, **15**, 872—877).—In a study of H_2S production from 64 yeasts from 12 genera in pure culture in grape juice under different conditions, large differences were observed between strains, ranging from no detectable production with Pb acetate to easy detection by smell. About five times as much was produced at 30° as at 15°. *Schizosaccharomyces malidevorans* produced the greatest quantity; *Candida* gave slow production over a long period. For commercial wine-making results emphasise the importance of selecting suitable yeasts with minimal production of H_2S for use as starter cultures, and of using sufficient of the starter to ensure that it dominates the yeasts naturally present in the crushed juice. E. M. J.

Estimation of hydrogen sulphide in beer. H. E. Jansen (*J. Inst. Brewing*, 1964, **70**, 401—404).—The method of Brenner for the determination of H_2S in beer has been shown to be unsatisfactory. The Cu content and time and temp. at which the Cu-containing beer is kept after bottling affect the value obtained by this method. S. A. Brooks.

Restriction of proteolysis in mashing by using a mixture of barley and malt. T-M. Enari, J. Mikola and M. Linko (*J. Inst. Brewing*, 1964, **70**, 405—410).—The use of a mixture of malt and barley reduced the amino-acid and total N content of wort caused by proteolytic inhibitors present in barley. A mixture of equal parts of barley and malt extracts gave a 50% inhibition of endopeptidase activity. (13 references.) S. A. Brooks.

Amine content of beer and its fluctuation during production. J. Hrdlička, J. Dyr and K. Kubíčková (*Brauwissenschaft*, 1964, **17**, 373—378).—The content and composition of volatile amines, determined by the methods of Drews *et al.*, varied in beers from three different breweries. The average content decreased from 4·8 (in the hot wort) to 1·9 (in the finished beer) as mg. of N/l., entailing a marked decrease in the monoamines, but a slight increase in the diamines. The major constituents were aliphatic mono- and diamines in the range C_1—C_6. (17 references.) P. S. Arup.

Determination of biochemical oxygen demand using dichromate oxidation. W. Wolfner (*J. Inst. Brewing*, 1964, **70**, 446—448).—Details are given of modifications which have been adopted to allow the dichromate technique to be used for measuring B.O.D. values as low as 10—40 mg./l. Colour comparison is by means of standardised colour disks. S. A. Brooks.

Bittering power of stored hops. G. A. Howard and P. A. Martin (*J. Inst. Brewing*, 1964, **70**, 424—439).—A series of brewing trials showed that the ratio of the α-acid content of a hop is estimated by conductometric titration with Pb acetate to that estimated by polarimetry is a reliable guide to its brewing value. Fractions obtained by thin-layer chromatography from old hops and bitterness extracts have been examined for bitterness and other properties. (19 references.) S. A. Brooks.

Measurement of bitterness in beers. Report of Analysis Committee of the European Brewery Convention. R. Bishop (*Brygmesteren*, 1964, **21**, 225—239).—Results obtained by the Committee and by the Isohumulones Sub-committee of the Amer. Soc. Brew. Chem. show agreement between values obtained by the Rigby and Bethune (RB) and the Moltke and Meigaard (MM) methods at 28 p.p.m. but above this limit the MM values become progressively higher and *vice versa*. Graphs of the relationship between the two sets of results are rectilinear. As the MM method is much more convenient than the RB method, tables have been drawn up for converting MM results or MM spectrophotometric extinction readings into RB values, viz., International Bitterness Units or the American Isohumulone Bitterness Units. In beers made from fresh hops the isohumulones are the sole bittering agents, but in beers made with old hops other bittering agents occur which are included in the MM but not in the RB measurements. The MM method should be employed if the use of old hops is suspected. P. S. Arup.

Determination of lead-titratable bitter acids in hop concentrate. E. Callesen, R. Djurtoft and B. Trolle (*Brygmesteren*, 1964, **21**, 201—204).—The accuracy of the conductimetric method of Trolle and Djurtoft (cf. *Brauwissenschaft*, 1958, **11**, 283) has been improved by carrying out the extraction of the mixture of the sample with 0·1N-H_2SO_4 with $CHCl_3$ in a globular separating funnel in which the phases are mixed by means of a Vibromixer. The acids determined by this method include, in addition to the true α-acids, initial oxidation products of hops such as humulinones and hulupones which also have a bittering effect. P. S. Arup.

Formation, nature and prevention of precipitates in frozen and thawed beers. G. J. Haas and A. I. Fleischman (*J. agric. Fd Chem.*, 1964, **12**, 409—411).—The flaky ppt. which forms on repeated freezing and thawing of beer is shown to be a carbohydrate polymer containing glucose, glucuronic acid and *N*-acetyl glucosamine. The source of the polymer is the malt. The formation of the ppt. may be prevented by adding the enzyme β-glucosaminidase to the beer. (20 references.) W. Elstow.

Treatment of liquor, e.g., beer. British Filters Ltd. (Inventor: A. G. Hobson) (B.P. 942,686, 20.4.61).—Haze in beer is removed or prevented by stirring it with a mixture of granular solid, diatomaceous earth (2—3) and polyamide particles (1 pt.) in a vessel having an unperforated retaining bottom; allowing the granular solid to settle on a perforated screen (of ~100 in.-mesh) above the vessel bottom and draining off the liquor. F. R. Basford.

Malting process. Kurth Malting Co. (B.P. 940,249, 26.4.61. U.S., 27.6.60).—The process, which gives improved yields of malt, comprises acidulating and adding a growth-promoting amount of gibberellic acid (e.g., 1—3 p.p.m. of barley) to a cereal grain within the period from initial steeping of the grain until before the commencement of significant germination of the steeped grain, then germinating the grain until it is modified to form malt, and drying the resulting green malt. Acidulation and addition of malt may be effected in each case before or after steep-out, and acidulation may be continued until the pH after 1 h. of a quantity of water to which an equal wt. of grain germinated for 1 day has been added, is <4. F. R. Basford.

Malt. K. Geys (B.P. 940,886, 16.11.61. Ger., 9.12.60 and 12.1.61).—High-grade malt is obtained in a short time, without need for steeping, by spraying green malt (which has already germinated during 2—3 days) with essentially O_2-free water during 24 h. (without allowing the water to come into contact with atm. air), allowing germination to continue during a further 2—3 days, then drying. F. R. Basford.

Continuous production of wort as hopped wort. Weigelwerk A.-G. (B.P. 942,623, 23.11.61. Ger., 23.11.60).—A continuously prepared mash is filled uninterruptedly between filter walls of a tower, and while moving therein downwards along the filter walls, the first wort is withdrawn through the filter walls, and the spent grains are sparged with water through the walls and passed at the lower end of the tower into a conveyer for discharge. Apparatus is figured and claimed. F. R. Basford.

Fruits, Vegetables, etc.

Apple aroma. J. Koch and H. Schiller (*Z. LebensmittUntersuch.*, 1964, **125**, 364—368).—Gas-chromatographic analyses of numerous samples of natural aromatic prep. and of their chief groups of components revealed the presence (in addition to previously detected aliphatic alcohols, carbonyl compounds and acids) of hexenol and hexenoic acid. Hexenal, the main aldehydic constituent, was established as being the chief contributor to the characteristic aroma of apples. P. S. Arup.

Occurrence of farnesene in the natural coating of apples. K. E. Murray, F. E. Huelin and J. B. Davenport (*Nature, Lond.*, 1964, **204**, 80).—Gas-chromatographic and i.r. analyses have established the presence of ~1% of farnesene (2,6-dimethyl-10-methylene-2,6,11-dodecatriene) in the cuticle oil fraction of Granny Smith apples. The farnesene in the separated fractions polymerises rapidly in air at 21°, but is stable for ~1 year in the entire ether-extracted apple coating. It may influence the development of 'superficial scald' during storage. W. J. Baker.

Use of cysteine to prevent browning in apple products. J. R. L. Walker and C. E. S. Reddish (*J. Sci. Fd Agric.*, 1964, **15**, 902—904).—A re-appraisal of the use of cysteine to prevent the enzymic browning of apple products following recent work on apple polyphenoloxidase was made. In comparison with ascorbic acid, smaller amounts of cysteine inhibited browning and the inhibition lasted longer. At optimum concentrations cysteine gave no adverse effects on flavour or tinplate. A simple method for estimating the cysteine requirement for a particular apple juice blend was devised. E. M. J.

Post-harvest sterilisation of oranges against Queensland fruit fly. D. Leggo, J. G. Gellatley, J. A. Seberry, I. D. Peggie, J. K. Long and E. G. Hall (*Food Pres. Quart.*, 1964, **24**, 15—19).—Research by the Australian citrus industry and State and Federal authorities on post-harvest sterilisation of oranges is reviewed. Fumigation of packed oranges with ethylene dibromide has proved acceptable against Queensland fruit fly. S. A. Brooks.

Prevention of browning during drying by the cold dipping treatment of sultana grapes. F. Radler (*J. Sci. Fd Agric.*, 1964, **15**, 864—869).—Polyphenol oxidase, mainly located in the skin of sultana grapes, is generally regarded as being responsible for browning reactions in grape products. Evidence is presented for an indirect mechanism by which the enzymic browning of grapes during drying is reduced by the use of dipping solutions that increase the drying rate. The enzyme is unspecifically inhibited by rising sugar concn. and not by components of the dipping solution mainly ethyl esters of fatty acids, (C_{14}—C_{18}) (2%) in 2·5% aq. K_2CO_3. (21 references.) E. M. J.

Influence of γ-irradiation of seeds on ascorbic acid content of the ripe vegetables. J. Kulesza, J. Kroh and L. Antoszewska (*Nahrung*, 1964, **8**, 599—600).—Irradiation of salad, spinach or onion seeds increased the ripe ascorbic acid content by 20—25% (on fresh wt.). Dosages of 500 rad. often sufficed for good results. Dosages of 10,000 rad. inhibited the growth of onions. P. S. Arup.

Discoloration in processed cauliflower. B. V. Chandler (*Food Pres. Quart.*, 1964, **24**, 11—14).—Investigations into the discoloration of processed cauliflower, particularly in canned products, were reviewed and discussed. Recommendations made to obviate discoloration are choice of good raw materials, elimination of metal pick-up, controlled use of ascorbic acid and SO_2, lacquered cans and avoidance of excessive heat treatment. (16 references.) S. A. BROOKS.

Carotene oxidation and off-flavour development in dehydrated carrot. M. E. Falconer, M. J. Fishwick, D. G. Land and E. R. Sayer (*J. Sci. Fd Agric.*, 1964, **15**, 897—901).—The carrots were washed, scrubbed and diced on the day after harvest. The dice were blanched, dried by the accelerated freeze-drying (AFD) process, canned under N_2 and stored at −20°. The off-flavour which develops is said to be due to the formation of β-ionone formed by the oxidation of β-carotene and accompanied by loss of colour. A direct relationship between loss of β-carotene and off-flavour was established in a series of storage tests, by carotene analyses and taste panel assessment for natural and off-flavour. E. M. J.

Greening of potatoes during marketing. R. E. Hardenburg (*Amer. Potato J.*, 1964, **41**, 215—220).—A review of factors affecting greening and research on greening prevention. A. H. CORNFIELD.

Nomograph for calculating the specific gravity of potato tubers. G. W. Hope (*Amer. Potato J.*, 1964, **41**, 221—222).—A nomograph is presented showing the relation between sp. gravity of tubers and their wt. in air and in water. A. H. CORNFIELD.

Potato quality. XXIV. Objective measurement of mealiness in potatoes. L. Lujan and O. Smith (*Amer. Potato J.*, 1964, **41**, 244—252).—The shear press method was able to detect differences in texture (mealiness) betwen tubers having sp. gr. differences of 0·002. Different varieties of potatoes of identical sp. gr. differed in mealiness. Within a variety mealiness was highly correlated with sp. gr. A. H. CORNFIELD.

Volatile sulphur compounds in potatoes. M. R. Gumbmann and H. K. Burr (*J. agric. Fd Chem.*, 1964, **12**, 404—408).—The S-containing volatiles from cooking potatoes were trapped as $HgCl_2$ complexes, regenerated and analysed by gas chromatography with H flame ionisation detectors. Methyl mercaptan and dimethyl sulphide were the major constituents of the regenerated vapour with smaller amounts of ethyl mercaptan, dimethyl sulphide, methyl ethyl sulphide and methyl isopropyl disulphide. Several other S-containing compounds including H_2S were also detected in smaller amounts. (39 references.) W. ELSTOW.

Ascorbic acid content of steam-peeled potatoes. E. Peppler and W. Feldheim (*Nahrung*, 1964, **8**, 597—599).—Although steam-peeled potatoes contain somewhat less ascorbic acid than do mechanically peeled potatoes, the use of steam-peeling would probably minimise subsequent losses because, owing to its comparative rapidity, it could be accomplished shortly before cooking. Steam-peeled potatoes do not undergo discoloration when kept under water during 1—2 days. P. S. ARUP.

Effects of lye-peeling and the food law. F. Günther and O. Burckhart (*Dtsch. LebensmittRdsch.*, 1964, **60**, 315—318).—Laboratory and technical scale experiments on the effects of lye-peeling on potatoes are reported. The pH was substantially unaltered by efficient lye-peeling and neutralisation with citric acid solution; vitamin C was destroyed in the outside of the peeled potato, but the loss was generally $\not>5\%$ of the total, and was insignificant compared with normal losses in storage and cooking. Necessary measures for the hygienic and technical control of the process are outlined. E. C. APLING.

Measurement of fibre content of asparagus. F. Kaufmann (*Nahrung*, 1964, **8**, 577—589).—The palatability of asparagus depends largely on its comparatively low fibre content. A close negative correlation is found between the (depth of penetration) results obtained with the Ap 4 VEB penetrometer (Feingeräte, Dresden) and the (ash-free) fibre content. This penetrometer is recommended for quality control. (27 references.) P. S. ARUP.

Lipid alterations during the fermentation of dill pickles. C. S. Pederson, L. R. Mattick, F. A. Lee and R. M. Butts (*Appl. Microbiol.*, 1964, **12**, No. 6, 513—516).—Lipid fractions of cucumbers and good and bloated dill showed marked changes during fermentation; the fall in the phospholipid fraction is the most noteworthy, there being a nearly fourfold increase in free fatty acids as well as a marked increase in the neutral fatty acids and unsaponifiables. In the analysis 41 esters were identified and 16 of these accounted for at least 95% of the acids. Among the marked changes were the increases in linoleic and linolenic acids in good pickles, this being in contrast to the increase of oleic acid in the bloated variety. The presence of tridecanoic in cucumber, its absence in pickles as well as the absence of caproic, caprylic and capric acids in pickles and cucumber is of considerably greater interest. Attention is drawn to the similar changes found in sauerkraut fermentation. (14 references.) C. V.

Pink discoloration in canned okra. M. Mahadeviah, L. V. L. Sastry and G. S. Siddappa (*Indian J. appl. Chem.*, 1964, **27**, 40—41).—The chemical composition of whole okra (a common vegetable in India), okra seeds and okra skins is presented. The seeds contain more protein, Fe, Ca, P and ascorbic acid and less crude fibre than the skin portion. The tannin-like constituents are present in the seeds only. Leucoanthocyanins were found in the seed, only traces in the skin, and leucocyanin was also present. I. DICKINSON.

Dehydrated mashed potatoes. J. & J. Colman Ltd. (B.P. 940,053, 2.10.61. U.S., 4.10.60).—The reconstitution properties of a dehydrated mashed potato product are improved by incorporation of 0·05—1 wt.-% of at least one natural gum (karaya, Na alginate). F. R. BASFORD.

Non-alcoholic beverages

Fruit juices and other fruit-based drinks; their designation and preparation. W. Zipfel (*Dtsch. LebensmittRdsch.*, 1964, **60**, 239—244).—Developments in methods of prep. and in the compositional range of commercial fruit-based drinks are reviewed and problems of their designation and classification for food law purposes are discussed. (29 references.) E. C. APLING.

Non-enzymic browning of lemon juice. K. M. Clegg (*J. Sci. Fd Agric.*, 1964, **15**, 878—885).—Of three possible modes of browning outlined, the sugar-amine reaction seems unlikely from findings with model systems at pH 2·5, but the active aldehyde theory appears more likely to apply to the highly acidic lemon juice with ascorbic acid being specifically involved. The principal rôle of amino-acids is to increase the browning potential after the oxidation of the ascorbic acid to reactive carbonyl compounds. The formation of melanoidin complexes originates from the polymerisation of carbonyl and α-amino-groups. In this study citric acid plays an important part; considerable browning occurs at the natural pH 2·5 but max. is obtained at pH 4·5; the furfural formed contributes little to browning of lemon juice under aerobic conditions. (14 references.) E. M. J.

Determination of ascorbic acid in blackcurrant and other coloured fruit-juice syrups. L. Kum-Tatt and P. C. Leong (*Analyst*, 1964, **89**, 674—677).—Ascorbic acid is determined by adding the syrup to $HgCl_2$, centrifuging the pptd. Hg_2Cl_2 and estimating Hg_2Cl_2 by dissolving in 0·01N-I_2 and 10% KI and titrating the excess I_2 with 0·01N-$Na_2S_2O_3$. Na_2SO_4, citric acid, glucose and sucrose do not interfere. E. C. DOLTON.

Detection of emulsifying agents in the basic materials for alcohol-free beverages. H. Rother (*Riechstoffe u. Aromen*, 1964, **14**, 329—335).—Methods for the detection and identification of emulsifying agents in the basic materials of alcohol-free beverages, e.g., fruit drinks, are reviewed. The main problem lies in isolating the emulsifiers from other possible interfering components in the basic materials and the literature on the methods for achieving this is reviewed together with that on identification of individual types and compounds. Emulsifiers discussed are fatty acid glycerides and polyoxyethylene compounds. Techniques involved include solvent extraction, ion exchange, paper chromatography and, for quant. determination, spectrophotometry. J. I. M. JONES.

Tea, coffee, cocoa

Examination of coffee and coffee substitutes. X. Cell-wall carbohydrates of roasted coffee. H. Thaler (*Z. LebensmittUntersuch.*, 1964, **125**, 369—375).—In continuation of previous work (cf. *ibid.*, 1959, **110**, 442) a holocellulose amounting to 20—30% of the roasted coffee was obtained after heating defatted grounds with aq. $NaClO_2$ and AcOH for 10 h. Evidence was also obtained of the presence of a water-sol. constituent of high mol. wt. that probably consisted of oxidation products of melanoids that had been adsorbed by the holocellulose. The content of both substances varied inversely with the degree to which the coffee had been roasted. P. S. ARUP.

Interference with determination of citric acid [in coffee] by chlorogenic and quinic acids. J. Schormüller and K. Rubach (*Nahrung*, 1964, **8**, 595—597).—Values found by the column chromatographic method of Marbrouk and Deatherage were much lower than values found by the pentabromoacetone method. The acids of roasted coffee and coffee extracts were separated on a column of Dowex 2X8; it was then found that the acids of the chlorogenic group yielded appreciable amounts of pentabromoacetone. P. S. ARUP.

Properties and determination of caffeic acid. I. Formation of esculetin from the *cis*-isomer of the acid. II. Determination after paper-chromatographic separation. R. Engst and J. Voigt (*Nahrung.*, 1964, **8**, 389—398, 399—404).—I. The partial transformation of *trans*-caffeic acid into the *cis*-acid during chromatographic analysis with 2% AcOH as developing solvent occurs in diffuse daylight as well as under u.v. light. The transformation of the *cis*-acid into esculetin occurs spontaneously in the presence of traces of Fe. These transformations can be prevented by using the developing solvent system BuOH–AcOH–water (4 : 1 : 5). (15 references.)

II. Caffeic acid was satisfactorily determined in potato extracts by spectrophotometry after chromatographic development with the solvent mentioned in the previous abstract. Recoveries of the acid were 96—108%. The standard deviation was ±4·0%, and the deviation range ±9·2%. P. S. ARUP.

Tea and coffee [of improved stability]. C. A. Longley and P. G. N. Ommanney (B.P. 940,867, 17.10.58).—There is claimed a composition comprising a vegetable substance having aromatic or narcotic properties derived from tea or coffee plants, especially a tea extract (78—96·5) and a stabiliser therefor (3·5—22 wt.-%) consisting of a mixture of oxidised tea leaf and tea gum (dried aq. extract of tea). The composition may also contain sweetening material. F. R. BASFORD.

Milk, Dairy Produce, Eggs

Analytical criteria for hygienic microbiological quality of raw milk. E. Pijanowski, M. Dłużewski and A. Miazga (*Nahrung*, 1964, **8**, 405—420).—Numerous samples from four herds were tested during the winter months. No correlation was found between the results of the Bruncke dirt test and the counts of total and psychrophilic bacteria or of *Escherichia coli*. Hand-milked samples from one farm showed smaller counts than did machine-milked samples from the three other farms. In the reductase test the *E. coli* group were much more active than the lactic acid group of bacteria. (19 references.) P. S. ARUP.

Quantitative electrophoretic analysis of milk proteins. F. Kiermeier and O. Kirchmeier (*Z. LebensmittUntersuch.*, 1964, **125**, 341—346).—Electrophorograms of the total proteins and of six different prep. of milk protein fractions have been obtained by a technique similar to that used by the Committee on Milk Protein Nomenclature, Classification and Methodology (cf. Jeness *et al.*, *J. Dairy Sci.*, 1956, **39**, 536). A review is given of the prep., properties and nomenclature of the component proteins of milk. (19 references.) P. S. ARUP.

Liberation of phosphoprotein-phosphorus from separated milk protein by heating. R. Thalacker (*Dtsch. LebensmittRdsch.*, 1964, **60**, 211—213).—The rate of liberation of phosphoprotein-P from separated milk protein on heating in water was measured for temp. between 80 and 120°, pH between 6·0 and 8·0 and concn. between 0·5 and 2·0 g. per 100 ml. The proportion of protein P liberated increased with duration of heating, temp. and pH, but varied only slightly with protein concn. E. C. APLING.

Occurrence of nucleotides and related compounds in milk and milk products. F. Kieffer, J. Solms and R. H. Egli (*Z. Lebensmitt-Untersuch.*, 1964, **125**, 346—350).—On examination of the effects of different technical treatments on the nucleotide content of milk, microbiological souring proved to be the only treatment to have any effect; in this case the content of orotic acid was reduced by ~65%, and that of hippuric acid to nil. (24 references.) P. S. ARUP.

Factors affecting the heat aggregation of proteins in selected skim milk sera. D. B. Kenkare, C. V. Morr and I. A. Gould (*J. Dairy Sci.*, 1964, **47**, 947—953).—Protein destabilisation in both acid-prepared (pH 4·6) and ultracentrifugal skim milk sera heated at temp. up to 118° was determined by N analysis of the sera and of the supernatants after centrifuging of the sera at 1000 × *g* for 30 min. Serum proteins of acid-prepared serum are much less stable to heat than those of ultracentrifugal serum. Reduction of the Ca phosphate content of the acid-prepared serum resulted in a reduction of heat-induced protein destabilisation. Addition of whole casein and various casein fractions to skim milk sera increased the stability of the proteins to heat. Sephadex G-100 gel filtration showed that heating of acid-prepared serum caused aggregation and destabilisation of all the protein components but that heating of ultracentrifugal serum resulted in the formation of intermediate-sized stable protein aggregates. M. O'LEARY.

Effect of certain salts on the stability of skim milk as determined by rennet coagulation time and alcohol test. J. M. DeMan and S. C. Batra (*J. Dairy Sci.*, 1964, **47**, 954—957).—The stability of skim milk was decreased by the addition of Ca ions in amounts as low as 1 mg. per 100 ml. milk. The addition of citrate increased the stability of skim milk and also counteracted the effect of added Ca. The addition of PO_4^{3-} had no effect on the stability of skim milk. A technique for measuring rennet coagulation time with an automatic blood clot-time is also described. (24 references.) M. O'LEARY.

Spatial distribution of milk constituents in powders made by different drying techniques. V. H. Holsinger, K. K. Fox, M. K. Harper and M. J. Pallansch (*J. Dairy Sci.*, 1964, **47**, 964—969).—Surface washings from five different types of powder granules were analysed for total solids, fat, lactose and protein, and f.p.; conductivities were also determined. The results indicated that foam-dried whole milk powder granules are the most uniform in composition. Migration of solutes of low mol. wt. towards the surfaces of the granules was demonstrated during both the foam- and spray-drying processes. No relationship could be established between the observed orientation of milk constituents within the powder granules and the dispersibility of the granules. A mathematical analysis of a model washing process is also presented. M. O'LEARY.

Factors related to the storage stability of foam-dried whole milk. IV. Effect of powder moisture content and in-pack oxygen at different storage temperatures. A. Tamsma and M. J. Pallansch (*J. Dairy Sci.*, 1964, **47**, 970—976).—Organoleptic tests were conducted on foam-dried whole milk powders with moisture contents ranging from 2 to 5%, packed in cans containing various levels of O_2, after storage at temp. ranging from 0 to 80°F for periods up to 6 months. Flavour deterioration at elevated temp. was reduced by low in-pack O_2 concn. and low powder moisture levels but high moisture contents led to greater flavour stability at moderately low storage temp. It is concluded that the various parameters used in the investigation could be manipulated to maintain an acceptable level of flavour in foam-dried whole milk during storage for 6 months. (11 references.) M. O'LEARY.

2,2′-Biphenyl-1-picrylhydrazyl as a reagent for the quantitative determination of hydroperoxides. B. G. Tarladgis, A. W. Schoemakers and P. Haverkamp Begemann (*J. Dairy Sci.*, 1964, **47**, 1011—1012).—A method of determining the peroxide value of fats by assaying their hydroperoxide contents is described. The method is based on spectrophotometric determination of the degree of reduction of 2,2′-biphenyl-1-picrylhydrazyl by the hydroperoxides. M. O'LEARY.

Specificity of milk lipase for a butyryl triglyceride. R. G. Jensen, J. Sampugna, R. L. Pereira, R. C. Chandan and K. M. Shahani (*J. Dairy Sci.*, 1964, **47**, 1012—1013).—The action of milk lipase on a mixture of glyceryl-1-palmitate 2,3-dibutyrate and triolein was studied. The results indicated that milk lipase possesses intermolecular specificity for butyryl triglycerides but does not possess intramolecular specificity. M. O'LEARY.

Procedure for determination of protein in ice milk and ice cream by formol titration. R. L. Hill and W. K. Stone (*J. Dairy Sci.*, 1964, **47**, 1014—1016).—A screening test for determining the protein content of ice milk and ice cream, based on a potentiometer formol titration procedure, is described. M. O'LEARY.

Ammonia content of egg-white and egg-yolk. I. Determination of free ammonia in egg constituents. II. Influence of storage on ammonia content and freshness of shell-eggs. H. Paulenz and R. Engst (*Nahrung*, 1964, **8**, 425—434, 567—576).—I. Satisfactory determinations in the range 0·01—0·2 mg. of NH_3 can be made by means of a modified form of the Conway and Byrne diffusion unit comprising a weighing bottle (of dia. 6 cm.) with interchangeable absorption cells. Interference by NH_3 released from amino-acids is negligible. (23 references.)

II. Numerous determinations were made on the whites and yolks of eggs stored at 0° or room temp. during periods up to 6 months by the method described in Part I. No increases occurred in the white-NH_3 in unoiled eggs; the increases observed in oiled eggs indicated that NH_3 probably escapes by diffusion through the shells of unoiled eggs. Increases occurred in the yolk NH_3, but owing to natural variations no reliance could be placed on single determinations. Statistical calculations gave the values (in mg./100 g.) ⩾3·3 for fresh eggs, 3·3—9 for stale eggs, and >9 for uneatable eggs. A 100-point grading system based on known tests is described. (24 references.) P. S. ARUP.

Influence of viscosity of liquid egg on its emulsifying properties. W. Wachs and A. Johnsson (*Dtsch. LebensmittRdsch.*, 1964, **60**, 279—282).—Curves of η (cP) in relation to rate of shear (sec.$^{-1}$) measured with the Rotavisko (Fa. Haake, Berlin-41) rotation viscometer are presented for various samples of fresh, preserved and pasteurised liquid egg and compared with the flow curves (shear rate

as a function of applied load) obtained with model emulsions prepared from egg, water and trans-decalin at 30° in a Schwingdüsen homogeniser. Initial η was low (~1100 cP) for preserved egg compared with fresh egg (~2500 cP), and generally high for pasteurised egg (generally 20,000—50,000 cP; extreme range 2,000—70,000 cP) and further increase in initial η resulted from storage of pasteurised egg for 4 weeks at −20°. The emulsions prepared with fresh and preserved egg showed little structural η (curvature of the flow curve), but curves for emulsions containing pasteurised egg showed a sharp break in the region of 1300 dynes per cm.^{2}, when the emulsions became free-running. Egg pasteurised after enzymic treatment with papain or ficin had similar structural η and emulsifying properties to fresh egg, with little change on deep-freeze storage.

E. C. APLING.

Factor for the estimation of egg-yolk content in foodstuffs from their lecithin-phosphorus pentoxide content. G. A. Van Stijgeren (*Dtsch. Lebensmitt-Rdsch.*, 1964, **60,** 277—279).—Methods for determining lecithin–P are critically reviewed. Most reproducible results were obtained by extraction with ethanol (95—96%) at room temp., followed by evaporation of the extract made alkaline with NaOH or $Mg(OAc)_2$, and determination of P in the ash by the gravimetric phosphomolybdate method. Lecithin–P_2O_5 found in eight samples of egg varied from 0·927 to 0·998% (mean 0·976%), corresponding to a factor for calculation to egg-yolk content of 102·4. For calculation of max. possible egg-yolk content, the factor 107 is proposed; the probable egg-content is then ~5% less than this.

E. C. APLING.

Powdered milk products. Koopmans Meelfabrieken N.V. (B.P. 939,935, 17.10.61. Switz., 17.10.60).—Milk powder of low % Ca and P is produced by treating skimmed milk with a proteolytic enzyme (rennin, ficin, bromelin) at pH >6 to effect pptn. of Ca phosphocaseinate. This is filtered off from the whey, suspended in water, the suspension is acidified to pH ~4·5 (whereby Ca or P are solubilised), casein is filtered off, washed and dispersed in the whey, and the dispersion is adjusted to pH 6—7·5, and the resulting homogeneous solution is dried (optionally after adding fat).

F. R. BASFORD.

Edible Oils and Fats

Physico-chemical characteristics for determination of quality of crude fats for rendering of lard. M. Stojtscheff (*Nahrung*, 1964, **8,** 591—594).—Lards of the best consistency and organoleptic quality have compartively high m.p. and f.p., and low I val. The qualities of the fat tissues decrease in the following order: kidney fat, back fat, abdominal fat, reticular tissue fat, tripe fat and skin fat.

P. S. ARUP.

Metabolism of saturated and unsaturated fatty acids. I. Weight curves and fat absorption. Z. Placer and Z. Slabochová (*Nahrung*, 1964, **8,** 291—303).—The growth and vigour of starved rats could be restored by a diet containing beef fat or a mixture of stearic or palmitic acid with 5% of unsaturated fatty acids, but not by a diet containing saturated fatty acids only. (44 references.)

P. S. ARUP.

Flavour volatiles of fats and fat-containing foods. I. Degradation of the peroxides of autoxidised sunflower and linseed oils. C. H. Lea and A. Hobson-Frohock (*J. Sci. Fd Agric.*, 1965, **16,** 18—27).—The production of volatile carbonylic compounds in the above named oils, specially treated (silicic acid and steam deodorisation) to remove preformed oxidation products, was measured after autoxidation of the oil at 37°. Further formation of volatile and non-volatile carbonyls was followed during thermal decomposition of the peroxides which are the major oxidation products at low temp. The peroxides of linseed oil (mainly linolenate) decomposed more readily than those (mainly linoleate) of the sunflower oil and produced 60 as compared with 40%, respectively, of carbonylic compounds. In both oils nearly half of the total carbonyl groups formed were in volatile compounds. (26 references.) E. M. J.

Determination of oxidation state of fats and fatty foods by the thiobarbituric acid test. A. Purr (*Dtsch. Lebensmitt Rdsch.*, 1964, **60,** 269—277).—A detailed review covering the chemistry of aldehyde formation in oxidised and irradiated fats, the reactions of 2-thiobarbituric acid (TBA), the constitution of the coloured compounds formed, and the factors influencing the results of the TBA test. Three alternative procedures for the TBA test are described and discussed, and reports of the application of TBA procedures to the examination of milk, meat and fish products, fats and oils, cereals and baked goods, and of correlations between the results of the TBA test, the Lea-peroxide test, and organoleptic examinations are reviewed and discussed. (72 references.) E. C. APLING.

Analysis of the fatty acid and triglycerides in cod-liver oil. H. P. Kaufmann and T. H. Khoe (*Fette Seif. Anstrichm.*, 1964, **66,** 590—597).—Cod-liver oil fatty acids were alkali isomerised and examined under u.v. light to determine their degree of unsaturation. Thin-layer chromatograms of the fatty acids were prepared using a gypsum plate. These are shown and compared with similar chromatograms made with the hydrogenated fatty acids. The hydrogenation allows fatty acids that did form critical pairs to be separated. The results of the gas-chromatographic analysis of the methyl esters of cod-liver oil fatty acids are shown. The thin-layer chromatographic separation of cod-liver oil triglycerides into 23 fractions on kieselguhr plates in the presence of paraffin oil is described. These fractions of triglyceride have been saponified and the fatty acids formed were analysed. Tables show the fatty acids which are present in the 23 fractions. (27 references.)

W. E. ALLSEBROOK.

Complete structural analysis of fatty acid mixtures by thin-layer chromatography. L. D. Bergelson, E. V. Dyatlovitskaya and V. V. Voronkova (*J. Chromatog.*, 1964, **15,** 191—199).—Complete separation of the acids, as their Me esters, is achieved according to chain length, structure and geometrical configuration. A thin layer of silica gel impregnated with dodecane is developed with methyl cyanide-acetone (1 : 1) in the first direction; the plate is then impregnated with $AgNO_3$ and developed in the second direction with propyl ether–hexane (2 : 3). The unsaturated acids may be identified by oxidative cleavage directly in the adsorbent layer.

A. R. ROGERS.

Thin-layer chromatography. E. Becker (*Getreide u. Mehl*, 1964, **14,** 103—105).—An introduction to the technique with some examples of its use in the separation of fats and lipids.

E. C. APLING.

Hydroxy-unsaturated oils and meal from *Dimorphotheca* and *Lesquerella* seed. R. E. Knowles, K. W. Taylor, G. O. Kohler and L. A. Goldblatt (*J. agric. Fd Chem.*, 1964, **12,** 390—392).—Castor is at present the only commercially available source of hydroxy-unsaturated oils. The extraction of oil from two alternative and potentially useful sources is described. These are *Dimorphotheca* seeds giving approximately 15% oil (on dry undecorticated wt.) containing 61% dimorphecolic acid and *Lesquerella* seeds giving approximately 24% oil (on moisture-free basis) containing 57% of lesquerolic acid.

W. ELSTOW.

Chemical composition of oils (glycerides, free fatty acids and unsaponified matter) during the development and ripening of fruit and seeds of some plants. M. G. Mirić (*Bull. sci. Yougosl.*, 1964, **9,** 81).—The chemical composition of the oils of maize, bitter oak, pumpkin, peach and nut was studied during development. Some conclusions are given. (In English.)

S. A. BROOKS.

Pilot-plant processing of Indian cottonseed. III. Effect of cooking conditions on the crushing of desi type (Farm variety) cottonseed. B. Appu Rao, S. D. Thirumala Rao, S. Kutumba Rao, M. Alla Baksh and K. S. Murti (*Indian J. appl. Chem.*, 1964, **27,** 16—20).—Cooking conditions and time of crushing, yields of oil and colour and quality of oil are examined. A 25-ton lot of seed was studied in 12 pilot plant runs. Oil yields of up to 12·2% were obtained from the seed, which contained 17·2% oil, in a Rosedown Max-Oil expeller. A better quality oil is obtained by humidifying and flaking the kernels followed by crushing, than by either flaking the raw kernels and humidifying them, or by crushing the raw kernels without humidification and without flaking. Equally good results were obtained both by low (80—100°) and high (115—125°) temp. cooking of kernels before crushing.

I. DICKINSON.

Hydrogenation characteristics of some linoleic-rich oils of Indian origin. H. N. Basu and M. M. Chakrabarty (*Indian J. appl. Chem.*, 1964, **27,** 24—31).—Niger seed, tobacco seed, tea seed, safflower seed and poppy seed oils are studied and relevant data on selectivity and iso-oleic acid formation are presented. The hydrogenation characteristics of these oils are similar to those of other oleic–linoleic oils. The main criticism levelled is the high iodine value (130—145) except for the tea seed oil. The replacement of groundnut oil for the manufacture of hydrogenated fats for edible and also for industrial purposes is shown to be economically feasible. (16 references.)

I. DICKINSON.

Journées d'Inform. Produits dérivés de l'Huilerie, 1964. *Rev. franç. Corps Gras Spec. no.*, 193 pp. [A] **Mucilages and lecithins: preparation, purification, presentation, rôle, usage.** J. P. Helme, pp. 15—33.—The following are reviewed: (i) prep. and uses of mucilages, especially of soya-bean oil; (ii) composition of various phospholipids; (iii) refining and purification of phospholipids; (iv) analytical and specific determinations of commercial lecithins; (v) properties and uses. The review is detailed and tables summarise various lecithins, uses in food and in industry. (35 references.)

[B] **Neutralisation pastes, acid oils and distilled fatty acids, treatments, purification properties, uses.** M. Pabst, pp. 34—40.—Pastes of neutralisation are formed when crude oil with a certain org. acidity is treated with an alkaline reagent. The contents of these pastes and uses, e.g., enrichment of animal feeds, are discussed. The following are reviewed: methods of preparing crude fatty acids and their uses, distillation, and various methods of fractionation.

[C] **Volatile products obtained in deodorisation.** Maurice Naudet, pp. 46—49.—The substances which give flavour (bouquet) to virgin fats are discussed, viz., the terpenes of vegetable oils and the tocopherols of oils of grains. The separation of these substances is reviewed, largely in relation to American Patent literature. (17 references.)

[D] **Non-glyceride constituents (I) eliminated during refining.** A. Uzzan, pp. 50—59.—In industrial refining, improvement in techniques preserves useful substances and reduces their elimination or transformation. This review discusses these problems including **I**, e.g., alcohols, sterols, triterpene alcohols, hydrocarbons, pigments, vitamins. (18 + 2 Jap. references.)

[E] **Shells of oil-containing seeds [groundnut and sunflower], treatment, uses.** R. François, pp. 60—67.—Detailed analyses are given including composition of the ash. After suitable preparation the uses are varied, e.g., for animal feeds, building material, manure. E. M. J.

Detection of emulsifiers in foodstuffs. V. E. Kröller (*Fette Seif. Anstrichm.*, 1964, **66**, 583—586).—The use of some polyethylene oxide emulsifiers in foods is not permissible and a method of detecting this type of emulsifier is required. The thin-layer chromatographic analysis and paper-chromatographic analysis of emulsifiers containing polyethylene oxide are discussed. Experimental details for the determination of this type of emulsifier, by paper-chromatographic analysis, in lemonade are given. W. E. ALLSEBROOK.

Physical and biological changes in an artificial fat emulsion during storage. J. Boberg and I. Håkansson (*J. Pharm. Pharmacol.*, 1964, **16**, 641—646).—100-ml. samples of a commercial soya-bean emulsion (' Intralipid '), used for parenteral nutrition, were stored in the dark at 4, 20 and 40° for up to 2 years. During storage the pH fell (more rapidly during the first 3 months and at higher temp.); free fatty acids were released (at higher levels at 40°, reaching 15 times the amount released at 4° after 700 days' storage); K_m and $V_{max.}$ (Michaelis–Menten constant) for the lipoprotein–lipase reaction increased significantly (40° samples); the dia. of particles in the emulsion increased from $<0{\cdot}5\mu$ to up to $0{\cdot}5$—$1{\cdot}0\mu$ and emulsion stored at 40° showed an increasing lethal effect on rabbits. The emulsion may be stored satisfactorily at 4° for up to 2 years. (11 references.) A. T. CARPENTER.

Biological influence of antioxidants. Z. Placer, Z. Slabochová and A. Veselková (*Nahrung*, 1964, **8**, 333—339).—A lecture. A protein-like antioxidant was discovered in association with the α_2- (and partly with the β-) globulin fraction of human blood serum. The inhibitor is thermolabile and non-dialysable; a concentrate of 300 times the normal strength has been prepared. The function of the inhibitor is considered. (13 references.) A. S. ARUP.

Meat and Poultry

Relationships of free and bound water to subjective scores for juiciness and softness and to changes in weight and dimensions of steaks from two beef muscles during cooking. S. J. Ritchey and R. L. Hostetler (*J. Fd Sci.*, 1964, **29**, 413—419).—Steaks (1 in.) from *longissimus dorsi* (**I**) and *biceps femoris* (**II**) were cooked to an internal temp. of 61, 68, 74 or 80°. **I** contained a higher % of free water at 74 and 80°. **I** became shorter, wider and thinner and **II** became longer, narrower and thinner at 61, 68 and 74°. A large % of the total change in free and bound water or in dimensions of steaks occurred between 74—80°. The meat became drier and harder at each increase in temp. (17 references.) E. M. J.

Detection and isolation of multiple myoglobins from beef muscle. J. R. Quinn, A. M. Pearson and J. R. Brunner (*J. Fd Sci.*, 1964, **29**, 422—428).—There appear to be at least four electrophoretically-distinct myoglobins in bovine muscle. They are separated on DEAE cellulose columns and show the same electrophoretic mobility before and after chromatography. (17 references.) E. M. J.

Characterisation studies of three myoglobin fractions from bovine muscle. J. R. Quinn and A. M. Pearson (*J. Fd Sci.*, 1964, **29**, 429—434).—The three fractions were compared with respect to absorption spectra, relative haem contents, susceptibility to acid cleavage of haem groups and autoxidation rates. All three had identical wavelength positions for min. and max. light absorption and had the same autoxidation rates. The differences in absorptivity values imply dissimilarities in the weaker haem linkages, i.e., the porphyrin–globin bonds. (21 references.) E. M. J.

Nitrogen factors for liver. Analytical Methods Committee (*Analyst*, 1964, **89**, 630—631).—N contents determined on a variety of liver samples are given. Average N factors of 3·55, 3·45 and 3·65 are respectively suggested for livers of unknown origin, ox livers and pig livers. E. C. DOLTON.

Reaction products of nitrite and nitrate in meat products. I. Determination of nitrite and nitrate. K. Möhler (*Z. Lebensmitt-Untersuch.*, 1964, **125**, 337—340).—The aq. extract is prepared by the method of Grau and Mirna (cf. *Anal. Abstr.*, 1958, **5**, 2390), and the determination is carried out by the method of Follett and Ratcliff (cf. J.S.F.A. Abstr., 1963, i, 327). P. S. ARUP.

Some calculations with sausages. E. Bohm (*Dtsch. Lebensmitt-Rdsch.*, 1964, **60**, 319—320).—Curves showing the connexion between fat % and moisture/protein ratio of sausages of various fat/protein ratios are presented and their use in the calculation of the original composition of sausages from determinations of fat, moisture and ash, or fat and protein on the sample as received are described with numerical examples The calculations are based on the assumption of an original moisture/protein ratio of 4·0 and ash content of 2·5%; the effects of differences in the actual orginal ash contents and moisture/protein ratios on the calculated original composition are discussed. E. C. APLING.

Chlorophyll catalysis of fat peroxidation. J. L. Hall and D. L. Mackintosh (*J. Fd Sci.*, 1964, **29**, 420—421).—Peroxide was apparently detected in freshly prepared sausage. No peroxide appeared in the extracts of pork fat alone or in extracts of sage alone. But mixtures of fat extract with sage extract developed peroxide; similarly extracts of green leafy material from other species and purified chlorophyll developed peroxides with fat extracts. This effect is probably due to the porphyrin structure in the chlorophyll mol. The incipient peroxide was developed not in the sausage itself but in the extracts exposed to light in the analytical laboratory. As long as chlorophyll remained an integral portion of the plant structure, it made insufficient contact with fat in the sausage to promote its oxidation when exposed to light. E. M. J.

Effect of inorganic polyphosphates on the solubility and extractability of myosin B. T. Yasui, M. Sakanishi and Y. Hashimoto (*J. agric. Fd Chem.*, 1964, **12**, 392—399).—The solubility of myosin B, and the extractability of proteins from myofibrils in the presence of various polyphosphates and chloride solutions were studied and compared. (33 references.) W. ELSTOW.

Specific interaction of inorganic polyphosphates with myosin B. T. Yasui, T. Fukazawa, K. Takahashi, M. Sakanishi and Y. Hashimoto (*J. agric. Fd Chem.*, 1964, **12**, 399—404).—The specific interaction of inorg. polyphosphates with myosin B was studied by different methods. The protein extracted from myofibrils is almost identical with myosin B. The possible rôle of inorg. polyphosphates in improving the binding properties and water-holding capacities of meat and meat products is discussed. (27 references.) W. ELSTOW.

Enzymic studies of bruised poultry tissue. W. E. Brown and M. K. Hamdy (*J. Fd Sci.*, 1964, **29**, 407—412).—In bruises on poultry, experimentally inflicted, the activity of acid phosphatase (**I**) increased and that of alkaline phosphatase (**II**) decreased. Activities of both enzymes were influenced by age of bruise, severity of muscular injury and previous bruise history. Activity of **I** in the bruise was approx. twice that of control activities by the 4th day. Activity of **II** decreased (5.%) within 24 h. of bruising. Under ordinary assay conditions appreciable **I** was bound in lysosomes whereas no bound activity was found in normal tissue. (33 references.) E. M. J.

Incidence of salmonellae in dressed broiler-fryer chickens. M. Woodburn (*Appl. Microbiol.*, 1964, **12**, 492—495).—Meat from the tail end and giblets was used in the sampling and salmonellae were isolated from 72 of 264 animals. An equal number of dressed whole and cut-up birds were positive for this organism; 13 different serotypes were identified and the methods used in typing are described. In general it was found that there were a larger number of positive specimens in locally produced and processed animals; this however was not true for the autumn period. (16 references.) C. V.

Polyphosphate inhibition of growth of pseudomonads from poultry meat. R. P. Elliott, R. P. Straka and J. A. Garibaldi (*Appl. Microbiol.*, 1964, **12**, 517—522).—Both commercial and chemically pure polyphosphates (**I**) inhibit the growth of non-fluorescent strains grown on synthetic media. Fluorescent strains grew after a short adaptation period. Inhibition does not arise from high pH but by chelation of essential metal ions such as Mg. Chilling chicken

carcasses overnight in slush ice containing 3—8% **I** lengthened subsequent shelf life 17—25%; if held in continuous contact with this concn. **I** during storage at 2·2° the time period was lengthened by 17—67%, likewise chickens held in antiseptic ice containing 8% **I** kept 60% longer than those preserved in water ice. (46 references.) C. V.

Sausage meats. Armour & Co. (B.P. 940,003, 13.3.62. U.S., 14.3.61).—A method of processing a meat component for a sausage product, which facilitates handling, transportation and storage, comprises mixing water, NaCl and finely ground meat fat and meat protein, to form a non-greasy emulsion (wherein the aq. phase is substantially continuous) and then freeze-drying the emulsion. There is thus obtained a dry, stable product which can be readily rehydrated to its original emulsified state. F. R. BASFORD.

Fish

Quality changes in stored refrozen cod fillets. W. J. Dyer, D. I. Fraser, D. G. Ellis, D. R. Idler, W. A. MacCallum and E. Laishley (*Fish. Res. Bd Can.*, 1963, *Studies*, 1964, No. 781, 10 pp.).—The organoleptic quality of round trap cod was not greatly affected by storage at −23° to −26° during periods up to 6 months, but considerable decreases in the organoleptic scores occurred in fillets prepared from this cod during the first two months in storage at −23° or (especially) at −18°; the rates of deterioration were similar for fillets from round cod that had been stored during 0—4 months. The results are considered with respect to possible chemical and physical changes in the stored fish. (12 references.) P. S. ARUP.

Examination and quality evaluation of fresh and preserved herrings. G. Hensel and J. Wurziger (*Dtsch. LebensmittRdsch.*, 1964, **60**, 311—315).—The significance of changes in the fat phase of herrings is discussed and typical results obtained from the examination of fresh and preserved (salted, marinaded, etc.) herrings by a modified alkali-colour method (cf. *Fette Seif. Anstrichm.*, 1950, **60**, 99) and the Lea peroxide test (Sully, *Analyst*, 1954, **79**, 86) are reported. The results by the two methods agreed well with organoleptic evaluation; for fish of good quality values were generally <10 and <5 respectively. An alkali-colour value >20 is a firm indication of taint. E. C. APLING.

Assessment of the quality of canned Northern Bluefin tuna *Kishinoella tonggol* (I). A. R. Prater and W. A. Montgomery (*J. Sci. Fd Agric.*, 1964, **15**, 885—889).—Canned packs of **I** were compared organoleptically with those of Southern Bluefin (**II**) and Yellowfin after ageing for 1 or 6 months. All were acceptable; **I** was not inferior to **II** (the species mainly used in commercial packs). The quality was not improved by ageing for 6 months. The similarity of the patterns in the gas-liquid aroma profiles for the three species as confirmed by statistical examination lends support to results of tasting test, that all three species are equally acceptable on the basis of flavour. E. M. J.

Spices, Flavours, etc.

Arylalkenones and their preparation and use. International Flavours & Fragrances (I.F.F.) (Inventors: M. G. J. Beets, W. Meerburg and W. J. Wiegers (B.P. 941,143, 9.4.61.).—The title compounds, which have the general formula $CMeR{\cdot}CH_2{\cdot}CO{\cdot}CH{:}CHR''$ (R is Ph or tolyl; R′ and R″ are H, Me or Et, but with a total of ⩾2C) are useful as perfume and flavouring ingredients. A typical product is 2,5-*dimethyl*-5-*phenylhexen*-3-*one*, b.p. 110°/0·3 mm., obtained in 75—80% yield by heating 2-acetoxy-2,5-dimethyl-5-phenylhexan-3-one for 4 h. at 400°/100—110 mm. in a glass tube packed with glass helices, then distilling the washed product. F. R. BASFORD.

Colouring matters

Sensitive method for determination of cadmium in food colouring matters. E. Kröller (*Z. LebensmittUntersuch.*, 1964, **125**, 401—405).—Improvements are made in the spectrophotometric micro-determination method of Chavanne and Geronimi (cf. *Anal. Abstr.*, 1959, **6**, 2890), based on the reaction of Cd with *p*-nitrophenyldiazoaminobenzene-*p*-azobenzene (Cadion). The modified method is accurate within ±10%, and permits of the detection of 0·04 mg./kg. of dye. P. S. ARUP.

Preservatives

Definitions of food additives in American food law. Conclusion. A. Schwarz (*Dtsch. LebensmittRdsch.*, 1964, **60**, 247—253).—A detailed assessment of American food law in its application to the control of food additives. (42 references.) E. C. APLING.

Physical and chemical characteristics of cellulose ethers used as food additives. II. Methods of testing cellulose ethers for identity and purity. F. Crössmann, W. Klaus, E. Mergenthaler and S. W. Souci (*Z. LebensmittUntersuch.*, 1964, **125**, 413—427).—In continuation of Part I (cf. Souci *et al.*, *ibid.*, 247), descriptions are given of the methods employed. The more specialised methods include the viscosimetric determination of mol. wt. and the degree of polymerisation, and determinations of methoxyl and hydroxyethoxyl, by the adaptation of known methods. P. S. ARUP.

Preservative medium for vegetable matter. P. R. Biggs (B.P. 939,932, 10.2.61. S.A., 12.2.60).—The medium consists of a water-sol. non-ionic org. nutrient medium permeable to protoplasm, water-sol. compounds producing SO_3^{2-}, SO_4^{2-}, PO_4^{3-}, K and B ions in solution and a fermentation- and mould-inhibitor from which heavy metals and synthetic halogenated compounds are excluded. It should have pH 2·5—5·5. In an example, to 13·5 lb. of sucrose dissolved in 12 pints of water are added 1·5 g. of K_2SO_4, 0·5 g. of H_3BO_3, 56 g. Na Benzoate and 40 g. of H_3PO_4 and the solution diluted 1 : 13. The fruit to be preserved is dipped into this solution and wrapped in polythene film. C. A. P.

Preservation of potato or potato pieces. J. R. Simplot Co. (Inventors: R. L. Shaw, jun., R. W. Kueneman and J. E. Conrad) (B.P. 940,802, 8.5.61).—Preservation is effected by defrosting frozen material (after parboiling and partly drying), boiling again, then immersing in an aq. starch solution (of >1% concn.) in a closed vessel. F. R. BASFORD.

Preservative compositions containing *p*-hydroxybenzoic acid esters R. Ueno (B.P. 940,379, 11.4.60. Jap., 9.4. and 2. and 23.10.59).—A liquid preservative composition is a mixture of two or more *p*-hydroxybenzoic acid esters or one or more *p*-hydroxybenzoic acid esters and one or more of 2-methyl-1,4-naphthaquinone, dehydroacetic acid, salicylic acid, sorbic acid, butylhydroxyanisole, butylhydroxytoluene, 2,4,5- or 2,4,6-trichlorophenol, pentachlorophenol, n-propyl gallate and isopentyl gallate, the proportions being such that the mixture is liquid at room temp. E. ENOS JONES.

Pesticides in Food

Use of thin-layer chromatography in food analysis. I. Detection of insecticides containing chlorine. II. Rapid detection of fat-soluble vitamins A, D and E. E. Ludwig and U. Freimuth (*Nahrung*, 1964, **8**, 559—561, 563—565).—I. The insecticides are extracted from fruits with C_6H_6, $CHCl_3$ or light petroleum; the conc. extracts are chromatographed on plates coated with Supergel VEB (Wolfen) containing ~15% of gypsum that has been well shaken with 0·04% aq. fluorescein (Na salt). The chromatograms, developed with CCl_4–light petroleum (1 : 1), are dried and then steamed for examination in u.v. light. R_F values are given for DDT, γ-HCH and Methoxychlor. The min. detectable amounts are 0·5 μg. Semi-quant. determinations can be made by comparison with standard solutions.

II. The vitamins can be obtained from pharmaceutical prep. by simple extraction, or from the carefully prepared unsaponifiable matter of biological fatty matter. The plates are prepared as described in Part I with water instead of the fluorescein solution. Cyclohexane–AcOEt is used as developing solvent. The spots are revealed by spraying with 20% ethanolic tungstophosphate and warming to 70—80° and identified by comparison with parallel runs made with standard solutions of the vitamins. The spots are scraped off and extracted with Et_2O for spectrophotometric determination of the vitamins. Recoveries are 75—90%. P. S. ARUP.

Quantitative polarographic determination of DDT. F. Fehér and H. Monieni (*Z. anal. Chem.*, 1964, **204**, 19—25).—The sample (⩾75 mg. DDT) is polarographed in a mixture of 5 ml. acetone/water 3 : 1 and 5 ml. 0·1M-tetramethylammonium bromide in 80% ethanol. In this medium *p,p′* DDT has a half-wave potential of −1·05 V; *o,p′* DDT −1·09 V; CTC −1·02 V, while DDD is not detected by this technique. T. R. ANDREW.

Labilisation of chlorine from polychlorinated dimethanonaphthalene insecticides with sodium monoethanolamine. R. V. Krishna, K. Krishnamurthy and S. K. Majumder (*Indian J. Technol.*, 1964, **2**, 212—213).—The effect of reaction time on the release of Cl from aldrin, dieldrin and endrin at 100—110° was studied using Na metal and mono-(**I**) and di-ethanolamine (**II**). With ~5 mg. of insecticide, 1 ml. of **I** or **II**, 0·2—0·5 mg. of Na metal and heating for 2 h. at 100—110° in stoppered glass tubes, 2 Cl atoms are rendered labile. The Cl released is determined potentiometrically. E. C. DOLTON.

Separation of chlorinated pesticides by loose-layer chromatography for further identification by gas-liquid chromatography. A. Taylor and B. Fishwick (*Lab. Pract., Lond.*, 1964, **13**, 525).—The sample in n-hexane (**I**) is chromatographed on a loose layer (20 cm. × 0·5 mm.) of 120-mesh unactivated Al_2O_3 with **I** as mobile solvent. A complete separation of DDE from dieldrin and of aldrin from heptachlor epoxide is obtained in ~5 min. By sectioning the layer and eluting the pesticides from each section (placed in small glass columns) with **I**, the eluates can be analysed by gas–liquid chromatography. W. J. BAKER.

Analysis of organo-phosphorus pesticide residues by gas chromatography. H. Egan, E. W. Hammond and J. Thomson (*Analyst*, 1964, **89**, 175—178).—The sample (50 g.) is macerated three times in a high-speed macerator with methyl ethyl ketone–hexane (3 : 1), the combined non-aq. phases are passed through anhyd. Na_2SO_4, and evaporated to ~20 ml. The residue in hexane is shaken twice with 5% aq. Na_2SO_4. The combined hexane extracts are evaporated to 5 ml. and applied to a column of activated alumina in hexane and the column is eluted with hexane. After suitable concentration, portions of the eluate are injected into a gas chromatograph. For malathion certain difficulties that occur in the elution from the alumina column are overcome by the use of a column of MgO. A. O. JONES.

Use of gas chromatography by food and drug administration for pesticide residue analysis. H. L. Reynolds (*J. Gas Chromatogr.*, 1964, **2**, 219—222).—A review, with 18 references, is presented. C. PEARCE.

Improved procedure for extraction of organo-choline pesticides from animal tissue. A. Taylor, R. E. Rea and D. R. Kirby (*Analyst*, 1964, **89**, 497—498).—The sample was macerated with anhyd. Na_2SO_4 and acetone (**I**) and macerated tissue was filtered under low pressure. The macerate was homogenised with more **I** and filtered. Water and n-hexane (**II**) were added to the filtrate and after shaking, the **II**-layer was run off. The **I**-extract was re-extracted with **II** and **II**-layers separated. After drying the **II**-layers were examined by gas-liquid chromatography. C. A. P.

Use of infra-red spectroscopy in analysis of pesticide residues. N. T. Crosby and E. Q. Laws (*Analyst.*, 1964, **89**, 319—327).—Unknown residues of organo-phosphorus pesticides are separated from co-extruded plant residues by column chromatography. Separated extracts were isolated by gas chromatography using a semi-preparative chromatograph. Columns are C-shaped stainless-steel tubes packed with glass beads coated with Epikote resin and Apiezon L grease. A diagram of the instrument is given. The effluent after treatment is transferred to a cavity cell and examined in an i.r. spectroscope using a beam condenser in the sample beam and a variable-path cell, filled with solvent, in the reference beam. A table gives the pesticides identified and estimated down to 0·1 p.p.m. level and comparison is made between i.r. and P methods. (10 references.) C. A. P.

Recovery of malathion from a range of stored products. A. N. Bates and D. G. Rowlands (*Analyst*, 1964, **89**, 288).—Recoveries from 15 plant products, with and without preliminary chromatography (**I**) of plant extracts, are given in a table. **I** is necessary when emulsions are encountered with unpurified plant extracts or when the recovery is raised by this means. C. A. P.

Recovery of malathion residues from pimento. D. G. Rowlands (*Analyst*, 1964, **89**, 498—500).—Satisfactory recoveries can be obtained by chromatography on acid-washed alumina but preliminary chromatography on polyethylene-coated alumina is essential to ensure effective running of columns. C. A. P.

Determination of parathion and related insecticides by gas-liquid chromatography. J. A. Dawson, L. Donegan and E. M. Thain (*Analyst*, 1964, **89**, 495—496).—A Pye Argon chromatograph with a β-ionisation detector fitted with a ^{90}Sr source modified for electron-capture detection was used. The column consisted of Epikote resin 1001 and silicone astatomer E301 supported on 100- to 120-mesh Celite. O_2-free N_2 was the carrier gas. Recovery of fenitrothion was 95% and limit of sensitivity 0·1 p.p.m. in the injected solution. Cocoa extract gave a decreased sensitivity due to extraneous extractives that emerge from the column at the same time as the fenitrothion. Related insecticides were studied and it was found that paraoxon behaved differently from the others, sensitivity of detection being only one-twentieth of that of parathion, chlorthion and fenitrothion. C. A. P.

Synthesis of α-methylbenzyl 3-(dimethoxyphosphinyloxy)-crotonate (I) labelled with phosphorus-32 and carbon-14. J. C. Potter and W. B. Burton (*J. agric. Fd Chem.*, 1964, **12**, 439—442).—The synthesis of **I** to facilitate the measurement of residues and metabolites is described in detail. W. ELSTOW.

Petroleum fractions as DDT solvents. A. M. Thomas, jun. (*J. agric. Fd Chem.*, 1964, **12**, 442—447).—A study has been made of the solubility of DDT in alkylbenzenes and condensed ring aromatic solvents in connexion with the prep. of emulsifiable concentrates. Total aromatics content, mol. wt. and the structure of aromatic compounds determine solvency for DDT. (14 references.) W. ELSTOW.

Identification of isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) with *m*-chloroaniline using thin-layer chromatography. R. Liebmann and H. Schuhmann (*Chem. Tech., Lpz.*, 1964, **16**, 267—268).—CIPC, used to prevent germination in stored potatoes, forms toxic *m*-chloroaniline on hydrolysis. Potatoes contain only ~0·4 mg. of CIPC/kg. and this can be analysed by thin-layer chromatography to a sensitivity of 375 μg. of residue in 500 mg. extract. Residues are extracted by mixing the potatoes with CH_2Cl_2 and chromatographing on Kieselgel G with n-hexane 40 vol.-%/CH_2Cl_2 60 vol.-%. The plate is developed with Mitchell's reagent, dried and irradiated with u.v. light, when CIPC appears as narrow blue stripes and *m*-chloroaniline as wider brown ones. No *m*-chloroaniline was found with a 10-fold overdose of CIPC. (14 references.) M. GREENAWAY.

Colorimetric determination of *o*-isopropoxyphenyl *N*-methylcarbamate. P. Bracha (*J. agric. Fd Chem.*, 1964, **12**, 461—463).—The *o*-isopropoxyphenyl-*N*-methylcarbamate was extracted from surfaces with methyl alcohol, hydrolysed with dil. NaOH and determined by reacting it with diazotised 3-nitroaniline-4-sulphonic acid and reading the extinction of the red water-sol. dye formed. The method was adapted to five other carbamic acid pesticides, Sevin, Dimetilan, Pyrolan, Isolan and *m*-isopropylphenyl-*N*-methylcarbamate. W. ELSTOW.

Determination of β-hydroxyethylhydrazine in pineapples. M. P. Thomas and H. J. Ackermann (*J. agric. Fd Chem.*, 1964, **12**, 432—433).—β-Hydroxyethylhydrazine (**I**) is extracted by blending the fruit with water and the extracted **I** is purified by passage through an OH^- resin column and absorption and subsequent elution from a H^+ resin column. The purified extract in dil. HCl is reacted with cinnamaldehyde and the extinction of the yellow colour produced measured at 420 mμ. W. ELSTOW.

Identification of metabolites of Zectran insecticide in broccoli. E. Williams, R. W. Meikle and C. T. Redemann (*J. agric. Fd Chem.*, 1964, **12**, 453—457).—The following metabolites were detected in the flower of broccoli plants treated with Zectran (4-dimethylamino-3,5-xylylmethyl carbamate): 4-dimethylamino-3,5-xylenol, 2,6-dimethylhydroquinone, 2,6-dimethyl-*p*-benzoquinone and 4-dimethylamino-3,5-dimethyl-*o*-benzoquinone. Evidence that some of the metabolites were incorporated into the lignin structure is given. (15 references.) W. ELSTOW.

Analysis of animal food products for chlorinated insecticide residues. I. Column clean-up of samples for electron-capture gas chromatographic analysis. B. E. Langlois, A. R. Stemp and B. J. Liska (*J. Milk Tech.*, 1964, **27**, 202—204).—The one-step method described can be applied to a variety of animal product samples with excellent success, 25—35 samples being analysed in an 8-h. day. Examples are given. C. V.

Extraction of Tinox and chlorinated hydrocarbons and their separation from one another. H. Ackermann and R. Knoll (*Nahrung*, 1964, **8**, 435—438).—The solids obtained by homogenising and centrifuging vegetables with CH_2Cl_2 or $CHCl_3$ followed by evaporation of the solvent are extracted with n-hexane (saturated with MeCN); on extraction of the C_6H_{14} solution with water, the colouring matters and the chlorinated hydrocarbons remain in the org. phase whilst the sulphoxides (metabolites of Tinox or other thiophosphoric esters) pass into the water from which they can be extracted with $CHCl_3$; the extracts are then concentrated and analysed separately by thin-layer chromatography (cf. Woggon, *ibid.*, 1963, **7**, 612). Recoveries of Tinox (as sulphoxide) are 91—94% and of HCH and heptachlor 80—90%. The min. detectable amounts are 0·3, 5 and 5 p.p.m., respectively. P. S. ARUP.

Automated analysis of phosphorus-containing compounds in biological materials. I. A quantitative procedure. L. H. Weinstein, R. F. Bozarth, C. A. Porter, R. H. Mandl and B. G. Tweedy (*Contr. Boyce Thompson Inst.*, 1964, **22**, 389—397).—The method uses a Technicon Autoanalyser and a continuous sample from the digestor unit is diluted with water and reacted with molybdate and 1-amino-2-naphthol-4-sulphonic acid. The colour is developed at 95° and the absorbence measured continuously at 810 mμ. The standard error is lower than with the u.v. spectroscope method. E. C. DOLTON.

Method for separating some triazine degradation products from plants. P. H. Plaisted and M. L. Thornton (*Contr. Boyce Thompson Inst.*, 1964, **22**, 399—403).—The degradation products are dissolved in 80% ethanol, partially purified by sorption on and elution from

a cation-exchange resin and the resultant solution sorbed on to a cation-exchange resin and eluted with dil. HCl. The results of preliminary tests with groundnuts, maize and soya-bean are given. E. C. DOLTON.

Food Processing, Refrigeration

Micro-waves [in food processing]. M. R. Jeppson (*Food Engng*, 1964, **36**, No. 11, 49—52).—The application to drying, thawing, cooking, pasteurising and baking are briefly reviewed and examples are given, e.g., raw potato slices can be dried to 2% moisture in 4 min., the resulting product being partially cooked and slightly foamed, and thawing times can be reduced from 20 h. or more with conventional methods to 20—30 min. in a continuous process. C. V.

Tentative classification of food irradiation processes with microbiological objectives. H. E. Goresline, M. Ingram, P. Macúch, G. Mocquot, D. A. A. Mossel, C. F. Niven, jun., and F. S. Thatcher (*Nature, Lond.*, 1964, **204**, 237—238).—The main food irradiation processes have been classified into three types: I consists of processes which reduce viable organisms to non-detectable limits, II reduce viable specific non-spore-forming pathogenic micro-organisms to non-detectable limits and III reduce the no. of viable specific spoilage micro-organisms sufficiently to enhance keeping quality. The names suggested for these types are radappertisation, radication and radurisation respectively. S. A. BROOKS.

Irradiation [and food products]. Anon. (*Food Engng*, 1964, **36**, No. 11, 53—54).—A brief review of the present position. It is pointed out that this treatment cannot replace canning, freezing or dehydration but can result in an extended shelf life and an overall improved standard of foods. C. V.

Dehydrated food products. Pillsbury Co. (B.P. 940,309, 20.12.61. U.S., 28.12.60).—Dehydration of moist food material (e.g., fruit, vegetable, meat, fowl, seafood, a condiment, cereal, etc.) is effected, to give a product which is readily rehydrated to substantially the original form, by enveloping particles of the food in a fluid medium under partial vacuum (the medium consisting of a foam-like mixture of vapour and hot oil at a temp. above the vaporisation point of moisture in the material at the applied partial vacuum), whereby moisture is rapidly evolved. The free oil is then separated from the dehydrated material. Apparatus is described. F. R. BASFORD.

Packaging

Packing and shelf-life of foodstuffs. W. Bartusch (*Brot u. Gebäck*, 1964, **18**, 169—172).—The desirable properties of packaging materials are discussed in relation to food properties (susceptibility to moisture change, sensitivity to O or light, etc.). The properties of available packaging films are reviewed and representative figures for permeability by moisture vapour and dry gases are tabulated. E. C. APLING.

Uptake of lead and tin by aqueous contents of tin plate cans. K. G. Bergner and H. Miethke (*Z. LebensmittUntersuch.*, 1964, **125**, 406—413).—Numerous trials in which fruit, vegetables and milk products were stored in experimental tins (lacquered and unlacquered) proved the uptake of Pb from the tin plate to be negligible; increases in the content of Pb derived almost entirely from exposed solder. Even in the case of spinach (the most aggressive material) the uptake of Pb was $\gtrsim$0·55 mg./kg. No relationship was found between the uptake of Pb and that of Sn. (25 references.) P. S. ARUP.

Testing of plastic objects. Detection of stabilisers for PVC by thin-layer chromatography. O. Korn and H. Woggon (*Nahrung*, 1964, **8**, 351—353).—Most stabilisers can be extracted from the comminuted samples with Et_2O. R_F values are given for phenylurea, diphenylurea, diphenylthiourea and 2-phenylindole, chromatographed on SiO_2-gel and Na silicate, with $CHCl_3$ as ascending solvent. Distinguishing colour reactions with two spraying reagents based on dimethylaminobenzaldehyde and on dichlorodiphenylmethane are tabulated. P. S. ARUP.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Improvement in the protein efficiency of soya-bean concentrates and isolates by heat treatment. J. B. Longenecker, W .H. Martin and H. P. Sarrett (*J. agric. Fd Chem.*, 1964, **12**, 411—412).—The protein efficiencies, as judged by tests with rats, of all but one of three soya-bean concentrates ($\simeq$60% protein) and four soya-bean isolates ($\simeq$90% protein) were improved by a mild heat treatment, i.e., in contact with live steam at 105° for 30 min. and drying at 100° for 1 h. (15 references.) W. ELSTOW.

Dried Torula yeast—a valuable product for human nutrition. H. Wittmann (*Dtsch. LebensmittRdsch.*, 1964, **60**, 308—310).—The nutrient composition of Waldhof-Torula dried yeast is reported and its use as a source of important amino-acid vitamins and minerals for the improvement of the nutritional quality of a variety of foodstuffs is advocated. (17 references.) E. C. APLING.

Method for determining 5′-nucleotides. H. G. Lento, J. A. Ford and A. E. Denton (*J. Fd Sci.*, 1964, **29**, 435—442).—A method is described for the separation and quant. determination in foods of the 5′-nucleotides: cytidine 5′-phosphate, adenosine-5′-phosphate, uridine-5′-phosphate, inosine-5′-phosphate and guanosine-5′-phosphate. A Dowex-1 ion-exchange resin (formate form) is used to adsorb and concentrate the nucleotides from an aq. extract of the food sample. The nucleotides are separated and eluted in the order previously given by a gradient elution system of water–formic acid–Na formate. Complete resolution from each other is obtained, but not from their 2′- and 3′-forms. After chromatographic separation, the 5′-nucleotides are determined colorimetrically in the presence of 2′- and 3′-nucleotides by oxidation with periodate and reaction of the oxidation products with 2,4-dinitrophenylhydrazine. (16 references.) E. M. J.

Effect of pretreatment on the extraction of proteins from plant sources. M. K. Rastogi and C. R. Krishna Murti (*Biotechnol Bioengng*, 1964, **6**, 167—171).—The effects of solvent extraction and heat pretreatments on the extraction of proteins by alkali peptisation and isoelectric pptn. was studied. The solubilisation is decreased by ethanol extraction and heating under pressure but is unaffected by n-hexane extraction. Heat treatment prior to trypsin digestion increases the degree of hydrolysis of the protein isolated from un-autoclaved materials. (10 references.) E. C. DOLTON.

Rheology of soya protein dispersions. Effect of heat and other factors on gelation. S. J. Circle, E. W. Meyer and R. W. Whitney (*Cereal Chem.*, 1964, **41**, 157—172).—Study of the rheological properties of aq. dispersions of a commercial edible isolated soya protein-ate prep. showed that in the absence of heat, η rose exponentially with concn. Rate of gelling and gel firmness were dependent on temp., time of heating and protein concn., with a temp. threshold of 65°. 8—14% gels are disrupted if overheated at 125° but above 16—17% the gels were less susceptible to heat disruption. Salts added to 10% dispersions lower the η before heating, but raise the η of the heated dispersion. η of both heated and unheated 10% dispersions were raised by additions of soya oil, soya lecithin, wheat starch, carboxymethylcellulose or carrageenan, but were profoundly lowered by additions of Na_2SO_3 or cysteine, with inhibition of gelation. (26 references.) E. C. APLING.

Rape meal. IV. Spectrophotometric detection of alterations in quality during industrial extraction of oil from rape. J. Pokorný, A. Rutkowski, J. Hrdlička and H. Kozlowska (*Nahrung*, 1964, **8**, 545—557).—In connexion with previous work (cf. *ibid.*, 537) extracts of meals prepared with 80% EtOH, water, 10% aq. NaCl and 0·2% aq. NaOH were prepared from meals that had been extracted by four different processes. The extinction values at five different λ of these extracts were individually compared with extracts prepared in a Soxhlet apparatus. The spectra of the aq. EtOH and aq. NaOH extracts were of interest in connexion with the formation of products of the Maillard reaction. P. S. ARUP.

Stability and stabilisation of ascorbic acid in raw-diet salads. M. Zobel (*Ernährungsforschung*, 1964, **9**, 248—265).—Advice based on a review of the literature is given on the prevention of losses of ascorbic acid in salads, vegetables and fruits. Treatments involving the prolonged exposure of the cut or comminuted tissues to air must be avoided. Losses in comminuted tissues can be retarded by immediate treatment with vinegar, fruit juice, sour milk, etc. (31 references.) P. S. ARUP.

Specific detection and quantitative determination of dehydroascorbic acid by paper chromatographic distribution. W. Fürtig and R. Pohloudek-Fabini (*Pharmazie*, 1964, **19**, 209—215).—Dehydroascorbic acid (**I**) and ascorbic acid are separated by paper chromatography with isopropyl alcohol–2% oxalic acid (7 : 3) as the solvent. When heated with *p*-thiocyanatophenylthiosemicarbazide, **I** gives a yellow colour which can be eluted with ethanol and measured in an absorptiometer. As little as 20 μg. of **I** can be determined. During the separation, some of the **I** is converted into 2,3-diketogulonic acid (**II**); the amount of **I** in the original sample is calculated from the sum of **I** and **II**. A. R. ROGERS.

Magnesium—a vitamin E synergist? H. Kronberger, O. Selisko

and H. Ackermann (*Ernährungsforschung*, 1964, **9**, 266—271).—Intramuscular injections of Mg adipate failed to relieve the effects of vitamin-E deficiency in chicks. P. S. ARUP.

Folic acid activity of Indian dietary articles and the effect of cooking on it. D. K. Banerjee and J. B. Chatterjea (*Food Technol.*, 1964, **18**, No. 7, 137—139).—Articles of food (54) of vegetable and animal origin were analysed, fresh materials being extracted in the raw, boiled and cooked states. In vegetables the folic acid activity, in descending order, was found in *Hinche sak* (*Enhydra fluctuens*), spinach, coconut; other vegetables were good sources; rice and pulses contained significant amounts. Germination of pulses resulted in an increase of the activity; a few fruits showed some activity. In the fish tested, folic acid activity was high, the largest amount being recorded in Rui (138 μg./100 g.), but the highest activity was in goat liver (255 μg./100 g.); in duck egg and cow's milk it was significant. Of 21 articles examined in raw and boiled states, 12 showed marked increase in folic acid activity from boiling; in general activity was less after cooking than after boiling. (15 references.) E. M. J.

Ubiquinone-40 and vitamin K_2 (40) in *Chromatium vinosum*. L. K. Osnitskaya, D. R. Threlfall and T. W. Goodwin (*Nature, Lond.*, 1964, **204**, 80—81).—The lipid extract (64 mg.) of cells (204 mg. dry-wt.) of the purple S bacteria *Chromatium vinosum* was chromatographed on Al_2O_3; the fraction (100 ml.) eluted with 1% ether–light petroleum contained vitamins K (1·1 mg.) and that with 5% ether–light petroleum contained ubiquinone (**I**). Concn. per g. of dry cell-material were estimated, from spectroscopic data, as 3—3·5 μM of vitamin K and 2·5 μM of **I**. Further purification of the two fractions by thin-layer chromatography permitted identification of the lipophilic quinones and chracterisation of the two substances as vitamin K_2 (40) and ubiquinone-40, respectively. The three strains of *Chromatium* so far examined each contain a different homologue of **I**. (Cf. *ibid.*, 1959, **184**, 1339; *Biochim. biophys. Acta*, 1963, **78**, 532.) W. J. BAKER.

Vitamin-A aldehyde complex. Eastman Kodak Co., Assee of C. H. Benton, jun. (B.P. 941,952, 28.9.60. U.S., 15.10.59).—A product suitable for fortifying animal feeds is the cryst. complex of 1 mol. each of 2,6-*trans*, *trans*-vitamin-A aldehyde and 2,4,5-butyrophenone. H. S. R.

Dry vitamin-containing preparations. Løvens Kemiske Fabrik ved A.Kongsted (B.P. 941,359, 21.10.59).—A solution of one or more fat-sol. vitamins in edible hydrophobic solvent (e.g., vegetable or animal oil or fat) is dispersed in aq. homogeneous phase containing materials which on drying afford a homogeneous matrix, and the mixture is dried in presence of *p*-aminophenol (or a salt thereof) and ground, or is divided into drops which are dried. There is thus obtained a highly stable vitamin prep. in digestible form. The aminophenol may be added before or during the dispersion stage. F. R. BASFORD.

Unclassified

Quantification of salmonellae in foods using the pre-enrichment method of North. A. E. Hall, D. F. Brown and R. Angelotti (*J. Milk Tech.*, 1964, **27**, 235—240).—Using laboratory prepared chicken pie-filling excellent recovery was achieved in the range of four to hundreds of thousands of salmonellae per 100 g. food. The technique is cumbersome with large no., hundreds per g. but exceedingly well adapted to smaller concn. The method was specific despite the presence of natural food flora or the addition of organisms normally associated with food. None of the natural food constituents interfered with the sensitivity or specificity of the method. C. V.

Bacterial response to the ionic environment. A. D. Brown (*Bact. Rev.*, 1964, **28**, 296—329).—A review in which the terminology is clarified, the structure and chemistry of the cell wall are considered; the association of bacterial properties with salt level is discussed and the response of individual species to changes in the ionic environment is examined. (148 references.) C. V.

Effect of moisture on ethylene oxide sterilisation. G. L. Gilbert, V. M. Gambill, D. R. Spiner, R. R. Hoffman and C. R. Phillips (*Appl. Microbiol.*, 1964, **12**, No. 6, 496—503).—Bacterial cells dehydrated beyond a critical point no longer react uniformly to this form of sterilisation. It is noted that the % of cells resistant to the lethal effect after desiccation may be as small as 0·1 to 0·001% but with an organism dried in broth a 5% resistance has been found. Org. material increases the resistance. This change in behaviour is not permanent and the resistance is lost if the cells are wetted with water, but mere exposure to R.H. 75—98% after desiccation requires 4—6 days to ensure a return to normality. (20 references.) C. V.

Malonaldehyde in aqueous solution and its rôle as a measure of lipid oxidation in foods. Tai-Wan Kwon and B. M. Watts (*J. Fd Sci.*, 1964, **29**, 294—302).—The kinetics of the acid hydrolysis of malonaldehyde (**I**) bis-(diethylacetal) and the ionisation and polymerisation of the product in aq. solution are described. The reaction patterns of **I** from mol. orbital data and an experimental exploration of several types of reactions that might lead either to improved procedures for isolating it from foods or to a better understanding of its reactivity in food systems are considered. (33 references.) E. M. J.

Influence of the oil content on the thermal resistance of *Bacillus mycoides*, *B. mesentericus*, *B. subtilis* and *Micrococcus candicans*. T. Kalinov (*Nauc. Trud. Viss. Inst. Chran. Vkus. Prom.*, 1962, [1964], **8**, 143—153).—Tests were made with cooked aubergine (puree consistency) to which was added oil at 4, 12 or 22%, these proportions representing foods with low and high fat contents and the third with fat content higher than that of any canned food on the market. These samples were sterilised and inoculated with *B. mycoides*, *B. mesentericus* or *B. subtilis* separately or mixed and cultured for 20 days; spores formed were approx. 90—95%. *M. candicans* was cultured on agar for 5 days. The thermal resistance of the bacteria as a function of the oil content was examined at 105 to 130° for 30 to 3 min. Results are detailed. *B. subtilis* showed the highest thermal resistance, *B. mesentericus* slightly lower, *M. candicans* the lowest. Increase in oil content of the medium reduces thermal conductivity; it takes longer for a product with high than with low oil content to reach a given sterilising temp. (From Bulgarian translation.) E. M. J.

Viability and heat resistance of anaerobic spores held 20 years at 40°F. C. Vinton (*J. Fd Sci.*, 1964, **29**, 337—338).—Under the conditions described there was very little change in the spore count over 20 years. E. M. J.

Heat-activation kinetics of endospores of *Bacillus subtilis*. F. F. Busta and Z. J. Ordal (*J. Fd Sci.*, 1964, **29**, 345—353).—*B. subtilis* strain 5230 endospores suspended in water at a concn. of 1×10^8 spores/ml. were heat-activated at eight temp. from 5 to 94° The response was measured by plate count and recorded as the heat-activated decimal fraction of the total viable count A medium used contained $CaCl_2$ and the Na_2 dipicolinate. A method was developed to estimate the contribution by the plate-count incubation to the total heat treatment. The exposure time required to achieve the response was extended as temp. was decreased. At a lower temp., a longer time was required for the response. The thermo-dynamic properties for the system were: $\Delta^{tH} = 27{\cdot}9$ kcal.; $\Delta^{Ft} = 25{\cdot}1 - 26{\cdot}4$ kcal.; $\Delta^{St} = 4{\cdot}6 - 8{\cdot}1$ cal./deg. (31 references.) E. M. J.

Influence of buffers and pH on the thermal destruction of spores of *Bacillus megatherium* and *Bacillus molymyxa*. H. W. Walker (*J. Fd Sci.*, 1964, **29**, 360—365).—The spores were heated at 100° in buffers adjusted to different pH values. Generally recovery of survivors was greatest in the neutral zone. Variations in recovery depended on the organism, buffer constituents and pH of the buffer system. Stability was greatest in a range of 0·005—0.05M-phosphate. Citrate, phthalate or NH_4^+ ion in the buffer reduced heat resistance of the spores below that in phosphate buffer. (27 references.) E. M. J.

Effect of inhibitors on sulphate-reducing bacteria: a compilation. A. M. Salem, R. Macpherson and J. D. A. Miller (*J. appl. Bact.*, 1964, **27**, 281—293).—The effects of some 200 bacteriostatics and bactericidal agents are tabulated. (42 references.) C. V.

3.—SANITATION, WATER, etc.

Combining of diphenyldithiophosphinic acid with simple aryl vinyl ethers. A. V. Kalabina, Lyu Mȳn-in' and N. V. Donskaya (*Zh. obshch. Khim.*, 1964, **34**, 1117—1121).—Diphenylphosphonodithioic acid easily combines with aryl vinyl ethers in exothermic reactions without catalyst. Introduction of electron-acceptor substituents in the benzene nuclei results in a lowering of reactivity of the aryl vinyl ethers because of reduced nucleophilicity of the double bonds. The character and position of the substituents does not affect the direction of reaction. Seven new α-aryloxyethyl and α-(n-butoxy)-ethyl esters of diphenylphosphonodithioic acid were prepared and examined. None of the materials possessed insecticide characteristics in relation to granary weevils (*Calandra granaria*). A. L. B.

Enzymic degradation of parathion in organophosphate-susceptible and -resistant houseflies. F. Matsumura and C. J. Hogendijk (*J. agric. Fd Chem.*, 1964, **12**, 447—453).—The metabolic fate of parathion and diazinon in one susceptible and two resistant strains of houseflies was studied by chromatography and radioisotope techniques. The main difference between the non-resistant and resistant strains was the superior ability of the latter to degrade parathion to diethyl phosphorothionate. (23 references.) W. ELSTOW.

Peet-Grady method [for flying insects]. Aerosol and pressurised space spray insecticide test method for flying insects. Cockroach aerosol test method. Cockroach spray test method. Textile resistance test [to insects]. Chemical Specialities Manufacturers' Ass. (*Soap, N.Y.*, 1964, Blue Book, 223—225, 226—228, 229—230, 231—232, 233—236).—The tests on insecticides are fully described including details of the apparatus, procedure, rearing of insects and the essential conditions for meaningful results. The insects studied in the textile resistance test were the black carpet beetle (*Attagenus piceus* Oliv.), the webbing clothes moth (*Tineola bisselliella* Hum.) and the furniture carpet beetle (*Anthranus flavipes* Lec.). C. V.

Estimation of pyrethrin II. A. Brierley and N. C. Brown (*Soap, N.Y.*, 1964, **40**, No. 5, 149, 151, 153, 294).—Chrysanthemic acid (**I**) is isolated by the P.B.K. procedure. The product is chromatographed on a silica gel–Supercel (2 : 1) column and eluted with Et_2O–light petroleum (1 : 1). When no more material is eluted the column is eluted with acetone–Et_2O (1 : 1) giving a further fraction The first fraction, 85% of the total is **I** mono- or di-carboxylic acid. If the extract is previously chromatographed on Al_2O_3 false pyrethrins are removed and the final result is only ~75% of that by the P.B.K. method. C. V.

DDVP in aerosol insecticides. J. H. Fales, O. F. Bodenstein and R. A. Fulton (*Soap, N.Y.*, 1964, **40**, No. 6, 99, 101, 105, 107, 119).—DDVP is not as effective as the Official Test Aerosol (OTA) against resistant or susceptible houseflies or female American cockroaches. It was more effective against American male, German or oriental cockroaches. In combination with allethrin DDVP was ineffective. In combination with pyrethrins or on substitution of 1% DDVP for 2% DDT in OTA DDVP showed good results. C. V.

Waterborne-disease outbreaks 1946—60. S. R. Weibel, F. R. Dixon, R. B. Weidner and L. J. McCabe (*J. Amer. Wat. Wks Ass.*, 1964, **56**, 947—958).—The occurrence of infectious hepatitis and typhoid, previous studies on waterborne diseases, the causes of outbreaks and seasonal distribution are reviewed. (17 references.) E. C. DOLTON.

Determination of chlorine dioxide, chlorine and sodium chlorite in drinking water. H. Karge (*Z. anal. Chem.*, 1964, **200**, 57—68).—Free Cl_2 and 1/5 of the ClO_2 are determined by releasing I_2 from KI at pH 7, and titrating with 0·001N-phenylarsine oxide. Free Cl_2, ClO_2 and ClO_2^- are determined by releasing I_2 from KI at pH 2·5, and titrating at pH 7. Free Cl_2 is determined colorimetrically with pyridine–barbituric acid reagent (Asmus and Garschagen, *Z. anal. Chem.*, 1953, **138**, 404). The method is suitable for water containing ~0·5 mg. of active Cl per litre. R. M. ROWLEY.

Determination of phosphine in air by gas chromatography. T. Dumas (*J. agric. Fd Chem.*, 1964, **12**, 257—258).—The determination of phosphine in air in the range 0·5 to 10 mg. PH_3/l. of air on samples of 0·05 to 1·0 ml. by gas chromatography is described. W. ELSTOW.

β-Cyclopropane-acrylic acids. Shell Internationale Research Mij N.V. (B.P. 940,140).—Methods are detailed for the prep. of the title compounds which have structures similar to that of chrysanthemum carboxylic acids and similarly useful as insecticides. One compound specified is *Et α-methyl-β-(2,2-diethoxycarbonyl-3-methylcyclopropyl)acrylate*, b.p. 127—128°/0·01 mm. H. S. R.

Trifluoromethyl salicylanilide. Cassella Farbwerke Mainkur A.-G. (B.P. 930,950, 23.3.62. Ger., 25.3.61).—Salicylic acid arylamides with ≤2 CF_3 groups are prepared as disinfectants for a no. of purposes, e.g., sanitisers for bottle washing and milk cans. A representative example is *5-chlorosalicyl*-N-*3,5-bis(trifluoromethyl)anilide*. H. S. R.

Fluorine-containing organ c compounds. E. I. Du Pont de Nemours & Co. (B.P. 939,592, 1.2.61. U.S., 9.2.59).—Preparative details are given for *trifluorothioacetyl fluoride*, b.p. −19° to −18°, a bright yellow fluid, which is claimed as a fumigant against (especially) houseflies. H. S. R.

Water wastes and sewage

Recovery of viruses from sewage. H. Malherbe (*J. Inst. Sew. Purif.*, 1964, 210—212).—A list is given of 62 human enteric viruses which have possible significance as sewage contaminants. Techniques for the detection of viruses by the inoculation of susceptible animals or tissue cultures and methods of sampling sewage for virus recovery are outlined. Factors influencing virus survival in sewage (effect of toxic substances in industrial wastes, adsorption on sludge in the activated-sludge process and on stone and sand filters, chlorination, stabilisation ponds), the significance of viruses in sewage effluents and the disposal of such effluents are briefly discussed. (13 references.) J. M. JACOBS.

4.—APPARATUS AND UNCLASSIFIED

An apparatus for continuous electrophoresis on a preparative scale. N. Blakebrough and R. Brookes (*Biotechnol. Bioengng*, 1964, **6**, 223—234).—The construction and operation of the apparatus is given and the theoretical implications are discussed. The method uses a large voltage gradient and the electrophoretic separation takes place in a thin, continuously flowing, horizontal layer of buffer. (15 references.) E. C. DOLTON.

Effect of temperature on the Kjeldahl digestion process. S. Jacobs (*Analyst*, 1964, **89**, 489—494).—Digestion at 47% of nicotinic acid or food diet hydrolysate as test substance with conc. H_2SO_4 was studied in the presence or absence of a mixture of HgO/K_2SO_4 (1 : 110) or a mixture of $K_2SO_4/CuSO_4/HgO/Se$ (15 : 5 : 5 : 1) as catalyst. Micro amounts of N in biological materials were successfully determined by the sealed-tube method in conjunction with the indanetrione hydrate method. Quant. recovery of N is temp. dependent but the presence of catalyst in the acid-digestion mixture is not critical if the digestion period is adequate. (22 references.) C. A. P.

Rapid new methods for the determination of nitrogen in fertilisers and other compounds. I. Description of the methods ; their accuracy and applicability. K. A. Potrafke, M. Kroll and L. Blom (*Anal. chim. Acta*, 1964, **31**, 128—138).—Apparatus and procedures are described which enable NH_3 distillations to be completed in ≃1 min. NH_3 is liberated by heating the moistened sample with Na_2CO_3 in a current of N_2, the vessel standing in an Al block heated to ≃380°. Amides are heated with 10M-NaOH with a block temp. of ≃250°. The NH_3 is absorbed from the gas stream and is titrated. NO_3^- is reduced with Devarda's alloy under conditions that generate sufficient heat to expel the NH_3, which is carried to the absorption vessel by the evolved H_2. When samples also contain NH_3, the NH_3 is oxidised with Na hypobromite before NO_3^- is determined. H. N. S.

Rapid new methods for the determination of nitrogen in fertilisers and other compounds. II. Automatic analysis. G. Kateman, L. L. M. Willemsen, J. B. G. Wijenberg and P. J. Stornebrink (*Anal. chim. Acta*, 1964, **31**, 139—146).—The apparatus and procedures described above are elaborated and adapted to the automatic determination of NH_3 and of the sum of NH_3 and NO_3^-. The analysis of 50 samples can be accomplished in 1 h. by one operator. H. N. S.

Rapid determination of important radionuclides (strontium-89, strontium-90, iodine-131 and caesium-137) in foodstuffs and water. II. Working details. K. G. Bergner and P. Jägerhuber (*Dtsch. Lebensmittl Rdsch.*, 1964, **60**, 207—210, 253—255).—Detailed routine working procedures are given for the separation and determination of : ^{131}I in liquid milk ; ^{89}Sr and ^{90}Sr in liquid milk ; ^{137}I,^{131} Cs, ^{140}Ba, ^{140}La and ^{40}K by γ-spectroscopy of milk ; ^{131}I in water ; ^{89}Sr, ^{90}Sr and ^{137}Cs in the acid-sol. and acid-insol. fractions of the ash of solid foodstuffs and water. (Cf. *Dtsch. Lebensmittl Rdsch.*, 1963, **59**, 359 ; 1964, **60**, 11.) E. C. APLING.

Rapid Titan Yellow method for determining magnesium in plant material in excess of manganese. E. M. Chenery (*Analyst*, 1964, **89**, 365—367).—A Titan Yellow reagent containing excess of triethanolamine, excess K ferricyanide and strong NaOH solution are added to an ash solution of the plant material. Mn produces a brown colour, the intensity being proportional to concn. ; >500 μg. of Mn will obscure the red Mg-complex until after the solution has been kept for 10—15 min. Mg content can then be estimated by measuring the optical *d* of the solution. Results are given which are in good agreement with those obtained by atomic-absorption spectrophotometry. (11 references.) C. A. P.

Polarographic determination of zinc in plant materials containing phosphate. G. Robertson (*Analyst*, 1964, **89**, 368—369).—Errors up to 40% Zn in ashes of plant material using a manual polarograph under alkaline conditions were due to ppt. containing $Zn_3(PO_4)_2$ and adsorbed Zn on $Ca_3(PO_4)_2$. The method removes PO_4^{3-} at an early stage and prevents formation of $Ca_3(PO_4)_2$ by pptn. with Zr oxychloride solution, evaporating to dryness and treating with dil. HCl to bring all Zn into the acid phase. Results are in good agreement with the dithizone colorimetric method. C. A. P.

Technique of detection of αε-diaminopimelic acid. C. Mund and H. Venner (*Naturwissenschaft*, 1964, **51**, 298—299).—Two minor modifications are introduced into the method of Primosigh *et al.* (cf. *Biochem. et Biophys. Acta*, 1961, **46**, 68).—The first consists in preparing the moist viscous culture of the stable L-form of *Proteus mirabilis* by the addition of 0·1M-Versene, 0·15M-NaCl, 25% aq. dodecyl sulphate, and a solution of 1-fluoro-2,4-dinitrobenzol in EtOH. The second modification concerns the technique of hydrolysis with Dowex 50-X8 at 100°. P. S. ARUP.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

APRIL, 1965

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.

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J. Sci. Fd Agric., 1965, Vol. 16, April

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Printed in Great Britain by Butler & Tanner Ltd, Frome and London