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BARIUM ISOTOPE METHOD FOR MEASURING CATION-EXCHANGE CAPACITY OF SOILS AND CLAYS

By B. W. BACHE

Cation-exchange capacity has been measured by isotopic exchange of ^{133}Ba with Ba^{2+}-saturated soils and clays in $2{\cdot}5 \times 10^{-3}$ M-$BaCl_2$ suspensions. The rate of isotopic dilution, the efficiency of displacement of soil cations by Ba^{2+}, and the precision of the method are critically assessed. The method is reliable and convenient, and can be used with both buffered and unbuffered systems.

Introduction

A variety of methods have been used for measuring the cation-exchange capacity (*CEC*) of clays and soils and the negative charge upon which it depends.[1] Many of these are experimentally tedious and entail theoretical uncertainties and practical difficulties.[2] Provided that suitable counting equipment is available, the use of radioactive isotope methods avoids most of these drawbacks and they are particularly appropriate for heterogeneous systems. The use of ^{45}Ca for exchange capacity measurements was introduced by Borland & Reitemeier,[3] and ^{24}Na and ^{42}K were also used by Deist & Talibudeen.[4] Divalent cations are more effective than monovalent ones for replacing soil cations, but Carlson & Overstreet[5] have shown that with Ca^{2+} significant amounts of the hydroxy-cation $CaOH^+$ may be adsorbed at neutral pH values thus overestimating the charge, but that this does not occur with Ba^{2+} provided the pH is below 9. Barium salts have frequently been used in conventional cation-exchange procedures and the use of its isotopes is a logical development. This paper describes such a method.

Experimental

Materials

As indicated in Table I, soils with various pH values and organic matter contents from three different parent materials, and two standard clay samples, were used to develop the method. These gave a wide range of values for *CEC*. To relate with other studies, all the *CEC* measurements were made on thoroughly mixed moist samples passed through a 3 mm sieve, but dried samples could equally well have been used.

Barium isotope method

Cation-exchange capacity may be considered as equivalent to the soil negative charge and from the theory of the diffuse double layer it is given by:

$$CEC = \gamma^+ + \gamma^-$$

where γ^+ is the adsorption of cations and γ^- is the negative adsorption (repulsion) of anions. Tests were made to measure the negative adsorption of chloride using the ^{36}Cl isotope and these showed that $\gamma^- = 0$ when the systems are Ba^{2+} saturated in equilibrium with $2{\cdot}5 \times 10^{-3}$ M-$BaCl_2$. Hence from the principle of isotopic dilution between two phases:

$$CEC = \gamma^+ = 2\,n\,(A_0/A_e - 1)\,\frac{100}{w}$$

where n = mmole Ba^{2+} in solution in equilibrium with w g of oven-dry material, A_0 is the measured radioactivity at zero time (i.e. the radioactive standard corrected for dilution to the test volume) and A_e is the radioactivity in solution at equilibrium. *CEC* is then expressed in mequiv./100 g.

Of the barium isotopes, ^{140}Ba was used by Popa *et al.*[7] but ^{133}Ba is more readily available and is probably most suitable. It has a half-life of 7·2 years and emits both β- and γ-radiation so providing a choice of counting methods. Scintillation counting with a 1-in dia. NaI crystal and annular geometry gave 2% efficiency whereas Geiger–Müller counting with a gas-flow counter gave 18% efficiency. The latter was therefore used.

A soil or clay sample containing up to 1 mequiv. *CEC* was shaken (240 rev/min) at 20 ± 1° for 10 min in a weighed 50 ml centrifuge tube with successive 25 ml aliquots of 0·5 M-$BaCl_2$ solution to saturate the exchange sites with

TABLE I

Results from different methods for measuring cation-exchange capacity (*CEC*)

Soil origin and designation	pH (in 10^{-2} M-$CaCl_2$)	Clay, %	Organic carbon, %	Estimates of *CEC* (negative charge), mequiv./100 g			Positive charge, mequiv./100 g
				^{133}Ba method	Sum of exchange cations	NH_4Cl adsorption	
Countesswells Series (granitic till, Aberdeenshire):							
topsoil, A	3·9	13·6	5·3	5·3 ± 0·37	6·5 ± 0·55	5·7 ± 1·16	0·6 ± 0·07
topsoil, B	5·7	13·5	4·0	10·9 ± 0·18	10·3 ± 0·16	10·6 ± 0·76	0·1 ± 0·05
topsoil, C	6·9	12·9	4·2	19·7 ± 0·68	18·6 ± 0·59	13·9 ± 0·43	<0·1
subsoil, D	4·6	12·6	2·5	2·6 ± 0·12	4·0 ± 0·39	4·4 ± 0·48	1·3 ± 0·07
Foudland Series topsoil, E (slate, Aberdeenshire)	5·9	14·8	6·6	17·0 ± 0·64	18·2 ± 0·88	16·2 ± 0·77	<0·1
Quorn Series topsoil, F (valley gravel, Derbyshire)	6·9	23·4	1·7	15·7 ± 0·34	16·0 ± 0·16	13·2 ± 0·21	<0·1
Kaolinite (St. Austell)	3·9	100	—	3·6 ± 0·23	5·5 ± 0·26	8·0 ± 0·39	2·4 ± 0·83
Montmorillonite (Belle Fourche)	8·8	100	—	86·6 ± 4·5	79·9 ± 11·4	73·4 ± 7·5	<0·1

Ba^{2+} (see below). The suspensions were cleared by centrifuging and the supernatant liquid was discarded. The sample was washed twice with water and once with $2 \cdot 5 \times 10^{-3}$ M-$BaCl_2$, weighed in the tube so that the final volume was calculable, and finally shaken for 30 min with 20 ml 10^{-3} M-$BaCl_2$ containing $0 \cdot 5$ μCi ^{133}Ba. After centrifuging, the Ba^{2+} concentration of the solution was accurately determined by EDTA titration (methyl thymol blue complexone indicator) and triplicate $0 \cdot 5$ ml aliquots were pipetted on to planchettes for radiation measurements. The sample and tube were dried at 105° and re-weighed to obtain the dry weight of the sample.

Other *CEC* measurements

Two other methods were used to estimate *CEC* using unbuffered salts: (i) the cations Al^{3+}, Ca^{2+}, Mg^{2+}, K^+ and Na^+ in the combined extract from four successive washings of soil with $1 \cdot 0$ M-NH_4Cl were determined. The charges of the adsorbed cations were assumed to be as indicated and the sum of their equivalents in the extract taken as the *CEC* of the soil; and (ii) the soil sample treated as in (i) was then brought to equilibrium with $0 \cdot 20$ M-NH_4Cl, weighed wet to allow the calculation of non-adsorbed NH_4Cl, and the salt was displaced by $1 \cdot 0$ M-KNO_3. Hence from NH_4^+ and Cl^- determined in the KNO_3 extract, both positive charge (if any) and negative charge were calculated.[6]

Results

The rate of isotopic dilution of ^{133}Ba with Ba^{2+}-saturated soils was estimated on samples withdrawn from rapidly stirred suspensions and quickly filtered under suction. It can be seen from Fig. 1 that reactions of different rates were involved, for while most of the isotopic exchange had occurred within the first minute final equilibrium was not reached until about 20 min. However, this shows that a 30 min period of shaking with the isotope is adequate for the method.

To ensure that isotopic dilution occurs with all the cations neutralising the negative charge, the soil cations must be completely displaced by the index cation Ba^{2+}. The extent to which this occurred is shown by the variation of apparent *CEC* with the number of $BaCl_2$ pre-treatments in Fig. 2. For acid soils (e.g. A and D) the *CEC* increased, approaching a maximum at about four treatments. Estimation of aluminium in the $BaCl_2$ extracts indicated that this increase was due to the gradual removal of strongly adsorbed Al, but the amount of Al released was 30–40% greater than would be expected from the increase in *CEC*, showing that the mean charge on the displaced Al was less than 3+ per atom. The *CEC* of the neutral soils (C and F) varied much less with $BaCl_2$ pre-treatments, in keeping with their low contents of exchangeable aluminium. Soil C was an acid soil which had been limed from a pH of about 5 to neutrality, and the apparent rise in *CEC* after the fourth $BaCl_2$ treatment probably reflected some disequilibrium within the organic complex. Four treatments with $0 \cdot 5$ M-$BaCl_2$ should be suitable for a standard method, but two should be sufficient if the soil has pH < 6 in 10^{-2} M-$CaCl_2$ solution, and leaching with the same total volume of $BaCl_2$ solution in a $\times 3$ porosity fritted filter tube was shown to be as effective as the centrifuging procedure.

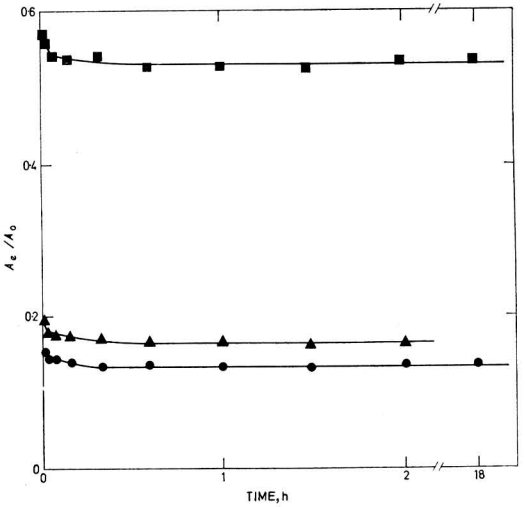

FIG. 1. *Rate of isotopic dilution of ^{133}Ba in contact with Ba^{2+}-saturated soils*

● Soil C; ■ soil D; ▲ soil E

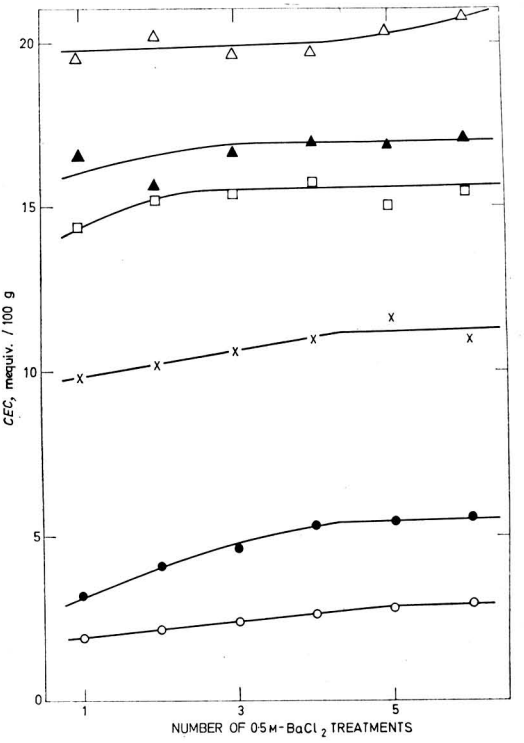

FIG. 2. *Variation of apparent cation-exchange capacity* (CEC) *with number of $0 \cdot 5$ M-$BaCl_2$ treatments*

● Soil A; × Soil B; △ Soil C; ○ soil D; ▲ soil E; □ soil F

Apart from sampling errors, the precision of the method depends on the accuracy with which the counting equipment will measure A_o/A_e and the accuracy of the titrimetric determination of n. As the equilibrium Ba^{2+} concentration increases, n can be determined with greater accuracy but the radioactivity ratio with less accuracy, and *vice versa*. These effects also depend on the magnitude of the *CEC*. It was found that $2{\cdot}5 \times 10^{-3}$M was the most suitable concentration for the equilibrium $BaCl_2$ solution, and 5 g a suitable sample for most soils; for soils with high *CEC* and for micaceous and montmorillonitic clays 0·5–1·0 g was necessary. Standard errors calculated from triplicate experiments were between 2 and 5% of the means (Table I); the errors would probably be lower if finely ground air-dried samples had been used instead of moist soil crumbs.

Discussion

The barium isotope method described is straightforward in principle, easy to use in practice and gives closely reproducible results over a wide range of *CEC* values. When the values are compared with those from summing the exchangeable cations (Table I) the agreement was excellent except for the strongly acid soils A and D, and for kaolinite, which gave somewhat higher values than for the barium method. This was not surprising, however, because these materials released exchangeable aluminium and the evidence given earlier indicates that the mean charge on the aluminium was less than the 3+ per atom assumed when summing the cation charges. The barium isotope method therefore gives the more reliable value.

In most cases the NH_4Cl adsorption method gave similar results to one or both of the other methods. For soil C however, it gave a much lower value, suggesting that not all of the adsorbed NH_4^+ was removed by the subsequent KNO_3 treatment. This again may possibly be due to disequilibrium in the organic fraction of this soil referred to earlier. For subsoil D and kaolinite, the NH_4Cl method gave higher values, especially compared with the barium method. These materials also hold positive charges, which suggests that repulsion of cations from the positive sites may be occurring in the barium method. Calculations from the double-layer theory showed, however, that this effect would be too small to account for the differences at the surface charge density and electrolyte concentration involved. Further work would be necessary to resolve these differences but is beyond the scope of this paper.

The object of the present work was to measure *CEC* at the natural acidity of the soil using unbuffered salts, but the method can be readily adapted to buffered solutions using barium acetate or $BaCl_2$–triethanolamine if required. Isotopic exchange of ^{133}Ba with Ba^{2+} adsorbed on carbonates may possibly occur in calcareous soils, but should not affect the results unless the carbonate has a large surface area.

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References

1. Chapman, H. D., 'Methods of Soil Analysis', 1965, p. 891 (Madison, Wis.: American Society of Agronomy)
2. Bascomb, C. L., *J. Sci. Fd Agric.*, 1964, **15,** 821
3. Borland, J. W., & Reitemeier, R. F., *Soil Sci.*, 1950, **69,** 251
4. Deist, J., & Talibudeen, O., *J. Soil Sci.*, 1967, **18,** 125
5. Carlson, R. M., & Overstreet, R., *Soil Sci.*, 1967, **103,** 213
6. Schofield, R. K., *J. Soil Sci.*, 1949, **1,** 1
7. Popa, A., Stoica, C., & Constantinescu, O., *Trans. 8th Int. Congr. Soil Sci.*, 1964, Vol. III, p. 489

EFFECT OF TIME OF APPLICATION OF FERTILISER NITROGEN ON THE DISTRIBUTION AND IDENTITY OF THE NITROGENOUS CONSTITUENTS OF YOUNG APPLE TREES

By D. G. HILL-COTTINGHAM and DENISE R. COOPER

The initial response to an application of fertiliser N appeared always as a rise in the level of asparagine. Following spring and summer N applications this high level of asparagine later decreased as those of arginine and the insoluble N increased, but after autumn N application both asparagine and arginine accumulated, especially in the roots. Leaves were exceptional both in containing a high proportion of insoluble N at all times and in containing a large amount of soluble glutamine soon after spring N application, in addition to the arginine and asparagine.

Direct analyses have been transformed on to a weight basis and the results are given diagrammatically as the total weight of N present in each tissue of the tree as each of the four components: insoluble N, soluble arginine, soluble asparagine, or the total of all other soluble compounds. This has enabled the movement of N within the whole tree and the interchange between compounds in response to season and to fertiliser application to be recognised more readily.

The possible significance of the relative mobilities of arginine and asparagine is also discussed, together with the possible merits of sampling leaves or other tissues, for example the roots, for predicting the future crop potential of apple trees.

Introduction

The influence of fertiliser nitrogen on the growth of apple trees is well established but more recently attention has been focused on its effects on flowering and fruit set and, in particular, on the variation in response to fertiliser applied at different times of year.[1,2] These results emphasised the potential benefits to flower quality of nitrogen applied after extension growth had ceased. Hence a collaborative study was made of the growth, flower bud development and fruit set, together with the distribution of total N, in young apple trees. From this it was concluded that the fertiliser N probably acted on the developing flower buds through a plant growth substance produced in the roots, although readily available N reserves would, no doubt, be essential to maintain the advanced growth of the blossom clusters the following year.[3] It was the trees from this experiment that have been analysed for their nitrogenous constituents with the results reported below.

Most of the soluble N in apple trees is in the form of amino acids and analyses of these constituents have been made by workers studying the build-up of reserves[4] and the problems of flower-bud initiation and biennial bearing.[5,6] In the present studies the advantage was that whole trees had been sampled and divided into their various tissues, and hence analyses could be expressed on a weight basis as has been done for the peach.[7]

Experimental

Plant material and fertiliser treatments

Full details of the management of this experimental material have already been published previously;[3] some essential facts only are given below.

Malling II rootstocks in pots of soil were budded with the cultivar Lord Lambourne in the summer of 1962 and, in the following winter, cut back to just above the inserted bud.

The trees were allocated into 4 groups for fertiliser treatment: 'minus N' or 'controls', given no additional N (group A); 'spring N', given N on 5 and 17 April 1963, prior to bud-burst (group B); 'summer N', on 2 and 9 August, after extension growth had ceased (group C); and 'autumn N', on 25 October and 1 November, at leaf fall (group D).

Fertiliser N was given as 2 applications of 25 g Nitrochalk, each equivalent to 5 g N/pot, with the exception of 'spring N' when double this amount was used.

Sampling and freeze-drying

When required whole plants were removed from the pots and their roots were washed free of soil as carefully as possible. The trees were then divided into morphologically-different parts which were weighed and dried separately. The parts were: (a) leaves plus petioles, and including flowers where present; (b) scion bark, i.e. all tissue outside the cambium; (c) scion wood; (d) stock bark; (e) stock wood; (f) old roots, i.e woody; and (g) young roots, i.e. those not woody.

Representative samples of each of these tissues from all the trees were freeze-dried by the method described previously.[8]

Methods of extraction and analysis

50 ml of extractant were added to 0·50 g of freeze-dried plant material in a 100 ml stoppered conical flask, which was then held at −15° for 24 h with occasional shaking.

The extractants used were: (a) ethanol/water/N-HCl (75 : 25 : 2 by vol.) for all tissues except leaves or (b) ethanol–chloroform–water (12 : 5 : 3 by vol.) for leaf tissue.

The methods of analysis used to determine the various N components have been described previously.[8]

Results

Insoluble nitrogen

The difference between the total N content of the original plant material and that of the corresponding extract is referred to below as the insoluble-N fraction. It is assumed to be largely protein. On all tissues other than leaves it accounted for from about 35–80% of the original total N content of the tissue depending on the N status of the tree. With leaves the corresponding figures were always higher, approximately 85–95% of the total N.

Soluble amino acids

In general the major soluble nitrogenous constituents found in all tissues and at all times of year were arginine and asparagine. Under certain circumstances glutamine also occurred in large amounts, notably in some leaves. Alanine, aspartic acid, glutamic acid, serine and threonine also occurred at times in small amounts, together with traces of most of the other protein amino acids. Other compounds which were recorded included γ-aminobutyric acid, ethanolamine and pipecolic acid, the latter only in leaves. Ninhydrin-positive spots in positions labelled by Bieleski & Turner[9] as peptides were found on the chromatograms of many extracts.

Effect of fertiliser N on the stock wood

The results of the analyses of all the stock wood samples are shown in Fig. 1 as the percentage N of the dry weight that was found as either of the three major components, soluble arginine, soluble asparagine or the insoluble fraction.

On the trees given no additional fertiliser the amounts of all three components fell rapidly in June, remained low during the summer but rose later in the autumn. Little or no soluble arginine or asparagine was detectable in this tissue in late summer.

Fertiliser N added to trees in spring, summer or autumn increased all three major components, but not in an identical manner. Following both 'spring ' and 'summer N' applications the level of asparagine rose rapidly but then fell away to be replaced by peaks in both the arginine and insoluble fractions. After 'autumn N', however, the asparagine level again rose rapidly but subsequently remained at a high level right through until May. Arginine and insoluble N levels also rose, but to a smaller extent than with the earlier applications.

Effect of fertiliser N on the leaves

The results of the analyses of all the leaf samples taken in 1963 are shown in Fig. 2 as the percentage N found as either soluble arginine, asparagine or glutamine, or as insoluble N.

The high proportion of the total N in the leaves that was insoluble is apparent immediately from the scale. The leaves of the 'minus N' trees contained very little soluble nitrogen and in fact only traces of alanine, asparagine and glutamic acid were detectable from July to September. The 'spring N' application greatly increased the level of arginine, asparagine and glutamine in the June sample, especially that of the latter compound. While the level of glutamine fell rapidly in the later samples, the peak of the arginine content came in July. Following 'summer N', however, the levels of arginine and glutamine were increased by much smaller amounts but there was a marked response to this fertiliser application in the level of asparagine.

Effect of fertiliser N on whole trees

'Spring N'

Table I shows the detailed analyses of one tissue, old roots, from trees sampled in June 1963, both from control trees and

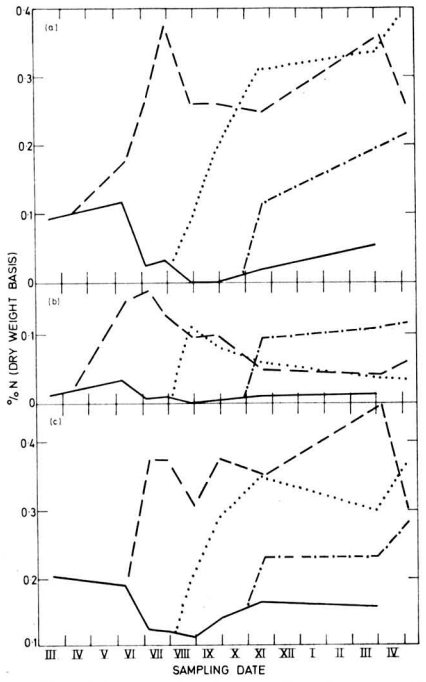

FIG. 1. *Effect of the time of application of fertiliser N on the stock wood tissue*

(a) Arginine; (b) asparagine; (c) insoluble N; ——— 'Minus N'; - - - - 'Spring N'; . . . . 'Summer N'; — · — · 'Autumn N'

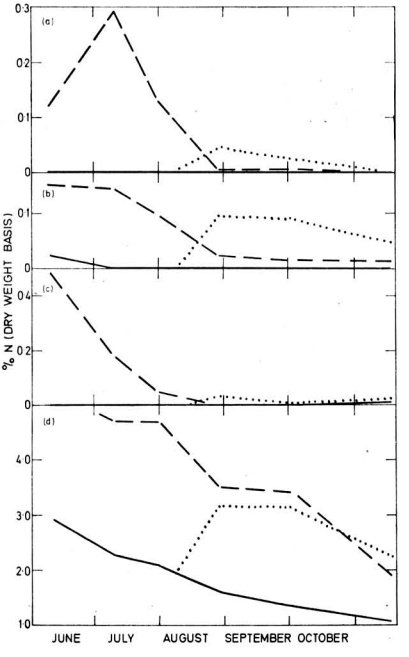

FIG. 2. *Effect of the time of application of fertiliser N on the leaf tissue*

(a) Arginine; (b) asparagine; (c) glutamine; (d) insoluble N ——— 'Minus N'; - - - - 'Spring N'; . . . . 'Summer N'

those given N fertiliser two months previously. The figures are those obtained from individual N determinations on the fractions specified or from colorimetric determination of the individual amino acids after chromatography.[8] Table I also shows the calculated percentage compositions, from which it will be seen that over 90% of the total N was accounted for as insoluble N, soluble arginine and asparagine. It can also be seen that the response to 'spring N' in this tissue was not uniform through all fractions, there being a greater increase in some soluble amino acids, particularly asparagine.

All other tissues of these trees have been analysed separately and the results prepared in a similar way. However direct analytical results, such as those in Table I, disguise the differences in weight between tissues in the whole tree and comparisons between the results from different tissues can give misleading impressions of the most significant quantitative changes in the tree as a whole. The analytical results, therefore, have been mutliplied by the total dry weight of the tissue as found at sampling to give the total weight of N present in its various forms in the tissue. These results can then be assembled to give the complete distribution of N within the whole tree. In order to simplify the presentation, the results from all fractions other than the three major components have been grouped together as 'other soluble N'.

Mean values from the trees sampled in June 1963 are shown in Table II. In terms of total weight of N most insoluble N was found in the young roots, most arginine in the stock wood and most asparagine in the old roots, irrespective of treatment. The largest single increase in response to 'spring N' was that of asparagine in the old roots.

Unfortunately the quantity of fertiliser applied as 'spring N' proved to be excessive in the confined space of the pots and some root damage resulted. For this reason a detailed study of the N economy of whole 'spring N' trees has not been continued.

'Summer N'

The analytical results from all the separate tissues of the trees given 'summer N' have been converted into the weights of N present as the major components in the various tissues, as was done for Table II. These weights have, in turn, been expressed in a simulated 3-dimensional diagram in order that the changes in tree composition with time may be seen more readily. In the diagrams the length of the vertical lines is in proportion to the weight of N present in that particular fraction, with the results from the 'control' or 'minus N' trees shown as a solid line and the increase over this value in the 'summer N' trees shown as the extended broken line.

TABLE I

Detailed analyses of old root tissue from trees sampled in June 1963

Results expressed in μg N present in the different fractions, as extracted from an original 0·50 g of freeze-dried plant material

Treatment	'Minus N'				'Spring N'			
	Tree 308		Tree 367		Tree 316		Tree 341	
	μg N	%	μg N	%	μg N	%	μg N	%
Total N content	6750	100	4975	100	13900	100	16600	100
Insoluble N	3027	44·8	2680	54·0	4868	35·0	5670	34·2
Chloroform-soluble N	48	0·7	50	1·0	80	0·6	66	0·4
Soluble protein	293	4·3	147	3·0	562	4·0	664	4·0
Soluble amino acids:								
Alanine	12	0·2	19	0·4	71	0·5	tr.	—
Arginine	1270	18·8	968	19·5	2919	21·0	3374	20·3
Asparagine	1950	28·9	1150	23·1	5200	37·4	6330	38·1
Aspartic acid	29	0·4	28	0·6	tr.	0	tr.	0
Glutamic acid	27	0·4	30	0·6	56	0·4	30	0·2
Glutamine	18	0·3	tr.	0	122	0·9	163	1·0
Serine	16	0·2	tr.	0	tr.	0	tr.	0
Threonine	34	0·5	19	0·4	tr.	0	tr.	0

tr. = trace present but quantity not measurable

TABLE II

Distribution of total nitrogen, as mg N, in whole trees harvested in June 1963

	'Minus N'					'Spring N'				
	Insoluble N	Soluble			Total	Insoluble N	Soluble			Total
		Arginine	Asparagine	Other compounds			Arginine	Asparagine	Other compounds	
Shoot	20·5	0·02	0·16	0·56	21·24	50·5	1·15	1·15	7·54	60·34
Stock bark	56·5	10·2	8·1	8·7	83·5	70·0	32·0	36·5	9·6	148·1
Stock wood	48·0	31·0	8·7	0·8	88·5	60·0	57·0	48·5	2·2	167·7
Old roots	38·5	15·3	20·0	4·7	78·5	68·5	40·8	76·5	12·6	198·4
Young roots	58·0	8·9	6·4	3·4	76·7	84·0	27·6	48·0	9·0	168·6
Whole tree	221·5	65·4	43·4	18·2	348·4	333·0	158·5	210·6	40·9	743·1

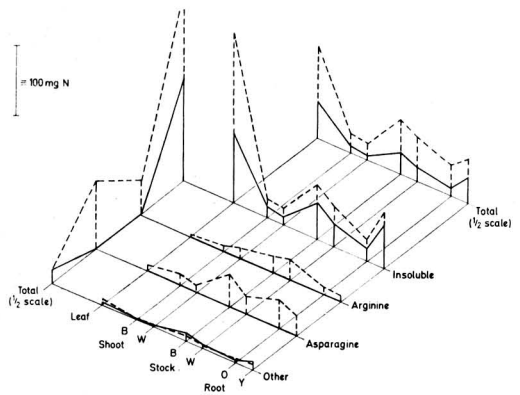

FIG. 3.

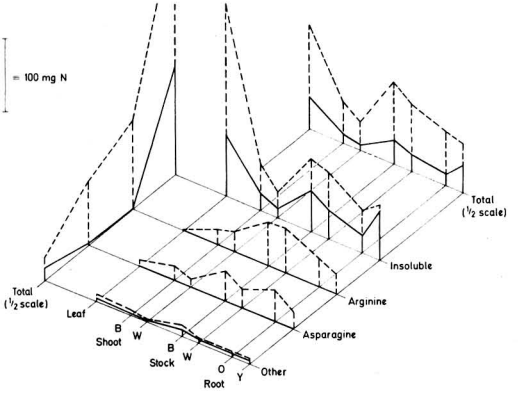

FIG. 4.

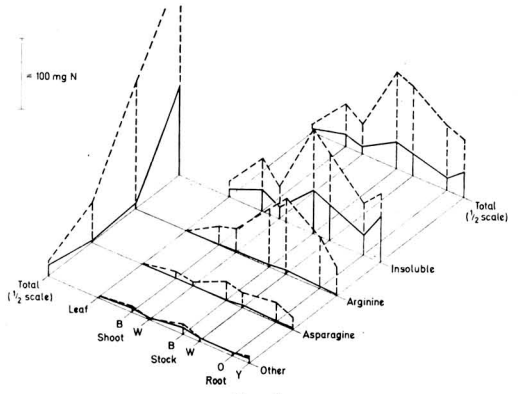

FIG. 5.

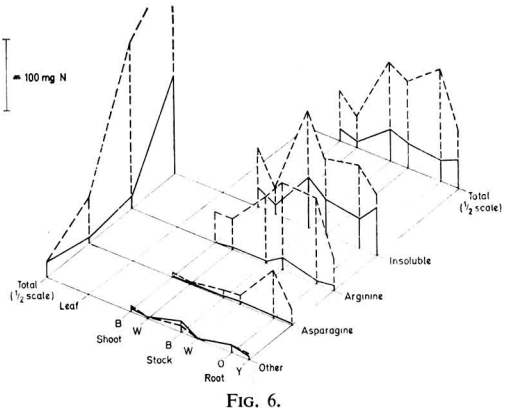

FIG. 6.

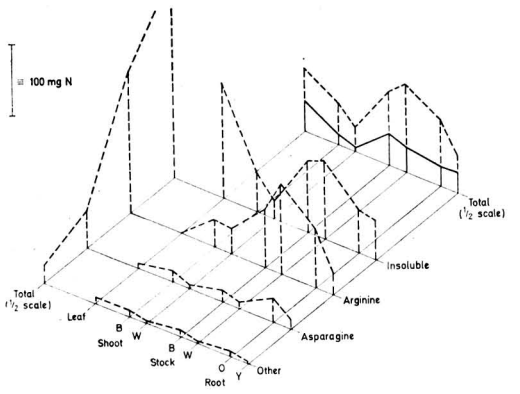

FIG. 7.

FIGS 3–7. *Distribution of the major components, in mg N, within trees given fertiliser nitrate on 2 and 9 August 1963 ('summer N') in comparison with that in untreated trees*

FIG. 3. Trees sampled 26–29 August 1963
FIG. 4. Sampled 30 September–2 October 1963
FIG. 5. Sampled 18–19 November 1963
FIG. 6. Sampled 31 March–2 April 1964
FIG. 7. Sampled 4–8 May 1964 (no fractionation possible on 'minus-N' trees)
B, bark; W, wood; O, old; Y, young
——— 'Minus N'; — — — + 'Summer N'

Figs 3–7 represent the composition of the trees given 'summer N' at intervals from August 1963 to May 1964, in comparison with untreated trees sampled at the same times. Fig. 3 shows that three weeks after fertiliser application the largest responses have been in leaf protein and in the soluble asparagine in the roots and stock. Comparison of Fig. 4 with Fig. 3, however, suggests that most of the additional N absorbed during September has been incorporated into insoluble N or soluble arginine in the tissues other than the leaves. Over the same period there has been very little change in the composition of the untreated trees. The next sampling was carried out in mid-November when most leaves had fallen (Fig. 5). It would appear that a considerable part of the leaf N had migrated into the scion and stock before leaf fall to be re-incorporated into the protein of these tissues. The other marked change in these 'summer N' trees was the increase in arginine, particularly in the stock and old roots, with a corresponding decrease in the amount of asparagine present. From November until the end of March the total N content of both the untreated and 'summer N' trees increased by about 20%, much of it accumulating as arginine in the roots and stock (Fig. 6). The final samples were collected in the first week of May when the trees were flowering. Unfortunately all the freeze-dried samples of the 'minus N' trees were spoilt accidentally during processing and had to be oven-dried. Hence no fractionation was possible with these tissues, only total N determinations. It would appear that the N for the new leaves and flowers on the 'summer N' trees was drawn largely from the previously insoluble fraction of the stock bark and old roots or from the soluble arginine accumulated in the old roots. Any change in the amounts of 'other soluble N compounds' did not appear to be significant in the total N economy of either the untreated or the 'summer N' trees during this experiment.

'Autumn N'

The results of the analyses of the trees given 'autumn N' have been prepared on a weight basis as above. Figs 8–10 represent the composition of these trees sampled from November 1963 until May 1964, in comparison with the results for untreated trees as before.

In the trees sampled three weeks after the 'autumn N' application (Fig. 8), the increases were largely confined to the roots and stock, particularly as insoluble N and soluble asparagine. Much more N had been absorbed by the treated trees by the end of March (Fig. 9), with the additional N again remaining in the roots and stock, but now appearing as all three major components, particularly as arginine For the new growth of leaves and flowers in May much of the N appears to have originated in the roots, from all three major components (Fig. 10).

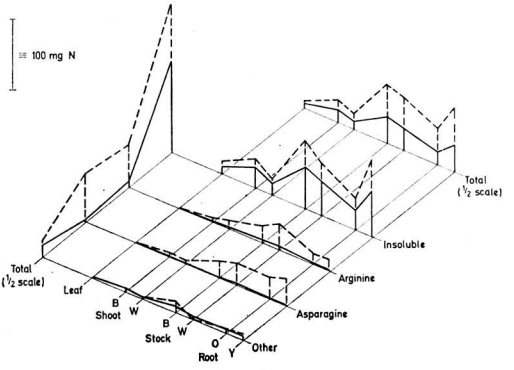

FIG. 8.

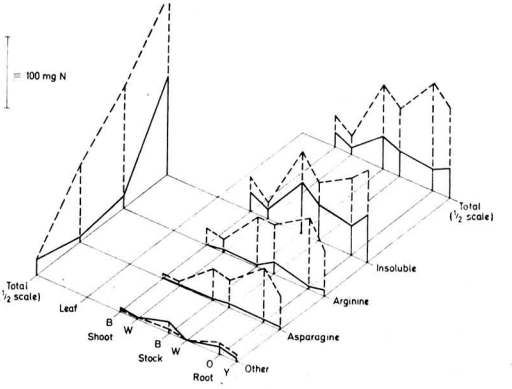

FIG. 9.

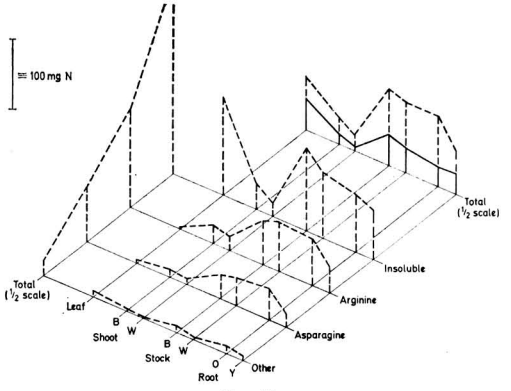

FIG. 10.

FIGS 8–10. *Distribution of the major components, in mg N, within trees given fertiliser nitrate on 25 October and 1 November 1963 ('autumn N') in comparison with that in untreated trees*

FIG. 8. Trees sampled 18–21 November 1963
FIG. 9. Sampled 31 March–8 April 1964
FIG. 10. Sampled 6–8 May 1964
(no fractionation possible on 'minus-N' trees)
B, bark; W, wood; O, old; Y. young
——— 'Minus N'; — — — + 'Autumn N'

Discussion

The range of soluble amino acids found is in general agreement with those reported by previous workers[4–6] who had also found that arginine and asparagine were the dominant soluble constituents in most tissues. However, some previous reports that normal apple leaves contain largely aspartic and glutamic acids[10,11] have not been confirmed.

The initial rapid rise in asparagine levels in most tissues following fertiliser N applications suggests that absorbed N may be translocated as this compound or as one closely related to it metabolically. It is thus of interest that the analysis of xylem sap from apple shoots showed that it contained largely aspartic acid and asparagine, with a smaller amount of glutamine during the spring and summer, and with some arginine also present in autumn and winter.[10]

Arginine is apparently a storage form of N which is formed from asparagine in tissues other than the leaves, for the concentration of asparagine often falls as that of arginine rises, as will be seen for 'spring' and 'summer N' in Fig. 1; also by comparing Figs 3 and 4. These results show that arginine can accumulate in certain tissues even during the spring and summer when the N supply exceeds the immediate growth requirements, although earlier work[4] had left the impression that arginine was formed only in the autumn as a winter storage form of N. On the contrary, these results suggest that the change of asparagine to arginine takes place most readily with a ready supply of photosynthates; hence the change would be restricted during the winter thus explaining the high level of asparagine maintained in the roots of the 'autumn N' trees over that period. It is possible that a high level of asparagine could be advantageous to the 'autumn N' trees in the following spring because this form of N is immediately available for translocation and metabolism, whereas other trees high only in arginine have their reserve N effectively unavailable to distant growing points until transformed into a more mobile form.[12] A potential disadvantage in 'autumn N' applications in some climates is the risk of winter injury associated with high asparagine levels.[13]

Apart from the possibility mentioned above that high asparagine levels could be advantageous to buds immediately prior to blossoming, there is no evidence from the results that increased flower initiation or improved development is associated with the presence of any particular amino acid. But during sampling the developing buds were mixed and analysed with the shoot bark and hence small changes in the buds alone could have been overlooked. However, Sahulka[6] from analyses of spur shoots has also concluded that there is no particular amino acid related to flower initiation.

Figs 3–10 represent the total response of whole trees to applications of fertiliser N as well as the subsequent redistribution of N within these trees, both qualitatively and quantitatively, in response to season and to growth. It is believed that this is the first attempt to present such comprehensive analyses for the tree as a whole. Taylor[14] has also stressed the importance of putting results on a weight basis so that concentration changes are not diluted by growth. Although these complete analyses are only practicable with small experimental trees, the results have a bearing on advisory work in mature orchards. The tissue most frequently sampled in order to assess the future crop potential of mature trees is the leaf, but these results raise doubts whether this is ideal. Leaf samples are most frequently taken only in late summer; hence the status of the tree at other times is unknown and any changes due to unconventional fertiliser application are missed. A more fundamental objection comes from the leaf analyses themselves, which show that leaves, unlike most other tissues, contain little soluble N and so do not reflect the amount of reserve or storage N within the tree. A theoretically better approach is the proposal by Baxter[15] to sample shoots and roots during the dormant season and to determine the level of soluble amino-N in these tissues, for he has shown that there are wider fluctuations in these results with N status than there are in leaf total N figures. The results would support this proposal, particularly for root extractions, and especially if both total amino-N and arginine were determined in order to give a measure of the relative availability of the reserve N.

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References

1. Hill-Cottingham, D. G., *J. hort. Sci.*, 1963, **38,** 242
2. Williams, R. R., *J. hort. Sci.*, 1965, **40,** 31
3. Hill-Cottingham, D. G., & Williams, R. R., *J. hort. Sci.*, 1967, **42,** 319
4. Oland, K., *Physiologia Pl.*, 1959, **12,** 594
5. Sahulka, J., *Biologia Pl.*, 1962, **4,** 278
6. Sahulka, J., *Biologia Pl.*, 1962, **4,** 291
7. Taylor, B. K., & May, L. H., *Aust. J. biol. Sci.*, 1967, **20,** 389
8. Hill-Cottingham, D. G., & Cooper, D. R., *J. Sci. Fd Agric.*, 1969, **20,** 662
9. Bieleski, R. L., & Turner, N. A., *Analyt. Biochem.*, 1966, **17,** 278
10. Bollard, E. G., *Aust. J. biol. Sci.*, 1957, **10,** 279
11. Sahulka, J., & Silova, A., *Biologia Pl.*, 1960, **2,** 70
12. Hill-Cottingham, D. G., & Lloyd-Jones, C. P., *Nature, Lond.*, 1968, **220,** 389
13. Romanovskaya, O. I., *Soviet Pl. Physiol.*, 1963, **10,** 581
14. Taylor, B. K., *Aust. J. biol. Sci.*, 1967, **20,** 379
15. Baxter, P., *J. hort. Sci.*, 1965, **40,** 1

ELECTROCHEMICAL ASPECTS OF ION TRANSPORT IN PLANTS*

By A. E. S. MACKLON

One of the fundamental problems of ion transport is to differentiate between those ion species which are transported by means of metabolically driven 'ion pumps' and those which enter or leave the cell by diffusion. Electrochemical theory proposes models which allow relatively precise analysis of the ionic relationships of the cell, and has led to considerable advances in distinguishing between actively and passively transported ions. A brief account of the theory adopted is given, and its use illustrated with examples of ion-uptake studies on potato tuber tissue, pea seedling stems and castor-bean root systems.

Introduction

Plants accumulate ions to levels of concentration much higher than are commonly found in the soil solution. It is clear that this process involves the expenditure of energy by the cell in 'active uptake', since the overall electrochemical activity gradient between the medium and the plant cell is uphill. However, that is not to say that nutrient ions are always transported by means of metabolically driven 'ion pumps', for the electrochemical activity gradient of a particular ion may allow its diffusive entry into the cell. Thus one of the fundamental problems of ion transport is to differentiate between ions which are actively accumulated and those which enter the cell by diffusion.

Plant cells are generally found to be negatively polarised with respect to the external medium. This transmembrane potential is commonly 50–150 mV. If ions are free to diffuse across the cell membrane, the rate and direction of diffusion will be influenced by this electrical potential gradient as well as by the chemical activity gradient. Thus an ion may move against its chemical concentration gradient if the electrochemical gradient is favourable.

Theory

A number of theories are used as a basis for describing the relationship of ion flux across a membrane to the electrical and chemical driving forces on an ion. The most widely used is the Goldman theory,[1] which makes the simplifying assumption that the electrical potential gradient through the membrane is linear. Then the net passive flux of an ion (e.g. K^+) can be expressed as

$$\varphi_K = \frac{-P_K z FE/RT}{1 - \exp(zFE/RT)} ([K_o] - [K_i] \exp(zFE/RT)) \quad .. \quad (1)$$

where φ_K is the net flux of K^+, P the permeability coefficient, E the electrical potential difference across the membrane, z the valency of the ion, F the Faraday constant, R the gas constant, T absolute temperature. Terms in square brackets are ion concentration inside (i) and outside (o) the membrane. For K^+, $z = +1$ and drops out, but for anions, terms including z become negative, e.g. for Cl^-

$$\varphi_{Cl} = \frac{P_{Cl} FE/RT}{1 - \exp(-FE/RT)} ([Cl_o] - [Cl_i] \exp(-FE/RT)) \quad (2)$$

From equations of this type can be obtained an expression for E, the Goldman equation for membrane potential:

$$E = \frac{RT}{F} \ln \frac{P_K[K_o] + P_{Cl}[Cl_i]}{P_K[K_i] + P_{Cl}[Cl_o]} \quad .. \quad .. \quad (3)$$

This would be the relationship for a system where K^+ and Cl^- are the only mobile ions. Where other mobile ions have to be considered, terms for these can be added. Thus a value for E, to be expected if all the ion movements are passive, can be calculated from ion concentrations and relative P values. (Strictly speaking, ionic activities, not concentrations, should be used but concentrations are considered an adequate approximation for most biological systems.)

The Goldman equation implies that the immediate source of the membrane potential is from the combined effects of an asymmetric distribution of passively moving ions, and their differing mobilities in the membrane (referred to as P, the permeability coefficient in the equations). The origin of the potential would seem to lie, therefore, in the cause of the asymmetric distribution of the ions in the first place. The major cause of this asymmetry is generally considered to be the so-called 'ion pumps', active mechanisms which can move ions against their respective electrochemical potential gradients.

The Goldman theory makes several assumptions about the characteristics of the cell membrane which are difficult to verify directly. However, there are many situations where criticism on these grounds can be avoided by using a different approach to distinguish between those ions moving passively down an electrochemical gradient and those which are being actively transported against the gradient.

By analogy with the flux equation for a non-electrolyte, (flux = mobility × concentration gradient) the flux equation for an ion j can be written:

$$\varphi_j = P_j (C_j^o - C_j^i \exp z_j FE/RT) \quad .. \quad (4)$$

i.e. net flux = mobility × electrochemical activity difference. This equation is of the same form as the Goldman equation, but it is of much more general validity, since it holds true whatever the assumption about the membrane may be. Use of radioactive tracers allows net flux to be resolved into influx and efflux, where influx is proportional to C_j^o and efflux to $C_j^i \exp z_j FE/RT$. So, for a system in which net accumulation is occurring:

$$\frac{\varphi_i}{\varphi_o} = \frac{C_j^o}{C_j^i \exp z_j FE/RT} = \frac{\bar{\mu}_j^o}{\bar{\mu}_j^i} \quad .. \quad .. \quad (5)$$

where $\bar{\mu}_j$ is the electrochemical activity of the ion j outside (o) and inside (i) the membrane. This formula, the Ussing flux ratio equation,[2] is quite general for the independent passive movement of ions and is a direct consequence of such movement being proportional to the electrochemical activity

* Presented at a joint meeting of the Agriculture group with the Physico-chemical and Biophysical Panel of the Pesticides Group, 18 February, 1969

difference, whatever the membrane model. Deviations from the equation indicate that active transport is occurring. Thus if the observed flux ratio is larger than can be accounted for by the electrochemical gradient, the ion would appear to be 'pumped in' across the membrane. If the flux ratio is smaller than predicted, it may be suspected that the ion is being 'pumped out'. However, caution must be exercised in interpretation to ensure that any deviation from the Ussing equation is not attributable to such factors as 'exchange diffusion' or the passage of water through the membrane causing 'solvent drag'.

When there is flux equilibrium, (i.e. when $\varphi_i = \varphi_o$) the Ussing equation reduces to this form:

$$E_j = \frac{RT}{zF} \ln \frac{C_j^o}{C_j^i} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

This is the Nernst equation. Testing for passive or active transport in these circumstances is much more straightforward since individual fluxes do not have to be determined. Instead, a measure of the driving force on an ion is obtained by comparing the Nernst potential E_j for the ion, calculated from Equation (6), with the observed electrical potential[3]:

$$\Delta E = E_{obs} - E_j \quad .. \quad .. \quad .. \quad .. \quad (7)$$

When ΔE is zero, it can be concluded that the ion is passively distributed in accordance with the electrochemical potential gradient, and enters the cell by diffusion. A negative ΔE indicates, for cations, an inward driving force, and for anions, an outward driving force. The converse holds for a positive ΔE.

Ion transport studies

The following examples, two concerning studies at the cellular level, and a third dealing with whole root systems, illustrate the use of the Ussing, Goldman and Nernst equations.

K^+ transport in pea epicotyl cells

The foregoing theoretical account refers to a single membrane separating an inner phase from an outer phase. In the plant cell, however, the situation is more complex than this since there are three phases to consider, separated by two membranes (Fig. 1). Therefore, a study of the mode of ion transfer from outside the cell to the vacuole requires the measurement of the electrochemical gradient and fluxes across both the plasmalemma and the tonoplast.

This has been done for potassium in the cortical cells which make up the bulk of segments cut from pea seedling epicotyls[4]. These segments show a rapid uptake of potassium when incubated in a dilute nutrient solution[5], and this capacity was utilised to load the segments with radioactively labelled K^+. From the subsequent appearance of the label in non-labelled washing solutions an efflux curve was constructed and analysed to provide estimates of K^+ influx and efflux at both the inner and outer membranes. The same analysis also provided the basis for estimates of the mean K^+ concentration in the cytoplasm and vacuole of the epicotyl cells. These results, together with values for electrical potential across the plasmalemma and tonoplast measured on a sample of single cells in the segments, were then used to test for passive diffusion according to the Ussing equation (Equation 5). The results of such an experiment are summarised in Table I. With 1 μ equiv. K^+/ml in the uptake solution, the flux ratio across the plasmalemma was found to be 14. The K^+ concentration in the cytoplasm was 145 μ equiv./ml and the membrane potential -128 mV. This yields an electrochemical activity ratio of only 1·1, so the great excess of K^+ influx over efflux across the plasmalemma must be due to a metabolically-driven uptake mechanism. However, across the tonoplast the electrochemical activity ratio is 2·6 compared with a flux ratio of 2·4. Thus, the flux ratio is very close to that expected for independent passive movement, and transfer of K^+ from the cytoplasm to the vacuole must be by passive diffusion. Much the same picture emerges when external K^+ concentration is 10 μ equiv./ml (Table I).

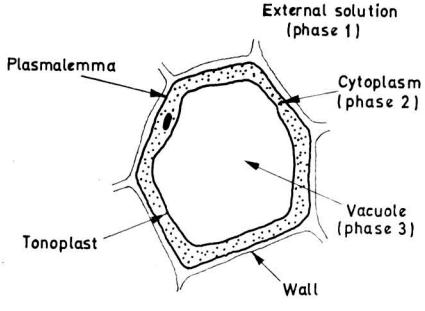

FIG. 1. *Diagram of a plant cell, indicating the three compartments separated by two membranes, the plasmalemma, and the tonoplast*

Cl^- uptake by potato tuber cells

The uptake of ions by washed discs of potato tuber is usually a hyperbolic function of the external ion concentration. However, it was found[6] that with freshly cut discs at room temperature, and with both fresh and aged discs at 0°, the Cl^- absorption isotherm was exponential (Fig. 2). This result was difficult to explain in terms of diffusion in response to external ion concentration alone, but was accounted for instead in terms of electrochemical theory as expressed in the Goldman equations. Making reasonable assumptions about the relative magnitudes of the permeability coefficients of K^+ and Cl^-, values for plasmalemma membrane potential at each external concentration of KCl used were calculated from Equation (3). These values of E were then used in the Goldman flux equation for Cl^- (Equation 2) to obtain the relative flux rates (φ_{Cl}/P_{Cl}), to be expected at each external concentration as a result of the electrochemical potential gradient. The resulting plot of relative Cl^- influx against KCl concentration gave a curve which showed a striking parallelism with the experimental Cl^- flux curve. This is illustrated by the results for fresh tissue at 0°, given in Fig. 2.

As external KCl concentration increases the transmembrane potential decreases (becomes less negative) and since Cl is negatively charged the real driving force on the ions increases more rapidly than the external concentration. Thus the exponential character of the Cl^- absorption isotherm for fresh discs and for aged discs at low temperature is explained in physical terms, as a process rate-controlled by diffusion down the electrochemical potential gradient.

This conclusion was subsequently confirmed by direct measurement of the electrical potentials on a sample of individual cells. Relative flux values calculated from these data gave an absorption isotherm closely matching the experimental and predicted curves (Fig. 2). This seems to confirm the validity of the Goldman theory.

TABLE I

Test of the Ussing flux ratio equation with experimentally determined ^{42}K fluxes, chemical concentrations and electropotentials in pea epicotyl segments

K^+ external concentration, μ equiv./ml	Cell compartment	Flux ratio $(\varphi_i/\varphi_o)^a$	K^+ internal concentration, μ equiv./ml	E, mV	$\bar{\mu}_{K^+o}{}^*/\bar{\mu}_{K^+i}$
1	cytoplasm	$\frac{42\cdot1}{3\cdot0} = 14\cdot0$	145	−128	1·1[b]
	vacuole	$\frac{68\cdot0}{28\cdot9} = 2\cdot4$	56	0	2·6[c]
10	cytoplasm	$\frac{54\cdot7}{2\cdot7} = 20\cdot3$	180	−108	4·0[b]
	vacuole	$\frac{91\cdot9}{39\cdot9} = 2\cdot3$	78	0	2·3[c]

[a] Fluxes in units of 10^{-5} μg/g fresh weight/sec
[b] The electrochemical activity ratio across the plasmalemma
[c] The electrochemical activity ratio across the tonoplast
* $\bar{\mu}_{K^+}$ is the electrochemical activity of K^+ inside (i) and outside (o) each membrane

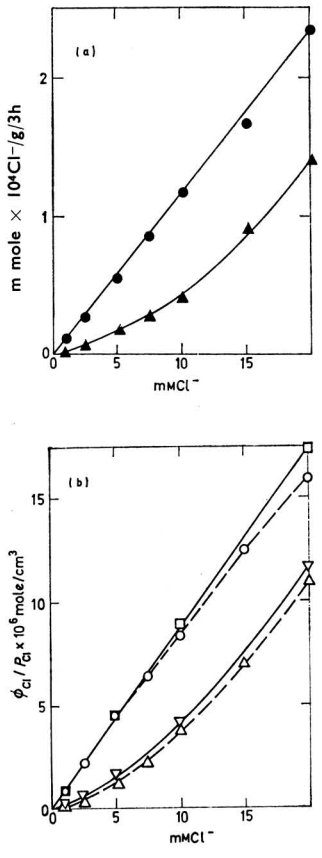

FIG. 2. *A comparison between* (a) *the observed uptake of Cl^- by freshly cut discs of potato tuber at 0° and* (b) *the passive influx to be expected from experimentally determined membrane potential values*

– – – – Theoretically derived flux curves
▲, ▽, △ increasing concentration of KCl
●, □, ○ increasing concentration of Cl^-, with K^+ constant at 40 mM

TABLE II

Values of E (the observed electrical potential difference) and ΔE (a measure of the driving force) between sap and external solution, for each nutrient ion supplied to detopped castor bean plants

	$\Delta E = E - E_j$ (mV)				
	K	Na	Ca	Mg	
E (mV) −52	+4	−32	−21	−25	
	NO_3	Cl	SO_4	H_2PO_4	HPO_4
	−105	−102	−64	−116	−69

In other experiments external Cl^- concentration was varied while K^+ was maintained at 40 mM by the addition of K_2SO_4. The Goldman theory predicts that changes in external anion concentration in the presence of a constant concentration of cation will alter the membrane potential very little. This was confirmed. Consequently the driving force on the Cl^- ion will vary directly with the external concentration so that the absorption isotherm is linear (Fig. 2).

With aged discs at higher temperatures the Cl^- uptake rate is much higher and is clearly unrelated to the physical driving force on the ion. Instead the absorption isotherm is hyperbolic, a pattern associated with processes rate-controlled by an active absorption mechanism.

Ion transport across whole root systems

An electrochemical approach to this problem involves the measurement of the electrical potential difference and ion concentration gradients between the xylem sap, exuding from the cut stem of the plant, and the nutrient solution bathing the root system.[3,7] Using the concentration gradient data the Nernst potential, E_j, for each ion can be calculated from Equation (6). A measure of the physical driving force, ΔE, on each ion is then obtained by comparing E_j with the observed potential according to Equation (7). Values of ΔE for each of the major nutrient ions, obtained with castor-bean roots[7] in a dilute nutrient solution, are given in Table II.

These figures seem to indicate that there is virtually no driving force on K^+, an inward driving force on the other cations and an outward driving force on each of the anions.

However since the system is not at flux equilibrium (a net influx causes exudation at the cut stump), it could be concluded that the anions are being actively transported into the xylem against their respective electrochemical potential gradients, and that the cations are diffusing in passively.

This interpretation implies that within the root there is a single barrier to diffusion behaving in the manner of a single cell membrane, but the root system is a much more complex structure than this and many objections can be raised to this rather naïve interpretation of the experimental data. How much the true picture may be obscured by ion interaction and exchange within the system has been indicated by Shone.[8] Nevertheless, since the electrochemical activity of all the anions appears to be higher in the xylem exudate than in the external medium, it seems reasonable to conclude that they are accumulated by active mechanisms. Study of the ionic relations of the root tissues at the cellular level may provide the evidence necessary to decide whether cation accumulation is also controlled by active mechanisms, or whether passive diffusion down a gradient arising from the activity of the anion pumps is the only factor in cation transport to the xylem sap.

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References

1. Dainty, J., *A. Rev. Pl. Physiol.*, 1962, **13,** 379
2. Ussing, H. H., *Acta physiol. scand.*, 1949, **19,** 43
3. Bowling, D. J. F., & Spanswick, R. M., *J. exp. Bot.*, 1964, **15,** 422
4. Macklon, A. E. S., & Higinbotham, N., *Pl. Physiol.*, in press
5. Macklon, A. E. S., & Higinbotham, N., *Pl. Physiol.*, 1968, **43,** 888
6. Macklon, A. E. S., & MacDonald, I. R., *J. exp. Bot.*, 1966, **17,** 703
7. Bowling, D. J. F., Macklon, A. E. S., & Spanswick, R. M., *J. exp. Bot.*, 1966, **17,** 410
8. Shone, M. G. T., *J. exp. Bot.*, 1968, **19,** 468

PROTEIN AND AMINO ACID COMPOSITION OF MAIZE GRAIN AS INFLUENCED BY VARIETY AND FERTILITY

By D. R. KEENEY

The protein and amino acid composition of maize grain from four Wisconsin single-cross hybrids grown on plots treated with 200 kg/ha of N or 200 kg/ha of N and 278 kg/ha of K fertiliser was determined. The yield increase due to N fertiliser was large, but K increased the yield of only one variety because of the adequate level of available K in the soil of the plot area. Little variation was found among varieties with respect to amino acid composition of the protein. The average protein concentration in maize grain was 7·0, 7·7 and 8·4% with the control, N and N–K treatments respectively. The results obtained indicated that the protein increase was due in large part to an increase in zein. The concentration in the maize grain of all essential amino acids, except methionine, was highest in the grain from the balanced N–K fertiliser treatment.

Introduction

Maize (*Zea mays* L.) protein has low contents of lysine, methionine and tryptophan.[1] Available evidence indicates that N fertilisation, which often increases the protein concentration of maize grain, does not increase and often decreases the relative composition of these essential amino acids in the maize grain protein.[2–4] Past investigations have demonstrated that such factors as soil moisture, planting rate, and variety will also influence the protein concentration in maize.[4] However, the influence of nutrient elements other than N on the concentration of protein in maize grain has not been investigated. The purpose of the work reported here was to evaluate the effect of N and K fertilisers on the protein and amino acid composition of four Wisconsin single-cross hybrid maize varieties.

Experimental

The maize grain samples investigated were obtained from a nitrogen and potassium fertiliser field trial conducted in 1966 on the University of Wisconsin Experimental Station located at Spooner, Wisconsin. The treatments selected were control (50 kg/ha of P as triple superphosphate), N (as in the control treatment but including 200 kg/ha of N as NH_4NO_3). and N–K (as in the N treatment but including 278 kg/ha of K as KCl). All of the P and K fertiliser and half of the N fertiliser was broadcast before ploughing; the remainder of the N being applied as a side-dressing treatment three and six weeks after planting. The soil was a Pence sandy loam (Alfic Haplothod) with a pH of 7·0 and 1·2% organic C. Soil test results indicated that the plot area contained 160 kg/ha available K, which is in the medium to high soil-test level for sandy Wisconsin soils. The Wisconsin single-cross maize hybrids were Wis. 1694 and 1718 (80-day relative maturity) and Wis. 273 and 1710 (85-day relative maturity). The plots were sown in rows 76·2 cm apart and the plant population was adjusted to 64,200 plants/ha about three weeks after emergence. Each treatment was replicated four times. The plot area was irrigated with 3·8 cm of water every two weeks from June 20 to July 30, and weeds were controlled by a pre-emergence treatment with atrazine. The maize was harvested in late September and ear samples were dried, weighed, and shelled, and a composite sample of each treatment and variety was obtained by combining the grain from the four replicate plots.

Amino acid patterns were obtained using the automated method of Spackman *et al.*[5] on a Beckman model 120C amino acid analyser. Total N was determined by the Kjeldahl procedure. All calculations are based on the moisture-free material.

Results

The grain yield of all four varieties was increased by N fertiliser but only with variety 1694 was yield further increased by addition of K fertiliser (Table I). The absence of a yield effect by K fertiliser was because the soil already contained an adequate level of available K. Nitrogen fertiliser increased the crude protein content of varieties 1710 and 1718. Applied K further increased the grain protein concentration of varieties 273 and 1718. The protein level in the other two varieties, however, was not affected by K treatment, and the protein concentration of variety 1694 was not influenced by either N or K treatments.

The amino acid composition of maize grain protein was similar among the varieties studied (Table II). With the varieties that increased in protein on addition of N or N–K, there was a concurrent decrease in lysine and glycine, which are not present in zein.[1] In contrast, the content of leucine and glutamic acid, which together constitute nearly half of the amino acids in zein,[1] increased with addition of N or N–K. These results are consistent with other findings[2,3] that

TABLE I

Maize grain yield and crude protein concentration of four Wisconsin varieties as influenced by N or N and K fertilisers

Variety Wis. No.	Fertiliser treatment	Grain yield, kg/ha	Crude protein,* %
273	Control	1900	7·9
	N	8800	7·6
	N–K	8700	9·8
1694	Control	1400	7·9
	N	7300	8·0
	N–K	8100	8·0
1710	Control	700	6·5
	N	8100	8·3
	N–K	8000	8·0
1718	Control	1600	5·8
	N	8000	6·8
	N–K	8000	7·7

* Total N × 6·25

increases in the protein content of maize grain are mainly due to increases in zein. This conclusion is further substantiated by the relationships between the amino acid composition of protein and protein concentration (Table III). Lysine and glycine were related inversely to protein content, and the relationships are highly significant, while the relationships obtained between leucine or glutamic acid and protein, while not significant, are positive.

It is noteworthy that of the nine essential amino acids determined in this investigation, (i.e., lysine, threonine, cystine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine) all but leucine and phenylalanine were related negatively to protein content. Thus, increasing the protein content of maize grain by fertiliser treatment appears to adversely affect the essential amino acid balance.

On average, the essential amino acid composition of maize grain protein (Table IV) did not vary widely among the varieties investigated. However, as the average protein level was increased by fertiliser treatments the content of lysine, threonine, cystine and methionine decreased in the protein and leucine and phenylalanine increased.

Also shown in Table IV are the R and variance of r ($V(r)$) values calculated as described by Kwolek & Van Etten.[6] These values compare the essential amino acid content of the protein source to be evaluated with those of a reference protein, in this case whole hen's egg. An R value of 0·513 with a $V(r)$ of zero would indicate perfect agreement with the hen egg pattern.[6] The R values obtained in this study ranged from 0·376 to 0·391 and are slightly lower than those reported for maize grain by Kwolek & Van Etten.[6] The $V(r)$ values are relatively large, and indicate that there is not a close agreement of the maize amino acid composition with that of the hen's egg. Varieties 273 and 1694 had higher R values than did the other two varieties. The control fertiliser treatment had the highest R and lowest $V(r)$ of the fertility levels studied.

The overall average protein concentration was 7·8% (Table V). Variety 1718 had the lowest average protein content (6·8%); this variety had the lowest protein concentrations in maize grain with all fertiliser treatments (Table I). On average, the N fertiliser treatment increased the protein level by 10%, while the balanced N–K treatment increased the protein concentration by an additional 14% (Table V). The varieties differed somewhat in their N and N–K responses, the average protein increase in the N treatments being due to marked protein increases with varieties 1710 and 1718, while varieties 273 and 1718 had large protein increases with K (Table I).

Table V also gives the average essential amino acid results calculated as a percentage of maize grain. The data calculated in this manner reflect changes in protein as well as amino acid content, and are perhaps more meaningful, as maize grain usually is supplemented with other protein sources in non-ruminant diets. There was little difference in the percentage of lysine, threonine or cystine among varieties or fertiliser treatments. However, varieties 273 and 1694 had higher per-

TABLE III

Relationship of amino acid composition of protein to protein content in maize grain

Amino acid	Correlation coefficient
Lysine	−0·904**
Threonine	−0·721**
Cystine	−0·556*
Valine	−0·546
Methionine	−0·480
Isoleucine	−0·230
Leucine	0·328
Tyrosine	−0·506
Phenylalanine	0·200
Arginine	−0·822**
Aspartic acid	−0·824**
Serine	−0·352
Glutamic acid	0·137
Proline	0·250
Glycine	−0·902**
Alanine	−0·147

* Significant at the 5% level of probability
** Significant at the 1% level of probability

TABLE II

Amino acid composition of maize grain protein in four Wisconsin varieties as influenced by N or N–K fertiliser
g amino acid/100 g protein

Amino acid	Fertiliser treatment											
	Control	N	N–K	Control	N	N–K	Control	N	N–K	Control	N	N–K
	Variety 273			Variety 1694			Variety 1710			Variety 1718		
Lysine	3·07	3·28	2·65	3·59	3·17	3·23	3·43	3·12	3·45	3·78	3·45	2·58
Threonine	3·62	3·78	3·57	3·90	3·83	3·66	3·87	3·36	3·94	3·87	3·71	3·26
Cystine	1·59	1·59	1·28	1·64	1·32	1·53	1·61	1·40	1·64	1·61	1·62	1·48
Valine	4·80	5·13	5·11	5·10	5·01	5·12	5·04	4·47	5·13	4·95	5·23	4·31
Methionine	2·14	1·55	1·73	1·72	1·60	1·85	1·87	1·27	1·45	1·91	1·87	1·42
Isoleucine	3·38	3·54	3·62	3·40	3·52	3·52	3·49	3·04	3·68	3·37	3·43	3·15
Leucine	11·13	11·62	12·99	10·87	11·78	11·57	11·09	9·74	11·86	10·12	11·00	10·87
Tyrosine	4·42	4·40	4·52	4·49	4·55	4·38	4·43	3·89	4·44	4·53	4·39	3·97
Phenylalanine	4·55	4·68	4·84	4·69	4·91	4·60	4·57	4·03	4·95	4·31	4·37	4·49
Arginine	5·04	5·19	4·20	5·92	5·05	5·06	5·44	4·85	5·22	5·71	5·27	4·34
Aspartic acid	6·73	6·89	6·69	6·89	6·66	6·40	7·17	6·49	7·26	7·20	7·04	5·66
Serine	4·54	4·71	4·87	4·76	4·80	4·65	4·76	4·06	4·93	4·70	4·70	4·26
Glutamic acid	17·30	18·02	19·56	17·80	18·46	18·12	17·50	15·39	18·54	16·45	17·88	16·73
Proline	8·09	9·08	8·90	8·63	8·64	8·84	8·21	7·85	8·91	7·43	9·12	8·40
Glycine	4·06	4·03	3·69	4·47	4·03	4·15	4·27	3·74	4·21	4·68	4·50	3·30
Alanine	7·12	7·37	7·94	7·34	7·32	7·60	7·19	6·13	7·31	7·06	7·47	6·56
Total	91·58	94·86	96·16	95·21	94·65	94·28	93·94	82·83	96·92	91·68	95·05	84·75

TABLE IV

Average essential amino acid concentration (%) of maize grain protein

	Amino acid									R^*	$V(r)^* \times 10^3$
	Lys	Thr	Cys	Val	Met	Ile	Leu	Try	Phe		
					Mean of all treatments of all varieties						
	3·23	3·70	1·53	4·95	1·70	3·43	11·22	4·37	4·58	0·384	22·0
Variety:					Mean of all treatments of each variety						
273	3·01	3·66	1·49	5·01	1·81	3·51	11·91	4·44	4·69	0·390	24·0
1694	3·33	3·80	1·50	5·08	1·72	3·48	11·40	4·47	4·73	0·391	21·5
1710	3·33	3·72	1·55	4·88	1·53	3·40	10·90	4·25	4·52	0·377	18·9
1718	3·27	3·81	1·57	4·83	1·73	3·32	10·65	4·30	4·39	0·376	17·5
Treatment:					Mean of all varieties of each treatment						
Control	3·47	3·81	1·61	4·97	1·91	3·41	10·80	4·46	4·53	0·391	17·2
N	3·26	3·67	1·48	4·96	1·57	3·38	11·03	4·31	4·50	0·378	20·5
N–K	2·98	3·61	1·48	4·92	1·61	3·49	11·81	4·32	4·72	0·380	24·4

* Calculated as described by Kwolek & Van Etten[6]

TABLE V

Average protein and essential amino acid composition in maize grain

	Protein, %	Average amino acid concentration in grain, %								
		Lys	Thr	Cys	Val	Met	Ile	Leu	Tyr	Phe
					Mean of all treatments of all varieties					
	7·7	0·24	0·28	0·12	0·38	0·13	0·26	0·88	0·35	0·36
Variety:					Mean of all treatments of each variety					
273	8·4	0·24	0·31	0·12	0·42	0·15	0·30	1·02	0·38	0·40
1694	8·0	0·26	0·30	0·12	0·40	0·14	0·28	0·91	0·36	0·38
1710	7·6	0·25	0·28	0·12	0·37	0·11	0·26	0·82	0·35	0·34
1718	6·8	0·21	0·24	0·10	0·32	0·11	0·22	0·72	0·29	0·30
Treatment:					Mean of all varieties of each treatment					
Control	7·0	0·23	0·27	0·11	0·35	0·14	0·24	0·76	0·31	0·32
N	7·7	0·25	0·28	0·11	0·38	0·12	0·26	0·85	0·33	0·35
N–K	8·4	0·26	0·31	0·13	0·43	0·14	0·30	1·03	0·40	0·42

centages of valine, methionine, leucine, tyrosine and phenylalanine than did the other two varieties, and N–K fertiliser treatment increased the content of valine, isoleucine, tyrosine and phenylalanine to about 20% above the control.

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References

1. Block, R. J., & Bolling, D., 'The Amino Acid Composition of Proteins and Foods', 1951, 2nd edn, p. 104 (Springfield, Illinois: Charles C. Decker)
2. Sauberlich, H. E., Chang, W. Y., & Salmon, W. D., *J. Nutr.*, 1953, **51**, 241
3. MacGregor, J. M., Taskovitch, L. T., & Martin, W. P., *Agron. J.*, 1961, **53**, 211
4. Kurtz, T. L., & Smith, G. E., in 'Advances in Corn Production' (Eds Pierre, W. H., Aldrich, S. A., & Martin, W. P.), 1966, p. 197 (Ames, Iowa: Iowa State Univ. Press)
5. Spackman, D. H., Stein, W. H., & Moore, S., *Analyt. Chem.*, 1958, **30**, 1190
6. Kwolek, W. F., & Van Etten, C. H., *J. agric. Fd Chem.*, 1968, **16**, 496

ISOLATION OF FURTHER COMPONENTS FROM WHEAT GLIADINS

By M. R. BOOTH*

Ion-exchange chromatography on CM-Sephadex C-50 has enabled a wheat protein, the β_3-gliadin, to be isolated in a pure state for the first time from two widely differing varieties. The two proteins were shown to be almost identical in amino acid composition. The preparation of an athin from gliadin, and its analysis, is also reported.

Introduction

Gliadin, a major protein fraction of wheat flour and soluble in 70% ethanol solution, has been shown to be a highly complex mixture of components.[1-3] Numerous attempts have been made to isolate the individual proteins from the mixture, but these have been mostly unsuccessful. Three closely related proteins, γ_1-, γ_2- and γ_3-gliadin, were isolated from a Ponca wheat in 1967 by a combination of chromatography on sulphoethyl-cellulose and gel filtration.[4] More recently[5] three components have been isolated by CM-cellulose chromatography from Wichita wheat gliadin, together with a similar one from Cappelle gliadin. The latter (the athins) have a mol. wt. of ~ 73,000. They are noteworthy because of the absence of sulphur-containing amino acids, and because of a very high content of glutamine and proline. This paper reports the successful isolation for the first time of one of the β-gliadins which provisionally has been called β_3-gliadin (cf. Woychik *et al*[6]).

Experimental

Gliadin preparation

Purified gliadin was prepared by the technique previously described.[5] The flours employed were from a U.S.A. hard red winter wheat (cv. Wichita) and from an English soft red winter wheat (cv. Maris Ranger). Each gliadin was subjected to recycling gel filtration on a column (100 cm × 3·4 cm) of Sephadex G-100 using 0·01N acetic acid as eluant. Monitoring was with a Uvicord recording absorptiometer. The eluate was run through the column at least three times, with selective removal of unwanted protein between runs, to give material which was substantially β-gliadin. It was free from contaminating glutenin.

Ion-exchange chromatography

A column of CM-Sephadex C-50 (90 cm × 1·5 cm) was prepared by equilibriating the ion exchanger (10 g, Pharmacia Ltd.) in 0·05M sodium acetate, 1M in dimethylformamide, adjusted to pH 5·5 with 0·05N acetic acid. Freeze-dried β-gliadin concentrate (190 mg) was dissolved in 5 ml of the above pH 5·5 buffer and was loaded onto the column. After elution for 20 h with this buffer, a salt gradient was applied from two inter-connected flasks each containing 800 ml of the buffer, the second being also 0·1M in NaCl. Fractions, pooled according to their ultra-violet absorption, were dialysed vs. 0·01N acetic acid, freeze-dried, and examined by starch-gel electrophoresis using a sodium lactate buffer, pH 3·3.[7]

This technique yielded 25 mg of the pure β_3-gliadin component from the Maris Ranger wheat flour. Only 8 mg of the β_3-gliadin component was obtained from the Wichita wheat flour since a smaller quantity of β-gliadin concentrate (75 mg) was available.

Amino acid analysis

This was carried out by the technique described by Ewart,[8] corrections being made for slow liberation or destruction of the amino acids during hydrolysis. Performate-oxidised protein[8] was used for calculating the content of sulphur-containing amino acids. Tryptophan was determined from the tyrosine value by the spectrophotometric method of Goodwin & Morton[9] using the formula quoted by Booth & Ewart.[5]

Results and Discussion

Fig. 1 shows the starch-gel electrophoretic patterns of the two purified gliadin starting materials and the β_3-components

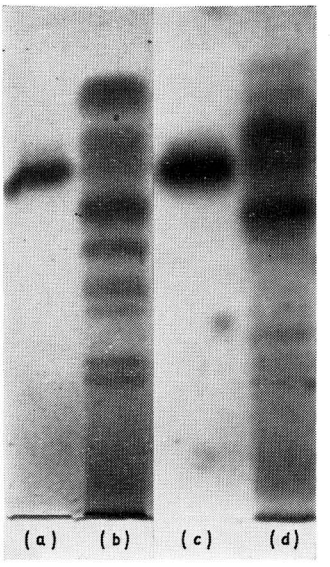

FIG. 1. *Wheat gliadins after starch-gel electrophoresis at pH 3·3*
(a) Wichita β_3-gliadin; (b) purified Wichita gliadin; (c) Maris Ranger β_3-gliadin (d) purified Maris Ranger gliadin
Cathode at the top, sample insertion slots at the bottom

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TABLE I

Amino acid compositions of components of wheat gliadins
Moles of amino acid in 10^5g of recovered anhydro amino acid

Amino acid	Wichita β_3	Maris Ranger β_3	Maris Ranger athin (SP2–2)
Asp	25·3	25·3	1·4
Thr	15·6	14·5	17·7
Ser	50·1	50·0	50·8
Glu	312·7	309·0	357·6
Pro	145·5	147·7	260·7
Gly	22·9	22·2	8·4
Ala	28·2	25·4	3·5
Val	40·6	45·8	3·3
CyS	19·6	21·2	0
Met	5·2	5·3	0
Ile	40·7	41·4	17·7
Leu	62·4	61·9	33·0
Tyr	27·7	29·3	11·7
Phe	32·1	31·3	76·0
Lys	4·4	4·1	2·9
His	14·0	13·5	6·1
Arg	14·1	15·8	3·5
Trp	5·5	3·5	2·7

which have been isolated from them. Background tailing in the starting materials is due to glutenin contamination which was removed in the recycling gel-filtration process.

It was of interest to compare the compositions of the two β_3-gliadins since earlier work on the athins[5] had shown a protein from Wichita wheat gliadin to be very similar in composition to the corresponding protein from Cappelle wheat gliadin (a wheat similar to Maris Ranger). Some minor differences between the two proteins were, however, noted in that work. The amino acid analyses of the β_3-gliadins are given in Table I. The most important feature is the extremely close similarity of the compositions of the two proteins, despite the fact that they have been isolated from two widely differing varieties of wheat. Table I also includes the analysis of an athin (SP2–2) which has been isolated from Maris Ranger flour by the techniques described previously.[5] The composition is almost identical to that of the two corresponding SP2-2 gliadins already characterised.[5]

Unlike the athins the β_3-gliadins contain cyst(e)ine and methionine and, moreover, differ significantly in their content of aspartic acid, proline, and amino acids with hydrocarbon side chains. The faster mobility of the β-gliadins during starch-gel electrophoresis at acid pH is due to their higher content of basic amino acids. Their composition is closer to that of the γ-gliadin[4] and resembles that of unfractionated gliadin.[8]

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References

1. Elton, G. A. H., & Ewart, J. A. D., *Nature, Lond.*, 1960, **187,** 600
2. Elton, G. A. H., & Ewart, J. A. D., *J. Sci. Fd. Agric.*, 1962, **13,** 62
3. Elton, G. A. H., & Ewart, J. A. D., *J. Sci. Fd Agric.*, 1964, **15,** 119
4. Huebner, F. R., Rothfus, J. A., & Wall, J. S., *Cereal Chem.*, 1967, **44,** 221
5. Booth, M. R., & Ewart, J. A. D., *Biochim. biophys. Acta*, 1969, **181,** 226
6. Woychik, J. H., Boundy, J. A., & Dimler, R. J., *Archs Biochem. Biophys.*, 1961, **94,** 477
7. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1966, **17,** 526
8. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1967, **18,** 111
9. Goodwin, T. W., & Morton, R. A., *Biochem. J.*, 1946, **40,** 628

RELATIONSHIP BETWEEN WHEAT PROTEINS

By M. R. BOOTH* and J. A. D. EWART

Recent evidence including amino acid analyses, immunological tests and fingerprinting suggests that the gliadins of wheat varieties have evolved from a common ancestral protein by a series of mutations. No evidence to support a relationship of this nature between the gliadin, albumin and globulin classes was found. Confirmation of the genetic link between wheat and barley is provided by the close structural affinity between purothionin and its barley counterpart. There are some similarities in amino acid composition between a barley trypsin inhibitor and a wheat albumin. Available evidence, though scanty, is significant enough to justify the generalisation that storage proteins of identical mobility in different wheat varieties have closely similar primary and tertiary structures. Although the individual gliadins within a given variety are mutationally linked, their tertiary structures as judged by immunological tests show characteristic differences.

Introduction

Several wheat proteins have now been isolated and analysed by groups throughout the world. It was thought instructive to discuss recent work on these proteins.

Experimental

Gliadin preparation

Wichita and Cappelle–Desprez flours were de-fatted essentially as described previously[1] except that the ratio of water-saturated n-butanol to flour was 10 : 1. Gliadin was prepared and purified by salt fractionation as related earlier[2] to give a material designated 'purified gliadin'.

Athin fraction

Wichita gliadin (900 mg) was dissolved in 15 ml of 0·01 N acetic acid, and loaded on a column (100 × 3·2 cm) of Sephadex G100 equilibriated with 0·01 N acetic acid. The L.K.B. 4900A ReCyChrom apparatus was used at a flow rate of 20 ml/h in conjunction with a Uvicord recorder and a Beaumaris fraction collector. Fractions were grouped, freeze-dried and tested by starch-gel electrophoresis. The one containing athins (20 mg) was run for 17 h at 24 mA in an L.K.B. 3340C column electrophoresis apparatus. The column was filled with sodium lactate buffer, pH 3·5, using Pevikon resin[3] as a supporting medium. After the run fractions were eluted from the column, dialysed vs. 0·01 N acetic acid and freeze-dried. Starch-gel electrophoresis was used to select the purest fraction.

γ-Gliadins

Purified gliadin (Cappelle–Desprez), after being subjected, in four batches, to column electrophoresis as above to reduce the level of glutenin, was separated by gel filtration on the ReCyChrom apparatus. A fraction containing substantially pure γ-gliadin was chosen.

Purified gliadin (Wichita) was run on the ReCyChrom apparatus in four batches, one of which had been previously treated by column electrophoresis. The crude γ-gliadin samples thus obtained were combined and re-run on the ReCyChrom column to give the γ-gliadin preparation which was used.

β-Gliadins

Purified gliadin (Wichita) was fractionated on the ReCyChrom column in five batches, two of which were products of previous column electrophoresis runs. The combined samples rich in the β-group were again separated by ReCyChrom gel filtration. A fraction containing only β-components when run at the normal concentration on starch-gel electrophoresis was chosen.

α-Gliadins

Four batches of purified gliadin (Wichita), including one from a column electrophoretic run, were separated by ReCyChrom gel filtration. The fractions containing α-gliadin were combined and re-run. The purest α-gliadin preparation was taken.

Double diffusion

This was carried out essentially as described earlier.[4] γ-Gliadin for injection was the purest fraction obtainable after ReCyChrom gel filtration of Wichita-purified gliadin. Gliadin for injection was prepared by column electrophoresis of Wichita gliadin in order to lower the level of glutenin contamination. This gliadin had not been precipitated with sodium chloride solution to remove water-soluble proteins, but these appeared to be absent in the fractions combined after column electrophoresis. Serum was prepared by Glaxo (Research) Limited. Rabbits were injected twice at a 20-day interval; Alhydrogel was added to the protein solutions. Sera taken 8 days after the second injection were concentrated ×5 by freeze-drying.

Starch-gel electrophoresis

The technique was as published previously.[5]

Results and Discussion

Nomenclature

The nomenclature of the gliadins in this paper is based on that devised by Woychik *et al.*[6] This scheme of referring to the fast-, medium- and low-mobility sections of the main gliadin group which runs ahead of the athin group[2] as α-, β-, and γ-gliadins respectively is useful. Unfortunately it was originally based on the limited resolution achieved on the Tiselius apparatus. If this nomenclature is intended to be

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permanent the only result will be the unwieldy complexity to which serum protein nomenclature has descended.

High-resolution starch-gel electrophoresis can reveal at least 30 components among the gliadin group alone. Until much more is known about the structures, properties and numbers of wheat proteins it is unlikely that a satisfactory system can be devised, and it is to be hoped that workers will not cling too rigidly to any particular system.

Comparison of amino acid compositions

In Table I are listed amino acid analyses of isolated wheat proteins, expressed as moles of amino acid/10^5 g of protein. The α-gliadin analysis quoted is not that of a pure protein because the preparation gives rise to at least a doublet on gel electrophoresis.[8] It is included in order to provide information at the higher mobility end of the gliadins. The analyses of Huebner & Rothfus[12] on four γ_1-, one γ_3-, and one *Triticum durum* γ-gliadins from six different varieties of wheats have not been included in Table I because values for cystine and tryptophan are missing and because they are close to the analyses for Ponca γ-gliadins as regards the other amino acids.

It is difficult and tedious to compare numbers in a table. The eye is, however, very sensitive to pattern and when the results have been plotted as histograms in Fig. 1, the relationships are readily apparent. The following observations can be made from Fig. 1.

Proteins of approximately equal mobility have very similar amino acid compositions, whether they are from the same variety (Ponca γ-gliadins) or from different varieties (SP2-2 of Wichita, Cappelle–Desprez and Maris Ranger; β_3 of Maris Ranger and Wichita). This also applies to two proteins of equal mobility, purothionin α and hordothionin α, taken from different genera, wheat and barley.

The close resemblances in the amino acid compositions of the athins assign these proteins to a distinct group. The individuals in this group have very probably arisen by mutations.

There is a significant resemblance in the profiles of the α-gliadin doublet, the β_3-gliadin and the γ-gliadins even though these are not from the same variety. This group may, with confidence, be regarded as the product of mutational divergence.

There is sufficient correspondence in patterns between the athins and the faster members of the gliadin group to suggest that the two groups may be linked. The general shape of the histogram should be compared and, in particular, the recurrent shape of the basic + tryptophan section in SP2-1 and γ_2 and γ_3.

The evidence from amino acid compositions does not indicate that there exists a genetic relationship between the albumins, globulins and gliadins. This is negative evidence, of course, and the point can only be settled by comparing their sequences.

The analysis of the barley trypsin inhibitor isolated by Mikola & Suolinna[10] shares a number of features with that of the wheat albumin. This may be seen better if the high valine content of the albumin is covered. The latter was shown by Skupin in these laboratories to possess some slight inhibitory activity towards trypsin. (The mobility of the barley protein has not been compared with the pattern of barley flour proteins, but its analysis suggests that it could lie in the albumin range.)

Ratio of polar and ionic to non-polar side chains

The low value of this ratio for Cappelle–Desprez SP2-2 (Table I) may be due to some uncertainty in the analyser value for proline and possibly glutamic acid as well when compared with the other two SP2-2 analyses. The analyses of these proteins are more prone to error owing to their unbalanced composition.

Contrary to what would be expected from the rough correlation of this ratio and solubility at the iso-electric point,[13] the ratio decreases significantly with increasing solubility in going from the gliadins to the albumins and globulins. Evidently the relationship between solubility and composition is not a simple one. It is probable that the highly crosslinked albumin and globulin molecules will be globular proteins, the approximately spherical shape imparting a minimum surface to volume ratio. Since polar groups are

TABLE I

Amino acid composition in moles of amino acid/10^5g of protein

Amino acid	Wichita SP2–3[2]	Wichita SP2–2[2]	Cappelle–Desprez SP2–2[2]	Maris Ranger SP2–2*	Wichita SP2–1[2]	Ponca γ_1[7]	Ponca γ_2[7]	Ponca γ_3[7]	Maris Ranger β_3*	Wichita β_3*	α-Gliadin[8]	Cappelle–Desprez albumin[9]	Barley trypsin inhibitor[10]	Hordothionin α[11]	Purothionin α[11]
Asp	1·3	1·4	1·2	1·4	5·4	25·0	17·6	16·7	25·3	25·3	26·9	68·6	71·2	47·8	42·4
Thr	13·7	15·9	13·2	17·7	14·9	18·8	17·6	16·7	14·5	15·6	13·4	22·9	49·8	54·8	52·1
Ser	49·3	53·5	53·7	50·8	51·4	50·0	47·1	38·9	50·0	50·1	44·8	60·9	56·9	92·0	101·0
Glu	369·9	362·3	334·1	357·6	336·6	350·0	335·3	344·4	309·0	312·7	322·3	99·0	99·6	23·9	23·9
Pro	260·1	267·7	316·2	260·7	290·2	131·3	158·8	161·1	147·7	145·5	116·4	68·6	78·3	44·3	42·0
Gly	9·4	10·2	8·4	8·4	9·7	18·8	23·5	22·2	22·2	22·9	22·4	76·2	71·2	84·2	90·7
Ala	3·9	3·7	3·4	3·5	5·6	31·3	23·5	27·8	25·4	28·2	22·4	76·2	71·2	49·0	43·7
Val	3·6	3·1	2·9	3·3	3·5	37·5	41·2	38·9	45·8	40·6	40·3	102·8	42·7	31·5	17·8
CyS	—	—	—	—	—	18·8	17·6	16·7	21·2	19·6	17·9	76·2	71·2	156·5	156·9
Met	—	—	—	—	—	6·3	11·8	11·1	5·3	5·2	9·0	22·9	14·2	—	—
Ile	17·9	16·1	14·8	17·7	15·6	43·8	41·2	38·9	41·4	40·7	40·3	15·2	35·6	—	10·1
Leu	32·2	32·0	29·1	33·0	35·1	62·5	64·7	55·6	61·9	62·4	71·6	68·6	64·0	92·7	93·8
Tyr	9·5	9·9	11·7	11·7	10·6	25·0	5·9	5·6	29·3	27·7	26·9	30·5	35·6	15·9	16·9
Phe	75·1	72·7	68·9	76·0	72·2	31·3	41·2	50·0	31·3	32·1	31·3	—	21·3	21·5	23·0
Lys	1·4	2·8	2·7	2·9	2·9	—	5·9	5·6	4·1	4·4	4·5	45·7	14·2	106·1	104·4
His	4·7	5·1	5·0	6·1	5·3	12·5	11·8	11·1	13·5	14·0	17·9	—	21·3	—	—
Arg	3·1	2·7	2·7	3·5	5·5	12·5	11·8	11·1	15·8	14·1	17·9	53·3	64·0	104·8	106·1
Trp	2·1	2·2	2·9	2·7	2·3	—	5·9	5·6	3·5	5·5	9·0	30·5	21·3	—	—
Total	857·2	861·3	870·9	857·0	866·8	875·4	882·4	878·0	867·2	866·6	855·2	918·1	903·6	925·0	924·8
Polar + ionic non-polar	1·12	1·11	0·95	1·11	1·00	1·29	1·05	10·5	1·14	1·15	1·25	0·71	0·84	0·93	0·93

* Analyses from Booth (J. Sci. Fd Agric. 1970, **21**)
Polar + ionic = Asp + Thr + Ser + Glu + Tyr + Lys + His + Arg
Non-polar = Pro + Gly + Ala + Val + Cys + Met + Ile + Leu + Phe + Trp

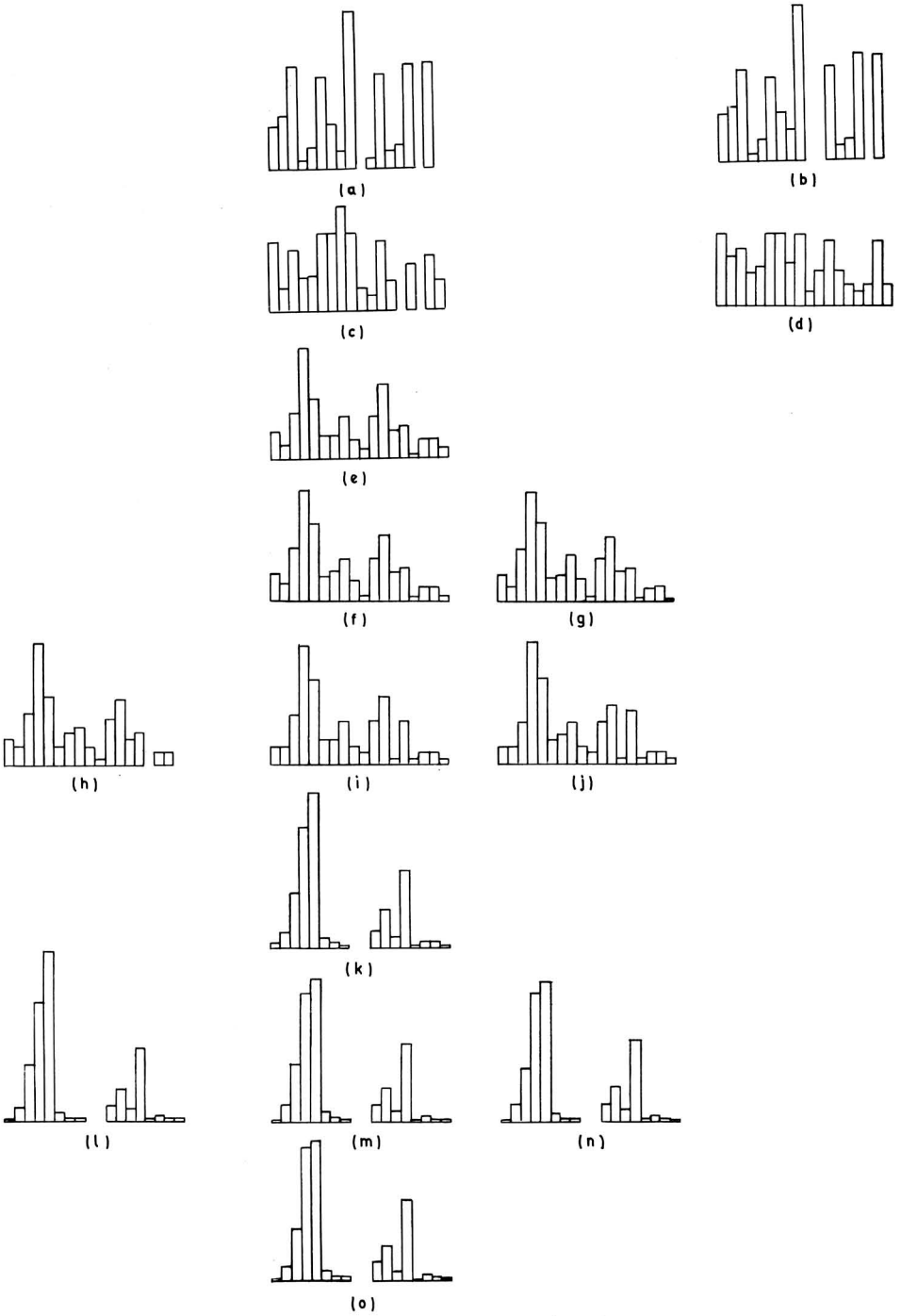

FIG. 1. *Histograms of the amino acid analyses of proteins*

The amino acids are from left to right: Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, CyS(½), Met, Ile, Leu, Tyr, Phe, Lys, His, Arg, Trp
Glu is reduced to ½ and Pro to ½

(a) Purothionin α; (b) hordothionin α; (c) Cappelle–Desprez albumin; (d) barley trypsin inhibitor; (e) α-gliadin; (f) Wichita β_3-gliadin; (g) Maris Ranger β_3-gliadin; (h) Ponca γ_1-gliadin; (i) Ponca γ_2-gliadin; (j) Ponca γ_3-gliadin; (k) Wichita SP2–1; (l) Cappelle–Desprez SP2–2; (m) Wichita SP2–2; (n) Maris Ranger SP2–2; (o) Wichita SP2–3

usually found at the surface and non-polar in the interior of a protein molecule, such a shape would be consistent with a low ratio. Possibly the gliadins may have a more elongated shape or a structure in which some hydrophobic side-chains are in contact with the liquid phase.

As expected, mobility at acid pH is well correlated with content of basic groups.

Immunological tests

Figs 2–4 show some of the double diffusion plates in which sharp gliadin fractions have been compared against rabbit anti-gliadin serum. The electrophoretic patterns of the samples used are shown in Fig. 5.

The athin fraction did not form a precipitin line, behaving similarly to the well filled with water (Fig. 2).

Wichita γ-gliadin formed three strong precipitin lines (Figs 2–4) although the fraction gave a single band on starch-gel electrophoresis (Fig. 5). This is in agreement with the finding of Huebner *et al.*[7] that γ-gliadin contains three proteins. Cappelle–Desprez γ-gliadin on the other hand gave a single precipitin line on one particular plate (Fig. 3), fusing with the inner two lines of Wichita γ-gliadin. A second outer line was, however, usually observed in other plates and

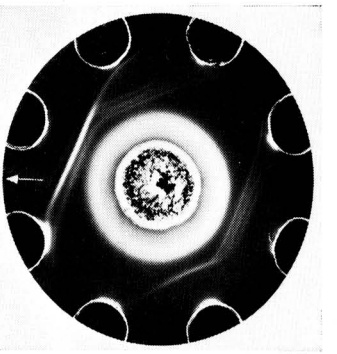

FIG. 3. *Double diffusion plate of gliadin fractions compared with rabbit anti-gliadin sera*

Clockwise from arrow: β; γ; H_2O; H_2O; γ; Cappelle–Desprez γ; H_2O; H_2O
Rabbit anti-Wichita gliadin serum is in the central well. Unless otherwise stated, all protein samples from Wichita

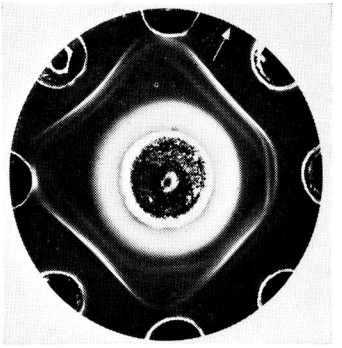

FIG. 2. *Double diffusion plate of gliadin fractions compared with rabbit anti-gliadin sera*

Clockwise from arrow: gliadin; α; β; γ; gliadin; athins; α; H_2O
Rabbit anti-Wichita gliadin serum is in the central well. All protein samples from Wichita

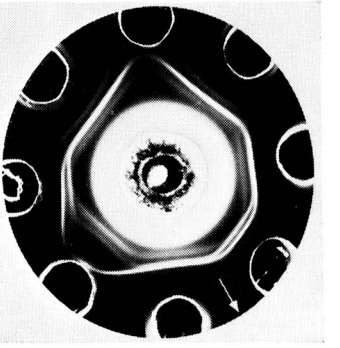

FIG. 4. *Double diffusion plate of gliadin fractions compared with rabbit anti-gliadin sera*

Clockwise from arrow: gliadin; γ; α; β; H_2O; β; gliadin; α
Rabbit anti-Wichita gliadin serum is in the central well. All protein samples from Wichita

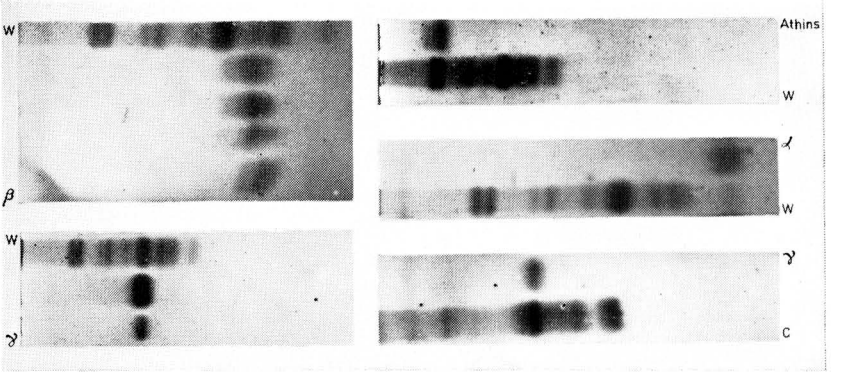

FIG. 5. *Starch-gel electrophoresis of fractions used in double diffusion experiments*

Unfractionated gliadin is designated by W (Wichita) or C (Cappelle–Desprez).
The fractions used are marked appropriately by α, β, γ, or athin

this fused with the outer line of Wichita γ-gliadin. This points to a similarity as regards portions of tertiary structure between components of γ-gliadin in two widely differing varieties. It had earlier been demonstrated that several common antigens were present in gliadins of a number of different wheat varieties and in rye.[4,14] The present result shows that at least two of these antigens have equal mobilities in two widely differing varieties. This is in agreement with the findings of Nimmo & O'Sullivan[15] who produced evidence from immunoelectrophoresis that reactions of identity occurred between components of like mobility in a *Triticum durum* and a *T. vulgare* wheat.

The γ-lines also appear opposite the β-wells. This is almost certainly due to the sensitivity of the test which responds to γ-impurities in the β-fraction. Owing to the incompleteness of the fractionation it is likely that the α-, β-, and γ-fractions are mutually contaminated.

Experience has shown that fractions which appear uncontaminated at normal electrophoretic loading can reveal impurities at higher concentrations. Trace quantities of impurities become sufficiently concentrated in narrow precipitation zones to give clearly visible lines, as described by Crowle.[16] Evidence for contamination was obtained when tests were run with anti-γ-serum. The lines opposite β were confluent with the γ-lines as would be expected if only impurities in the β-fraction were reacting. The α-gliadin gave only a faint line confluent with that of γ-gliadin suggesting that even lower concentrations of γ-gliadin were present. This agrees with Fig. 2 where the outer of the three γ-lines runs farther to the periphery, indicating falling concentration, as it passes through the β- and finally the α-well.

In the cases of the main precipitin lines of α and β it is probable that the inner line opposite the α-wells corresponds to α-gliadin and the outer line to β-impurities. The outer line is confluent with the main β-line as can be seen in Fig. 4. This explanation seems more likely than one which assigns the two α-lines to two α-proteins; the difficulty then arises of explaining why the α-components exhibit reactions of identity with the β-line but not with one another.

It would appear that α-, β-, and γ-gliadin contain immunologically distinct proteins. This is not unexpected because a few mutational changes in amino acid sequence could easily change the tertiary structures of the molecules. Such changes are likely to be tolerated since the conditions to be met by the structures of storage proteins, are presumably less exacting than for enzymes.

This evidence would seem to be to some extent in conflict with that of Grabar and co-workers[17,18] who claimed that all gliadin constituents were identical as judged by immunoelectrophoresis in agar gels at pH 8·2. It is possible that the inferior electrophoretic resolution of gliadin proteins in agar gel may account for the discrepancy.

Conclusions

From published data[19] the number of possible sequences for the amino acids in a molecule of human cytochrome *c* (mol. wt. ~ 12,000) can be shown to be $1{\cdot}414 \times 10^{111}$. It is possible to estimate from data of Hoyle[20] that the observable universe with a radius of ~ 10^9 light years contains matter equivalent in mass to 10^{79} hydrogen atoms (see also Butler[21]). Therefore to make one molecule of all the possible sequences of even a small protein would require a quantity of matter more than 10^{36} times the entire mass of the observable universe!

As long as isomorphous changes in sequence do not involve alterations in charge or chromatographic properties or affect the sites of enzyme attack, it is likely that they will not disturb the appearance of a fingerprint. Bearing this in mind it is nevertheless true that Ingram's fingerprinting technique[22] is a most powerful method of probing sequential changes since, in spite of the astronomical figures just quoted, a change in sequence of a single amino acid can often be detected.

Fingerprinting of purothionin α and hordothionin α indicates that the two molecules possess regions of similar sequence, while immunological tests demonstrate that they have areas of tertiary structure in common.[11] In the case of the athins of unequal mobility close sequential similarity was observed, and for the two which were of equal mobility, even though they came from widely differing varieties, no significant differences in the fingerprints were apparent.[2]

These results suggest firstly that wheat and barley have a common evoluntionary origin; it is exceedingly unlikely that the sequential similarities observed by these workers could be a chance occurrence. Secondly, bearing in mind the odds against two unrelated proteins of similar amino acid composition having the same sequence, the very close sequential relationship of two athins of equal mobility from Wichita and Cappelle–Desprez leads at once to the tentative generalisation that storage proteins of equal mobility from different wheat varieties will have closely similar amino acid compositions and sequences.

It is also suggested that the α-, β-, and γ-gliadins will prove to be related sequentially. They have already been shown to resemble one another in amino acid composition. In the case of components of storage protein in the same variety this is very likely to indicate sequential relationship in view of the precise way that protein biosynthesis is directed. The possibilities for variation in protein structure are so enormous that the observed differences in tertiary structures of the α-, β-, and γ-gliadins are quite compatible with only small differences in sequence. Taking the argument one stage further, in view of sequential similarities between glutenin and gliadin[23] it may be postulated that intervarietal differences in the important glutenin fraction are likely to be determined by small sequential changes.

Whether the sort of changes which are envisaged are likely to have much influence on the rheological properties of proteins is still unknown. It is pertinent to add that there appears to be little variation in tertiary structure of gliadins with variety as judged by immunological reaction. Rheological properties might be expected to depend on intra-molecular and inter-molecular forces[24] and changes in the former would be expected to influence the tertiary structure. Since such changes have not manifested themselves to any appreciable extent, the inference is that the contribution to the rheological properties of dough of the intrinsic protein, after allowance has been made for factors such as quantity, SH content, damaged starch, water content and mechanical treatment, may not be very variable.

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References

1. Elton, G. A. H., & Ewart, J. A. D., *J. Sci. Fd Agric.*, 1966, **17,** 34
2. Booth, M. R., & Ewart, J. A. D., *Biochim. biophys. Acta*, 1969, **181,** 226
3. Müller-Eberhard, H. J., *Scand. J. clin. Lab. Invest.*, 1960, **12,** 33
4. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1966, **17,** 279
5. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1966, **17,** 526
6. Woychik, J. H., Boundy, J. A., & Dimler, R. J., *Archs Biochem. Biophys.*, 1961, **94,** 477
7. Huebner, F. R., Rothfus, J. A., & Wall, J. S., *Cereal Chem.*, 1967, **44,** 221
8. Bernardin, J. E., Kasarda, D. D., & Mecham, D. K., *J. biol. Chem.*, 1967, **242,** 445
9. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1969, **20,** 730
10. Mikola, J., & Soulinna, E.-M., *Europ. J. Biochem.*, 1969, **9,** 555
11. Redman, D. G., & Fisher, N., *J. Sci. Fd Agric.*, 1969, **20,** 427
12. Huebner, F. R., & Rothfus, J. A., *Cereal Chem.*, 1968, **45,** 242
13. Fox, F. W., & Foster, J. F., 'Introduction to Protein Chemistry', 1957, p. 240 (New York: John Wiley & Sons)
14. Elton, G. A. H., & Ewart, J. A. D., *J. Sci. Fd Agric.*, 1963, **14,** 750
15. Nimmo, E. C., & O'Sullivan, M. T., *Cereal Chem.*, 1967, **44,** 584
16. Crowle, A. J., 'Immunodiffusion', 1961, p. 83 (New York: Academic Press)
17. Benhamou-Glynn, N., Escribano, M.-J., & Grabar, P., *Bull. Soc. Chim. biol.*, 1965, **47,** 141
18. Grabar, P., Escribano, M.-J., Benhamou, N., & Daussant, J., *J. agric. Fd Chem.*, 1965, **13,** 392
19. Matsubara, H., & Smith, E. L., *J. biol. Chem.*, 1963, **238,** 2732
20. Hoyle, F., 'The Nature of the Universe', 1953, p. 86 (Oxford: Blackwell)
21. Butler, J. A. V., 'Inside the Living Cell', 1959, p. 157 (London: Allen & Unwin Ltd.)
22. Ingram, V. M., *Nature, Lond.*, 1956, **178,** 792
23. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1966, **17,** 30
24. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1968, **19,** 617

CHLOROPHYLLS AND THEIR DERIVATIVES DURING DRYING OF SULTANA GRAPES

By D. E. BOTTRILL and J. S. HAWKER

Untreated berries of *Vitis vinifera* and berries treated with commercial dipping solution were dried in the dark, full shade or in the sun. Dipping treatment increased the drying rate.

The major pigments, namely, chlorophyll *a*, chlorophyll *b*, pheophytin *a*, pheophorbide *a* and the carotenoids, decreased in all treatments where berries received light. Only the chlorophylls and carotenoids decreased in berries dried in the dark, but not to the same extent as in berries exposed to light during drying. Pigment loss was not directly related to berry moisture content.

Berries dried in the dark remained green while berries dried in the light changed to brown if untreated or pale golden-brown if treated with commercial dipping solution. *In situ* removal of the magnesium from the magnesium porphyrin compounds demonstrated that the green coloration was due to the chlorophylls and chlorophyllides.

Introduction

Grape berries of *Vitis vinifera* L. cv. Sultanina (Sultana, Thompson Seedless) are dried quickly in Australia to produce pale golden brown sultanas, whereas the same variety is sun-dried to produce dark brown raisins in the U.S.A. or dried slowly to produce 'green naturals' or 'Soyagi' in Afghanistan.[1] In Australia the golden-brown dried sultanas are obtained by immersing fresh berries in commercial dipping solution to increase their drying rate,[2] drying them on a tiered rack to approximately 13% moisture content and spreading them in the sun for several days to remove any residual green coloration. However, under some conditions sultanas dried commercially retain an unwanted green tinge which could be due to residual chlorophyll. This paper reports changes in the chlorophyll content of sultanas dried under a range of conditions.

Experimental

Drying conditions

Bunches of mature sultana grapes were harvested from a commercial irrigated vineyard in South Australia and divided into six 3 kg samples. Three samples were immersed in a commercial cold dip[2] for 3 min, allowed to drain and either hung in the dark at 25–29°, laid on wire mesh outside in full sunlight or laid on wire mesh outside in full shade. Undipped samples were put in similar environments.

Sampling procedure, pigment extraction and assay

Samples were taken at intervals during the drying, and uniformity between samples was achieved by taking single berries from the apex centre and base of a number of primary branches leading from the main bunch axis. Berries (80–200) were weighed individually and divided into 3 samples. Each sample (16–25 g) was homogenised for 2 min in a Sorval Omnimix at 240 V with 2 g calcium carbonate and 80 ml of acetone and water, depending on the moisture content of the berries, to make the final concentration of acetone to 80%. Excess calcium carbonate was allowed to settle and the homogenate was filtered through a fine cloth into a separatory funnel containing 50 ml diethyl ether and shaken. After being shaken with 200 ml 1% aqueous sodium chloride the ethereal phase separated, and the ethereal layer was washed twice with 50 ml 1% aqueous sodium chloride. The ethereal layer was adjusted to 25 ml of which 15 ml was used for the analysis of chlorophylls *a* and *b*, chlorophyllides *a* and *b*, pheophytins *a* and *b* and pheophorbides *a* and *b*.[3] Carotenoids were measured in the remaining 10 ml fraction which was saponified with 10 ml freshly prepared methanolic potassium hydroxide (2·3 ml 60% potassium hydroxide in 7·7 ml methanol), washed twice with 50 ml 4% aqueous sodium chloride, dried with anhydrous sodium sulphate and the extinction measured at 442 nm. An average extinction coefficient of 240 l/g cm was used.[4]

Spectra of all solutions were examined between 400 and 700 nm on a Beckman DB-G recording spectrophotometer. There was no evidence of the presence of significant quantities of contaminating pigments.

Acid treatment of dried berries

After storage for 3 months at 4° in an air-tight container samples of 30 berries of the dark-dried dipped treatment were washed in chloroform to remove waxes, placed in 3 N hydrochloric acid for 2 h, washed with H_2O, blotted dry and allowed to stand overnight in an open vessel at room temperature. The pigments were then extracted and assayed as above.

Results

Extraction of pigments

Grape berry homogenates have a low pH which could result in pigment conversions during storage or extraction. The results of determinations made on freshly picked berries and berries frozen overnight and thawed just prior to pigment extraction were compared. The results (Table I) indicate that two reactions took place on freezing and thawing berries.

TABLE I

Effect of freezing on pigment distribution in fresh berries

Pigment	n moles/berry	
	Fresh	Frozen
Chlorophyll *a*	6·192	2·102
„ *b*	2·558	1·469
Chlorophyllide *a*	0	0·689
„ *b*	0	0·468
Pheophytin *a*	1·079	3·108
„ *b*	0	0
Pheopharbide *a*	1·746	2·160
„ *b*	0	1·025
Total chlorophyll and chlorophyll derivates	11·021	11·575

Chlorophyll was converted to chlorophyllide and the chlorophyll and chlorophyllide were converted to pheophytin and pheophorbide respectively.

Routinely, berries were extracted as soon after sampling as possible to avoid inter-conversions of pigments.

Effect of treatment on berry chlorophyll and chlorophyll derivatives

Dipping treatment increased the drying rate of berries in all environments (Fig. 1). Final harvests were made when berries had reached a moisture content of about 12%. The sum of the chlorophylls, pheophytins, chlorophyllides and pheophorbides are shown plotted against time in Fig. 2. Berries in the full sun lost the pigments most rapidly and to the greatest extent while berries maintained in the dark showed the slowest and smallest loss of these pigments. Undipped berries lost pigments more rapidly than dipped berries in the dark while the reverse occurred in the shade. Dipping had no effect on the pigment loss in the sun.

The relationship between total pigment and berry weight was different for each treatment (Fig. 3) indicating that factors other than water loss are important in pigment breakdown.

The distribution of the various components in the pigment for the six treatments is shown in Fig. 4. The standard error was similar for all components and an average standard error at each time is indicated on the top curve of each group.

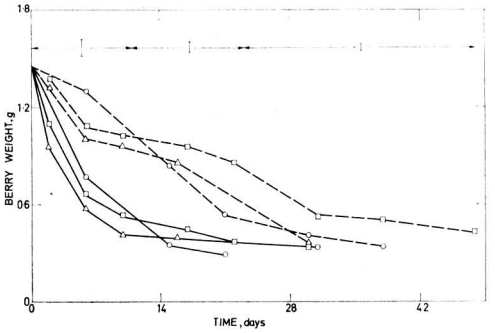

FIG. 1. *Drying rates of dipped* (———) *and undipped* (— — —) *sultana berries*

○ Dark-dried; □ shade-dried; △ sun-dried

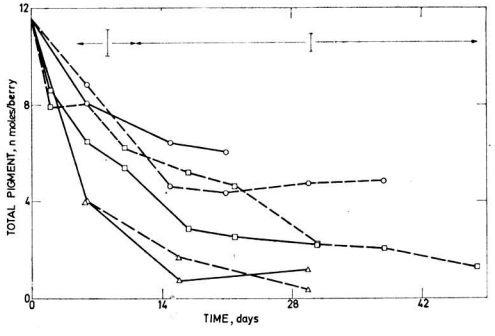

FIG. 2. *Rate of loss of the total pigment (chlorophylls* a *and* b, *chlorophyllides* a *and* b, *pheophytins* a *and* b *and pheophorbides* a *and* b) *in dipped* (———) *and undipped* (— — —) *sultana berries*

○ Dark-dried; □ shade-dried; △ sun-dried

Chlorophylls *a* and *b*, pheophytin *a* and pheophorbide *a* were the major components of the pigment. Berries maintained in the dark had constant levels of pheophytin *a* and pheophorbide *a* throughout the drying period while chlorophylls *a* and *b* decreased; no pheophytin *b* was detected. In all other treatments all major components decreased.

The colour of dried berries and the Munsell colour notation[5] for representative berries from each treatment are shown in Table II. In the dark, dipping treatment resulted in a much more uniform colour. Undipped berries dried in the dark ranged in colour from green to brown, the majority of berries being green. In full sun and shade undipped berries resulted in dark brown 'naturals' with tough skins, while dipped berries remained light in colour. A higher proportion of shade-dried dipped berries retained a green tinge than sun-dried dipped berries.

Effect of treatment on berry carotene

Carotenoids were degraded under all conditions (Fig. 5). The pattern of carotenoid loss in each treatment was similar to the chlorophyll loss (Fig. 3) although at any berry weight a larger proportion of the carotenoids was lost indicating a more rapid rate of breakdown of carotenoids.

Effect of acid treatment on dried berries

Dark-dried dipped berries stored in the dark at 4° for 3 months retained both colour and total pigment and little change occurred in the distribution of the pigments (Table III). Acid treatment changed the colour of the berries from green to golden-brown. Chlorophyll and chlorophyllide decreased, pheophytin and pheophorbide increased, but no change in either the sum of these pigments or in carotenoid content occurred (Table III).

Discussion

In brining cucumbers chlorophyll loss was accompanied by increases in chlorophyllides, pheophytins and pheophorbides and it was suggested that these changes were due to a combination of chlorophyllase action and a replacement of magnesium by hydrogen.[3] Similar changes occurred when fresh sultana berries were frozen and thawed (Table I) and when dried berries were treated with acid (Table III), in which case the berries lost their green coloration. Chlorophyll was

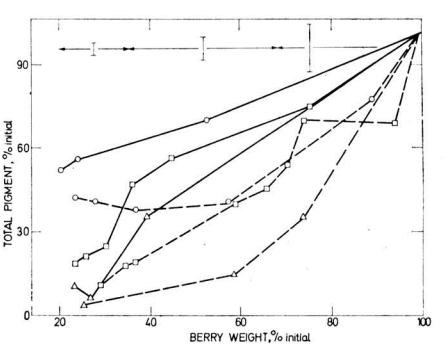

FIG. 3. *Relationship between total pigment loss and loss of berry weight for dipped* (———) *and undipped* (— — —) *sultana berries*

○ Dark-dried; □ shade-dried; △ sun-dried

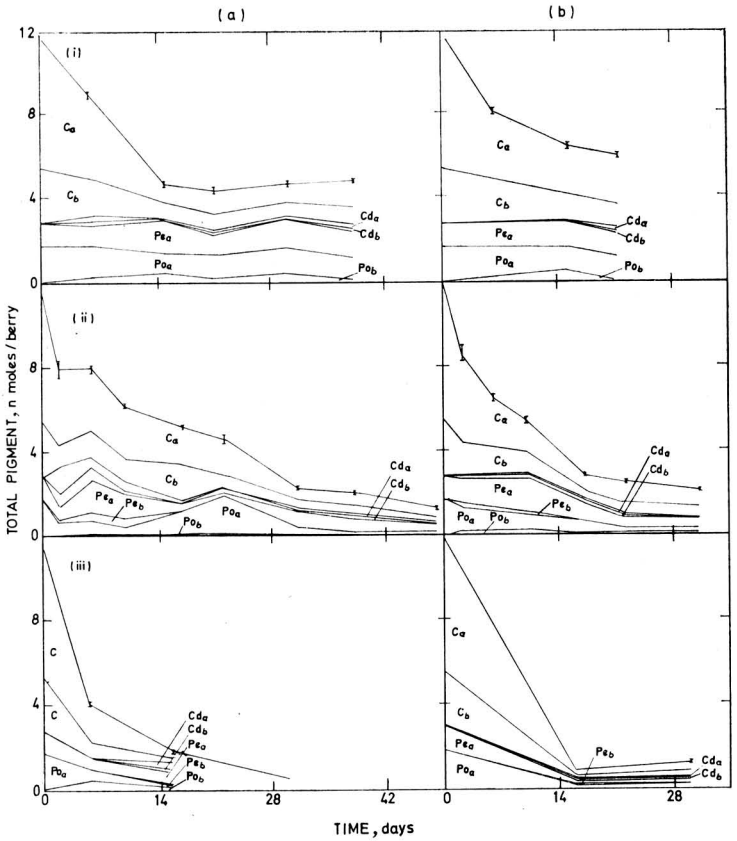

FIG. 4. *Composition of the total pigments for* (a) *dipped and* (b) *undipped sultana berries dried in* (i) *the dark*, (ii) *shade and* (iii) *sun*

The amount of each pigment is represented by the distance between lines; the upper line therefore represents the total pigment.
C_a and C_b, chlorophyll *a* and *b*; Cd_a and Cd_b, chlorophyllide *a* and *b*; Pe_a and Pe, pheophytin *a* and *b*; Po_a and Po_b, pheophorbide *a* and *b*

lost to varying degrees in all drying treatments but there was not a concomitant increase in chlorophyllides, pheophytins or pheophorbides (Fig. 4). Possibly other reactions account for the chlorophyll breakdown in drying sultanas.

TABLE II

Effect of treatment on berry colour

Treatment	Visual assessment of colour	Munsell colour notation for representative berries	
Dipped: dark-dried	Green	10Y	5/6
shade-dried	Golden-brown with slight green tinge	2Y	5/6
sun-dried	Golden-brown	10YR	5/6
Undipped: dark-dried	Green	10Y	5/5
shade-dried	Dark brown	5YR	2·5/2
sun-dried	Dark brown	5YR	3/2

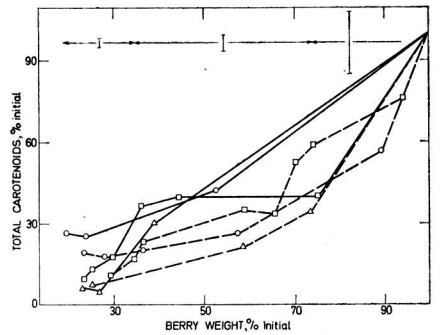

FIG. 5. *Relationship between carotenoid loss and loss of berry weight for dipped* (———) *and undipped* (— — —) *sultana berries*

○ Dark-dried; □ shade-dried; △ sun-dried
Initial carotenoid content was 3·4 μg/berry

TABLE III

Effect of storage and acid treatment on pigment distribution in dried berries

Each value is the mean of 3 replicates. Mean standard error of individual pigments was ± 0·135

Pigment nmoles/berry	Freshly dried sultanas*	After 3 months' storage†	Acid treated
Chlorophyll *a*	2·247	2·254	0·550
„ *b*	1·105	1·193	0·335
Chlorophyllide *a*	0·225	0·048	0·022
„ *b*	0·116	0·136	0·038
Pheophytin *a*	0·999	1·820	4·020
„ *b*	0	0	0·543
Pheophorbide *a*	1·195	1·268	2·035
„ *b*	0·118	0·454	0·494
Total chlorophyll and chlorophyll derivates	6·005	7·173	8·037
Carotenoids, μg/berry	0·888	0·834	0·605
Colour	Green	Green	Golden-brown

* Dipped berries were dried in the dark
† The berries were stored in air-tight containers in the dark at 4°c

Chlorophyll bleaching can take place in both the absence and presence of light by oxidation reactions.[6,7] In the dark chlorophyll bleaching is thought to be initiated by the disintegration of the photosynthetic apparatus[8] while in the light bleaching is due to reaction between photosensitised chlorophyll and molecular oxygen.[7] Carotenoids are usually degraded more rapidly than chlorophyll and are thought to protect chlorophyll from photochemical bleaching.[9] In drying sultanas loss of carotenoids was more rapid than chlorophyll loss but the difference between residual carotenoids in sultanas dried in the presence or absence of light was relatively small to the total loss of carotenoids (Fig. 5). Other factors than light must be involved in carotenoid breakdown.

The present results show that chlorophyll and chlorophyll derivatives are not completly broken down during the drying of sultanas and the amount remaining depends on the drying conditions. The intensity of green colour in the dried sultanas was correlated with the amount of chlorophyll remaining Both chlorophyll and green colour were preserved during storage of dried fruit and acid treatment reduced both chlorophyll and green colour (Table II). These facts together, with the absence of other obvious green pigments in the berries, show that chlorophyll can cause green tinge in dried sultanas.

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References

1. Grncarevic, M., *Am. J. Enol. Vitic.*, in the press
2. Radler, F., *J. Sci. Fd Agric.*, 1964, **12,** 864
3. White, R. C., Jones, I. D., & Gibbs, E., *J. Fd Sci.*, 1963, **28,** 431
4. Boardman, N. K., & Anderson, J. M., *Biochim. biophys. Acta*, 1967, **143,** 187
5. Munsell, A. H., 'Munsell Book of Color', 1929 (Baltimore, Md: Hoffman Bros. Co.)
6. Kirk, J. T. O., in 'The Plastids', (Kirk, J. T. O., & Tilney-Bassett, R. A. E., eds) 1967, p. 562 (London: W. H. Freeman and Co.)
7. Bonner, J., 'Plant Biochemistry', 1952, p. 447 (New York: Academic Press Inc.)
8. Shibuya, I., & Hase, E., *Pl. Cell Physiol.*, 1965, **6,** 267
9. Krinsky, N. I., in 'Biochemistry of Chloroplasts' (Goodwin, T. C., ed.) 1966, Vol. I, p. 423 (New York: Academic Press Inc.)

EFFECT OF PROCESSING VARIABLES ON THE STABILITY OF β-CAROTENES AND XANTHOPHYLLS OF DEHYDRATED PARSLEY

By M-D. NUTTING, H. J. NEUMANN and J. R. WAGNER

Curled- and plain-leaf parsley were analysed for β-carotene, xanthophyll and the stereo-isomers of β-carotene, before and after various combinations of freezing, blanching, sulphiting and air-drying. Heating of parsley by blanching or by dehydration of unblanched samples generally resulted in an increased retention of β-carotene. Dried samples contained a higher percentage of the *cis*-isomer and neo-β-carotene *b* but a lower percentage of the all-*trans*-isomer. Freezing unblanched parsley reduced total carotene content 8–13%. Xanthophyll changes were similar to those for total carotene except that dried samples contained 2–28% less xanthophyll than fresh parsley. Sulphited samples retained 8–30% more carotenoid pigments than the non-sulphited samples. Total β-carotene of dried parsley stored for 3 months in air and in nitrogen at 22° decreased 10–15%, and 3·5–10%, respectively, while that stored at −18° showed no change. The stereo-isomer ratio did not change. Blanching had no apparent effect on carotene stability in storage. Carotene loss in fresh parsley, additional extractions, and enzymic destruction of added carotene were also investigated.

Introduction

Early experiments in this laboratory by Thompson *et al.*[1] indicated that increased temperature is the principal factor responsible for the formation of *cis*-isomers of β-carotene during drying of lucerne (alfalfa). Deuel *et al.*[2,3] found that, when fed to rats, the *cis*-isomers neo-β-carotene *u* and neo-β-carotene *b* had respectively, 38 and 53% of the growth-promoting activity of all-*trans*-β-carotene. Therefore, variation in β-carotene and its isomers during the processing and storage of parsley were followed as one measure of quality change and storage stability. The decision to include total xanthophylls as another measure of processing effects was based on the observation of Thompson & Maclay[4] that xanthophylls were retained better than carotene during processing and storage of dehydrated lucerne. Later Livingston *et al.*[5] found xanthophylls to be less stable during dehydration.

Experimental

Materials and Processing

Two types of parsley were used: curled leaf (samples 1 and 2) purchased at a local market and freshly cut plain leaf (sample 3) obtained from the grower in Mountain View, California. The parsley was washed, drained, and trimmed to separate the cluster of leaf and adjacent stem from the larger stem material. The leaves were spread on trays at 0·5 lb/ft^2.

Part of each sample of parsley was steam-blanched on trays at 100° for 25 sec, and was cooled with water sprays and air blasts. This blanch was adequate to inactivate peroxidase according to the semi-quantitative Masure & Campbell[6] test. Sub-samples of unblanched and blanched parsley from sample 1 were sulphited in trays with spray solution containing 1000 ppm SO_2 and 0·25% $NaHCO_3$. The blanched parsley was sulphited without cooling; the unblanched parsley was at room temperature when sulphited. After sulphiting, all trays were inclined about 30° and allowed to drain for 3 min.

Assay samples were collected for each pre-dehydration variable, placed in Mason* jars with tight-fitting lids and held at 1° while being analysed over a period of 2–3 days. Portions of each sample, unblanched and blanched, were frozen on trays in an air-blast freezer at −32°. These frozen samples were stored in tightly closed containers held at −18°.

After sampling, the sub-samples were dried in a cabinet with cross-flow air at 66° to moisture levels of 6·3–9·6%, except one sub-sample of plain-leaf parsley which was dried to 17·5% moisture. After samples were removed for analysis, the remainder of each sub-sample was bin-dried overnight at 47 ± 2° to final moisture contents ranging from 2·5 to 4·7%. The processing data are listed in Table I.

Comments by members of the dehydration industry indicate that carotene in parsley can be lost rapidly after harvest. Because completion of all analyses on fresh parsley sometimes extended to 72 h in the early experiments an additional sample of curled-leaf parsley was harvested in order to follow post-harvest changes in carotene content prior to processing. This was separated into 10 sub-samples of about 600 g each. One sub-sample was placed between slabs of dry ice. The other sub-samples were assayed for total β-carotene after being held in perforated plastic bags for 3 h at room temperature or for approximately 1, 2, 5, 7 and 12 days at 1°. One sub-sample of unfrozen parsley and one frozen in the field were put into a Stokes vacuum plate dryer about 4·5 h after harvest and dried at 38° and 900 μm pressure for 19 h. Another sub-sample, 5 h after harvest, was air-dried in a through-flow dryer at 63° for 45 min and then at 49° for 130 min. All parsley was trimmed by uniformly removing leaf material just below the first fork of stem before assay.

Analytical

Total solids and alcohol-insoluble solids

Slurries (80% alcohol) were prepared by blending 100 g of parsley and 450 g of 95% ethanol. Total solids and alcohol-insoluble solids (*AIS*) of unfrozen and frozen parsley were determined by procedures described by Makower *et al.*[7] except that samples were dried in a vacuum oven at 70° for 40 h instead of 48 h.

Water in dried parsley was determined by the Fischer method[8] except that methyl Cellosolve replaced methanol in the preparation of the Fischer reagent.[9]

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable

Sulphite

Sulphite was determined by the Monier–Williams[10] method of distillation into hydrogen peroxide. The peroxide solution was then titrated with 0·1 N-NaOH standardised with bromophenol blue.

Sugars

The A.O.A.C.[10] procedures used for the determination of reducing and total sugars as glucose were from sections 6.074, 6.076. 6.083 and (1) 29.056.

Extraction of carotenoids

A 10 g or 20 g sample of fresh or frozen parsley was ground and extracted for 2 min with anhydrous acetone in a Waring blender. The slurry was transferred to a volumetric flask, made to 100 ml, swirled for 1 min and allowed to settle for 1 h. Analytical methods of Bickoff *et al.*[11] sometimes slightly modified, were used for the separation of β-carotene. A 5 ml aliquot of the clear supernatant acetone solution was added to 5 ml of Skellysolve B (petroleum ether b.p. 67°) plus 5 ml of water in a 50 ml separating funnel. The mixture was swirled for 1 min to avoid forming an emulsion and to transfer the carotenoid pigments into the upper layer of petroleum ether. The lower acetone–water layer was drawn off and discarded. The stem of the separating funnel was wiped dry with a cotton swab, and the entire upper layer was transferred to a column for separation of the carotenoids.

Extraction of carotenoids from dried material was carried out according to the method of Thompson *et al.*[12] The dried parsley leaves were ground through a 40-mesh screen in a Wiley mill. A 2·0 g sample was extracted overnight with 30 ml of acetone–Skellysolve B (3 : 7 by vol.) in a 100 ml volumetric flask. The next morning the extract was made to volume with Skellysolve B, shaken well, and allowed to settle for 1 h. A 2–5 ml aliquot was added to a chromatographic column for separation.

Preparation of columns

For the carotene and xanthophyll separations a glass column (20 × 1·5 cm) with a 4 cm funnel top was firmly packed to a height of 7·5 cm with a mixture of equal weights of Sea Sorb 43 and Hyflo Supercel while under a vacuum of 5–6 in. of Hg. A layer of anhydrous sodium sulphate was added to the column to remove any moisture remaining in the parsley extract.

For the separation of the β-carotene stereo-isomers a glass column (20 × 1 cm) was packed with calcium hydroxide which had been dried overnight at 100°. A small amount of powder, about 1 cm at a time, was tightly packed under vacuum. After each addition, the top 3 mm was scraped loose before adding a second portion of powder to avoid the formation of visible layers in the column and to produce more even bands of the pigments. The column height was 4·5 cm.

Separation of carotenoids

The β-carotene was eluted from the columns under vacuum (5–6 in. of Hg) with Skellysolve B–acetone (9 : 1 by vol.). The yellow eluate was completely removed from the column and the flask was replaced by a second 25 ml volumetric flask. The column was then washed with 15–20 ml of Skellysolve B–acetone–methanol (88 : 10 : 2 by vol.) (White, D. & Kohler, G. O., 1965, unpublished data). When the orange-yellow xanthophyll had moved about halfway down the column, the clear eluate in the flask was discarded and the band of xanthophyll was eluted. Both carotenoid samples were made to volume for measurements on a Cary 15 spectrophotometer. No attempt was made to separate the various xanthophylls.

Separation of stereo-isomers

A measured amount of the 25 ml β-carotene sample was evaporated just to dryness on a roto-evaporator at 20°. The vacuum was slowly increased to 30 in. of Hg in order to prevent foaming and consequent loss of sample. The dried β-carotene was dissolved in exactly 1·0 ml of Skellysolve C and a measured amount was added to the dry $Ca(OH)_2$ column. The solution was allowed to soak in to the level of the powder. A 2·5% solution of *p*-methyl anisole in Skellysolve C was quickly added to the column and the light yellow band of *cis*-isomer, neo-β-carotene *b*, was eluted under slight vacuum and collected in a 10 ml flask. A few drops of clear solution were eluted and discarded and the remaining *cis*-isomer, neo-Δ-carotene *u*, was washed off the column with a 5% solution of actone in Skellysolve C. This last portion of pigment, collected in a 5 ml volumetric flask, appeared to consist of several diffuse bands.

Some difficulty was experienced in consistently separating the band of all-*trans*-β-carotene from the neo-β-carotene *u*. A blue filter (Corning No. 4308) was placed over the aperature of a microscope light to illuminate the column. This caused the bands of pigment to appear a deeper yellow with more clearly discernible edges and resulted in better separation.

All of the above separations were carried out at 22° and the chromatographic columns were kept under dim light throughout the experiment to avoid degradation. Samples were made to volume and spectra were run immediately. The stereo-isomers were calculated as percentage of the total β-carotene previously determined.

Stored samples

Dried unblanched and blanched samples were stored for three months under nitrogen and under air at 22 and −18°. These samples were also analysed for total β-carotene and its stereo-isomers.

Statistical procedure

Least-squares analysis of variance, by the procedures of Harvey[13] for unbalanced designs, was used to determine levels of significance of β-carotene in non-sulphited samples.

Results and Discussion

The total β-carotene for each of the 3 samples of parsley, unblanched and blanched, before and after freezing and after cabinet and subsequent bin-drying (Table I) showed similar changes influenced by processing. These changes are reflected in the percentage difference between the carotene contents of the treated parsley and the starting material.

Total β-carotene values for each sample (Table II), representing the means of all analyses, were significantly ($P = 0{\cdot}01$) different from each other. After freezing, unblanched parsley had significantly ($P = 0{\cdot}01$) less carotene than before freezing; β-carotene in blanched parsley was more stable with no significant change due to freezing. De Felice & Fellers[14] noted a large loss of carotene in spinach after freezing. Zscheile *et al.*[15] reported that β-carotene of blanched peas increased 29–35% after 6 days at −20° and explained the increase as due to better extraction.

TABLE I

Effect of processing on β-carotene content in parsley

Treatment	β-carotene					
	M.f.b.,* ppm			% difference from unfrozen, unblanched		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Unblanched:						
Unfrozen	371 ± 9	354 ± 36	425 ± 65	—	—	—
Frozen	342	307 ± 34	373 ± 6	−7·8	−13·3	−12·2
Dried (cabinet)	457 ± 27	426 ± 8	482 ± 1	+23·2	+20·3	+13·4
Dried (bin)†	458 ± 30	397 ± 11	527 ± 28	+24·4	+12·1	+24·0
Blanched:						
Unfrozen	420 ± 38	389 ± 37	483 ± 7	+13·2	+9·9	+13·6
Frozen	418 ± 44	363 ± 36	483 ± 23	+12·7	+2·5	+13·6
Dried (cabinet)	448	402 ± 14	533	+20·8	+13·6	+25·4
Dried (bin)†	423	330 ± 37	454 ± 18	+14·0	−6·8	+6·8

* M.f.b.: Moisture-free basis. Mean values ± standard deviation; averages of 1–11 samples with 2–3 assays per sample
† After being cabinet-dried

Before dehydration, blanched parsley contained significantly ($P = 0·01$) more β-carotene than the corresponding unblanched sample; however, after bin-drying the unblanched parsley had significantly ($P = 0·01$) more β-carotene than the blanched material. Similar changes in carotene content have been reported in the literature. Greater retention of β-carotene in various plant materials after blanching has been attributed generally to inactivation of enzymes. However, Griffith & Thompson[16] noted that blanching lucerne destroyed the natural antioxidants and thus could decrease the stability of carotenes. Loss of these natural antioxidants could account for the lower carotene values in the blanched parsley after drying.

Three stereo-isomers of β-carotene were determined in all samples of samples 2 and 3 (Table III). The neo-β-carotene *b* fraction increased with each successive processing step (except the undried samples of sample 2), while the all-*trans* decreased. The changes in neo-β-carotene *u* showed no definite trend.

Xanthophyll changes in unblanched parsley followed the same pattern as for total carotene, except that the bin-dried samples contained 2–7% less than the unfrozen material (Table IV). Blanched parsley contained about one-third more xanthophyll than the unblanched parsley before freezing. Xanthophyll content in blanched parsley decreased with each successive processing procedure, and after bin-drying the blanched parsley contained 10–28% less than the starting material.

Sulphited samples (sample 1 only) followed the same pattern of carotenoid pigment change as unsulphited parsley except that the carotene content was 8–30% higher than similarly processed unsulphited material (Table V). Xanthophyll values also were higher in sulphited samples. These higher carotenoid values in sulphited parsley, particularly in the dried material, reflect the antioxidant properties of sulphate dioxide.

The percentage moisture ranged from 2·5 to 3·5% in bin-dried unsulphited parsley, with a maximum of 0·3 percentage units between blanched and unblanched samples from the same sample. When these bin-dried samples were stored at 22° for 3 months in air and in nitrogen, total β-carotene values decreased 10–15% and 3·5–10%, respectively; blanching gave no significant effect on carotene stability in storage. At −18° total carotene content did not change in any of the samples held in air or nitrogen. Under these storage conditions the stereo-isomers in parsley did not show any significant change in content. Schillinger & Zimmerman,[17] determining the carotene content and stability of numerous vegetables, found β-carotene to be particularly stable in freeze-dried parsley that was stored in vacuum at ambient temperatures (5–28°) for up to 3 years.

TABLE II

Effect of treatment on β-carotene content in parsley

Number of samples in parentheses

Comparison	β-carotene		Significance level
	ppm	m.f.b.	
	(20) (46) (24)		
Samples 1, 2, 3	423	370 473	*
Unblanched:			
	(18)	(9)	
Unfrozen & frozen	380	344	*
	(10)	(9)	
Cabinet & bin-dried	456	462	n.s.
	(27)	(19)	
Undried & dried	362	459	*
Blanched:			
	(10)	(15)	
Unfrozen and frozen	442	431	n.s.
	(13)	(6)	
Cabinet and bin-dried	457	401	*
	(25)	(19)	
Undried & dried	437	429	n.s.
	(46)	(44)	
Unblanched & blanched:	410	433	n.s.
	(27)	(25)	
Undried	362	437	*
	(10)	(13)	
Dried (cabinet)	456	458	n.s.
	(9)	(6)	
Dried (bin)	462	401	*

* $P = 0·01$; n.s. = not significant

TABLE III

Effect of processing on the relative amounts of β-carotene stereo-isomers in parsley

Treatment	β-carotene							
	Total m.f.b., ppm		Stereo-isomers, %					
			neo-β *b*		All-*trans*		neo-β *u*	
	Sample		Sample		Sample		Sample	
	2	3	2	3	2	3	2	3
Unblanched:								
Unfrozen	354	425	8·0	5·7	76·1	80·7	15·9	13·6
Frozen	307	373	4·7	6·9	79·2	80·3	16·1	12·7
Dried (cabinet)	426	482	10·5	10·2	75·6	79·7	13·8	10·1
Dried (bin)	397	527	13·5	13·2	67·0	74·2	19·5	12·8
Blanched:								
Unfrozen	389	516	6·5	6·6	70·4	77·8	23·1	15·6
Frozen	363	483	4·5	8·0	79·6	79·0	15·9	13·2
Dried (cabinet)	402	533	13·9	13·4	67·0	75·9	19·4	10·7
Dried (bin)	330	454	19·5	19·4	64·6	67·9	15·9	12·7

TABLE IV

Effect of processing on xanthophyll content in parsley

Treatment	Xanthophyll					
	M.f.b.,* ppm			% difference from unfrozen, unblanched		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Unblanched:						
Unfrozen	772	615	948	—	—	—
Frozen	963	581	904	+24·7	−5·9	−4·6
Dried cabinet)	787	768	1125	+1·9	+24·9	+18·7
Dried (bin)	717	591	931	−7·1	−3·9	−1·8
Blanched:						
Unfrozen	1028	810	1227	+33·2	+31·7	+29·4
Frozen	906	752	1131	+17·4	+22·3	+19·3
Dried (cabinet)	883	661	1212	+14·4	−24·2	+27·8
Dried (bin)	623	440	854	−19·3	−28·5	−9·9

* Average of 2–3 samples

TABLE V

Effect of sulphite of β-carotene and xanthophyll levels

Treatment	β-carotene		Xanthophyll	
	Sulphited m.f.b., ppm	Change from unsulphited (Sample 1 Table I) %	Sulphited m.f.b., ppm	Change from unsulphited (Sample 1 Table IV) %
Unblanched:				
Unfrozen	402	+8·4	743	−3·8
Frozen	446	+30·4	989	+2·7
Dried (cabinet)	528	+15·5	1059	+34·6
Dried (bin)	528*	+15·3	929*	+29·6
Blanched:				
Unfrozen	483	+15·0	1043	+1·5
Frozen	475	+13·6	1051	+16·0
Dried (cabinet)	546	+21·9	1061	+20·2
Dried (bin)	506†	+19·6	879†	+41·1

* 96 ppm SO_2
† 2576 ppm SO_2

TABLE VI

Effects of steam-blanching and sulphite solution sprays on the moisture, alcohol-insoluble solids and sugar contents of parsley*

Treatment	H_2O, %	*AIS*, % m.f.b.	Total sugar,† % m.f.b.
Sample 1, curled leaf:			
Unblanched	86·7	62·5	3·83
Unblanched, sulphited**	90·4	62·0	4·28
Blanched‡	85·3	59·4	7·21
Blanched, sulphited**,‡	88·5	60·4	5·30
Sample 2, curled leaf:			
Unblanched	84·8	56·8	12·3
Blanched‡	85·5	56·6	11·6
Sample 3, plain leaf:			
Unblanched	86·7	58·6	10·5
Blanched‡	88·3	61·5	8·06

* Means of 2–3 samples
† As glucose
** Sulphited bin-dried, 96 ppm SO_2; blanched bin-dried 2576 ppm SO_2
‡ Steam-blanched at 99–100°C for 25 sec with tray loading of 0·5 lb/ft²

TABLE VII

β-Carotene content of parsley 1 h to 12 days after harvest

Acetone extract	β-carotene, m.f.b.*†, ppm						
	1 h 25°C	3 h 25°C	1 day 1°C	2 days 1°C	5 days 1°C	7 days 1°C	12 days 1°C
1	457	435	505	501	476	458	547
	460	436	505	482	465	454	526
	447	431	514	509	465	461	513
2	452	496	486	501	480	466	532
	477	506	496	514	475	485	526
	452	498	490	509	487	498	542
S.D.	10·6	36·3	10·5	11·3	8·6	17·4	12·3

* The groups of 3 carotene values represent separate determinations on 3 aliquots from each acetone extract
† No significant difference at the 5% level

The lower carotene values of unblanched parsley (sample 1) before drying and (sample 2) after freezing are not readily explained. Bailey & Dutton[18] noted 5–35% increases in the carotene content of dehydrated carrots over the original fresh material. They explained this increase as being due to loss of soluble solids which ranged from 16·4 to 24·5% in the various processing steps. Lee [19] found that the use of *AIS* as a basis for calculating vitamin values of carrots gave consistent results. Monica & McDowell[20] also found apparent increases in β-carotene after blanching (2–25%) and after cooking, (57–85%), when compared to the original fresh carrots; 50–66% of these increases were due to loss of soluble solids. In the present studies, however, the *AIS* and total sugars in parsley (Table VI) did not show sufficient differences to indicate that loss of soluble solids during blanching or sulphiting contributed to higher carotene values.

Booth[21] noted that low carotene values could result from losses before, during, and after extraction, while high values could arise from incomplete removal of other pigments, chromogenesis, and isomerisation accelerated by heat. Booth[22] also suggested that poor extraction may be due to a protein–carotene linkage. Thompson *et al.*[1] listed several possible sources of error including re-isomerisation, partial destruction and incomplete removal of carotene during hot extraction. The assays in the present studies were all carried out at 22°.

To clarify some inconsistencies in the data several possibilities were explored: rate of carotene loss in freshly harvested parsley; incomplete extraction of carotene from the acetone–parsley slurry; enzymic destruction; and effect of heating parsley in boiling acetone before slurrying.

No significant difference was found at the 5% level between carotene values of parsley assayed in the field or after 3 h at ambient temperature (about 25°) and after approximately 1, 2, 5, 7 and 12 days at 1° (Table VII).

The comparison of air-dried, vacuum-dried and freeze-dried samples showed that the freeze-dried samples were significantly lower than either of the other two at the 1% level (Table VIII). These lowered values parallel the carotene values of frozen, unblanched parsley from other samples. The air-dried parsley showed more carotene than the fresh parsley assayed 3 h after harvest, and carotene in air-dried unblanched parsley of 3 other samples was also more than in fresh parsley.

A second acetone extraction of pigment at room temperature from parsley residues of original parsley–acetone slurries increased carotene values for fresh, frozen and dried (unblanched or blanched) samples by about 4, 2 and 2% respectively. These additional amounts of carotene would not resolve the unexplained differences encountered in the various treatments.

TABLE VIII

Effect of drying procedures on β-carotene levels of unblanched parsley

Acetone extract	β-carotene, m.f.b.,* ppm		
	Air-dried, 4·33% H_2O	Vacuum-dried, 4·67% H_2O	Freeze-dried,† 5·54% H_2O
1	529	505	415
	530	505	416
	529	505	418
2	514	505	408
	510	509	426
	513	505	427
S.D.	9·4	1·6	7·2

* The groups of 3 carotene values represent separate determinations on 3 aliquots from each acetone extract
† Significantly different at the 1% level from the air-dried and vacuum-dried parsley

Bergstrom & Holman[23] have reviewed the involvement of lipoxidase in the oxidation of carotene. Booth[24] reported the presence of a carotene-destroying enzyme system and the loss of carotene when the green tissue of many plants was damaged; holding Sicilian parsley 1/2 h after maceration resulted in a 29% loss of carotene, while no carotene was lost if the parsley was boiled or dried immediately.

The anomalous carotene values of unblanched parsley samples found in the present studies could be due to varying degrees of lipoxidase activity; the disruption of the unblanched tissue during blending or as a result of freezing could facilitate this enzyme action.

To determine if significant amounts of carotene were destroyed during blending of unblanched parsley, known concentrations (1–10 ppm) of pure carotene were added before blending with acetone and were assayed with a minimum of delay. In 4 different lots of parsley 89·4–99·6% of the added carotene was recovered, indicating no

significant enzymic action. However, Booth[21] noted that recovery of added carotene was no assurance that all of the indigenous carotene had been extracted from a sample.

The higher carotene values obtained from blanched vegetable tissue when compared with the unheated has generally been attributed to enzyme inactivation and better extraction. Heating below the temperature required for lipoxidase inactivation might be sufficient to increase the amount of carotene extracted. However, heating parsley samples in acetone at 60° for 2 min prior to blending did not significantly change the carotene content.

Results of this study indicate that more β-carotene is retained in unfrozen and frozen parsley given blanching pre-treatment than in the unblanched unfrozen samples. This has been attributed to inactivation of enzymes. However, in the dried material, the blanched samples show lower values in three of the four treatments. These decreases could be due to the absence of natural antioxidants which were destroyed by the blanching process since the drying of the unblanched material appears to favour retention or possibly improves extraction of the carotenoid pigments. As with other plant materials, heating increased the percentage of the *cis*-isomer, neo-β-carotene *b*, but had little effect on the all-*trans*- and neo-β-*u*-isomers.

The antioxidant effect of the sulphur dioxide treatment is shown in the increased percentages of carotene and xanthophyll compared with the unsulphited samples.

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References

1. Thompson, C. R., *et al.*, *J. Ass. off. agric. Chem.*, 1951, **34,** 219
2. Deuel, H. J., jun., Johnston, C., jun., Summer, E., Polgar, A., & Zechmeister, L., *Archs Biochem.*, 1944, **5,** 107
3. Deuel, H. J., jun., Johnston, C., jun., Meserve, E. R., Polgar, A., & Zechmeister, L., *Archs Biochem.*, 1945, **7,** 247
4. Thompson, C. R., & Maclay, W. D., *Feed Age*, 1952, **2** (10), 22
5. Livingston, A. L., Knowles, R. E., Israelsen, M., Nelson, J. W., Mottola, A. C., & Kohler, G. O., *J. agric. Fd Chem.*, 1966, **14,** 643
6. Masure, M. P., & Campbell, H., *Fruit Prod. J.*, 1944, **23,** 369
7. Makower, R. U., Boggs, M. M., Burr, H. K., & Olcott, H. S., *Fd Technol., Champaign*, 1953, **7,** 43
8. Mitchell, J., & Smith, D. D., 'Aquametry', 1948, (New York: Interscience)
9. Peters, E. D., & Jungnickel, J. L., *Analyt. Chem.*, 1955, **27,** 450
10. 'Official Methods of Analysis', 1960, (9th edn) (Washington D.C.: Association of Official Agricultural Chemists)
11. Bickoff, E. M., Livingston, A. L., & Van Atta, G. R., *J. Ass. off. agric. Chem.*, 1952, **35,** 826
12. Thompson, E. R., Bickoff, E. M., & Maclay, W. D., *Ind. Engng Chem.*, 1951, **43,** 126
13. Harvey, W. R., 'Least-squares analysis of data with unequal subclass numbers', 1960, ARS-20-8 (Beltsville, Md.: U.S. Dept. of Agriculture)
14. De Felice, D., & Fellers, C. R., *Proc. Am. Soc. hort. Sci.*, 1938, **35,** 728
15. Zscheile, F. P., Beadle, B. W., & Kraybill, H. R., *Fd Res.*, 1943, **8,** 299
16. Griffith, R. B., & Thompson, C. R., *Bot. Gaz.*, 1949, **111,** 165
17. Schillinger, A., & Zimmermann, G., *Dt. Lebensmitt. Rdsch.*, 1965, **61(2),** 45; *Chem. Abstr.*, 1965, **62,** 15350e
18. Bailey, G. F., & Dutton, H. J., *Fruit Prod. J. Am. Fd Mfr*, 1945, **24,** 138
19. Lee, F. A., *Ind. Engng Chem. analyt. Edn*, 1945, **17,** 719
20. Della Monica, E. S., & McDowell, P. E., *Fd Technol., Champaign*, 1965, **19,** 1597
21. Booth, V. H., *Trans. J. Soc. chem. Ind.*, 1945, **64T,** 162
22. Booth, V. H., *Analyt. Chem.*, 1949, **21,** 957
23. Bergstrom, S., & Holman, R. T., 'Advances in Enzymology', 1948, Vol. VIII, p. 425 (New York: Interscience)
24. Booth, V. H., *J. Sci. Fd Agric.*, 1960, **11,** 8

ISOPRENOID FATTY ACIDS IN ANTARCTIC KRILL (*EUPHAUSIA SUPERBA*)

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In an attempt to elucidate the origin of phytanic (3,7,11,15-tetramethylhexadecanoic), pristanic (2,6,10,14-tetramethylpentadecanoic) and 4,8,12-trimethyltridecanoic acids found in small quantities in whales, the fatty acid composition of the lipids from Antarctic krill (*Euphausia superba* Dana) was determined by gas–liquid chromatography. Phytanic acid was found to be present to the extent of 1·4% of the total fatty acids, and was isolated and identified by mass and infra-red spectrometry. Two other isoprenoid fatty acids, pristanic acid (0·04%) and 4,8,12-trimethyltridecanoic acid (0·05%) were detected by gas–liquid chromatography. *E. superba* constitute almost exclusively the diet of whales inhabiting Antarctic waters, and the phytanic acid in whale oils is probably derived from ingested krill which presumably biosynthesise this acid from the phytol moiety of chlorophyll present in the diatoms on which these planktonic crustaceans live.

Introduction

Antarctic krill (*Euphausia superba* Dana) are small planktonic crustaceans which constitute almost entirely the diet of Antarctic whales.[1] As the diet of Antarctic krill is unicellular diatoms, particularly *Fragilariopsis antarctica*,[1] which is rich in chlorophyll, and as trace amounts of the isoprenoid acids phytanic (3,7,11,15-tetramethylhexadecanoic), pristanic (2,6,10,14-tetramethylpentadecanoic) and 4,8,12-trimethyltridecanoic acids have been found in whale oil,[2–5] it was considered pertinent to investigate the fatty acids of the lipids from Antarctic krill (*E. superba*) to determine if the isoprenoid acids in whale oil could have come from the diet and presumably have originated from the phytol moiety of chlorophyll in diatoms.

In recent years, particular interest has been focused on the multibranched isoprenoid fatty acids and their corresponding hydrocarbons, because their distribution in nature appears to provide links in a chain of evidence relating to the genesis of crude petroleum.[6–8]

Another purpose in carrying out this investigation, was to obtain information on the nature of the oil from Antarctic krill, for any assessment of the economics of harvesting and utilising the abundant swarms of these crustaceans, would be based on the composition of the oil, as well as that of the protein. Examination of the amino acids of this sample of krill, has already been carried out (Gilberg, Y. C., personal communication).

Prior to this investigation, the presence of phytanic and other isoprenoid fatty acids in Antarctic krill does not appear to have been reported, but concurrent with this work, Ackman *et al.*[9] have independently carried out a comprehensive examination of the lipids of North Atlantic krill (*Meganyctiphanes norvegica* and *Thysanoessa inermis*), and have found that these species also contain small proportions of isoprenoid fatty acids. Earlier, however, Avigan & Blumer[10] demonstrated the occurrence of phytanic acid, together with pristane, in two species of planktonic *Calanus* copepods which had been fed labelled phytol adsorbed on algae cultures.

A note reporting the isolation of phytanic acid in this investigation, was published earlier.[11]

Experimental

Samples

The sample of Antarctic krill (*E. superba*) investigated was collected at location 136° 59′W and 66° 58′S, and was taken from a very large swarm at the surface of the sea. The average length of the krill in the sample was about 4 cm. 500 g of krill (sample P897), which had been stored in deep freeze, were extracted in a Sorvall Omni-mixer using chloroform and methanol as solvents. Four extractions were made, each of the first three being with 1000 ml chloroform–methanol (2 : 1 by vol.), and the fourth with 500 ml chloroform. The solvent extract (lipid) weighed 25·86 g (5·17% of the weight of wet krill), and the dried fibrous material weighed 71·90 g (14·36% of the weight of wet krill). The lipid was refluxed with ethanolic KOH, and the unsaponifiable matter was extracted. As the ethereal extract of unsaponifiable matter was cloudy, it was centrifuged, and the bottom layer (which was soluble in chloroform) was discarded (unsaponifiable matter, 1·41 g; 5·5% of total weight of lipid). The alkaline solutions were acidified and the fatty acids were extracted (18·35 g). Esterification of the fatty acids was carried out by refluxing for 4 h with 2½ vol. methanol containing 3% (wt./vol.) H_2SO_4 (methyl esters, 18·54 g).

Gas–liquid chromatography

Gas–liquid chromatographic (g.l.c.) analyses of the methyl esters indicated the presence of small amounts of phytanic, pristanic and 4,8,12-trimethyltridecanoic acids.

The preparative g.l.c. and the 40% ethylene glycol adipate (EGA) and 20% Apiezon L columns used in this investigation, have been described earlier.[13]

Analytical g.l.c. was carried out with an F & M instrument with a hydrogen flame-ionisation detector. The 20% EGA and the 5% Apiezon L columns used, were prepared as previously described.[13]

Isolation of phytanic acid

Part (15·9 g) of the methyl ester mixture was hydrogenated, and crystallised from 40 vol. acetone at −45°, yielding 3·49 g 'liquids' and 12·30 g 'solids'. The 'liquid' fraction was applied to a column (45 × 2 cm) containing a mixture of 21 g urea and 4·2 g acid-washed celite, and the column was eluted with 80 ml urea-saturated methanol.[12] The eluted non-adduct was treated with dilute HCl and extracted with diethyl ether (urea non-adduct, 0·39 g). G.l.c. analysis of the non-adduct indicated a high content of phytanic acid (~ 76%), together with small proportions of pristanic and 4,8,12-trimethyltridecanoic acids. The fraction was injected into the preparative g.l.c. fitted with a 20% Apiezon L

column at 210°, and the phytanic acid component was collected (0·09 g). As this was shown by analytical g.l.c. to be contaminated with a small proportion of 16-methylheptadecanoic acid (C_{18} *iso*), it was accordingly rechromatographed on the preparative g.l.c. using a 40% EGA column at 196°. This final operation furnished phytanic acid (sample P897C, 0·06 g).

Equivalent chain length (*ECL*) values and retention volumes (V_R) relative to methyl stearate, were determined on the methyl esters of the fatty acids. Response factors for individual fatty acids were not used. Total fatty acid composition was determined by g.l.c. analyses alone, and was based on *ECL* values obtained with both polar and non-polar columns. For the saturated and more common unsaturated fatty acid constituents, the *ECL* values were determined in this laboratory on standard fatty acid methyl esters. For the less readily available unsaturated acids, assignment was based on the *ECL* values recorded by Hoffstetter *et al.*[14] with due consideration being given to proven fatty acid constituents of marine oils as reported by workers in this field.[15–19] As an aid in the identification of unsaturated components, and as a check on their chain length, a sample of the mixed methyl esters from *E. superba* was hydrogenated and then re-examined by g.l.c.

An A.E.I. MS_9 mass spectrometer was used for the analysis of the sample of phytanic acid methyl ester (sample P897C). The infra-red examination was carried out on thin films of the methyl ester between KBr discs, using a Perkin-Elmer model 137E spectrophotometer.

Results and Discussion

Fatty acid composition

G.l.c. analyses of the fatty acid methyl esters from this krill sample (Table I), showed that its constituents were characteristic of most marine oils.[15–19]

The major components were 18 : 1 (20·6%), 16 : 0 (18·0%), 20 : 5 (16·2%) and 22 : 6 (9·3%). Of the unsaturated fatty acids, 20 : 5 had a higher content than is found in most marine oils. The lipids of *E. superba* have been examined by Nonaka & Koizumi,[20] but they recorded only an incomplete analysis of the fatty acids. A fatty acid composition analysis has, however, been made by Yamada[21] for *E. pacifica*, and this was not unlike that of *E. superba* (Table I), except that it reported an appreciably higher content of 20 : 5 (25·9%) and of 22 : 6 (14·7%). Branched-chain fatty acids were not reported by these investigators, but in the present work they constituted 3% of the total fatty acids. As in ruminant fats, the branched-chain fatty acids were found to be comprised of small amounts of phytanic, pristanic and 4,8,12-trimethyltridecanoic acids, together with odd and even-numbered iso acids and only odd-numbered *ante*-iso acids. Among the branched-chain acids detected, was a small proportion (0·2%) of a component corresponding with an acid isolated in this laboratory from sheep fat and butterfat, which appears to be comprised of a series of C_{17} monomethyl isomers.[22] Whereas in ruminant fats the odd-numbered *ante*-iso acids generally preponderate in amount over the corresponding iso acids, in *E. superba* (Table I), as in most marine oils,[5] the opposite appears to apply. The fatty acid composition now reported for a sample of Antarctic krill, is similar overall, to that determined by Ackman *et al.*[9] for a sample of Atlantic krill (*M. norvegica*).

TABLE I

Percentage fatty acid* composition of the oil from *E. superba*

Fatty acid	%	Fatty acid	%
n-Saturated		Mono-enoic:	
10 : 0	tr.	14 : 1	0·2
12 : 0	0·4	15 : 1	0·2
13 : 0	0·1	16 : 1	6·5
14 : 0	12·1	17 : 1	0·2
15 : 0	0·4	18 : 1	20·6
16 : 0	18·0	19 : 1	0·2
17 : 0	0·5	20 : 1	0·8
18 : 0	0·9	22 : 1	0·5
19 : 0	tr.	24 : 1	0·4
20 : 0	0·2		
Total:	32·6	Total:	29·6
		Di-enoic:	
Branched		16 : 2	0·4
Saturated:		18 : 2	4·2
13 : 0 iso	tr.	20 : 2	0·1
14 : 0 iso	0·1	Total:	4·7
16 : 0 4,8,12-TMTD**	0·05		
		Tri-enoic:	
15 : 0 iso	0·5	16 : 3	0·2
15 : 0 *ante*-iso	tr.	18 : 3	1·2
16 : 0 iso	0·2	20 : 3	0·4
19 : 0 pristanic	0·04	Total:	1·8
17 : 0 isomers†	0·2		
17 : 0 iso	0·2	Tetra-enoic	
17 : 0 *ante*-iso	0·1	18 : 4	0·8
20 : 0 phytanic	1·4	20 : 4	1·3
18 : 0 iso	0·3	22 : 4	tr.
Total:	3·0	Total:	2·1
		Penta-enoic:	
		20 : 5	16·2
		21 : 5	0·3
		22 : 5	0·4
		Total:	16·9
		Hexa-enoic:	
		22 : 6	9·3

* In the designation of fatty acids, the figures represent the number of carbon atoms and the number of double bonds

** 4,8,12-Trimethyltridecanoic acid

† Corresponds in V_R with a series of C_{17} monomethyl isomers[22]

G.l.c. analysis of isoprenoid fatty acids

ECL and V_R values obtained from g.l.c. analysis of the sample of phytanic acid isolated in this work (sample P897C) are recorded in Table II. Also shown are the g.l.c. constants measured for the pristanic and 4,8,12-trimethyltridecanoic acid components. These values are all in accord with those of corresponding authentic acids earlier isolated and identified from butterfat and from sheep fat.[23–25]

Mass spectrum of phytanic acid

The mass spectrum of the methyl ester of phytanic acid (sample P897C) isolated in this project (see Fig. 1) corresponded closely with that of methyl phytanate from butterfat[23,26,27] and when interpreted according to the observations of Ryhage & Stenhagen,[28] this constituent was identified as the methyl ester of 3,7,11,15-tetramethylhexadecanoic acid.

Infra-red spectrum of phytanic acid

The infra-red spectrum of the methyl ester of phytanic acid (sample P897C) was very similar to that reported for methyl phytanate isolated from butterfat,[23] fish oil,[29] and whale oil,[2] and that synthesised from phytol.[2] The main features of significance were as follows: (i) a doublet at about 1366 and 1379 cm^{-1}, and a strong band at about 1170 cm^{-1}, absorptions characteristic of a terminal isopropyl grouping. Whereas in the butterfat sample of methyl phytanate,[23] the 1170 cm^{-1} band exhibited a distinct shoulder at 1152 cm^{-1}, in fraction P897C from Antarctic krill, this shoulder was only faintly apparent, as also it was in methyl phytanate from whale oil;[2] and (ii) a strong band at about 735 cm^{-1} indicating a regular $-(CH_2)_3-$ sequence, and the absence of the accompanying shoulder at 727 cm^{-1} which is reported to be characteristic of a $-(CH_2)_4-$ grouping.

Based on the foregoing analyses by g.l.c. and mass and infra-red spectrometry, the identity of fraction P897C was established as phytanic acid (3,7,11,15-tetramethylhexadecanoic acid).

TABLE II

G.l.c. constants for phytanic acid isolated from *Euphausia superba* oil, and for pristanic and 4,8,12-trimethyltridecanoic acids detected in the same sample

Figures in brackets indicate V_R and *ECL* values obtained with previously isolated fatty acid methyl esters under corresponding conditions

Isoprenoid fatty acid	20% EGA at 207°C		5% Apiezon L at 200°C	
	V_R	*ECL*	V_R	*ECL*
Phytanic	0·71 (0·72)	16·75 (16·80)	0·81 (0·81)	17·5 (17·5)
Pristanic	0·49 (0·50)	15·4 (15·5)	0·51 (0·51)	16·4 (16·4)
4,8,12-Trimethyltridecanoic	0·32 (0·32)	14·0 (14·0)	0·22 (0·22)	14·4 (14·4)

Diastereoisomerism

The phytanic acid methyl ester (fraction P897C) was examined by Ackman using open-tubular g.l.c. capillary columns (0·01 in i.d.) coated with butanediol succinate (BDS) polyester, and was found to exhibit diastereoisomerism characteristic of phytanic acid from other natural sources.[30] The diastereoisomers identified were LDD and DDD, and the ratio of their peak areas was approximately 18 : 1. Ackman & Hooper[31] reported an LDD : DDD ratio of about 8 : 1 for a sample of phytanic acid from Antarctic whale oil. By contrast, phytanic acid synthesised from phytol[32] gave an LDD : DDD ratio of 0·9 : 1, and that isolated from New Zealand butterfat,[23] a ratio of about 0·5 : 1, when examined under corresponding conditions.[30]

These results indicate that Antarctic krill (*E. superba*) has a fatty acid composition not unlike that of most marine oils, particularly those of zooplankton. One distinguishing feature, however, is its appreciable content (1·4%) of phytanic acid.

It has been shown in recent years, that phytanic acid, together with smaller proportions of pristanic and 4,8,12-trimethyltridecanoic acids, is of widespread occurrence in animal lipids, although in most analyses reported, it constitutes less than 0·1% of the total fatty acids.

It is generally accepted that phytanic acid is derived from phytol (3,7,11,15-tetramethylhexadec-*trans*-2en-1-ol) which is part of the chlorophyll molecule. Antarctic krill live on a diet of unicellular diatoms, mainly *F. antarctica*,[1] which are rich in chlorophyll, and it is considered that enzymes within the krill convert part of the phytol to phytanic acid. That such conversion does take place in some zooplankton, has been demonstrated by Avigan & Blumer.[10] These investigators fed radioactively labelled phytol adsorbed on algae cultures to two species of *Calanus* copepods, and found that after 48 h the animals contained radioactive phytanic acid and radioactive pristane. Similarly, experiments with rats, mice, rabbits and chinchillas[33] have shown that dietary

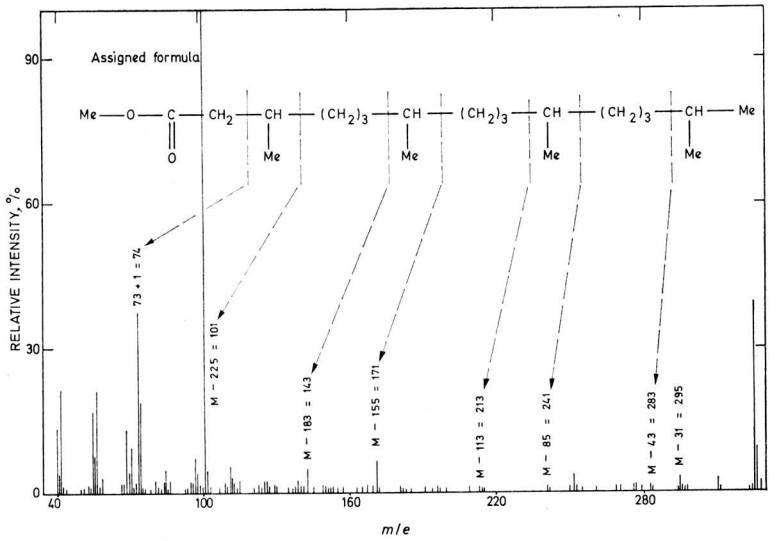

FIG. 1. *Mass spectrum of methyl ester of phytanic acid isolated from Antarctic krill* (Euphausia superba)

phytol is readily converted, in part, to phytanic acid. It has been demonstrated that phytanic acid, when administered at high levels to rats, accumulates in the tissues, blood, urine and faeces, but at low levels it is readily catabolised via pristanic acid and 4,8,12-trimethyltridecanoic acid.[33–36] Corresponding observations have been made with normal humans.[33,37] The trace amounts of phytanic, pristanic and 4,8,12-trimethyltridecanoic acid in the blubber, bone oil and milk fat of whales,[2–5] are probably not of endogenous origin within the whale, but have probably been assimilated into the fatty tissues from ingested krill. Antarctic whales live almost exclusively on the planktonic crustacean *E. superba*, and the presence of 1·4% phytanic, 0·04% pristanic, and 0·05% 4,8,12-trimethyltridecanoic acid in the total fatty acids of this species of zooplankton, suggests that the phytanic acid was biosynthesised from phytol, and then part of it was enzymically degraded by successive α -and β-oxidation steps to pristanic and 4,8,12-trimethyltridecanoic acids. The occurrence of small proportions of these branched-chain isoprenoid fatty acids in whale oil and in fish oils, may well be attributed to the repetitive processes of ingestion and assimilation in the food cycle of marine fauna. Always, however, the original precursor appears to be the phytol moiety of chlorophyll present in unicellular phytoplankton.

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References

1. Slijper, E. J., 'Whales', 1962 (London: Hutchinson)
2. Sano, Y., *J. Japan Oil Chem. Soc.*, 1966, **15,** 456
3. Sano, Y., *J. Japan Oil Chem. Soc.*, 1967, **16,** 8
4. Sano, Y., *J. Japan Oil Chem. Soc.*, 1967, **16,** 56
5. Ackman, R. G., Eaton, C. A., & Hooper, S. N., *Can. J. Biochem.*, 1968, **46,** 197
6. Cason, J., & Graham, D. W., *Tetrahedron*, 1965, **21,** 471
7. Eglinton, G., Douglas, A. G., Maxwell, J. R., & Ramsay, J. N., *Science, N.Y.*, 1966, **153,** 1133
8. Blumer, M., & Cooper, W. J., *Science, N.Y.*, 1967, **158,** 1463
9. Ackman, R. G., Easton, C. A., Sipos, J. C., Hooper, S. N., & Castell, J. D., *J. Fish. Res. Bd Can.*, in the press
10. Avigan, J., & Blumer, M., *J. Lipid Res.*, 1968, **9,** 350
11. Hansen, R. P., *Aust. J. Sci.*, 1969, **32,** 160
12. Peters, H., & Wieske, T., *Fette Seifen Anstr-Mittel*, 1966, **68,** 947
13. Hansen, R. P., & Meiklen, S. M., *N. Z. Jl Sci.*, 1969, **12,** 324
14. Hofstetter, H. H., Sen, N., & Holman, R. T., *J. Am. Oil Chem. Soc.*, 1965, **42,** 537
15. Ackman, R. G., & Burgher, R. D., *J. Fish. Res. Bd Can.*, 1964, **21,** 319
16. Ackman, R. G., & Eaton, C. A., *J. Fish. Res. Bd Can.*, 1966, **23,** 991
17. Ackman, R. G., Eaton, C. A., & Ke, P. J., *J. Fish. Res. Bd Can.*, 1967, **24,** 2563
18. Ackman, R. G., & Eaton, C. A., *Can. J. Biochem.*, 1966, **44,** 1561
19. Sen, N., & Schlenk, H., *J. Am. Oil Chem. Soc.*, 1964, **41,** 241
20. Nonaka, J., & Koizumi, C., *Bull. Jap. Soc. scient. Fish.*, 1964, **30,** 630
21. Yamada, M., *Bull. Jap. Soc. scient. Fish.*, 1964, **30,** 673
22. Ryhage, R., *J. Dairy Res.*, 1967, **34,** 115
23. Hansen, R. P., Shorland, F. B., & Morrison, J. D., *J. Dairy Res.*, 1965, **32,** 21
24. Hansen, R. P., & Morrison, J. D., *Biochem. J.*, 1964, **93,** 225
25. Hansen, R. P., *J. Dairy Res.*, 1969, **36,** 77
26. Stenhagen, E., *Z. analyt. Chem.*, 1961, **181,** 462
27. Abrahamsson, S., Ställberg-Stenhagen, S., & Stenhagen, E., 'Progress in the Chemistry of Fats & Other Lipids', 1963, Vol. VII (London: Pergamon Press)
28. Ryhage, R., & Stenhagen, E., *J. Lipid Res.*, 1960, **1,** 361
29. Sen Gupta, A. K., & Peters, H., *Fette Seifen Ansr-Mittel*, 1966, **68,** 349
30. Ackman, R. G., & Hansen, R. P., *Lipids*, 1967, **2,** 357
31. Ackman, R. G., & Hooper, S. N., *Comp. Biochem. Physiol.*, 1968, **24,** 549
32. Hansen, R. P., Shorland, F. B., & Prior, I. A. M., *Biochim. biophys. Acta*, 1966, **116,** 178
33. Steinberg, D., Herndon, J. H., Uhlendorf, B. W., Mize, C. E. Avigan, J., & Milne, G. W. A., *Science, N.Y.*, 1967, **156,** 1740
34. Shorland, F. B., Hansen, R. P., & Prior, I. A. M., *7th Int. Congr. Nutrition, Hamburg*, 1966, Vol. V, p. 399
35. Hansen, R. P., Shorland, F. B., & Prior, I. A. M., *Biochim. biophys. Acta*, 1968, **152,** 642
36. Mize, C. E., Steinberg, D., Avigan, J., & Fales, H. M., *Biochem. biophys. Res. Commun.*, 1966, **25,** 359
37. Eldjarn, L., Stokke, O., & Try, K., *J. Clin. Lab. Invest.*, 1966, **18,** 694

INFRA-RED IDENTIFICATION OF SYNTHETIC FOOD COLOURS

By W. H. EVANS, J. A. MACNAB and D. F. WARDLEWORTH

The solid-state infra-red spectra of 55 permitted and formerly permitted synthetic food colours have been recorded, and their polymorphism has been investigated. 31 of these, within the wide context of available spectra, are readily distinguishable; a further 15 are polymorphic but may be consistently converted to a form with a unique spectrum. The remaining nine give spectra indistinguishable from at least one other closely related dyestuff. With the aid of chromatographic techniques the latter, within the group of 55 food colours investigated, should be identifiable with the exception of two pairs of food colours.

Introduction

Initial consideration of the methods for the identification of synthetic food colours suggests a number of possibilities. Chromatographic techniques allied to visual comparison of colour[1-3] permit satisfactory separation and identification within the limited context of food colours. Similarly, visible and ultra-violet absorption spectroscopy may be employed. Most individual members, however, belong to groups of structurally related compounds, and the large number of dyestuffs in common use make specific identification of food colours difficult using these techniques. For a similar reason it would appear that the same difficulty of related structures would be encountered in obtaining discrete infra-red (i.r.) absorption spectra. The relative insolubility of the majority of these dyestuffs in solvents other than water and alcohols precludes investigation in solution, so they must be examined in the solid state where the effect of lattice interactions on molecular vibrations are introduced; these may give rise to characteristic absorptions which may improve the likelihood of obtaining unique spectra.

Many complex molecules when examined in the solid state exhibit the phenomenon of polymorphism which may be due to inter- or intra-molecular bonding or to the lattice vibrations mentioned. There are only isolated references to the few unsulphonated food colours[4,5] and the lack of literature on the infra-red identification and polymorphism of synthetic food colours suggested this investigation; the results indicate the possibility of positive identification within the framework of available spectra of dyestuffs. These include the spectra recorded for a number of azo dyestuffs other than food colours and over a thousand reference spectra contained in the Sadtler Commercial collection of dyes, pigments and stains.

Experimental

Materials

Commercial samples of unspecified purity from several wholesale sources were used, and normally one sample of each dyestuff was examined. Where major differences from standard spectra occurred, alternative samples were examined; a number of errors of description were encountered. Many samples contained appreciable amounts of inorganic salts, e.g. sodium chloride, carbonate, sulphate or acetate, or starch, ranging in content from a few % to over 30%. Solvent extraction gave satisfactory samples for further work. The purity of each dyestuff sample was determined by a paper chromatographic examination using systems 5 and 6 recommended by the Association of Public Analysts.[1] Four of the food colours are described as mixtures, but many of the remainder contained varying quantities of coloured impurities. After the separation of these impurities and elution with de-ionised water from the paper chromatograms spectra of the dyestuffs were obtained, together with those of the major impurities which were identified where possible. In two instances only, Orange RN and Yellow RFS, was the level of impurity sufficient to alter the character of the true spectrum; the required food colour was obtained by extraction into ethanol (99%) and methanol, respectively.

Chloroform, acetone and methanol used in the solvent treatments of these food colours were of Analar grade. Two grades of ethanol, 95% and 99% (absolute), were employed while water was of de-ionised grade.

Infra-red absorption spectra

Spectra of the original materials were recorded as Nujol mulls in liquid paraffin (B.P.) and as 0·25% potassium bromide discs (spectroscopic grade: E. Merck A.-G., Darmstadt). All spectra were recorded over the range 2·5–15 μm on a Grubb Parsons Spectromaster grating instrument. Generally, spectra of material after solvent treatment were recorded as Nujol mulls; discrete stable polymorphic forms were also recorded as alkali halide discs. For the small samples of dyestuff eluted from paper chromatograms, microdiscs prepared with the aid of cardboard formers, 11 mm × 2 mm, enabled spectra of samples of ~ 200 μg to be obtained without loss of sensitivity.

Solvent treatments

Separate small portions of the dyes were dissolved in solvent, filtered and evaporated to dryness on a water bath or evaporated in a current of air at room temperature to dryness. Where materials were relatively insoluble in a particular solvent a spectrum of the insoluble material after extraction was often also recorded. All samples were subjected to solvent treatment with de-ionised water and ethanol and when possible with methanol and acetone. In the few instances of unsulphonated dyestuffs, recovery from chloroform solution was substituted for recovery from aqueous solution. Of significance were the occasional polymorphic differences encountered using 95% and 99% ethanol.

Results

Table I lists the 55 dyestuffs examined sub-divided by the nature of their colour and listed in order of their Colour Index number. The Table also includes the structural type of the molecule where not mono- or di-azo, whether spectrally unique or not, and the number of solid polymorphic forms encountered.

TABLE I

Descriptions of 55 dyestuffs examined

Colour Index No.	Description	U.K. permitted	No. of polymorphic forms encountered	Spectrum unique	Comments
12150	Sudan Red G	—	1	Yes	Unsulphonated
12156	Citrus Red 2	—	3	Yes	Unsulphonated
14700	Ponceau SX	—	1	Yes	
14720	Carmoisine	Yes	1	Yes	
14780	Red FB	Yes	1	Yes	
14815	Scarlet GN	—	1	Yes	
16045	Fast Red E	Yes	1	Yes	
16150	Ponceau MX	Yes	1	No	
16155	Ponceau 3R	—	1	No	
16180	Bordeaux B	—	1	Yes	
16185	Amaranth	Yes	1	Yes	
16255	Ponceau 4R	Yes	1	No	
16290	Ponceau 6RA	—	1	Yes	
17200	Red 10B	Yes	1	Yes	
18050	Red 2G	Yes	2	Yes	
18055	Red 6B	Yes	1	Yes	
45170	Rhodamine B	—	2	Yes	Xanthene
45380	Eosine	—	1	Yes	Xanthene
45430	Erythrosine	Yes	1	Yes	Xanthene
10316	Napthol Yellow S	—	2	Yes	Nitro
11380	Yellow AB	—	2	Yes	Unsulphonated
11390	Yellow OB	—	1	Yes	Unsulphonated
11920	Oil Yellow GG	Yes	2	Yes	Unsulphonated
12740	Oil Yellow XP	Yes	1	Yes	Unsulphonated
13011	Yellow RFS	—	1	Yes	
13015	Acid Yellow	—	3	Yes	
13445	Yellow 27175 N	—	1	Yes	
14270	Chrysoin S	—	3	Yes	
14330	Yellow RY	—	2	Yes	
15985	Sunset Yellow FCF	Yes	1	Yes	
18965	Yellow 2G	Yes	7	Yes	
19140	Tartrazine	Yes	1	Yes	
47005	Quinoline Yellow S	—	1	Yes	Quinoline
14600	Orange I	—	1	Yes	
15970	Orange RN	Yes	3	Yes	
15980	Orange GGN	—	1	Yes	
16230	Orange G	Yes	6	Yes	
20285	Chocolate Brown HT	Yes	1	No	
28440	Black PN	Yes	1	Yes	
27755	Black 7984	Yes	1	Yes	
	Brown FK	Yes	1	Yes	
	Chocolate Brown FB	Yes	1	No	
42045	Blue VRS	—	2	Yes	Triarylmethane
42051	Patent Blue V	—	2	Yes	,,
42053	Fast Green FCF	—	1	Yes	,,
42085	Guinea Green B	—	1	Yes	,,
42090	Brilliant Blue FCF	—	1	No	,,
42095	Light Green SF Yellowish	—	1	Yes	
42535	Methyl Violet	—	1	No	,,
42581	Violet BNP	Yes	1	Yes	,,
42640	Violet 6B	—	1	No	,,
42650	Violet 5BN	—	1	No	,,
44090	Green S	Yes	1	Yes	,,
69800	Indanthrene Blue	—	1	Yes	Anthraquinonoid
73015	Indigo Carmine	Yes	3	Yes	Indigoid

Substances exhibiting polymorphism are discussed below together with other anomalies when compared with reference spectra of the Sadtler Commercial collection of dyes, pigments and stains hereafter referred to as the reference spectra. Where recovery from both grades 95% and 99% ethanol gave identical spectra the grade has been omitted.

Citrus Red 2

Form A, as received as a Nujol mull; B, evaporation of an ethanolic (99%) solution on a water bath; C, evaporation of a chloroform or acetone solution on a water bath or a chloroform solution at room temperature. These forms were interconvertible, B being the most stable as a potassium bromide disc. Other recoveries gave mixtures of B and C.

Red 2G

Form A, evaporation of a methanolic solution at room temperature; B, evaporation of an aqueous solution on a water bath. Samples as received were a mixture of the two forms when recorded as Nujol mulls, but were converted to form A exclusively as a potassium bromide disc. Other recoveries gave mixtures of the two forms. Reference spectra are form A or a mixture of forms; spectrum described as Amido Naphthol Red G is not acceptable.

Rhodamine B

Form A, samples as received and recovery from hot ethanol or acetone; B, all other recoveries. The relevant reference spectra are form A.

Naphthol Yellow S

Form A, as received and recovery from ethanol on a water bath; B, evaporation of an aqueous solution on a water bath or evaporation of an ethanolic solution at room temperature. Of the two, form B was the most stable as an alkali halide disc; reference spectra are a mixture of the two forms.

Yellow AB

Form A, as received and evaporation of an ethanolic (99%) solution on a water bath; B, recovery from hot ethanol (95%) or cold chloroform or ethanol (95%). These forms were interconvertible and A was the most stable as an alkali halide disc; other recoveries gave mixtures of the two forms. Reference spectra represent a mixture of the two forms and in one case B.

Oil Yellow GG

Form A, as received and recovery from cold chloroform on occasion; B, recovery from a cold ethanol (99%) or acetone solution or a hot ethanolic solution. These forms were interconvertible.

Acid Yellow

Form A, sometimes recovered from aqueous solution on a water bath in original form; B, occasionally obtained from ethanol (99%) but usually in admixture with form C from ethanol (99%) or methanol on a water bath; C, unstable form, sometimes obtained from hot ethanolic (99%) solution and converts to form B on standing or in alkali halide discs. Forms A and B were stable as alkali halide discs. The reference spectra available did not agree with any of these forms but X 314 of the Sadtler commercial collection, described as Acid (Resorcine) Yellow approximates to Form B. The samples examined contained a small amount of impurity identified as the corresponding monosulphonated compound, Yellow RFS.

Chrysoin S

Form A, samples as received recorded as a potassium bromide disc and on occasion from evaporation of a hot ethanolic (95%) solution; B, samples as received recorded as a Nujol mull and recovered from hot aqueous solution, cold acetone or cold ethanolic (95%) solution; C, obtained on evaporation of a hot ethanolic (99%) solution, but usually in admixture with one of the other two forms. All forms were interconvertible, forms B and C were wholly or partly converted to form A as an alkali halide disc. Only one of the reference spectra, described as Tropaeolin, agrees with form A.

Yellow RY

Form A, as received and recovery from aqueous solution on a water bath; B, obtained on boiling with ethanol or methanol and removing solvent on a water bath.

Yellow 2 G

Form A, samples as received recorded as potassium bromide discs and recoveries from methanol or ethanol at room temperature; B, samples as received recorded as Nujol mulls; C, evaporation of an aqueous solution on a water bath. D, recovered from ethanolic (95%) solution on a water bath on occasion, but generally with form F from hot methanolic solution; E, insoluble residue after extraction with ethanol; F, obtained sometimes from hot methanol, but usually in admixture with form D; G, insoluble residue from methanol extraction usually admixed with form D. All forms were interconvertible and forms B and C tended to give form A as potassium bromide discs. The reference spectrum is unacceptable.

Orange RN

Form A, samples as received recorded as Nujol mulls and obtained from hot water or hot acetone; B, evaporation of an ethanolic (99%) solution on a water bath; C, evaporation of hot or cold methanolic solutions or cold acetone solution. Both forms A and B as alkali halide discs yielded form C. The available samples were heavily contaminated with Ponceau MX or Ponceau 3R; the authentic material was obtained by extraction with absolute ethanol. The reference spectrum is unacceptable.

Orange G

Form A, as received recorded as a Nujol mull and usually recovered from ethanol (99%) on a water bath; B, evaporation of a methanolic solution at room temperature; C, sometimes recovered from hot aqueous solution; D, potassium bromide discs of forms A, B and F and on occasion from hot ethanol (99%); E, occasionally recovered from aqueous solution on a water bath; F, residue after extraction with ethanol (99%); unstable form which converts to form B on standing. These forms were interconvertible, most recoveries giving mixtures of these forms. The reference spectra are mixtures of forms A and D.

Blue VRS

Form A, all recoveries and potassium bromide disc of material as received; B, as received recorded as a Nujol mull. The reference spectra are form A.

Patent Blue V

Form A, samples as received as potassium bromide discs and most solvent recoveries; B, samples as received recorded as Nujol mulls. The sample spectra did not agree with the reference spectrum, which on structural grounds is rejected.

Indigo Carmine

Form A, samples as received; B, recovery of a hot or cold ethanolic solution; C, usually recovered from aqueous solution on a water bath. Most reference spectra are mixtures, but Sadtler X 775 is form B and No. 6219 of the Sadtler standard collection is form A.

Yellow RFS

While this did not exhibit polymorphism both samples examined contained only approximately 30% of the described material which agreed with the Sadtler standard collection spectrum No. 18153. The major impurity appeared to be a disulphonated secondary amine.

Discussion

Methods of extracting colours from various foodstuffs and purifying them for chromatographic identification have been described in the First Report of Trace Materials (Colour) Committee and elsewhere.[1,6] I.r. spectroscopic techniques would be a useful adjunct for the positive identification of the extracted dyestuffs after chromatographic separation. Removal from paper or from thin-layer plates, with chloroform for the water-insoluble food colours and with de-ionised water for the remainder, followed by evaporation to dryness would give relatively small amounts of dyestuffs. The most satisfactory method of recording spectra is as potassium bromide discs or microdiscs. The normal 0·25% alkali halide disc requires 1 mg for a suitable spectrum, but using cardboard formers with a 11 mm × 2 mm aperture similar spectra may be obtained without loss of sensitivity at the 200 μg level. Decreasing the aperture to 5 mm × 1 mm enables the level of detection to be decreased to 50 μg but at this level some loss of instrument sensitivity is inevitable and trace impurities would have a disproportionate effect. Satisfactory spectra may be obtained, however, without reference to more sophisticated instrumentation such as ordinate scale expansion or a beam condenser at the 200 μg level. It must be emphasised that this investigation has not been extended to the application of the identification of food colours extracted from a variety of foodstuffs, but is primarily concerned with i.r. identification of food colours.

Spectra for 41 of the food colours are available in the Sadtler Commercial collection of spectra of dyes, pigments and stains or in the Sadtler standard collection. Owing to errors of description, impurities or quality of the spectra these were not always found to be authenticated, particularly where only one published spectrum existed. Allowing for the differences due to polymorphic forms agreement was obtained in 36 cases. For the remaining five, Indanthrene Blue, Patent Blue V, Orange RN, Acid Yellow and Yellow 2G, samples of each from different sources gave identical spectra which disagreed with the corresponding reference spectra; the latter four have been commented on in the results section. A number of other cases also reflect the poor quality and description of commercial dyestuffs rather than the standard of the reference spectra: Ponceau 4R, only the reference spectrum described as Cochineal Red A is authentic; Red 6B, spectrum described as Hidacid Fast Fuchsine 6B is heavily contaminated with sodium acetate; Orange I, of the reference spectra, X 145 of the Sadtler commercial collection is correct, X 328 is Sudan Red G and the Sadtler standard collection No. 8439 is Orange II; Violet 5BN, spectrum is contaminated with inorganic sulphate.

Recovery of polymorphic substances from many solvents at varying temperatures give non-reproducible forms or a mixture of forms. It is therefore necessary to specify solvent recovery and conditions which consistently give a standard form for any one material. The individual solvent treatments are listed below for recording as potassium bromide discs; where the use of ethanol is advocated the 99% grade (absolute) is implied.

Citrus Red 2	:	Dissolve in ethanol and evaporate to dryness on a water bath.
Red 2G	:	Dissolve in methanol and evaporate to dryness at room temperature.
Rhodamine B	:	Dissolve in water and evaporate to dryness on a water bath.
Naphthol Yellow S	:	Recover from hot aqueous solution.
Yellow AB	:	Recover from ethanolic solution on a water bath.
Oil Yellow GG	:	Dissolve in ethanol and evaporate to dryness on a water bath.
Acid Yellow	:	Recover from ethanol on a water bath.
Chrysoin S	:	Recover from hot aqueous solution, grind material well before recording as an alkali halide disc.
Yellow RY	:	Recover from hot aqueous solution.
Yellow 2G	:	Dissolve in methanol and evaporate to dryness at room temperature, grind material well with potassium bromide before preparing alkali halide disc.
Orange RN	:	Recover from aqueous solution on a water bath.
Orange G	:	Recover from ethanolic solution on a water bath.
Blue VRS	:	Recover from aqueous solution on a water bath.
Patent Blue V	:	Recover from aqueous solution on a water bath.
Indigo Carmine	:	Dissolve in ethanol and evaporate to dryness on a water bath.

These solvent treatments may not coincide with those used for recovery from paper chromatograms or thin-layer plates and so may entail a further recovery from the chosen solvent. Ideally, recovery of standard material of known purity from both the chromatographic separation and solvent treatment should be made for comparison and reliance on reference spectra should be minimal. Accordingly, reference spectra of the standard forms obtainable are not included, nor are spectra of food colours which are unavailable in the references quoted. It is noteworthy that food colours which exist as polymorphs exhibit distinct gradations in colour in the solid state. This may be due to the crystalline texture or the crystalline state and in the latter case would not be unexpected on structural examination of the molecules.

Of these food colours, 46, which include the 15 polymorphic food colours, gave unique spectra after reference to a number of other azodyestuffs recorded and the Sadtler Commercial Collection of dyes, pigments and stains. This applies only under ideal conditions; in the isolation of dyestuffs from food materials it is doubtful whether this degree of uniqueness would be retained particularly in the case of the triarylmethane dyes. The remaining nine food colours gave indistinguishable spectra from at least one other closely related dyestuff. These are: Ponceau 4R, not distinguishable from the published spectrum of Direct Blue 71, CI 34140, which is different in colour and unlikely to be authentic; Ponceau MX and Ponceau 3R, two food colours separable by chromatographic techniques; Brilliant Blue FCF which is similar to Fast Green Extra Bluish, CI 42038, a non-permitted dyestuff; Methyl Violet accepted to be a mixture similar to many Acid Blues and Violets; Violet 6B and Violet 5BN, the latter no longer permitted, which are identical in structure except for a diethylamino- substituted for a dimethylamino- group and which are not separable by paper chromatography; and Chocolate Brown FB and Chocolate Brown HT also indistinguishable by paper chromatography.

Conclusions

Some 85% of the permitted or formerly permitted food colours can be distinguished from other dyestuffs in common use by the i.r. technique. Within the framework of these 55 food colours investigated with the aid of chromatographic techniques, only two pairs of food colours remain difficult to resolve. These are the two violet dyes and the pair of chocolate browns for which no solution is presented at this stage.

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References

1. *Rep. Ass. publ. Analysts*, 1960, p. 10
2. Griffiths, M. H. E., *J. Fd Technol.*, 1966, **1**, 63
3. Dickes, G. J., *J. Ass. publ. Analysts*, 1965, **3**, 49
4. Dolinsky, M., and Jones, J. H., *J. Ass. off. agric. Chem.*, 1954, **37**, 167
5. Morgan, K. J., *J. chem. Soc.*, 1961, p. 2151
6. *Analyst, Lond.*, 1963, **88**, 864

NUTRITIONAL EVALUATION OF TUBERS OF *CYPERUS ESCULENTUS* L.

By SH. MOKADY and A. DOLEV*

In three feeding experiments with rats, the nutritional value of tubers of *Cyperus esculentus* L. was determined. Contents of carbohydrate, fat and protein, and protein composition are reported. It was shown that the low quantity and quality of the protein and the high cellulose content, as well as the protein/fat imbalance, all contributed to a low nutritional value of a whole tuber diet.

Introduction

Chufa tubers ('tiger nuts', *Cyperus esculentus* L.) are daily ingredients of the diet of many people in North Africa and Spain.[1] In North Africa the tubers are consumed in their natural form just as groundnuts in the U.S.A. In Spain the tubers are consumed mainly as a drink called locally 'horchata de chufa' (milk of chufa). Although the microbiological aspects of chufa milk have been investigated, no work has been done on the nutritional evaluation of either whole tubers or the milk.[2–9] Various chemical analyses performed on chufa tubers differ considerably.[1] Since no analytical procedure describing the methods used is quoted, the analytical data presented could not be adapted by the authors.

In European countries, in the U.S.A. and in Israel, chufa tubers have been promoted in recent years by vegetarian organisations as a complete diet. Chufa milk has been recommended as a whole milk substitute for babies allergic to mother's and cow's milk.[10] The purpose of this investigation was therefore to determine the nutritional value of whole chufa tubers, the contents of fat, carbohydrate and protein and the protein composition.

Materials

Animals

The rats used were of the Charles River C.D. strain, divided into groups of six, three males and three females, each group having the same average weight. The rats were kept in individual steel cages with wire-mesh bottoms in an air-conditioned room (24°, 55 ± 5% relative humidity) for 28 days. Fresh water was provided *ad libitum*.

Food consumption, animals' weight and weight of air-dried faeces were determined weekly. At the end of the test period, in addition to the above, the weight of the individual liver, brain, spleen and kidneys was determined.

Test substances

Chufa tubers were grown in Amirim, Israel, and obtained immediately after harvesting without any additional treatment.

Vitamin-free casein was obtained from Difco Laboratories, U.S.A. and contained 90% protein based on N × 6·30.[11] It was used in all control diets.

Vitamin and mineral mix was prepared in accordance with A.O.A.C. recommendations.[12] The details of the composition are given in Table I. Choline was added separately from the vitamin mixture because of its hygroscopic characteristic. Fat was added to the diets as soyabean oil extracted with commercial solvent, deodourised and bleached.

Crude fibre was added as finely ground cellulose (Alphacel Nutritional Biochemical Corporation, Cleveland, Ohio).

The starch used was commercial maize starch (Galam, Israel). It was used as a carbohydrate ingredient in the diets.

Preparation of diets

As can be seen from Tables I–III, eight different diets were used, including the control diet (diet A). Diets to be used were stored under a nitrogen atmosphere at 4°. Test diets were prepared as follows.

Whole, ground chufa-tuber diet (diet B)

The chufa tubers (10–11% water content) were ground in a Waring blendor and sieved through a 25 mesh stainless-steel screen. The residue was blended again until all the ground powder passed through the screen.

Peeled, whole, ground chufa-tuber diet (diet C)

Chufa tubers were peeled by being steeped in a 2% NaOH solution at 90° for 2 min. The tubers were then rinsed in cold water, dried at 70° to a water content of 10–11% and ground by the same manner as the whole ground chufa-tuber diet.

Fat-extracted, ground chufa diet (diet D)

Chufa tubers in batches of 100 g were blended for 2 min in a Waring blender at high speed with 300 ml petroleum ether (A.R. grade 40–60° b.p. range, Frutarom Laboratory Chemicals, Israel). The slurry was filtered and the residue was blended again as before. Both filtrates were combined, dried with Na_2SO_4, the solvent was evaporated under reduced pressure in a Büchi rotary evaporator at 45°, and the dried oil was stored under nitrogen at 4° for further use. The residue was air-dried and screened as the whole, ground chufa-tuber diet. It still contained 0·5% lipids which was ignored. 8% of soyabean oil was added to the ground meal for use as the diet.

Normal-protein chufa diet (diet E)

In this diet the protein level was adjusted to that of the control diet. To the whole ground chufa-tuber diet 4·9% of casein protein was added to replace some of the carbohydrate content.

Enriched, whole, ground chufa tubers (diet G)

The whole, ground chufa-tuber diet was enriched with vitamins and minerals at the same level as the control diet.

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Low-protein chufa-oil diet (diet H)

This diet was composed of the same ingredients as the control diet, but with only 5·0% casein protein and with 22·0% chufa oil diet (obtained during the preparation of the fat-extracted, ground chufa-tuber diet) to replace some of the carbohydrate content.

High-protein chufa diet (diet I)

To the whole, ground chufa-tuber diet 10·0% of casein protein was added.

Normal-protein enriched chufa diet (diet J)

The diet was adjusted so that the whole ground chufa contributed 8·9% fat, by the addition of 32% chufa to the diet. The protein was completed to 10·0% with casein protein, and the normal amount of vitamins and minerals as in the control diet was added.

Methods

Fatty acid composition

The extracted oil was transmethylated,[13] and methyl esters were determined on a dual-column Packard gas chromatograph, equipped with a hydrogen flame detector, disc integrator, 8 ft × 4 mm i.d. glass column packed with 10% EGSS-X (Applied Science Laboratory, Pa., U.S.A.) on Chromosorb W (100–120 mesh). The temperature for the injection port was 210°, for the oven 185°, and for the detector 200°. Nitrogen flow rate was 40 ml/min. A standard mixture of fatty-acid methyl esters (Applied Science Laboratory, Pa., U.S.A.) was used for peak identifications. Results were expressed as mole %.

Phosphorus determination

Phosphorus was determined so that the phospholipids content of the oil[14] could be found.

Cellulose determination

Cellulose was determined according to the crude-fibre determination method.[15]

Results were analysed statistically according to Brownlee.[16]

Results and Discussion

The nutritional evaluation of the chufa tubers was divided into three interrelated feeding experiments.

The composition of the diets tested in Experiment 1 is listed in Table I. From the composition of the chufa tubers it appeared that three factors might impair nutritional value, namely excess of cellulose, excess of fat, and lack of protein. Therefore three test diets in addition to the control (diet A) and whole chufa-tuber diets (diet B) were added to the experiment. The peeled chufa-tuber diet (diet C) was thought to be lower in cellulose, but in fact the cellulose was concentrated in the outer peel, but rather distributed throughout the tuber. The fat-extracted chufa-tuber diet (diet D) was used as a check on the effect of the high fat content in the tuber. The natural fat was replaced with soyabean oil at the same level as the control diet. The normal-protein chufa diet (diet E) was the whole, ground chufa-tuber diet enriched with casein protein to the same level of protein as the control diet. The weight gain of the experimental animals grown on the test diets of Experiment 1 is shown in Fig. 1. As the reduction of cellulose content was unsuccessful, there was no difference in growth rate between the animals grown on whole, ground chufa tubers (diet B) and the peeled, whole, ground tubers (diet C), nor was there a difference between the former and the fat-extracted, ground chufa tubers (diet D). The replacement of 22% chufa oil with 8% of soyabean oil had no effect. Only the protein-enriched diet (diet E) had a significant effect in that it increased the weight gain of the test animals, which was still significantly lower than that of the control (diet A).

The composition of the diets tested in a second experiment is given in Table II. Since it was noticed during the first experiment that the food intake of all the groups on any kind of chufa-tuber diet was considerably lower than that of the control group, a 'pair-fed' group was introduced; they received the control diet and the whole, ground chufa diet alternately. In addition, a diet based on the same ingredients as the control diet but with 22% soyabean oil in place of carbohydrates was tested (diet F). In order to check if a lack of vitamins or minerals causes the impaired growth, a whole, ground chufa-tuber diet was enriched with vitamins and minerals mix (diet G) with the same level as the control (diet A). The last diet tested in that experiment was a control

TABLE I

Composition of diets used in Experiment 1

Ingredients	Control, % (diet A)	Whole ground chufa tubers, % (diet B)	Peeled whole ground chufa tubers, % (diet C)	Fat extracted ground chufa tubers, % (diet D)	Normal protein chufa diet, % (diet E)
Protein	10·0[a]	5·1[g]	5·2[g]	6·0[g]	10·0[f]
Carbohydrates	74·8[b]	60·0[g]	60·4[g]	70·6[g]	57·0[g]
Cellulose	2·0	13·0	12·3	15·4	12·4
Fat	8·0	21·9	22·1	8·0[e]	20·6
Vitamin mix	1·0[c]				
Choline chloride	0·2				
Mineral mix	4·0[d]				

[a] Casein; [b] Starch

[c] The vitamin mix provided the following per 100 g diet (in international units): vitamin A, 2000; vitamin D, 200; vitamin E, 10 (mg): vitamin K, 0·5; thiamin, 0·5; riboflavin, 0·8; niacin, 4·0; pyridoxin, 0·5; calcium pantothenate, 4·0; folic acid, 0·2; biotin, 0·04; vitamin B_{12}, 0·003

[d] Mineral mix provided the following per 100 g diet (mg): NaCl, 557; KI, 32; KH_2PO_4, 1555; $MgSO_4 \cdot 7H_2O$, 229; $CaCO_3$, 1525; $FeSO_4 \cdot 7H_2O$, 10·8; $MnSO_4 \cdot H_2O$, 16; $ZnSO_4 \cdot 7H_2O$, 2·2; $CuSO_4 \cdot 5H_2O$, 2·0; $CoCl_2 \cdot 6H_2O$, 0·1

[e] Soyabean oil added; [f] 5·1% protein present in the original chufa + 4·9% protein added as casein; [g] Contributed by the chufa only

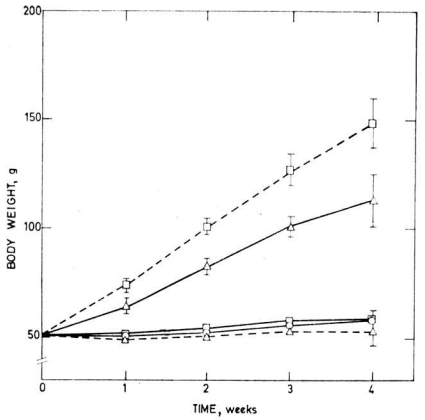

FIG. 1. *Effect of various chufa and control diets on growth of weanling rats*

□— — — —□ Control (diet A), □————□ whole, ground chufa (diet B), △————△ chufa completed to 10% protein (diet E), ○————○ fat-extracted chufa (diet D), △— — — —△ peeled chufa (diet C).
Vertical bars represent the standard deviations.

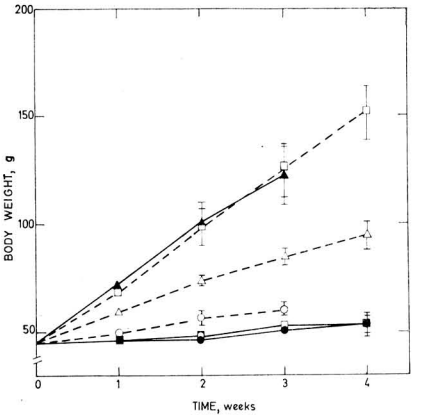

FIG. 2. *Effects of various chufa and control diets on growth of weanling rats*

□— — — —□ Control diet (diet A), □————□ whole, ground chufa (diet B), ●————● whole ground + vitamin and minerals (diet G), △— — — —△ control pair-fed with whole ground chufa, ▲————▲ fat control diet (diet F), ○— — — —○ low protein chufa oil diet (diet H).
Vertical bars represent the standard deviations.

TABLE II

Composition of diets used in Experiment 2*

Ingredient	Fat control diet (diet F)	Enriched whole, ground chufa tubers (diet G)	Low-protein chufa oil diet (diet H)
Protein	10·0[a]	4·9[e]	5·0[a]
Carbohydrates	60·8[b]	57·0[e]	65·8[e]
Cellulose	2·0	12·3	2·0
Fat	22·0[c]	20·6[e]	22·0[d]
Vitamin mix	1·0	1·0	1·0
Choline chloride	0·2	0·2	0·2
Mineral mix	4·0	4·0	4·0

*Control diet (diet A); pair-fed diet, whole, ground chufa-tuber diet (diet B). Vitamin and mineral mix is identical to that described in Table No. I;
[a] Added as casein; [b] Added as starch; [c] Soyabean oil; [d] Chufa oil; [e] Contributed by the chufa only

diet containing casein protein at the protein level of whole chufa tubers and the 8% soyabean oil in place of the carbohydrate content (diet H). The weight gain of the test animals grown on the test diets of Experiment 2 is shown in Fig. 2. The vitamin and mineral enrichment of the whole, ground chufa tubers (diet G) had no effect on the weight gain. The increase of fat in the control diet from 8 to 22% (diet F) showed no effect and the animals developed just as well as on the control (diet A). The 'pair-fed' animals demonstrated a much faster growth rate than those on the whole chufa (diet B). Changing the protein : lipid ratio in the control diet to 1 : 4·4 by lowering the casein protein to 5% and increasing the fat content to 22% chufa oil (diet H) decreased the weight gain of the animals almost to the level of those on the whole ground chufa (diet B). The only reason that the weight gain here was still significantly higher was the comparatively low cellulose content of that diet.

The test diets of a third experiment are given in Table III. Since it appeared from Experiment 1 that it was not only the protein/fat imbalance which was responsible for the impaired growth, the protein level and quality had to be checked. In one diet, therefore, the protein content of the whole, ground

TABLE III

Composition of diets used in Experiment 3*

Ingredients	High-protein chufa diet (diet I)	Normal-protein enriched chufa diet (diet J)
Protein	15·1[a]	10·0[b]
Carbohydrates	54·4[d]	72·0[e]
Cellulose	11·3	4·8
Fat	19·2	8·0[c]
Vitamin mix		1·0
Choline chloride		0·2
Mineral mix		4·0

* Control diet (diet A); whole, ground chufa tubers (diet B); normal-protein chufa diet (diet E). Vitamin mix and mineral mix are identical to that described in Table I.
[a] 5·1% protein present in the original chufa + 10·0% protein added as casein
[b] 1·8% protein contributed by the chufa + 8·2% casein protein
[c] Contributed wholly by adding 32% chufa to the diet
[d] Contributed only by chufa
[e] Contributed part by the 32% chufa in the diet and the rest by starch

chufa tubers was ignored and 10% casein protein was added to the diet (diet I). Another diet was a repetition of a test diet in Experiment 1, where the protein content was completed to 10% with casein protein (diet E). A third test diet consisted of 32% whole, ground chufa tubers that contributed 8% fat to the diet (diet J). Vitamins, minerals, casein, protein, and carbohydrate were completed to that of the control diet. The growth rates of the animals in Experiment 3 are presented in Fig. 3. The diet in which the fat was contributed by chufa tubers but in which most of the protein came from casein (diet J) resulted in the same growth rate as the control diet, even though the cellulose content amounted to 4·8%. On the other hand, addition of 10% casein protein, which resulted in a diet with 5% more protein (diet I) than in the control diet, caused growth rates significantly lower. Since the high fat content of the diet is not responsible for this effect, it was concluded that the high cellulose content

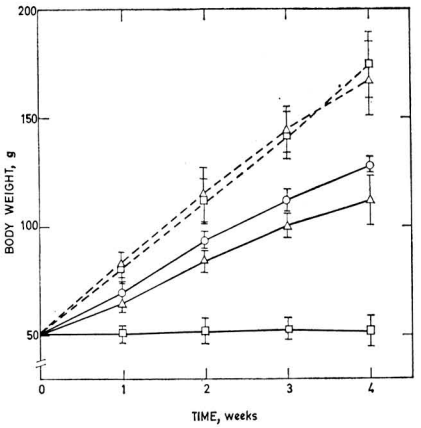

FIG. 3. *Effects of various chufa and control diets on growth of weanling rats*

□— — — — □ Control (diet A), □————□ whole, ground chufa (diet B), △————△ chufa completed to 10% protein (diet E), ○————○ chufa + 10% protein (diet I) △— — — —△ normal-protein enriched chufa (diet J). Vertical bars represent the standard deviations.

and low protein quality of chufa tubers are responsible for the growth impairment.

At the end of each of the three experiments, in addition to the weight of the animals, the weights of the liver, brain, kidneys and spleen were determined. A fatty liver was observed in every case when the diet contained whole chufa tubers. This point is being further investigated. However, when expressed as percentage of body weight no significant differences were found in the liver, kidney and spleen of animals on the different diets. No significant differences were found in the absolute weights of the brains of animals raised on the different diets.

Once a week at each weighing period, feed efficiency (food consumption per weight gain) was determined. Feed efficiencies of the various diets corresponded exactly to the gain in weight on the various diets expressed in Figs 1–3.

The lipid composition of chufa tuber oil is given in Table IV.

TABLE IV

Fatty acid composition of chufa oil*

Fatty acid	mole %
14 : 0	tr.
16 : 0	14·8
16 : 1	tr.
18 : 0	3·7
18 : 1	70·5
18 : 2	11·0
18 : 3	tr.

* Oil contained 2% phospholipids
tr. = trace (less than 0·5%)

The fatty acid composition of those tubers grown in Israel is only slightly different from the composition reported previously.[1] The oil itself is a yellowish odourless stable oil, resembling olive oil in its composition.

The authors cannot recall any other vegetative edible oil source containing such a large amount of oil and as little protein.

Conclusions

It was shown that whole chufa tubers could by no means be substituted for a complete diet. The low protein content was demonstrated to be of low quality. The cellulose level of over 11% seems to have a detrimental effect on the diet efficiency. Even though the oil by itself is of a high nutritional value, it is difficult to overcome the extreme imbalance between protein and fat in a whole chufa diet. Vitamins and minerals do not seem to be the limiting factor of a chufa tuber diet.

Acknowledgments

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References

1. Bayer, G., 'Die Erdmandel', 1959, Heft 35 (Berliner Gärtner Bücher: Deutscher Bauerverlag)
2. Ferriols, B. L., Assensio, A., & Gimeney, E. H., *Agroquim Technolog Aliment*, 1962, **2,** 142
3. Gimenez, E. H., & Aguilar, R. V., *Agroquim. Technol Aliment*, 1963, **3,** 143
4. Hernandez, E., Alonso, I., & Lafuente, B., *Agroquim. Technolog Aliment*, 1963, **3,** 17
5. Gimenez, E. H., Aguilar, R. V., & Ferriols, B. L., *Agroquim. Technolog Aliment*, 1963, **3,** 49
6. Pinaga, F., & Lafuente, B., *Agroquim. Technolog Aliment*, 1965, **5,** 99
7. Anguilar, R. V., & Gimenez, E. H., *Agroquim. Technolog Aliment*, 1965, **5,** 467
8. Hernandez, E., & Vila, R., *Agroquim. Technolog Aliment*, 1967, **7,** 119
9. Primo, E., Lafuento, B., & Pinago, F., *Agroquim. Technolog Aliment*, 1967, **7,** 354
10. Personal communications of the authors with the Head of the Pediatric Department at Zahalon Hospital, Jaffa, Israel and Shearey Zedek Hospital, Jerusalem, Israel
11. Orr, M. L., & Watt, B. K., 'Amino Acid Content of Foods', 1957 (Washington: U.S. Gov. Print. Office)
12. 'A.O.A.C. Methods of Analysis', 1965, p. 785 (Washington D.C.: Association of Official Agricultural Chemists)
13. 'Official and Tentative Methods of the American Oil Chemists', Society Method Ce-2-66', 1966
14. 'Official and Tentative Methods of the American Oil Chemists' Society Method Ca-12-55', 1966
15. 'A.O.A.C. Methods of Analysis', 1965, (Washington D.C.: Association of Official Agricultural Chemists)
16. Brownlee, K. A., 'Industrial Experimentation', 1953, p. 27 (New York: Chemical Publishing Co., Inc.)

COMPOSITION OF EDIBLE WILD GREENS

By S. R. SENGUPTA and B. PAL

Eleven edible wild greens of West Bengal (*Enhydra fluctuans, Hydrocotyle asiatica, Herpestis monneira, Oxalis corniculata, Mollugo spergula, Mollugo hirta, Cephalandra indica, Hygrophila spinosa, Amaranthus spinosus, Rumex vesicarius* and *Trianthema monogyna*) were analysed for moisture, protein, fat, carbohydrate, ash, crude fibre, calcium, phosphorus, iron, nicotinic acid, ascorbic acid and calories. The nutritive value of the leaves of these species was generally low.

Introduction

Varieties of wild greens are consumed by the country people of West Bengal. The nutritive value of many such vegetables is yet to be properly studied, although much is already known about their pharmacologic and therapeutic properties.[1] The present authors are reporting here the nutrient composition of eleven edible wild greens.

Experimental

The methods of the Association of Official Agricultural Chemists,[2] and of Fiske & Subba Raw[3] and Elvehjem[4] were followed for the estimation of calcium, total acid-soluble phosphorus and iron, respectively. Total ascorbic acid was determined on fresh material by the 2,4-dinitrophenylhydrazine method of György.[5] Nicotinic acid was assayed by the method of James *et al.*[6] Calorie values were calculated by multiplying the protein, fat and carbohydrate (other than crude fibre) values by 4, 9 and 4 respectively, and adding them.

Results and Discussion

The results given in Table I show that none of the wild greens studied was rich in calories or proteins. However, *Cephalandra indica* and *Trianthema monogyna* contained fair amounts of protein (4·58% and 4·2%, respectively). *Amaranthus spinosus* was the richest in ash and calcium contents (2·6% and 0·56%). *Hygrophila spinosa, Trianthema monogyna, Mollugo hirta* and *Amaranthus spinosus* were found to be fairly rich in iron, with values of 41·0, 34·0, 32·6 and 30·5 mg/100 g respectively. Ascorbic acid content was not very high in any of the leaves analysed, but relatively high amounts were present in *Hygrophila spinosa, Oxalis corniculata, Cephalandra indica* and *Herpestis monneira*, (79, 78, 72 and 63 mg/100 g respectively).

Although, in the present study, ash and calcium contents of *Amaranthus spinosus* were found to be very high, still higher values have been reported by Patwardhan & Ranganathan.[7] The ascorbic acid content of *Oxalis corniculata, Enhydra fluctuans, Hydrocotyle asiatica* and *Trianthema monogyna* (*Boerhaavia repens*) obtained by the present authors are similar to the values reported by Basu *et al.*[8] Iengar & Rau[9] found in *Oxalis corniculata* a high percentage of calcium (5·6% on dry basis); but the value found by the present authors, although much lower (0·15% on fresh basis or 1·07% on dry basis) is comparable to that reported by Dua & Puri.[10]

Acknowledgments

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References

1. Chopra, R. N., Chopra, I. C., Handa, K. L., & Kapur, I. D., 'Chopra's Indegenous Drugs of India', 2nd edn., 1958 (Calcutta: U. N. Dhur & Sons)
2. Association of Official Agricultural Chemists, 'Official Methods of Analysis', 10th edn., 1965, p. 96 (para 6.011) (Washington, D.C.: Association of Official Agricultural Chemists)
3. Fiske, C. H., & Subba Raw, Y., *J. biol. Chem.*, 1925, **66,** 375
4. Elvehjem, C. A., *J. biol. Chem.*, 1930, **86,** 463
5. György, P., 'Vitamin methods', 1950, Vol. I, p. 274 (New York: Academic Press)
6. James, E. M., Norris, F. W., & Wokes, F., *Analyst, Lond.*, 1947, **72,** 327
7. Patwardhan, V. N., & Ranaganathan, S., *Hlth Bull. Malar. Inst. India*, 1941, No. 23, p. 32
8. Basu, N. M., Ray, G. K., & De, N. K., *J. Indian chem. Soc.*, 1947, **24,** 358
9. Iengar, N. G. C., & Rau, Y. V. S., *Ann. Biochem. exp. Med.*, 1952, **12,** 41
10. Dua, S., & Puri, B., 'Studies in nutrition', 1967, p. 19 (New Delhi: Lady Irwin College)

TABLE I

Nutrient composition of wild greens/100g edible material

Botanical name*	Nature of edible material (analysed)	Proximate composition, g						Minerals, mg			Vitamins, mg		Calories
		Moisture	Protein (N × 6·25)	Fat (ether extractives)	Carbohydrate (by difference)	Ash	Crude fibre	Calcium	Phosphorus	Iron	Nicotinic acid	Ascorbic acid	
Enhydra fluctuans	Tender leaves	85·05	3·72	1·25	6·61	2·15	1·22	107	55	5·0	0·5	39	53
Hydrocotyle asiatica	,, ,,	87·22	1·66	0·69	4·76	2·30	3·37	176	72	12·0	0·8	42	32
Herpestis monneria	Leaves and tender stalk	88·41	2·12	0·62	5·90	1·90	1·05	202	16	7·8	0·3	63	38
Oxalis corniculata	,, ,,	86·00	2·30	0·80	8·20	1·30	0·90	150	78	8·0	0·6	78	49
Mollugo spergula	,, ,,	88·78	2·00	0·65	5·27	2·15	1·15	136	40	11·5	0·7	32	35
Mollugo hirta	,, ,,	89·20	2·36	0·50	4·84	1·90	1·20	82	72	32·6	0·2	26	33
Cephalandra indica	Leaves	81·00	4·58	0·95	7·77	2·50	3·20	520	93	17·0	0·4	72	58
Hygrophila spinosa	,,	82·53	3·97	1·20	8·75	2·10	1·45	251	55	41·0	0·3	79	62
Amaranthus spinosus	Leaves and tender stalk	84·50	3·00	0·50	8·10	2·60	1·30	560	65	30·5	0·3	30	49
Rumex vesicarius	,, ,,	86·31	3·60	1·15	5·57	1·85	1·52	156	44	12·0	1·0	33	47
Trianthema monogyna	,, ,,	85·24	4·20	0·83	6·00	2·48	1·25	300	67	34·0	0·7	45	48

*Local names in descending order: Hincha, Thankuni, Brahmi, Amrul, Gima, Kakdima, Telakucha, Kulekhara, Kanta nate, Chuk palang, Swet Punarnava

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

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ABSTRACTS

APRIL, 1970

1.—AGRICULTURE AND HORTICULTURE

Soils and Fertilisers

Soil Formation, Classification, Constituents

Numerical classification of some Minnesota forested soils. D. F. GRIGAL and H. F. ARNEMAN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 433–438. 20 ref.).—Forty upland Minnesota forest soil profiles were classified by a non-numerical method (7th Approximation) and 5 numerical methods based on properties measured in the field, moisture-related properties, nutrient-related properties, horizon texture and thickness, and all 22 properties measured. The numerical classifications did not heavily weight 'diagnostic horizons' and the results generally differed from those of the non-numerical classification. A. H. Cornfield.

Chemical and thermal characteristics of allophane in andosols of the tropics. K. H. TAN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 469–472. 20 ref.).—Profile characteristics, chem. properties and clay mineralogy showed that tropical andosols could be divided into two groups, namely, young soils containing pro-allophane and intergradient vermiculite material, and older soils with thicker AC or ABC profiles containing allophane. The clay fractions of the two groups exhibited marked differences in thermal and chem. behaviour. A. H. Cornfield.

Study of some biosequences and lithosequences in the zone of Brown Forest soils in Northern Greece: morphological, physical and chemical properties. N. J. YASSOGLOU, C. NOBELI and S. C. VRAHAMIS (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 291–296. 23 ref.).—Characteristics of 8 soils, differing in parent material and vegetation and developed under similar climatic, topographic, and age conditions, are presented. A. H. Cornfield.

Distribution of forms of nitrogen and carbon in some soil profiles. II. Characterisation of nitrogen and carbon constituents in water extracts. K. VLASSAK, L. M. J. VERSTRAETEN and J. LIVENS (*Soil Sci.*, 1969, **108** (3), 188–192. 10 ref.; cf. *Idem. ibid.*, 1969, **108** (2), 127).—The amounts of water-sol. N and C diminished with increasing depth in the profile. 50% of the total sol. C was in the form of carbohydrate. Both vegetation and soil type influenced the distribution of the principal components of C and N fractions in the profile. A. G. Pollard.

Opal phytoliths in *Bouteloua eriopoda* roots and soils. D. S. PEASE and J. U. ANDERSON (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 321–322. 5 ref.).—Phytoliths were identified in the roots of black grama. The distinctive, rectangular phytoliths found were also the most common form in the A horizons of several soils and paleosols. A. H. Cornfield.

Genetic evaluation of profile distribution of aluminium, iron and manganese oxides. H. P. BLUME and U. SCHWERTMANN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 438–444. 25 ref.).—The contents of oxides of Al, Fe and Mn in the profiles of great soil groups of the temperate region were determined and great soil groups could be differentiated by profile distribution of these oxides. In some cases intergrades between different soil groups, which could not be recognised in the field, had distinctly different profile distributions of one or more of the oxides. A. H. Cornfield.

Thorium movements in morainal soils of the High Sierra, California. R. O. HANSEN and G. L. HUNTINGTON (*Soil. Sci.*, 1969, **108** (4), 257–265. 16 ref.).—In a sequence of soil profiles, the distribution was examined by *X*-ray spectrometry. The Th contents varied in the range 10·8–24·0 ppm. It appeared that Th accumulated immediately beneath horizons of high org. matter content. The latter formed complexes with Th as well as with Fe. Leached Fe subsequently tended to accumulate in horizons of pH $\lesssim$ 5·5; leached Th was more widely distributed and Ra moved to even greater depths. Some factors influencing the distribution of Ra are suggested. Concn. of K were generally greater in A_2 than in A_1 horizons. A. G. Pollard.

Lanthanum and scandium distribution in three glacial soils of western Wisconsin. J. R. KLINE, J. E. FOSS and S. S. BRAR (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 287–291. 12 ref.).—The total La content (determined by neutron activation) in the < 250 μ fraction of 3 loess-capped glacial till soils (Hapludalfs) averaged 23·7 ppm in the loess and 28·5 ppm in the glacial till. Total Sc averaged 6·5 and 9·0 ppm in the loess and glacial till respectively. Translocation by pedogenic processes was indicated by accumulation of these elements in the argillic B horizons. Clay content of the profiles was highly correlated with La and Sc contents. A. H. Cornfield.

Chemical and mineralogical characteristics of eutrophic lake sediments. C. R. FRINK (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 369–372. 21 ref.).—The contents of clay, org. matter, N and P in sediments of a lake increased significantly with depth of water above the sediments, and were higher than in the watershed soils. The Al-interlayered vermiculite entering the lake was converted by chem. weathering to illite. The lake sediments were proportionately higher in Ca- than in Fe- or Al-bound phosphate than were the watershed soils. A. H. Cornfield.

Interlayer forces in montmorillonite and vermiculite. J. A. KITTRICK (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 217–222. 20 ref.).—Oriented *X*-ray spacings were obtained for vermiculite and montmorillonite, saturated with Li, Na, Mg, Ca, Sr and Ba, as sample temp. was changed at a const. rate (20–200°). Changes in spacing with temp. are discussed. A. H. Cornfield.

Quantitative evaluation of the strong-force model for expansion and contraction of vermiculite. J. A. KITTRICK (*Proc. Soil. Sci Soc. Am.*, 1969, **33** (2), 222–225. 14 ref.).—The model used and the thermodynamic estimates obtained suggest a mechanism for fixation of ions by vermiculite. A. H. Cornfield.

***X*-ray emission spectroscopic determination of potassium in sub-milligram mica samples after sample treatment.** S. B. WEED, O. D. PHILEN, JUN. and R. A. LEONARD (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 318–320. 7 ref.).—A thin-specimen *X*-ray emission technique, utilising Ti in the sample as an internal standard, provided a rapid, accurate method of determining total K in parent and expanded micas. A. H. Cornfield.

***X*-ray diffraction technique for direct determination of interlayer spacing of expanded clay minerals at various swelling pressures.** J. D. RHOADES, R. D. INGVALSON and H. T. STUMPF (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 473–475. 14 ref.).— A. H. Cornfield.

Physical Properties of Soils

Construction, operation and accuracy of the gray hydrocal sensor. G. J. BOUYOUCOS and R. L. COOK (*Soil Sci.*, 1969, **108** (1), 79–82. 5 ref.).—Descriptive details and experimental data of the sensor, made from the gray hydrocal material previously described (*Idem, ibid.*, 1967, **104**, 297) are given. A. G. Pollard.

Influence of western hemlock, *Tsuga heterophylla*, and western red cedar, *Thuja plicata*, on soil properties. D. H. ALBAN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 453–457. 18 ref.).—The A2 and B2 horizons were generally more strongly developed under hemlock than under cedar, and more mixing of org. and mineral horizons occurred under cedar. Soil pH, total N and org. C, cation exchange capacity, and exchangeable Ca, K and Mg were higher under cedar than under hemlock. There were only small differences in soil properties between the two tree species at depths greater than 15 cm. A. H. Cornfield.

Nature of aggregation in two tropical soils of Puerto Rico. R. P. ESCOLAR and M. A. LUGO LOPEZ (*J. Agric. Univ. P. Rico*, 1968,

52 (3), 227–232. 4 ref.).—The org. matter content of the 2 acid clay soils was as high as that found in many soils of the same texture in temperate zones. Org. matter had a very definite influence as a cementing agent between clay particles. Aggregates of all sizes had high water stability. The highest proportion of aggregates were in the > 5 mm class. The similarity of particle-size distribution in aggregates of different sizes indicates that aggregates are formed mainly by breakdown of large, massive units of soil.
A. H. Cornfield.

Effects of soil physical properties, rainfall characteristics and wind velocity on clod disintegration by simulated rainfall. L. LYLES, L. A. DISRUD and N. P. WOODRUFF (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 302–306. 9 ref.).—Studies in a wind tunnel raintower showed that at a particular wind velocity, high intensity rain for a short period resulted in as much clod disintegration as did low intensity rain for a longer period, even though total rainfall was larger in the latter case. Disintegration was not related to clod density. Small clods were more susceptible to disintegration by rain drops than were large clods. For a particular rain intensity and clod size, disintegration was greater when the rain was wind-driven compared with vertical rain. A. H. Cornfield.

Interdependence of water drop energy and clod size on infiltration and clod stability. W. C. MOLDENHAUER and W. D. KEMPER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 297–301. 17 ref.).—Infiltration rate into clods of various sizes from soils ranging in texture from loam to clay loam was much higher initially for 8–20 mm clods than for smaller clods, but infiltration rate decreased much more rapidly in the larger than in the smaller clods as water drop energy was increased on successive days. Final intake rates were not correlated with aggregate stability, as measured by wet sieving. Final intake rates after distintegration of large clods were in many cases less than when initial clod sizes were small. There was a negative correlation between clay contents and final intake rates. A. H. Cornfield.

Relation of water application to evaporation and storage of soil water. H. R. GARDNER and W. R. GARDNER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 192–196. 7 ref.).—Evaporation (E) was measured from columns of loam and loamy sand to which water was applied at different rates and after various time intervals. E loss ranged from 100% of that applied at 0·25 cm per day to 31% of that applied at 10·2 cm every 20 days. The cumulative curves were scaled to dimensionless variables and compared with a theoretical solution of the diffusivity equation for finite media.
A. H. Cornfield.

Chemical interactions of waste water in a soil environment. B. F. HAJEK (*J. Wat. Pollut. Control Fed.*, 1969, **41** (10), 1775–1786. 20 ref.).—Methods available for predicting the assimilative capacity of soil for chem. contaminated waste water are discussed. These include: waste water chem. characterisation parameters, e.g., pH, pollutant form and concn., complementary or accompanying ion concn., disposal variations, temp.; characterisation of soil by means of phys. characteristics, e.g., bulk *d*, grain *d*, grain size distribution; mineralogical characteristics, e.g., *X*-ray diffraction, surface area detn. of soil colloids; chem. characteristics, e.g., cation exchange capacity, resident exchangeable ions; and chem. interactions of soil–waste systems, e.g., equil. and soil column techniques, with dynamic column and hydrodynamic dispersion analysis for field prediction. J. M. Jacobs.

Moisture characteristics of some Rothamsted, Woburn and Saxmundham soils. P. J. SALTER and J. B. WILLIAMS (*J. agric. Sci., Camb.*, 1969, **73** (1), 155–158. 7 ref.).— M. Long.

Osmotic tensiometer for measuring capillary potential [of soil water]. A. J. PECK and R. M. RABBIDGE (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 196–202. 9 ref.).—The design and use of the instrument is described. A. H. Cornfield.

Independent measurement of matric and osmotic potential of soil water. J. D. OSTER, S. L. RAWLINS and R. D. INGVALSON (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 188–192. 15 ref.).—The technique uses the operating principles of the thermocouple psychrometer and the porous plate apparatus to measure the matric and osmotic potentials of soil water without extraction of a significant amount of soil soln. The standard error of the measurement was ± 0·04 bar. The method also allowed measurement of the partial molar vol. of soil water with an accuracy of ± 1%. A. H. Cornfield.

Absence of threshold gradients in clay–water systems. R. J. MILLER, A. R. OVERMAN and J. H. PEVERLY (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 183–187. 20 ref.).—The transient pressure transducer technique was used to test for threshold gradients in clay–water pastes containing 9–50% w/w montmorillonite and < 2 μ and 2–20 μ size fractions of kaolinite. Threshold gradients were not found in any of the samples tested. A. H. Cornfield.

Adsorption of non-ionic surfactants by soil materials. N. VALORAS, J. LETEY and J. F. OSBORN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 345–348. 9 ref.).—The extent of adsorption of 5 non-ionic surfactants applied in soln. to soils, clay, peat and vermiculite varied considerably. This has a bearing on the relative effectiveness of different surfactants in improving initial and long-term wetting properties of soils. A. H. CORNFIELD.

Locational stability of hexadecanol in soil and its effect on modulus of rupture. M. A. MYHRMAN and D. D. EVANS (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 203–205. 9 ref.).—Addition of 0·4% of $C_{16}H_{33}OH$ (**I**) powder decreased the modulus of rupture (M) of a sandy loam 65% and of a silt loam 57%. Emulsified **I** was less effective. Commercial tallow alcohol was somewhat less effective than **I** in decreasing M. There was virtually no movement of **I** in the soils during 55–70 days of either satd. or unsatd. water movement. A. H. Cornfield.

Influence of the copper, zinc, iron, and aluminium salts of some microbial plant polysaccharides on aggregation and hydraulic conductivity of Ramona sandy loam. J. P. MARTIN and S. J. RICHARDS (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 421–423. 17 ref.).—In general the Fe and Al salts of the polysaccharides (**I**) were less effective in influencing soil aggregation and hydraulic conductivity than were the corresponding metal-free **I**. Effects of the Cu and Zn salts of **I** depended on the source of **I**.
A. H. Cornfield.

Influence of soil moisture tension on nitrate accumulation in soils. B. R. SABEY (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 263–266. 12 ref.).—Over the moisture tension (MT) range 0·1 to 15 bar, max. NO_3^- accumulation during incubation (with non-limiting amounts of NH_4-N present) of silt loams occurred at 0·1 bar and declined with increasing MT until at 15 bar it was only 13% of the rate at 0·1 bar. An equation is presented which enables NO_3^- accumulation to be calc. under varying conditions of temp. and MT.
A. H. CORNFIELD.

Environmental factors influencing sorption of atmospheric ammonia by soils. R. B. HANAWALT (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 231–234. 9 ref.).—When subjected in the lab. to air containing a concn. of NH_3 comparable with that in the atm., 6 soils (ranging in texture from loamy sand to clay loam) sorbed the equiv. of 55–74 kg NH_3-N per ha per yr. NH_3 sorption rate increased with atm. [NH_3] and, to a small extent, with temp. (4·2–20·1°). Calc. energies of activation suggested that sorbed NH_3 was held by chemical bonds rather than by physical adsorption. Sorption of NH_3 was increased by increasing movement of air above the soils. A. H. Cornfield.

Influence of partial pressure of carbon dioxide on the pH values of aqueous carbonate systems. F. N. PONNAMPERUMA, R. U. CASTRO and C. M. VALENCIA (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 239–241. 5 ref.).—The pH values of dil. soln. of Na_2CO_3 and aq. suspensions of $CaCO_3$, $MnCO_3$, and $FeCO_3$, equilibrated with CO_2 at different partial pressures, conformed to the theoretical equations (*Soil Sci.*, 1966, **101**, 90). Sodic and carbonate soils behaved like the corresponding carbonate systems whilst reduced ferruginous soils behaved like $Fe_3(OH)_8$. A. H. Cornfield.

Interactions of pH-dependent and permanent charges of clays. I. Use of specific ion electrodes for measuring calcium and rubidium activities in bentonite and illite suspensions. E. O. MCLEAN and G. H. SNYDER. **II. Calcium and rubidium bonding to bentonite and illite suspensions: clay-phase retention.** G. H. SNYDER, E. O. MCLEAN, and R. E. FRANKLIN. **III. Calcium and rubidium activities *versus* soyabean root uptake from bentonite and illite suspensions.** E. O. MCLEAN, G. H. SNYDER and R. E. FRANKLIN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 388–392. 22 ref.; 392–396. 13 ref.; 397–400. 15 ref.).— A. H. Cornfield.

An explanation for the variability in strontium–calcium exchange selectivity of soils, clays, and humic acid. A. S. R. JUO and S. A. BARBER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 360–363. 20 ref.).—The relative strengths of adsorption by the clay minerals were largely governed by the relative energy of hydration of the two cations, so that Sr^{2+}, the more weakly hydrated, was adsorbed more strongly than Ca^{2+}. On humic acid, in which the COOH group was mainly responsible for exchange adsorption, the field strength of the exchange site was more important, so that Ca^{2+} was adsorbed more strongly than Sr^{2+}. Exchange selectivity in soils varied according to whether the exchange sites were mainly org. or inorg. A. H. Cornfield.

Rate of cation exchange on clay minerals as determined by specific-ion electrode techniques. R. L. MALCOLM and V. C. KENNEDY (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 247–253. 23 ref.).—The rates of exchange of Mg^{2+}, Ca^{2+}, and Ba^{2+} for Na^+ and K^+ on clay minerals are reported. A. H. Cornfield.

Equilibrium constants between chloride salt solutions and freshly prepared and aged hydrogen-montmorillonite. A. E. FOSCOLOS and I. BARSHAD (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 242–247. 24 ref.).—Equil. const. were obtained between freshly-prepared montmorillonite (*M*) and dil. soln. of chlorides of Na, K, Mg and Ca. With aged H-*M*, equil. const. were not obtained because the N-KCl extraction technique did not yield the correct amount of adsorbed H^+. Even when another method of determining adsorbed H^+ was used, contradictory results for equil. const. using aged *M* were obtained, because it was not possible to assess the exact amount of adsorbed H^+ in presence of hydroxyaluminium compd. A. H. Cornfield.

Effect of exchangeable aluminium on conductance and diffusion activation energies in montmorillonite. R. J. MILLER and D. S. BROWN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 373–378. 27 ref.).—A.c. conductance and D_2O diffusion activation energies for Na^+- and Al^{3+}-montmorillonite mixtures increased with % of exchangeable Al^{3+} when this was > 50%. Possible structural changes in adsorbed water at high Al^{3+} concn. are suggested as an explanation. A. H. Cornfield.

Quasi-crystals in sodium–calcium systems. G. A. O'CONNER and W. D. KEMPER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 464–469. 10 ref.).—Quasi-crystals (oriented bundles of clay platelets) were formed by drying Ca-satd. bentonites. Formation of quasi-crystals favoured the adsorption of Ca^{2+} from mixed Na^+–Ca^{2+} soln. and resulted in greatly reduced rates of exchange of Ca^{2+} associated with the internal surfaces of the bundles. The quasi-crystals affected exchange capacities, adsorption ratios and swelling properties of bentonites and soil clays. A. H. Cornfield.

Biological Aspects, Available Nutrients, Soil Analysis

Reactions of triammonium pyrophosphate with soils and soil minerals. I. HASHIMOTO, J. D. HUGHES and O. D. PHILEN, JUN. (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 401–405. 12 ref.).—Hydrolysis of $P_2O_7^{4-}$ to PO_4^{3-} was greatly accelerated by soil micro-organisms, but only slightly by soil clays and goethite. $P_2O_7^{4-}$ was adsorbed more strongly on soils and clay minerals than was PO_4^{3-}. A. H. Cornfield.

Proton transfer reactions at clay mineral surfaces. K. V. RAMAN and M. M. MORTLAND (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 313–317. 9 ref.).—Ammonium, pyridinium, ethylammonium, methylammonium and protonated-urea montmorillonite and nontronite complexes were equilibrated over NH_4OH, pyridine, ethylamine, or with 3-aminotriazole. Proton transfer or retention by a species depended on the relative basicities of the two interacting compd. and the relative concn. or activities of the reactants and products. Similar reactions may occur in other clay–organic systems such as humus complexes and the application of NH_3 to soil is discussed in this light. A. H. Cornfield.

Separation and functional group analysis of soil organic matter. J. A. LEENHEER and P. G. MOE (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 267–269. 16 ref.).—No loss of org. matter resulted from separation of sand and silt fractions using dispersion by ultrasonic vibration in water at pH 10, followed by sedimentation. 12–37% of the org. matter was lost when the residual mineral matter in the clay fraction was removed by HCl–HF treatment. Functional group analysis indicated that the org. matter of a silty clay loam prairie soil (Typic Argiaquolls mesic) was in a greater state of humification (oxidn.) than was that in a silt loam forest podzol (Typic Fragiudalf mesic). A. H. Cornfield.

Relation between the fulvic acid–humic acid ratio and the extractable organic iron in sandy soils. H. WIECHMANN (*Z. PflErnähr. Bodenk.*, 1969, **123** (1), 64–69. 5 ref.).—The extractability of org. Fe (**I**) by 0·01 N- and 0·1 N-NaOH was studied in relation to the fulvic acid–humic acid ratio *F*/*H*, using 105 sandy soils. Soils in which the amount of **I** per g C increased with rising [NaOH] frequently contained a higher proportion of *H*; these soils included only a few B_t and B_{tv} horizons. On the other hand, where the amount of **I** per g C decreased with rising [NaOH], a higher *F* fraction was found in the soil and these soils were frequently found in the B_t and B_{tv} horizons. The reasons for the dependence of the amount of **I** per g extracted C on *F*/*H* are discussed. M. Long.

Stimulation of soil respiration by volatiles from alfalfa [lucerne]. R. G. GILBERT and G. E. GRIEBEL (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 270–273. 11 ref.).—Addn. to soils, before incubation, of conc. aq. lucerne volatiles (obtained by distillation of chopped lucerne hay in water suspension) increased the respiration rate of 12 soils 3–8 fold. Enhancement of respiration increased with temp. from 15 to 36° and declined at higher temp. The enhancement occurred within 2 h of addn. of the volatiles and persisted for at least 72 h providing a sufficient quantity of these was added. Low R.Q. values for treated soils indicated that micro-organisms were utilising substrates low in O_2. A. H. Cornfield.

Effect of certain fungi on the composition of natural microflora of the rhizosphere and root zone of winter wheat. V. F. PAVLENKO (*Mikrobiologiya*, 1969, **38** (2), 329–335. Russ., 26 ref.).—The effect of the fungi, *Trichoderma lignorum*, *Penicillium lanosum*, *Zygorhynchus moelleri* and *Aspergillus niger* on winter wheat, Belotserkovskii 198, was examined. All the fungal species became readily acclimatised and grew among the natural microflora, mainly in the rhizosphere. *T. lignorum* grew most abundantly. Fungal compn. changed because of the presence of added species. *T. lignorum* induced the greatest effect and decreased considerably the genera *Penicillium*, *Fusarium* and *Mucor*, *Fusarium* disappearing from the root system. Of 5 species of *Fusarium* genus, only *F. gibbosum* was recorded after the introduction of *T. lignorum*. L. A. Haddock.

Sensitivity of *Rhizobium meliloti* to ultra-violet light and to nitrosomethylurea. A. N. PARIĬSKAYA and L. M. TITAREVA (*Mikrobiologiya*, 1969, **38** (2), 313–315. Russ., 4 ref.).—The effect of u.v. light and nitrosomethylurea (NMU) (both mutagenic to many bacteria) on *R. meliloti* was investigated. Dose response curves for lethal action were obtained. *R. meliloti* cells in the exponential phase, were more sensitive to u.v. lethal action at low dosages than in the lag or stationary phase. LD_{90} of u.v. light was 900–1100 erg/mm² for old cultures; for most bacteria it was ⩽ 500. The lethal effect of u.v. light was much reduced in soil extracts. Old cultures of *R. meliloti* were more stable to 0·2% NMU than young ones. L. A. Haddock.

Contribution of some soil fungi to natural and heat-induced water repellancy in sand. S. M. SAVAGE, J. P. MARTIN and J. LETEY (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 405–409. 15 ref.).—Certain species of fungi, isolated from water-repellant soil and incubated with silica sand, caused the sand to become water-repellant, particularly after it had been heated briefly. This water repellancy was destroyed by extraction with MeOH or water. Sand treated with the MeOH extracts and then dried and heated also exhibited water repellancy. A no. of org. compd. caused water-repellancy in sand after heating. A. H. Cornfield.

Rapid changes in nitrite after gamma irradiation of fresh soils. P. A. CAWSE and T. WHITE (*J. agric. Sci., Camb.*, 1969, **73** (1), 113–118. 28 ref.).—The increase in soil NO_2^- content after γ-irradiation was investigated in the range 0·25–2·5 Mrad under aerobic conditions. 115 ppm of NO_2^--N accumulated after 3 days in a soil contg. 4·0% of inorg. C. Formation of NO_2^- correlated with % of soil inorg. C and incubation time. Very acid soils did not produce NO_2^-; formation was poor for soils with pH < 7. Microbial reduction of NO_3^- appeared to be the cause of NO_2^- formation, rather than inhibition of its oxidn. M. Long.

Steady state studies of nitrification in soil: theoretical considerations. A. D. MCLAREN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 273–276. 17 ref.).—The reactions $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$ in an idealised soil column with max. no. of nitrifying organisms are considered mathematically with respect to time and depth within the column under steady state conditions. A. H. Cornfield.

Formation of nitrate from ammonium nitrogen in soils. IV. Use of the delay and maximum rate phases for making quantitative predictions. B. R. SABEY, L. R. FREDERICK and W. V. BARTHOLOMEW (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 276–278. 10 ref.).—Max. NO_3^- accumulation (*NA*) rates and delay periods in the temp. range 0 to 25° are shown relative to values at 25° taken as 1·0. Whereas the actual max. rates and delay periods of *NA* varied greatly due to temp. among different soils, the relative rates and delay periods were more nearly const. at a particular temp. An equation is presented for estimating *NA* at any temp. providing *NA* rate and delay period are known at a particular temp. and that the supply of NH_4^+-N is non-limiting. A. H. Cornfield.

Instrumental analysis of neutron irradiated soils. J. R. KLINE and S. S. BRAR (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 234–238. 9 ref.).—Soils from many different parts of the world were analysed

by γ-ray spectrometry following thermal neutron activation. Radionuclides of Br, Hf, Zr and Na were detected; those of La, Sc, Fe, Co, Eu and Sm were measured quant. The amounts of Sc were correlated with those of Fe, Co and Eu in soils of highly diverse origin. Elemental relationships sometimes resulted in similar γ-ray spectra for soils of dissimilar origin. Without other supporting evidence it is doubtful whether the technique would be useful for identifying soil specimens or tracing their place of origin.
A. H. Cornfield.

Potentiometric estimation of chloride in water extracts of soils. B. G. Davey and M. J. Bembrick (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 385–387. 5 ref.).—The method, which uses a Ag–AgCl electrode, covers the range 5×10^{-5} M- to 10^{-3} M-Cl^- and gave results, for Cl^- in water extracts of soils, very similar to those obtained by using electrometric, conductometric or volumetric methods. A. H. Cornfield.

Effect of pH on labile and soluble phosphate in soils. R. P. Murrmann and M. Peech (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 205–210. 23 ref.).—The amount of labile phosphate (detd. by isotopic exchange) and the phosphate concn. in the soil soln. both reached min. values at pH 5·5 and increased rapidly as pH increased or decreased from this value. This indicates that the phosphate concn. in the soil soln. is detd. by the amount of labile phosphate rather than by the solubility of some cryst. phosphate.
A. H. Cornfield.

Fractionation of phosphorus in lake sediments: analytical evaluation. C. R. Frink (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 326–328. 9 ref.).—Methods used for determining Al-P and Fe-P in soils gave satisfactory results when applied to lake sediments, but modifications were required in the detn. of reductant-sol. and Ca-P. Org. P obtained by difference between total P and the sum of the inorg. P fractions, was more closely related to the org. matter content of the sediments than was org. P determined by conventional methods. A. H. Cornfield.

New method for characterisation of available soil phosphorus. A. K. Sacheti and S. N. Saxena (*J. Indian Soc. Soil Sci.*, 1968, **16** (1), 91–93. 5 ref.). The gradient elution method of Avnimelech and Hagin (*Proc. Soil Sci. Soc. Am.*, 1965, **29**, 393) is examined with a view to its possible adaptation for assessing available P in soils. In glass tubes of 25 mm dia., 5 g-samples of each of 5 soils were placed between two glass wool plugs and leached (150 ml/h) with soln. of increasing acidity (HCl < 0·1 N). Inorg. P was detd. in 15 successive fractions (total 450 ml). The inorg. P in the first 5 fractions represented the first peak of the gradient elution curve and was correlated with the P uptake of maize and sorghum plants (correlation coeff. 0·9867 and 0·9584). A. G. Pollard.

Plant uptake and chemical extractions for evaluating potassium release characteristics of soils. E. S. Conyers and E. O. McLean (*Proc. Soil Sci. Soc., Am.* 1969, **33** (2), 226–230. 13 ref.).—Five silt loams and silty clay loams were treated with varying levels of K or diluted with varying proportions of sand and cropped with millet followed by lucerne. The soils differed greatly in the amounts of K released to the plants and K extracted by various reagents. Amounts of K extracted by N-NH_4OAc (pH 7), boiling N-HNO_3, 0·5 N-HCl, and Na tetraphenylboron (2 mequiv. in 30 ml N-NaCl per g of soil) were highly correlated with plant uptake of K. The energy of exchange ($\triangle F$) between K and Ca + Mg was poorly correlated with plant K uptake because of the unusually high release of K from non-exchangeable form in soil–sand mixtures. Cropping reduced considerably the levels of exchangeable and N-HNO_3-extractable K. A. H. Cornfield.

Reduction of nitrite deficits by alkaline metal carbonates. L. H. Wullstein (*Soil Sci.*, 1969, **108** (3), 222–226. 10 ref.; c.f., *Idem., Agric. Sci. Rev.*, 1967, **5**, 8).—Loss of N from soil by volatilisation from NO_2^--N (N deficit) was examined in a number of soils of varied pH (5·4–9·4). The N deficits were greatly reduced by addition of Ca, Mg or K carbonates or by $FeSO_4$ alone or mixed with $CaCO_3$. The mechanism of these effects is discussed.
A. G. Pollard.

Evaluation of tests for predicting availability of soil manganese to plants. M. G. Browman, G. Chesters and H. B. Pionke (*J. agric. Sci., Camb.*, 1969, **72** (3), 335–340. 22 ref.).—The ability of NH_4 acetate, $Mg(NO_3)_2$, H_3PO_4, hydroquinone, 3M-$NH_4H_2PO_4$, 1·5 M-$NH_4H_2PO_4$ and EDTA extractants to predict the Mn uptake of *Zea mays* was investigated on a variety of soils. Regression equations were calc. for each extractant in combination with soil pH; the most successful prediction was obtained with the first extractant in combination with pH. Of the remainder, EDTA and H_3PO_4 were the most successful. Of the soil variables investigated, only total Mn and soil pH were of any use in improving the accuracy of prediction. Org. C and clay contents were only of significance in determining EDTA- and $Mg(NO_3)_2$-extractable Mn, resp. M. Long.

Fertilisers

Effect of farmyard manure on the fertiliser requirement of sugar-beet. A. P. Draycott (*J. agric. Sci., Camb.*, 1969, **73** (1), 119–124. 12 ref.).—The effect of farmyard manure (*FYM*) on the economic fertiliser requirements of sugar-beet was examined. In one trial where *FYM* was applied, N, P, K, with and without NaCl, were added; in a second trial, N and NaCl were added with and without *FYM*. Whilst higher levels of added NPK fertiliser increased yields when 12 ton/acre of *FYM* were applied, the av. economic level for N was 0·6, for PO_5 0·3 and for K_2O 0·5 cwt/acre. NaCl could largely replace K requirements. Analysis of *FYM* used gave no reliable guide to fertiliser requirements. NaCl, at 4 cwt/acre, increased yields at most sites. Soil analysis for Na was not a guide to sugar yield. M. Long.

Granulated lime fertilisers. C. M. J. Sluijsmans and H. Loman (*Landbouwvoorlichting*, 1969, **26** (7/8), 281–284. Dut.).—The properties of granulated and pulverised lime fertilisers are compared. In the 1st yr, the action of granulated lime fertiliser was unfavourable but after 3 yr there was no difference in efficiency between the two types of fertiliser. J. C. T. Nieuwenhuis.

Granulation of mixed complex fertilisers. V. Narayana Rao and E. H. Hohmann (*Chem. Age India*, 1969, **20** (3), 236–245. Engl., 8 ref.).—Optimum water content (*OWC*) was detd. for the granulation of ten formulations of compounded N-P-K fertilisers. *OWC* decreased linearly with effective urea content.
K. Graupner.

Effects on barley and kale of NPK fertilisers containing differing proportions of urea and ammonium nitrate, and either triple superphosphate or monourea phosphate. F. V. Widdowson and A. Penny (*J. agric. Sci., Camb.*, 1969, **73** (1), 125–132. 3 ref.).—Field trials, using fertilisers with the same N : P_2O_5 : K_2O ratio of 2 : 1 : 1, but of different constitutions, are reported. In a wet yr (after drilling), barley yields from each fertiliser were the same but in a dry yr, all fertilisers checked growth and those contg. urea (**I**) killed plants. Yields from fertilisers contg. urea phosphate (**II**) were higher than those with **I** alone. Kale yields were similar with all 8 fertilisers. **II** was safer than **I** alone and plants recovered more N from NH_4NO_3. M. Long.

Long term effects of fertilisers on grassland. III. Effects on some soil properties. R. G. Heddle and K. Simpson (*J. agric. Sci., Camb.*, 1969, **73** (1), 49–55. 7 ref.).—The effects of NPK fertilisers after 14 yr were investigated on a grass/clover sward. Changes in soil characteristics and herbage minerals are discussed.
M. Long.

Influence of drying on determination of seasonal variations of mineral nitrogen in soils. J. Chabannes (*C.r. hebd. Séanc. Acad. agric. Fr.*, 1969, **55** (11), 783–791. Fr., 18 ref.).—Bare and cultivated areas of loamy soil were continually treated with N-contg. manure. Surface soils (0–25 cm) and subsoils (25–40 cm) were analysed both fresh and after drying (quickly by i.r. heating and slowly in air). Results confirmed that drying of soil increased the mineral N content, esp. NH_4-N. Drying of soil greatly increased the mineral N of samples taken in June from soils cultivated for 3 yr with 120 kg/ha of 'ammonitrate'/yr. This effect was much less marked on soils without N manure. M. T. Rawnsley.

Polarographic determination of titanium in urea. R. M. Bhatnagar, B. N. Singh and A. K. Roy (*Technology, Q. Bull. Fertil. Corp. India*, 1969, **5** (4), 264–267. 7 ref.).—A polarographic study was made of the reduction of Ti in urea–H_2SO_4 in order to investigate the determination in urea fertilisers of insol. Ti or TiO_2 which originates due to corrosion or erosion of the reactor lining.
M. Dudley.

Phosphorus availability to maize as affected by granulating manganese with ortho- and pyro-phosphate fertilisers. P. M. Giordano and J. J. Mortvedt (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 460–463. 9 ref.).—Maize forage yields and P uptake were decreased when 10% Mn (as SO_4^{2-}) was incorporated into granular NH_4 polyphosphate (APP) and $(NH_4)_3HP_2O_7$ (TPP) when compared with application of Mn and P materials separately. Incorporation of Mn in the P materials decreased the solubility of both Mn and P, mainly due to the formation of $Mn(NH_4)_2P_2O_7.2H_2O$.
A. H. Cornfield.

Granulation of superphosphate with binders. V. NARAYANA RAO and E. H. HOHMANN (*Chem. Age India*, 1969, **20** (3), 231–235. Engl., 6 ref.).—The effect of binding agents, starch, bentonite, $Al_2(SO_4)_3$ and H_3PO_4, in the granulation of superphosphate, contg. 19% water, was examined. Bentonite, at 0·75% addn., gave the max. granulating efficiency. K. Graupner.

Long term trials with different phosphates. T. REICHARD (*Z. Pflernähr. Bodenk.*, 1969, **123** (1), 22–32. 9 ref.).—Field trials lasting several years were carried out on an acid, a neutral and a calcareous soil with a variety of rotations. The effects of 'hyperphosphate,' basic slag and superphosphate were compared. On the acid soil, all 3 phosphates were equally effective, whilst on the neutral and calcareous soils only superphosphate and basic slag improved yields. The double lactate method for phosphate detn. correctly estimated the P status of the acid soil fertilised with hyperphosphate, but over-estimated it in the other two cases. M. Long.

Soil pH, phosphate fertilisers and soil investigations [on available phosphorus]. R. LIBISELLER (*Z. PflErnähr. Bodenk.*, 1969, **123** (1), 33–48. 24 ref.).—The thermodynamics of change of pure Ca phosphates were studied to predict reactions to be expected at different soil pH values. Fractionation of soil phosphates in plots in a long-term fertiliser trial, involving hyperphosphate (*HP*), basic slag (*BS*) and superphosphate (*SP*), showed that the predicted phosphates were actually formed. The apatite of *HP* yielded Fe- and Al-phosphates in an acid soil, but in neutral and alkaline soils insufficient transformation occurred and the fertiliser gave inadequate P. The P of *BS* and of *SP* were partly transformed to Fe- and Al-phosphates in all soils. Analytical methods for detn. of P are discussed and their shortcomings with regard to *HP* are explained. M. Long.

The CAL-method, a new method for determining available soil phosphate. H. SCHÜLLER (*Z. PflErnähr. Bodenk.*, 1969, **123** (1), 48–63. 2 ref.).—The new method has been used to deal with typical Austrian arable, calcareous soils. The technique involves a 0·1 M-Ca lactate, 0·1 M-Ca acetate and 0·3 M-acetic acid extractant. Various methods for detn. of available soil P were compared, using samples from a long term phosphate fertiliser trial; no single extractant was found suitable for all soils. The CAL method neglects apatitic P and is therefore suitable for neutral or alk. soils, in which apatitic P is not available. M. Long.

Effects of phophorus carriers and zinc source on phosphorus–zinc interactions in corn [maize]. R. B. GANIRON, D. C. ADRIANO, G. M. PAULSEN and L. S. MURPHY (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 306–309. 15 ref.—P accumulated in both roots and shoots of Zn-deficient plants grown in soln. culture at 30°, but was depleted from the roots when Zn was supplied. At 20°, P distribution between roots and shoots was not affected by Zn deficiency. Seedling growth was slightly better when P was supplied as $NH_4H_2PO_4$ (MAP) than as NH_4 polyphosphate (APP), but P uptake was greater with the latter source. ZnEDTA was slightly more effective than $ZnSO_4$ as a source of Zn. On a silt loam (pH 5·7), application of P decreased maize grain yields when soil Zn was low, and application of Zn increased grain yields only to levels produced without P fertilisation. Leaf P% varied inversely with rate of Zn fertilisation, but Zn% was not affected. APP and ZnEDTA were only slightly better sources than MAP and $ZnSO_4$, respectively. A. H. Cornfield.

Water solubility of zinc in a granular mixed fertiliser as affected by zinc source and method of addition. G. E. RICHARDS (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 310–313. 16 ref.).—Most of the Zn added in chelated form to an 8–16–8 (N–P_2O_5–K_2O) fertiliser remained in water-sol. form when incorporated before or after ammoniation of the fertiliser or in coated form. Less than 10% of the Zn in sol. forms remained water-sol. when incorporated before ammoniation. When incorporated after ammoniation, water solubility of Zn was higher, especially when applied as coatings. Solubility of fritted Zn was unaffected by method of incorporation. Sol. Zn from non-chelated sources increased with increasing water-solubility of P and with decreasing fertiliser pH. A. H. Cornfield.

Plant Physiology, Nutrition and Biochemistry

Light, Air and Water Relationships

Comparison of the rates of apparent photosynthesis of the cranberry and the common lowbush blueberry. B. BONN, F. R. FORSYTH and I. V. HALL (*Naturaliste can.*, 1969, **96** (5), 799–804. Engl., 8 ref.).—At a flow rate of 800 cm³/in and a light intensity of 1,000 ft candles, cranberry leaves had a rate of assimilation of 40·0 mg of CO_2/dm²/h compared with 14·4 in the blueberry (*Vaccinium angustifolium*). At 2,000 ft candles, apparent photosynthesis was below satn. level for cranberry shoots but above level for blueberry. E. G. Brickell.

Light filtration by foliar canopies: significance for light-controlled weed seed germination. R. B. TAYLORSON and H. A. BORTHWICK (*Weed Sci.*, 1969, **17** (1), 48–51. 4 ref.).—The spectral quality of light filtered through tobacco, maize, and soyabean leaves showed that much more of the incident red than far-red energy was absorbed. The effect of this light on the phytochrome-controlled germination of 6 weed species was generally to inhibit germination of seeds given a stimulatory pre-irradiation of red light. Germination of seeds with no pre-irradiation was either not promoted or was promoted to various degrees according to species. Unfiltered light at intensities equiv. to those under the leaf filters, caused no comparable effects. A. H. Cornfield.

Cytological effect of laser radiation on the growth points of *Allium fistulosum* L. V. V. RODIONOVA and V. A. TARASOV (*Dokl. Akad. Nauk SSSR*, 1969, **188** (3), 692–693. Russ., 10 ref.).—Coherent monochromatic radiation affects germinating power cytogenetically; specificity of action with wavelength and mechanisms require investigation. The standard method of counting chromosome aberrations in the anaphase and telophase in meristematic cells from *Allium fistulosum* was used; the laser wavelengths were 4880, 5682, 6328 and 106,000Å. The power used was 1×10^{-2} to $1 \cdot 2 \times 10^{-2}$ J/cm². Two mechanisms may be involved in the relationship between the no. of chromosome aberrations and the dose of radiation. With radiation of 10·6 μm, no statistically significant increase in aberrations occurred with dosage. The cytogenetic effect normally depends on the wavelength and laser radiation probably inflicts fundamental injuries on the chromosomes. L. A. Haddock.

Effect of prometryne on $^{14}CO_2$ fixation in cotton and soyabean. H. C. SIKKA and D. E. DAVIS (*Weed Sci.*, 1969, **17** (1), 122–123. 9 ref.).—Photosynthetic fixation of ^{14}C-labelled CO_2 was significantly reduced in excised leaves of cotton and soyabean from plants which had been treated with 0·5-5·0 ppm prometryne (*P*); The extent of reduction increased with herbicide concn. *P* had little effect on the types and amt. of labelled C compd. produced in the leaves and produced no significant effect on $^{14}CO_2$ fixation in the dark in either species, except for changes in the concn. of aspartic and glyceric acids in soyabean. A. H. Cornfield.

Plant Nutrition and Metabolism

Iron–manganese inter-relationship in soil and plant. S. N. TEWARI, M. DAS and A. K. DEKA (*J. Proc. Instn Chem., India*, 1969, **41** (4), 135–137. Engl., 10 ref.).—Results of a pot culture expt. with paddy, grown on an acid soil (pH 5·5) with 77 ppm of available Mn, support the concept that it is the ratio of available Fe and Mn in the medium that determines the growth of plants. E. G. Brickell.

Responses of 'Acadia' strawberry to two forms and three rates of nitrogen at two pH levels. C. R. BLATT (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 346–353. 27 ref.).—The effects of $NH_4^+ + NO_3^-$-N, compared with NO_3^--N alone, at 28, 84 or 140 ppm in the nutrient at pH 5·5 or 6·5, on the content of major elements in strawberry leaves and roots, top and root yields, and no. of crowns and runners were studied in sand culture. A. H. Cornfield.

Influence of potassium on the utilisation of ammonium by tomato plants. D. N. MAYNARD, A. V. BARKER and W. H. LACHMAN (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 537–542. 22 ref.).—The incidence of stem lesions in tomato plants increased with level of supply of $(NH_4)_2SO_4$ when K supply was low, but lesions were absent when K supply was high, irrespective of the rate of application of NH_4^+. The incidence of stem lesions increased with NH_4^+/K^- mequiv. ratio in the leaf. A. H. Cornfield.

Effect of nitrite and phosphorus levels in nutrient solutions on growth of vegetable crops. T. OSAWA and O. A. LORENZ (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 595–602. 8 ref.).—Increasing the level of NO_2^- (5–15 mequiv. per l) in the nutrient culture soln. resulted in decreased growth of bean, mustard and potato, and in darker green leaves. NO_2^- decreased growth to a greater extent when PO_4^{3-} in the nutrient was high (1 mequiv. per l) than when low (0·05 mequiv.). Low P in the nutrient resulted in decreased growth and dull green leaves. P % in the tops was decreased by

high NO_3^- supply in bean, but not in mustard and potato. Ca % in the tops of all species was decreased by high NO_3^- supply. A. H. Cornfield.

Effects of water table, pH and nitrogen fertilisation upon growth and nutrient content of highbush blueberry plants. H. M. E. HERATH and G. W. EATON (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 274–283. 15 ref.).—Pot tests with one-year-old blueberry plants grown in peat from a blueberry-growing area showed that a 3-in water table, compared with freely draining pots, decreased leaf size and resulted in a lower content of major elements in the leaves. Growth was better where N (20–60 lb per acre) was applied as NH_4^+ than as NO_3^-. Growth was better at pH 4·3–6·0 than at pH 3·4, but foliar nutrient content was little affected by pH. A. H. Cornfield.

Salinity effects on growth and salt uptake of seedlings of date, *Phoenix dactylifera* L., J. R. FURR and C. L. REAM (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 268–273. 21 ref.).—Leaf growth on date seedlings in nutrient culture was not affected by concn. of NaCl > 3000 ppm, but decreased linearly with increasing level of NaCl up to 24,000 ppm. Even at 12,000 ppm growth was still about 60% of that of the control, indicating a high salt tolerance for date. Although Na% and Cl% in tops and roots increased with level of NaCl in the nutrient, the uptake of both ions increased relatively slowly with increasing NaCl concn. of the nutrient > 3000 ppm. A. H. Cornfield.

Variation in leaf nutrient content of *Hevea* with clone and age of leaf. M. M. GUHA and R. NARAYANAN (*J. Rubb. Res. Inst. Malaya*, 1969, **21** (2), 225–239. Engl., 11 ref.).—Changes with age in N, P, K, Mg, Ca and Mn contents of leaves were studied using 5 clones of *Hevea brasiliensis*, using top-light leaves of 6-year-old trees. N, P, K and Mg leaf contents dropped sharply in the 3 weeks after emergence, then altered more slowly for 8 months (N, P and K concn. reduced, whilst Ca and Mn increased; Mg content was not affected by leaf age). The nutritional status of the trees is discussed in relation to the extensive data presented. C. A. Finch.

Pot culture technique for study of *Hevea* nutrition. J.-P. POLINIERE and H. VAN BRANDT (*J. Rubb. Res. Inst., Malaya*, 1969, **21** (2), 250–257. Engl., 5 ref.).—A simple, small-scale method for orientation of field studies of *Hevea* nutrition is described. It is shown that the mineral constituents of the leaves with greatest diagnostic value are (in order of decreasing significance): Mn, Ca, K, Mg, Fe, S, N, P (for differences between the treatments) and Mn, Mg, Ca, K, Fe, S, P, N (for differences between the soils). C. A. Finch.

Comparative ^{137}Cs content of agricultural crops grown in a contaminated soil. E. J. EVANS and A. J. DEKKER (*Can. J. Pl. Sci.*, 1968, **48** (2), 183–188. 5 ref.).—In a greenhouse expt. crops were grown in ^{137}Cs-contaminated soil to which additional known amt. of ^{137}Cs were added. Uptake of ^{137}Cs varied between different crops; uptake of added ^{137}Cs dose was small (max. by sugar beet of 0·13%). In general, concn. were in the order, vegetable crops > forage crops > cereals. Concn. in edible parts was lower than in non-edible parts of the plant. ^{137}Cs/K ratios were not const., correlations being noted only for cereals. A. G. Pollard.

Boron content of tissues and roots of rutabagas (*Brassica napobrassica*) and of soil as associated with brown-heart condition. U. C. GUPTA and D. C. MUNRO (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 424–426. 10 ref.).—The B content of the leaf (dry basis) of plants showing moderate to severe brown-heart symptoms ranged from 6 to 20 ppm, but symptoms were absent from plants having leaf-B of 38–140 ppm. Leaf contents of B > 250 ppm were considered to be toxic. The B content of rutabaga tissue was significantly correlated with hot water-sol. B (B_{hws}). Brownheart symptoms occurred in plants growing on soils having 1·3 ppm B_{hws}; no symptoms occurred with B_{hws} of 1·3–1·8 ppm and toxicity symptoms occurred when B_{hws} was > 3·1 ppm. A. H. Cornfield.

New flavonoid in alfalfa [lucerne]. N. R. BRADNER and V. C. BRINK (*Can. J. Pl. Sci.*, 1968, **48** (1), 97–99. 10 ref.).—The compd. extracted from lucerne seed ('Rhizoma') was probably a flavone with empirical formula $C_{16}H_{12}O_8$, and m.p. 261–262°. Characteristics are recorded and a structural formula is suggested. A. G. Pollard.

Biochanin A and formononetin content in red clover varieties at several maturity stages. W. DEDIO and K. W. CLARK (*Can. J. Pl. Sci.*, 1968, **48** (2), 175–181. 15 ref.).—Methods for estimating isoflavones (I) in red clover are described. Paper chromatograms were scanned with a densitometer for biochanin A and with a fluorimeter for formononetin. Varietal differences in I contents were noted. Max. contents (up to 1% of dry wt. in each case) occurred in leaves prior to flowering, and subsequently declined. Stems and petioles contained only small amt. of I throughout the growth of the plant. A. G. Pollard.

Acid and sugar changes during ripening in Wolcott blueberries. L. J. KUSHMAN and W. E. BALLINGER (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 290–295. 20 ref.).—The main changes from the unripe to the overripe stage of blueberry fruit was an increase in the ratio of total sugars to acids, accompanied by increases in glucose and fructose and a decrease in citric acid. Very small changes in quinic and malic acid occurred, and there were no changes in the amounts or types of amino acids. A. H. Cornfield.

***In vitro* effects of silicon on the action patterns of sugar-cane acid invertase.** A. G. ALEXANDER (*J. Agric. Univ. P. Rico*, 1968, **52** (4), 311–322. 21 ref.).—Incorporation of Si (SiO_4^{4-}) into invertase digests at 3 μmole per ml immediately stopped reactions against sucrose and raffinose and stachyose. At Si concn. > 27 μmole/ml, sucrose and raffinose substrates yielded a large variety of products resembling fragments of hydrolysed starch. The mechanism of Si in inhibiting sucrose hydrolysis is discussed. A. H. Cornfield.

Controlled temperature studies of growth, enzymology, and sucrose production by two sugar-cane varieties in Puerto Rico. A. G. ALEXANDER and G. SAMUELS (*J. Agric. Univ. P. Rico*, 1968, **52** (3), 204–217. 42 ref.).—The performances of a high dry-matter producing variety and a high sucrose variety were studied during growth in environments of 55–60°F and 80–85°F. A. H. Cornfield.

Thermal stability of protein synthesis in leaves according to age. N. L. KLYACHKO and O. N. KULAEVA (*Dokl. Akad. Nauk SSSR*, 1969, **188** (1), 230–232. Russ., 8 ref.).—Expt. on leaves have shown that ageing is accompanied by a fall in sensitivity of protein synthesis as well as inhibition by pyromycin, streptomycin, tetracycline and biomycin. Old and young leaves (*Nicotiana rustica* L.) were heated at 22–50° for 5 min, sometimes on four occasions. Leaves were placed in a soln. of ^{14}C-lysine (1 μCi/ml) for 3 h at 22° under lamps of 3000 lux. The leaves were chilled to −70° and the ^{14}C in the protein was measured. Protein synthesis in young leaves was more sensitive to high temp. than in old leaves. The same observation applied to the presence of antibiotics in young and old leaves. L. A. Haddock.

Germination, Growth Regulation, Senescence

Effect of ethylene on growth and maturation of fig fruit. E. C. MAXIE and J. C. CRANE (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 255–267. 33 ref.).—When the fig fruit on the tree was subjected to an atm. concn. of 5 ppm C_2H_4, growth of the fruit was inhibited during Period I (normally a period of rapid growth), but growth and maturation were stimulated when the treatment was given during Period II (normally of slow growth) or Period III (normally the 2nd rapid growth stage). Fruit treated during the last half of Period II or during Period III developed normal flavour, but that treated during the early part of Period II did not. The rôle of 2,4,5-T in stimulating growth and maturation of fig fruit is considered to be due to the effect of the hormone in producing endogenous C_2H_4. A. H. Cornfield.

Influence of growth regulators on ethylene production by apple leaves. J. R. HICKS and D. S. BROWN (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 755–762. 21 ref.).—Application of Alar spray (3000 ppm) decreased rate of C_2H_4 evolution and respiration in leaves after 1 day, but stimulated both in leaves collected 101 days after treatment. Spraying with 100 ppm 2,4,5-T enhanced leaf C_2H_4 production and respiration initially, but the effects gradually disappeared. When the chemicals were applied together, Alar delayed the acceleration in respiratory rate caused by 2,4,5-T, but C_2H_4 production was not affected. A. H. Cornfield.

Metabolism of iodoacetic acid (IOAC) by orange leaves. T. J. FACTEAU, C. H. HENDERSHOTT and R. H. BIGGS (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 195–202. 10 ref.).—Studies with IOAC in which different C atoms were labelled and with I-labelled IOAC and NaI showed that a major portion of the IOAC entering the leaf was metabolised in 2 varieties of orange within 24 h of application. The major action of IOAC on abscission of citrus leaves and fruits is probably due to I^-, or to products of subsequent metabolism of I^-, formed from IOAC. A. H. Cornfield.

Apical dominance in the lowbush blueberry altered by indolebutyric acid. I. V. HALL, L. E. AALDERS and A. D. CROWE (*Hortscience*, 1969, **4** (1), 27–28).—The administration of this compd. to the midvein of the leaves stimulated lateral shoot development in what is normally an unbranched stem. C. V.

Factors influencing root initiation in easy- and difficult-to-root chrysanthemum varieties. L. P. STOLTZ (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 622–626. 14 ref.).—Total carbohydrates were higher in the stem tissue of the easy- than of the difficult-to-root variety and both sugar and starch concn. followed the same trend. Rooting cofactor 4 was lower in the stems but higher in the leaves of the 'difficult' compared with the 'easy' variety. An auxin corresponding to indoleacetic acid was found in approx. equal amounts in both varieties. A. H. Cornfield.

Effects of kinetin and auxin on tissue formation in separate cells from tobacco cultured *in vitro*. M. K. PAVLOVA and R. G. BUTENKO (*Dokl. Akad. Nauk SSSR*, 1969, **188** (5), 1186–1188. Russ., 14 ref.).—Tissue cultures of tobacco (*Nicotiana tabacum* L. var. Trapezond), 7 and 35 days old, were employed. 2,4-Dichlorophenoxyacetic acid (2,4-D), (1 mg/l) and adenine (1 mg/l) or β-indolylacetic acid (IAA) (0·1 mg/l) and kinetin (0·5 mg/l) were added. The auxins and kinetin are capable of modifying the type of differentiation of the separate cells and hence to cause cross-programming in them without reference to the tissue mass. The first effect of auxin administration leads to rearrangement of cell metabolism by some system of division, leading to the creation of fine-mesh tissue. L. A. Haddock.

Effects of two hormones applied to pineapples during the formation of the fruit. A. POIGNANT (*Fruits d'outre mer*, 1969, **24** (7–8), 353–364. Fr., 4 ref.).—Studies were made of the effects of the Na salt of naphthaleneacetic acid (**I**) and β-naphthoxyacetic acid. Over-application damaged fruit. Results showed that although size of fruit was increased with **I** treatment, there was a loss of sugar content, and sometimes an increase in acidity and cracks in the surface appeared. These hormones should be used with caution. M. T. Rawnsley.

Plant growth regulator activity of optical isomers of *O*-(2,4-dichlorophenyl) *O*-methylisopropylphosphoroamidothioate (DMPA). T. W. HOLMSEN (*Weed Sci.*, 1969, **17** (2), 187–188. 10 ref.).—Concn. of DMPA in soln. required for 50% inhibition of growth of fescue seedlings were 1·5, 4·0 and 36·5 ppm for the *l*-, *dl*- and *d*-isomers, resp. Root growth of wheat seedlings was increased by 0·2–0·4 ppm *dl*-DMPA in the nutrient. A. H. Cornfield.

Growth, enzyme and sugar response of immature sugar-cane to foliar treatment with 6-azauracil and gibberellic acid. A. G. ALEXANDER (*J. Agric. Univ. P. Rico*, 1968, **52** (4), 295–310. 29 ref.).—Foliar sprays, containing 0·04% or more of 6-azauracil (**I**), damaged immature sugar-cane plants, but increased sucrose % of storage tissue. The activity of 6 types of enzyme decreased, whilst water-sol. protein increased, with increasing concn. of **I** in the sprays. Gibberellic acid (*GA*) (0·1%) sprays partially counteracted the growth suppression due to **I**. GA alone increased sugar% and retarded enzyme action. A. H. Cornfield.

Effects of foliar combinations of gibberellic acid (GA) and silicon on sucrose production by sugar-cane. A. G. ALEXANDER (*J. Agric. Univ. P. Rico*, 1968, **52** (3), 218–226. 14 ref.).—Foliar applications of aq. GA (K salt) or Si (Na_2SiO_3) increased sucrose% of sugar-cane leaf, and the effects of combined treatments were usually additive, max. sucrose% being obtained with 0·01% GA + 100 ppm Si. Effects of treatment on enzymology of the plant are also reported. A. H. Cornfield.

Wheat root tip bioassay for gibberellins. J. J. CHINOY, C. K. SHAH, HEMLATA D. PATEL and H. K. SUTHAR (*Proc. Indian Acad. Sci. 'B'*, 1969, **70** (2), 98–103. Engl., 13 ref.).—The effects of indole-3-acetic acid (IAA), gibberellic acid (GA) and ascorbic acid (AA) on the elongation of excised plants were examined. Addn. of a 1% soln. of sucrose was compared with addn. of IAA, GA and AA alone, or in combination. Sucrose resulted in accelerated root tip elongation, indicating that it has not only nutritional value. It can be useful in bioassay for comparing extension growths and thus leading to evidence for gibberellin-like substances in growing parts of the plant. E. G. Brickell.

Biosynthesis and physiological activity of indolylacetic acid and gibberellin in the presence of phenolic growth inhibitors and morphactine. V. I. KEFELI, G. S. MUROMTSEV, V. N. AGNISTIKOVA *et al.* (*Dokl. Akad. Nauk SSSR*, 1969, **188** (5), 1182–1185. Russ., 17 ref.).—Studies were made of (*a*) the action of natural phenolic growth inhibitors and of the structural gibberellin antagonist, morphactine IT-3456 (chlorofluorophenol), on growth in a series of biotests, in the presence and the absence of auxin and gibberellin; (*b*) the synthesis of indolylacetic acid (**I**) in a culture of the fungus *Taphrina sadebeckii* in the presence of phenolic inhibitors; and (*c*) the effect of phenolic growth inhibitors and of morphactine on gibberellin synthesis by the fungus *Fusarium moniliforme*. The actions of the inhibitors on synthesis and the functions of phytohormones, **I** and gibberellin, are not specific; they must not be considered merely as anti-auxin or anti-gibberellin. L. A. Haddock.

Effect of low temperature and growth regulators on germination of seed of 'Tokay' grapes. KANG YEOU-DER, R. J. WEAVER and R. M. POOL (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 323–330. 11 ref.).—Although exposure of grape seed to 0° for 3 days increased germination (*G*) % slightly, optimum *G* required 63 days of exposure to 0° before sowing. *G* % of seed soaked in aq. K gibberellate (GA) for 24 h increased with concn. of GA up to 100% where 8000 ppm GA was used. When seed was scarified, soaking in 10 ppm GA gave high *G*. Soaking seed in 10–8000 ppm benzyladenine had little effect on *G*. A. H. Cornfield.

Effect of certain metabolic inhibitors on the ability of gibberellin to induce growth in the leaf base of maize. M. A. STROGANOVA (*Dokl. Akad. Nauk SSSR*, 1968, **183** (5), 1217–1218. Russ., 5 ref.).—Chloramphenicol had the sharpest inhibiting effect (47%) on the stimulating action of gibberellin. J. T. Greaves.

Other Aspects

Potentiometric determination of boron in agricultural samples. R. M. CARLSON and J. L. PAUL (*Soil Sci.*, 1969, **108** (4), 266–272. 2 ref.).—The method previously described (*Idem., Analyt. Chem.*, 1968, **40**, 1292) is improved to provide a technique for handling acid extracts of plant tissue. A series of three exchange columns constructed (details given) of polyethylene parts, and containing, respectively, Amberlite XE-249, Dowex 50W-X8 and Bio Rex 70 resins are required; operational detail is described. A. G. Pollard.

Automated procedure for determination of boron in plant tissue. W. D. BASSON, R. G. BÖHMER and D. A. STANTON (*Analyst, Lond.*, 1969, **94**, (1125), 1135–1141. 12 ref.).—Soln. of plant ash, contg. 1–10 μg of B/ml, are introduced into a Standard Technicon Auto-Analyser together with azomethine H soln. (prepn. given); the colours are measured (15 mm flow cell and 410 nm filter used) and compared to those of known standards. Interfering elements, Cu, Fe and Al, are removed with EDTA soln. S. S. Chissick.

Variation in content of microelements in the foliage of yellow birch, *Betula alleghaniensis*, due to season and soil drainage. M. C. HOYLE (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 458–459. 7 ref.).—The contents (%) of Al, Mn, Zn, Cu and B in yellow beech foliage usually decreased from May to June and increased thereafter. Leaf Mn% and Zn% were higher on poorly than on well-drained soil, but drainage did not affect leaf levels of the other elements. Nutrient content per leaf was higher in poorly than in well-drained soils for Al, Mn and Zn, but not for Cu and B. A. H. Cornfield.

Transforming data in the evaluation of tissue analysis for micronutrients. D. P. GOWING (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 721–725. 3 ref.).—Results from many experiments showed that the coeff. of variation (CV) values for Zn, B, Mo, Cu, Al and S in sugar-cane tissue were higher than CV values for N, P, K, total sugars and moisture. Transformation of the data for micronutrients to logarithms before statistical analysis greatly reduced CV values, increased 'F' values in about 66% of the cases, and raised these to significant values in a few cases. The CV values for major nutrients were not improved by using logarithmic transformation of data. This indicates that the field distribution of major elements was normal, whilst that of micronutrients was not normal in the statistical sense. A. H. Cornfield.

Rind oil components of intergeneric *Citrus-Poncirus* hybrids and their parents. R. W. SCORA, J. W. CAMERON and A. B. ENGLAND (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 221–226. 8 ref.).—The contents of 19 compd. in the rind oil of 4 *Citrus* parent varieties, 2 *Poncirus* selections and 17 hybrids between the two genera were determined. A. H. Cornfield.

Chromatographic analyses of leaf oils in the genus *Lycopersicon*. R. K. SOOST, R. W. SCORA and J. J. SIMS (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 568–571. 7 ref.).—Vapour phase chromatography was used to determine the compn. of leaf oil of *Lycopersicon esculentum*, *L. hirsutum*, and *L. hirsutum* f. *glabratum*. A. H. Cornfield.

I. Gas chromatographic analysis of fruit tissue polysaccharides. II. Relation of trimethylsilyl derivatives of fruit tissue polysaccharides to apple texture. M. TAVAKOLI and R. C. WILEY (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 772–779. 11 ref. 780–787, 8 ref.).—I. The hydrolysates of alcohol-insol. solids in apple fruit cell wall tissue were converted to volatile trimethylsilyl deriv. and analysed by g.c.

II. Pectic substances and hemicelluloses in apple fruit were characterised as trimethylsilyl deriv. of their respective hydrolysates. The fruit of a relatively firm variety (York Imperial) contained greater amounts of water-insol. pectinic acids, galactans, and cellulose than did the fruit of intermediate (Stayman) and soft (Golden Delicious) varieties. Changes in firmness in relation to time of harvest or period of storage could be accounted for to the extent of 87–95% by changes in content of polysaccharide materials. Firmness changes were most highly correlated with changes in glucan, galactan, and pectinic acid. A. H. Cornfield.

I. Determination of cyanidin and pelargonidin in *Poinsettia* by differential absorptiometry. II. Effects of temperature on anthocyanin content and colour of *Poinsettia bracts*. F. J. MAROUSKY (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 672–677. 8 ref.; 678–684. 20 ref.).—I. The method can be used to determine 0·1 mg of pelargonidin and cyanidin providing the concn. of each anthocyanin is $\leqslant$ 0·2 mg per 50 ml solvent.

II. The lower the temp. during growth the greater was the intensity of red coloration and the concn. of pelargonidin and cyanidin glycosides in the bracts. Cyanidin glycosides were the major anthocyanins found at all temp. (55–70°F). A. H. Cornfield.

Anthocyanins from *Hemerocallis*. S. ASEN and T. ARISUMI (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 641–645. 7 ref.).—The nature of the principal anthocyanins present in the flowers of a number of cultivars of *Hemerocallis* was determined. A. H. Cornfield.

Inheritance of *Verticillium* wilt resistance and correlation of resistance with performance traits of the strawberry. R. S. BRINGHURST, P. E. HANSCHE and V. VOTH (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 369–375. 13 ref.).—Data over 11 years on 1544 genotypes from 118 families showed that *Verticillium* wilt resistance is controlled by genes highly additive in their effect. Heritability was estimated at 0·73 $\pm$ 0·03. There was a significant positive correlation between wilt resistance and firmness and a significant negative correlation between wilt resistance and fruit yield. A. H. Cornfield.

Physiological studies of gamma-irradiated tomato fruits. I. Effect on respiratory rate, ethylene production, and ripening. A. S. ABDEL-KADER, L. L. MORRIS and E. C. MAXIE (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 553–567. 29 ref.).—The respiratory rate of tomato fruit increased very rapidly following γ-irradiation. The magnitude and duration of the response increased with dose (100–700 krad). Doses of $>$ 600 krad depressed C_2H_4 production; $\leqslant$ 500 krad stimulated C_2H_4 production, but this was not accompanied by increased ripening rate. The extent of inhibition of colour development due to irradiation decreased with advancing fruit maturity at time of treatment. A. H. Cornfield.

Effects of temperature and light on Arabica coffee. P. K. RAMAJAH and N. H. GOPAL (*Indian Coff.*, 1969, **33** (8), 254–259, 263. 44 ref.).—A general review dealing with the effects upon germination, vegetative growth, bud initiation, flowering, fruit set, berry development and physiological processes. C. V.

Technical significance of air quality standards. D. C. MCCUNE (*Contr. Boyce Thompson Inst. Pl. Res.*, 1969, (2169); *Envir. Sci. Technol.*, 1969, **3** (Aug), 720, 727–728, 731–732, 735).—The development of fluoride criteria for vegetation is discussed. E. G. Brickell.

Crops and Cropping

Survey of the chemical composition of *Potamogeton* and *Myriophyllum* [aquatic plants] in New Jersey. D. N. RIEMER and S. J. TOTH (*Weed Sci.*, 1969, **17** (2), 219–223. 9 ref.).—The high total N content of all species ($\sim$ 1·5 to 3·5% N, dry basis) indicated that no extra N need be added if the materials are to be composted. A. H. Cornfield.

Field Crops

Effect of the freezing of seeds after sowing on the development of winter crop cereals. G. V. ZABLUDA and M. I. PROSTEVA (*Dokl. Akad. Nauk SSSR*, 1968, **183** (5), 1213–1216. Russ., 6 ref.).—Plants grown from seeds subjected during swelling and germination to the action of a negative temperature developed more quickly and more uniformly than plants grown from seeds which had been vernalised before sowing. J. T. Greaves.

Variations in tolerance of some Rhodesian wheat varieties to high soil acidity. I. B. EDWARDS (*Rhodesia agric. J.*, 1969, **66** (5), 120–122. Engl., 3 ref.).—Varieties F1516 and Tokwe were tolerant of pH 4·8; Devuli and Mtilikwe were extremely sensitive. 50 days after planting, 3000 lb/acre of lime was applied by banding between the rows; beneficial effects were observed three weeks after application. E. G. Brickell.

Comparison of semi-dwarf and standard height wheat varieties at two levels of water supply. J. R. SYME (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (40), 528–531. 13 ref.).—Four varieties, viz., Mexico 120, late-Mexico 120 (both semi-dwarfs), Sunset and Heron (Australian standard heights), were subjected to normal and irrigation water supply and 0 and 65 lb of N [as $(NH_4)_2SO_4$] per acre. No response to N in any variety was noticed, and lodging occurred only rarely in Mexico 120. This variety also gave larger ears, and had a higher ratio of grain to post-anthesis leaf area. These factors suggest that this variety could be included with advantage in Australian wheat programmes. M. T. Rawnsley.

Study of the cycle and yields of wheat by quantitative analysis of its growth. J.-F. LEDENT (*C.r. hebd. Séanc. Acad. agric. Fr.*, 1969, **55** (10), 730–735. Fr., 12 ref.).—Two varieties of wheat (Cappelle and Felix) were tested using the methods employed by Whitehead and Myerscough (*New Phytol.*, 1962, **61**, 314–321) for studies on *Epilobium roseum* and *Impatiens parviflora*. During Apr.–June, α (ratio of mean values of R and R_L, where R is the relative rate of growth and R_L the relative increase of leaf surface) was positive, while in June it became negative, increasing later. With active growth, α was $>$ 1. The relationship between this factor and seasonal influences is discussed. The parameter S (from total dry wt. at various times and α) dropped sharply in June at the time when ears appeared, thus showing the use of reserves for ear formation. Prediction of future yields is discussed. M. T. Rawnsley.

Effect of soil potassium, potassium fertiliser, and method of fertiliser placement upon maize yields. W. L. PARKS and W. M. WALKER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 427–429. 18 ref.).—A quadratic regression model was fitted to yields of maize grown on a loam having varying soil-K test levels and K fertiliser applications in the row or broadcast. Over 3 yr there was no interaction between year and the two K parameters, although there were significant effects and interaction of the two K parameters on yields. Row placement of K increased yields to a greater extent than did broadcasting, but the difference due to method of placement decreased with increasing rate of applied K. A. H. Cornfield.

Soya seed 'nitraginisation' in mixed sowing with maize. P. F. STADNICHUK, L. F. SEKRETEVA and G. K. TIMURDZHI (*Mikrobiologiya*, 1969, **38** (3), 497–498. Russ.).—In mixed sowing with maize for silage, treatment of soya seed with 'nitragin' increases the yield of soya green biomass and maize cobs. The increase in yield depends on the variety of soya. The greatest yields occurred with Ussurriiskaya-154 soya (3900 kg/ha); the lowest with Primorskaya-539 (1300 kg/ha). The rise in yield of maize cobs was similarly related to the variety of soya. L. A. Haddock.

Growth and yield of progeny of nitrogen and phosphorus fertilised barley plants. LIN-YING CHANG and J. A. ROBERTSON (*Can. J. Pl. Sci.*, 1968, **48** (1), 57–66. 14 ref.).—Effects of N and P in factorial expt. were compared at five localities using seed graded according to size. N increased but P had little effect on seed size. Larger seeds produced more vigorous seedlings, fewer smutted heads and greater yields of grain and straw; they had higher contents of total carbohydrates and of Kjeldahl-recoverable N. There was no difference in rate of germination of seeds of different sizes. A. G. Pollard.

Growth and development of some barley varieties in response to irrigation and nitrogen fertiliser. E. J. M. KIRBY (*J. agric. Sci., Camb.*, 1969, **72** (3), 467–474. 6 ref.).—The effect of N fertiliser and irrigation, both at two levels, on barley varieties, Procter, Deba Abed, Algérie 48, Philip and Yokozuna was investigated. Irrigation increased tillering and growth rate. Max. leaf area index was greater with irrigation at the high N level. Irrigation and variety affected the rate at which the N content of the shoot fell with time. N uptake early in the season was an important factor determining the total N uptake. Differences in leaf growth, rather than net assimilation rate, led to varietal differences in growth rate. M. Long.

Fluctuations in available mineral nitrogen in a flooded rice soil on the sub-coastal plains of the Adelaide River, N.T. R. W. Strickland (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (40), 532–540. 32 ref.).—The author (*ibid.*, 1968, **8**, 212) has shown that yellowing of rice in heavy clay soils is caused by ferrous iron toxicity rather than a deficiency of available soil N. This work showed that the addn. of ammonium fertiliser to a slightly acid soil resulted in increased levels of available mineral N in the top 12 in of soil for only 4–6 weeks. Mineralisation of native soil N increased available N for 8 weeks. It was shown that rice fields must be flooded as soon as possible after fertilising, and that short duration varieties are of value in these conditions. M. T. Rawnsley.

Manuring methods in cultivation of potatoes. C. H. Henkens (*Landbouwvoorlichting*, 1969, **26** (7/8), 268–272. Dut.).—For the best financial results from manuring, different manuring methods for cultivation of potatoes for consumption, industrial potatoes, cereals, peas and sugar-beet are discussed. Good quality potatoes for consumption require a high % of K in the tuber. Therefore the K no. of the soil should be ~ 25, corresponding to ~ 200 kg of K_2O/ha/yr. Industrial potatoes (with a K no. of 12) need ~ 100 kg of K_2O/ha/yr. J. C. T. Nieuwenhuis.

Relation between germination and number of main stalks in potatoes. A. Schepers and R. F. Hoogland (*Landbouwvoorlichting*, 1969, **26** (7/8), 276–280. Dut., 7 ref.).—In potato production, the no. of stalks of the crop per unit area is important. The relation between the no. and quality of the 'eyes' on the seed-potatoes and the no. of stalks was examined by expt. with pre-germinated seed-potatoes. The results showed that only part of the existing 'eyes' grow to a stalk. J. C. T. Nieuwenhuis.

Effects of dimethyl sulphoxide (DMSO) and 3-indolebutyric acid (IBA) on plant production by three varieties of sweet-potato roots. B. T. Whatley, S. O. Thompson and M. Mayes (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 523–525. 2 ref.).—Soaking *Ipomoea batatas* roots for 5–15 min in aq. DMSO before planting increased the no. of plants (sprouts) as DMSO concn. increased (4–12%). Treatment had no effect on the av. wt. of plants appearing during the first 30 days. The 3 varieties tested responded similarly to DMSO. Soaking in 1000 ppm IBA had no effect on plant production. A. H. Cornfield.

Turnip growth and mineral composition as influenced by soil temperature and two fertility levels. C. G. del Valle and S. A. Henson (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 578–582. 6 ref.).—Over the soil temp. range 45 to 95°F, max. top growth of turnip occurred at 75–85°F and max. edible root yield at 55–75°F. Max. total uptake of P and K by tops occurred at 75–85°F and of Ca and Mg at 75°F. Max. total uptake of all minerals by the edible portion of roots occurred at 55–75°F. Top and root growth and total uptake of P, K, Ca and Mg were greater where an 8–8–8 (N–P_2O_5–K_2O) than where a 6–12–6 fertiliser (both at 2000 lb per acre) had been applied. A. H. Cornfield.

Interaction between nitrogen and water in the growth of grass swards. II. Leaf area index and net assimilation rate. M. J. D'Aoust and R. S. Tayler (*J. agric. Sci., Camb.*, 1969, **72** (3), 437–443. 24 ref.).—The effect of N and irrigation treatments on leaf area index (*LAI*) and net assimilation rate (*E*) was investigated in a factorial trial with 2 levels of irrigation and 3 levels of N up to 4 cwt/acre. Growth of the *Lolium multifolium* sward was followed fortnightly. Response to N arose from increases in both *LAI* and *E*, whereas response to irrigation was largely due to increase in *LAI*. The periodic harvesting caused new growth which led to new tillers, and was probably the reason for the lack of seasonal decline in *E*. M. Long.

Carry-over effects of high fertiliser rates on native sedge bog vegetation in interior British Columbia. W. L. Pringle and A. L. van Ryswyk (*Can. J. Pl. Sci.*, 1968, **48** (1), 49–55. 10 ref.).—N, P_2O_5 and K_2O, in all combinations, were applied at rates of 224 or 448 kg/ha to a sedge bog over 5 yr. Yields of grass were greatest with N : P : K ratios 1 : 1 : 2. The most limiting element was P, followed by K. N fertilisers showed positive effects only in the first yr. Annual variations in yield and fertiliser effects were related to rainfall. Digestibility of the forage was unaffected by differences in fertiliser treatment. Hay quantity increased after using complete fertilisers; N : P : K ratio was as important in increasing yields as the rate of application. A. G. Pollard.

Research on plants for cellulose usable in paper making. M. Arnoux, A. Cotte, J. Castiaux *et al.* (*C.r. hebd. Séanc. Acad. agric. Fr.*, 1969, **55** (12), 879–891. Fr., 8 ref.).—Broom sorghum, kenaf and ramie were shown to be useless, but hybrids of hemp, and Provencal cane will probably be useful. Further pilot scale tests are required. M. T. Rawnsley.

A search for new fibre crops. XI. Compositional characteristics of Illinois kenaf at several population densities and maturities. T. F. Clark and I. A. Wolff (*TAPPI*, 1969, **52** (11), Pt. 1, 2111–2116. 9 ref.).—Properties of kenaf, planted at different population *d* (17,800–149,000 plants/acre) and different ages (90–138 days after planting) were investigated. The av. proportion of bark increased with population *d*; bast and core fibres were unaffected by maturity and population *d*; α-cellulose, lignin and pentosan contents in stalk bottoms increased with maturity. Results suggest that kenaf for pulp should be grown to max. maturity. Max. efficiency at harvest can be achieved by selecting the appropriate planting *d*. M. Allsebrook.

Horticultural Crops

Stimulation of pear rooting by pre-plant treatment of nursery stock with indole-3-butyric acid (IBA). N. E. Looney and D. L. McIntosh (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 150–154. 1 ref.).—Treatment of roots of pear nursery trees with IBA (either by inserting toothpick sections impregnated with IBA into the roots or by dusting with 0·8% IBA powder) significantly increased root wt., but had no effect on shoot wt., during the first season. A. H. Cornfield.

Effects of Alar [succinic acid mono(2,2-dimethylhydrazide] sprays used to control growth of 'Bartlett' pear trees planted in hedgerows. W. H. Griggs and B. T. Iwakiri (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 155–166. 12 ref.).—Shoot and internode lengths of pears planted in rows were decreased by increasing concn. (1000–3000 ppm) of Alar applied at petal fall. At harvest, control fruit was smaller and greener than fruit from sprayed trees. Flesh firmness was not affected, but picked pears from sprayed trees softened more slowly than did control pears. The treatments had no effect on flavour. A. H. Cornfield.

Effects of soil management, pollination and nitrogen fertilisers on Williams' pear trees. A. Selemi and J. C. Keatley (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (40), 553–559. 25 ref.).—This long term trial showed that clover sward can be used for Williams' pear trees, provided that cross-pollination is encouraged, light pruning is used, the tree line is free of sod, and there is only moderate application of N. Fertiliser should be non-acidifying. This method is much less costly than straw cultivation. M. T. Rawnsley.

Assessment of winter hardiness in peach cultivars by electric impedance, scion diameter and artificial freezing studies. G. M. Weaver, H. O. Jackson and F. D. Stroud (*Can. J. Pl. Sci.*, 1968, **48** (1), 37–47, 11 ref.).— A. G. Pollard.

Effect of naphthaleneacetic acid (NAA) on abscission of peach fruits in relation to endosperm development. S. J. Leuty and M. J. Bukovac (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 124–134. 21 ref.).—The cytokinetic stage of endosperm development in peach varieties represented an area of demarcation between fruit sensitive and non-sensitive to NAA. Although pericarp length was an accurate index of cytokinesis, this parameter varied among 8 cultivars. Max. thinning by NAA applied in 30 ppm sprays was obtained when fruit length averaged 30–31 mm in Redhaven and 27–30 mm in Loring. A. H. Cornfield.

Influence of amount of fruit and time of harvest on macronutrient concentrations in 'Valencia' orange leaves. W. W. Jones and T. W. Embleton (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 191–194. 7 ref.).—A 5-yr study with Valencia orange, which produces low and high yields in alternate years, showed that leaf N% was not related to crop load, whilst leaf P% and K% were inversely related to crop load only in the heavy-cropping years. Time of harvest did not influence crop load, but delayed harvest decreased leaf P% and K%. A. H. Cornfield.

Iron chlorosis on orange trees induced by irrigation with saline water in the Tunis region. P. Saglio (*C.r. hebd. Séanc. Acad. agric. Fr.*, 1969, **55** (10), 741–751. Fr., 7 ref.).—12 yr work on the behaviour of orange trees treated with brackish water showed that widespread occurrence of withering is not directly due to excess salt in the soil, since the most saline parts suffered least. Nor is it due to excess absorption of Cl or Na, as was previously thought. The water seems to create a medium in which strong iron chlorosis is caused, especially in soil with a high Ca content. M. T. Rawnsley.

Further studies on growth regulator-induced parthenocarpy in the 'Bing' cherry. J. C. Crane and J. R. Hicks (*Proc. Am. Soc. hort.*

Sci., 1968, **92**, 113–118. 7 ref.).—To produce parthenocarpic cherries it was necessary to apply combined sprays of gibberellic acid and auxins (2,4-D, 2,4,5-T, NAA, PDPA or ATCP). All treatments markedly stimulated the spurs to form long shoots which were devoid of flower buds the following year. No combination or concn. of materials overcame this undesirable vegetative response. A. H. Cornfield.

Relation of gibberellin (GA) treatment to fruit-set, berry development and cluster compactness in *Vitis vinifera* grapes. A. J. CHRISTODOULOU, R. J. WEAVER and R. M. POOL (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 301–310. 10 ref.).—Application of 20–40 ppm GA during the bloom period decreased berry set in two of three grape varieties. GA application after anthesis had little effect on berry set. Bloom sprays resulted in elongated berry growth, loose clusters and increased berry wt. A. H. Cornfield.

Fruit quality of fresh strawberries as influenced by nitrogen and potassium nutrition. G. K. SAXENA and S. J. LOCASCIO (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 354–362. 25 ref.).—Total N% of fruit and shear resistance increased with rate of applied N (60–180 lb per acre), whilst titrable acidity decreased. Total K% and titrable acidity in the fruit increased with level of applied K (60–180 lb per acre), but shear resistance was not affected. Compression resistance tended to increase with N rate and decrease with K rate. Quality changes were little affected by source of K (SO_4^{2-}, NO_3^-, or Cl^-). Titratable acidity was significantly correlated with K% and Ca% in the fruit. A. H. Cornfield.

Effect of Alar on winterhardiness of raspberries. R. L. GRANGER and E. J. HOGUE (*Can. J. Pl. Sci.*, 1968, **48** (1), 100–101. 4 ref.)—Two varieties of raspberries were sprayed on fixed dates in June, July or Aug. (Quebec, Can.) with Alar (succinic acid mono[2,2-dimethylhydrazide]) at 0, 1000 or 2000 ppm in amt. sufficient to thoroughly wet the foliage. Reduction in the amt. of die-back, measured in the following spring was greater with early than with later treatments. A. G. Pollard.

Induced parthenocarpic fruit development in highbush blueberry. C. M. MAINLAND and P. ECK (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 284–289. 8 ref.).—Application of 500 ppm K gibberellate (KG) or naphthalene-acetic acid (NAA) or a combination of 500 ppm KG with 5–500 ppm NAA in lanolin paste to emasculated blueberry flowers resulted in better fruit set than did hand pollination. Berry dia., using the higher level of either material, was only slightly less than that of hand-pollinated fruit, whilst the combined treatment at the highest levels of KG and NAA promoted the earliest ripening and highest sol. solids in the fruit. A. H. Cornfield.

Effect of delayed harvest and storage on pigment development in cranberries. R. M. DEVLIN, B. M. ZUCKERMAN and I. E. DEMORANVILLE (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 793–796. 4 ref.).—The anthocyanin% (fresh wt. basis) of cranberry fruit left on the vine for 15 days after apparent maturity increased to a greater extent than when the berries were picked and stored (17–24°) for 15 days. High-quality berries contained $< 0{\cdot}67$ mg anthocyanin per g of fresh wt. A. H. Cornfield.

Cranberry fruit set, growth and yield as influenced by gibberellic acid (GA) alone and in combination with Alar [succinic acid mono (2,2-dimethyl hydrazide)]. C. M. MAINLAND and P. ECK (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 296–300. 5 ref.).—Application of 50–500 ppm GA at bloom in 1965 and 1966 increased fruit set and yield in the first year. Flower bud initiation was decreased, which resulted in fewer flowers and much lower yields in 1966. Berries that were seedless or had low seed count were smaller than berries with high seed count. In 1966 uprights were considerably longer due to two years of treatment with GA, and the uprights developed very few flower buds. Application of 2000 ppm Alar, in combination with 500 ppm GA, did not overcome the effect of the latter in increasing length of the uprights. A. H. Cornfield.

Effects of phosphorus sources and copper rates on watermelons. S. J. LOCASCIO, P. H. EVERETT and J. G. A. FISKELL (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 583–589. 15 ref.).—In two sandy soils, low in Cu and P, $CuSO_4$ (2–4 lb Cu^{2+} per acre) increased watermelon fruit yields and tissue Cu %. Application of P increased tissue P% but decreased tissue Cu %. Where no Cu^{2+} was applied, P applications decreased yields, whilst where Cu^{2+} was applied yields increased with level of P application (superphosphate, conc. superphosphate, and ammoniated superphosphate). Max. yields were obtained with 70–105 lb P per acre. A. H. Cornfield.

Marginal soil salinity in cantaloupe production. R. J. MILLER and R. M. DAVIS, JUN. (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 455–460. 5 ref.).—Characteristics of a clay loam (pH 8·3) in which salinity caused some variation in the pattern of cantaloupe fruit production are described. Naturally occurring levels of Na^+, Cl^-, and B in the soil were not toxic, although Na in plant tissue increased when N and P were applied and Cl^- uptake increased when the soil was leached. KCl decreased early fruit yields when applied alone, but not when N and P were applied. A. H. Cornfield.

Nutritional studies with Chinese waterchestnuts, *Eleocharis dulcis*. H. T. DERIGO and H. F. WINTERS (*Proc. Am. Soc. Hort. Sci.*, 1968, **92**, 394–399. 3 ref.).—Field trials with waterchestnuts (grown under waterlogged conditions) showed that N was the main nutrient limiting yields, although source of N [urea or $(NH_4)_2SO_4$] was not important. Responses to P and K usually only occurred where N was also applied. In sand culture tests in the glasshouse, omission of N, P, or Mg from the nutrient reduced growth, whilst omission of Ca increased growth of culms. Yield of corms was reduced by omission of N or Ca, but not by omission of P, K, or Mg. A. H. Cornfield.

Application of tissue analysis to production of commercial greenhouse tomatoes. K. S. MACLEAN, H. A. L. MCLAUGHLIN and M. H. BROWN (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 531–536. 17 ref.).—The N, P, K, Ca, Mg, Mn, Fe and B % in the 5th leaf from the top of the main stem were determined at weekly intervals for 17 weeks, starting 10 weeks after sowing, in samples from 12 commercial glasshouses. Antagonistic reactions between K, Ca, and Mg occurred and indicated the need for a carefully regulated feeding programme. Visual deficiency symptoms due to lack of Ca, Mg, or B were verified by leaf analysis and remedied by appropriate nutrient addition. A. H. Cornfield.

Nutrient content of field-grown peas. K. S. MACLEAN and D. L. BYERS (*Can. J. Pl. Sci.*, 1968, **48** (2), 155–160. 19 ref.).—The annual av. nutrient content of field-grown pea leaves (var. Pride) showed little variation over 3 yr. N, K, Fe, Mn, Mo and Zn contents diminished as the season advanced, whereas P, Ca, Mg and B contents remained relatively constant. Soil texture had little influence on nutrient contents or crop yield. Four varieties, showing similar seasonal variations and yields, differed in amt. of nutrients absorbed at various growth stages. Notably higher yields in one season were attributed partly to lower temp. and higher moisture conditions in that yr. A. G. Pollard.

Fibre development in snap bean, *Phaseolus vulgaris*, as influenced by *N*-dimethyl succinamic acid [succinic acid mono (2,2-dimethylhydrazide)] (DMAS) sprays and moisture stress. A. E. NIGHTINGALE, E. T. GRAHAM and H. T. BLACKHURST (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 426–431. 8 ref.).—Application of 1000 ppm DMAS spray to snap bean at first bud, first bloom, or first fruit set decreased pod fibre %, whilst 2500–4000 ppm sprays had no effect. Results were similar, irrespective of time of application. Pod fibre content was no different between well-watered plants and those receiving sufficient water to induce incipient wilting during the day. A. H. Cornfield.

Effect of nitrogen levels and micronutrients on yield, chlorophyll and mineral content of spinach. J. K. GREIG, J. E. MOTES and A. S. AL-TIKRITI (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 508–515. 10 ref.).—Yield and chlorophyll % of spinach leaves increased with level of applied N (40–120 lb as NH_4NO_3), whilst application of Fe, Mg or Zn had no effect. Leaf Ca, Mg and K % were increased by the highest level of N, whilst application of Fe, Mg or Zn had no consistent effect on the content of major elements or of Fe or Zn. A. H. Cornfield.

Supplemental carbon dioxide and growth of *Chrysanthemum morifolium*. R. S. LINDSTROM (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 627–632. 14 ref.).—When glasshouse chrysanthemum plants were grown in an atm. supplemented with 1500–4000 ppm CO_2, starting in Jan., the plants were taller, showed increased dry wt. and were of better quality than control plants. A. H. Cornfield.

Relationship of water salinity and fluorides to keeping quality of chrysanthemum and gladiolus cut-flowers. W. E. WATERS (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 633–640. 12 ref.).—In general the vase-life of chrysanthemum and gladiolus flowers and foliage decreased with increasing concn. of salts and F^- in the water. Equations relating vase-life to the concn. of salts and F^- are presented. A. H. Cornfield.

Plantation Crops

Preharvest changes in the physical and chemical properties of plantains. F. SANCHEZ NIEVA, G. COLOM COVAS, I. HERNANDEZ, *et al.* (*J. Agric. Univ. P. Rico*, 1968, **52** (3), 241–255. 10 ref.).—Physical

characteristics, pulp texture, pulp : peel ratio, starch, total and reducing sugars, total acidity, and pH were measured in plantain fruit harvested 70 to 110 days after inflorescence. A. H. Cornfield.

Effects of α-naphthalene acetic acid (ANA) on the processing quality of pineapples. R. P. BOWDEN (*Fd Technol. Aust.*, 1969, **21** (9), 454–457. 8 ref.).—Fruit size and wt. were increased by spraying ANA on to pineapple fruits 2 months before harvesting, but the total sol. solids and flesh colour rendered the fruit less acceptable to the palate and affected the relationship between skin colour and internal ripeness. ANA increased breakstrength and, in some cases, reduced porosity. I. Dickinson.

Action of improving plants in pineapple cultivation. II. Pedological aspects. J. GODEFROY (*Fruits d'outre mer.* 1969, **24** (7–8), 380–386. Fr., 3 ref.).—The action of *Flemingia congesta*, *Stylosanthes gracilis* and *Crotalaria usaramoensis* on the chem. and phys. characteristics of a soil exhausted by several cycles of pineapples was studied. The only beneficial effect was an increase in the proportion of mineral N in the soil. M. T. Rawnsley.

Response to magnesium of intensively managed sun-grown coffee. E. H. MEDINA, F. ABRUNA and R. DEL VALLE (*J. Agric. Univ. P. Rico*, 1968, **52** (3), 185–194. 12 ref.).—Application of 30–90 lb Mg^{2+} per acre to a clay (pH 5·1) increased coffee bean yields by 36–50% and eliminated visual leaf-deficiency symptoms. Bean yields were highly correlated with leaf Mg % in summer and autumn. A. H. Cornfield.

Effects of different levels of major nutrients and lime on coffee yield in Puerto Rico. S. J. RODRIGUEZ, R. BOSQUE LUGO, E. G. BONETA GARCIA and A. MORALES MUNOZ (*J. Agric. Univ. P. Rico*, 1968, **52** (3), 195–203. 10 ref.).—Coffee yields (cv. Columnaris) on a clay (pH 4·5) were not affected by application of N, P and K in various combinations with or without CaO to raise soil pH to 6·5. Yields of the cv. Puerto Rico were increased by N, K and NPK but not by liming. Leaf K% increased with level of applied K (100–300 lb K_2O per acre), but leaf P and N levels were little affected by application of N or P. A. H. Cornfield.

Yield and quality of irrigated tobacco under subhumid conditions. M. CAPIEL and A. S. BRACERO (*J. Agric. Univ. P. Rico*) 1968, **52** (4), 281–294. 5 ref.).—The effects of different irrigation frequencies on yield and quality of two cigar-filler tobacco varieties are reported. Yields were increased (to different extents in the 2 varieties) by both irrigation regimes, but were unaffected by frequency of irrigation. Mosaic which developed in one of the varieties decreased the uptake of nutrients. A. H. Cornfield.

Response in growth and yield of *Hevea brasiliensis* to fertiliser application on Regnam series soil. E. PUSHPARAJAH. **Relative importance of fertiliser application during pre- and post-tapping phases of *Hevea*.** A. J. JEEVARATNAM. **Effect of fertiliser applications on latex properties.** H. M. COLLIER and J. S. LOWE. **Effects of minerals introduced directly into the wood and of acetylene applied to the bark of *Hevea*.** Y. BANCHI and J.-P. POLINIERE. **Recent advances in fertiliser usage for rubber in Malaya.** M. M. GUHA (*J. Rubb. Res. Inst. Malaya*, 1969, **21** (2), 165–174 (24 ref.); 175–180 (18 ref.); 181–191 (11 ref.); 192–206 (14 ref.); 207–216 (12 ref.). C. A. Finch.

Ground covers and performance [of *Hevea brasiliensis*]. (*J. Rubb. Res. Inst. Malaya*, 1969, **21** (2), 107–164).—After an introduction and summary (B. S. GRAY, 107–112. 5 ref.) of four papers, the papers are presented: **Residual effects of ground cover and nitrogen fertilisation of *Hevea* prior to tapping.** B. J. MAINSTONE (113–125. 8 ref.). **Manuring of rubber in relation to covers.** E. PUSHPARAJAH and K. CHELLAPAH (126–139. 11 ref.). **Effects of cover plants.** P. R. WYCHERLEY and M. M. CHANDAPILLAI (140–157. 23 ref.). **Cover plant trials.** S. M. WARRIAR (158–164). C. A. Finch.

Chemical retardation of tung blossoming. B. G. SITTON, W. A. LEWIS and W. W. KILBY (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 383–393. 8 ref.).—A no. of chemicals (0·02–5 g/l) tested on excised shoots in humid chamber expt. retarded tung blossom-bud development, usually without causing any apparent injury. In the tung orchard the use of thiouracil, glycerol, and propylene glycol (which were effective in humid chamber tests) gave inconsistent results, but glycerol or propylene glycol applied with thiouracil in Oct. or early Mar. retarded blossoming and reduced fruit set. A. H. Cornfield.

Forest Crops

Effect of nutrient mist on propagation of selected woody ornamental plants. D. C. SORENSON and G. D. COORTS (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 696–703. 21 ref.).—Although addition of N+P+K to the nutrient mist applied during rooting of cuttings of *Buxus*, *Ilex*, *Juniperus* and *Taxus* increased the N, P and K% in the cuttings at the time of callus and rooting, the treatment decreased the no. of roots formed and the % of rooted cuttings in all species except *Buxus*. A. H. Cornfield.

Evaluating control of wood quality through breeding. G. NAMKOONG, A. C. BAREFOOT and R. G. HITCHINGS (*TAPPI*, 1969, **52** (10), 1935–1938. 34 ref.).—Work on the genetic gain potential in wood quality is reviewed and efforts to increase sp. gr. and fibre length are discussed. For loblolly pine, it would be more profitable to breed for gross fibre yield per acre than for an increase in wood sp. gr. Geneticists require reliable estimates of the future value of wood qualities before planning a rational selection programme. M. Allsebrook.

Forest fertilisation. (*Proc. Vth Colloquium int. Potash Inst.*, 1967, 379 pp.).—Papers are presented on the following topics: Evolution of forest fertilisation; growth factors and assessment of fertiliser needs; fertilising young trees; effects of fertilisers; quality and economic aspects. P. P. R.

Animal Husbandry

Feedstuffs

Distribution of miserotoxin in varieties of *Astragalus miser*. M. C. WILLIAMS and F. A. NORRIS (*Weed Sci.*, 1969, **17** (2), 236–238. 9 ref.).—A method for determining miserotoxin (poisonous to livestock) in *A. miser* (timber milkvetch) is described. The % in 8 varieties varied considerably; it occurred mainly in the leaves, with smaller amounts in roots, flowers and seeds. The poison level in leaves and stems decreased rapidly after dispersal of the seed. A. H. Cornfield.

Effects of supplementing predominantly roughage rations with oats grass, trace minerals, or B-vitamins on nutrient utilisation. T. W. PERRY and R. J. HILLIER (*J. Dairy Sci.*, 1969, **52** (11), 1786–1791. 24 ref.).—Results of digestion trials with lambs showed that the availability of nutrients in a low-energy urea-supplemented ration was not significantly affected by supplementation of the ration with oats grass, trace minerals, or B-vitamins plus vitamin E. Addn. of oats grass altered rumen volatile fatty acid patterns, but neither blood NH_3 nor urea levels were affected by any of the treatments. M. O'Leary.

Effect of maturity and leafiness on the intake and digestibility of alfalfas (lucernes) and grasses fed to sheep. J. E. TROELSEN and J. B. CAMPBELL (*J. agric. Sci., Camb.*, 1969, **73** (1), 145–154. 36 ref.).—The effect of maturity on the nutritional quality of two lucerne spp. (*L*) and four grass (*G*) hays was investigated. Each hay was cut at six stages of growth and fed to sheep at a rate 10% in excess of their voluntary intake. Rate intake of *L* was 10% greater than that of a *G* of similar digestibility. Differences in nutritional quality, expressed as voluntary intake per $kg^{0.75}$ of body wt., and time of harvest as day no. in the yr, showed little or no variation between the *G* species at immature stages but were apparent at maturity. *L* hay declined in value more slowly than the *G*, and stopped at the mature stage. On av., voluntary intake of digestible org. matter fell 0·29 g per day delay in harvesting and a 1% decline in leafiness reduced intake by 0·58 and 0·73 g for *L* and *G*, resp. Time of harvest accounted for 77–89% of quality variation and the combined effect of leafiness and growth stage accounted for 75%. The relationship between intake and digestibility of the hays was used to illustrate how voluntary intake of metabolisable energy from hay of pure or mixed species may be predicted from *in vitro* digestibility. M. Long.

Progress in defining the differences in nutritive value to sheep of perennial ryegrass, short-rotation ryegrass and white clover. M. J. ULYATT (*Proc. N.Z. Soc. Anim. Prod.*, 1969, **29**, 114–123. 17 ref.).—Romney marsh sheep were used and the grass was nitrolimed at 4 cwt/month. Results confirmed previous work that liveweight gain decreased in the order clover (*C*) > short rotation ryegrass (*S*) > perennial ryegrass (*P*). There was a lower rumen digestibility, quicker turnover and greater passage of org. matter from the rumen with *S* compared with *P*. *P* can be digested more completely. The good results from *C* are probably due to low cellulose content and a high ratio of readily fermentable to structural carbohydrate. M. T. Rawnsley.

Survival in South Australia of Hunter River, African, and [two] creeping lucernes after extended periods of severe grazing. G. J.

LEACH (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (40), 517–520. 13 ref.).—Tests were carried out on the lucernes on red-brown earth soil in a Mediterranean climate. Severe grazing, with 3-week recovery periods, was carried out for 2 yr. None of the strains could resist severe, extended grazing. The creeping strains gave lower yields than the Hunter River cultivar. The results were different from those obtained at Canberra, probably because of environmental variations. M. T. Rawnsley.

Potential digestibility of cellulose in forage and faeces. R. J. WILKINS (*J. agric. Sci., Camb.*, 1969, **73** (1), 57–64. 35 ref.).—Potential cellulose digestibility (*CD*) (i.e. max. digestibility when conditions and duration of digestion are not limiting factors) was examined. *In vitro CD* reached a max. after 5 days; *in vivo CD* was lower than that found *in vitro*. Where the differences were appreciable, digestibility of cellulose in the faeces was high. Plant factors limited further digestion and the residue, after prolonged digestion *in vitro*, consisted only of lignified and cutinised tissue. M. Long.

Relative rate of *in vitro* disappearance as a possible estimator of digestible dry matter intake. S. S. GILL, H. R. CONRAD and J. W. HIBBS (*J. Dairy Sci.*, 1969, **52** (10), 1689–1690. 12 ref.).—A correlation of 0·993 was shown to exist between the relative rate of digestible cellulose disappearance *in vitro* and digestible dry matter intake of cows fed high dry matter legume-grass silage. This observation may lead to the elaboration of a method for estimating digestible dry matter intake of forages. M. O'Leary.

Effects of calcium sources and urea on corn silage fermentation. F. N. OWENS, J. C. MEISKE and R. D. GOODRICH (*J. Dairy Sci.*, 1969, **52** (11), 1817–1822. 24 ref.).—Addn. of urea (**I**) and Ca [as $CaCO_3$ (**II**) or dicalcium phosphate (**III**)] to maize silage was shown to increase and extend fermentation. The additives stimulated fermentation in the order **I** > **II** > **III**. M. O'Leary.

Effect of recutting and plant maturity on kernel passage and feeding value of corn [maize] silage. G. R. BUCK, W. G. MERRILL, C. E. COPPOCK and S. T. SLACK (*J. Dairy Sci.*, 1969, **52** (10), 1617–1623. 14 ref.).—Recutting of maize to produce a finely chopped material for silage was shown to have no beneficial effects on the digestibility of the silage, dry matter (*DM*) intake or milk production. Increasing plant maturity resulted in increased silage *DM* intake up to about 35% *DM*. Stage of maturity had little effect on digestibility or milk production and it is concluded that *DM* yield considerations suggest 30–35% *DM* as a desired range for harvesting maize for silage. M. O'LEARY.

Growth, intake and digestibility from formic acid silage *versus* hay. D. R. WALDO, L. W. SMITH, R. W. MILLER and L. A. MOORE (*J. Dairy Sci.*, 1969, **52** (10), 1609–1616. 19 ref.).—The growth, feed intake and digestibility of 0·5% HCO_2H-preserved unwilted silage, when fed as the sole ration component, were determined in expt. with Holstein heifers, using hay prepared from a common forage as control. Mean daily gains were 692 g for the silage and 620 g for the hay. Digestibility of energy from the formic silage was 67·1 while that of the hay was 59·4. The formic silages were shown to be lower in pH, $PrCO_2H$, HOAc and NH_4-N and higher in lactic acid than unwilted silages without added HCO_2H. M. O'Leary.

***In vitro* digestibility of forage species as affected by fertiliser application, stage of development and harvest dates.** F. W. CALDER and L. B. MACLEOD (*Can. J. Pl. Sci.*, 1968, **48** (1), 17–24. 12 ref.).—Single-species blocks of *Bromus inermis* L., *Dactylis glomerata* L., and *Medicago sativa* L. were grown for 2 yr with various mixtures of N and K fertilisers. Two cuttings per season were harvested; *in vitro* digestibility (*IVD*) and digestible dry matter (*DDM*) were detd. *IVD* of the grasses was unaffected by N + K at all rates of application; for lucerne, the *IVD* was increased by K and the *DDM* was increased by both N and K. Observations at 4-day intervals showed that *IVD* values of all species declined from June onwards. The effect was most rapid in *D. glomerata* and least in *B. inermis*. A. G. Pollard.

Fermentation of various soluble carbohydrates by rumen micro-organisms. J. W. CZERKAWSKI and G. BRECKENRIDGE (*Proc. Nutr. Soc.*, 1969, **28** (2), 52A–53A).—Glucose, fructose and sucrose (Goup A) fermented rapidly; L(+)-arabinose, xylose, galactose, mannose, cellobiose, maltose, lactose, raffinose, inulin, xylan and pectin were fermented at appreciable rates but significantly lower than group A. D-(−)arabinose, trihalose, starch and fucose were fermented slowly, if at all, while rhamnose and flucosamine were fermented but no CH_4 was produced. C. V.

Calorimetric studies on effect of dietary energy source and environmental temperature on the metabolic efficiency of energy utilisation by mature Light Sussex cockerels. D. W. F. SHANNON and W. O. BROWN (*J. agric. Sci., Camb.*, 1969, **72** (3), 479–489. 27 ref.).—The net availabilities of the metabolisable energy (*NAME*) of a cereal based diet and a maize oil diet for maintenance and lipogenesis and the effect of environmental temp. on *NAME* were investigated, using four 1–2 yr old birds. *ME* intake and energy retention were related linearly for both diets. *NAME* values for the cereal diet were lower than those for the maize oil diet, the latter diet leading to an increase in the energy retained as fat. The mean fasting heat production at 28° was 15% lower than at 22°. M. Long.

Flavomycin. First antibiotic developed exclusively for animal nutrition. G. L. LEITNER (*Allg. prakt. Chemie*, 1969, **20** (12), 403–408. Ger.).—After a discussion of the nutritive value of antibiotics in animal feed, medical and hygienic aspects are considered, particularly the problem of micro-organism resistance. Flavomycin is obtained from *Streptomyces* strains. It is not resorbed by the animal body and is excreted intact so that residues in animal organs are small; no uptake by plants was observed. It is very stable in feeds and shows good compatibility; no allergising properties were found. M. Sulzbacher.

Quantitative antibiotic sensitivities of ruminal bacteria. C.-L. WANG, B. B. BALDWIN, R. S. FULGHUM and P. P. WILLIAMS (*Appl. Microbiol.*, 1969, **18** (4), 677–679. 10 ref.).—The disc method was used. Effects of 10 antibiotics against 17 bacteria are shown, the min. inhibitory concn. being recorded. C. V.

Effects of Diet and Environment on Livestock

Some factors affecting yellow fat colour in cattle. J. H. L. MORGAN, F. S. PICKERING and G. C. EVERITT (*Proc. N.Z. Soc. Anim. Prod.*, 1969, **29**, 164–175. 12 ref.).—Jersey, Friesian, Aberdeen Angus, Jersey/Friesian, Jersey/Charolais and Jersey/Hereford cattle were examined for fat colour intensity, blood and fat carotene levels. It was shown that genetic manipulation can reduce both fat colour and the marketing problem of yellow fat. The Charolais sire had some effect on reducing colour, but no closely correlated results were found. M. T. Rawnsley.

Genetic and environmental effects on beef production. G. P. EVERITT, S. T. EVANS and M. FRANKS (*Proc. N.Z. Soc. Anim. Prod.*, 1969, **29**, 147–163. 16 ref.).—500 steers and heifers of Aberdeen Angus, Friesian (steers only), and Friesian/Jersey, Hereford/Jersey and Charolais/Jersey breeds were studied. Friesians were best in growth rate, carcass wt. and meat production, but carcass grading favoured Aberdeen Angus. However, cross-breds, esp. Charolais/Jersey, gave more high-priced cuts than the others, and were more profitable. M. T. Rawnsley.

Effect of grain and urea supplements fed with maize forage for fattening cattle. P. N. THURSON and L. WINKS (*Queensland J. agric. Anim. Sci.*, 1968, **25** (1), 19–28. 26 ref.).—Twelve groups of five steers were group-fed *ad lib.* on six rations for 100 days. Two comparable groups were used as controls. The rations were green maize forage (*GMF*) harvested daily and *GMF* with grain. All rations were fed with and without a supplement of 85 g urea per head daily. Urea, with or without grain, led to an increase in dry-matter intake and efficiency of feed conversion. With both grain–forage rations, the addition of urea showed significant increase in body wt. gain (*BWG*), carcass gain and dressing % (*D*). *BWG* and *D* were not significantly influenced by the size of ration. C. V.

Intensive beef production from sorghum grain and sorghum stubble. Effects of methods of processing the grain and stubble, vitamins A, E and K, and selenium supplementation and anthelmintic treatment on rate and efficiency of body-weight gain and carcass composition. J. G. MORRIS and P. M. PEPPER (*J. agric. Sci., Camb.*, 1969, **73** (1), 41–48. 24 ref.).—Intensive finishing of steers on rations contg. 10% of sorghum stubble and 90% of grain was investigated. Fine grinding of grain reduced intake but increased efficiency of feed conversion. Coarse chaffing of stubble (compared with hammer milling), reduced intake but had no other effects. Supplements of either 1 mg of Se, 200 mg of vitamin E, with or without vitamin K, had no effects on rate or efficiency of body wt. gain. Vitamin A injections maintained $\lessdot$ 33 μg/g hepatic vitamin A concn, although they had no effect on feed conversion, rate of body wt. gain or time to attain slaughter wt. Thiabendazole drenching had no effect on body wt. gain. M. Long.

Effect of pregnancy in heifers on voluntary intake, total rumen contents, digestibility and rate of passage. J. L. LAMBERTH (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (40), 493–496. 18 ref.).—

Results for heifers fed mainly on *Paspalum dilatatum* pastures showed that depression of dry matter intake occurred, but depression of dry matter digestibility did not agree entirely with other work. It is suggested that intake and digestibility effects are of biological significance. The results also showed that compression of the digestive tract by the foetus is not yet established.
M. T. Rawnsley.

Influence of feeding below the starch equivalent standards on liveweight and production of dairy cows. N. D. DIJKSTRA (*Versl. landbouwk. Onderz. Ned.*, 1969, (723), 85 pp., Dut., 7 ref.).—To analyse these effects of feeding below the Dutch starch equiv. standards (*SES*) three feeding trials were carried out with two groups of cows. The control cows were fed, on average, 4·2% above the *SES* and the exptl. cows 11·8% below the *SES*. Feeding below the *SES* showed a decrease in production (on average 1·37 kg standard milk or 1·23 kg fat-corrected milk for the first kg starch equiv. below the standards), a decrease in protein and the solids-not-fat % of milk and a fall in liveweight of almost 1 kg week. These data apply to the exptl. period, when the cows were in the 3rd and 4th months of lactation. J. C. T. Nieuwenhuis.

Sodium acetate and sodium propionate as additives to all-in-one silage rations for milk production. M. E. MCCULLOUGH, L. R. SISK and W. W. G. SMART (*J. Dairy Sci.*, 1969, **52** (10), 1605–1608. 9 ref.).—The feeding of 600 g of NaOAc per cow significantly increased total and 4% fat-corrected milk production and caused small increases in milk fat and total solids production of cows receiving a total ration consisting of 65% maize silage and 35% concentrate mixture. The addition of a similar quantity of Na propionate to the ration decreased milk fat and increased *SNF* but did not affect total milk production. M. O'Leary.

Accuracy of sampling procedures for estimating lactation yields. B. T. MCDANIEL (*J. Dairy Sci.*, 1969, **52** (11), 1742–1761. 60 ref.).—Methods of estimation of lactation yields are reviewed. Reports show that av. error in lactation yields is primarily a function of the length of interval between tests. At least 90% of milk yields, estimated from a single day's yield once a month, were shown to be within ± 5% of true production. Errors in milk yields estimated from bimonthly samples were shown to be ~ 30% greater than those based on monthly samples. M. O'Leary.

Complete diets given *ad libitum* to dairy cows: the effect of the level of inclusion of milled straw. J. B. OWEN and E. L. MILLER (*J. agric. Sci., Camb.*, 1969, **72** (3), 351–357. 8 ref.).—The effect of feeding dairy cows, over a whole lactation, diets containing 16, 24, 32 and 40% coarsely milled barley straw *ad lib.* was studied. Dry matter and digestible energy and intake were depressed at the higher levels of straw, and milk butterfat content was depressed at the lowest level. The net efficiency of conversion of metabolisable energy (*ME*) into milk was highest with the higher levels of straw. Complete diets for the self feeding of cows should contain ~ 24% coarsely milled straw. M. Long.

Calcium metabolism of cows at parturition and during milk production. B. F. SANSOM (*J. agric. Sci., Camb.*, 1969, **72** (3), 455–458. 11 ref.).—^{45}Ca was given orally to estimate gastro-intestinal absorption of Ca, and ^{89}Sr given i.v. to estimate endogenous excretion of Ca into the gut, the accretion rate of Ca into the skeleton, and size of the exchangeable pool of Ca. Dairy cow metabolism was studied 2 weeks before, 2 weeks after parturition and at peak lactation. Only bone accretion rate changed significantly, falling at parturition and rising after to pre-calving levels. % Ca absorption from the diet did not increase after calving but total absorption rose due to increased dietary intake. M. Long.

Changes in fatty acid patterns of milk fat resulting from pasture quality. H. VOGTMANN, A. L. PRABUCKI, R. BIEDERMANN and A. SCHÜRCH (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1968, **59** (5), 441–445. Ger., 3 ref.).—Fatty acid composition of milk fat was determined by g.l.c. and mass spectrometry. The compositions are quoted for milk produced from pastures fertilised in a number of different ways. J. B. Woof.

Effect on early lactation of feeding a milk fat-depressing ration preparation. C. K. WALKER and J. M. ELLIOT (*J. Dairy Sci.*, 1969, **52** (10), 1582–1587. 20 ref.).—The results of trials with Holstein cows showed that animals fed a milk fat (I)-depressing ration for 5–7 weeks before parturition and continued on this feed after calving, exhibited a steadily declining I content during the first 6–8 weeks of lactation. These changes were accompanied by a significant reduction in the ratio of acetate to propionate in the rumen fluid. Feed consumption was greater and I content was significantly lower in a group of cows receiving supplementary vitamin B_{12} or treated with cobalt 'bullets' in the rumen.
M. O'Leary.

Factors affecting whole- and part-lactation milk yield and fat percentage in a herd of Holstein cattle. R. H. MILLER and N. W. HOOVEN, JUN. (*J. Dairy Sci.*, 1969, **52** (10), 1588–1600. 20 ref.).—Relationships among milk and fat-corrected milk yield, fat %, wt. change, body wt. and days open were studied in 1004 lactations collected over a 14-yr period in a herd of Holstein cattle. Least square analyses of 31–60, 121–150, and of 181–210 day part lactations and total lactation were performed on an intra-sire basis. The regressions of milk and fat-corrected milk on wt. change were consistently large, negative and linear. The regressions of yield on average lactation body wt. were significant and curvilinear; production increased up to average or above-average wt., then declined. Effects of the no. of days open were small, accounting for less than 2% of the variance in all cases. Years and age were the factors most closely associated with variation in fat %. M. O'Leary.

Influence of sucrose on rumen fermentation pattern and milk fat content of cows fed a high-grain ration. D. W. KELLOGG (*J. Dairy Sci.*, 1969, **52** (10), 1601–1604. 18 ref.).—Neither milk yield nor solids-corrected milk yield was altered ($P > 0{\cdot}05$) by the addition of sucrose (at levels up to 15% of the dry matter) to high-grain rations fed to Holstein cows. Similar results were obtained for milk fat and solids-not-fat contents and production efficiency. Rumen pH was decreased and rumen lactate concn. increased by the addition of sucrose to the ration. Mean proportions of rumen volatile fatty acids were not significantly affected by the sucrose.
M. O'Leary.

Additive nature of sodium bicarbonate and magnesium oxide on milk fat concentrations of milking cows fed restricted-roughage rations. J. W. THOMAS and R. S. EMERY (*J. Dairy Sci.*, 1969, **52** (11), 1762–1769. 12 ref.).—Addn. of $NaHCO_3$ (272 or 363 g/day) and/or MgO (136 or 181 g/day) to a low-roughage high-grain ration, fed to dairy cows, resulted in an increase in fat test and rumen pH and a decrease in molar proportion of valerate (**I**) and propionate (**II**) in the rumen. These effects were additive when both supplements were fed together. $NaHCO_3$ increased daily milk and fat production, whereas MgO caused a slight decrease. Milk fat concn. was shown to be negatively related to rumen I and II proportions; I was the more important when the supplements were fed. M. O'Leary.

Effects of the addition of bentonite to high-grain dairy rations which depress milk fat percentage. R. B. RINDSIG, L. H. SCHULTZ and G. E. SHOOK (*J. Dairy Sci.*, 1969, **52** (11), 1770–1775. 19 ref.).—Addn. of 5% of bentonite (*B*) to pelleted high-grain rations, resulted in a significant increase in milk fat test and in milk production of Holstein cows. No differences were observed between 5% and 10% addn. of *B*. M. O'Leary.

Effect of abrupt ration changes on milk and blood components. L. D. SATTER and A. N. BRINGE (*J. Dairy Sci.*, 1969, **52** (11), 1776–1780. 6 ref.).—An abrupt change between a normal and a fat-depressing ration was accomplished by simultaneously switching rumen contents and rations between two cows. Feed intake was not affected; five or six days were necessary to affect complete change in the level of milk fat. Blood glucose was significantly increased and blood acetate significantly decreased within two days of changing from normal to fat-depressing ration. The extent of milk fat synthesis is considered to be controlled through slow metabolic adaptations rather than by the immediate availability of rumen acetate, propionate, or butyrate. M. O'Leary.

Chronic copper toxicity of ruminants. J. R. TODD (*Proc. Nutr. Soc.*, 1969, **28** (2), 189–198. 49 ref.).—The importance of Cu as a nutritional hazard is reviewed and is established, passive accumulation varying from a few weeks to > 1 yr. The toxic phase is acute and death may occur in a few hours, jaundice being the predominant symptom. British breeds rarely survive the crisis, but Merinos are more resistant. C. V.

Influence of time of lambing in south-eastern South Australia on the reproductive rate of the Merino ewe and on survival and growth. P. E. GEYTENBEEK (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (40), 508–512. 12 ref.).—No significant conclusions could be drawn, although winter-born lambs were heavier. Survival of progeny was not affected by time of lambing. M. T. Rawnsley.

Lactation of Persian Blackhead ewes and the growth of lambs. Effect of three different nutritional regimes during gestation on subsequent growth. M. H. BUTTERWORTH and T. W. D. BLORE (*J. agric. Sci., Camb.*, 1969, **73** (1), 133–137. 12 ref.).—42 ewes were divided between three nutritional regimes; good grazing

with concentrate (high, *H*), good grazing without supplement (medium, *M*) and poor grazing without supplement (low, *L*). Pangola grass was successively grazed by calves, *H* and *M* ewes together and finally *L* ewes. Each group grazed for one week. Analysis of the pasture changed only slightly. *H* ewes gained more wt. during the last 6 weeks of gestation than the others. Wt. losses during early lactation were similar. Birth wt. were higher at higher planes of nutrition. Differences between live wt. gains at 4 weeks were a result of plane of nutrition, maturity of dam, sex and type of birth. At 12 weeks the first factor was still apparent. M. Long.

Seasonal and age changes in the calcium, phosphorus and magnesium content of the blood of Scottish Blackface ewes, as influenced by calcium and phosphorus supplementation. R. G. Gunn (*J. agric. Sci., Camb.*, 1969, **73** (1), 159–160. 3 ref.).—Seasonal changes were greater than treatment effects, although P values were less affected than Ca by season. Ca values fell during the winter; P values also fell but rose markedly when supplementary feeding started. Mg values showed much smaller fluctuations, the greatest fall occurring during lactation periods. Ca values were highest generally in the Ca-supplemented group; the control group on pasture, however, were significantly lower. Compared with other elements Ca fell dramatically with age. M. Long.

Effects of calcium and phosphorus supplementation on performance of Scottish Blackface hill ewes, with particular reference to the premature loss of permanent incisor teeth. R. G. Gunn (*J. agric. Sci., Camb.*, 1969, **72** (3), 371–378. 19 ref.).—The effects of 12 g $CaCO_3$ or 13 g NaH_2PO_4, administered orally 3 times a week to ewes grazing reseeded pasture in April and May, were compared with those receiving no supplement and with those receiving no supplement on hill pastures throughout. Neither supplement affected lamb no. or wt. Both improved ewe live-wt. gain, the firmness and permanence of incisor teeth and serum Ca levels during lactation. The use of hill pastures during late pregnancy and early lactation without mineral supplements was undesirable, as the Ca content of the pasture was not high enough to prevent a sub-clinical Ca deficiency. M. Long.

Utilisation of perennial ryegrass and white clover by young sheep. P. V. Rattray and J. P. Joyce (*Proc. N.Z. Soc. Anim. Prod.*, 1969, **29**, 102–113. 12 ref.).—Sheep were fed with either pure or 50/50 diets of the two feeds, indoors. Wool growth was much stimulated by white clover, probably due to S-contg. amino acids. Overall white clover was better at levels higher than maintenance. Nutritive value, esp. of ryegrass, is probably better in spring than in autumn. M. T. Rawnsley.

Some effects of an increased stocking level on wool growth. R. M. W. Sumner and G. A. Wickham (*Proc. N.Z. Soc. Anim. Prod.*, 1969, **29**, 208–217. 16 ref.).—In general, more intensive stocking leads to more fibre entanglement, wool of lower tensile strength, and more depression of wool growth M. T. Rawnsley.

Effect of dietary copper on the fatty acid composition and physical properties of pig adipose tissues. J. H. Moore, W. W. Christie, R. Braude and K. G. Mitchell (*Br. J. Nutr.*, 1969, **23** (2), 281–287. 13 ref.).—Control pigs were given a basal diet and a treated group received additionally 250 ppm of Cu. Animals were 10 weeks old and weighed 90 kg (live wt.) when killed. The ratio oleic (*OA*) to stearic acids (*SA*) was higher in pigs given Cu and the m.p. of the back fat was ~ 10° lower in the Cu-treated group. Separate analysis of inner and outer layers of back fat showed the *OA* : *SA* ratio in outer layers of the control group and inner and outer layers of the Cu-treated group to be higher than in the inner fat layer of the controls. The changes were in the positional distribution of the fatty acids within the glycerides of the back fats rather than in gross fatty acid composition, and this was responsible for the observed differences in the physical properties. C. V.

Protein, lysine and feed intake level effects on pig growth. I. Main effects. R. Blair, J. B. Dent, P. R. English and J. R. Raeburn (*J. agric., Sci., Camb.*, 1969, **72** (3), 379–400. 18 ref.).—Live-wt. gain was not improved by increasing the protein level above 16, 14, 12 and 12% for the 50–100, 100–150, 150–200 and 200–250 lb live-wt. stages, resp., although gain in lean meat and efficiency of conversion of feed to lean meat were improved by increases in protein %. Live-wt. gain was likewise not improved by increasing the lysine content above 1·04, 0·74, 0·70 and 0·59% resp., for the same stages. Rate and efficiency of lean meat gain were also unaffected by increases in lysine. Live-wt. gain was improved by raising feed intake to near *ad lib.* levels. M. Long.

Comparison between barley and sorghum when combined with soyabean meal or meat and bone meal in rations for growing pigs. R. M. Beames and J. O. Sewell (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (40), 482–489. 18 ref.).—In the first expt., barley or sorghum was fed with four levels of soyabean meal. No significant differences in feed efficiency or daily wt. gain were noticed, but barley gave greater eye muscle index and appraisal score, and smaller backfat thickness. Decrease in soyabean meal level from 16·6 to 7·4% decreased feed efficiency and growth rate and also appraisal scores. In the second expt., various levels of meat and bone meal were tried. These were generally not as good as soyabean meal. M. T. Rawnsley.

Liver weight and its N and vitamin A contents in piglets from sows fed two levels of protein and food. D. L. Frape, K. L. Wolf, J. Wilkinson and L. G. Chubb (*J. agric. Sci., Camb.*, 1969, **73** (1), 33–40. 27 ref.).—Investigations, on piglets killed at birth, were carried out using sows receiving either low or high protein diet at high or low daily intake. Vitamin A was detected only in the liver and not in lung or kidney. Storage of vitamin A in the piglet was increased in the first 2 yr period by raising either total intake or protein content of the diet. Liver vitamin A and N concn. were negatively correlated with liver wt. The vitamin A level, of 4800 i.u./kg during the breeding life of the sows and 8600 i.u./day during gestation, was adequate on the basis of stored liver contents of the sows at the end of 4 yr trial. M. Long.

Dietary protein, energy, and volume in pullet grower diets as related to growing and laying performance. J. D. Wolf, E. W. Gleaves, L. V. Tonkinson, *et al.* (*Poult. Sci.*, 1969, **48** (2), 559–574. 5 ref.).—The effects of feeding 3 levels of dietary protein, 3 levels of dietary energy, and 3 levels of dietary vol. in factorial combination on period to sexual maturity, body wt. gain, egg production and wt., and feed consumption were studied. A. H. Cornfield.

Studies with maize–soya laying diets. VIII. Requirements for limiting amino acids—the basal diet and the requirements for isoleucine, lysine and tryptophan. D. J. Bray (*Poult. Sci.*, 1969, **48** (2), 674–684. 22 ref.).—The isoleucine, lysine, and tryptophan requirements of laying pullets were 0·397, 0·493 and 0·110% of the diet resp. corresponding to a daily intake of 0·472, 0·522 and 0·117 g per bird per day. These results are discussed in relation to calc. requirements based upon the needs for maintenance and egg protein synthesis. A. H. Cornfield.

Some nutritional aspects of feeding sorghum grain of high tannin content to growing chickens. J. K. Connor, I. S. Hurwood, H. W. Burton and D. E. Fuelling (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (40), 497–501. 16 ref.).—High tannin content appeared to depress feed intake and also had a toxic effect. Liver fat content was less, but this was overcome by use of methionine and choline. These results do not agree completely with previous work. M. T. Rawnsley.

Sodium requirement of the chick. H. Nott and G. F. Combs (*Poult. Sci.*, 1969, **48** (2), 660–665. 8 ref.).—The Na requirement of broilers, on a diet containing 3190 kcal metabolisable energy per kg, to 8 weeks of age was 0·11–0·13% in the diet. Addn. of Na (NaCl) up to 0·4% had no effect on wt. gains or feed efficiency, but resulted in poor litter condition. A. H. Cornfield.

Availability of sodium (to chicks) in defluorinated rock phosphate. H. Nott and G. F. Combs (*Poult. Sci.*, 1969, **48** (2), 482–485. 6 ref.).—A chick assay test showed that the Na present in defluorinated rock phosphate was 82·9% as available as the Na in NaCl. A. H. Cornfield.

Influence of dietary alterations on the utilisation of soft phosphate in broiler diets. P. W. Waldroup, W. W. Abbott, T. E. Bowen and V. E. Tollett (*Poult. Sci.*, 1969, **48** (2), 578–585. 17 ref.).—When added to an all-plant diet (0·36% total P) to supply 0·45% inorg. P, soft phosphate was as effective as $Ca(H_2PO_4)_2$ in improving wt. gains and feed efficiency from 4 to 8 weeks of age providing the Ca content of the diet was increased by 1·05%, by adding ground limestone. Although there were no differences in tibia ash and incidence of leg weakness between use of soft phosphate and $Ca(H_2PO_4)_2$, the former source produced bones of lower breaking strength and higher flexibility. A. H. Cornfield.

Phosphorus in the nutrition of the adult hen. III. Influence of phosphorus source and level on cage layer osteoporosis (cage layer fatigue). E. P. Singsen, C. Riddell, L. D. Matterson and J. J. Tlustohowicz (*Poult. Sci.*, 1969, **48** (2), 394–401. 16 ref.).—The incidence of mortality due to osteoporosis in caged layers was less when birds received 0·76% than when they received 0·34% total P in the diet. Mortality was lower on both diets when $CaHPO_4$ than when Curacao rock phosphate was the source of added P. Birds receiving the same diets on wire floors or litters showed little or no cage layer osteoporosis. A. H. Cornfield.

Manganese requirements of laying hens as related to dietary calcium. A. C. Cox and S. L. Balloun (*Poult. Sci.*, 1969, **48** (2), 745–747. 5 ref.).—The Mn requirement of White Leghorn hens for maintenance of high egg production, egg size and good shell quality was ⩾ 20 ppm in the diet irrespective of dietary Ca level (2·5–3·5%). A. H. Cornfield.

Short-term chick assay for unidentified growth factors. K. K. Bhargava and M. L. Sunde (*Poult. Sci.*, 1969, **48** (2), 694–697. 5 ref.).—A diet based on pure amino acids is described for use in a chick assay test for unidentified growth factors. Addition of 2% fish solubles or 2% maize fermentation condensed solubles to this diet resulted in significantly better wt. gains and feed efficiency of chicks to 7 days of age. A. H. Cornfield.

Effect of type of dietary carbohydrate on tissue cholesterol levels in the adult male chicken. N. J. Daghir (*Poult. Sci.*, 1969, **48** (2), 739–741. 9 ref.).—There were no significant differences in serum-, aorta- and liver-cholesterol levels of adult male chickens fed diets containing 50% glucose, sucrose, or maize starch. A. H. Cornfield.

Effect of exogenous oestrogen and/or androgen on performance, egg shell characteristics and blood plasma changes in laying hens. R. D. Brahmakshatriya, D. C. Snetsinger and P. E. Waibel (*Poult. Sci.*, 1969, **48** (2), 444–451. 27 ref.).—Addition of 66–110 ppm diethylstilboestrol (*DES*) to the diet of old hens increased egg production but had no consistent effect on egg shell quality. The treatment had no effect on egg production or egg shell quality from young hens. Methyltestosterone (*MT*) (110 ppm) decreased egg production in both young and old hens, and improved egg shell quality in young hens. *DES* with or without *MT* increased plasma triglycerides and cholesterol in old hens. A. H. Cornfield.

Effect of dietary melengestrol acetate (*MGA*) on body weight gain, percentage carcass fat and fatty acid composition of roasting chickens. N. G. Sauer, D. A. Cramer and J. V. Shutze (*Poult. Sci.*, 1969, **48** (2), 543–548. 20 ref.).—*MGA* (a synthetic progestin used as a fattening agent), added to chicken diets, caused growth depression, increased fat deposition and altered fatty acid composition toward increased saturation. Sex, level of *MGA* fed, and growth period during which the drug was supplied affected the degree of response. A. H. Cornfield.

Colour of poultry meat as influenced by dietary nitrates and nitrites. G. W. Froning, J. Daddario, T. E. Hartung, *et al.* (*Poult. Sci.*, 1969, **48** (2), 668–674. 12 ref.).—Addn. of 150–600 ppm NO_3^- and 25–50 ppm NO_2^- to the diet of chickens and 300–600 ppm NO_3^- and 100–200 ppm NO_2^- to the diet of turkeys generally altered the colour of white and dark meat before and after cooking. A. H. Cornfield.

Effect of iodinated casein and thiouracil on free amino acids in the blood plasma of chicks. Tsang-Cheng Shao and D. C. Hill (*Poult. Sci.*, 1969, **48** (2), 697–700. 5 ref.).—Addn. of 0·2% thiouracil (**I**) to a wheat–maize–soyabean meal diet decreased wt. gains and feed efficiency of chicks and plasma levels of 12 amino acids, but had no effect on plasma aspartic acid, glycine, or phenylalanine. Iodinated casein (**II**) (0·04%) added to the diet had little effect on wt. gains, feed consumption or plasma amino acid levels. When **II** was added to the diet containing 0·2% **I**, wt. gains and plasma amino acids were increased to control levels or above. 0·35% of **II** was toxic, particularly when added together with 0·2% **I**. A. H. Cornfield.

Vitamin K deficiency in chicks fed practical diets. J. C. Fritz (*Poult. Sci.*, 1969, **48** (2), 736–737. 5 ref.).—Vitamin K deficiency, as indicated by plasma prothrombin time, occurred at 14 days of age when chicks were fed from hatching with two practical-type maize meal–soyabean meal diets even in absence of vitamin K antagonists in the diet. The deficiency was prevented by addn. of 1·5% lucerne meal. A. H. Cornfield.

Incorporation of lycopene in egg yolk. D. Suarez (*Poult. Sci.*, 1969, **48** (2), 733–735. 1 ref.).—Studies of the pigmentation of egg yolk of hens receiving xanthophylls (lucerne flour) or lycopene (powdered tomatoes) indicated that lycopene is transformed by the hen to other carotenoids or xanthophylls and then incorporated into the egg yolk. A. H. Cornfield.

Depletion and repletion of body xanthophylls' reserves as related to broiler pigmentation. I. Bartov and S. Bronstein (*Poult. Sci.*, 1969, **48** (2), 495–504. 15 ref.).—Factors studied were the level of dietary xanthophylls, the duration of their administration, and the age of the chicks. A. H. Cornfield.

Analysis and Other Aspects

Non-protein nitrogen composition of grass silages. I. Estimation of basic amino acids and non-volatile amines by chromatography on a weak cation exchange resin. A. D. Hughes (*J. agric. Sci., Camb.*, 1969, **72** (3), 459–466).—The use of a strong cation exchange column to determine the compn. of grass silages led to difficulties in detn. of ethanolamine in presence of high concn. of NH_3, and of histidine in presence of δ-amino n-valeric acid. An alternative column of Amberlite CG 50, type III resin, overcame this difficulty. Histamine, putrescine and cadaverine were not eluted from the column under the same conditions as were the amino acids but bringing up the ionic strength of the buffer by addn. of NaCl to give a soln. 1·4 N with respect to Na^+ gave a satisfactory separation. M. Long.

Analysis of rumen fluid volatile fatty acids by chromatography with Porapak QS. D. W. Kellogg (*J. Dairy Sci.*, 1969, **52** (10), 1690–1692. 11 ref.).—A description is given of a g.c. method for the analysis of volatile fatty acids in rumen fluid using Porapak QS, a packing material consisting of ethylvinylbenzene–divinylbenzene polymer with silane treatment. The method eliminates solvent and acid peak tailing and the ghosting effect. M. O'Leary.

Distribution and origin of nitrogen in sheep faeces. V. C. Mason (*J. agric. Sci., Camb.*, 1969, **73** (1), 99–111. 44 ref.).—Methods for detn. of undigested dietary N, based on neutral or acid detergent or ultrasonic techniques, are described and compared. Similar estimates were obtained of the true digestibility of dietary N. Two other methods based on treatment with PhOH–HOAc–H_2O or lysozyme–trypsin were found to be unsuitable. Quant. distribution of N between undigested dietary, bacterial and endogenous debris residues, and the water-sol. fraction was chem. detd. 57–81% of the non-dietary faecal N was associated with bacterial matter. Indirect evidence suggested that bacterial N originated in the rumen. M. Long.

Absorption of ^{57}Co- and ^{60}Co-labelled vitamin B_{12} by the laying hen and mature rooster. H. M. Edwards, jun. (*Poult. Sci.*, 1969, **48** (2), 414–420. 2 ref.).—There were considerable differences among individual birds in the extent of retention of labelled vitamin B_{12} supplied orally or intramuscularly. The highest % of the vitamin supplied appeared in eggs laid 6–8 days after dosing by either method. The av. biological half-life of vitamin B_{12} in the laying hen was 14 days and in the mature rooster 35 days. A. H. Cornfield.

Effect of hypophysectomy on the radioactive phosphorus uptake of chick adrenals. D. B. King (*Poult. Sci.*, 1969, **48** (2), 459–464. 17 ref.).— A. H. Cornfield.

2.—FOODS

Cereals, Flours, Starches, Baking

Relation between chemical composition and bread-making potentialities of wheat flour. Y. Pomeranz (*Adv. Fd Res.*, 1968, **16**, 335–445. ~350 ref.).—A review, covering flour strength and baking test; proteins; carbohydrates; lipids; and enzymes. P. C. W.

Brightness determination of flour and bakery goods with the Leucometer. R. Zimmermann (*Jena Rev.*, 1969, **14** (3), 183–187. Engl., 4 ref.).—A bubble-free, homogeneous mixture of flour, breadcrumbs, etc., and water is introduced into the Leucometer and the brightness compared with reference standards. The method is useful for production and quality control extending over several flour mills and results are obtained in ~ 10 min. S. S. Chissick.

Test for irradiation of maize starch based on the use of 2-thiobarbituric acid. G. Berger and L. Saint-Lèbe (*Stärke*, 1969, **21** (8), 205–211. Fr., 16 ref.).—Starch samples were irradiated using a ^{60}Co source with various dose rates; sol. substances formed were extracted with water at room temp. The filtered soln. was reacted with an equal vol. of thiobarbituric acid in 30% $CCl_3 \cdot CO_2H$ at 100°. Absorption spectra revealed two peaks, one at 440–450 nm which depended on the purity of the sample and its previous treatment, and the other at 530 nm for which intensity was proportional to dose rate. Dried and autoclaved starches did not show this absorption max. Intensity of colour produced from irradiated samples decreased on storage at a rate depending on temp. and moisture. T.l.c. indicated that the substance responsible was malonaldehyde, produced by irradiation via the desoxyglucose intermediate. J. B. Woof.

Microscopic characterisation of heat-treated starches. A. CIMERMAN, M. BLINC and D. STUCIN (*Stärke*, 1969, **21** (8), 211–214. Ger., 10 ref.).—Maize, wheat and sorghum starches, with moisture contents of 11–20%, were heated at 100 and 120° for 3 or 5 h. Typical damage to the centre of the starch granules was detected microscopically. The birefringence characteristics were retained in each case; wheat was the most resistant to this damage. Starch η were reduced by heat treatment and solubility at 90° increased. J. B. Woof.

Synthetic glycosylglycerides in breadmaking. Y. POMERANZ and H. P. WEHRLI (*Fd Technol., Champaign*, 1969, **23** (9), 1213–1215. 11 ref.).—Effects of synthetic glycosylglycerides were compared with those of phospholipids, sucroesters and wheat flour and soyabean polar lipids. The capacity to restore breadmaking potentialities of defatted wheat flour, and to increase loaf vol. of soya-enriched bread, varied with the lipid classes. The improvement depended on carbohydrate and lipid composition (chain length and degree of fatty acid unsaturation). I. Dickinson.

Production of new baker's yeast, and its use in bakery. KONINKLIJKE NEDERLANDSCHE GIST-EN SPIRITUSFABRIEK N.V. (Br. Pat. 1,152,286, 13.11.67. Neth., 16.11.66).—A baker's yeast having a powerful and const. gas production in dough, good keeping quality, suspensibility and output is claimed, together with its production from, e.g., beet molasses, using *Saccharomyces carlsbergensis* Ng 3010 (C.B.S. 5755) and its use in bakery products. S. S. Chissick.

Sugars, Syrups, Confectionery

Reaction at limited water concentration. I. Sucrose hydrolysis. T. SCHOEBEL, S. R. TANNENBAUM and T. P. LABUZA (*J. Fd Sci.*, 1969, **34** (4), 324–329. 19 ref.).—Hydrolysis of satd. soln. of sucrose obeys kinetics the same as those predicted from dil. soln. Catalysis by inert solids is negligible. With very low concn. of adsorbed water (freeze-dried system), results showed that similar kinetics are obeyed. M. T. Rawnsley.

Enzymic and acid hydrolysis of sucrose as influenced by freezing. D. B. LUND, O. FENNEMA and W. D. POWRIE (*J. Fd Sci.*, 1969, **34** (4), 378–382. 31 ref.).—Freezing resulted in a marked decrease in invertase activity. Ice crystals were not responsible, and all the decrease was due to the concentrating effect and secondary temp. effect. Reaction mechanisms are discussed and a new method for studying reactions was developed. M. T. Rawnsley.

Food laws relating to dextrose and glucose syrup. K. A. SCHROETER (*Stärke*, 1969, **21** (8), 214–220. Ger., 49 ref.).—The existing legal situation in Germany and drafts for international legislation are surveyed. The definition of these products and their use in foods is considered. J. B. Woof.

Effect of various substances on the blooming of chocolate. J. CERBULIS (*J. Fd Technol.*, 1969, **4** (2), 133–140. 22 ref.).—Bile acids, cholesterol, other sterols and choline promoted the blooming of chocolate. Tripalmitin, added in good dispersion, made chocolate very resistant to fat bloom and the chocolate had a high gloss. Hydrogenated fats made chocolate very difficult to temper and it had a waxy taste. Only Delft 37 (specially treated vegetable fat, m.p. 37°) and some Edelfette improved the resistance of chocolate, but they made it waxy. Butylated hydroxyanisole (BHA) had undesirable effects. 15–20% of anhyd. glucose increased the resistance to blooming; other sugars were either inert or diminished the resistance. Glycerol had a strikingly unfavourable effect on both quality and resistance. Amino acids had no special influence. I. Dickinson.

Malting, Brewing and Alcoholic Beverages

Malting of water-sensitive and non-sensitive barleys using various steeping techniques. K. ZASTROW (*Mschr. Brau.*, 1969, **22** (11), 325–329. Ger., 9 ref.).—Six barleys of different varieties, crops and origins, and with differing water sensitivities, were malted by two methods; (*i*) a malting period of 9 days involving steeping under aerated water to a moisture content of 43%, and (*ii*) the moisture content was increased to 37% and a 2-h dry rest given before re-steeping to 45%. A 0·1% higher malting loss, a reduction of 0·2% in extract and a slightly lower modification were given by (*ii*). The beneficial effect of (*ii*) was not conclusively established for water-sensitive barleys. It is considered that the importance of a rapid start to germination is often overrated. J. B. Woof.

Small scale brewing trials on the effect of different decoction mashing procedures on the properties of beer. I. Examination of the barleys. II. Investigation of malt. L. NARZISS and L. HEIDEN (*Brauwissenschaft*, 1969, **22** (11), 452–464. 32 ref.; (12), 493–505. 24 ref. Ger.).—I. Winter (*WB*) and spring barleys (*SB*) grown in Irlbach between 1964 and 1967, were compared. *WB* were more dormant and appeared to be much more dependent on weather conditions; they had a higher husk content and ~15% more anthocyanogen (I). *WB* contained more water-sol. material which tended to increase acidity and buffering capacity; this tendency was enhanced in wet seasons. The I complex varied in compn. In *SB*, it was mainly in the bound form but essentially in the free state in *WB*. Enzymic activities, with the exception of α-amylase, were generally higher in *WB* but again were dependent on weather conditions.

II. *WB* differed from *SB* also in the quality of malts prepared from them. Hot water extract was 2·5% lower; this improved in wet seasons and the Lundin fractionation procedure revealed differences in compn. of the nitrogenous components. Friability was poorer and I content higher in the *WB*. Lack of gum-degrading enzymes led to higher η. J. B. Woof.

Small scale brewing trials on the effect of different decoction mashing procedures on the properties of beer. III. Modifications in the two-mash and abbreviated processes. IV. General comparison of the mashing procedures examined. L. NARZISS and H. HEISSINGER (*Brauwissenschaft*, 1969, **22** (11), 442–452. 27 ref.; (12), 482–488. 17 ref. Ger.).—III. Four further series of brews were carried out with variations in the temp. programmes. In all cases the worts were analysed for fermentability, attenuation limit, compn. of nitrogenous constituents, η, pH, chill haze, head retention and flavour; the effects of the temp. programme on each of these are discussed in detail.

IV. From the results in the previous three papers, it is considered that (*i*) the last two methods have advantages over the first in respect of clarification, head retention and flavour, (*ii*) with malt of av. modification or better, the three-mash system offers few advantages, (*iii*) increased degradation resulting from increased no. of stages is offset by the greater denaturation of the enzymes in the portion taken out for boiling, and (*iv*) the more complex systems could only be justified in special cases where the malt was deficient in enzymes. J. B. Woof.

Estimation of proteases in brewing. P. DE CEUSTER and J. STRUYVELT (*Brauwissenschaft*, 1969, **22** (12), 488–493. Ger., 13 ref.).—A modified method is described in which bactohaemoglobin was used as substrate. 0·5 g of substrate was carefully suspended in 2 g of glycerine and, after homogenisation, dil. with 20 ml of acetate buffer (pH 4·1). After incubation at 40° for 60 min in the presence of the enzyme, $CCl_3 \cdot CO_2H$ was added and the suspension filtered. Absorption of the filtrate was measured at 275 nm and activity was read from a standard curve. Relative standard deviations of 1·1–5·5% were attained. J. B. Woof.

Structure, analysis and synthesis of glycogen, glucan and mannan of brewing yeast. B. MÄNDL, F. WULLINGER, R. HENRICH and A. PIENDL (*Brauwissenschaft*, 1969, **22** (11), 433–442. Ger., 110 ref.).—A review of the occurrence and structure of the yeast polysaccharides includes a discussion of the evidence for their location and function in the cell. An extraction and fractionation scheme is described, based on differences of solubility in alkali and acid. Concn. are detd. by the phenol–H_2SO_4 method which gives the same colour yield and absorption max. for glucose and mannose. Synthetic pathways leading to these carbohydrates are discussed. J. B. Woof.

Determination of fusel oils in alcoholic beverages and spirits. M. DEVITTORI (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1968, **59** (5), 490–512. It., 4 ref.).—The Komarowsky reaction, in which fusel oils (alcohols with 3–5 C atoms) give a red colour with dimethylaminobenzaldehyde in acid soln., was examined. In order to obtain reproducible results, the strength of the acid (31 N), reaction temp. (20°) and reaction time (20 min) must be carefully controlled. The absorption max. of the product is 500 nm. J. B. Woof.

Gas chromatographic determination of fermentation products. B. MÄNDL, F. WULLINGER, W. BINDER and A. PIENDL (*Brauwissenschaft*, 1969, **22** (12), 477–482. Ger., 19 ref.).—An automatic gas chromatograph, incorporating an electropneumatic loading system, integrator and print out, was used for rapid and reproducible head-space analysis of beers. Using a Carbowax 1500 column

and flame ionisation detector, the following compd. were measured with the precision indicated: MeCHO (± 2·4), EtOAc (± 0·8), Pr^nOH (± 3·1), Bu^iOH (± 1·1), isoamyl acetate (± 2·1) and amyl alcohols (± 0·8%); on a glycerine column with *FI* detector: 2-methylbutan-1-ol (± 1·0), 3-methylbutan-1-ol (± 0·7%); and on a glycerine column with electron capture detector: diacetyl (± 1·5) and 2,3-pentanedione (± 3·1%). J. B. Woof.

Hop rate and chill stability of beer. G. W. Haase and E. Wolter (*Mschr. Brau.*, 1969, **22** (11), 309–311. Ger., 10 ref.).—Beers were brewed using different levels of hops with high and low α-acid contents so that concn. of bitter substances in the worts and beers were the same. At lower hop rates, levels of tannins (**I**) and anthocyanogens (**II**) were decreased. Levels of total, coagulable and $MgSO_4$-N were increased in most cases, whilst the chill haze was reduced. Comparison between hop extracts and natural hops indicated that the former showed less haze and had reduced levels of **I** and **II**. Protein levels were not affected as much. J. B. Woof.

Large scale tests of the effect on beer quality of the addition of calcium sulphate or calcium chloride to the mash. F.-W. Schimpf, W. Rinke and H. Janssen (*Mschr. Brau.*, 1969, **22** (11), 316–321. Ger., 8 ref.).—$CaSO_4$ and $CaCl_2$ were added at mashing-in, at levels which increased the original non-CO_3^{2-} hardness from 0·2 to 35° (Ger.). The wort pH was reduced by 0·21 and 0·34, resp., and the beers by 0·08 and 0·11. Tasting tests revealed a preference for Cl^--treated beers but no difference between SO_4^{2-}-treated ones and the controls. The increased extract yield observed (1%) was due to sol. salts and to increased breakdown and extraction of non-carbohydrate compd. at the lower pH. J. B. Woof.

Cobalt in beer and raw materials. A review. H. G. Schultze-Berndt (*Mschr. Brau.*, 1969, **22** (11), 322–324. Ger., 25 ref.).—Co addn. to beer improves foam stability and prevents gushing. The normal level in beers is ~ 0·05 mg/l which is below the detection limit of some analytical methods but addn. of Co increases the level to ~ 1 mg/l. Yeast absorbs Co rapidly during fermentation. Various spectrographic and complexometric methods for detn. of Co are reviewed. J. B. Woof.

Precipitation of flavonols in a dry red table wine. G. Ziemelis and J. Pickering (*Chemy Ind.*, 1969, (49), 1781–1782. 4 ref.).—Preparative t.l.c. and *X*-ray diffraction patterns revealed that the sediment (mainly cryst.) causing rapid clogging of the pads during filtration of the young wine consisted of quercetin (71), kaempferol (24) and myricetin (5%). These aglycones are absent in red grapes but are present in the wine because their glycosides are hydrolysed during fermentation. Previously reported concn. range from 37 to 97 mg/l, and in this instance the wine (11·8% EtOH, titratable acid 79 mequiv./l) was presumably satd. with the aglycones. The quality of the wine was unaffected. W. J. Baker.

Natural alcoholic beverages. I. Determination of the composition of natural plum brandy by means of gas chromatography. J. Đuković (*Kemija Ind.*, 1969, **18** (4), 241–245. Serbo-Cr., 15 ref.).—MeCHO, Et formate, EtOAc, MeOH, EtOH, Pr^nOH, Bu^sOH, Bu^iOH, n-amyl alcohol, iso-amyl alcohol and other amyl alcohols were found in plum brandy. The quality of the plum brandy was inversely proportional to the ratio of the contents of higher alcohols to EtOH. J. T. Greaves.

Fruits, Vegetables and Their Products

High resolution vapour analysis for fruit variety and fruit product comparisons. R. A. Flath, R. R. Forrey and R. Teranishi (*J. Fd Sci.*, 1969, **34** (4), 382–386. 13 ref.).—Large bore open-tubular g.c. columns were evaluated for fresh and canned apple products. It is suggested that they can be used for distinguishing between varieties without identification of components, for juice blending and for evaluation of aroma loss and change during processing. M. T. Rawnsley.

Removal of bitter flavour from citrus fruits, especially by enzyme methods. P. Dupaigne (*Fruits d'outre mer*, 1969, **24** (9–10), 445–450. Fr., 76 ref.).—A review of recent work, with emphasis on naringin and limonin as bitter flavour components. M. T. Rawnsley.

Method for determining gas flow characteristics in apple fruit. J. E. Hoff and H. C. Dostal (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 763–771. 4 ref.).—Porosity in 'Red Delicious' fruit increased with time of storage and decreased with increasing fruit tissue temp. About 50% of the total resistance to gas flow in the fruit is due to the peel tissue. There were differences in fruit porosity among varieties. A. H. Cornfield.

Factors influencing the ethanol content of harvested apple fruit. G. D. Blanpied, R. M. Smock and L. C. Frank (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 748–754. 9 ref.).—The extent of accumulation of EtOH in the flesh of harvested apple fruit was affected by level of atm. O_2 during storage, storage temp., date of harvesting, and variety. A. H. Cornfield.

Identification and characterisation of the pectic enzymes of the McFarlin cranberry. O. A. Arakji and H. Y. Yang (*J. Fd Sci.*, 1969, **34** (4), 340–342. 7 ref.).—Pectic enzymes were extracted by modified methods of Badran (*Nature, Lond.*, 1965, **206,** 622) and Loomis (*Phytochemistry*, 1966, **5,** 423). Endopolygalacturonase activity was found, and it was shown that this enzyme can randomly hydrolyse pectin substances with high and low methoxyl contents, with optimum pH ~ 5·0. Pectin esterase activity was low, but was optimum with 0·15M-NaCl. M. T. Rawnsley.

Quality characteristics of canned grapefruit segments. I. Factors affecting the drained weight and texture. A. Ludin, Z. Samish, A. Levi and E. Hershkowitz. **II. Additives improving their drained weight and texture.** A. Levi, Z. Samish, A. Ludin and E. Hershkowitz (*J. Fd Technol.*, 1969, **4** (2), 171–177. 7 ref.; 179–183. 6 ref.).—I. Storage of the fresh fruit for up to 9 days decreased firmness of the fresh segments and the loss in firmness increased with progressing maturation. Time and temp. of pasteurisation significantly affected the firmness of the segments as well as their tendency to break. Storage of the canned product for up to 1 yr had little effect on drained wt., whereas texture and colour of the segments were significantly affected.

II. An increase in syrup concn. lowered the drained wt. and altered the firmness of the segments. Addition of $CaCl_2$ to the syrup improved textural properties of the product. Addition of low-methoxyl pectin increased the drained wt. and improved the firmness and other textural properties without influencing the taste or the colour even after 180 days of storage. I. Dickinson.

Observations on strawberry texture. A three-pronged approach. A. S. Szczesniak and B. J. Smith (*J. Texture Stud.*, 1969, **1** (1), 65–89. 88 ref.).—The textural parameters of fresh, frozen and rehydrated freeze-dried strawberries were investigated using sensory, instrumental and microscopic methods of evaluation. There was good qual. agreement between the methods. S. S. Chissick.

Anthocyanins of strawberry, rhubarb, radish and onion. T. Fuleki (*J. Fd Sci.*, 1969, **34** (4), 365–369. 37 ref.).—Paper chromatography was used to detect the anthocyanins, with heavy grade (Whatman No. 3 MM) paper and Bu^nOH–C_6H_6–HCO_2H–H_2O (100 : 19 : 10 : 25) and Bu^nOH–HCO_2H–H_2O (100 : 25 : 60) as developing solvents. New anthocyanins found were: in strawberry, 2, rhubarb 1, radish 8, and onion 5. Tentative identifications have been made. M. T. Rawnsley.

Drying characteristics of several sultana clones. H. C. Barrett, G. H. Kerridge and A. J. Antcliff (*Fd Technol. Aust.*, 1969, **21** (10), 516–517. 4 ref.).—Ten varieties of sultana were dried in outdoor shade and changes in wt., flavour, colour, size, and rate of wt. loss with time are recorded. All samples were subjected to high r.h. when nearly dried. Changes in flavour were small but 2 varieties acquired an attractive, slightly acidulous flavour. Rate of loss of wt. was related to sol. solids content. I. Dickinson.

Some properties of mitochondria from irradiated tomato fruit. S. R. Padwal-Desai, E. M. Ahmed and R. A. Dennison (*J. Fd Sci.*, 1969, **34** (4), 332–335. 25 ref.).—Mitochondria were isolated from Homestead tomato fruit by Ku's method (*Pl. Physiol.*, 1968, **43,** 883–887). The fruit were irradiated with 300 krad of ^{60}Co γ-radiation when mature green, and at 2 and 6 days from colour break. O_2 consumption by mitochondria in succinate substrate was twice that in malate. It was greatly suppressed immediately after irradiation, but increased to a max. during the next 2 days, later declining like non-irradiated fruit. Consumption was reduced during ripening of both treated and untreated 6-day breakers. M. T. Rawnsley.

Colorimetry of foods. I. Correlation of raw, transformed and reduced data with visual rankings for spinach purée. F. M. Clydesdale and F. J. Francis (*J. Fd Sci.*, 1969, **34** (4), 349–352. 11 ref.).—Good correlations were obtained from data from a General Electric Recording Spectrophotometer, a Hunterlab Model D25

Color Difference Meter, and a Colormaster Differential Colorimeter Model V, *versus* visual rankings. Conversion of Colormaster data to Adams data gave poor correlations.
M. T. Rawnsley.

Effect of blanching on mineral and oxalate content of spinach. B. L. Bengtsson (*J. Fd Technol.*, 1969, **4** (2), 141–145. 5 ref.).—Spinach was shown to take up Ca from the water during blanching and the increase in Ca content of the spinach had a direct influence on the ratio between the sol. and insol. form of oxalate. Loss of nitrate was higher than that of the other water-sol. constituents. An attempt was made to increase the nutritional value of spinach by pptn. of all sol. oxalate by the addition of excess $CaCl_2$ to processed, chopped spinach. A taste panel rated the enriched sample as inferior owing to its colour. A small blanching trial with tap, distilled and softened water showed that the amount of Ca in tap water had no such undesirable effect on colour.
I. Dickinson.

Investigations on the lipids of potatoes. A. Fricker (*Fette Seifen AnstrMittel*, 1969, **71** (10), 889–892. Ger., 6 ref.).—The fatty acid distribution, present in the lipids of freeze-dried potatoes, was investigated by g.l.c. 90% of the 31 individual acids detected consist of linolenic, linoleic, palmitic and stearic acids. Noticeable amt. of odd-numbered acids, and of $> C_{20}$ acids were detected. It was found that potato lipids contain cephalin 28, lecithin 25, triglyceride 10–12, sterol esters 3–4, free sterols 1 and free fatty acids 2·5%, expressed as % of the total lipid content.
G. R. Whalley.

Texture studies on mushrooms. T. R. Gormley (*J. Fd Technol.*, 1969, **4** (2), 161–169. 3 ref.).—Dry matter contents and textural properties of mushrooms varied under different conditions. Shearing a given fresh wt. of sliced mushrooms gave an incomplete picture of texture and it was necessary to express texture differences on a dry wt. basis as well. In this way, one can distinguish between primary (caused by variations in dry matter content) and secondary texture differences (caused by variations in the nature of the dry matter content). Secondary differences seem to develop to the greatest extent in covered (prepacked) mushrooms, possibly due to the properties of the covering film, e.g., its ability to reduce water loss and modify the atm. in the pack. I. Dickinson.

Non-alcoholic Beverages

Potentiometric determination of fluoride in beverages by means of the ion selective solid state electrode. W. P. Ferren and N. A. Shane (*J. Fd Sci.*, 1969, **34** (4), 317–319. 9 ref.).—The method described allows 2–0·02 ppm of F^- to be detected. Commercial carbonated beverages were found to be practically free of F^-; packaged beverages made up with F^--contg. water retained the free F^-; milk and orange juice as received had $< 0{\cdot}019$ ppm of free F^-. M. T. Rawnsley.

Determination of moisture content of freeze-dried instant coffee by i.r. spectrophotometry. F. Chassevent, M. d'Ornano and G. Dalger (*Café-Cacao-Thé*, 1968, **12** (3), 250–260. Fr.).—Because of the hygroscopicity, classical oven methods cannot be used. If homogenised in MeOH, an extract is obtained after centrifuging which shows max. absorption at 1·94 μm. Background disturbance due to other substances are sufficiently weak and regular to enable the moisture content to be determined after correction.
C. V.

Milk, Butter, Other Dairy Products, Eggs

Cleaning of milk machines and milk quality. A. Twomey (*Proc. N.Z. Soc. Anim. Prod.*, 1969, **29**, 48–53).—Machines should be cleaned with an iodophor detergent before milking, then with cold water contg. non-ionic detergent, then with hot alk. detergent. This is not always done, and infection may arise, although milk grading is not always affected. Most important is the presence of dissimilar metals, esp. Cu-based alloys and stainless steel. Electrolytic cells are formed, pptg. milk solids. Butterfat is affected, and slimy deposits may be formed. Alternatives to the methylene blue reductase test for grading milk are suggested. M. T. Rawnsley.

Effect of freezing raw milk on Standard Plate Count. R. B. Read, jun., J. G. Bradshaw and D. W. Francis (*J. Dairy Sci.*, 1969, **52** (11), 1720–1723. 9 ref.).—Freezing of raw milk at temp. −20 to −196°, followed by storage at −20° for up to 28 days, resulted in a significant, though variable, reduction in the Standard Plate Count. M. O'Leary.

Effect of freezing and length of storage on milk properties. S. J. Weese, D. F. Butcher and R. O. Thomas (*J. Dairy Sci.*, 1969, **52** (11), 1724–1726. 4 ref.).—Studies with individual cow milks showed that milk fat, total solids and dye-binding estimates are significantly affected by freezing and thawing of milk. Period of frozen storage, at −20°, affected milk fat, Golding bead, freezing point, micro-Kjeldahl, lactose and dye-binding estimates. Chloride estimations were not affected by freezing of the milk.
M. O'Leary.

Influence of milk sample agitation on abnormal milk test scores. R. B. Read, jun., J. G. Bradshaw and A. R. Brazis (*J. Dairy Sci.*, 1969, **52** (10), 1682–1684. 10 ref.).—Bacteriological shaking procedures for milk had no effect on results obtained with the Wisconsin Mastitis Test and Direct Microscopic Somatic Cell Count. Precise Electronic Somatic Cell Count scores depended on the milk sample being shaken 25 times through a 30-cm arc within 7 sec. Subsequent shakings had no farther effect on the Electronic Cell Count. M. O'Leary.

Inhibition of heat-induced browning of milk by L-cysteine. R. G. Arnold (*J. Dairy Sci.*, 1969, **52** (11), 1857–1859. 6 ref.).—L-Cysteine (free base) was shown to be inhibitory to browning of milk, heated to 121°, at concn. as low as 0·01%. L-cysteine hydrochloride also inhibited browning but produced an objectionable sulphide flavour. L-cystine was not inhibitory to milk browning at concn. up to 0·04%. M. O'Leary.

Influence of mastitis on properties of milk. II. Acid production and curd firmness. O. Hampton and H. E. Randolph. **III. Lactic culture inhibitory activity and inhibition titres.** H. E. Randolph. **IV. Hydrolytic rancidity.** P. T. Tallamy and H. E. Randolph (*J. Dairy Sci.*, 1969, **52** (10), 1562–1565. 10 ref.; 1566–1568. 12 ref.; 1569–1572. 13 ref.).—II. Results showed that the firmness of curds produced by single-strain cultures of *Streptococcus lactis* C2 and *S. cremoris* R1 was > 70% lower in skim-milk with a positive Wisconsin Mastitis Test than in that with a negative test.

III. Expt. are described which showed inhibition titres of mastitis-positive skim-milks for two *Streptococcus* strains, susceptible to the natural inhibitory action of milk, to be twice that of mastitis-negative skim-milks.

IV. The acid degree values and lipase activity of mastitis-positive milk were shown to be significantly higher than those of mastitis-negative milk. M. O'Leary.

Method for determining the contribution of methyl ketones to flavours of sterile concentrated milks. C. Allen and O. W. Parks (*J. Dairy Sci.*, 1969, **52** (10), 1547–1551. 12 ref.).—A method for determining the concn. of the C_5–C_{15} odd-C-numbered methyl ketones (I) in fluid milk is described. The method is based on the free C_{13} I content and the I potential remaining in the fat phase of the product. Application of the method to commercial evaporated milk indicated that the rôle of I in off-flavours of this product is dependent on total I potential of the milk fat, composition of the I potential (especially the heptanone-2 potential), and the degree of hydrolysis and decarboxylation of β-keto acids as determined by the initial heat treatment of the product, storage temp. and storage time. M. O'Leary.

Calcium ion concentration in milk, whey and β-lactoglobulin as influenced by ionic strength, added calcium, rennet concentration and heat. B. J. Demott (*J. Dairy Sci.*, 1969, **52** (10), 1672–1675. 10 ref.).—Additions of up to 2·5 mmole of $CaCl_2$/l of milk resulted in a linear response curve with about a third of the added Ca remaining in the ionic state. The addition of rennet to milk caused the Ca^{2+} concn. to decrease until coagulation occurred, after which the concn. in the curd–whey mixture rose gradually. Heating β-lactoglobulin to 65 or 75° for 30 min had no effect on its Ca-binding capacity. M. O'Leary.

Action of rennet on casein as influenced by hydrogen peroxide–catalase treatment. R. H. Schmidt, H. A. Morris and C. V. Morr (*J. Dairy Sci.*, 1969, **52** (11), 1727–1732. 16 ref.).—H_2O_2–catalase treatment of skimmed milk (*SM*) and caseinate–ultrafiltrate (*C–U*) systems, retarded the release of glycomacropeptide from κ-casein by rennin. Treatment also reduced the rate and completeness of casein clotting by rennin in the same systems. H_2O_2–catalase treatment of *SM* reduced the methionine content of isoelectric casein by ~19% but did not affect that of rennet curd prep. from *SM* or *C–U* systems. Peroxide oxidn. of methionine is a possible cause of the retardation of the primary action of rennin on casein. M. O'Leary.

Proteose-peptone fraction of bovine milk: distribution in the protein system. C. K. KOLAR and J. R. BRUNNER (*J. Dairy Sci.*, 1969, **52** (10), 1541–1546. 12 ref.).—The proteose-peptone fraction of bovine milk was shown to consist of three heat stable components (Components 3, 5 and 8). Components 5 and 8 appeared to be distributed throughout the protein system, whereas Component 3 appeared to be restricted to the serum fraction. On starch–urea gel electrophoresis, Component 5 consisted of two principal zones, whereas Components 3 and 8 appeared as single zones. M. O'Leary.

Effect of sodium acetate on protease activity. R. M. BECHTLE (*J. Dairy Sci.*, 1969, **52** (11), 1733–1737. 11 ref.).—NaOAc stimulated the hydrolysis of milk proteins by some proteases at acid pH values. The effect was at a min. in neutral media; an inhibitory effect was observed at alk. pH values under some conditions. M. O'Leary.

Accuracy of the micromethod of estimating milk fat concentration by high-speed centriguation in capillary tubes. J. L. LINZELL and I. R. FLEET (*J. Dairy Sci.*, 1969, **52** (10), 1685–1687. 5 ref.).—The accuracy of a previously described micromethod for milk fat estimation was examined. A summary of results obtained with milk of 14 different animals is presented. Significant variations in accuracy were obtained both between different species and between individuals within a species. The method gave the best results with goat, cow, sheep and horse milk. The chief advantage of the method is its ability to deal with very small samples ($< 0{\cdot}1$ ml). M. O'Leary.

Orotic acid in yoghurt. P. OKONKWO and J. E. KINSELLA (*J. Dairy Sci.*, 1969, **52** (11), 1861–1862. 5 ref.).—A decrease in concn. (8·2 to 4·6 mg/100 ml) of orotic acid, occurring during the prepn. of commercial yoghurt, is attributed to the metabolic activity of the *Lactobacillus* spp. in the yoghurt culture. M. O'Leary.

Food texture measurements with the penetration method. J. M. DE MAN (*J. Texture Stud.*, 1969, **1** (1), 114–119. 3 ref.).—Penetration tests on cheese, butter and margarine using (*a*) punches of const. area and variable perimeter and (*b*) punches of const. perimeter and variable area, indicate that for products with a strongly bound network, e.g., processed cheese, shear and flow are the main factors which affect penetration. For products with a weakly bound network, e.g., plastic fat, flow is the only major factor involved. S. S. Chissick.

Effect of mechanical treatment on the hardness of margarine and butter. J. M. DE MAN (*J. Texture Stud.*, 1969, **1** (1), 109–113. 5 ref.).—The effect of the passage of fat products through perforated discs containing pores of various size but with equiv. areas of perforation, was investigated at 4 and at 15°. There was a partly reversible reduction of hardness which was not much affected by reduction in pore size. The final hardness of samples worked at 4° and stored at 15° was about the same as that of samples worked and stored at 15°. It is concluded that energy is wasted by mechanically treating fats at temp. lower than necessary. S. S. Chissick.

Comparison of methods to determine the free fatty acid content of butter. E. S. HUMBERT and R. C. LINDSAY (*J. Dairy Sci.*, 1969, **52** (11), 1862–1864. 4 ref.).—Extraction of fat from intact butter with Et_2O after acidification, resulted in higher measurable levels of volatile short chain fatty acids than did separation of butteroil by centrifugation of a warmed sample. M. O'Leary.

Chemistry and biochemistry of cheese ripening. J. SCHORMÜLLER (*Adv. Fd Res.*, 1968, **16**, 231–334. ~ 450 ref.).—A review, covering substances in cheese ripening and enzymic processes. P. C. W.

[A]. Effect of lactic starter culture on pink discoloration and oxidation–reduction potential in Italian cheese. E. L. SHANNON, N. F. OLSON and J. H. VON ELBE. **[B]. Rapid screening test to predict the tendency of lactic starter cultures to produce pink discoloration in Italian cheese.** E. L. SHANNON and N. F. OLSON (*J. Dairy Sci.*, 1969, **52** (10), 1557–1561. 14 ref.; 1678–1680. 5 ref.).—[A]. An evaluation of 15 cultures of high temp. streptococci and lactobacilli is described which showed that only certain strains of lactobacilli were associated with the occurrence of pink discoloration in Romano cheese. Evidence was obtained that changes in the redox potential during the early stages of cheese curing are a significant factor in the development of discoloration. It is considered that oxidative metabolism of some *Lactobacillus* strains may be important in affecting the discoloration.

[B]. A rapid screening test for the ability of lactic starter cultures to produce pink discoloration in Italian cheese is described. The test culture is incubated in a milk–$CaCO_3$ or milk–phosphate medium for 5–10 days at 37°, during which cultures associated with the pink discoloration produce a dark brown colour in the whey of the milk–$CaCO_3$ medium or a pink band in the milk–phosphate medium. M. O'Leary.

Colloidal stability of ice cream mix. P. SHERMAN (*J. Texture Stud.*, 1969, **1** (1), 43–51. 8 ref.).—The stability was studied with reference to the effects exerted by oil globule size (*OGS*), emulsifying agent, hydrocolloid stabiliser and temp. It was found that the globules coalesce in (*A*) one ($OGS > 0{\cdot}95$ μm) or (*B*) two ($OGS < 0{\cdot}95$ μm) stages. *A* is slow; *B* is initially fast and becomes the same as *A* when all the small globules have coalesced. Stability in the rapid phase of *B* is primarily due to milk protein. Glycerol monostearate exerts only a small effect and acts mainly as a synergist. S. S. Chissick.

Flavour modifications produced in ice cream mix made with corn syrup. I. Occurrence of syrup flavour, flavour masking and browning reaction. A. A. EOPECHINO and J. G. LEEDER (*Fd Technol., Champaign*, 1969, **23** (9), 1215–1220. 43 ref.).—A method of evaluating the extent of the browning reaction, based on quant. determination of 5-hydroxymethyl-2-furfural, was shown to be applicable to ice cream mix. High conversion syrup was most active in promoting the reaction in the mix. The initial pH of the mix and the time of pasteurisation, at const. holding temp., did not significantly affect the extent of browning. $K_2S_2O_5$ apparently inhibited the browning reaction while causing a significant drop in the initial mix pH. The type of syrup used and its concn. also influenced the initial mix pH. The method developed by Keeney *et al.* (*J. Dairy Sci.*, 1959, **42**, 945–960) was shown to be suitable for use with ice cream mix and results indicated the significance of the saccharide distribution in corn syrup and its relationship to mix flavour and the extent of the browning reaction. I. Dickinson.

Effect of dietary fats on some chemical and functional properties of eggs. R. D. PANKEY and W. J. STADELMAN (*J. Fd Sci.*, 1969, **34** (4), 312–317. 24 ref.).—Laying pullets were fed maize oil (**I**), soyabean oil (**II**), olive oil (**III**), hydrogenated coconut oil (**IV**) or safflower oil (**V**), as supplements to a low fat diet. Lipids were extd., lipoproteins fractionated, and fatty acids prepd., and all were analysed. Linoleic acid concn. increased and oleic acid decreased with **I**, **II** and **V**, but **III** had the reverse effect. Changes in shorter chain fatty acids occurred with **IV**. Lipoproteins varied according to the oil. Emulsification capacity of egg yolk was not affected but oil supplement gave better sponge cake volume. Flavour was not affected. M. T. Rawnsley.

Quantitative determination of formic, acetic, propionic and butyric acids in frozen whole eggs by gas–liquid chromatography. J. E. STEINHAUER and L. E. DAWSON (*J. Fd Sci.*, 1969, **34** (4), 359–364. 19 ref.).—Whole eggs contg. known amt. of the acids were evaluated by g.l.c. with flame ionisation detection and compared with results of an A.O.A.C. procedure. The methods gave recoveries of similar accuracy, and those from the g.l.c. method were less variable. Recoveries varied according to the concn. of the acids, but were generally > 96%. M. T. Rawnsley.

Edible Oils and Fats

Foreign fat determination in butter pastries by gas chromatography of fatty acid methyl esters. H. HAHN and H. KEDING (*Dt. Lebensmittl Rdsch.*, 1969, **65** (10), 320–322. Ger., 14 ref.).—Lauric, myristic, palmitic, stearic, oleic and linoleic acids in butter and in petroleum ether/diethyl ether extracts of pastries were compared by g.l.c. A ratio value of lauric/palmitic or linoleic/palmitic > 0·15, indicated adulteration with fat other than butter. J. B. Woof.

Detection of adulteration of beef fat with horse fat. H. J. LANGNER (*Fette Seifen Anstr Mittel*, 1969, **71** (10), 893–896. Ger., 13 ref.).—Beef and horse fats, and their mixtures were examined by g.l.c. 50-m Golay columns were used with both Apiezon L and butanediol succinate polyester as liquid phases. By comparing the amt. of C_{18} and higher unsatd. acids with the amt. of other fatty acids, ~ 5% horse fat adulterant was detd. G. R. Whalley.

Meat, Poultry, Fish

Meat emulsions. R. L. SAFFLE (*Adv. Fd Res.*, 1968, **16**, 105–160. 79 ref.).—A review, covering theory; model systems; factors affecting production; texture; colour; and casings. P. C. W.

Emulsifying power of animal fats in production of Frankfurter-type sausages. III. Effect of meat proteins. J. SCHUT (*Fleischwirtschaft*, 1969, **49** (1), 67–70, 73. Ger.).—It is shown that the presence of a high emulsifying, high stabilising protein (milk) is advantageous. C. V.

Determining glucose and glycogen from a single sample of meat. P. M. HEFFERAN and K. C. GOODNIGHT (*J. Fd Sci.*, 1969, **34** (4), 353–354. 4 ref.).—A non-reducing org. solvent (e.g., tetrahydrofuran) precipitates glycogen from raw meat extracts. The ppt. is hydrolysed to glucose and detd. by Cu reduction. The supernatant is deproteinised and the glucose detd. by Cu reduction without interference from the org. solvent. Temp. must be kept low (< 5°) to avoid formation of Maillard addn. products. Addn. of glucose to samples showed a recovery of 92·6–100% by the new method. M. T. Rawnsley.

Volatile components of irradiated beef and pork fats. J. R. CHAMPAGNE and W. W. NAWAR (*J. Fd Sci.*, 1969, **34** (4), 335–339. 26 ref.).—A large no. of hydrocarbons (n-alkanes, 1-alkenes and internally unsatd. alkenes and alkadienes) were found, which were not present before irradiation and of which the last two types have not been reported previously. Hexadecanal, octadecanal, and octadecenal were also detected. There is a relationship between the volatile pattern and the fatty acid compn. Flavour thresholds were tested, and those of the unsatd. hydrocarbons were much lower than those of the n-alkanes. M. T. Rawnsley.

Relationship between the subjective and objective measurement of pork colour. R. J. ELLIOTT (*J. Fd Technol.*, 1969, **4** (2), 147–156. 39 ref.).—Results obtained by use of a spectrophotometer and a simple colour reflectometer and by a panel of ten untrained graders were compared with the Munsell V value of the samples, calculated from the reflection spectra. Visual colour grading produced an almost linear correlation with the Munsell V value. Dark samples tended to be underestimated and pale samples overestimated by the panel. There was considerable variation between visual and instrumental grading. Results obtained from the colour meter were reproducible, and the error in comparison with the spectrophotometer was small. The colour meter provides a convenient portable and inexpensive method of measuring the colour lightness of pork muscle. The determination of colour quality requires both subjective and objective measurements. I. Dickinson.

Connective tissues from normal and pale, soft and exudative (*PSE*) porcine muscles. II. Physical characterisation. P. E. MCCLAIN and A. M. PEARSON (*J. Fd Sci.*, 1969, **34** (4), 306–308. 10 ref.).—Shrinkage temp., determination, d.t.a. and stress–strain and swelling measurements were carried out. Results showed that connective tissue from *PSE* pigs was altered compared with normal pigs, and the epimysium from *PSE* muscles had a lower or changed ground substance content; the collaginous proteins were less mature. Epimysium from *PSE* tissues had lower onset and recovery temp. and contained a higher % of components melting at lower temp. than that from normal tissues. M. T. Rawnsley.

Turbidity, viscosity and ATPase activity of fibrillar protein extracts of rabbit muscle. P. D. WEINER, A. M. PEARSON and B. S. SCHWEIGERT (*J. Fd Sci.*, 1969, **34** (4), 303–305. 20 ref.).—Actomyosin from muscle in rigor was more easily dissociated than that from pre-rigor muscles. Addn. of $MgCl_2$ decreased the rate of ATP hydrolysis, as did pyrophosphate in the presence of Mg. Hydrolysis of ATP caused increased viscosity of extracts, but samples cleared with pyrophosphate retained their low viscosity characteristics. M. T. Rawnsley.

Influence of tension on pre-rigor excised bovine muscle. W. A. GILLIS and R. L. HENDRICKSON (*J. Fd Sci.*, 1969, **34** (4), 375–377. 18 ref.).—The effects of 0, 1000, 2500 and 5000 g pull treatments on the fibre dia., sarcomere length, kinkiness and shear value of semitendinosus and semimembranosus muscle were assessed. The relationship of these effects to tenderness is discussed. M. T. Rawnsley.

Free amino acids in bovine muscles and their relationship to tenderness. R. A. FIELD and Y. CHANG (*J. Fd Sci.*, 1969, **34** (4), 329–331. 22 ref.).—The muscles used were *longissimus dorsi* and *biceps femoris*, from cattle of various age, sex and breeding. Results showed that greater quantities of free amino acids are associated with more tender meat, but variation in type of beast and amount of free amino acids showed that simple correlations cannot be found. M. T. Rawnsley.

Effect of automobile exhaust fume inhalation by poultry, immediately prior to slaughter, on colour of meat. G. W. FRONING, F. B. MATHER, J. DADDARIO and T. E. HARTUNG (*Poult. Sci.*, 1969, **48** (2), 485–487. 8 ref.).—The cooked, dark meat of chickens and turkeys which had been exposed to automobile exhaust fumes just before slaughter showed an extremely reddened condition and appeared to be raw and unappetising. The condition was characteristic of some colour defects reported by poultry processers. A. H. Cornfield.

Composition of fish. J. MURRAY and J. R. BURT (*Torry advis. Note*, 1969, No. 38, 16 pp., Engl.).—Tabular data are presented on simplified chem. compn. (water, fat and protein) of commercially important fish landed or imported into Britain, chem compn. of fish products, mineral constituents of fish muscle and vitamins A, B, and D in fish. E. G. Brickell.

Fish protein concentrate. M. L. WINDSOR (*Torry advis. Note*, 1969, No. 39, 10 pp., Engl.).—Three types of concentrate are described together with methods of prepn. and some of the technical problems. E. G. Brickell.

Quality in frozen cod and limiting factors on its shelf life. T. R. KELLY (*J. Fd Technol.*, 1969, **4** (2), 95–103. 11 ref.).—Organoleptic assessments of toughness, dryness and overall texture and flavour acceptability were made on cod fillets stored at −7°. Inter-relationships between these parameters, muscle pH and time were derived, which showed that in high pH cod, flavour development during cold storage limits the shelf life, while low pH fish become tough before the flavour becomes acceptable. A 'phase diagram' is presented which specifies the limiting storage times for fish of different pH on the basis of both flavour and texture. I. Dickinson.

Reaction of free fatty acids with protein in cod muscle frozen and stored at −29°C after ageing in ice. M. L. ANDERSON and E. M. RAVESI (*J. Fish Res. Bd Can.*, 1969, **26** (10), 2727–2736. Engl., 23 ref.).—Freezing and holding cod muscle in the frozen state, favoured the association process that involves protein-free fatty acid complex formation and begins during ageing in ice. Results were consistent with a reaction rate that was greater at −29° than at temp. a few degrees above zero. E. G. Brickell.

Effect of EDTA treatment on spoilage characteristics of petrale sole and ocean perch fillets. G. A. PELROY and J. P. SEMAN, JUN. (*J. Fish. Res. Bd Can.*, 1969, **26** (10), 2651–2657. Engl., 11 ref.).—Ethylenediaminetetra-acetic acid (EDTA), used in 1% soln. as a dip, extended the shelf life of petrale sole and ocean perch fillets by repressing growth of *Pseudomonas* organisms and formation of trimethylamine. Na_2EDTA and Na_4EDTA were superior to $Na_2CaEDTA$ and vac. packing enhanced the effectiveness. E. G. Brickell.

Food Additives

Preservatives, Colouring Matter

No abstracts

Spices, Flavours, Other Additives

Caking of onion powder. Y. PELEG and C. H. MANNHEIM (*J. Fd Technol.*, 1969, **4** (2), 157–160. 7 ref.).—Caking of onion powder at the 4–5% moisture level was greatly affected by storage temp. when stored without adjuncts. Caking was strongly accelerated by higher storage temp. The effects of 15 different anti-caking agents on the occurrence of caking at 35° were tested, and only Ca and Mg stearate, Al silicate, Santocel 62 and Cab-O-Sil were effective. The addition of Al silicate had a beneficial effect on the colour of the powder. When onion powder contained 7% moisture, there was no advantage in the addition of anti-caking agents. Caking occurred after 72 h at 35°. Powder, dried to 3% moisture, remained free-flowing even after 30 days at 35° without anti-caking agents. I. Dickinson.

Detection of emulsifiers in foodstuffs. IX. E. KRÖLLER (*Fette Seifen AnstrMittel*, 1969, **71** (10), 896–898. Ger., 1 ref.).—A method for the isolation of Ca stearyl lactate (I) from foodstuffs is described. It involves preliminary extraction with MeOH, pptn. with Pb acetate, removal of Pb by boiling with $2N-H_2SO_4$, followed by paper chromatog. Characteristic colour reactions of I are listed; various chromatog. separation procedures are discussed. G. R. Whalley.

Food Processing, Refrigeration, Packaging and Storage

Heat exchange in the food industry. R. FULLER (*Fd. Technol. Aust.*, 1969, **21** (9), 448–453).—A range of equipment for the heating and cooling of liquid and semi-liquid food is discussed. Single-phase heat transfer, plate, tubular and scraped-surface heat exchangers, vacuum pans and film, centrifugal and wiped film evaporators are described. I. Dickinson.

Some methods of preparation of high value foodstuffs on drum dryers. M. PREGER (*Industrie chim. belge*, 1969, **34** (11), 965–968. Fr.).—Processes and plants for the drum drying of foodstuffs, especially sweet and acid milk serum, cereal starches and dry yeasts rich in vitamins, are briefly described, and diagrams of the process equipment are given. M. Sulzbacher.

Solvent drying: a new food preserving method. ANON. (*Chem. Engng*, 1968, **75** (4), 60–62).—EtOAc (**I**) is the solvent, a min. boiling azeotrope with the water in the food being formed. **I** has been cleared by the Food and Drug Administration for use with foods, and following solvent removal, **I** is present at permissible levels. The advantages of the method over freezing for food preservation are discussed. Rehydration occurs in 15 min, well maintained colour, shape, texture and flavour being found. When colour and flavour are solvent-extractable, these can be recovered. C. V.

Comparisons of short-time holding procedures to determine thermal resistance of *Staphylococcus aureus*. E. A. ZOTTOLA and J. J. JEZESKI (*J. Dairy Sci.*, 1969, **52** (11), 1855–1857. 1 ref.).—The submerged sealed tube technique, either with thermal death time tubes or capillary tubes, and the tubular high-temp. short-time pasteuriser were shown to give comparable F and z_F values for two strains of *Staphylococcus aureus*. M. O'Leary.

Effect of short-time sub-pasteurisation treatments on the destruction of *Staphylococcus aureus* in milk for cheese manufacture. E. A. ZOTTOLA, J. J. JEZESKI and A. N. AL-DULAIMI (*J. Dairy Sci.*, 1969, **52** (11), 1707–1714. 8 ref.).—Heat resistance of 236 *S. aureus* isolates, obtained in a cheese plant, was detd. High-temp. short-time (*HTST*) heat treatment of milk at 147 to 152°F, for a 21 sec total equiv. exposure time, reduced the no. of staphylococci in raw milk to such an extent that detection of *S. aureus* in subsequent cheese was at a min. M. O'Leary.

Respiration, internal atmosphere, and ethylene evolution of citrus fruit. H. M. VINES, W. GRIERSON and G. J. EDWARDS (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 227–234. 19 ref.).—The effects of thickness of waxing, freezing for various periods, and mechanical damage on respiration, internal CO_2 and O_2 contents and evolution of C_2H_4 in 4 citrus species are reported. A. H. Cornfield.

Processing and preserving horseradish. I. Frozen and refrigerated storage stability. II. Use of blanched root. F. E. WEBER, A. I. NELSON, M. P. STEINBERG and L. S. WEI (*Fd Technol., Champaign*, 1969, **23** (9), 1207–1210. 13 ref.; 1211–1212. 8 ref.).—When stored for 6 months at 0°F, prepared horseradish only lost a slight amount of pungency, but it darkened noticeably. Frozen storage did not affect the rate of pungency and colour changes during subsequent holding for 8 weeks at 45°F, but pungency loss and darkening were much greater during the short time at 45°F than during the longer time at 0°F. Addition of dairy cream improved pungency retention during frozen storage. Propylene glycol alginate and gum tragacanth improved pungency retention and reduced syneresis on storage at 45°F. Prepared horseradish made with 98% blanched and 2% raw root had very high pungency which was retained during storage at 45°F for 8 weeks. Use of blanched root made the prepared product appear translucent and less white, but discoloration which develops with 100% fresh root was inhibited. Addition of sulphite decreased pungency retention but improved the colour. I. Dickinson.

Progress of food irradiation work. ANON. (*Fd Irrad.*, 1968–9, **9** (3), 2–30; (4), 2–10).—A review. C. V.

Effect of radiation on ethylene production of bananas. I. KHAN and A. MUHAMMED (*Fd Irrad.*, 1968–9, **9** (4), 34–40. 24 ref.).—Irradiation of 'Basri' bananas with low doses of γ-radiation (15–35 kr) showed little effect on ethylene production (*EP*) and there was no delay in fruit ripening. *EP* was stimulated at 50–100 kr dosages and ripening was hastened. The results showed that this technique cannot be used to extend shelf-life. C. V.

Effect of gamma irradiation on ripening of banana fruit. E. C. MAXIE, R. AMEZQUITA, B. M. HASSAN and C. F. JOHNSON (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 235–254. 19 ref.).—Gamma irradiation (35–50 krad) inhibited the ripening of pre-climacteric bananas without harming fruit quality. The max. tolerable dose of γ-rays for banana fruit was approx. doubled 1 day after onset of the respiratory climacteric. Mechanical injury stimulated ripening, but the stimulus was partially overcome by irradiation with 35–50 krad. A. H. Cornfield.

Blast freezing and cold storage of bakery products. ANON. (*Mod. Refrig. Air Condit.*, 1968, **71** (845), 55–58, 61).—The freezer can cool 1 ton bread from 27 to −21° in 1 h. In the batch-type recovery room, the bread is dry-air heated (38–71°) for ~ 10 min; steam is then injected to bring the r.h. to 50–70%, this being followed by dry heating for 10 min. C. V.

Liquid nitrogen freezing of soft fruit. H. DAVIDGE (*Aust. Fd Mf.*, 1968, **37** (7), 34–36).—Immersion of strawberries, raspberries, peaches and orange segments for 30 sec in liquid N_2 was carried out. On thawing, the flavour is claimed to be similar to that of the fresh fruit. C. V.

Refrigeration in the meat industry. G. B. MORGAN (*Aust. Refrig. Air Condit. Heat.*, 1968, **22** (3/4), 23–26, 27–29).—Boning of meat must take place at < −10°. NH_3 is not the ideal refrigerant because of leaks, and the gradual change-over to Freons is stressed. There is no significant difference in drip or thawing between meat frozen for 24 or 48 h, the choice being dependent on economics and space. Blast and plate freezing are compared. C. V.

Current trends in fish handling and processing. Aspects of fish freezing in relation to processing. G. S. SIDHU and W. A. MONTGOMERY (*Fd Technol. Aust.*, 1969, **21** (10), 510–515, 517. 34 ref.).—Handling before freezing, effect of freezing and the stage of rigor on quality, effect of freezing and pH on texture, freezing methods at sea, super-chilling vs. storage in ice, thawing of frozen fish, handling and processing on shore, polyphosphates and processing of frozen fish, and frozen storage temp. are discussed. I. Dickinson.

Effect of low temperature freezing on quality changes in cold stored cod. T. R. KELLY and J. S. DUNNETT (*J. Fd Technol.*, 1969, **4** (2), 105–115. 13 ref.).—Cod fillets were frozen between −7 and −195° and stored at −7° to establish whether the freezing temp. affects the fillets when they are stored at the same temp. Objective and organoleptic tests showed that quality changes occurred at a rate which was independent of freezing temp. I. Dickinson.

Canning fruit and vegetable products in aluminium containers. A. LOPEZ and M. A. JIMENEZ (*Fd Technol., Champaign*, 1969, **23** (9), 1200–1206. 16 ref.).—Six different enamel systems and uncoated Al cans were tested, in addition to tin-plate control cans. The canned products were stored at 75 and 100°F for 2 yr and examined periodically. Tin-plate cans performed better with acid foods than the coated Al cans. With low acid foods, coated Al and tin-plate containers were about equal in performance. Uncoated Al cans were rapidly corroded by most of the foods tested. Low-acid foods such as peas and maize caused the least corrosion. Corrosion was not a problem with coated Al cans, but some coatings imparted objectionable taste or caused discoloration of foods during storage. I. Dickinson.

Changes during storage of foods rich in fat. A. FRICKER (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1968, **59** (5), 428–440. Ger., 23 ref.).—A review dealing with oxidation and hydrolysis reactions undergone by simple and complex lipids and their deriv. The production of off-flavours in vegetables when stored at different temp. and under inert gas is considered. J. B. Woof.

Observations on prolonged grain storage with forced aeration in Israel. S. NAVARRO, E. DONAHAYE and M. CALDERON (*J. stored Prod. Res.*, 1969, **5** (1), 73–81. 10 ref.).—Over a 2-yr period it was found that aeration was effective in cooling grain and maintaining temp. Grain storage over long periods in sub-tropical climates with a cool season is thus a practicable proposition. C. V.

Storage of coffee in producing countries. E. M. LAVABRE and B. DECAZY (*Café-Cacao-Thé*, 1968, **12** (4), 321–342. Fr., 31 ref.).—*Avaecerus fasciculatus* is probably the most destructive predator and the susceptibility of Arabica (**I**) and Robusta (**II**) were compared at 80% r.h. and 25°. After the 3rd month of storage, **I** was 80% infected while **II** was unaffected during the first nine months. To prevent attack, clean storage areas and well dried and cleaned coffee are necessary and r.h. must be < 80%. C. V.

Nutrition, Proteins, Amino Acids, Vitamins

Aspects of allergy to food proteins. G. J. H. MELROSE (*Fd Technol. Aust.*, 1969, **21** (8), 398–401. 19 ref.).—Symptoms and causes of food allergy are discussed. Use of bradykinin and alanine-bradykinin and of ^{32}P-phosphanilic acid to study the inflammatory responses is described. I. Dickinson.

Processed vegetable protein mixtures for human consumption in developing countries. R. BRESSANI and L. G. ELIAS (*Adv. Fd Res.*, 1968, **16**, 1–103. ~500 ref.).—A review, covering vegetable protein sources; factors affecting protein value; improvement of nutritive value; and utilisation of vegetable proteins. P. C. W.

Nutritive value of 1,2-dichloroethane-extracted fish protein concentrate. B. H. ERSHOFF and P. G. RUCKER (*J. Fd Sci.*, 1969, **34** (4), 355–359. 5 ref.).—The nutritive value and also the toxicity of these concentrates depends on the time and temp. of extraction. Under optimum conditions, these concentrates are suitable protein nutrients. Conditions for removal of toxicity are discussed. M. T. Rawnsley.

Unclassified, Tobacco

The aromagram: image of aroma. J. HULSTKAMP (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1968, **59** (5), 471–481. Fr., 1 ref.).—In order for g.l.c. peaks to represent the aroma of the sample, all the aromatic components must be resolved and detected and no peak must result from a substance without aroma. In practice, this is difficult to achieve. However, substances without odour because of their low volatility can be eliminated by restricting the column temp. It was shown that substances with a very high level of detection are the most sol. in water so that they can be eliminated by extraction from water with pentane before fractionation. J. B. Woof.

Mechanical properties, rheology and heptaesthesis of food. H. G. MULLER (*J. Texture Stud.*, 1969, **1** (1), 38–42, 13 ref.).—A philosophical analysis of the terms rheology (*R*) and heptaesthesis (*H*) leads to the conclusion that *R* is the science of deformation of matter and is a branch of physics, while *H* is the perception of the mechanical behaviour of materials and is a branch of psychology. S. S. Chissick.

Objective measurements for texture in foods. E. E. FINNEY, JUN. (*J. Texture Stud.*, 1969, **1** (1), 19–37. 79 ref.).—The concepts of rheology important in understanding the mechanical behaviour of food materials are reviewed and certain experimental techniques (e.g., food crushing sounds, optical *d*, vibrational techniques) and instruments (e.g., shear devices, farinograph, texturometer) are discussed under the headings meat, dairy products, bakery products and fruits and vegetables. S. S. Chissick.

Sensory evaluation of food firmness. A. S. SZCZESNIAK and M. C. BOURNE (*J. Texture Stud.*, 1969, **1** (1), 52–64. 49 ref.).—Nine different pairs of foods (milk puddings, bread, carrots, etc.) were presented to 131 different people, who were asked to determine by non-oral methods which sample in each pair was the most firm. The results indicated that, from a sensory point of view, firmness of a food can be its consistency, deformability, puncturability or flexibility, and these criteria can be used in this order as the degree of firmness increases from a low (whipped toppings) to a very high (carrots) level. S. S. Chissick.

Food quality as determined by metabolic byproducts of microorganisms. M. L. Fields, B. S. RICHMOND and R. E. BALDWIN (*Adv. Fd Res.*, 1968, **16**, 161–229. ~200 ref.).—A review, covering definitions and criteria for chem. indicators; and chem. indicators of quality for foods with high protein, high fat or high carbohydrate content. P. C. W.

Enzyme-catalysed reactions as influenced by inert gases at high pressures. J. R. BEHNKE, O. FENNEMA and W. D. POWRIE (*J. Fd Sci.*, 1969, **34** (4), 370–375. 18 ref.).—Tyrosinase, invertase, trypsin and chymotrypsin were exposed to N_2 or Ar at 5000 psig or N_2O at 600 psig in fluid and non-fluid systems. The results indicated that the use of inert gases under pressure to inhibit enzyme activity does not have many possibilities. M. T. Rawnsley.

Applications of enzymes in the food industry. E. GRAMPP (*Dt. LebensmittRdsch.*, 1969, **65** (11), 343–347. Ger., 8 ref.).—The review deals with the use and effects of pectinase, cellulase and amylases in the treatment of fruits and vegetables. J. B. Woof.

A rapid selection method for pectolytic enzyme generators. N. M. VERBINA and O. I. TSŸTSURA (*Mikrobiologiya*, 1969, **38** (2), 372–375. Russ., 6 ref.).—A rapid method is described for selecting fungal generators of pectolytic enzymes. The method is based upon the colour change of methyl red, induced by galacturonic acid (**I**) in the medium; **I** is produced by the action of pectolytic enzymes upon pectin. Results agree well with chem. methods used for aq. extracts of fungi. 22 strains of fungi were examined, the most active being *Aspergillus niger* P, mutant *A. niger 57* and *Botrytis cinerea*. L. A. Haddock.

Fat destabilisation in frozen desserts containing low dextrose equivalent maize [corn] sweeteners. S. R. MAHDI and R. L. BRADLEY, JUN. (*J. Dairy Sci.*, 1969, **52** (11), 1738–1741. 17 ref.).—It was shown that α_s- and β-casein complexed with low dextrose-equiv. maize sweeteners and fluidity starch in frozen desserts mixes during pasteurisation. With subsequent freezing the complex was stripped from the fat globule surfaces, resulting in aggregation. Frozen desserts contg. 10-dextrose-equiv. maize sweetener, made from milo, did not have this defect. This suggests that the amylose fraction of maize sweeteners is involved in the complex with α_s- and β-casein. M. O'Leary.

Metal chelates of L-ascorbic acid. K. PFEILSTICKER (*Dt. LebensmittRdsch.*, 1969, **65** (11), 348–352. Ger., 30 ref.).—U.v. and i.r. spectral data are quoted for some 1 : 1 metal complexes with stability const. of log $k_1 = 3$–10. Their rôle in affecting rates of metal-catalysed oxidn. in foods is discussed. Complexing with L-ascorbic acid is comparable to complexing with citric acid. J. B. Woof.

Food plants as a possible factor in fertility control. A. SHARAF (*Qualitas Pl. Mater. veg.*, 1969, **17** (2), 153–159. Engl., 24 ref.).—Oestrogenic activity of *Glycyrrhiza glabra* extract, *Pimponella anisum* oil, pomegranate seed oil, *Trifolium alexandrinum* and palm kernel extracts was studied and compared with that of oestradiol. The relationship between the natural hormones and phyto-oestrogens was demonstrated. C. V.

Palm kernels as a growth promoting substance. A. SHARAF (*Qualitas Pl. Mater. veg.*, 1969, **17** (2), 148–152. Engl., 11 ref.).—Palm kernels possess antispasmodic, hypotensive, oestrogenic and growth promoting properties. When powdered and fed to chickens (10 g per day for 3 weeks), they give a significant increase in growth rate. C. V.

3.—PEST AND DISEASE CONTROL, SANITATION

Plant Diseases, Pests and Weeds

Pesticides. R. A. E. GALLEY and T. A. MELROSE (*Inst. Petrol. Review*, 1969, **23** (275), 291–295.).—Current adverse criticism of the use of pesticides is discussed. Their function in combating food shortage and their applications in the fields of agriculture, public health and industry are reviewed together with the development of biol. control of pests. F. C. Sutton.

Quality of clay materials used in pesticidal preparations. M. KOTAKEMORI (*Bentonite, Kyoto*, 1968, (9), 11–18. 41 ref.).—The many required characteristics of the clays are examined with special reference to the degradation rates of organophosphates. With Meobal (3,4-dimethylphenyl-*N*-methyl-carbamate) dusts, pH and Fe were the main cause of degradation. The rôle of carriers in the production of granular and wettable powder is also studied and it is stressed that the influences of these additions on the degree of pest control is not made sufficiently clear. (From Engl. summ.) C. V.

Fluorescent particle spray droplet tracer method. C. M. HIMEL (*J. econ. Ent.*, 1969, **62** (4), 912–916. 5 ref.).—A known number of micron-sized, insol. fluorescent Zn-Cd sulphide particles are suspended in a known vol. of a liquid, non-volatile carrier (dioctylphthalate). The spray droplets formed contain a predictable number of fluorescent particles according to the dia. of the droplets at the moment of formation. Droplet size counting is undertaken in u.v. light on the actual insects and foliage treated. The method can also be used to mark arthropods for other studies. C. M. Hardwick.

Herbicides

Weed problems in developing countries. L. HOLM (*Weed Sci.*, 1969, **17** (1), 113–118).—An address. A. H. Cornfield.

Infra-red studies of the mechanism of adsorption of [labelled] urea, methylurea and 1,1-dimethylurea by montmorillonite. W. J. FARMER and J. L. AHLRICHS (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 254–258. 7 ref.).—I.r. studies of the reaction of the ureas with Ca-, Ni-, and Al-montmorillonite indicated a carbonyl co-ordination to the exchangeable cation in all systems. The NH_2 group appeared relatively inactive. The results related to the adsorption of herbicidal ureas by soil. A. H. Cornfield.

Influence of herbicides on photosynthetic activity and transpiration rate of intact plants. J. L. P. VAN OORSCHOT (*Pestic. Sci.*, 1970, **1** (1), 33–37. 24 ref.).—The effect of various herbicides on photosynthesis, respiration and transpiration of intact plants was studied. The herbicide was applied during measurement, so that the effect could be related to the photosynthetic activity of the same plants before treatment. The selectivity of various herbicides was studied by determining the capacity of a plant species to inactivate a herbicide absorbed by the roots. These and other differential effects of various herbicides on photosynthetic activity of different plant species coincided with the selective properties in the field. Such differences were also observed after leaf sprayings. Bean plants were studied at various r.h., light intensities and temp., resulting in different transpiration rates. The decrease in photosynthetic activity due to the presence of a herbicide in the nutrient soln. at a standard concn was more rapid at the higher transpiration rates. The total transpiration during treatment up to 50% inhibition of photosynthesis was constant under the various exptl. conditions. P. C. W.

Effect of light environment on the activity and behaviour of diquat and paraquat in plants. R. C. BRIAN (*Pestic. Sci.*, 1970, **1** (1), 38–41. 5 ref.).—The importance of controlling the light environment of expt. involving diquat and paraquat was shown in expt. where activity was markedly dependent on the light quality and intensity before treatment and on the time of day the treatment was carried out. Activity and uptake were not directly related since treatments after low light intensities gave increased activity associated with reduced uptake; following afternoon treatments, reduced activity was associated with increased uptake. Uptake increased when plants were darkened after treatment but the increase was not directly related to its duration, because after a time, uptake decreased. Three possible explanations for this decrease are considered: diquat exudation from the leaves, downward movement into the roots, and adsorption of diquat in plant tissue. Evidence did not support exudation from leaves or downward movement into the roots. P. C. W.

Influence of light on paraquat activity in the tropics. D. W. R. HEADFORD (*Pestic. Sci.*, 1970, **1** (1), 41–42. 7 ref.).—The effect of light on the herbicidal activity of paraquat against the tropical perennial weed *Paspalum conjugatum* was studied in rubber and oil palm plantations in Malaya. Results showed that paraquat (at 0·5 lb/acre) gave a more rapid desiccation when applied in full sunlight but gave more persistent control when applied under a 70% shade of older trees or in the evening compared with other times of day. A similar two-fold improvement in the duration of weed control was achieved by the addition of the photosynthetic inhibitor bromacil (at 0·25 lb/acre) to paraquat. The response to the mixture suggested a synergistic reaction between the two compd. P. C. W.

Herbicide activity involving light. J. CASELEY (*Pestic. Sci.*, 1970, **1** (1), 28–32. 44 ref.).—The influence of light on the activity of herbicides after they have entered the plant and have been translocated to their site of action within the cell is surveyed. Herbicides that are affected by light are mainly those that exert part of their phytotoxicity by inhibiting photosynthesis. Aspects covered include micro-organism and isolated chloroplast studies, light-induced damage not associated with lack of photosynthates, action of bipyridylium herbicides (diquat, paraquat), and herbicides with activity reduced by light (dinitro compd.). P. C. W.

Influence of soil properties on phytotoxicity of atrazine, ametryne, prometryne and diuron in Puerto Rican soils. L. C. LIU and H. CIBES VIADÉ (*J. Agric. Univ. P. Rico*, 1968, **52** (4), 269–280. 19 ref.).—The ED_{50} values for oats (dosage required to decrease fresh wt. of oats by 50%) varied with nature of herbicide and soil type (13 soils tested). Phytotoxicity decreased in the order atrazine, ametryne, diuron, prometryne. Soil org. matter content (*OMC*) was the main variable affecting the degree of phytotoxicity, which decreased with increasing *OMC*. A. H. Cornfield.

Importance of root, shoot, and seed exposure on the herbicidal activity of EPTC. R. A. GRAY and A. J. WEIERICH (*Weed Sci.*, 1969, **17** (2), 223–229. 7 ref.).—The comparative effects of root, shoot, and seed treatment of several crop and weed species on the herbicidal effect of EPTC are reported. Seed treatment caused severe damage to some species, thus differing from previous reports that only shoot exposure to EPTC leads to damage. A. H. Cornfield.

Phytotoxicity and movement of amiben derivatives in soil. J. J. LINSCOTT, O. C. BURNSIDE and T. L. LAVY (*Weed Sci.*, 1969, **17** (2), 170–174. 5 ref.).—When applied pre-emergence to maize in field tests the NH_4 salt of amiben (chloramben) (**I**) (2–4 lb per acre) and the Me and butoxyethyl esters (4 lb) gave the best weed control, whilst the amide was ineffective. NH_4-**I** caused early stunting of maize, but did not reduce yields. Where excessive rainfall occurred after application of **I**, the best deriv. for weed control in soyabean were the amide and the esters. A. H. Cornfield.

Metabolism of trifluralin in groundnuts and sweetpotatoes. P. K. BISWAS and W. HAMILTON, JUN. (*Weed Sci.*, 1969, **17** (2), 206–211. 22 ref.).—Several degradation products of trifluralin (**I**) were detected in groundnut and sweetpotato plants; all those isolated inhibited root growth of cucumbers. A. H. Cornfield.

Basis for selectivity of linuron on carrot and common ragweed. H. KURATLE, E. M. RAHN and C. W. WOODMANSEE (*Weed Sci.*, 1969, **17** (2), 216–219. 5 ref.).—When carrot plants, tolerant to linuron (*L*) and common ragweed plants (susceptible) were treated with *L*, 4 deriv. were detected in both species; two of these deriv. were toxic to ragweed, but none was toxic to carrot. Of the applied *L*, 87% was metabolised to non-phytotoxic deriv. in carrot and 13% in ragweed. A. H. Cornfield.

Weed control in carrots with linuron and prometryne. H. KURATLE and E. M. RAHN (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 465–472. 2 ref.).—Post-emergence application of linuron (*L*) and prometryne (*P*), when the carrot plants had 1–2 true leaves, was the most effective. The optimum rate for broadleaf weed control was 1·3 lb per acre in April and 0·8 lb in May. For annual grass control 1·2 lb *L* and 2·2 lb *P* were required in April and 3·5 lb *L* and 4·0 lb *P* in May. Carrots became less tolerant to herbicides with increasing age. Injury occurred when daily max. temp. was > 90°F from 2 days before to 2 days after application. Although shading carrots before or after applications increased injury, final crop yields were not decreased. A. H. Cornfield.

Selective control of hoary alyssum, [*Berteroa incana*] in alfalfa [lucerne]. C. A. KUST (*Weed Sci.*, 1969, **17** (1), 99–101. 3 ref.).—Hoary alyssum was selectively controlled in lucerne by simazine (2 lb/acre), 3-t-butyl-5-bromo-6-methyluracil (DuPont 733, 0·5–1 lb), 2,4-DB (0·5–1 lb), and Terbacil (3-t-butyl-5-chloro-6-methyluracil, 0·5–1 lb) applied after the last forage harvest in early autumn. Treatment over 3 yr had no effect on lucerne yields except that simazine reduced first-harvest hay yields in the first 2 yr. A. H. Cornfield.

Precision placement of herbicides for weed control in seedling alfalfa [lucerne]. D. L. LINSCOTT and R. D. HAGIN (*Weed Sci.*, 1969, **17** (1), 46–47. 3 ref.).—When planting lucerne, EPTC was applied in subsurface bands beneath the seed and each of 3 triazine herbicides (simazine, atrazine and 2-isopropylamino-4-methoxy-ethylamino-6-methylmercapto-*s*-triazine) or EPTC was placed in subsurface bands between the rows. EPTC applied between and within rows, as well as simazine between rows and EPTC within rows gave superior weed control, with little chronic effect on lucerne. A. H. Cornfield.

Effects of chlorpropham vapours on dodder (*Cuscuta* spp.) seedlings. C. H. SLATER, J. H. DAWSON, W. R. FURTICK and A. P. APPLEBY (*Weed Sci.*, 1969, **17** (2), 238–241. 11 ref.).—Seedlings of dodder were killed when exposed to chlorpropham (*C*) vapours. In a field trial, application of *C* (6 lb per acre in attapulgite granules) when lucerne stubble was 3 in high, reduced by 50% the emergence of dodder seedlings and almost prevented the occurrence of seedlings wrapped around lucerne stems. A. H. Cornfield.

Placement of EPTC (ethyl *N*,*N*-dipropylthiocarbamate)–fertiliser when sowing birdsfoot trefoil. D. L. LINSCOTT and R. D. HAGIN (*Weed Sci.*, 1969, **17** (1), 108–109. 7 ref.).—Annual grasses were reduced and yields of birdsfoot trefoil increased when EPTC (1·5–3·5 lb/acre) was incorporated in granular fertiliser applied at sowing time, or sprayed on the soil surface and incorporated by disking before sowing. When perennial grasses and sedges were abundant, a combination of the two treatments was more effective. A. H. Cornfield.

Selective grass control with siduron. H. D. KERR (*Weed Sci.*, 1969, **17** (2), 181–186. 8 ref.).—When used at suitable rates, siduron (*S*) controlled weed grasses without injury to forage and turf grasses. Incorporation of *S* in the soil before planting, spraying seed on the soil surface, and mixing seed with *S* some time before sowing were all effective. A. H. Cornfield.

Downy brome control by herbicides for re-vegetation of rangelands. R. A. EVANS, R. E. ECKERT, JUN., B. L. KAY and J. A. YOUNG (*Weed Sci.*, 1969, **17** (2), 166–169. 5 ref.).—Of 18 herbicides tested over a no. of years and at several locations for control of *Bromus tectorum* and associated broadleaf weeds, atrazine (1 lb per acre) was the most effective with respect to length of weed control activity and spectrum of weeds. A. H. Cornfield.

Growth characteristics and herbicide susceptibility of Texas panicum [*Panicum texanum*]. J. M. CHANDLER and P. W. SANTELMANN (*Weed Sci.*, 1969, **17** (1), 91–93. 2 ref.).—The best germination of Texas panicum seed was obtained after storage at 21·1° or at temp. varying from −12·2 to 37·8°. The weed was controlled by pre-plant application of trifluralin (0·75–1·5 lb) and by pre-emergence applications of amiben (4 lb), fluometuron (3 lb) or prometryne (5 lb per acre). A. H. Cornfield.

Control of green rabbitbrush, *Chrysothamnus viscidflorus*, and big sagebrush, *Artemisia tridentata* [with picloram and 2,4-D]. P. T. TUELLER and R. A. EVANS (*Weed Sci.*, 1969, **17** (2), 233–235. 5 ref.).— A. H. Cornfield.

Effects of foliarly applied desiccants on some species under tropical environment. R. W. BOVEY (*Weed Sci.*, 1969, **17** (1), 79–83. 7 ref.).—Of a number of herbicides tested for desiccating leaves of cucumber, sorghum, mango and guava, PCP (pentachlorophenol) and DNBP (4,6-dinitro-*o*-s-butylphenol) consistently acted the fastest. Paraquat, diquat, and 2,4-D : 2,4,5-T were equally effective but took up to 3 days to cause complete desiccation. A. H. Cornfield.

Effect of simulated rainfall on herbicide performance. R. W. BOVEY and J. D. DIAZ-COLON (*Weed Sci.*, 1969, **17** (2), 154–157. 4 ref.).—The effect of simulated rainfall 1 min to 2 h after application of 7 herbicides to mango, guava, sorghum and dioscorea on the % desiccation of the plants is reported. A. H. Cornfield.

Effect of nitrogenous materials on uptake of triazine herbicides. W. H. MINSHALL (*Weed Sci.*, 1969, **17** (2), 197–201. 7 ref.).—Application of urea or KNO_3 to the soil increased uptake and translocation of triazine herbicides into the stump exudate of detopped tomato plants. Atrazine applied to the soil was detected within 10 min in stump exudate and approached its max. concn. in 3 h. Concn. of 4 triazines in the exudate increased with their solubility in water. A. H. Cornfield.

Herbicidal control of western ironweed [*Vernonia baldwini*]. M. K. MCCARTY and C. J. SCIFRES (*Weed Sci.*, 1969, **17** (1), 77–79. 8 ref.).—Picloram (0·5–2·0 lb/acre) was the most effective material tested for controlling ironweed. An initial treatment (2 lb) followed by another 2 yr later resulted in complete elimination of ironweed after 4 yr. A. H. Cornfield.

Matricaria and lambsquarter control in brassicas with analogues of propachlor. J. MUKULA and W. A. GENTNER (*Weed Sci.*, 1969, **17** (1), 124–125. 4 ref.).—Five analogues of propachlor were tested for control of *Matricaria inodora* and *Chenopodium album* in soln. cultures at 10^{-4} to 10^{-8} M concn. *N*-(butoxymethyl)-2-chloro-2′,6′-diacetanilide and 2-chloro-*N*-(propoxymethyl)-2′,6′-acetoxylidide were promising for matricaria control without excessive toxicity to brassicaceous crops. All materials tested were less effective against *C. album*, the margin of safety between the weed and crop plants being too small to be of practical use. A. H. Cornfield.

Control and ecological studies of Scotch thistle [*Onopordum acanthium*]. J. A. YOUNG and R. A. EVANS (*Weed Sci.*, 1969, **17** (1), 60–63. 8 ref.).—Application of picloram (0·03–2 lb/acre) in June, or picloram (0·25 lb) or 3-amino-1,2,4-triazole (6 lb) at flowering, killed the weed. A. H. Cornfield.

Effects of herbicides on yews and Japanese maples. L. L. DANIELSON and C. MAY (*Weed Sci.*, 1969, **17** (2), 142–144. 16 ref.).—Chlorpropham, dinoseb, sesone (2,4-DES), simazine, DCPA, trifluralin, and amitrole had no significant effect on growth of either species when applied at herbicidal rates to control broadleaf weeds and grasses. A. H. Cornfield.

Effects of direct contact of pine seeds or young seedlings with commercial formulations, active ingredients, or inert ingredients of triazine herbicides. T. T. KOZLOWSKI and S. SASAKI (*Can. J. Pl. Sci.*, 1968, **48** (1), 1–7. 10 ref.).— A. G. Pollard.

Response of woody species to 2,4-D, 2,4,5-T, and picloram as a function of treatment method. H. D. COBLE, R. P. UPCHURCH and J. A. KEATON (*Weed Sci.*, 1969, **17** (1), 40–46. 17 ref.).—2,4,5-T (propyleneglycol butyl ether ester), as dormant stem and basal applications, gave the most consistent control of 12 woody species. The triethanolamine salt was generally as effective as the ester when applied as foliar sprays. Foliar sprays of picloram controlled all except 4 species, but none was effectively controlled by dormant stem or basal applications. A. H. Cornfield.

Economic evaluation for weed control in field-lined woody ornamental nursery crops. S. W. BINGHAM (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 704–712. 23 ref.).—Hand labour for weed control was reduced more than 55% by a single application of simazine + amitrole (banded in the row or broadcast). Granular simazine remained effective longer than did a wettable powder formulation. Areas treated twice during spring and autumn required less labour for weeding than when treated only once. In general weed control with herbicides was more economical than hand hoeing. Growth of ornamentals was better where chemicals were applied than where hand hoeing was used. A. H. Cornfield.

Effects of 2,4,5-T during the approach of woody plant dormancy. R. P. UPCHURCH, J. A. KEATON and H. D. COBLE (*Weed Sci.*, 1969, **17** (2), 229–233. 21 ref.).—Application of 2,4,5-T (butoxyethanol ester or triethylamine salt) to turkey oak (*Quercus laevis*) and red maple (*Acer rubrum*) on Oct. 11 gave significantly better control of both species than did application on Sept. 13. A. H. Cornfield.

Response of conifer seedlings and weeds to seed-bed fumigants. R. GROVER (*Can. J. Pl. Sci.*, 1968, **48** (2), 189–196. 9 ref.).—Soil fumigants were applied, at several different rates, to seed beds prior to sowing. The fumigants [allyl alcohol, dazomet, metham (Na salt) and MeBr contg. 2% chloropicrin], generally gave up to 90% control of weeds esp. at medium and high applications; weeds escaping fumigants subsequently grew well. Hand weeding facilitated germination of additional weed seeds. Neither emergence nor stands of the conifers were affected by the fumigants. Emergence of two spruce species was somewhat reduced in unweeded and in hand weeded plots when the weed density was high. A. G. Pollard.

Woody plant [red maple and persimmon] shoot management and response to herbicidal treatment [with 2,4-D or 2,4,5-T]. R. P. UPCHURCH, J. A. KEATON and H. D. COBLE (*Weed Sci.*, 1969, **17** (2), 175–180. 17 ref.).— A. H. Cornfield.

Rainfall effects following herbicidal treatment of woody plants. R. P. UPCHURCH, H. D. COBLE and J. A. KEATON (*Weed Sci.*, 1969, **17** (1), 94–98. 14 ref.).—Shoots of turkey oak, *Quercus laevis*, and red maple, *Acer rubrum* were, treated in Aug. with 3 herbicides followed by simulated rainfall (0 to 1 in) 5–120 min after treatment. The rainfall did not affect the extent of control after 10–13 months where 2,4,5-T (ester or amine formulation) was used, but reduced control where picloram + 2,4-D was used. A. H. Cornfield.

Influence of time and method of application on turkey oak response to picloram and 2,4-D. H. D. COBLE, R. P. UPCHURCH and J. A. KEATON (*Weed Sci.*, 1969, **17** (1), 87–91. 8 ref.).—The best control of *Quercus laevis* was obtained by basal leaf application, in Aug., of picloram (1 lb) + 2,4-D (4 lb) as isopropanolamine salts. Other methods of application were less effective. Soil applications of pelleted picloram (2·5–10 lb/acre) were most effective when applied in early spring or summer. A. H. Cornfield.

Herbicidal treatment effect on carbohydrate levels of alligatorweed [*Alternanthera philoxeroides*]. L. W. WELDON and R. D. BLACKBURN (*Weed Sci.*, 1969, **17** (1), 66–69. 11 ref.).—Application of silvex or 2,4-D (each at 4–8 lb/acre 5 times during the growing season) to floating alligatorweed depleted readily acid-hydrolysable carbohydrates by 24% in a tidal area and by 15% in a non-flowing area. Silvex was more effective than was 2,4-D and control was better in the tidal than in the non-flowing area. A. H. Cornfield.

Fungicides

Fungicidal activity and chemical constitution. XV. The activity of 4-(1-substituted n-alkyl)-2,6-dinitrophenols. R. J. W. BYRDE, D. M. FIELDGATE, V. W. L. JORDAN, *et al.* (*Ann. appl. Biol.*, 1969, **64** (1), 119–130. 19 ref.).—Several 4-(1-phenylalkyl)-, 4-(1-cyclohexylalkyl-, 4-(1-cyclopentylalkyl)- and 4-(1-cyclobutylalkyl)-2,6-dinitrophenols, together with some deriv., were tested against powdery mildews of apple, blackcurrant, and rose in glasshouse and field trials and against *Venturia inaequalis* in spore germination tests. Highest activity in the glasshouse was shown by members of

the cyclobutyl series with 4–6 C atoms in the alkyl group. No compd. was phytotoxic to healthy foliage. A. H. Cornfield.

Some factors affecting the activities of dinitrophenol fungicides. D. R. CLIFFORD, E. C. HISLOP and M. E. HOLGATE (*Pestic. Sci.*, 1970, **1** (1), 18–23. 23 ref.).—The vapour phase and protectant activities of members of seven homologous series of alkyldinitrophenols against cucumber powdery mildew (*Oidium* sp.) are discussed. No correlation existed between vapour activities *in vitro* or *in vivo* and vapour pressure, but a positive correlation between 'vapour' protection *in vivo* and conventional protectant activity was evident. Although vapour activity occurs with some homologues, results obtained for zone assays *in vivo* are probably better explained in terms of easy movement of the compd. in the leaf surface than in terms of vapour transmission. Alkyldinitrophenylcrotonates showed no zone activity *in vivo* but often good protectant activity. The possible existence of two optimum π-values for protectant activity is suggested. No appreciable systemic activity was found with these compd. P. C. W.

Preparation and properties of 5-arylazo-5-nitro-2-phenyl-1,3,2-dioxaboracyclohexane derivatives. R. KOWALIK, B. WYDŹGA, Z. EJMOCKI and Z. ECKSTEIN (*Bull. Acad. pol. Sci. Sér. Sci. chim.*, 1969, **17** (4), 209–213. Engl., 9 ref.).—Sixteen 2-phenyl-5-R-5-nitro-1,3,2-dioxaboracyclohexane compd. were synthesised as potential fungicides, where R particularly contained an aryldiazo function, and their activities against *Fusarium culmorum*, *Alternaria tenuis* and *Rhizoctonia solani* were compared with those of the corresponding 2-nitro-2-R-1,3-propanediol compd. Max. activity was observed with 5-(2′,5′-dichlorophenylazo)-5-nitro-2-phenyl-1,3,2-dioxaboracyclohexane. A. Lewin.

Mixed ligand chelates of copper(II) with 8-quinolinol and arylhydroxycarboxylic acids. III. Rôle of stability constants in antifungal action. H. GERSHON, S. G. SCHULMAN and D. OLNEY (*Contr. Boyce Thompson Inst. Pl. Res.*, 1969, **24** (8), 167–171. 12 ref.).—The first dissociation const., log k_2, varied from 11·01 to 6·5 among the active compd. The second, log k_1, ranged from 11·95 to 10·20 whereas antifungal activity varied only from 5- to 13-fold between the least and most active compd., with respect to the organism inhibited. Fungitoxicity appears to be more correlated with k_1 than with k_2 or the overall const. β_2. E. G. Brickell.

7-Nitro-8-quinolinols and their copper(II) complexes. Implications of the fungal spore wall as a possible barrier against potential antifungal agents. H. GERSHON (*Contr. Boyce Thompson Inst. Pl. Res.*, 1968, (2127), *J. mednl Chem.*, 1968, **11**, 1094–1096. 13 ref.).—Antifungal activity of the 5-halo deriv. of these compd. (prepn. described) appears to be directly dependent on whether the spore wall of the fungi acts as a barrier or not. The activity is in the order 5-I > 5-Br > 5-Cl > 5-F compd. and the Cu(II) bis complexes were inactive. It is suggested that holes in the spore walls are elliptical or hexagonal thus excluding the bis(7-nitro-8-quinolinolato) copper(II) complexes from the spore. E. G. Brickell.

Anthracnose caused by *Colletotrichum coffeanum* Noack on Robusta and Excelsa coffee in the Central African Republic. A. M. SACCAS and J. CHARPENTIER (*Café-Cacao-Thé*, 1969, **13** (2), 131–150. Fr., 205 ref.; (3), 221–230.).—This disease was first noted in 1949. The symptoms as shown by leaves, branches, shoots, berries and flowers are described, and details of resulting damage and method of control are discussed. Bordeaux mixture (1·0–1·5% $CuSO_4$ or equiv. concn. of other Cu salt) is effective. C. V.

Control of Arabica coffee berry disease in Kenya. R. A. MULLER (*Café-Cacao-Thé*, 1968, **12** (1), 39–52. Fr.).—The choice of fungicide, seasonal spraying programme and spraying equipment are discussed. A species of *Colletotrichum coffeanum* Noack is involved. In another series, excellent results were obtained in the Cameroons using cupric oxychloride + Cu_2O (50%), this mixture being used at 1% or 0·5% strength. C. V.

Effect of fungicidal treatments on sporulating capacity in relation to control of coffee berry disease. F. J. NUTMAN and F. M. ROBERTS (*Ann. appl. Biol.*, 1969, **64** (1), 101–112. 8 ref.).—Fungicidal effectiveness in controlling the disease, due to *Colletotrichum coffeanum*, differed according to the epidemiology of the disease. Good control occurred when the main source of inoculum was from the wood and poor control when it was from diseased berries. Sprayed branches re-infected from the berries had a higher sporulating capacity than did unsprayed branches. Correct timing of early-season spray schedules is important. A. H. Cornfield.

Chemical control of decaying of apples during preservation: efficiency of Benomyl and thiabendazole. G. BOMPEIX, F. MORGAT, B. POIRET and J.-P. PLANQUE (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (11), 776–783, Fr., 12 ref.).—Orchards of 12-yr old Golden Delicious were treated in spring and summer with Benomyl [Me 1-(butylcarbamoyl)-2-benzimidazole carbamate,] (**I**), thiabendazole (**II**), 8-hydroxyquinoline sorbate (**III**), captan (**IV**), carboxine (**V**), and thiram (in oil). **I** and **II** were very effective. After harvest, fruit from untreated infested orchards was treated with **I**–**V**. Both **I** and **II** were suitable for preventing decay in fruit. M. T. Rawnsley.

Persistence in soil of the fungicidal seed dressings captan and thiram. R. L. GRIFFITH and S. MATTHEWS (*Ann. appl. Biol.*, 1969, **64** (1), 113–118. 8 ref.).—When thoroughly mixed with moist soil, both fungicides had a half-life of 1–2 days but when glass beads covered with a thin film of fungicides were placed in soil there was little decomp. even after 21 days. These results explain why coatings of captan or thiram on seed are effective in controlling soil-borne fungi despite their apparently low persistence in soil. A. H. Cornfield.

Field control of loose smut in barley with the systemic fungicides Vitavax and Plantvax. E. REINBERGS, L. V. EDGINGTON, D. R. METCALFE and V. M. BENDELOW (*Can. J. Pl. Sci.*, 1968, **48** (1), 31–35. 2 ref.).—Dressing seed barley with Vitavax (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin) (*V*) at 113 g per 45·4 kg of seed, gave complete control of smut; yields increased without affecting malting quality. Plantvax (4,4-dioxide deriv. of *V*) (*P*), applied to soil at 5·6 or 11·2 kg/ha gave effective control of loose smut but did not increase yields. At the higher rate, *P* lowered yields of some varieties and caused slightly adverse effects on height, date of maturity, wt. of seed per hectolitre, and wt. per 1000 kernels. A. G. Pollard.

Treatment of oranges against *Penicillium* with formulations based on thiabendazole. J. CUILLÉ and L. BUR-RAVAULT (*Fruits d'outre mer*, 1969, **24** (9–10), 421–424. Fr., 20 ref.).—Soaking in 600 ppm of thiabendazole (I) for 3 min was completely successful. The **I** remained on the skin and continued its antifungal activity. It is possible to incorporate **I** into normal pesticide applications. M. T. Rawnsley.

Spectrophotometric determination of the fungicide dithianon in aqueous formulation Delan-Col with 2,4-dinitrophenylhydrazine. S. H. YUEN (*Analyst, Lond.*, 1969, **94** (1125), 1095–1098. 13 ref.).—The sample was extracted with a $CHCl_3$–Me_2CO mixture and the org. layer evaporated to dryness; the residue was refluxed with neutral methanolic-2,4-dinitrophenylhydrazine. The resulting soln. was made alk. with methanolic M-NaOH and the optical *d*, measured at 500 nm, was compared with those of known standards. The error was 2·1% (other carbonyl compd. must be absent.). S. S. Chissick.

Insecticides and Others

Degradation of DDT and DDE by cheese micro-organisms. R. A. LEDFORD and J. H. CHEN (*J. Fd Sci.*, 1969, **34** (4), 386–388. 10 ref.).—The growth of streptococci and micrococci (found on the surface of cheeses) did not degrade DDT or DDE, but the growth was not inhibited. A Gram-positive short rod (16AE2), however, dechlorinated DDT. This has not previously been reported in food micro-organism metabolism. M. T. Rawnsley.

Use of the multiple regression equation in the prediction of insecticidal activity of anticholinesterase insecticides. R. L. JONES, R. L. METCALF and T. R. FUKUTO (*J. econ. Ent.*, 1969, **62** (4), 801–808. 20 ref.).—With *m*-substituted phenyl *N*-methylcarbamates, cholinesterase inhibition was more significant than liposolubility in predicting synergised LD_{50} values. With *p*-substituted compd., both cholinesterase inhibition and lipophylicity were significant. There was no correlation of any parameters with the detoxication of these compd. C. M. Hardwick.

Residues of diazinon on field corn treated with granular, capsular, and *ULV* formulations for control of the European corn borer. J. A. HARDING, C. CORLEY, M. BEROZA and W. G. LOVELY (*J. econ. Ent.*, 1969, **62** (4), 832–833. 5 ref.).—Residues from *ULV* application of diazinon were greatest on the leaves while heaviest deposits in the whorl and axils came from capsular formulations, with granular formulations being intermediate in effect. As the amount present in the whorl is the critical factor for control of *Ostrinia nubilalis*, *ULV* applications were not effective. C. M. Hardwick.

Control of *Aphis fabae* on spring-sown field beans, *Vicia faba*. H. J. GOULD and C. W. GRAHAM (*Ann. appl. Biol.*, 1969, **64** (1),

1-10. 7 ref.).—Disulfoton (I) or phorate (II) granules or demeton-S-methyl (III), menazon, and vamidothion sprays, applied once in early June as preventive treatments before heavy aphid colonies appeared, gave good control on spring-sown field beans. Eradicant treatments on heavily infested plants during flowering were effective where III and dimethoate sprays or I or II granules were applied. A. H. Cornfield.

Aphids on leafy vegetables. W. J. REID, JUN. and F. P. CUTHBERT, JUN. (*Fmrs' Bull., U.S. Dep. Agric.*, 1969, No. 2148, 16 pp. Engl.).—*Myzus persicae*, *Brevicoryne brassicae*, *Hyadaphis pseudobrassicae* and *Aphis fabae* are briefly described. Control by cultural practice and insecticides is discussed. E. G. Brickell.

Some experiments with different formulations of organophosphorus insecticides as seed dressings to control wheat bulb fly (*Leptohylemyia coarctata*). D. C. GRIFFITHS, K. A. LORD and G. C. SCOTT (*Pestic. Sci.*, 1970, **1** (1), 3-4. 3 ref.).—In expt. on the control of wheat bulb fly larvae by seed dressings, comparisons were made between four organophosphorus compd. in standard siliceous earth formulations (*SEF*) and special formulations (*SF*) of the same compd. in polyvinyl acetate, polypropylene or wax. The *SF* allowed more insecticide to be placed on the seeds without affecting germination. Counting shoots damaged by wheat bulb fly larvae showed that: *SEF* of diazinon gave good control which was not improved by *SF*; control with *SEF* of dimethoate was poor and was improved only little by the *SF*; and control with *SEF* of parathion and dichlofenthion was moderate and was improved by *SF*, especially polyvinyl acetate and polypropylene. The results suggest that *SF* are of most value with compd. that are moderately effective but where the amount of insecticide in standard seed dressings cannot be increased without damaging the plants. P. C. W.

Toxicity of some systemic, granular insecticides to Arabica coffee. G. I. D'SOUZA and G. H. VENKATARAMIAH (*Indian Coff.*, 1969, **33** (5), 156).—Of the 5 compd. tested only Terracur (a monothiophosphate deriv.) and Rogor (≡ dimethoate) were promising for control of coffee pests (green bug) at 2 g per seedling; the other 3 compd. caused phytotoxic symptoms. C. V.

Pests of the coconut palm. R. J. A. W. LEVER (*F.A.O. agric. Stud.*, 1969, No. 77, 190 pp., Engl., 274 ref.).—An account is presented of the species, both invertebrate and vertebrate, that are harmful to the coconut palm, *Cocos nucifera*. Insect pests of copra and some practical aspects of pesticide application are discussed. E. G. Brickell.

Laboratory and field tests in 1966–67 on chemical control of wireworms (*Agriotes* spp.). D. C. GRIFFITHS, G. C. SCOTT, J. R. LOFTY and P. F. ROBERTS (*Ann. appl. Biol.*, 1969, **64** (1), 21-29. 11 ref.).—In lab. tests only 5 of 16 org compd. tested killed wireworms; in further tests with the same soils only 3 compd. were effective. In field trials organo-P insecticides, e.g., phorate, were almost as effective as was aldrin in increasing plant stands of barley, although they killed fewer wireworms and were less effective than aldrin in protecting potatoes from wireworm damage. The organo-P materials were as effective as γ-BHC in killing wireworms and protecting sugar-beet seedlings. A. H. Cornfield.

Distribution of a chemical following its application at a point source in an irrigation system. J. M. OSGERBY (*Pestic. Sci.*, 1970, **1** (1), 5-9. 3 ref.).—The development of large, complex irrigation systems presents problems of pest control which are likely to become increasingly difficult to solve by established application methods. By applying a chemical at one point in such a system, use can be made of the flow of water through the system to effect the necessary distribution. This technique has been used most effectively with the molluscicide Frescon (*N*-tritylmorpholine). However, the complexity of many irrigation systems precludes the optimisation of the necessary rate of addition of molluscicide by any simple method. A mathematical model of an irrigation system is described which enables a detailed study of this type of application to be made with speed and economy. An analogue computer was used to solve the equations subject to the operating conditions of the irrigation system. P. C. W.

Effects of spider mites on tomato yield and fruit quality. A. K. STONER and F. F. SMITH (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 543-552. 2 ref.).—Defoliation of tomato plants by the carmine spider mite, *Tetranychus cinnabarianus*, resulted in increased earliness, lower fruit yields, more sun-scalded fruit, and lower sol. solids. The main effect on yield was reduced fruit size. A. H. Cornfield.

Flame photometric analysis of bromine [insecticides]. B. GUTSCHE and R. HERRMANN (*Dt. LebensmittRdsch.*, 1969, **65** (11), 352-355. Ger., 10 ref.).—Using a Br-sensitive flame photometric detector, 4 Br-contg. insecticides were determined. Petroleum ether extracts were injected into the burner and concn. were then calc. from the peak height. Standard deviations were 0·91-2·44%. J. B. Woof.

Alternatives to insecticides. R. H. WRIGHT (*Pestic. Sci.*, 1970, **1** (1), 24-27. 13 ref.).—Results of work in progress on the elucidation of insect responses to attractant substances of various kinds is reviewed, and the possibility of developing practical alternatives to insecticides is discussed. P. C. W.

Releases of unsexed gamma-irradiated codling moths for population suppression. L. D. WHITE, R. B. HUTT and B. A. BUTT (*J. econ. Ent.*, 1969, **62** (4), 795-798. 11 ref.).—Release of 500 irradiated *Laspeyresia pomonella* daily in a small apple and pear orchard, following one pre-release spray of parathion, reduced fruit damage from 50% to 1·57%. Moths were sterilised at 40 krad; this dose caused loss of vigour in the females. Sex and light traps showed an av. ratio of 23 sterile to 1 native male. C. M. Hardwick.

Combating weeds. N. V. PHILIPS' GLOEILAMPENFABRIEKEN (Br. Pat. 1,152,185, 19.5.66. Neth., 22.5.65).—Crops, particularly cereals, are protected from weeds by compn. contg. 0·5-2·5 kg of 2,6-dichlorobenzonitrile (I) and 3·0-4·0 kg of 2-methyl-4,6-dinitrophenol (II), or its salts together with a solid carrier, wetting agent and dispersing agent. In an example, I is mixed with dolomite, Na dodecylbenzene sulphonate and Na_2 dinaphthyl methane disulphonate and ground to an av. particle size of 10 μ. The resulting mixture is mixed with II (NH_4 salt) contg. a small amt. of lignin sulphonate and the total compn. stirred with water and then further diluted for use. S. D. Huggins.

Diseases and Pests in Livestock; Veterinary Treatments

Control of Exogenous Pests

No abstracts.

Other Treatments

Water kinetics in enteric disease of neonatal calves. R. W. PHILLIPS and K. L. KNOX (*J. Dairy Sci.*, 1969, **52** (10), 1664-1668. 12 ref.).—By means of jugular injections of ^3H_2O, normal calves were shown to have a water space of 87% of body wt. compared to 76% for diarrhoeic calves. Dehydration during diarrhoea was evenly distributed throughout the body compartments and turnover of the body water pool increased sharply with increasing severity of diarrhoea. M. O'Leary.

Common liver fluke in sheep. ANON. (*Leafl. U.S. Dep. Agric.*, 1969, No. 492, 8 pp., Engl.).—The mode of ingress and life cycle of *Fasciola hepatica* are described, together with symptoms in sheep, treatment, and control. E. G. Brickell.

The parasitic life cycle of the swine kidney worm, *Stephanurus dentatus* Diesing. A. H. WADDELL (*Aust. J. Zool.*, 1969, **17** (4), 607-618. 36 ref.).—Detailed expt. showed that more larvae migrate through the intestine than the stomach, and relatively few across the peritoneal cavity, from which they move at random. Large numbers reach the liver, and some can escape. *S. dentatus* was shown to be very similar to other skin-penetrating nematodes. M. T. Rawnsley.

Furaltadone and tylosin tartrate in the control of experimentally induced chronic respiratory disease complex of chickens. N. MATZER (*Poult. Sci.*, 1969, **48** (2), 701-705. 22 ref.).—Addn. of 1·75 g of furaltadone per 1 of drinking water of hens which had been inoculated with *Mycoplasma gallinarum* and *Escherichia coli* decreased the no. and extent of lesions in chickens compared with non-medicated controls, whereas 0·066 g/l of tylosin tartrate was ineffective. A. H. Cornfield.

Metabolism of clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) in chickens. G. N. SMITH (*Poult. Sci.*, 1969, **48** (2), 420-436. 9 ref.).—Studies with ^{36}Cl-labelled clopidol (anti-coccidial drug) supplied to chickens showed that the drug was relatively unaffected during passage through the bird. A. H. Cornfield.

Control of European foulbrood disease of the honey bee. H. SHIMANUKI, T. LEHNERT, D. A. KNOX and E. W. HERBERT, JUN. (*J. econ. Ent.*, 1969, **62** (4), 813-814. 6 ref.).—Hive bodies from

colonies infected by *Streptococcus pluton* were treated with ethylene oxide (I) or Terramycin (II). Both reduced the extent of the disease; I also seems to have controlled some unknown factor since the bee colonies were larger than those from the II-treatment.
C. M. Hardwick.

Household Pests, Sanitation, Food Hygiene

General Sanitation

Biological interaction between plasticisers and insecticides. E. P. Lichtenstein, K. R. Schulz, T. W. Fuhremann and T. T. Liang. (*J. econ. Ent.*, 1969, **62** (4), 761–765. 10 ref.).—Of the 11 polychlorinated biphenyl plasticisers (*P*) tested, those with the lower amounts of chlorine showed greater toxicity to *Drosophila melanogaster* and houseflies. In combination with DDT and dieldrin, the *P* were synergistic against houseflies.
C. M. Hardwick.

Detoxification of diazinon by subcellular fractions of diazinon-resistant and susceptible houseflies. J. B. Lewis (*Nature, Lond.*, 1969, **224** (5222), 917–918. 13 ref.).—Three detoxification mechanisms observed in six strains of housefly are discussed and each mechanism is related to the genetics of diazinon (I)-resistance of the flies. The first, which required O_2 and occurred entirely in the microsomal fraction, was cleavage of I to Et_2 phosphorothioic acid and of diazoxon (II) to Et_2 phosphoric acid. All strains used this degradative path, which is probably a common mode of detoxification. The second, which required neither O_2 nor NADPH, was desethylation of I and II confined entirely to the sol. fraction of strains with gene *a* and requiring reduced glutathione as cofactor. The third mechanism, which required O_2 and NADPH, occurred in the microsomal fraction of the strains with gene for resistance on chromosome V and consisted of degradation of II, but not I, to two metabolites. The first and third mechanisms were inhibited by sesamex, but the second was unaffected. The results explain some effects of sesamex and the synergist *S,S,S*-Bu_3 phosphorotrithionate on the toxicity of I and II to the different strains.
W. J. Baker.

Dioxane and dioxaspiro derivatives as attractants for male yellow-fever mosquitoes. G. G. Grant (*J. econ. Ent.*, 1969, **62** (4), 786–789. 9 ref.).—Of 70 compd. volatilised from aq. soln., only 2 were significantly attractive to *Aedes aegypti*; both were deriv. of 2-phenyl-*m*-dioxane. Out of 32 dioxane or dioxaspiro deriv. tested, 19 were significantly attractive; diethyl dioxaspiroundecane was 4× as attractive as the control. CO_2 concn., temp. and humidity affected the results. The most active compd. attracted males to a height of 5 in or more above the dish. C. M. Hardwick.

Chemosterilants in rodent control. J. E. Brooks and A. M. Bowerman (*Soap chem. Spec.*, 1969, **45** (10), 58, 60, 62, 64, 82, 83. 11 ref.).—The use of chemosterilants in reducing rat populations is reviewed with special reference to their mode of activity. Steroid compd. are described which interfere with the female reproductive processes, and the lack of success with many of them is attributed to the strong bait shyness that is induced. Such compd. also have a slow-acting effect. Mestranol is a compound which inhibits ovulation and permanently sterilises newly born rats that have received a sufficient dose through their mother's milk. Non-steroid compounds such as clomiphenes and diphenylindene deriv. are also effective in inhibiting reproduction, although the problem of bait rejection has still to be solved. Male anti-fertility drugs such as nitrofurans, thiophenes, halogenated diamines and dinitropyrroles, which are direct acting antispermatogenic agents, are also discussed. An unnamed compd. is being tested which is capable of sterilising male rats in a single dose. G. R. Whalley.

***Salmonella* in wastes produced at commercial poultry farms.** D. J. Kraft, C. Olechowski-Gerhardt, J. Berkowitz and M. S. Finstein (*Appl. Microbiol.*, 1969, **18** (5), 703–707. 13 ref.).—Samples from 91 poultry houses were found to be 29% positive. This sampling was based on 36 farms of which 50% yielded one or more positive samples. High densities were found in wastes from cage houses but not in the waste from floor houses (litter or wire floors). A total of 15 *Salmonella* serotypes were identified.
C. V.

***Arthrobacter luteus* nov. sp. isolated from brewery sewage.** T. Kaneko, K. Kitamura and Y. Yamamotu (*J. gen. appl. Microbiol., Tokyo*, 1969, **15** (3), 317–326. Engl., 26 ref.).—The characteristics are described. C. V.

Food Hygiene

Food hygiene. R. M. Blood (*Fd Process. Ind.*, 1969, **38** (457), 37–40. 17 ref.).—Hazards, control measures and plant hygiene are discussed. P. C. W.

Carcinogenic hydrocarbons in coffee-substitute products. H. G. Maier and W. Stender (*Dt. LebensmittRdsch.*, 1969, **65** (11), 341–343. Ger., 9 ref.).—Aq. extracts of barley, rye, chicory and sugar-beet, heated to various temp. up to 240°, were analysed for 3,4-benzopyrene (I), 1,12-benzoperylene and fluoranthene. Soln. were extracted with cyclohexane, the solvent was removed and the residue was then chromatographed on Al_2O_3–acetylated cellulose with Et_2O. Individual components were estimated fluorometrically. Recovery was 65–88% and the relative standard deviation was 3–8%. Hydrocarbon content increased with roasting temp., esp. > 175°. Final levels, however, were not high compared with other common foodstuffs. (I, 0·04–0·40 μg/kg).
J. B. Woof.

Release of dipicolinic acid from spores of *Bacillus stearothermophilus* NCA 1518. Y. Rotman and M. L. Fields (*J. Fd Sci.*, 1969, **34** (4), 343–344. 9 ref.).—The effects of pH, heat and heating time were studied. Max. release of dipicolinic acid (I) occurred at pH 14 when spores of two variants (rough and smooth) were autoclaved at 250°F for 15 min. With the smooth variant, max. release of I was achieved at pH 7·0. Loss of viability of spores of both variants followed complete release of I in contrast to *B. megaterium*.
M. T. Rawnsley.

Chemical composition and heat resistance of *Bacillus stearothermophilus* spores. Y. Rotman and M. L. Fields (*J. Fd Sci.*, 1969, **34** (4), 345–346. 12 ref.).—The rough variant was shown to be more heat tolerant than the smooth variant. No direct relationship was found between dipicolinic acid (I), mineral concn. (Ca, Mg, Mn, Zn) and heat resistance. The rate of I release from spores is considered to be of great importance in estimating heat tolerance.
M. T. Rawnsley.

Sporulation of rough and smooth variants of *Bacillus stearothermophilus*. Y. Rotman and M. L. Fields (*J. Fd Sci.*, 1969, **34** (4), 346–349. 9 ref.).—Sporulation was studied in nutrient agar, nutrient broth and trypticase soy agar, and yeast extract, dextrose, $Ca(NO_3)_2$, $Co(NO_3)_2$ and $MnSO_4$ were added to assess the effects of minerals. The rough variant sporulated best on nutrient agar enriched with 1 ppm of Mn with or without yeast extract. The smooth variant sporulated best on aerated broth fortified with yeast extract and 1 ppm of Mn. Very pure cultures can be obtained by manipulation of these techniques. M. T. Rawnsley.

Lethality of radio-frequency energy upon micro-organisms in liquid, buffered and alcoholic food systems. D. E. Carroll and A. Lopez (*J. Fd Sci.*, 1969, **34** (4), 320–324. 11 ref.).—The organisms used were *Saccharomyces cerevisiae*, *Bacillus subtilis* and *Escherichia coli* in an aq. buffered medium (pH 7·0, 5·0 and 3·0). R.F. (60 Mc/s) alone had no effect, and with heat there was no synergistic effect, although organisms were killed. A synergistic effect of EtOH was observed with *S. cerevisiae*. No lethality was observed when cultures were grown on tomato juice, etc. Thus, R.F. energy was shown not to be effective, which contradicts previous work in some respects. M. T. Rawnsley.

Survival of *Salmonella montevideo* on wheat stored at constant relative humidity. M. H. Crumrine and V. D. Foltz (*Appl. Microbiol.*, 1969, **18** (5), 911–914).—11 samples were inoculated and placed in a const. r.h. chamber, the r.h. being held at 7, 11, 27, 33, 43, 53, 62, 75, 84, 92 and 98%. Viable counts of samples held at 7, 11, 22% r.h. decreased from an initial 10^6 cells/g of wheat to a final count of 10^4/g for each sample. Those stored at 33–62% r.h. decreased to 20 viable cells/g and none were detected in the 75–98% r.h. groups after 22 and 16 weeks. C. V.

Microbiological evaluation of Pacific shrimp processing. J. M. Harrison and J. S. Lee (*Appl. Microbiol.*, 1969, **18** (2), 188–192. 16 ref.).—Observations on *Pandalus jordani* were made at five processing points, the microbial count being $1·3–3·0 \times 10^6$. The initial flora, in order of predominance, were *Acinetobacter–Moraxella*, *Flavobacterium*, *Pseudomonas*, Gram-positive cocci and *Bacillus* spp. No yeasts were isolated. Differences in processing practices influenced the findings but the bacterial load always increased after peeling and sorting and decreased after washing and brining. Significantly Gram-positive cocci were recovered with increasing frequency after each processing step, reaching 76% of the total in the final product, but most were coagulase-negative.
C. V.

Evaluation of the botulism hazard in vacuum packed smoked fish. G. HOBBS, D. C. CANN and B. B. WILSON (*J. Fd Technol.*, 1969, **4** (2), 185–191. 28 ref.).—Measurements of time and temp. during boil-in-the-bag cooking, and inactivation of toxin during this process, showed that boiling time > 5 min was necessary to ensure that no toxin survived the cooking process. Manufacturers' cooking instructions are adequate for the destruction of *Clostridium botulinum* type E toxin. I. Dickinson.

Variations in the composition of the flora on a Wiltshire cured bacon side. G. A. GARDNER and J. PATTON (*J. Fd Technol.*, 1969, **4** (2), 125–131. 16 ref.).—Three sites were investigated, singed rind, unsinged rind and meat. There were wide variations in counts on any one site both between sides from one factory and between factories. The most frequently isolated bacteria from all sites were micrococci. The meat samples differed from the rind samples by a marked increase in the proportions of Gram-negative bacteria such as *Acinetobacter* spp. and *Vibrio* spp. The composition of the flora from each of three factories was slightly different, and there was no appreciable difference attributable to the methods of maturation. I. Dickinson.

Chemical preservatives to inhibit the growth of *Staphylococcus aureus* in synthetic cream pies acidified to pH 4·5 to 5·0. E. W. SCHMIDT, JUN., W. A. GOULD, and H. H. WEISER (*Fd Technol., Champaign*, 1969, **23** (9), 1197–1199. 14 ref.).—K sorbate (**I**) and Na benzoate (**II**) are effective in controlling the growth of *S. aureus* inoculated into synthetic cream pies and incubated at 22 and 37°. At 22°, I was more effective than II, but at 37°, the reverse occurred. Effective control was accomplished only in synthetic creams which were acidified to pH 4·5–5·0 and not at higher pH. I. Dickinson.

Formaldehyde levels on and in chicken eggs following pre-incubation fumigation. J. E. WILLIAMS and H. S. SIEGEL (*Poult. Sci.*, 1969, **48** (2), 552–558. 7 ref.).—When freshly-laid eggs were fumigated for 20 min with HCHO, bactericidal levels persisted on the egg shell for 15 min, then concn. declined rapidly and was very low after 21-days storage at 24°. The concn. of HCHO was $\geq 3{\cdot}7$ ppm in the shell membrane and $\geq 0{\cdot}16$ ppm in the albumen. A. H. Cornfield.

Natural and synthetic materials with insect hormone activity. VI. Juvenile hormone effects of some alkyl ethers derived from aliphatic β-hydroxy acids. J. RATUSKÝ, K. SLÁMA and F. ŠORM (*J. Stored Prod. Res.*, 1969, **5** (2), 111–117. 14 ref.).—The substances exhibited this effect on *Tenebrio*, *Pyrrhocoris* and *Dysdercus* where the acetyl radical is geranyl or farnesyl. Hydrogenation of the double bond of these radicals was followed by loss of activity, whereas hydrochlorination and epoxidation conferred increased activity. The effects are weaker than those obtained with farnesenic acid deriv. but the new compd. can be synthesised from more readily accessible sources. C. V.

Control of insects in bagged grain by injection of dichlorvos. A. A. GREEN and D. R. WILKIN (*J. Stored Prod. Res.*, 1969, **5** (1), 11–19.).—The control of *Oryzaephilus surinamensis* (L.) and *Sitophilus granarius* with dichlorvos in CCl_4 soln. was studied; good results were obtained. Residues were higher on wheat than on barley and were greatest where dust and frass had collected. C. V.

Toxicity to *Tribolium castaneum* of mixtures of pyrethrins and piperonyl butoxide: fitting a mathematical model. P. S. HEWLETT (*J. Stored Prod. Res.*, 1969, **5** (1), 1–9. 13 ref.).—The beetles were sprayed with the mixture in volatile oil and the findings fitted a proposed equation. For indefinitely large doses of piperonyl butoxide, the mixtures were estimated to be 3·04 times as toxic as pyrethrins alone. C. V.

Effect of gamma radiation on insecticidal efficiency of malathion deposits on wheat and kraft paper. R. R. COGBURN and P. G. MAHANY (*J. econ. Ent.*, 1969, **62** (4), 829–831. 3 ref.).—Emulsifiable malathion was applied at 10 ppm and samples were irradiated at 25 and 100 krad. Chem. and biol. analysis was carried out for 6 months; irradiation did not affect the toxicity of malathion to *Tribolium castaneum*, neither did it cause chem. breakdown of deposits on paper, even after very heavy irradiation (4·3 Mrad). C. M. Hardwick.

Effect of carbon dioxide on toxicity of hydrocyanic acid and methyl bromide to adults of the confused flour beetle and granary weevil, at two different temperatures. M. T. ALINIAZEE and D. L. LINDGREN (*J. econ. Ent.*, 1969, **62** (4), 904–906, 9 ref.).—The addn. of CO_2 to HCN increased its effectiveness against *Tribolium confusum* at 60° and 80°F. and to a lesser extent against *Sitophilus granarius*. CO_2 only slightly increased the effectiveness of MeBr against both species. C. M. Hardwick.

Contamination by Pesticides

Pesticide drift. I. High-clearance vs. aerial application of sprays. G. W. WARE, B. J. ESTESEN, W. P. CAHILL, P. D. GERHARDT and K. R. FROST. **II. Mist-blower vs. aerial application of sprays.** G. W. WARE, E. J. APPLE, W. P. CAHILL, P. D. GERHARDT and K. R. FROST (*J. econ. Ent.*, 1969, **62** (4), 840–843. 6 ref.; 844–846. 2 ref.).—I. Aerial application resulted in greater drift than ground application at all distances as shown by deposits on glass plates or by Andersen air sampler.

II. Mist blowers resulted in greater drift than aerial application. Results from glass plate droplet analysis agreed with those from lucerne residues. C. M. Hardwick.

Arsenic content of soil and crops following use of methanearsonate herbicides. L. R. JOHNSON and A. E. HILTBOLD (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 279–282. 11 ref.).—The As content of a sandy loam under turf which had received methanearsonate (Na, Na_2, and NH_4 salts) at up to 9 kg a.i. per ha for 4 yr decreased with increasing depth. Yields of subsequent crops of cotton, soyabeans, sorghum–Sudan hybrid, maize, oats, vetch, and crimson clover were not affected by the previous herbicide treatments. The As content of seeds was greatest in cotton and soyabean. About 90% of the total soil As occurred in the clay fraction and was associated with Al. Much of the residual As was extractable with 0·5 N-NH_4 Cl, and negligible amounts of org. As were found. A. H. Cornfield.

Soil, Air, Water

Dissipation of herbicides at three soil depths. C. I. HARRIS, E. A. WOOLSON and B. E. HUMMER (*Weed Sci.*, 1969, **17** (1), 27–31. 7 ref.).—Open-ended tubes filled with atrazine or fenac (chlorfenac) were placed at 3-, 9-, and 15-in depths at various locations. After 3 months, 61% more atrazine and 41% more fenac, on average, were found in the 15-in than in the 3-in depth-placed tubes. Samples in northern areas contained more than twice as much residue of both herbicides as did those from southern areas. Persistence of the herbicides, in general, increased with soil org. matter content and depth of placement, and decreased with increasing temp. A. H. Cornfield.

Bioassay for Bensulide [*N*-(2-mercaptoethyl)benzenesulphonamide *S*-(*O*,*O*-di-isopropyl phosphorodithioate)], DCPA (dimethyl 2,3,5,6-tetrachloroterephthalate), and siduron in turfgrass. A. R. MAZUR, J. A. JAGSCHITZ and C. R. SKOGLEY (*Weed Sci.*, 1969, **17** (1), 31–34. 13 ref.).—Of 22 species evaluated at the seedling stage, barnyard grass, browntop millet, crabgrass, oats and Sudan grass were the most sensitive. These species were nearly always reliable in detecting the presence and movement of the herbicides in soils from field-treated plots. None of the herbicides accumulated in soil treated with 4 annual applications of the materials. A. H. Cornfield.

Microbial degradation of five substituted urea herbicides. D. S. MURRAY, W. L. RIECK and J. Q. LYND (*Weed Sci.*, 1969, **17** (1), 52–55. 12 ref.).—*Aspergillus niger*, *A. sydowi*, and *A. tamarii* were most tolerant to fenuron and least tolerant to diuron. The order of tolerance to norea (noruron), fluometuron, and monuron varied with fungal species. Oat bioassay for residual herbicide activity indicated differences in herbicide degradation rates between the 3 fungi in a sand. Diuron was degraded more rapidly than monuron, with the other materials showing intermediate rates. *A. niger* was most and *A. sydowi* least effective in degrading the herbicides. High org. N in soils favoured increased rates of degradation. A. H. Cornfield.

Soyabean injury from triazine residues in soil. R. J. FINK and O. H. FLETCHALL (*Weed Sci.*, 1969, **17** (1), 35–36. 6 ref.).—Yields of soyabean forage pot-grown in soil treated 6 months previously with atrazine (*A*) were unaffected where 2·5–5·0 lb/acre had been applied but were reduced where 10 lb had been applied. Yields were reduced by all rates of *A* 6 and 12 months after a second application. Yields were reduced where simazine (*S*) had been applied at all rates and after all periods. Yields of soyabeans and height of plants in field trials were unaffected where either *A* or *S* was applied at 2·5–5·0 lb/acre 1 and 2 years previously, but were reduced where 10 lb of either herbicide had been applied. A. H. Cornfield.

Inactivation of herbicides by activated carbon and other adsorbents. D. L. COFFEY and G. F. WARREN (*Weed Sci.*, 1969, **17** (1), 16–19. 27 ref.).—The possibility of inactivating persistent herbicides by addn. of adsorbents was investigated by determining the concn. of herbicide required to cause 50% inhibition of cucumber roots in silica sand culture. Of 8 herbicides tested CIPC, trifluralin, 2,4-D, diphenamid, DCPA, and amiben (chloramben) were more strongly adsorbed by activated C than by anion and cation exchange resins, muck soil, or bentonite clay. Paraquat activity was reduced by clay and cationic resin, but not by activated C. DNBP was more strongly adsorbed by anionic resin than by activated C.
A. H. Cornfield.

Effect of activated carbon on phytoxicity of herbicides in a tropical soil. R. W. BOVEY and F. R. MILLER (*Weed Sci.*, 1969, **17** (2), 189–192. 5 ref.).—In glasshouse tests with a silty clay, the toxic effects of propazine to beans were eliminated when 66 times as much activated C was added, whilst picloram required 3600 times its own wt. of C. In field trials, application of 600 lb activated C per acre reduced the extent of injury of propazine (2·5 lb per acre) to oats, beans and cucumbers. A. H. Cornfield.

Persistence pattern for diuron and linuron in Norfolk and Duplin sandy loam soils. R. P. UPCHURCH, F. T. CORBIN and F. L. SELMAN (*Weed Sci.*, 1969, **17** (1), 69–77. 9 ref.).—Residues of diuron, applied for weed control in cotton, were greater on a Duplin than a Norfolk sandy loam, but residues on the former soil were less toxic to succeeding crops, probably due to its higher org. matter content. Residues of diuron, applied pre-emergence to Norfolk soil at 1 lb/acre, plus 0·6 lb 'layby', frequently injured subsequent crops of wheat, groundnuts, and tobacco. Soyabeans were sometimes injured, but maize and cotton were unaffected. Linuron was less damaging than diuron in these respects.
A. H. Cornfield.

Effects of herbicides on growth of soil algae. C. LOEPPKY and B. G. TWEEDY (*Weed Sci.*, 1969, **17** (1), 110–113. 17 ref.).—Growth of *Chlamydomonas reinhardi* was completely inhibited by 1 ppm metobromuron (*M*) and 0·5 ppm atrazine (*A*), but was stimulated by 1–5 ppm diphenamid (*D*). *M*, 5 ppm, was toxic to *Chlamydomonas eugametos*, whilst even 10 ppm *A* and *D* had little effect on growth. Growth of *Chlorella vulgaris* was partially inhibited by 2–10 ppm *A* and stimulated by 1–10 ppm *D* and *M*. Growth of *Chlorella pyrenoidosa* was reduced by the higher levels of *A* and *M* and stimulated by all levels of *D*. These expt. were carried out in relation to herbicide persistence in soil.
A. H. Cornfield.

Response of Kentucky bluegrass to soil residues of pre-emergence herbicides. G. S. SMITH and L. M. CALLAHAN (*Weed Sci.*, 1969, **17** (1) 13–15. 8 ref.).—Kentucky bluegrass sod-plugs were grown in soil freshly treated with one of 11 herbicides and in soil collected from the 0–2 and 2–4 in depths of field plots treated 10 months previously with the same herbicides. Of those freshly applied, simazine and atrazine were the most and siduron the least toxic. Treatments which caused the least significant reduction in root regrowth (considering their effect at both depths) were siduron (12 lb/acre), benefin (*N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine) (2 lb/acre) and DCPA (dimethyl 2,3,5,6-tetrachloroterephthalate) (12 or 24 lb/acre). A. H. Cornfield.

Disappearance of trifluralin from field soils. S. J. PARKA and J. B. TEPE (*Weed Sci.*, 1969, **17** (1), 119–122. 7 ref.).—Analysis of soils from 107 field locations (U.S.A.) where 1–10 lb/acre trifluralin, spread over 1–4 yr, had been applied, showed that in most cases < 10% of the applied trifluralin remained in the soil. The herbicide did not accumulate with repeated annual application and the decline level decreased steadily with time. A. H. Cornfield.

Soil degradation of malathion. J. G. KONRAD, G. CHESTERS and D. E. ARMSTRONG (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (2), 259–262. 8 ref.).—The rate of chem. degradation of malathion in soil is so rapid that no significant microbial degradation has a chance to occur. Chem. degradation of malathion was an adsorption-catalysed hydrolysis. Final products were thiomalic acid, dimethylthiophosphoric acid, and EtOH. A. H. Cornfield.

Atrazine degradation in two soil profiles. F. W. ROETH, T. L. LAVY and O. C. BURNSIDE (*Weed Sci.*, 1969, **17** (2), 202–205. 17 ref.).—Degradation of atrazine (*A*), measured by bioassay with soyabeans, was 2–3 times faster in the top than in the sub soils of a silty clay loam and a silt loam. Degradation rate increased 2–3 times with each 10° temp. increase over the range 15–35° ,and up to 6 times when soil moisture content was increased from 40% to 80% of field capacity. *A* is detoxified relatively rapidly in soil and this is followed by slow degradation of the detoxified mol.
A. H. Cornfield.

Adsorption, volatility, and migration of thiocarbamate herbicides in soil. E. KOREN, C. L. FOY and F. M. ASHTON (*Weed Sci.*, 1969, **17** (2), 148–153. 13 ref.).—The rate of adsorption of thiocarbamate herbicides by 5 soils was highly correlated with soil org. matter content (*OMC*) and cation exchange capacity. Pebulate was adsorbed to the greatest extent. The downward movement of the materials in leached soil columns was directly related to their solubility in water and inversely related to soil *OMC*. Vapour losses from soil were correlated with v.p. of the materials.
A. H. Cornfield.

Effect of cation exchange capacity (*CEC*) on retention of diquat and paraquat by three-layer type clay minerals. I. Adsorption and release. S. B. WEED and J. B. WEBER. **II. Plant availability of paraquat.** J. B. WEBER, R. C. MEEK and S. B. WEED (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (3), 379–382. 11 ref.; 382–385. 13 ref.).—I. Both diquat (*D*) and paraquat (*P*) were adsorbed to the extent of 100% of the *CEC* by Na^+-, Ca^{2+}-, and Mg^{2+}-saturated bentonite. Adsorption by Na^+-saturated vermiculite (*V*), biotite, and phlogopite was also high but that by expanded muscovite was low; adsorption by the corresponding minerals saturated with Ca^{2+} or Mg^{2+} was relatively low. Less than 10% of the *P* and *D* were released from montmorillonite (*M*) equilibrated with 0·005 N-Cl^- solutions of 4 cations, but 20–80% was released from *V*. Release increased in the order K^+-, Mg^{2+}-, Ca^{2+}-, Al^{2+}-Cl^-.

II. Approx. 5–10% of the *P* adsorbed on a Ca^{2+}-*M* was available to cucumber seedlings in sand culture tests. Ca^{2+}-*M* was more effective than Ca^{2+}-*V* in decreasing the phytotoxicity of *P* to the seedlings. A. H. Cornfield.

Influence of simazine on apparent photosynthesis of aquatic plants and herbicide residue removal from water. D. L. SUTTON, D. A. DURHAM, S. W. BINGHAM and C. L. FOY (*Weed Sci.*, 1969, **17** (1), 56–59. 12 ref.).—Addn. of 0·12 to 1 ppm simazine to nutrient cultures of *Lemna minor*, *Elodea canadensis*, and *Myriophyllum brasiliense* reduced dissolved O_2 within 24 h, to an extent dependent on dose. *M. brasiliense* exhibited the greatest reduction in apparent photosynthesis. The plants reduced the concn. of simazine in soln. within 48 h of treatment. Inactivation of simazine by resistant aquatic plants could be important for the removal of the herbicide from an aquatic environment. A. H. Cornfield.

Crops, Livestock, Food

DDT and related chlorinated hydrocarbon residues on alfalfa [lucerne] hay exposed to drying by sunlight, ultra-violet light and air. T. E. ARCHER (*J. Dairy Sci.*, 1969, **52** (11), 1806–1811. 8 ref.).—Lucerne, sprayed with large concn. of DDT, was (*a*) air-dried in the dark, (*b*) dried in sunlight and (*c*) in u.v. light. (*a*) Resulted in 49% loss of the original residues, (*b*) after four days, resulted in 67% loss and (*c*) after six days, in 72% loss. M. O'Leary.

Effects of organic arsenical herbicides [methanearsonic acid] on cotton response and chemical residues. R. S. BAKER, H. F. ARLE, J. H. MILLER and J. T. HOLSTUN, JUN. (*Weed Sci.*, 1969, **17** (1), 37–40. 9 ref.).—Cotton plants were highly tolerant to sprays of methanearsonic acid (Na or Na_2 salts) at 2–3 lb/acre applied 2–3 times to cover the small weeds in drill rows, with min. contact of cotton leaves 2–3 in. above the soil. Topical application to cotton plants (2–4 in high) reduced yields only slightly, but applications made at later stages caused progressively severe reductions and delayed maturity. The As content of cottonseed from treated plots was not significantly higher than that from checks provided that applications were restricted to pre-bloom stages. The treatments had little effect on As content of the soils.
A. H. Cornfield.

Determination of lindane residues in peas by gas chromatography. M. ŽIGIĆ (*Kemija Ind.*, 1969, **18** (4), 245–247. Serbo-Cr., 14 ref.).—Lindane residues in peas were extracted and purified by g.c. on a Silicon fluid MS/Chromosorb W column at 184°. Recoveries were 91–120%. J. T. Greaves.

Effects of dichlobenil on two fishpond environments. O. B. COPE, J. P. MCCRAREN and L. ELLER (*Weed Sci.*, 1969, **17** (2), 158–165. 10 ref.).—Application of dichlobenil (*D*) at 10–40 ppm in wettable powder form or 10 lb per acre in granular form effectively controlled water weeds, but regrowth occurred after 3 months. *D* was present in bottom sediments up to 312 days after treatment. No mortality of fish occurred immediately after treatment, but day-to-

day mortality was serious, reproduction was affected and pathological symptoms were found in liver, kidney, and pancreas.
A. H. Cornfield.

Pollen gathering of honey bees reduced by pesticide sprays. F. E. Todd and C. B. Reed (*J. econ. Ent.*, 1969, **62** (4), 865–867. 6 ref.).—Measurement of the amount of pollen collected daily by *Apis mellifera* was made, using special traps. Application of naled spray to safflower and a mixture of toxaphene, endosulfan and trichlorfon to lucerne in the early morning caused a drop in pollen samples due to losses of field bees; the effects of the 2 sprays could not be separated, but naled appeared to cause most of the damage. The amount of brood in the colony 3 weeks before spray damage, had a great effect on the colony's recovery. C. M. Hardwick.

Residual free methyl bromide in fumigated commodities. K. A. Scudamore and S. G. Heuser (*Pestic. Sci.*, 1970, **1** (1), 14–17. 12 ref.).—The extent to which MeBr (**I**) was retained by fumigated material after treatment was followed in lab. studies on a range of commodities exposed to the vapour at atm. pressure. Amounts of **I**, recovered by solvent extraction and determined by g.l.c. were related to the temp., moisture content and manner of post-treatment storage. Immediately after exposure, the initial amt. of free **I** was more dependent on the gas concn. used than on the time of exposure. Under the exptl. conditions of exposure, the residual free **I** in all commodities fumigated at 25°, except cocoa beans and groundnuts, fell to <1 ppm within a few days when they were held at that temp., whether spread in thin layers on trays or kept sealed in glass bottles. At lower temp., the rate of loss was slower, small amounts of **I** being extracted from several commodities one month after treatment. The disappearance of fumigant from wheat and sultanas was more rapid from samples with higher moisture contents. A mathematical treatment of the data is presented, to assist in prediction of the behaviour of residual fumigant under storage conditions before processing. It is concluded that the risk of ingestion of harmful quantities of free **I** by the consumer is small and that the occasions when relatively high residues might occur can be predicted. P. C. W.

Pesticide residues in the total diet in England and Wales, 1966–1967. III. Organophosphorus pesticide residues in the total diet. D. C. Abbott, S. Crisp, K. R. Tarrant and J. O'G. Tatton (*Pestic. Sci.*, 1970, **1** (1), 10–13. 17 ref.).—Details are given of the methods of analysis used in the determination of organophosphorus pesticide residues in each of the food groups into which the total diet samples were divided. Residues of only six pesticides were detected. Malathion was the most commonly found, mainly in the cereal foods group of the diet, but all the residues were at low levels. P. C. W.

4.—MISCELLANEOUS

Agricultural accidents: study of 132 patients seen at Addenbrooke's Hospital, Cambridge, in 12 months. D. R. C. Cooper (*Br. med. J.*, 1969, **4** (5677), 193–198).—Machinery and implements were concerned in 50% of the accidents and animals in 10%. Immunity of the patients against tetanus was extremely low, only 9% being fully immunised and 56% never having received a course of prophylactic adsorbed tetanus toxoid. Tables give fuller information relating to the site, nature and cause of the injuries. C. V.

Rapid determination of nitrate in crops, soils, drainage and rainwater by a simple field method using diphenylamine or diphenylbenzidine with glass fibre paper. R. J. B. Williams (*Chemy Ind.*, 1969, (48), 1735–1736. 7 ref.).—The method is based on oxidn. of diphenylamine (**I**) or diphenylbenzidine (**II**) to a blue quinoid imonium ion by HNO_3 generated from the nitrate by action of conc. H_2SO_4. This spot test is made on a glass fibre filter paper impregnated with the sample plus reagent. Only ~20 mg of tissue, < 0·5 g of soil and one drop of rain- or drainage-water are required as sample. With **I**, a blue colour indicates > 25 ppm of NO_3-N and standard colour charts permit detn. of concn. of > 1000 ppm. In absence of colour, the test is repeated with **II**, which is sensitive for 0·5–10 ppm of NO_3-N. Estimated concn. are in agreement with detn. with the AutoAnalyzer. Fe (III), CrO_4^{2-} and MnO_4^- interfere. W. J. Baker.

New fluorometric method for determining ascorbic acid concentration and its application to blood plasma. B. Hubmann-Ballabey, D. Monnier and M. Roth (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1968, **59** (5), 482–484. Fr.).—When ascorbic acid reacts with dihydroxynaphthalenesulphonic acid in the quinone form (non-fluorescent), the oxidation–reduction system results in the formation of the fluorescent phenol form. The reaction is most rapid at pH 4 and the fluorescence max. occurs at 465 nm when excited at 330 nm. The detection limit is 0·1 μg/ml and the standard error ± 6%. J. B. Woof.

5.—RECENT BOOKS AND JOURNALS

Functioning of terrestrial ecosystems at the primary production level. Ed. F. E. Eckardt. 1968, 516 pp. (Paris: U.N.E.S.C.O.). —Proceedings of a Symposium held at Copenhagen, July 1965. S. C. H.

New approaches to breeding for improved plant protein. International Atomic Energy Agency and Food and Agricultural Organisation. 1969, 193 pp.,£2 1 8d. (Vienna: International Atomic Energy Agency).—Proceedings of a Panel Meeting organised by the joint FAO/IAEA Division of Atomic Energy in Food & Agriculture, held at Rostanga, Sweden, in June 1968. S. C. H.

Sisal. (Tropical Agric. Series) (G. W. Lock. 1969, 2nd edition, xix + 365 pp., 63 plates. Price: 60/-. (London: Longmans, Green and Co.).—The book is divided into 15 chapters. An outline is given of the history of sisal growing together with detailed descriptions of the sisal plant and other agaves. The account is based on 25-yr research and includes detailed results relating to: planting material and nursery technique; planting prepn.; spacing; cultivation systems; cutting and manurial trials; plant nutrition and deficiency diseases; pests and diseases; soils in sisal-growing areas; breeding long fibre agaves; characteristics of sisal fibre; decortication and fibre prepn. Estate planning and economic aspects are also discussed. (184 ref.). W. J. G.

Fish population dynamics: the biological background for rational exploitation and management of fishery resources. G. V. Nikolskii, transl. J. E. S. Bradley, ed. R. Jones. 1969, 323 pp., £8 10s. (Edinburgh: Oliver & Boyd). S. C. H.

Byproducts of the cane sugar industry: an introduction to their industrial utilisation. J. M. Paturau. 1969, 274 pp. (Amsterdam, etc.: Elsevier Publishing Co.). S. C. H.

Milk pasteurisation. C. W. Hall and G. M. Trout. 1968, 234 pp. (Westport, Conn.: AVI Publishing Co. Inc.). S. C. H.

Food processing plant. Vol. 1. F. H. Slade. 1967, 381 pp., 110/-. (London: Leonard Hill Books). S. C. H.

Fluorescent protein tracing. Ed. R. C. Nairn. 1969, 3rd edn., 503 pp., 60/-. (Edinburgh & London: E. & S. Livingstone Ltd.). S. C. H.

Enzymes of the bacterial wall. J. E. Kirk. 1969, 494 pp. (New York & London: Academic Press). S. C. H.

Energy metabolism. A. T. Miller, jun. 1968, 186 pp., 42/-. (Philadelphia: F. A. Davis Co.). S. C. H.

Viruses in plant hosts: form, distribution and pathologic effects. K. Esau. 1968, 225 pp., $10. (Madison, Milwaukee & London: Univ. of Wisconsin Press).—The 1968 John Charles Walker Lectures. S. C. H.

Pests of protected cultivation: biology and control of glasshouse and mushroom pests. N. W. Hussey, W. H. Read and J. J. Hesling. 1969, 404 pp., 8 gns. (London: Edward Arnold (Publishers) Ltd.). S. C. H.

Capillary methods of investigating micro-organisms. B. V. Perfil'ev and D. R. Gabe, transl. J. M. Shewan. 1969, 627 pp., £9 10s. (Edinburgh: Oliver & Boyd) S. C. H.

The bacterial spore. Ed. G. W. Gould and A. Hurst. 1969, 724 pp., 180/-. (London & New York: Academic Press). S. C. H.

Toxic constituents of plant foodstuffs. Ed. I. E. Liener. 1969, 500 pp. (New York & London: Academic Press). S. C. H.

Natural antinutritive substances in foodstuffs and forages. I. Gontzea and P. Sutzescu. 1968, 2nd edition, viii + 184 pp., 8 fig., 18 tables. Price 87/-. (New York: S. Karger AG. Basel.). —The book is divided into sections: **I. General consideration of the nutritive value of foodstuffs. II. Antinutritive substances of natural origin.** This includes general concepts and classification. **III. Group A. Substances depressing utilisation of proteins** (264 ref.).

This includes chapters on: the antiprotease of egg white; trypsin inhibitor in colostrum and milk; inhibitors in Leguminosae seeds; antipyretic effect of wheat flour; gossypol in cotton-seeds. **IV. Group B. Substances depressing utilisation of minerals** (349 ref.). This includes chem. structure, distribution in foodstuffs, nutritional effects, mode of action and methods of control of phytic acid, oxalic acid and natural antithyroid substances. **V. Group C. Substances inactivating vitamins.** Similar aspects are dealt with for ascorbic acid, thiaminase, avidin, dicoumarol, antipyroxidine and antinutritive substances in maize. W. J. G.

New horizons for chemistry and industry in the 1990s. SOCIETY OF CHEMICAL INDUSTRY. 1970, 194 pp. (London: Society of Chemical Industry).—Proceedings of a Soc. Chem. Ind. Symposium held at Lancaster Univ., 7–11th July 1969. Six subject groups included one on food and agriculture (pp. 43–86), where topics covered were the use of chemicals to increase soil productivity, the relationship between pesticides and crop yields, separation of protein concentrates from green leaves and seeds, animals and micro-organisms as food producers, and synthesis, preparation and presentation of food. P. C. W.

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