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

Published by the Society of Chemical Industry

Volume 19 No. 2 February, 1968

SOCIETY OF CHEMICAL INDUSTRY

FOUNDED IN 1881 INCORPORATED BY ROYAL CHARTER 1907

Offices of the Society: 14 Belgrave Square, S.W.1
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DETERMINATION OF ALKYL DINITROPHENYL CARBONATES AND OTHER ESTERS, WITH PARTICULAR REFERENCE TO DINOBUTON, IN FRUIT AND VEGETABLES*

By H. CROSSLEY and V. P. LYNCH

A method for determining residues of dinobuton in crops has been developed. It is based on the extraction of the crop with hexane followed by a clean-up involving acid potassium permanganate solution, and chromatography on neutral alumina. Dinobuton is converted on the alumina column to an aluminium salt of dinoseb, which is eluted with aqueous acetone containing butylamine, and determined spectrophotometrically at 378 mμ. The method is applicable to other alkyl dinitrophenyl esters.

Introduction

With the introduction of dinobuton[1,2] for control of mites and mildew, it became necessary to develop a method for determining its residues in various crops. Consideration was given to published methods for the related compound dinocap. The most suitable approach appeared to be to measure the intensely coloured dinitrophenate ion produced on hydrolysis of the compounds in aqueous media.

Rosenthal *et al.*[3] hydrolysed dinocap with aqueous pyridine and measured the resultant colour at 442 μ. They separated dinocap from interfering plant extract by steam distillation. Careful control of the distillation was needed and recoveries varied from 55 to 100% of those obtained in the absence of plant extract.

Baker & Skerrett[4] modified the method of Rosenthal *et al.* by hydrolysing the benzene washings of the fruit or leaves with tetraethylammonium hydroxide. They were, however, only concerned with surface deposits and it was considered that a more stringent clean-up would be required if the method was to be applicable to residue determination on the whole fruit. It was therefore decided to adopt a chromatographic clean-up.

Experimental

A study was made of the behaviour of dinobuton [2-(1-methyl-n-propyl)-4,6-dinitrophenyl isopropyl carbonate] on several types of chromatographic adsorbents. It was found that when a solution of this compound in hexane or benzene was applied to a neutral grade I alumina column, a tightly held yellow band was formed. The yellow band was found to be unaffected by such solvents as chloroform, acetonitrile and some alcohols, but could be eluted with water or aqueous acetone. The absorption spectrum of the resulting aqueous eluate corresponded to that of the potassium salt of dinoseb (the parent dinitrophenol of dinobuton) (Fig. 1). It was therefore assumed that dinobuton had become converted to an aluminium salt of the dinitrophenol and was held as such on the column. Further experiments confirmed that the adsorption and subsequent decomposition was quantitative and took place from either dilute or concentrated solution (Fig. 2), the only limiting factor being column dimensions. Presence of plant extract did not inhibit adsorption but merely led to a more diffuse band. Examination of a number of related compounds (Table I) including dinocap and binapacryl showed that these substances behave similarly. As was expected the parent alkyl dinitrophenols behave similarly in that they are quantitatively adsorbed on the alumina column.

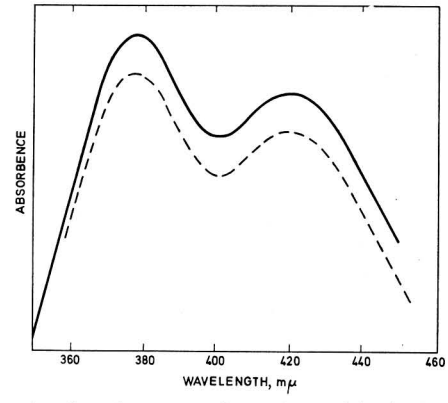

FIG. 1. *Absorption spectra of potassium- and butylammonium dinoseb*

———— Absorption spectrum of potassium dinoseb in aqueous acetone (pure specimen)
– – – – Absorption spectrum of the butylamine salt of dinoseb derived from dinobuton on an alumina column

*The substance of this paper was presented at the Vth International Pesticides Congress, London, 1963.

TABLE I

Structures of compounds mentioned in this paper
2,4,6-$R^1.C_6H_2(NO_2)_2.O.CX.R$

R^1	X	R
H	O	OEt
Me	O	OEt
Bu^s	O	OEt
Bu^s	O	SEt
Bu^s	O	$OCHMe_2$
Bu^s	O	OPh
Bu^s	O	OC_8H_{17}
Bu^s	S	OEt
Bu^t	O	OEt
Bu^t	O	SEt
Bu^t	O	$OCHMe_2$
Bu^t	O	OPh
Bu^t	O	OC_8H_{17}
CHMePr	O	OEt
CHMePr	O	$OCHMe_2$
CHMeHexyl	O	OEt
CHMeHexyl	O	OMe
CHMeHexyl	O	SMe
Cyclohexyl	O	OEt
Ph	O	OEt
Bu^s	O	Me
Bu^s	O	$CH : CMe_2$
CHMeHexyl	O	CH : CHMe

The method described below is primarily for dinobuton but with slight modifications can be applied to other esters of alkyl dinitrophenols.

The sample is extracted with hexane using sodium sulphate *in situ* as dehydrating agent. The filtered extract is evaporated to dryness on a rotary film evaporator and the residue is dissolved in benzene. The benzene extract is subjected to an acid potassium permanganate wash and, after destruction of excess permanganate, phase separation and drying, the benzene solution, or an aliquot of it, is chromatographed on a grade I neutral alumina column. Interfering plant pigments are removed by washing the column with chloroform followed by a mixture of acetonitrile and propan-2-ol, and the parent dinitrophenol of dinobuton is eluted as its salt with aqueous acetone. The absorbence is measured against a reagent blank at 378 mμ.

Apparatus

The apparatus consisted of: a macerator, a 1 litre Buchner flask, a Buchner sintered glass funnel, a chromatographic column comprising a glass tube 10 cm long, 1·4 cm i.d. fitted with a tap at the lower end and a ground joint (B24) at the upper end to which is attached a 100 ml vessel serving as a reservoir.

Reagents

Benzene, reagent grade
Hexane b.p. 67–69°c.
Chloroform, reagent grade
3 : 2 Acetonitrile : propan-2-ol v/v
Neutral alumina (grade I Woelm)
1 : 1 Acetone : water v/v containing 1% n-butylamine
Potassium permanganate 0·1M in N sulphuric acid
Hydrogen peroxide 20 vol.
Sodium sulphate anhydrous, *neutral.*

Procedure

Preparation of column

Weigh 8 g neutral grade I alumina; place a cotton wool support in the column, add the alumina and tamp it to a depth of about 5 cm. Place a cotton wool pad on top of the alumina and tamp it firmly with a glass rod.

Preparation of standard curve

Prepare a benzene solution of dinobuton to contain 10 μg per ml. Transfer aliquots containing solute in the 0–200 μg range to 500 ml pear-shaped separating funnels. Adjust the volume in each funnel to 200 ml with benzene. Add 50 ml 0·1 M potassium permanganate solution and shake the funnels vigorously for 5 minutes. Add sufficient 20 vol. hydrogen peroxide to destroy the excess potassium permanganate. Allow the phases to separate, and run off the lower, aqueous, layer into a 250 ml separating funnel. Extract this aqueous solution with 50 ml benzene and bulk the benzene extracts. Wash the combined benzene extract with 25 ml water and discard the washing. Dry the benzene solution over sodium sulphate, filter and concentrate it to about 20 ml on a rotary-film evaporator. Transfer the concentrate to the chromatographic column, wash the flask with 3 × 5 ml benzene and add each wash to the column. Allow the benzene to drain through the column and then rinse the walls of the reservoir with about 5 ml benzene and allow the rinsing to drain through. Add 50 ml chloroform, allow it to wash through and then add 25 ml acetonitrile–propanol mixture and allow it to wash through. Elute the coloured bands with the aqueous acetone containing 1% n-butylamine and start collecting the eluates when the coloured bands reach a point 1 cm from the cotton-wool support. Collect a total volume of 10 ml. Read the absorbence of the solutions against a reagent blank, similarly prepared, at 378 mμ. Plot absorbence versus concentration.

Extraction procedure for apples and cucumbers

Weigh 100 g finely shredded sample into a macerator jar and add 250 ml hexane. Macerate the sample thoroughly for 2–3 minutes and then add sufficient anhydrous sodium sulphate to break any emulsion formed. Decant the supernatant liquid on to a sintered glass funnel containing a 1 cm layer of sodium sulphate. Repeat the extraction with a further 250 ml hexane. Finally transfer the residue from the macerator jar on to the filter funnel and wash it with about 50 ml hexane. Evaporate the extract to incipient dryness on a rotary film evaporator and remove final traces of solvent under a gentle stream of nitrogen. Dissolve the residue in 200 ml benzene and then proceed as for the standard curve starting at the addition of permanganate stage.

Extraction procedure for blackcurrants

The extraction procedure used for apples and cucumbers is inapplicable to blackcurrants. Instead of hexane as extracting solvent a 1 : 4 acetone–hexane mixture is used.

Weigh 100 g fruit into the macerator jar, add 200 ml acetone–hexane and macerate the mixture thoroughly for 2–3 minutes. Add 300 g anhydrous sodium sulphate and mix it well in. Filter the supernatant liquid through a sintered glass funnel containing a 1 cm layer of sodium sulphate. Repeat the extraction with a further 200 ml acetone–hexane. Finally transfer the residue to the filter funnel and wash it with about 50 ml solvent mixture.

Evaporate the extract to incipient dryness on a rotary film evaporator and remove the last traces of solvent under a gentle stream of nitrogen. Dissolve the residue in 200 ml benzene and proceed as for the standard curve starting at the addition of permanganate stage.

Additional clean-up technique

Crop blanks tend to vary with variety and maturity of the crop and, where the control values are too high, the following additional clean-up may be employed.

Collect the acetone–water eluate in a 50 ml separating funnel. Acidify with hydrochloric acid, add 20 ml hexane and shake it for one minute. Allow the phases to separate and discard the aqueous layer. Extract the hexane with 2 ml and then 1 ml 2% n-butylamine in acetonitrile. Collect each extract (lower layer) in a 5 ml volumetric flask and make it up to volume with acetone. Read the absorbence of the solution against a reagent blank at 378 mμ.

Refer to a standard calibration curve prepared by this method (Fig. 2).

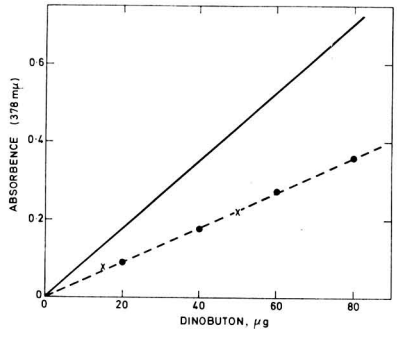

FIG. 2. *Calibration curve for dinobuton.*

– – – – Final volume 10 ml.
● dinobuton applied to column in 20 ml benzene
× dinobuton applied to column in 200 ml benzene
——— Final volume 5 ml (after additional clean-up procedure)

Recovery experiments

To determine the efficiency of extraction and overall recovery, the finely shredded crop samples were treated with hexane solutions of dinobuton at suitable levels. The samples were then extracted as described above. Examples of recovery values obtained are given in Table II.

TABLE II

Recovery of dinobuton from apples, blackcurrants and cucumber

Amount of dinobuton added (ppm) in recovery experiments	Recovery of dinobuton %		
	apples	blackcurrants	cucumber
0·20	109, 100	93	83
0·30			98, 95
0·40	94, 100		
0·60		97	98, 86
0·80	96		
0·90			93
1·00		85, 85, 84	
2·00	86, 90		

Control values gave <0·05 ppm 6 determinations except for blackcurrants for which they gave 0·06 ppm.

Discussion

Hexane was chosen as extracting solvent since it removed a minimum of interfering substances from crops and yielded satisfactory recovery values. Emulsion problems normally encountered with solvents such as benzene rarely occurred with hexane. With blackcurrants the acetone–hexane mixture proved to be more satisfactory than hexane alone.

The clean-up step using potassium permanganate was first tried on the hexane extract but this led to low recoveries since some degradation of dinobuton occurred. Benzene was chosen instead, because under the prescribed conditions, dinobuton is unaffected by potassium permanganate.

In certain cases, e.g. surface deposits, the hexane extract or washing may be chromatographed directly on the alumina column without the preliminary potassium permanganate clean-up. The absorption spectrum (Fig. 1) shows an absorption at 378 mμ and 425 mμ. The former wavelength was chosen since natural background contributed little to the absorbence.

Acknowledgments

We are grateful to Mr. D. R. Pritchard for his technical assistance and to Dr. M. Pianka for supplying the compounds in Table I.

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Received 3 July, 1967

References

1. Pianka, M., & Smith, C. B. F., *Chemy Ind.*, 1965, p. 1216
2. Pianka, M., *J. Sci. Fd Agric.*, 1966, **17**, 47
3. Rosenthal, I., Gordon, C. F., Stanley, E. L., & Perlman, M. H., *J. agric. Fd Chem.*, 1957, **5**, 914
4. Skerrett, E. J., & Baker, E. A., *Analyst, Lond.*, 1962, **87**, 228

STRUCTURES AND PESTICIDAL ACTIVITIES OF DERIVATIVES OF DINITROPHENOLS

VIII.*—Effects of substitution with C_5 to C_{13}-s-alkyl groups and of esterification on the acaricidal activity of dinitrophenols

By M. PIANKA and J. D. EDWARDS

38 methyl-, 37 ethyl- and 19 other alkyl-carbonates, 37 crotonates, 10 acrylates and 17 other esters, and 15 methyl ethers of 2-(C_5 to C_{13}-s-alkyl)-4,6-dinitro- and 4-(C_4 to C_{13}-s-alkyl)-2,6-dinitrophenols were synthesised, and their activities against *Tetranychus telarius* (greenhouse red spider mite) were investigated. 2-s-Alkyl-4,6-dinitrophenols and esters were more active than their 4-s-alkyl-2,6-dinitro- analogues, acaricidal activity remaining high with the 4,6-dinitrophenols up to 2-(C_{11}-s-alkyl). Generally compactness of the 2-s-alkyl group aided activity. Methyl ethers had very low activity. Esters of 2-(C_3 to C_7-s-alkyl)-4,6-dinitrophenols were more acaricidal than the parent phenols, but the reverse was the case with C_8 to C_{13}-s-alkyl compounds. Crotonates and other esters were generally less active than methyl carbonates. The methyl carbonates of 2-(1-ethylhexyl)- and 2-(1-propylpentyl)-4,6-dinitrophenols were found to be of particular economic interest as acaricides.

Introduction

In Part I[1] the acaricidal activity of dinitrophenols and their carbonates was examined and in Part III[2] that of dinitro-*m*-cresols and their carbonates with nuclear normal and α-methyl alkyl groups containing up to five carbon atoms was examined. High activity was given by phenols and *m*-cresols containing nitro-groups in the 4- and 6-positions and an alkyl group with an α-methyl substituent in the 2-position.

In a series of ethyl carbonates of 2-alkyl-4,6-dinitrophenols, the C_3-alkyl (isopropyl) compounds showed moderate activity, the C_4-alkyl (s-butyl and t-butyl) and C_5-alkyl (1-methylbutyl) compounds high activity, and the C_8-alkyl (1-methylheptyl) ones lower activity.[3,4]

4-s-Butyl- and 4-t-butyl-2,6-dinitrophenols[1] and their carbonates[1] and 4-s-butyl-5-methyl-2,6-dinitrophenyl carbonates[2] had low to moderate activity.

The effects on acaricidal activity of size, shape and position of the alkyl groups containing 5 to 13 carbon atoms in 2-alkyl-4,6-dinitro- and 4-alkyl-2,6-dinitrophenols and the effect of their esterification and etherification have been examined.

Experimental

Derivation of compounds

Chloroformates

All the chloroformates used here have been described in the literature. Methyl-, ethyl- and isopropyl-chloroformates were obtained commercially. Methyl thiolo-, propyl-, butyl-, s-butyl-, isobutyl-, hexyl-, octyl-, 1-methylheptyl- and decyl-chloroformates were prepared by the method described in Part I.[1]

Acid chlorides

These were prepared by methods described in the literature.

Phenols

2-Isopropyl-4,6-dinitrophenol (Compound No. 220), 2-s-butyl-4,6-dinitrophenol (No. 1), 4-s-butyl-2,6-dinitrophenol (No. 108) and 2-(1-methylbutyl)-4,6-dinitrophenol (No. 4) have been described in the literature. The compounds shown in Table I were prepared as described in Part IV.[5]

TABLE I

Compounds prepared as described in Part IV[5]

2-(1-R)-4,6-dinitrophenols		4-(1-R)-2,6-dinitrophenols	
Compound no.	R	Compound no.	R
7	ethylpropyl	111	methylbutyl
10	methylpentyl	112	ethylpropyl
13	ethylbutyl	115	methylpentyl
16	methylhexyl	116	ethylbutyl
19	ethylpentyl	119	methylhexyl
22	propylbutyl	122	ethylpentyl
25	methylheptyl	125	propylbutyl
30	ethylhexyl	126	methylheptyl
37	propylpentyl	131	ethylhexyl
40	methyloctyl	144	propylpentyl
43	ethylheptyl	149	methyloctyl
46	propylhexyl	152	ethylheptyl
49	butylpentyl	155	propylhexyl
52	ethyloctyl	158	butylpentyl
55	propylheptyl	160	ethyloctyl
58	butylhexyl	163	propylheptyl
61	propyloctyl	166	butylhexyl
64	butylheptyl	169	methyldecyl
67	pentylhexyl	170	ethylnonyl
70	pentylheptyl	171	propyloctyl
73	hexylheptyl	172	butylheptyl
		175	pentylhexyl
		176	ethyldecyl
		177	propylnonyl
		178	butyloctyl
		179	pentylheptyl
		180	propyldecyl
		181	butylnonyl
		182	pentyloctyl
		183	hexylheptyl

*Part VII, *J. chem. Soc.*, (c), 1967, 2618.

TABLE II

2-Alkyl-4,6-dinitrophenyl carbonates

No. of compound	Alkyl	Carbonate	M.p.	Appearance and n_D^{20} of oil or appearance of solid	Formula	Found: N, %	Required: N, %
8	1-Ethylpropyl	Methyl	66–69°	Orange pink crystals	$C_{13}H_{16}N_2O_7$	9·3	9·0
9	1-Ethylpropyl	Ethyl	43–46°	Orange pink crystals	$C_{14}H_{18}N_2O_7$	8·9	8·6
11	1-Methylpentyl	Methyl		Yellow, 1·5222	$C_{14}H_{18}N_2O_7$	8·6	8·6
12	1-Methylpentyl	Ethyl		Yellow, 1·5170	$C_{15}H_{20}N_2O_7$	8·4	8·2
14	1-Ethylbutyl	Methyl[a]	52·5–54°	Off-white prisms	$C_{14}H_{18}N_2O_7$	8·8	8·6
15	1-Ethylbutyl	Ethyl		Yellow, 1·5177	$C_{15}H_{20}N_2O_7$	8·0	8·2
17	1-Methylhexyl	Methyl		Yellow, 1·5197	$C_{15}H_{20}N_2O_7$	8·6	8·2
18	1-Methylhexyl	Ethyl		Yellow, 1·5145	$C_{16}H_{22}N_2O_7$	8·2	7·9
20	1-Ethylpentyl	Methyl		Yellow, 1·5210	$C_{15}H_{20}N_2O_7$	7·9	8·2
21	1-Ethylpentyl	Ethyl		Yellow, 1·5151	$C_{16}H_{22}N_2O_7$	7·9	7·9
23	1-Propylbutyl	Methyl	57–58°	Brown crystals	$C_{15}H_{20}N_2O_7$	8·6	8·2
24	1-Propylbutyl	Ethyl		Pale brown, 1·5137	$C_{16}H_{22}N_2O_7$	7·8	7·9
26	1-Methylheptyl	Methyl		Yellow, 1·5159	$C_{16}H_{22}N_2O_7$	7·9	7·9
27	1-Methylheptyl	Ethyl		Yellow, 1·5120	$C_{17}H_{24}N_2O_7$	7·5	7·6
28	1-Methylheptyl	Isopropyl		Yellow, 1·5075	$C_{18}H_{26}N_2O_7$	7·3	7·3
29	1-Methylheptyl	Methyl thiolo-		Yellow, 1·5404	$C_{16}H_{22}N_2O_6S$	7·6	7·6
31	1-Ethylhexyl	Methyl		Yellow, 1·5170	$C_{16}H_{22}N_2O_7$	7·8	7·9
32	1-Ethylhexyl	Ethyl		Yellow, 1·5133	$C_{17}H_{24}N_2O_7$	7·6	7·6
33	1-Ethylhexyl	Propyl		Orange, 1·5100	$C_{18}H_{26}N_2O_7$	7·2	7·3
34	1-Ethylhexyl	Isopropyl		Orange, 1·5088	$C_{18}H_{26}N_2O_7$	6·7	7·3
35	1-Ethylhexyl	Hexyl		Orange, 1·5041	$C_{21}H_{32}N_2O_7$	6·9	6·6
36	1-Ethylhexyl	Decyl		Orange, 1·4951	$C_{25}H_{40}N_2O_7$	5·8	5·8
38	1-Propylpentyl	Methyl[b]	66–68°	Fine white needles	$C_{16}H_{22}N_2O_7$	8·2	7·9
39	1-Propylpentyl	Ethyl		Red-brown, 1·514	$C_{17}H_{24}N_2O_7$	7·5	7·6
41	1-Methyloctyl	Methyl		Light red, 1·5139	$C_{17}H_{24}N_2O_7$	8·0	7·6
42	1-Methyloctyl	Ethyl		Light red, 1·5100	$C_{18}H_{26}N_2O_7$	7·6	7·3
44	1-Ethylheptyl	Methyl		Light red, 1·5159	$C_{17}H_{24}N_2O_7$	7·9	7·6
45	1-Ethylheptyl	Ethyl		Yellow, 1·5112	$C_{18}H_{26}N_2O_7$	6·5	7·3
47	1-Propylhexyl	Methyl		Orange, 1·5142	$C_{17}H_{24}N_2O_7$	7·5	7·6
48	1-Propylhexyl	Ethyl		Orange, 1·5098	$C_{18}H_{26}N_2O_7$	7·2	7·3
50	1-Butylpentyl	Methyl	47–48°	Cream coloured crystals	$C_{17}H_{24}N_2O_7$	7·8	7·6
51	1-Butylpentyl	Ethyl		Light red, 1·5112	$C_{18}H_{26}N_2O_7$	7·3	7·3
53	1-Ethyloctyl	Methyl		Yellow, 1·5128	$C_{18}H_{26}N_2O_7$	7·4	7·3
54	1-Ethyloctyl	Ethyl		Yellow, 1·5092	$C_{19}H_{28}N_2O_7$	7·2	7·1
56	1-Propylheptyl	Methyl		Yellow, 1·5134	$C_{18}H_{26}N_2O_7$	7·5	7·3
57	1-Propylheptyl	Ethyl		Yellow, 1·5094	$C_{19}H_{28}N_2O_7$	7·5	7·1
59	1-Butylhexyl	Methyl		Pale brown, 1·5123	$C_{18}H_{26}N_2O_7$	7·3	7·3
60	1-Butylhexyl	Ethyl		Pale brown, 1·5082	$C_{19}H_{28}N_2O_7$	6·9	7·1
62	1-Propyloctyl	Methyl		Yellow, 1·5102	$C_{19}H_{28}N_2O_7$	6·9	7·1
63	1-Propyloctyl	Ethyl		Yellow, 1·5071	$C_{20}H_{30}N_2O_7$	6·7	6·8
65	1-Butylheptyl	Methyl		Brown, 1·5112	$C_{19}H_{28}N_2O_7$	6·8	7·1
66	1-Butylheptyl	Ethyl		Brown, 1·5072	$C_{20}H_{30}N_2O_7$	7·3	6·8
68	1-Pentylhexyl	Methyl		Yellow, 1·5100	$C_{19}H_{28}N_2O_7$	6·6	7·1
69	1-Pentylhexyl	Ethyl		Yellow, 1·5063	$C_{20}H_{30}N_2O_7$	6·7	6·8
71	1-Pentylheptyl	Methyl		Yellow, 1·5087	$C_{20}H_{30}N_2O_7$	7·2	6·8
72	1-Pentylheptyl	Ethyl		Yellow, 1·5051	$C_{21}H_{32}N_2O_7$	6·8	6·6
74	1-Hexylheptyl	Methyl		Orange-yellow, 1·5069	$C_{21}H_{32}N_2O_7$	6·9	6·6
75	1-Hexylheptyl	Ethyl		Orange-yellow, 1·5048	$C_{22}H_{34}N_2O_7$	6·6	6·4

4-(1,1,3,3-Tetramethylbutyl)-2,6-dinitrophenol (*Compound No. 147*). A mixture of commercial *p*-t-octylphenol [described as containing 94% 4-(1,1,3,3-tetramethylbutyl)phenol] (20·6 g; 0·1 mole), ethylene dichloride (37 ml) and nitric acid (36%; 48 ml) was heated under reflux for 1·5 h. The organic layer was separated and washed thoroughly with 3% aqueous sodium sulphate. The yellow solid, m.p. 112–113°, that separated out after each washing was filtered off. Ethylene dichloride was then removed from the organic layer leaving a dark oil which was dissolved in light petroleum, b.p. 60–80°, then shaken with 20% aqueous sodium carbonate. The red solid that separated was filtered off, dissolved in hot water and acidified. The oil that separated was extracted with light petroleum, b.p. 40–60°, treated with charcoal and anhydrous sodium sulphate and filtered. The solid that was obtained on adding cyclohexylamine was filtered off. The *cyclohexylamine salt* of the phenol melts at 215° (from aqueous propan-2-ol). When a solution of the salt in methanol was acidified with conc. hydrochloric acid the *phenol* was obtained as light brown prisms, m.p. 50–51° (from methanol) (Found: N, 9·5. $C_{14}H_{20}N_2O_5$ requires N, 9·4%.)

Dinitrophenyl carbonates

The general method of preparation was essentially as described in Part I.[1] Alkyl dinitrophenol, in acetone, and potassium carbonate (1·1 equivalent), were heated under reflux for 1 h. The chloroformate (1·1 equivalent) was then added and the mixture was heated under reflux for 3 h. The solid was filtered off and washed with acetone. The volatile matter was then removed from the combined filtrate and washings on a steam-bath at 10–20 mm pressure. The residue was dissolved in benzene or light petroleum, washed

with 1% aqueous sodium carbonate and then with water. The organic layer was separated, dried (sodium sulphate) and the solvent was removed on the steam-bath at 1–2 mm pressure. When appropriate the residue was crystallised from a suitable solvent. The oils were not distilled. Yields ranged from 80 to 100%. The physical data of the carbonates are shown in Tables II and III.

2-s-Butyl-4,6-dinitrophenyl methyl and ethyl carbonates (Compounds Nos. 2 & 3), 2-(1-methylbutyl)-4,6-dinitrophenyl methyl and ethyl carbonates (Nos. 5 & 6) and 2-isopropyl-4,6-dinitrophenyl ethyl carbonate (No. 221) were prepared as described in Part I.[1]

Dinitrophenyl esters

The general method of preparation was essentially as described in Part VI.[6] Alkyldinitrophenol, in acetone, and potassium carbonate (1·1 equivalent) were heated under reflux for 1 h. To the stirred ice salt-cooled mixture the acid chloride (1·1 equivalent) was slowly added. The mixture was kept stirred at 0–5° for a further hour, allowed to stand for 16 h at room temp., then heated under reflux for 3 h. The solid was then filtered off and washed with acetone, and the combined filtrate and washings were worked up in the same way as the carbonates. When appropriate the residue was crystallised from a suitable solvent. The oils were not distilled. Yields ranged from 80 to 100% and were generally higher for the compounds with unsaturated ester chains. Yields of compounds with saturated ester chains could be improved by adding, after one hour's heating, a further 0·1 equivalent of potassium carbonate, then 0·1 equivalent of the acid chloride and proceeding as described above. The physical data of the esters are shown in Tables IV and V.

TABLE III

4-Alkyl-2,6-dinitrophenyl carbonates

No. of compound	Alkyl	Carbonate	M.p.	Appearance and n_D^{20} of oil or appearance of solid	Formula	Found: N, %	Required: N, %
109	s-Butyl	Methyl[c,d]	59·5–60°	Off-white prisms	$C_{12}H_{14}N_2O_7$	9·1	9·4
110	s-Butyl	Ethyl	33–34°	Brown crystals	$C_{13}H_{16}N_2O_7$	9·1	9·0
113	1-Ethylpropyl	Methyl[c]	66°	Cream coloured prisms	$C_{13}H_{16}N_2O_7$	9·4	9·0
114	1-Ethylpropyl	Ethyl		Yellow, 1·5183	$C_{14}H_{18}N_2O_7$	8·8	8·6
117	1-Ethylbutyl	Methyl		Red-brown, 1·5210	$C_{14}H_{18}N_2O_7$	8·3	8·6
118	1-Ethylbutyl	Ethyl		Red-brown, 1·5155	$C_{15}H_{20}N_2O_7$	8·2	8·2
120	1-Methylhexyl	Methyl		Brown, 1·5172	$C_{15}H_{20}N_2O_7$	8·0	8·2
121	1-Methylhexyl	Ethyl		Red-brown, 1·5131	$C_{16}H_{22}N_2O_7$	7·8	7·9
123	1-Ethylpentyl	Methyl		Gold coloured, 1·5188	$C_{15}H_{20}N_2O_7$	8·1	8·2
124	1-Ethylpentyl	Ethyl		Orange, 1·5148	$C_{16}H_{22}N_2O_7$	8·2	7·9
127	1-Methylheptyl	Methyl		Pale brown, 1·5129	$C_{16}H_{22}N_2O_7$	8·0	7·9
128	1-Methylheptyl	Ethyl		Pale brown, 1·5070	$C_{17}H_{24}N_2O_7$	7·7	7·6
129	1-Methylheptyl	Isopropyl		Yellow, 1·5040	$C_{18}H_{22}N_2O_7$	7·3	7·3
130	1-Methylheptyl	Methyl thiolo-		Pale brown, 1·5360 (n_D^{25})	$C_{16}H_{22}N_2O_6S$	7·6	7·6
132	1-Ethylhexyl	Methyl		Yellow, 1·5133 (n_D^{25})	$C_{16}H_{22}N_2O_7$	7·4	7·9
133	1-Ethylhexyl	Ethyl		Orange, 1·5084 (n_D^{25})	$C_{17}H_{24}N_2O_7$	7·8	7·6
134	1-Ethylhexyl	Propyl		Orange-brown, 1·5068	$C_{18}H_{26}N_2O_7$	7·3	7·3
135	1-Ethylhexyl	Isopropyl		Dark brown, 1·5062 (n_D^{20})	$C_{18}H_{26}N_2O_7$	7·4	7·3
136	1-Ethylhexyl	Butyl		Brown, 1·5049	$C_{19}H_{28}N_2O_7$	7·1	7·1
137	1-Ethylhexyl	s-Butyl		Orange, 1·5030	$C_{19}H_{28}N_2O_7$	7·0	7·1
138	1-Ethylhexyl	Isobutyl		Orange-brown, 1·5035	$C_{19}H_{28}N_2O_7$	6·9	7·1
139	1-Ethylhexyl	Hexyl		Orange-red 1·5012	$C_{21}H_{32}N_2O_7$	6·9	6·6
140	1-Ethylhexyl	Octyl		Orange, 1·4930	$C_{23}H_{36}N_2O_7$	6·2	6·2
141	1-Ethylhexyl	1-Methylheptyl		Orange, 1·4927	$C_{23}H_{36}N_2O_7$	6·7	6·2
142	1-Ethylhexyl	Decyl		Orange, 1·4926	$C_{25}H_{40}N_2O_7$	5·8	5·8
143	1-Ethylhexyl	Methyl thiolo-[d]	56–58°	Pale green prisms	$C_{16}H_{22}N_2O_6S$	7·6	7·6
145	1-Propylpentyl	Methyl		Orange, 1·5131	$C_{16}H_{22}N_2O_7$	7·9	7·9
146	1-Propylpentyl	Ethyl		Orange, 1·4995	$C_{17}H_{24}N_2O_7$	7·8	7·6
148	't-Octyl'*	Isopropyl[d]	57–58°	Cream prisms	$C_{18}H_{26}N_2O_7$	7·2	7·3
150	1-Methyloctyl	Methyl		Yellow, 1·5108	$C_{17}H_{24}N_2O_7$	7·9	7·6
151	1-Methyloctyl	Ethyl		Yellow, 1·5050	$C_{18}H_{26}N_2O_7$	6·9	7·3
153	1-Ethylheptyl	Methyl		Red-brown, 1·5136	$C_{17}H_{24}N_2O_7$	7·3	7·6
154	1-Ethylheptyl	Ethyl		Red-brown, 1·5091	$C_{18}H_{26}N_2O_7$	7·0	7·3
156	1-Propylhexyl	Methyl		Orange-red, 1·5126	$C_{17}H_{24}N_2O_7$	7·8	7·6
157	1-Propylhexyl	Ethyl		Orange-red, 1·5085	$C_{18}H_{26}N_2O_7$	7·4	7·3
159	1-Butylpentyl	Methyl	40–42°	Brown crystals	$C_{17}H_{24}N_2O_7$	8·1	7·6
161	1-Ethyloctyl	Methyl		Yellow, 1·5094	$C_{18}H_{26}N_2O_7$	6·7	7·3
162	1-Ethyloctyl	Ethyl		Yellow, 1·5054	$C_{19}H_{28}N_2O_7$	6·7	7·1
164	1-Propylheptyl	Methyl		Yellow, 1·5088	$C_{18}H_{26}N_2O_7$	7·5	7·3
165	1-Propylheptyl	Ethyl		Yellow, 1·5050	$C_{19}H_{28}N_2O_7$	7·1	7·1
167	1-Butylhexyl	Methyl		Yellow, 1·5102	$C_{18}H_{26}N_2O_7$	7·3	7·3
168	1-Butylhexyl	Ethyl		Yellow, 1·5063	$C_{19}H_{28}N_2O_7$	7·0	7·1
173	1-Butylheptyl	Methyl		Yellow, 1·5078	$C_{19}H_{28}N_2O_7$	7·5	7·1
174	1-Butylheptyl	Ethyl		Yellow, 1·5038	$C_{20}H_{30}N_2O_7$	6·8	6·8
184	1-Hexylheptyl	Methyl		Yellow, 1·5040	$C_{21}H_{32}N_2O_7$	6·6	6·6
185	1-Hexylheptyl	Ethyl		Yellow, 1·5009	$C_{22}H_{34}N_2O_7$	5·8	6·4

*Substantially 1,1,3,3-tetramethylbutyl

2-s-Butyl-4,6-dinitrophenyl crotonate (No. 76) and 2-(1-methylbutyl)-4,6-dinitrophenyl crotonate (No. 77) were prepared as described in Part VI.[6]

Dinitrophenyl methyl ethers

The method of preparation was essentially as described in Part I.[1] The potassium dinitrophenoxide (0·01 mole) was dehydrated by addition of toluene (20 ml) and then removal of toluene (10 ml) and any water present by distillation. Dimethyl sulphate (0·011 mole) was then added and the mixture was heated under reflux (oil-bath) for 6 h and occasionally shaken. It was cooled to room temp. and shaken with 10% aqueous sodium hydroxide (4 × 10 ml), then with water (2 × 10 ml). The organic layer was separated and treated with sodium sulphate and charcoal. The solvent was then removed (steam-bath) at 0·2 mm. The alkyldinitroanisoles were obtained as orange-brown or reddish brown oils in 82–93% yields. Lower yields were obtained when the reaction mixtures were heated under reflux for shorter times. No apparent methylation occurred when the potassium dinitrophenoxide was heated with methyl iodide in acetone under reflux for 1 h. The physical data of the anisoles are shown in Table VI.

Formulation of compounds

The compounds were formulated as 10–25% w/v emulsifiable concentrates in a suitable non-phytotoxic solvent (such as heavy naphtha) and emulsifier. The solutions were then diluted with water to the required concentrations.

Biological Results

The tests were carried out as described in Part I.[1]

Results from the different biological tests would be expected to be comparable and valid, since the tests were carried out over a short period of time.

The results are presented in Tables VII–XI. For completeness, the activity of the crotonates of 2-alkyl-4,6-dinitro- and 4-alkyl-2,6-dinitro-phenols is included in Table IX. The results obtained with carbonates other than methyl- and ethyl-carbonates are presented in Table X.

2-(1-Ethylhexyl)- and 2-(1-propylpentyl)-4,6-dinitrophenyl methyl carbonates

The differences in acaricidal activity of the three isomeric phenols of the C_8-alkyl series (Nos. 25, 30, 37) were not significant. Esterification of 2-(1-methylheptyl)-4,6-dinitrophenol to the methyl- (No. 26) or ethyl- (No. 27) carbonates, acrylate (No. 89) or crotonate (No. 90) (Tables VII & IX, Fig. 2), of 2-(1-ethylhexyl)-4,6-dinitrophenol (No. 30) to the ethyl carbonate (No. 32), butyrate (No. 92), acrylate (No. 93) or crotonate (No. 94) (Tables VII & IX, Fig. 3), and of 2-(1-propylpentyl)-4,6-dinitrophenol (No. 37) to the crotonate (No. 95) (Table VII) reduced the acaricidal activity of the phenols. However, esterification of 2-(1-ethylhexyl)- and 2-(1-propylpentyl)4,6-dinitrophenols to the methyl carbonates (No. 38) either had little effect on activity (No. 31) or brought about an increase in acaricidal activity (Table VII). 2-Alkyl-4,6-dinitrophenyl methyl carbonates were generally less phytotoxic than the parent phenols. 2-(1-Ethylhexyl)- and 2-(1-propylpentyl)-4,6-dinitrophenyl methyl carbonates thus combine high acaricidal activity with low phytotoxicity.[8] In extensive laboratory testing a mixture consisting substantially of the two methyl carbonates gave rapid kills of the motile stages of *Tetranychus telarius*, specially selected for its resistance to organophosphate insecticides. In field

TABLE IV

2-Alkyl-4,6-dinitrophenyl esters

No. of compound	Alkyl	Ester	Appearance and n_D^{20} of oil	Formula	Found: N, %	Required: N, %
78	1-Ethylpropyl	Crotonate	Orange, 1·5432	$C_{15}H_{18}N_2O_6$	8·8	8·7
79	1-Methylpentyl	Acetate	Pale green, 1·5261	$C_{14}H_{18}N_2O_6$	9·3	9·0
80	1-Methylpentyl	Acrylate	Pale green, 1·5362	$C_{15}H_{18}N_2O_6$	9·0	8·7
81	1-Methylpentyl	Crotonate	Yellow, 1·5352	$C_{16}H_{20}N_2O_6$	8·0	8·3
82	1-Ethylbutyl	Crotonate	Orange-yellow, 1·5386	$C_{16}H_{20}N_2O_6$	7·9	8·3
83	1-Methylhexyl	Acetate	Pale green, 1·5211	$C_{15}H_{20}N_2O_6$	8·4	8·6
84	1-Methylhexyl	Acrylate	Pale yellow, 1·5332	$C_{16}H_{20}N_2O_6$	8·4	8·3
85	1-Methylhexyl	Crotonate	Yellow, 1·5328	$C_{17}H_{22}N_2O_6$	7·6	8·0
86	1-Ethylpentyl	Crotonate	Yellow, 1·5350	$C_{17}H_{22}N_2O_6$	8·1	8·0
87	1-Propylbutyl	Crotonate	Yellow, 1·5304	$C_{17}H_{22}N_2O_6$	7·6	8·0
88	1-Methylheptyl	Acetate	Yellow, 1·5195	$C_{16}H_{22}N_2O_6$	8·5	8·3
89	1-Methylheptyl	Acrylate	Yellow, 1·5278	$C_{17}H_{22}N_2O_6$	8·2	8·0
90	1-Methylheptyl	Crotonate	Yellow, 1·5306	$C_{18}H_{24}N_2O_6$	7·5	7·7
91	1-Ethylhexyl	Acetate	Orange, 1·5216	$C_{16}H_{22}N_2O_6$	8·3	8·3
92	1-Ethylhexyl	Butyrate	Orange, 1·5160	$C_{18}H_{26}N_2O_6$	7·7	7·6
93	1-Ethylhexyl	Acrylate	Pale brown, 1·5295	$C_{17}H_{22}N_2O_6$	7·6	8·0
94	1-Ethylhexyl	Crotonate	Yellow, 1·5282	$C_{18}H_{24}N_2O_6$	7·7	7·7
95	1-Propylpentyl	Crotonate	Red-brown, 1·5330	$C_{18}H_{24}N_2O_6$	7·7	7·7
96	1-Methyloctyl	Crotonate	Pale brown, 1·5249	$C_{19}H_{26}N_2O_6$	7·3	7·4
97	1-Ethylheptyl	Crotonate	Pale red, 1·5283	$C_{19}H_{26}N_2O_6$	6·9	7·4
98	1-Propylhexyl	Crotonate	Orange, 1·5241	$C_{19}H_{26}N_2O_6$	6·9	7·4
99	1-Butylpentyl	Crotonate	Pale red, 1·5278	$C_{19}H_{26}N_2O_6$	7·5	7·4
100	1-Ethyloctyl	Crotonate	Yellow, 1·5234	$C_{20}H_{28}N_2O_6$	6·6	7·1
101	1-Propylheptyl	Crotonate	Yellow, 1·5253	$C_{20}H_{28}N_2O_6$	7·1	7·1
102	1-Butylhexyl	Crotonate	Yellow, 1·5260	$C_{20}H_{28}N_2O_6$	6·9	7·1
103	1-Propyloctyl	Crotonate	Yellow, 1·5220	$C_{21}H_{30}N_2O_6$	7·1	6·9
104	1-Butylheptyl	Crotonate	Pale brown, 1·5236	$C_{21}H_{30}N_2O_6$	7·4	6·9
105	1-Pentylhexyl	Crotonate	Yellow, 1·5214	$C_{21}H_{30}N_2O_6$	6·9	6·9
106	1-Pentylheptyl	Crotonate	Dark yellow, 1·5181	$C_{22}H_{32}N_2O_6$	6·7	6·7
107	1-Hexylheptyl	Crotonate	Orange-yellow, 1·5162	$C_{23}H_{34}N_2O_6$	6·6	6·5

TABLE V

4-Alkyl-2,6-dinitrophenyl esters

No. of compound	Alkyl	Ester	M.p.	Appearance and n_D^{20} of oil or appearance of solid	Formula	Found: N, %	Required: N, %
186	s-Butyl	Crotonate[c,d]	65°	Cream prisms	$C_{14}H_{16}N_2O_6$	8·7	9·1
187	1-Ethylpropyl	Crotonate		Yellow, 1·5394	$C_{15}H_{18}N_2O_6$	8·8	8·7
188	1-Ethylbutyl	Crotonate		Red-brown, 1·5370	$C_{16}H_{20}N_2O_6$	7·7	8·3
189	1-Methylhexyl	Crotonate		Yellow, 1·5257	$C_{17}H_{22}N_2O_6$	8·3	8·4
190	1-Ethylpentyl	Crotonate		Orange, 1·5236	$C_{17}H_{22}N_2O_6$	7·7	8·0
191	1-Propylbutyl	Acetate[e]	60–61°	White needles	$C_{15}H_{20}N_2O_6$	8·6	8·6
192	1-Propylbutyl	Acrylate		Yellow, 1·5284	$C_{16}H_{20}N_2O_6$	8·2	8·3
193	1-Methylheptyl	Acrylate		Yellow, 1·5255	$C_{17}H_{22}N_2O_6$	8·1	8·0
194	1-Methylheptyl	Crotonate		Pale brown, 1·5236	$C_{18}H_{24}N_2O_6$	7·7	7·7
195	1-Ethylhexyl	Acetate[e]	51·5–53°	Yellow prisms	$C_{16}H_{22}N_2O_6$	8·5	8·3
196	1-Ethylhexyl	Butyrate		Yellow, 1·5110	$C_{18}H_{26}N_2O_6$	7·5	7·6
197	1-Ethylhexyl	Isobutyrate		Red-brown, 1·5100	$C_{18}H_{26}N_2O_6$	7·7	7·6
198	1-Ethylhexyl	Octanoate		Red-brown, 1·5040	$C_{22}H_{34}N_2O_6$	6·4	6·6
199	1-Ethylhexyl	Chloroacetate		Pale brown, 1·5278	$C_{16}H_{27}ClN_2O_6$	7·5	7·5
200	1-Ethylhexyl	2-Chloropropionate		Orange-brown, 1·5202	$C_{17}H_{23}ClN_2O_6$	Cl, 9·7 N, 7·0	Cl, 9·2 N, 7·2
201	1-Ethylhexyl	3-Chloropropionate		Orange-brown, 1·5243	$C_{17}H_{23}ClN_2O_6$	Cl, 9·6 N, 6·9	Cl, 9·2 N, 7·2
202	1-Ethylhexyl	Acrylate		Orange, 1·5220	$C_{17}H_{22}N_2O_6$	8·0	8·0
203	1-Ethylhexyl	Crotonate		Yellow, 1·5250	$C_{18}H_{24}N_2O_6$	7·6	7·7
204	1-Ethylhexyl	Methacrylate	44–45°	Cream coloured crystals	$C_{18}H_{24}N_2O_6$	7·5	7·7
205	1-Ethylhexyl	3-Methylcrotonate		Orange-brown, 1·5297	$C_{19}H_{26}N_2O_6$	7·4	7·4
206	1-Ethylhexyl	Benzoate		Brown, 1·5585	$C_{21}H_{24}N_2O_6$	7·0	7·0
207	1-Ethylhexyl	*p*-Chlorobenzoate		Orange, 1·5604	$C_{21}H_{23}ClN_2O_6$	Cl, 8·1 N, 6·3	Cl, 8·2 N, 6·4
208	1-Propylpentyl	Crotonate		Dark orange, 1·5272	$C_{18}H_{24}N_2O_6$	7·3	7·7
209	1-Methyloctyl	Crotonate		Yellow, 1·5229	$C_{19}H_{26}N_2O_6$	7·2	7·4
210	1-Ethylheptyl	Crotonate		Red-brown, 1·5255	$C_{19}H_{26}N_2O_6$	7·3	7·4
211	1-Propylhexyl	Crotonate		Orange-red, 1·5240	$C_{19}H_{26}N_2O_6$	7·3	7·4
212	1-Ethyloctyl	Acrylate		Orange, 1·5197	$C_{19}H_{26}N_2O_6$	7·8	7·4
213	1-Ethyloctyl	Crotonate		Yellow, 1·5210	$C_{20}H_{28}N_2O_6$	6·8	7·1
214	1-Propylheptyl	Acrylate		Orange, 1·5200	$C_{19}H_{26}N_2O_6$	7·5	7·4
215	1-Propylheptyl	Crotonate		Yellow, 1·5216	$C_{20}H_{28}N_2O_6$	7·4	7·1
216	1-Butylhexyl	Acrylate		Yellow, 1·5202	$C_{19}H_{26}N_2O_6$	7·5	7·4
217	1-Butylhexyl	Crotonate		Pale yellow, 1·5211	$C_{20}H_{28}N_2O_6$	7·2	7·1
218	1-Butylheptyl	Crotonate		Yellow, 1·5201	$C_{21}H_{30}N_2O_6$	7·1	6·9
219	1-Hexylheptyl	Crotonate		Yellow, 1·5152	$C_{23}H_{24}N_2O_6$	6·6	6·4

Solvents for crystallisation used for certain compounds in Tables II–V:
[a] light petroleum, b.p. 40–60°; [b] propan-2-ol; [c] ethanol; [d] methanol; [e] light petroleum, b.p. 60–80°

TABLE VI

2-Alkyl-4,6-dinitrophenyl methyl ethers

No. of compound	Alkyl	n_D^{20}	Formula	Found: N, %	Required: N, %
222	1-Methylheptyl*	1·5303	$C_{15}H_{22}N_2O_5$	9·2	9·0
223	1-Ethylhexyl*	1·5298	$C_{15}H_{22}N_2O_5$	8·9	9·0
224	1-Propylpentyl*	1·5324	$C_{15}H_{22}N_2O_5$	9·1	9·0
225	1-Ethylheptyl	1·5273	$C_{16}H_{24}N_2O_5$	8·8	8·6
226	1-Propylhexyl	1·5282	$C_{16}H_{24}N_2O_5$	8·8	8·6
227	1-Butylpentyl	1·5798	$C_{16}H_{24}N_2O_5$	8·7	8·6
228	1-Hexylheptyl	1·516	$C_{20}H_{32}N_2O_5$	7·3	7·4
	4-Alkyl-2,6-dinitrophenyl methyl ethers				
229	1-Ethylpentyl	1·5246	$C_{14}H_{20}N_2O_5$	9·6	9·5
230	1-Methylheptyl*	1·5206	$C_{15}H_{22}N_2O_5$	9·3	9·0
231	1-Ethylhexyl*	1·5225	$C_{15}H_{22}N_2O_5$	9·1	9·0
232	1-Propylpentyl*	1·5222	$C_{15}H_{22}N_2O_5$	8·9	9·0
233	1-Methyloctyl	1·5184	$C_{16}H_{24}N_2O_5$	9·0	8·6
234	1-Propylhexyl	1·5190	$C_{16}H_{24}N_2O_5$	9·0	8·6
235	1-Butylpentyl	1·515	$C_{16}H_{24}N_2O_5$	9·2	8·6
236	1-Butyloctyl	1·5215	$C_{19}H_{30}N_2O_5$	7·9	7·7

*Clifford, Watkins and Woodcock[9] referred to the compound, but did not report its preparation, physical data or analysis

TABLE VII

Contact acaricidal activity of 2-alkyl-4,6-dinitrophenols, their methyl- and ethyl-carbonates and their crotonates

Alkyl	No. of carbon atoms in alkyl group	Phenols					Methyl carbonates					Ethyl carbonates					Crotonates				
		No. of compound	Kill of *T. telarius* (%) at ppm				No. of compound	Kill of *T. telarius* (%) at ppm				No. of compound	Kill of *T. telarius* (%) at ppm				No. of compound	Kill of *T. telarius* (%) at ppm			
			100	30	10	3		100	30	10	3		100	30	10	3		100	30	10	3
Isopropyl	3	220	99	34	30	–	–	–	–	–	–	221	98	80	75	–	–	–	–	–	–
s-Butyl	4	1	100	50	35	–	2	100	100	98	56	3	100	100	99	96	76	100	100	97	95
1-Methylbutyl	5	4	100	70	65	–	5	100	100	100	91	6	100	100	99	99	77	100	100	100	91
1-Ethylpropyl	5	7	100	100	92	65	8	100	100	96	37	9	100	100	100	95	78	100	100	100	99
1-Methylpentyl	6	10	100	96	59	–	11	100	98	80	61	12	100	89	83	–	81	100	81	61	–
1-Ethylbutyl	6	13	100	100	73	–	14	100	100	98	69	15	100	99	99	79	82	100	100	100	98
1-Methylhexyl	7	16	99	75	47	–	17	99	95	78	–	18	99	75	58	–	85	94	65	70	–
1-Ethylpentyl	7	19	65	53	36	–	20	92	84	81	–	21	98	83	57	–	86	99	74	70	–
1-Propylbutyl	7	22	100	100	91	53	23	100	100	98	81	24	100	100	92	56	87	100	95	85	–
1-Methylheptyl	8	25	99	99	87	33	26	81	78	61	–	27	96	57	38	–	90	73	59	23	–
1-Ethylhexyl	8	30	100	100	88	58	31	100	94	73	61	32	96	80	60	–	94	85	74	38	9
1-Propylpentyl	8	37	100	100	61	–	38	99	100	98	57	39	100	95	83	–	95	92	79	69	–
1-Methyloctyl	9	40	100	100	90	38	41	100	71	52	–	42	78	55	47	–	96	80	64	35	–
1-Ethylheptyl	9	43	100	83	61	–	44	72	32	38	–	45	47	38	39	–	97	40	29	20	–
1-Propylhexyl	9	46	100	100	99	52	47	100	97	92	84	48	100	90	58	–	98	84	49	47	–
1-Butylpentyl	9	49	100	100	92	77	50	100	86	84	60	51	100	92	29	–	99	64	40	51	–
1-Ethyloctyl	10	52	100	56	48	–	53	57	0	0	–	54	40	40	38	–	100	44	0	0	–
1-Propylheptyl	10	55	100	100	85	74	56	87	70	40	–	57	87	75	40	–	101	45	24	24	–
1-Butylhexyl	10	58	100	100	96	87	59	87	80	45	–	60	77	56	38	41	102	59	40	36	–
1-Propyloctyl	11	61	100	100	86	–	62	80	60	54	–	63	77	46	24	–	103	60	39	21	–
1-Butylheptyl	11	64	100	90	61	–	65	67	70	49	–	66	33	17	12	–	104	66	39	37	–
1-Pentylhexyl	11	67	100	100	84	77	68	88	66	35	–	69	74	73	67	–	105	39	40	36	–
1-Pentylheptyl	12	70	90	77	57	–	71	52	0	0	–	72	44	29	24	–	106	61	49	34	–
1-Hexylheptyl	13	73	100	84	55	–	74	26	32	16	–	75	24	13	–	–	107	16	12	3	–

TABLE VIII

Contact acaricidal activity of 4-alkyl-2,6-dinitrophenols, their methyl- and ethyl-carbonates and their crotonates

Alkyl	No. of carbon atoms in alkyl group	Phenols					Methyl carbonates					Ethyl carbonates					Crotonates				
		No. of compound	Kill of *T. telarius* (%) at ppm				No. of compound	Kill of *T. telarius* (%) at ppm				No. of compound	Kill of *T. telarius* (%) at ppm				No. of compound	Kill of *T. telarius* (%) at ppm			
			300	100	30	10		300	100	30	10		300	100	30	10		300	100	30	10
s-Butyl	4	108	–	55	43	39	109	57	58	42	43	110	44	40	32	23	186	89	64	29	–
1-Methylbutyl	5	111	72	36	25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Ethylpropyl	5	112	–	14	3	–	113	–	55	48	45	114	–	49	29	–	187	–	82	71	66
1-Methylpentyl	6	115	76	43	39	29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Ethylbutyl	6	116	65	58	50	41	117	52	42	36	27	118	47	30	28	–	188	77	60	50	18
1-Methylhexyl	7	119	92	54	39	–	120	63	55	–	–	121	65	37	–	–	189	79	67	42	–
1-Ethylpentyl	7	122	95	86	71	–	123	65	43	51	–	124	54	53	47	–	190	61	50	45	–
1-Propylbutyl	7	125	90	33	28	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Methylheptyl	8	126	57	46	44	–	127	67	32	34	–	128	45	25	18	–	194	28	29	15	–
1-Ethylhexyl	8	131	92	43	36	–	132	42	36	18	–	133	32	18	–	–	203	67	61	36	–
1-Propylpentyl	8	144	62	35	38	36	145	26	31	33	–	146	48	49	42	33	208	41	36	36	34
't-Octyl'	8	147	–	66	44	25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Methyloctyl	9	149	66	59	38	20	150	56	–	–	–	151	46	–	–	–	209	87	–	–	–
1-Ethylheptyl	9	152	96	79	51	–	153	45	66	46	–	154	80	66	42	–	210	54	37	29	–
1-Propylhexyl	9	155	98	67	55	29	156	25	0	0	0	157	60	65	23	11	211	44	16	11	2
1-Butylpentyl	9	158	89	71	34	33	159	45	37	36	20	–	–	–	–	–	–	–	–	–	–
1-Ethyloctyl	10	160	–	46	45	45	161	81	–	–	–	162	82	–	–	–	213	69	–	–	–
1-Propylheptyl	10	163	97	–	–	–	164	74	–	–	–	165	46	–	–	–	215	56	–	–	–
1-Butylhexyl	10	166	83	50	14	2	167	46	18	20	14	168	36	18	18	15	217	45	36	26	15
1-Methyldecyl	11	169	67	55	44	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Ethylnonyl	11	170	77	57	46	40	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Propyloctyl	11	171	75	57	52	38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Butylheptyl	11	172	84	–	–	–	173	37	–	–	–	174	56	–	–	–	218	50	–	–	–
1-Pentylhexyl	11	175	81	33	37	36	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Ethyldecyl	12	176	91	55	49	54	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Propylnonyl	12	177	81	62	59	61	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Butyloctyl	12	178	54	66	40	51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Pentylheptyl	12	179	69	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Propyldecyl	13	180	84	56	59	38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Butylnonyl	13	181	78	51	41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Pentyloctyl	13	182	74	56	51	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Hexylheptyl	13	183	–	52	38	35	184	–	45	37	0	185	–	24	13	–	219	–	38	27	–

TABLE IX

Contact acaricidal activity of certain 2-alkyl-4,6-dinitrophenyl esters (I) and 4-alkyl-2,6-dinitrophenyl esters (II)

Alkyl	No. of carbon atoms in alkyl group	Ester	No. of compound (I)	Kill of *T. telarius* (%) at ppm 300	100	30	10	3	No. of compound (II)	Kill of *T. telarius* (%) at ppm 300	100	30	10
s-Butyl	4	Crotonate	76	–	100	100	97	95	186	89	64	29	–
1-Ethylpropyl	5	Crotonate	78	–	100	100	100	99	187	–	82	71	66
1-Methylpentyl	6	Acetate	79	100	100	100	100	86	–	–	–	–	–
1-Methylpentyl		Acrylate	80	100	100	100	100	84	–	–	–	–	–
1-Methylpentyl		Crotonate	81	–	100	81	61	–	–	–	–	–	–
1-Ethylbutyl	6	Crotonate	82	–	100	100	100	98	188	77	60	50	18
1-Methylhexyl	7	Acetate	83	100	100	100	98	73	–	–	–	–	–
1-Methylhexyl		Acrylate	84	100	100	96	97	–	–	–	–	–	–
1-Methylhexyl		Crotonate	85	–	94	65	70	–	189	79	67	42	–
1-Ethylpentyl	7	Crotonate	86	–	99	74	70	–	190	61	50	45	–
1-Propylbutyl	7	Acetate	–	–	–	–	–	–	191	80	40	32	–
1-Propylbutyl		Acrylate	–	–	–	–	–	–	192	88	–	58	56
1-Propylbutyl		Crotonate	87	–	100	95	85	–	–	–	–	–	–
1-Methylheptyl	8	Acetate	88	100	100	94	88	–	–	–	–	–	–
1-Methylheptyl		Acrylate	89	96	40	–	–	–	193	61	58	32	8
1-Methylheptyl		Crotonate	90	–	73	59	23	–	194	28	29	15	–
1-Ethylhexyl	8	Acetate	91	–	100	96	95	–	195	13	10	3	0
1-Ethylhexyl		Butyrate	92	–	88	87	44	–	196	51	35	24	24
1-Ethylhexyl		Isobutyrate	–	–	–	–	–	–	197	42	27	27	0
1-Ethylhexyl		Octanoate	–	–	–	–	–	–	198	60	45	40	30
1-Ethylhexyl		Chloroacetate	–	–	–	–	–	–	199	59	32	21	–
1-Ethylhexyl		2-Chloropropionate	–	–	–	–	–	–	200	82	53	36	–
1-Ethylhexyl		3-Chloropropionate	–	–	–	–	–	–	201	21	–	11	–
1-Ethylhexyl		Acrylate	93	100	95	51	38	–	202	36	24	15	7
1-Ethylhexyl		Crotonate	94	–	85	74	38	9	203	67	61	36	–
1-Ethylhexyl		Methacrylate	–	–	–	–	–	–	204	40	32	32	31
1-Ethylhexyl		3-Methyl crotonate	–	–	–	–	–	–	205	71	62	55	–
1-Ethylhexyl		Benzoate	–	–	–	–	–	–	206	32	32	30	29
1-Ethylhexyl		*p*-Chlorobenzoate	–	–	–	–	–	–	207	36	29	7	–
1-Propylpentyl	8	Crotonate	95	–	92	79	69	–	208	41	36	36	34
1-Methyloctyl	9	Crotonate	96	–	80	64	35	–	209	87	–	–	–
1-Ethylheptyl	9	Crotonate	97	–	40	29	20	–	210	54	37	29	–
1-Propylhexyl	9	Crotonate	98	–	84	49	47	–	211	44	16	11	2
1-Ethyloctyl	10	Acrylate	–	–	–	–	–	–	212	65	35	21	–
1-Ethyloctyl		Crotonate	100	–	44	0	0	–	213	69	–	–	–
1-Propylheptyl	10	Acrylate	–	–	–	–	–	–	214	39	36	32	–
1-Propylheptyl		Crotonate	101	–	45	24	24	–	215	56	–	–	–
1-Butylhexyl	10	Acrylate	–	–	–	–	–	–	216	22	34	18	–
1-Butylhexyl		Crotonate	102	–	59	40	36	–	217	45	36	26	15
1-Butylheptyl	11	Crotonate	104	–	66	38	37	–	218	50	–	–	–
1-Hexylheptyl	13	Crotonate	107	–	16	12	3	–	219	–	38	27	–

TABLE X

Contact acaricidal activity of certain carbonates of 2-alkyl-4,6-dinitrophenols (III) and carbonates of 4-alkyl-2,6-dinitrophenols (IV)

Alkyl	No. of carbon atoms in alkyl group	Carbonate	No. of compound (III)	Kill of *T. telarius* (%) at ppm 300	100	30	10	3	No. of compound (IV)	Kill of *T. telarius* (%) at ppm 300	100	30	10
1-Methylheptyl	8	Isopropyl	28	49	38	21	24	–	129	33	34	16	11
1-Methylheptyl		Methyl thiolo-	29	93	63	46	33	–	130	47	38	36	–
1-Ethylhexyl	8	Propyl	33	–	95	61	45	–	134	66	30	10	–
1-Ethylhexyl		Isopropyl	34	–	100	87	57	–	135	29	0	9	–
1-Ethylhexyl		Butyl	–	–	–	–	–	–	136	38	39	33	25
1-Ethylhexyl		s-Butyl	–	–	–	–	–	–	137	34	33	34	16
1-Ethylhexyl		Isobutyl	–	–	–	–	–	–	138	37	8	14	–
1-Ethylhexyl		Hexyl	35	–	100	99	26	–	139	29	22	–	–
1-Ethylhexyl		Octyl	–	–	–	–	–	–	140	22	26	–	–
1-Ethylhexyl		1-Methylheptyl	–	–	–	–	–	–	141	88	52	51	33
1-Ethylhexyl		Decyl	36	–	96	92	15	–	142	37	37	39	29
1-Ethylhexyl		Methyl thiolo-	–	–	–	–	–	–	143	38	23	23	–
't-Octyl'	8	Isopropyl	–	–	–	–	–	–	148	13 (59% at 1000 ppm)			

TABLE XI

Contact acaricidal activity of certain 2-alkyl-4,6-dinitrophenols and 4-alkyl-2,6-dinitrophenols and their methyl ethers

Alkyl	No. of carbon atoms in alkyl group	Phenols			Methyl ethers		
		No. of compound	Kill of *T. telarius* (%) at ppm 300	100	No. of compound	Kill of *T. telarius* (%) at ppm 300	100
2-Alkyl-4,6-dinitrophenols and their methyl ethers							
1-Methylheptyl	8	25	100	99	222	30	15
1-Ethylhexyl	8	30	100	100	223	45	34
1-Propylpentyl	8	37	100	100	224	57	34
1-Ethylheptyl	9	43	100	100	225	32	32
1-Propylhexyl	9	46	100	100	226	44	20
1-Butylpentyl	9	49	100	100	227	30	33
1-Hexylheptyl	13	73	100	100	228	20	17
4-Alkyl-2,6-dinitrophenols and their methyl ethers							
1-Ethylpentyl	7	122	95	86	229	16	18
1-Methylheptyl	8	126	57	46	230	31	26
1-Ethylhexyl	8	131	92	43	231	42	26
1-Propylpentyl	8	144	62	35	232	37	17
1-Methyloctyl	9	149	66	59	233	35	29
1-Propylhexyl	9	155	98	67	234	41	27
1-Butylpentyl	9	158	89	71	235	42	31
1-Butyloctyl	12	178	54	66	236	52	26

trials the mixture at 250–500 ppm gave rapid kills of active stages of red spider mites: *Panonychus ulmi* on apples, pears, and peaches; *Tetranychus telarius* on beans; and *Tetranychus urticae* on beans, egg plants, vines, and chrysanthemums.

Mammalian toxicity

The acute oral toxicity to rats was determined as follows. The compounds were suspended in distilled water with 25% propylene glycol and 0·5% Tween 80 to give a 300 mg/ml concentration. Thirty female Wistar rats (bodyweight 140–170 g) were used per determination. The doses were administered by stomach tube. Mortalities were observed after 7 days. Median lethal doses are presented in Table XII.

TABLE XII

Toxicity to rats of certain alkyldinitrophenyl esters
2,4,6-RR′.$C_6H_2(NO_2)$.O.CO.R″

No. of compound	R	R′	R″	Acute oral LD_{50} mg/kg
26	1-Methylheptyl	NO_2	OCH_3	1400
31	1-Ethylhexyl	NO_2	OCH_3	> 3000
38	1-Propylpentyl	NO_2	OCH_3	> 3000
127	NO_2	1-Methylheptyl	OCH_3	> 3000
132	NO_2	1-Ethylhexyl	OCH_3	1750
202	NO_2	1-Ethylhexyl	$CH{:}CH_2$	1075
145	NO_2	1-Propylpentyl	OCH_3	1800

Discussion

Nuclear substitution in phenols

In Part I[1,3] it was reported that 2-s-butyl- and 2-t-butyl-4,6-dinitrophenols and their aliphatic carbonates had much higher acaricidal activity than 4-s-butyl- and 4-t-butyl-2,6-dinitrophenols and corresponding carbonates. For maximum acaricidal activity phenols required nitro-groups in the 4- and 6-positions and an s-alkyl group in the 2-position. The present investigation using s-alkyldinitrophenols with C_5 to C_{13}-s-alkyl groups confirms this postulate since these 2-s-alkyl-4,6-dinitrophenols and their esters were much more active against *Tetranychus telarius* adults than the corresponding 4-s-alkyl-2,6-dinitrophenols and their esters (Tables VII–X), with the notable exception of the 1-ethylpentyl-dinitrophenols (Nos. 19, 122).

An examination of the activity of the 2-s-alkyl-4,6-dinitrophenols (Table VII) shows that in the C_5-, C_6-, C_7- (except for the ethylpentyl-isomer, No. 19) and C_{10}-alkyl series the more compact the s-alkyl group the higher the activity (Nos. 4 & 7; 10 & 13; 16 & 22; 52, 55 & 58). In the C_8-alkyl series the ethylhexyl- (No. 30) was only marginally more active than the methylheptyl-isomer (No. 25) (cf. Kirby *et al.*[7]). In the C_9-alkyl series the more compact propylhexyl- (No. 46) and butylpentyl- (No. 49) isomers were more active than were the methyloctyl- (No. 40) and ethylheptyl- (No. 43) isomers. Activity was still high at C_{11}-alkyl (Nos. 61, 64, 67), and dropped at C_{12}- (No. 70) and C_{13}- (No. 73) alkyl.

Except for 2-(1-methylalkyl)- the plots of the activity of 2-(1-ethylalkyl)-, 2-(1-propylalkyl)- and 2-(1-butylalkyl)-4,6-dinitrophenols at 10 ppm against *T. telarius* adults show a maximum when the alkyl was a hexyl group (Fig. 1).

The ester chain

In Part I[1] it was reported that the toxicity to spider mites of alkyl carbonates of 2-s-butyl- and 2-(1-methylbutyl)-4,6-dinitrophenols was higher than that of the parent phenols. It was assumed that the un-ionised carbonates penetrated more readily to the vital sites of action, thus yielding a greater concentration of the toxicants in the organism. Activity was considered to be due to the hydrolytic products, i.e. the alkyldinitrophenols. Figs. 2–5 show comparisons of percentage mortalities of *T. telarius* adults caused by concentrations of 10 ppm 2-s-alkyl-4,6-dinitrophenols, their methyl carbonates, ethyl carbonates and crotonates. With 1-methyl-

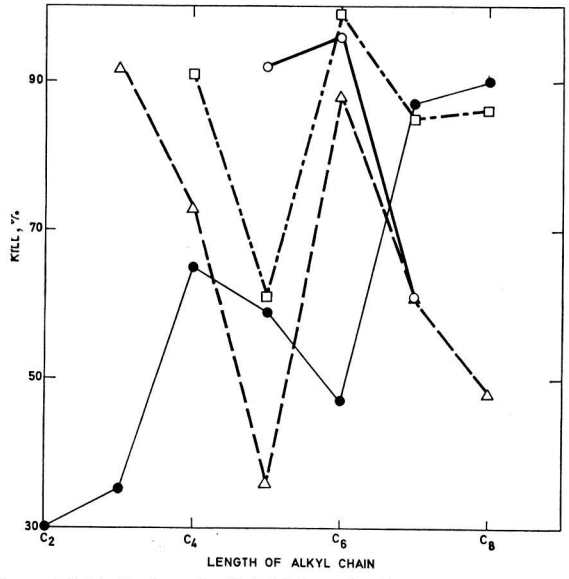

FIG. 1. *Activity of some* 2-(1-*Me, Et, Pr or Bu alkyl*)-4,6-*dinitrophenols at* 10 *ppm against* Tetranychus telarius *adults*
●——● 1-Methylalkyl △- - - - -△ 1-Ethylalkyl □—·—·—·□ 1-Propylalkyl ○——○ 1-Butylalkyl

alkyl-compounds (Fig. 2) up to 1-methylhexyl- (total chain of 7 carbon atoms), with 1-ethylalkyl-compounds (Fig. 3) up to 1-ethylpentyl- (total chain of 7 carbon atoms), with 1-propyl-alkyl-compounds (Fig. 4) up to 1-propylpentyl- (total chain of 8 carbon atoms) the esters were more active than the phenols. With the longer alkyl chains: heptyl and octyl (Fig. 2); hexyl (except for No. 31), heptyl and octyl (Fig. 3); hexyl, heptyl and octyl (Fig. 4) and pentyl, hexyl and heptyl with 1-butylalkyl-compounds (Fig. 5) the phenols were more active than their ester derivatives. High polarity of 2-(C_3 to C_7-alkyl)-4,6-dinitrophenols may impede their penetration to the vital sites of action; esterification may aid it. The more lipophilic properties of 2-(C_8 to C_{13}-alkyl)-4,6-dinitrophenols may aid their penetration and render these phenols more active. Esterification of these higher alkylphenols proved detrimental to activity perhaps because: increase in bulk may interfere with penetration; and the bulky alkyl group may hinder hydrolysis of the esters to the phenols. Significantly, the bulkier crotonates of 4,6-dinitrophenols with 2-s-alkyl groups of seven or more carbon atoms were less active than the corresponding methyl carbonates (Table VII, Figs 2–5),

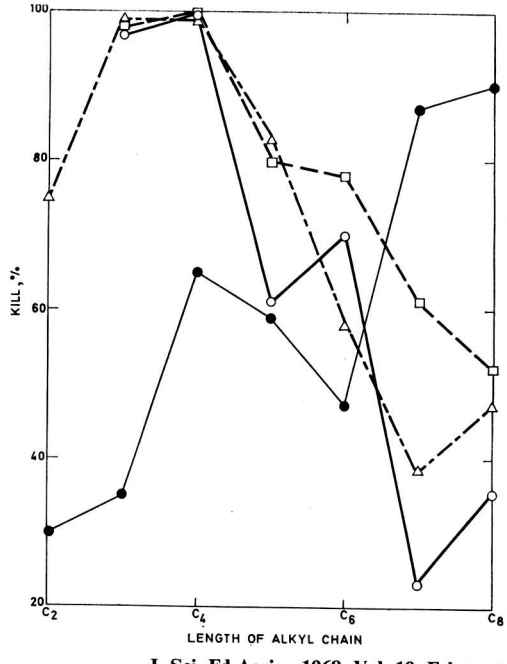

FIG. 2. (right). *Activity of* 2-(1-*methylalkyl*)-4,6-*dinitrophenols, their methyl carbonates, ethyl carbonates and crotonates at* 10 *ppm against* Tetranychus telarius *adults*
●——● Phenols
□- - - - -□ Methyl carbonates
△—·—·—△ Ethyl carbonates
○——○ Crotonates

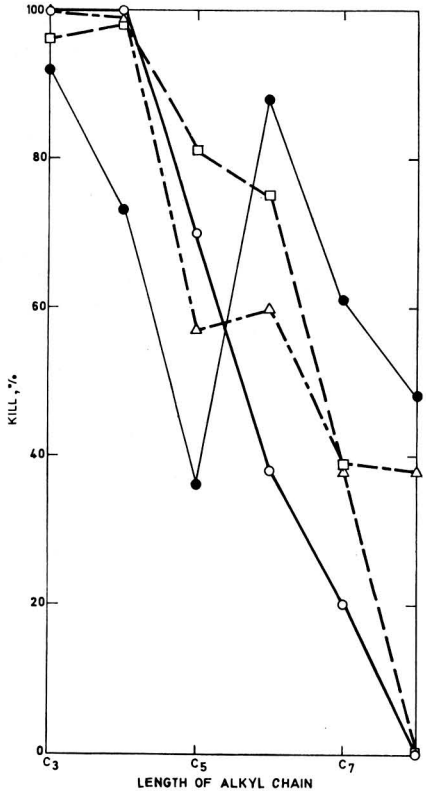

FIG. 3. *Activity of* 2-(1-*ethylalkyl*)-4,6-*dinitrophenols, their methyl carbonates, ethyl carbonates and crotonates at* 10 *ppm against* Tetranychus telarius *adults*

Phenols
Methyl carbonates
Ethyl carbonates
Crotonates

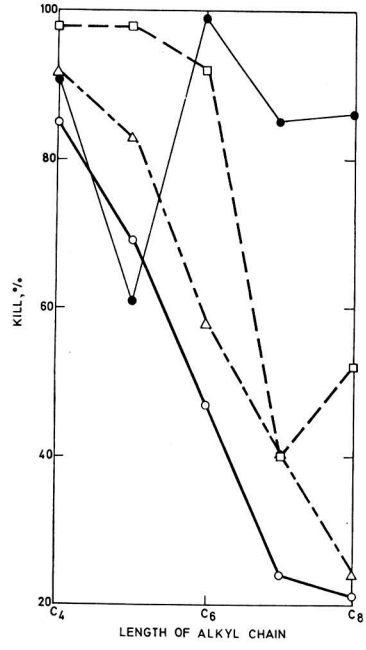

FIG. 4. *Activity of* 2-(1-*propylalkyl*)-4,6-*dinitrophenols, their methyl carbonates, ethyl carbonates and crotonates at* 10 *ppm against* Tetranychus telarius *adults*

Phenols
Methyl carbonates
Ethyl carbonates
Crotonates

the crotonates were less active than the acetates (Table IX; Nos. 81 & 79; 85 & 83; 90 & 88; 94 & 91), and 2-(1-ethylhexyl)-4,6-dinitrophenyl butyrate (No. 92) was less active than the acetate (No. 91).

Extension of the carbonate chain of 2-(1-ethylhexyl)-4,6-dinitrophenol (Table X) to propyl (No. 33) and isopropyl (No. 34), hexyl (No. 35) and decyl (No. 36) had surprisingly little effect on activity. 2-(1-Methylheptyl)-4,6-dinitrophenyl isopropyl- (No. 28) and methylthiolo- (No. 29) carbonates were considerably less active than methyl- (No. 26) or ethyl- (No. 27) carbonates.

As with the 2-s-alkyl-4,6-dinitrophenols, esterification generally reduced the activity of 4-s-alkyl-2,6-dinitrophenols containing an s-alkyl group of seven or more carbon atoms (Tables VIII–X).

Etherification

The activity of methyl ethers of 2-alkyl-4,6-dinitro- and 4-alkyl-2,6-dinitro-phenols was found to be very low, compared with that of the parent phenols (Table XI). 2-s-Butyl- and 2-t-butyl-4,6-dinitroanisoles[1] and 2-t-butyl-5-methyl-4,6-dinitroanisole[2] also had very low activity, compared with that of the parent phenols. The view that the low activity of anisoles may be due to their hydrolytic stability[1] gains thus a broader basis.

Conclusions

The following tentative conclusions may be drawn about the structure-activity relationship in the dinitroalkylphenols and their esters examined: 2-s-alkyl-4,6-dinitrophenols are more toxic than 4-s-alkyl-2,6-dinitrophenols to spider mites; with s-alkyl groups whose total number of carbon atoms is 5, 6, 7, 9 or 10 within a series of 2-s-alkyl-4,6-dinitrophenols containing the same total number of carbon atoms, the more compact the isomers the higher is their acaricidal activity; acaricidal activity remains high up to 2-(C_{11}-s-alkyl)-4,6-dinitrophenols, beyond which it drops; generally esters of 2-(C_3 to C_7-s-alkyl)-4,6-dinitrophenols are more acaricidal

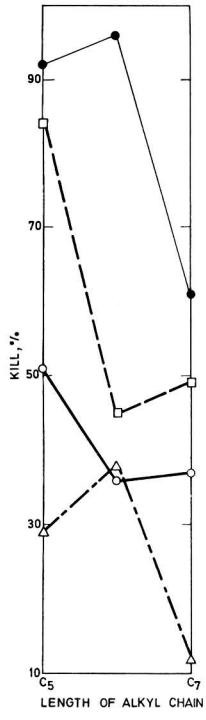

FIG. 5. *Activity of* 2-(1-*butylalkyl*)-4,6-*dinitrophenols, their methyl carbonates, ethyl carbonates and crotonates at* 10 *ppm against* Tetranychus telarius *adults*

than the parent phenols, but 2-(C_8 to C_{13}-s-alkyl)-4,6-dinitrophenols are more acaricidal than their esters, and a similar relationship holds for 4-s-alkyl-2,6-dinitrophenols and their esters; crotonates of 2-(C_7 to C_{13}-s-alkyl)-4,6-dinitrophenols are less acaricidal than methyl carbonates or acetates; 2-s-alkyl-4,6-dinitrophenols are more phytotoxic than their esters; etherification of the toxiphoric phenols substantially reduces acaricidal activity; and methyl carbonates of 2-(1-ethylhexyl)- and 2-(1-propylpentyl)-4,6-dinitrophenols are of particular practical and economic interest for their activity against various resistant spider mites.

Acknowledgments

Thanks are due to Messrs. W. S. Catling and P. J. Jeffery for the assessment of the acaricidal activity of the compounds, and Mr. T. R. Middleton of Glaxo Laboratories Ltd. for the acute oral LD_{50} determination in rats.

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Received 3 July, 1967

References

1. Pianka, M., *J. Sci. Fd Agric.*, 1966, **17**, 47
2. Pianka, M., & Edwards, J. D., *J. Sci. Fd Agric.*, 1967, **18**, 355
3. Pianka, M., Vth International Pesticides Congress, London, 1963
4. Pianka, M., *Chemy Ind.*, 1967, p. 1625
5. Pianka, M., & Edwards, J. D., *J. chem. Soc.*, (C), 1967, 2281
6. Pianka, M., & Browne, K. M., *J. Sci. Fd Agric.*, 1967, **18**, 447
7. Kirby, A. H. M., Frick, E. L., & Gratwick, M., *Ann. appl. Biol.*, 1966, **57**, 211
8. Pianka, M., Second Crop Protection Symposium, Magdeburg, October, 1966
9. Clifford, D. R., Watkins, D. A. M., & Woodcock, D., *Chemy Ind.*, 1965, p. 1655

AVAILABILITY OF ESSENTIAL AMINO ACIDS FROM PROTEINS. I.—Beef serum albumin

By Z. DVOŘÁK

Available essential amino acids from beef serum albumin were determined microbiologically, after total enzymic hydrolysis of the protein by papain, leucine aminopeptidase and prolidase. Samples of pure beef serum albumin of different moisture content were heat-treated with glucose at 90° or without it at 121°. In the enzymic digest of the protein, available essential amino acids were determined using *Streptococcus faecalis*, *S. zymogenes* and *Lactobacillus arabinosus*. The content of essential available amino acids was correlated with the degree of *in vitro* digestibility of heat-treated albumin.

The amount of lysine determined by Carpenter's method with fluordinitrobenzene did not correspond to the values found for lysine by microbiological analysis with *S. faecalis*. Lysine reactive with fluordinitrobenzene was found in the non-digestible residue of the protein, so that the values obtained by the chemical method are probably higher than the actual amount of available lysine. The results for the trypsin digest of the albumin were similar by both methods. The availability of lysine as well as that of amino acids in general depended on the enzymic digestibility.

Introduction

The biological value of proteins is given primarily by their content of essential amino acids, and the concept of availability was introduced after it had been shown that the biological value of proteins was also influenced by the limited digestibility of proteins, due to the formation of enzymo-resistant complexes of amino acids. Knowledge of the availability of amino acids may be important with proteins damaged by heat, or by interaction with sugars or with peroxy fats. By determination after acid or enzymic hydrolysis, the quantity of amino acids in these damaged proteins was found to be reduced.[1-7]

Some study of the availability of amino acids *in vitro* has been possible, and available lysine can be determined on the basis of the reaction with fluordinitrobenzene[8,9] of the free ε-amino group of lysine bound in the protein.

Ford[4] determined available methionine, leucine, isoleucine, arginine, histidine, tryptophan and valine in a series of protein concentrates, by using the proteolytic organism, *Streptococcus zymogenes*, NCDO 592. These samples have to be predigested by papain. At the higher concentrations of papain, the amounts of available methionine and tryptophan found were correlated closely with the digestibilities of the proteins and also with their biological values.[10] Miller *et al.*[11] confirmed the correlation between available methionine and biological values determined by chick assay. According to Ford[10] other enzymes, e.g. pepsin, or trypsin and erepsin, did not bring about any further increase in available methionine value, but he showed[5] that, in pepsin and papain digests of heat-treated protein meals, peptides of relatively high molecular weight in which essential amino acids are not completely available for micro-organisms are present. This may have been caused by the proteolytic capacity of the bacteria used for the determination being inadequate to split all bonds in the larger peptides so that the amino acids from them could not be used for growth.

Total enzymic digestion would guarantee that all α-amino peptide bonds were broken before microbiological determination of the amino acids. Hill & Schmidt[12] obtained total hydrolysis of proteins by using papain, leucine aminopeptidase and prolidase. By this procedure it should be possible to determine the maximum theoretically possible amount of available amino acids and to use Ford's technique[10] for determination of essential amino acids which cannot be determined by *S. zymogenes*, i.e. lysine, phenylalanine and threonine.

Complexes of amino acids that are enzyme-resistant, e.g. products of the Maillard reaction, but not resistant to acid hydrolysis, can be present in such enzymic digests, and these bound amino acids may not be available to micro-organisms for growth.

The purpose of this communication is to ascertain the amount of available essential amino acids in pure beef serum albumin. In native beef serum albumin the theoretical amount[13] of all essential amino acids may be determined microbiologically after total enzymic hydrolysis. Also the amount of available essential amino acids in heat-treated beef serum albumin was determined, in relation to enzymic hydrolysis of the proteins, by the method of Hill & Schmidt.[12]

Experimental

The beef serum albumin used was a preparate from Armour Pharmaceuticals, London, Nr. 2293, V. fraction of Cohn fractionation, and it was used without further purification.

Albumin was kept at room temperature before use, to reach constant moisture content. The content of water in the protein was 6·6%. Native serum albumin was dissolved in water in a container, adjusted to pH 7·0, and denaturated by being dipped in a boiling-water bath for 5 min before enzymic hydrolysis. For heat-treatment of the protein, water was added to give a content of 14, 40 or 80% and the preparates were autoclaved at 121° in sealed tubes in air. To one batch of the same preparates, D-glucose was added, in an amount corresponding to 5% of the protein. After being mixed, the contents were also sealed in tubes, which were placed in a drying-thermostat at 90°. The time of heat-treatment varied but in most cases was 24 hours. During this time the consistency and colour of the preparates changed

according to the amount of water and the temperature; the original white powder changed to creamy brown, which was more marked in the preparates with glucose. The product was dehydrated with ethyl alcohol and diethyl ether and dried in air. After milling to a powder, nitrogen was determined in every preparate.

For total enzymic hydrolysis the method of Hill & Schmidt[12] was used. An amount of serum albumin corresponding to 50 mg N was solved or suspended in 60 ml water. To the solution or suspension 20 ml 0·2 M acetate buffer, pH 5·2, and 8 ml 0·1 N-NaCN (pH 5·2) were added. Purified preparate of papain from Meer Corp., New York, was used. It was added in an amount corresponding to 5% of proteins, in 2 ml 0·2 M acetate buffer, pH 5·2. To each sample 0·5 ml toluene was added. The mixture was incubated for 24 hours at 40° in Dubnoff's incubator, with constant shaking. After this time the solution was adjusted to pH 2 with 1 N-HCl for inactivation of papain and evaporated in the rotary evaporator. The dry matter was suspended in 10 ml water and the pH was adjusted to 8·0. To the mixture 30 ml 0·02 M veronal–acetate buffer, pH 8·0, then 0·125 ml 0·2 M $MnCl_2$, 6 ml of the solution of leucine aminopeptidase with the activity of $C_1 = 40$ and 3 ml of the solution of prolidase, $C_1 = 20$, were added. Leucine aminopeptidase and prolidase were prepared by purification from acetone extract from swine kidneys.[14,15] Control experiments showed that the concentration of enzymes used did not influence the amount of amino acids determined by enzymic digest. The mixture with exopeptidases was incubated with 0·5 ml toluene for 24 hours at 40° in Dubnoff's incubator. After this time the digests were adjusted to pH 2, boiled for 5 min in water and centrifuged. The residue was washed twice with distilled water. The supernatants were pooled with the original digests, which were then made up to 100 ml. For microbiological determination of amino acids each digest was diluted with water so that 1 ml corresponded to 0·1 mg N of the original albumin. The undigested residue was dehydrated with ethyl alcohol and weighed after being dried for 4–5 hours at 105°. The weight of the residue of the control sample, with the enzyme but without albumin, was subtracted from the weight of samples incubated with the enzymes. It represents the undigested protein.

For hydrolysis of native beef serum albumin with hydrochloric acid an amount equivalent to 50 mg N of the protein was weighed. The hydrolysis was made with 30 ml 6 N-HCl (constantly boiling), in a sealed tube *in vacuo* for 24 hours at 110°. Hydrochloric acid was evaporated off in rotary evaporator and the residue was removed by double evaporation with a little water. The hydrolysate was diluted with water to 500 ml after neutralisation.

For hydrolysis of heat-treated albumin with trypsin, 1 g of the sample was weighed and incubated in 50 ml 0·05 M-Na_2HPO_4–HCl buffer, pH 8·1, with 20 mg trypsin. The enzyme was crystalline obtained from Lachema, CSSR. After the addition of toluene to the mixture it was incubated for 24 hours at 38° in Dubnoff's incubator, after which the pH was adjusted to 6·5 with 1 N-HCl. Trypsin was inactivated by being boiled in a water bath for 5 min. After centrifugation and washing of the residue twice with water a solution with water was made up to 100 ml. In the solution lysine was determined chemically and microbiologically. The undigested protein remained in the centrifugation tubes and was treated as above. An aliquot of part of the residual lysine was determined chemically.

From the trypsin digest 25 ml was used for further digestion by carboxypeptidases, after adjustment of the pH to 8·1. A mixture of carboxypeptidases A and B pancreatine (acetone extract from beef pancreas, prepared by the method of Keller *et al.*[16]) was used, at 1 ml 5% solution. For hydrolysis with crystalline carboxypeptidase A, the preparate of Reanal, Budapest, was solubilised with 10% LiCl.[17] To the trypsin digest 1 ml of the enzyme solution was added. This volume corresponds to 2 mg of the solubilised carboxypeptidase A. The digestion in both cases was for 24 hours at 23°, after which the pH was adjusted to 4·0 with acetic acid, and the enzymes were inactivated in boiling water for 5 min, before centrifugation. The supernatant was used for microbiological determination of lysine.

Nitrogen in proteins was determined by semimicro-Kjeldahl analysis.[18]

The amino acids were determined microbiologically with *Streptococcus faecalis* R (ATCC 8043), *S. zymogenes* NCDO 592, and *Lactobacillus arabinosus* 17/5 (ATCC 8014). *S. zymogenes* was a gift of Dr. Ford from Shinfield, Reading. For *S. faecalis* a technique of Stokes *et al.*[19] and Stokes & Gunnes[20] was used. For *S. zymogenes* a technique of Ford,[4] with enrichment of K_2HPO_4[10] and with titrimetric measurement of the growth of micro-organisms was applied. For *L. arabinosus* a technique of Sauberlich & Baumann[21] was used. The results of the titration were evaluated by a method of Wood.[22]

Relative error of the measurements of leucine in beef serum albumin after acid hydrolysis was calculated from 4 observations, being a mean of values for growth responses in duplicate tubes at four dosage levels for each of the micro-organisms used. It was about $\pm 6{\cdot}4\%$ ($P = 0{\cdot}05$). For particular amino acids, determined in the total enzymic digests, this error might be higher. Each result represents the average of at least 2 determinations. No statistical assessment of the errors of the particular measurements was attempted.

Lysine was determined chemically by the method of Carpenter,[8] with fluordinitrobenzene. Results represent the average of at least two determinations.

Results

Availability of essential amino acids

The results of the determination of essential amino acids in total enzymic digests from native and heat-treated beef serum albumin, in the presence of D-glucose or without it, are given in Table I. The content of amino acids in native protein, obtained by acid hydrolysis agreed with those by enzymic digestion. Ratios of variances, calculated from differences between the values obtained by using different micro-organisms for the same amino acid, show that in general results are not influenced by the micro-organism sort of the organism used. However, with decreasing amounts of amino acids the difference between values increases, especially for methionine and tryptophan.

Table II gives the average values for each amino acid, the percentage availability of particular amino acids, and the percentage of the digested proteins.

Although albumin in the presence of glucose was treated at a lower temperature than albumin without it, the percentage availability of amino acids was lower in the former case, at least in the proteins with 40 and 80% moisture contents. Temperature is not the only factor affecting the degree of availability. In addition to the probable interaction of

glucose with proteins, the moisture of heat-treated preparates is also important.

The amount of essential amino acids found in the enzymic digest of the albumins corresponds to the degree of heat treatment of them. The content of amino acids determined decreases with the decreasing portion of the digest, but for particular amino acids different degrees of availability were found. For lysine the presence of glucose in the preparate with 14% moisture substantially lowered the availability to 24·9% instead of the 51% expected.

The rate of decrease of availability of essential amino acids during heat-treatment of beef serum albumin in the presence of glucose is shown in Fig. 1. In this case also, analysis of enzymic digest of the protein after 6 hours of treatment at 90° was made. The loss of availability is greatest in those amino acids which are most abundant in the protein. The decrease is greater after the first 6 hours of heat-treatment than after a further 18 hours.

Availability of lysine

Loss of the availability of lysine in beef serum albumin, treated at 90° in the presence of glucose, was studied by

TABLE I

Essential amino acids, determined microbiologically, in digest of albumins

(in g/16 g N; F = *Streptococcus faecalis*, Z = *S. zymogenes*, A = *Lactobacillus arabinosus*)

	Isoleucine			Leucine			Lysine	Phenyl-alanine	Methionine			Threo-nine	Tryptophan			Valine		
	F	Z	A	F	Z	A	F	A	F	Z	A	F	F	Z	A	F	Z	A
Native albumin:																		
HCl hydrolysis	2·61	2·25	2·48	11·85	11·85	12·00	12·73	6·38	0·89	0·83	0·85	5·68	–	–	–	5·50	5·50	5·78
Enzymic hydrolysis	2·39	2·61	2·35	11·74	11·79	11·89	12·79	6·68	0·89	0·80	0·77	5·49	0·58	0·53	0·61	5·85	5·54	5·76
Albumin heat-treated for 24 hours at 121°																		
14% moisture	1·29	1·22	1·38	5·95	5·88	5·94	7·20	4·48	0·46	0·40	0·38	4·11	0·29	0·23	0·28	4·26	3·83	4·16
40% ,,	1·20	1·39	1·24	6·17	6·40	6·72	5·59	3·92	0·46	0·48	0·42	3·20	0·32	0·30	0·35	3·32	3·05	3·20
80% ,,	0·92	1·04	0·80	5·34	5·80	5·47	5·59	3·45	0·36	0·32	0·35	2·74	0·32	0·34	0·37	3·08	3·05	3·20
Albumin heat-treated in the presence of 5% glucose 24 hours at 90°																		
14% moisture	0·92	0·87	1·10	5·18	4·94	5·03	3·20	3·84	0·46	0·44	0·38	2·52	0·36	0·25	0·28	2·67	2·56	2·56
40% ,,	0·92	1·04	1·03	3·19	2·91	3·20	2·80	2·91	0·34	0·32	0·32	1·83	0·27	0·25	0·30	2·13	1·83	2·08
80% ,,	0·55	0·70	0·55	3·20	3·20	2·97	3·20	2·69	0·29	0·24	0·19	1·83	0·17	0·25	0·22	2·07	1·98	1·92

TABLE II

Digestibility and average content of available essential amino acids in albumins

(In parenthesis theoretical amount in g/16g N is given;[13] first number is average amount, obtained from values of Table I, in g/16g N; second number is the availability of particular amino acid in %)

	Digestibility %	Iso-leucine (2·61)	Leucine (12·27)	Lysine (12·83)	Methionine (0·81)	Phenyl-alanine (6·59)	Threonine (5·83)	Trypto-phan (0·58)	Valine (5·92)
Native albumin									
HCl hydrolysis	100	2·45	11·90	12·73	0·85	6·38	5·68	–	5·59
		93·8	97·1	99·2	106·2	96·8	97·5	–	94·5
Enzymic hydrolysis	100	2·45	11·81	12·79	0·82	6·68	5·49	0·57	5·72
		93·8	96·5	99·5	101·1	101·4	94·3	98·4	96·7
Albumin heat-treated for 24 hours at 121°									
14% moisture	56·7	1·30	5·95	7·20	0·41	4·48	4·11	0·27	4·08
		49·8	48·5	56·1	50·6	68·1	70·5	46·6	69·0
40% ,,	59·0	1·28	6·43	5·59	0·45	3·92	3·20	0·32	3·19
		49·0	52·5	43·6	55·5	59·5	55·0	55·2	53·8
80% ,,	55·7	0·93	5·54	5·59	0·34	3·45	2·74	0·34	3·01
		35·7	45·2	43·6	42·0	52·4	47·0	58·6	50·8
Albumin heat-treated in the presence of 5% glucose 24 hours at 90°									
14% moisture	51·0	0·96	5·05	3·20	0·43	3·84	2·52	0·30	2·60
		36·8	41·2	24·9	53·1	58·3	43·2	51·7	43·9
40% ,,	18·0	1·00	3·10	2·80	0·33	2·91	1·83	0·27	2·01
		38·3	25·3	21·8	40·7	44·2	31·3	46·6	34·0
80% ,,	26·7	0·60	3·12	3·20	0·24	2·69	1·83	0·21	1·99
		23·0	25·5	24·9	29·6	40·8	31·3	36·2	33·6

chemical analysis with fluordinitrobenzene (FDNB). Results are given in Fig. 2. Decrease of available lysine is linear for the first 12 hours of heat treatment, after which it was less. Only 70% of the original content of lysine was found after 24 hours. A stoicheiometric ratio 1 : 1 is suggested for the reaction of glucose with the free ε-amino group of lysine bound in the protein, in which case the loss of availability found after 24 hours is 94·3% of the theoretically possible loss in given experimental conditions.

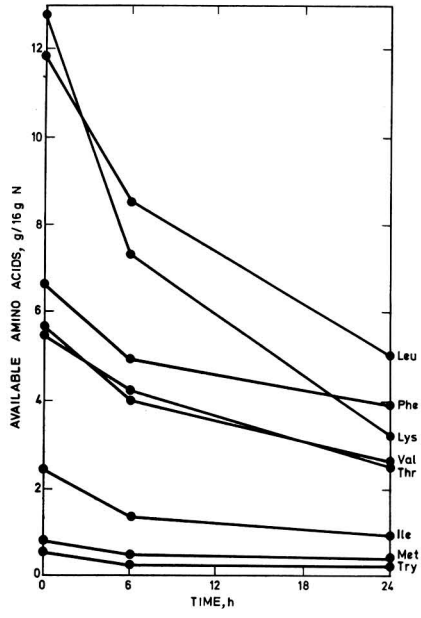

FIG. 1. *Available amino acids in albumins heated with glucose at* 14% *moisture and* 90°C

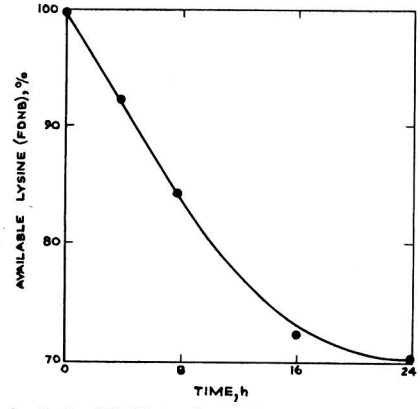

FIG. 2. *Lysine (FDNB) in albumin heated with glucose at* 90°C
(Mixture contained 100 parts by wt of protein, 5 parts by wt of D-glucose and 14 parts by vol. of water)

In Fig. 2 the availability of lysine decreases to 88% after 6 hours of heat treatment. This value can be compared with lysine microbiologically determined. Availability of the latter is shown in Fig. 1 and reaches 57% after 6 hours and 24·9% after 24 hours of heat treatment. This can be explained by the fact that lysine (by FDNB) shows losses by the formation of complexes with glucose derivative, irrespective of their presence in the enzymic digest of the albumin or in its non-digestible part. On the other hand microbiological determination takes into consideration only the hydrolysable part of the protein, excluding lysine bound in the complex and eventually present in the digest.

For comparison of both methods, hydrolysis of beef serum albumin by trypsin was used. Albumin with 40% moisture and 5% of glucose was treated for 24 hours at 90°. The other preparate was made under the same conditions, but without glucose, and treated at 121°. Trypsin is specific for attacking the bonds at carboxyl ends of basic amino acids. At 85 lysine and arginine residues from a total of 602 amino acid residues[23] in beef serum albumin, approximately 14% of all peptide bonds ought to be split. Peptides with basic amino acids as C-terminal would be developed. Determination of lysine (by FDNB) in the trypsin digest and in the non-digestible residue of albumin was used, and microbiological determination of lysine was made on the trypsin digest. Lysine C-terminal from peptides was also released by carboxypeptidases and determined microbiologically.

The results are given in Tables III and IV. From Table III it can be seen that the content of lysine (FDNB) found in heat-treated albumins is decreased, in comparison with its content in native albumin, by 21·6% and 36·8% for albumin treated with glucose or untreated, respectively. In the protein with glucose, 78·1% and 18·3%, respectively, of lysine (FDNB) was found in the non-digestible fraction and in the digestible part. In the protein without glucose, 48·6% and 50·8%, respectively, of lysine (FDNB) was determined in the non-digestible fraction and in the digestible part. These values are approximately correlated with the percentage distribution of hydrolysable fraction in each preparate.

From Table IV it can be seen that in trypsin digest alone lysine can be determined by *S. faecalis*, without the use of carboxypeptidases. The activity of these enzymes in the micro-organisms is sufficient for releasing C-terminal lysine from peptides. The values obtained are the same as those found after hydrolysis of the digest by carboxypeptidases. The average amount of lysine found in these hydrolysates is 17·5 mg and 36·0 mg, respectively, in the albumin with glucose and without it. Less lysine was determined also in the non-digestible fraction by trypsin, using pancreatine. The total amount of available lysine, calculated in relation to the lysine in native beef serum albumin, is 2·22 g/16 g N and 4·46 g/16 g N for albumin with glucose and without it, respectively. These results can be compared with the values of similar preparates, given in Table II, where the protein was hydrolysed by papain, leucine aminopeptidase and prolidase. The differences are small, considering the different efficiencies of the enzymes used.

The results indicate that the values for lysine determined chemically (by FDNB) and microbiologically in trypsin

TABLE III

Lysine (FDNB) in heat-treated albumins with 40% moisture and its distribution after digestion by trypsin

	Trypsin digestion %	Lysine mg/100 mg	Lysine %	Lysine g/16g N	Lysine availability %
Albumin with glucose, heat-treated for 24 hours at 90°					
Total preparate		87·4	100	10·08	78·4
Non-digestible part	80·7	68·2	78·1	7·86	61·2
Digest	19·3	16·0	18·3	1·84	14·4
Albumin without glucose, heat-treated for 24 hours at 121°					
Total preparate		70·8	100	8·11	63·2
Non-digestible part	44·5	34·4	48·6	3·94	30·7
Digest	55·5	36·0	50·8	4·12	32·1

TABLE IV

Lysine determined microbiologically in heat-treated albumins with 40% moisture, after digestion by trypsin and carboxypeptidases

	Lysine			Differences,* compared with values in Table III %
	In total fraction mg	In total preparate g/16g N	Availability %	
Albumin with glucose, heat-treated for 24 hours at 90°				
Trypsin digest	17·4	2·01	15·7	109·0
Trypsin and pancreatine digest	17·0	1·96	15·3	106·2
Trypsin and carboxypeptidase A digest	18·1	2·09	16·3	113·1
Average	17·5	2·02	15·8	109·4
Residue not digestible by trypsin after pancreatine hydrolysis	1·76	0·20	1·6	2·6
Total	19·26	2·22	17·3	22·9
Albumin without glucose, heat-treated for 24 hours at 121°				
Trypsin digest	38·8	4·44	34·6	107·8
Trypsin and pancreatine digest	36·8	4·22	32·8	102·2
Trypsin and carboxypeptidase A digest	32·4	3·71	28·9	90·0
Average	36·0	4·12	32·1	100·0
Residue not digestible by trypsin after pancreatine hydrolysis	2·93	0·34	2·6	8·5
Total	38·93	4·46	34·7	55·6

*Availability of lysine (FDNB) is taken to be 100%

digest, are almost identical. Greater differences occur in the undigestible part.

The available lysine determined microbiologically represents only 22·9% and 55·6%, respectively, of the total lysine (FDNB) in the heat-treated albumin with glucose and without it.

Discussion

For complete availability of amino acids from proteins total release of peptide bonds in a relative short time is suggested.[24] In the digestive system of mammals and birds there is a system of endopeptidases and exopeptidases efficient enough to digest the proteins to free amino acids, unless other than α-amino peptide bonds are present in the protein molecule.

Total enzymic hydrolysis of native proteins *in vitro* can be carried out by the method of Hill & Schmidt.[12] In this system leucine aminopeptidase is used to hydrolyse all peptide bonds that remain intact in papain hydrolysates, except those which contain the imino nitrogen of proline; proline peptides are hydrolysed by prolidase. The resulting digest would probably be similar to the end products of the digestion in the animal, although hydrolysis would take longer than in physiological conditions. In this case total enzymic hydrolysis and suitable analysis of the end products can give a useful indication of the availability of amino acids, at least in the native protein.

Erepsin, as a source of intestinal exopeptidases, has been used[3,10] for similar purposes. Nevertheless, Ford[10] did not obtain increased values of available methionine by using erepsin. The failure is probably caused by inactivation of prolidase when stored dry, as is done with commercial preparates. The other reason of the failure might be the absence of competent activators.

Results of this study show that the availability of amino acids in native beef serum albumin, microbiologically determined after total enzymic digestion, corresponds to the theoretical amount in the protein. The same values were obtained irrespective of the micro-organisms used.

The problem of how much papain, leucine aminopeptidase and prolidase is sufficient to release all α-amino peptide bonds in those peptides, in which amino acid with enzyme

resistant bond is present near the N-terminal amino acid of the protein, is still unsolved. Release of peptide bonds from N-terminal amino acid would probably be stopped. Release of other peptide bonds from the C-terminal end by carboxypeptidases and prolinase would perhaps be necessary. In micro-organisms used for analysis some activity of carboxypeptidases could be expected and *S. faecalis* showed a capacity to release C-terminal lysine. The necessity of introducing carboxypeptidases and prolinase to enzymic system for total hydrolysis of heat-treated proteins has not been examined.

According to the results, the main loss of availability of amino acids occurred during first 6 hours of heat treatment of albumin. Baldwin *et al.*[6] treated casein with dextrose at ~120° and showed that the greatest loss of amino acids occurred during first hour, and it is probable that a heat-treated albumin is similar. This occurred with all essential amino acids to approximately the same degree as their percentage distribution in albumin.

It was ascertained that determination of available lysine by Carpenter's method with fluordinitrobenzene and by microbiological analysis give different results. In the non-digestible residue after enzymic hydrolysis of heat-treated albumin some lysin, capable of reacting with fluordinitrobenzene, was present. Nevertheless, similar values of lysine were obtained in trypsin digest by both methods. Ford[5] found similar results in whale-meat meals. It is probable that 'available' lysine determined chemically includes also some lysine, which cannot be considered to be available. For these reasons, microbiological determination after total enzymic digestion seems to give more real values of lysine availability.

Carpenter *et al.*[25] recommended adding hydroxylysine to the starting medium for microbiological analysis of lysine. In the present experiment this was not done, but it is thought that no great error is likely to have arisen because of this.

The lower digestibility of heat-treated albumins by trypsin may be explained by the substitution of ε-amino groups of lysine. Twice as much protein was digested in the preparate heat-treated at 121° without glucose than in the albumin with glucose, treated at the lower temperature. However this lowered digestibility cannot be explained solely by substitution of the ε-amino group of lysine by carbonyl compounds because only 55·5% of the albumin treated without glucose was hydrolysed. Substitution with other reactive groups from the protein can be considered. Crosslinks within or between molecules may be formed by heat treatment, as shown by Mecham & Olcott;[26] these probably cause resistance of treated proteins against proteolytic enzymes. Lysine with free ε-amino group as well as lysine with the ε-amino group blocked by a carbonyl compound, may be included in the part of protein, resistant to the action of enzymes.

A small amount of lysine was released by carboxypeptidases in addition from non-digestible residue of the protein after hydrolysis of trypsin. This C-terminal lysine could not be set free by trypsin.

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Received 18 April, 1967;
Amended manuscript 25 August, 1967

References

1. Miller, E. L., Hartley, A. W., & Thomas, D. C., *Brit. J. Nutr.*, 1965, **19,** 565
2. Donoso, G., Lewis, O. A. M., Miller, D. S., & Payne, P. R., *J. Sci. Fd Agric.*, 1962, **12,** 192
3. Beuk, J. F., Chornock, F. W., & Rice, E. E., *J. biol. Chem.*, 1949, **180,** 1243
4. Ford, J. E., *Brit. J. Nutr.*, 1962, **16,** 409
5. Ford, J. E., *Brit. J. Nutr.*, 1965, **19,** 277
6. Baldwin, R. R., Lowry, J. R., & Thiessen, R., *Fd Res.*, 1951, **16,** 107
7. Roubal, W. T., & Tappel, A. L., *Archs. Biochem. Biophys.*, 1966, **5,** 113.
8. Carpenter, K. J., *Biochem. J.*, 1960, **77,** 604
9. Rao, S. R., Carter, F. L., & Frampton, V. L., *Analyt. Chem.*, 1963, **35,** 1927
10. Ford, J. E., *Brit. J. Nutr.*, 1964, **18,** 449
11. Miller, E. L., Carpenter, K. J., Morgan, C. B., & Boyne, A. W., *Brit. J. Nutr.*, 1965, **19,** 249
12. Hill, R. L., & Schmidt, W. R., *J. biol. Chem.*, 1962, **237,** 389
13. Stein, W. H., & Moore, S., *J. biol. Chem.*, 1949, **178,** 79
14. Smith, E. L., in "Methods in Enzymology", 1955, Vol. II, p. 83 (Colowick, S. P., & Kaplan, N. O., Ed.) (New York: Academic Press)
15. Smith, E. L., in "Methods in Enzymology", 1955, Vol. II, p. 93 (Colowick, S. P., & Kaplan, N. O., Ed.) (New York: Academic Press)
16. Keller, P. J., Cohen, E., & Neurath, H., *J. biol. Chem.*, 1956, **223,** 457
17. Neurath, H., in "Methods in Enzymology", 1955, Vol. II, p. 77 (Colowick, S. P., & Kaplan, N. O., Ed.) (New York: Academic Press)
18. Block, R. J., in "Analytical Methods of Protein Chemistry", 1960, Vol. II, p. 3 (Alexander, P., & Block, R. J., Ed) (Oxford: Pergamon Press)
19. Stokes, J. L., Gunnes, M., Dwyer, I. M., & Caswell, M. C., *J. biol. Chem.*, 1945, **160,** 35
20. Stokes, J. L., & Gunnes, M., *J. biol. Chem.*, 1945, **157,** 651
21. Sauberlich, H. E., & Baumann, C. A., *J. biol. Chem.*, 1946, **166,** 417
22. Wood, E. C., *Nature, Lond.*, 1945, **155,** 632
23. Putnam, F. W., in "The Proteins", 2nd Edn., 1965, Vol. III, p. 153 (Neurath, H., Ed.) (New York: Academic Press)
24. Melnick, D., Oser, B. L., & Weiss, S., *Science, N.Y.*, 1946, **103,** 326
25. Carpenter, K. J., Hartley, A. W., & Ward, L. D., *Proc. Nutr. Soc.*, 1964, **23,** xl
26. Mecham, D. K., & Olcott, M. S., *Industr. Engng Chem.*, 1947, **39,** 1023

AVAILABILITY OF ESSENTIAL AMINO ACIDS FROM PROTEINS. II.*—Food proteins

By Z. DVOŘÁK

The availability of leucine from L-leucinamide hydrochloride, L-leucyl-glycyl-glycine and glycyl-L-leucine as substrates for leucine aminopeptidase and glycyl-leucine dipeptidase was studied with *Streptococcus faecalis*, *S. zymogenes* and *Lactobacillus arabinosus*. The results indicate that these micro-organisms have their own activity of exopeptidases through which they may utilise amino acids of peptides, released from the proteins by hydrolysis by endopeptidases. The availability of leucine from the proteins of beef muscle, hydrolysed by pepsin, trypsin, pancreatin or papain was also studied. The amount of available leucine found differed according to the enzyme and micro-organism used. Most efficient were pancreatin and papain. The calculated total amount of leucine present in the proteins studied was not found experimentally by digestion with any of these enzymes. Further hydrolysis by leucine aminopeptidase increased the availability of leucine to 84·6–100%. By using leucine aminopeptidase simultaneously with prolidase, complete availability with all micro-organisms was reached. It is suggested that the availability of essential amino acids in the proteins may be determined microbiologically after total enzymic hydrolysis by papain, leucine aminopeptidase and prolidase. By this method the proteins of beef muscle freeze-dried and stored for 4 years, and those of meat bone meal and blood meal were analysed.

Introduction

The availability of essential amino acids in bovine serum albumin after enzymic hydrolysis was studied in a previous communication.[1] The results confirmed that the digestibility of proteins decreases with the degree of denaturation[2] which also causes changes in the nutritive value of proteins. As a cause of lowered digestibility, changes in bonds between amino acids were considered. If the only amino acids considered to be available are those released to the greatest extent possible by proteolytic enzymes of the digestive tract and are not bound in enzyme-resistant complexes, then their availability may be determined *in vitro* by the use of these same enzymes. For this purpose pepsin,[3,4] pancreatin[5,6] or papain[7] has usually been used. These enzymes, as endopeptidases, split the protein to a different number of peptides but do not give complete hydrolysis. Quantitative hydrolysis was not obtained even by a combination of pepsin, trypsin and chymotrypsin with erepsin as the mixture of duodenal peptidases.[8] Ford[7] used microbiological determination of some amino acids with *Streptococcus zymogenes* NCDO 592 after hydrolysis of the protein by papain. If the enzymic system of this micro-organism is efficient enough to completely split peptides arising by papain hydrolysis, then the amount of amino acids determined corresponds to their availability. However other work by Ford[9] shows that in the papain digest of heat-treated proteins, peptides of higher molecular weight are present, and that in these the availability of the amino acids is low.

The purpose of this communication is partly to determine the availability of leucine from simple peptides to certain micro-organisms. Also presented is the degree of availability, in microbiological determinations, of leucine from the proteins of beef muscle after hydrolysis by some endopeptidases, and a comparison is made with the values obtained after total enzymic hydrolysis of the proteins by papain, leucine aminopeptidase and prolidase.[10] In this way the availability of essential amino acids in some food proteins is determined.

*Part I: Preceding paper

Experimental

Materials

Peptides: L-leucinamide hydrochloride and glycyl-L-leucine were synthetised in usual manner.[11] L-Leucyl-glycyl-glycine was a preparate from Medika, C.S.S.R.

Samples of beef muscle, *psoas major*, were taken from 7 mature animals of the red-mottled breed, without regard to their sex and age. Composite samples were dehydrated after being ground three times with ethyl alcohol and twice with ethyl ether at room temperature. The tissue was again ground after being dried in air, and the powder obtained was used for analysis.

Some samples of beef muscle, *psoas major*, were ground and freeze-dried, and the material was stored in a bottle for 4 years at room temperature. It was pulverised before analysis.

Meat-bone meal was obtained from the meal factory in Blatec. It was ground to pass a 40-mesh sieve and stored for 1 year at room temperature before use.

Blood meal was obtained by drying the blood of slaughtered animals in a Niro-Atomizer spraying system in the slaughterhouse in Brno.

Hydrolysis

Hydrolysis by pepsin

A preparate of beef muscle, containing 50·0 mg N, was suspended in 0·1 N-HCl. Further HCl was added to make up a volume of 19 ml. After addition of 1 ml 1% or 5% pepsin solution (Léčiva, C.S.S.R., 10,000 units/g) in 0·1 N-HCl, the mixture was incubated at 37° with constant shaking in Dubnoff's incubator. At appropriate intervals 2 ml were taken from the digest. In these aliquots pepsin was inactivated on a boiling-water bath for 5 min. After amendment of the pH to 7·0 the solution was diluted with water to 50 ml. This solution was taken for microbiological

determination of leucine (1 ml was calculated to contain 0·1 mg N). Control determinations with pepsin without addition of proteins were also made in the same way.

Hydrolysis by trypsin

This was carried out as for pepsin, except that the preparates of beef muscle and trypsin (crystalline, Lachema, C.S.S.R.) were suspended and dissolved in a buffer Na_2HPO_4–HCl 0·05 M, pH 8·1. Toluene, 0·5 ml, was added to the mixture before incubation.

Hydrolysis by pancreatin

This again was as above. The preparate of beef muscle was suspended in water and the pH was corrected to 7·8 with 0·1 N-NH_4OH. The solution of pancreatin (acetone extract from beef pancreas, prepared according to Keller *et al.*[12]) was prepared by 30 min extraction of 1 g pancreatin with 20 ml water at +2°. After filtration and amendment of the pH to 7·8 this filtrate was used (5% solution) or diluted 5 times with water (1% solution).

Hydrolysis by papain

For hydrolysis of beef muscle Ford's procedure[7] was used. The incubation mixture had the same composition as for the hydrolysis by pepsin, but the proteins were hydrolysed in a solution, the composition of which was per 1 litre: 5 g sodium citrate, 30 mg NaCN. The pH was brought to 7·0 with 1 N-H_3PO_4. Papain was a preparate of Meer Corp., New York. The incubation was run at 56°.

Hydrolysis by leucine aminopeptidase and prolidase

This was carried out only after previous digestion by endopeptidases. Such a digest represents 5 mg N of the original proteins in 2 ml. In this volume endopeptidases were inactivated by boiling on a water bath for 5 min (digests with papain were previously acidified to pH 2). After correction of the pH to 8·5, 1 ml veronal–acetate buffer, 0·02 M, pH 8·5 and 0·1 ml $MnSO_4$, 0·04 M, was added. Leucine aminopeptidase, the proteolytic coefficient of which was approximately $C_1 = 40$ (enzyme was obtained by purification from acetone extract of pig kidneys[13]) was added at 0·3, 0·6 or 0·9 ml, and the volume was made up to 5·0 ml with veronal–acetate buffer. Control measurements were made by incubation with leucine aminopeptidase without proteins. Incubation ran for 24 hours at 40° in Dubnoff's incubator. After being boiled on a water bath for 5 min the pH was amended to 7·0 and the digests were filtered and diluted to 50 ml with water. 1 ml of the digest corresponds to 0·1 mg N of the original proteins. This solution was taken for analysis.

When prolidase was used with leucine aminopeptidase, the procedure differed only in so far as 0·1, 0·2 or 0·3 ml of the solution of prolidase was added to the incubation mixture. Prolidase was purified from acetone extract of pig kidneys[14] and had the average proteolytic coefficient $C_1 = 20$.

Total enzymic hydrolysis

Total enzymic hydrolysis of the proteins of beef muscle, freeze-dried muscle, meat-bone meal and blood meal by papain, leucine aminopeptidase and prolidase was made in the same way as before.[1] For digestion with exopeptidases greater aliquots of the papain digest and of exopeptidases were taken so that the determination of all essential amino acids was possible.

Acid hydrolysis of the proteins of meat-bone and blood meals

Proteinaceous samples corresponding to 50 mg N were refluxed with 40 ml 6 N-HCl for 16 hours. After evaporation of hydrochloric acid in the rotary evaporator, the digest was diluted with water and the pH was corrected to 7·0. After filtration the solution was diluted with water so that 1 ml corresponded to 0·1 mg N of the original sample. This solution was used for analysis.

Microbiological analysis

Analysis of nitrogen and essential amino acids microbiologically, using *Streptococcus faecalis* ATCC 8043, *S. zymogenes* NCDO 592 and *Lactobacillus arabinosus* ATCC 8014 was described in the previous communication.[1]

Results

Availability of leucine from synthetic peptides to *Streptococcus faecalis, S. zymogenes* and *Lactobacillus arabinosus*

The degree of hydrolysis of peptides known to be substrates for determination of the activity of leucine aminopeptidase and glycylleucine dipeptidase was studied with the micro-organisms used. To culture media deficient in leucine was added, L-leucinamide hydrochloride, L-leucyl-glycyl-glycine or glycyl-L-leucine in an amount equivalent to 0·05 mg L-leucine in 1 ml of the solution. The growth of the micro-organisms was compared with their growth in a medium containing L-leucine (0·05 mg/ml), and the results are given in Fig. 1. It is seen that all 3 micro-organisms use these peptides but to different extents. It is suggested that before they are utilised they are hydrolysed by competent enzymes. The highest activity for aminopeptidase and glycyl-leucine dipeptidase was shown by *S. faecalis*, for which 100% hydrolysis of the substrates for aminopeptidase was found after only 24 hours. Glycyl-L-leucine was 93% hydrolysed after 72 hours of incubation. *L. arabinosus* showed lower activity, and with this, L-leucinamide HCl was hydrolysed more quickly than L-leucyl-glycyl-glycine. *S. zymogenes* hydrolysed all 3 peptides to more than 60% in 3 days.

Availability of leucine from the proteins of beef muscle after hydrolysis by endopeptidases

The proteins of beef muscle were hydrolysed by pepsin, trypsin, pancreatin and papain. The course of hydrolysis was studied by the microbiological determination of leucine in the digests, using *S. faecalis*, *S. zymogenes* and *L. arabinosus*. These proteins of beef muscle contained 8·00 g leucine/16 g N, the mean of all values obtained with all micro-organisms after hydrolysis of the proteins with 6 N-HCl.

The course of hydrolysis by pepsin is indicated in the Fig. 2. If 0·05% or 0·25% pepsin is used, only the initial rate changes. It is seen that the availability of leucine from peptic peptides varies according to the micro-organism used; the most active is *S. zymogenes*, with which 80% of the total leucine was determined. The availability of leucine is approximately 50% for other micro-organisms.

The availability of leucine from trypsin digests of beef muscle is shown in Fig. 3. It is similar with all micro-organisms and low in comparison with the hydrolysis by pepsin. Constant values were reached after 24 hours and represent 34–45% of total leucine.

Hydrolysis by pancreatin on the other hand is rapid. The results in Fig. 4 indicate that *S. zymogenes* used 89% of

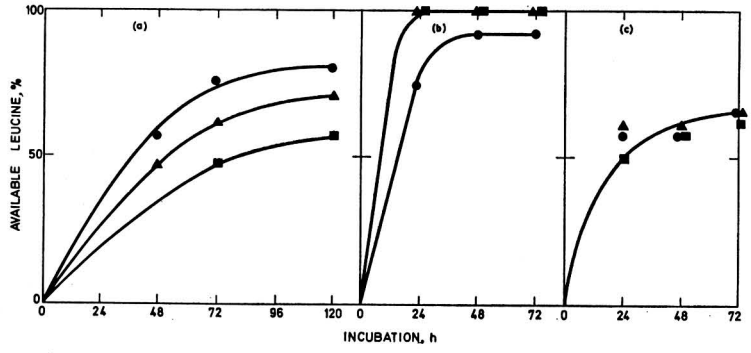

FIG. 1. *The availability of leucine from peptides determined by* Lactobacillus arabinosus, Streptococcus faecalis *and* S. zymogenes
(a)—*L. arabinosus*; (b)—*S. faecalis*; (c)—*S. zymogenes*;
●—L-leucinamide HCl; ■—L-leucyl-glycyl-glycine; ▲—glycyl-L-leucine

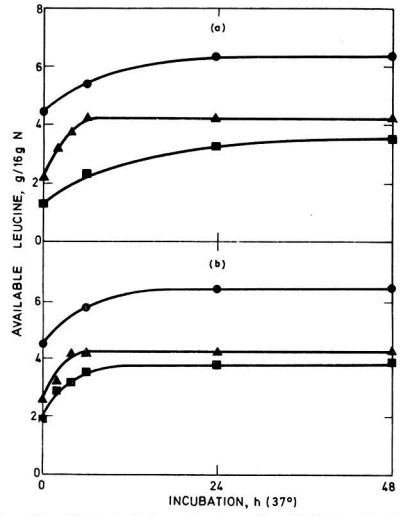

FIG. 2. *Microbiological determination of available leucine from the proteins of beef muscle after hydrolysis by pepsin*
(a)—0·05% pepsin in the solution; (b)—0·25% pepsin in the solution;
●—*S. zymogenes*; ■—*S. faecalis*; ▲—*L. arabinosus*

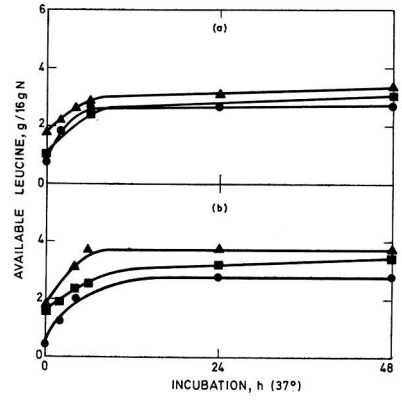

FIG. 3. *Microbiological determination of available leucine from the proteins of beef muscle after hydrolysis by trypsin*
(a)—0·05% trypsin in the solution; (b)—0·25% trypsin in the solution;
●—*S. zymogenes*; ■—*S. faecalis*; ▲—*L. arabinosus*

leucine from the digest; *S. faecalis* and *L. arabinosus* used 67% and 73%, respectively.

The amount of leucine from digestion with papain is given in Fig. 5. There is greater variability according to the micro-organisms used, the most efficient being *S. zymogenes*. The initial rate of digestion with this micro-organism is so rapid that a constant value for leucine was obtained after only 2 hours' or 4 hours' incubation with the concentration of the enzyme at 0·25% or 0·05%, respectively. For *S. zymogenes* 80% leucine is available; for *S. faecalis* and *L. arabinosus*, constant availability with the concentration of papain at 0·05% is not reached even after 48 hours' digestion, and the values are 52% and 69%, respectively.

Availability of leucine from enzymic digest of beef muscle after further hydrolysis by leucine aminopeptidase and prolidase

The digests obtained by action of endopeptidases on the proteins of beef muscle were further hydrolysed by purified leucine aminopeptidase in progressively increasing quantities. Leucine in the digest was determined with *S. faecalis* and *S. zymogenes*. The results are given in the Table I. Hydrolysis by aminopeptidase increases the amount of leucine found in all digests to 92–96% of total leucine. An exception is the trypsin digest with *S. zymogenes*, where only 84·6% total leucine was found. Also leucine determination with *S.*

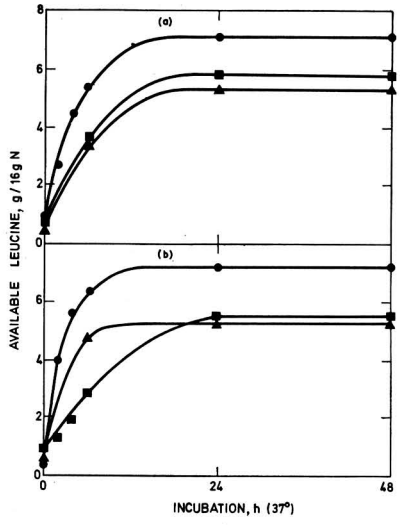

FIG. 4. *Microbiological determination of available leucine from the proteins of beef muscle after hydrolysis by pancreatin*
(a)—0·05% pancreatin in the solution; (b)—0·25% pancreatin in the solution;
●—*S. zymogenes*; ■—*S. faecalis*; ▲—*L. arabinosus*

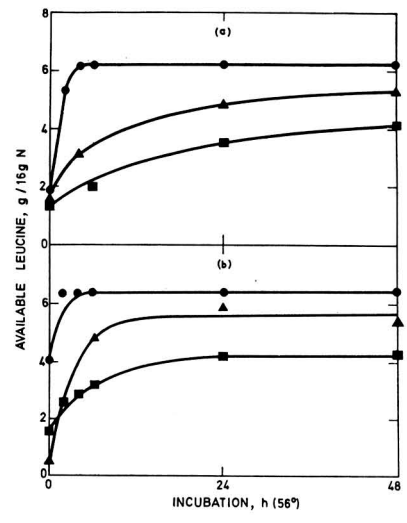

FIG. 5. *Microbiological determination of available leucine from the proteins of beef muscle after hydrolysis by papain*
(a)—0·05% papain in the solution; (b)—0·25% papain in the solution;
●—*S. zymogenes*; ■—*S. faecalis*; ▲—*L. arabinosus*

TABLE I

Available leucine in the proteins of beef muscle after hydrolysis by endopeptidases, followed by leucine aminopeptidase
(Z—*Streptococcus zymogenes*; F—*S. faecalis*)

		Leucine after				% of total leucine (max. value = 8·00 g/16 g N)
		24 hours of hydrolysis by endopeptidase g/16 g N	further 24 hours of hydrolysis by leucine aminopeptidase g/16 g N			
			0·3 ml	0·6 ml	0·9 ml	
Pepsin	Z	6·77	7·38	7·38	7·38	92·4
	F	4·00	7·11	7·57	7·11	94·5
Trypsin	Z	3·08	5·54	6·15	6·77	84·6
	F	3·52	7·11	7·57	7·11	94·5
Pancreatin	Z	7·38	8·00	8·00	8·00	100
	F	5·77	6·22	7·73	7·11	96·5
Papain	Z	6·15	7·38	7·38	7·38	92·4
	F	4·45	7·11	7·57	7·57	94·5

zymogenes on the original digest by pancreatin was quantitative.

To release all the leucine from the proteins it was necessary to hydrolyse the peptides by both leucine aminopeptidase and prolidase. Therefore leucine determined after hydrolysis by endopeptidase and leucine aminopeptidase was compared with leucine determined after hydrolysis by the same endopeptidase and aminopeptidase with simultaneous addition of prolidase, the amount of which was progressively increased. From the results given in the Table II it may be seen that, with prolidase, quantitative determination of leucine may be attained by using any of the organisms cited.

Available essential amino acids in some food proteins

Eight available essential amino acids were determined in some food proteins after total enzymic hydrolysis by papain, leucine aminopeptidase and prolidase. The results are shown in Table III. Muscle proteins of beef fillet, prepared from fresh tissue and used as mentioned above, compared with the same sort of muscle, freeze-dried and stored 4 years in air at the room temperature, showed higher availability of particular amino acids. For comparison also the results obtained by hydrolysis of this sample by papain only are given. These values are lower than after the preceding hydrolysis by leucine aminopeptidase and prolidase. Except for phenylalanine,

TABLE II

Available leucine in the proteins of beef muscle after hydrolysis by pepsin, leucine aminopeptidase and prolidase

	Leucine after					% of total leucine (max. value = 8·00 g/16 g N)
	24 hours of hydrolysis by pepsin g/16 g N	further 24 hours of hydrolysis by l. amino-peptidase (0·6 ml) g/16 g N	further 24 hours of hydrolysis by l. aminopeptidase (0·6 ml) + prolidase g/16 g N			
			0·1 ml	0·2 ml	0·3 ml	
Streptococcus zymogenes	6·68	7·34	8·00	8·00	8·00	100
S. faecalis	4·37	7·16	8·05	8·05	8·05	100·5
Lactobacillus arabinosus	4·67	6·66	7·98	—	7·98	99·8

TABLE III

Essential amino acids in some protein foods, determined after enzymic and acid hydrolysis (g/16 g N)

	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Beef muscle—fillet, fresh								
Total enzymic hydrolysis	6·16	8·00	9·15	2·73	4·52	4·57	1·44	6·00
Beef muscle—fillet, freeze-dried and stored 4 years								
Total enzymic hydrolysis	5·54	7·04	7·25	2·56	4·07	3·20	0·90	5·90
Hydrolysis by papain	5·33	6·39	6·64	2·40	3·78	3·20	0·75	4·42
Meat-bone meal								
Acid hydrolysis	3·20	6·62	5·33	1·28	3·62	2·67	—	4·43
Total enzymic hydrolysis	2·74	4·97	3·66	0·96	2·91	2·00	0·42	3·41
Availability, %	85·6	75·1	68·6	75·0	80·4	75·3	—	77·0
Blood meal								
Acid hydrolysis	2·29	11·50	9·43	1·60	6·75	2·49	—	8·00
Total enzymic hydrolysis	1·97	8·53	7·31	0·96	6·22	1·80	0·63	6·18
Availability, %	86·0	74·2	77·5	60·0	92·2	72·3	—	77·2

threonine and lysine, they represent the availability obtained by Ford's method[7] with *S. zymogenes*.

The availability of essential amino acids in meat-bone and blood meals, compared with the total amount obtained after acid hydrolysis, was always lower and represented 60·0–92·2% of the total amount of particular amino acids. It appears that the least loss of availability occurs with phenylalanine and isoleucine, and the greatest loss with lysine and methionine.

Discussion

The determination of the availability of essential amino acids in the proteins *in vitro* by the method discussed is based on 2 suppositions: firstly, in the hydrolysis of the proteins by endopeptidase, which releases different peptide bonds, and by exopeptidases, which are able to split all usual peptide bonds in peptides from their N-terminal end, only those parts of the proteins, which contain a structure, which is not attacked by any of proteolytic enzymes of the digestive tract, are not split; secondly, in the digest the micro-organisms used only free amino acids for growth.

Although results with native beef serum albumin[1] indicate that the amount of available essential amino acids released coincides with the theoretical amount, a single digestion by papain, leucine aminopeptidase and prolidase does not always give quantitative hydrolysis. Haley *et al.*[15] digested some proteins by viocase, pronase and then by leucine aminopeptidase with prolidase and did not obtain complete hydrolysis. Further digestion by carboxypeptidases increased the degree of hydrolysis, but still not to 100%.

For this reason, micro-organisms used for the determination of essential amino acids were tested on the availability of leucine from peptides which are used as substrates for leucine aminopeptidase and glycyl-leucine dipeptidase. From all peptides the availability of leucine was greater than 50% after 72 hours with all micro-organisms examined. Results obtained by Kuiken *et al.*[16] for the availability of leucine from glycyl-L-leucine with *L. arabinosus* agree with this. Krehl & Fruton[17] examined the availability of leucine from different peptides with *S. faecalis* and *L. arabinosus*. They found lower availability for L-leucinamide acetate than for L-leucyl-glycyl-glycin, which agrees with the present results for *S. faecalis*, but with *L. arabinosus* they found 100% availability after 72 hours for glycyl-L-leucine, in contrast to 72% in the present experiment, and also lower availability for glycyl-L-leucine with *S. faecalis*. These differences may be caused by the different composition of the cultivation media. From the results *S. faecalis*, *S. zymogenes* and *L. arabinosus* can utilise leucine from the peptides for growth, and can therefore influence the substrate by their own system of peptidases. *S. faecalis* also shows carboxypeptidase, and it is probable that carboxypeptidases are also present in other micro-organisms. It may be suggested that, during the determination of the availability of essential amino acids, the micro-organisms used take a part on the splitting of some peptides, which had not been completely hydrolysed by previous enzymic digestion.

The importance of the choice of a suitable endopeptidase for the initial splitting of the proteins was shown. Pepsin and trypsin were chosen as enzymic individua of the digestive

tract. Pancreatin represents a mixture of enzymes, present in the pancreas. These enzymes were compared with papain, used by Hill & Schmidt[10] for total enzymic hydrolysis. The amount of leucine shown depended on the micro-organism and enzyme used. The least variable values were obtained for hydrolysis by trypsin, where a maximum of 3·7 g leucine/16 g N from a total 8·00 g/16 g N. Trypsin attacks only peptide bonds with carboxyl of basic amino acids and gives limited availability of leucine from high-mol. wt. peptides. (Also inactivation of trypsin after 6 hours' incubation cannot be excluded.) Hydrolysis by pepsin makes about 50% leucine available with *S. faecalis* and *L. arabinosus*. *S. zymogenes* with its greater endopeptidases activity is probably most suitable for determining leucine after digestion by pancreatin and papain. With *S. zymogenes* 7·2 g leucine/16 g N were found in the pancreatin digests; with *S. faecalis* and *L. arabinosus* only about 5 g leucine/16 g N were found. The complex of endopeptidases and exopeptidases in pancreatin evidently influences the degree of hydrolysis of muscle proteins. The results obtained with pancreatin are higher than those with papain, and with *S. zymogenes* values obtained with papain are nearly the same as with pepsin. The availability of leucine with *S. faecalis* and *L. arabinosus* after 48 hours is 4·15 and 5·33 g/16 g N, respectively. In all cases digestion is almost complete after 24 hours at a concentration of enzymes in the incubation mixture of 0·25%; a concentration of 0·05% for 24 hours of hydrolysis is usually sufficient. Although pancreatin appears to be the most active enzyme system for initial digestion of proteins, papain was chosen for these purposes, because it is easier to characterise.

Hydrolysis of the proteins by endopeptidases with digestion by exopeptidases principally increases the availability of leucine. When only leucine aminopeptidase was used, the availability shown depended on the micro-organism used; in the case of pancreatin and leucine aminopeptidase the complete amount of leucine present in the sample was shown, and in the other cases, more than 90% of the total leucine could usually be determined. Leucine aminopeptidase alone is not sufficient for total enzymic hydrolysis, because 4–15% of the leucine remains bound in peptides for which leucine aminopeptidase is inactive. Peptide bonds with proline may be split by the use of prolidase, and with this enzyme the proteins were completely hydrolysed, so that the amount of leucine found corresponded to the total amount. After total enzymic hydrolysis in this way, the amount of leucine found was the same whichever micro-organism was used for its determination.

In the analysis of available amino acids in freeze-dried beef muscle, Ford's method,[7] using hydrolysis by papain and followed by the determination of amino acids microbiologically, gives lower values of the availability in comparison with total enzymic hydrolysis. To what extent hydrolysis of the protein by only papain is sufficient for determination of the availability of particular amino acids, depends on the sequence of amino acids and the total quantity of particular amino acids in the protein, so total enzymic hydrolysis may be the more suitable.

Determination of the availability of essential amino acids in proteins of beef muscle, meat-bone and blood meals showed that decreased availability differed for particular amino acids and varied according to the type of protein and its treatment. Proteins freeze-dried but stored for a long time were denatured in such a way that they contained only 68% of available threonine. Drying of blood and storage for one year decreased the availability of methionine to 60%. These were also in the proteins of meat-bone meal, which were presumably denatured most by heat treatment. The lowest availability was found for lysine, 68·6%. Loss of the availability in the protein sources studied concerned methionine, threonine and lysine mainly, and was less concerned with phenylalanine, isoleucine and valine.

Differentiation into 'available' and 'unavailable' is arbitrary and cannot be exactly interpreted in terms of animal or human nutrition, although the concept may be used for nutritional evaluation of proteins. Some revision may be necessary if in the present method the enzyme system requires the addition of other enzymes, such as carboxypeptidases. Complete judgment of the results obtained must be withheld until tests on laboratory animals are reported.

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Received 16 May, 1967

References

1. Dvořák, Z., *J. Sci. Fd Agric.*, 1968, **19,** 71
2. Rice, E. E., & Beuk, J. F., *Adv. Fd Res.*, 1953, **4,** 246
3. Sheffner, A. L., Eckfeldt, G. A., & Spector, H., *J. Nutr.*, 1956, **60,** 105
4. Jacquot, R., & Abraham, J., *Nutritio Dieta*, 1965, **7,** 43
5. Akeson, W. R., & Stahmann, M. A., *J. Nutr.*, 1964, **83,** 257
6. Menden, E., & Cremer, H. D., *Nutritio Dieta*, 1966, **8,** 188
7. Ford, J. E., *Br. J. Nutr.*, 1962, **16,** 409
8. Frankel, E. M., *J. biol. Chem.*, 1916, **26,** 31
9. Ford, J. E., *Br. J. Nutr.*, 1965, **19,** 277
10. Hill, R. L., & Schmidt, W. R., *J. biol. Chem.*, 1962, **237,** 389
11. Smith, E. L., in 'Methods in Enzymology', 1957, Vol. III, p. 528 (Colowick, S. P., & Kaplan, N. O., Ed.) (New York: Academic Press)
12. Keller, P. J., Cohen, E., & Neurath, H., *J. biol. Chem.*, 1956, **223,** 457
13. Smith, E. L., in 'Methods in Enzymology', 1955, Vol. II, p. 83 (Colowick, S. P., & Kaplan, N. O., Ed.) (New York: Academic Press)
14. Smith, E. L., in 'Methods in Enzymology', 1955, Vol. II, p 93 (Colowick, S. P., & Kaplan, N. O., Ed.) (New York: Academic Press)
15. Haley, E. E., Corcoran, B. J., Dorer, F. E., & Buchanan, D. L., *Biochemistry*, 1966, **5,** 3229
16. Kuiken, K. A., Norman, W. H., Lyman, C. M., & Blotter, L., *J. biol. Chem.*, 1943, **151,** 615
17. Krehl, W. A., & Fruton, J. S., *J. biol. Chem.*, 1948, **173,** 479

REMOVAL OF LIMONIN FROM BITTER ORANGE JUICE

By B. V. CHANDLER, J. F. KEFFORD and G. ZIEMELIS

Polyamides are shown to be effective adsorbents for removal of the bitter principle, limonin, from pasteurised Washington Navel orange juice. The extent of limonin removal varies with different juices, but sufficient can be removed by one or two treatments to bring the concentration below the organoleptically detectable level. The commercial use of polyamides to prepare non-bitter juices from Navel oranges is discussed.

Introduction

The bitterness that develops in many orange juices on standing or heating is due to the dilactone limonin, a tetranortriterpenoid of formula $C_{26}H_{30}O_8$.[1,2] This bitter principle, initially present in the albedo of the orange, passes into the juice from incorporated albedo particles, until at concentrations of 8–12 ppm the juice becomes detectably bitter.[3] Owing to high limonin contents in the albedo, bitterness can become particularly objectionable in juice from Washington Navel oranges, which are not normally processed for consumption as pure orange juice or concentrate. Two methods have been patented for 'de-bittering' Navel juice, one involving lengthy selective extraction and adsorption procedures,[4] and the other an adjustment of juice pH both after extraction and immediately before consumption.[5] These processes do not appear to have received commercial application.

A recent report[6] on the use of polyamide as an adsorbent in the thin-layer chromatography of various lactones, mainly sesquiterpenoid, prompted a study of its suitability as an adsorbent for removing limonin from bitter orange juice. Since polyamide is also an effective adsorbent for flavanones,[7] it may simultaneously remove the bitter flavanone neohesperidosides, such as naringin, from orange juice. Therefore, to determine whether polyamide removed bitterness from the juice by limonin adsorption or by flavonoid adsorption, bitterness removal was followed by chemical analysis of the juice for limonin,[3] as well as by organoleptic assessment. Since polyamide can be used in the thin-layer chromatography of ascorbic acid,[6] tests were also made on its ability to adsorb this important orange juice constituent.

Experimental

Three polyamides were used in this work: a polycaprolactam type, prepared as a dry powder in the laboratory[8] from Ultramid BM pellets (Badische Anilin-Soda-Fabrik A.G., Ludwigshafen, Germany), a Nylon-66 type, commercially marketed as Polypenco Moulding Powder (Polymer Corporation, Reading, Pa., U.S.A.), and an insoluble polyvinylpyrrolidone, commercially marketed as Polyclar (General Aniline and Film Corporation, New York City, U.S.A.).

The juice was extracted from Washington Navel oranges on an F.M.C. Model 091 in-line juice extractor. It was processed in lacquered cans held for 40 seconds in a spin-cooker in an atmosphere of steam, and stored at 0° for varying periods.

Except where otherwise noted, the treatments with polyamide were carried out as follows. The juice was centrifuged for 20 minutes at 800 g to separate the cloud particles, the clear decanted serum was treated with 1% (w/v) butylated hydroxyanisole to prevent atmospheric oxidation of limonin[3] from interfering in the analysis, and gently stirred on a magnetic stirrer with varying amounts of dry polyamide for varying periods of time. The polyamide was removed by centrifugation, and the resultant serum was analysed for limonin[3] and tasted for bitterness by two experienced tasters.

In determinations of the removal of ascorbic acid from orange juice by polyamide, samples of clarified juice were stirred with and without the adsorbent, and a comparison was made of the 2 : 6 dichlorophenolindophenol titres of the supernatant sera; both samples contained butylated hydroxyanisole as above.

Juice reconstitution was achieved by stirring the polyamide-treated serum for 5 minutes with the previously separated pulp; cloud stability was assessed by visual comparison of the resultant product with a sample of the original juice that had been given no treatment other than cloud resuspension by shaking.

Results

From preliminary experiments it was apparent that polyamide adsorbed limonin from orange juice serum; when a slightly bitter juice containing 12·8 ppm limonin was shaken for one hour with Nylon-66 powder (5 g per 100 ml) it gave a non-bitter serum containing 4·8 ppm limonin (62·5% removal). Under this treatment the juice, apparently as a result of aeration, developed an off-flavour which was not evident in a juice gently (magnetically) stirred for one hour with Nylon-66 powder in the same proportion. In this case the limonin content was lowered from 21·7 ppm (strongly bitter) to 10·7 ppm (slightly bitter) without production of off-flavour, whereas the shaking procedure with a sample of the same juice resulted in an almost identical limonin removal (50·7%) with concomitant off-flavour production.

The three types of polyamide tested, polycaprolactam, Nylon-66 and insoluble polyvinylpyrrolidone were found to be equally effective (Table I), but Nylon-66, as the material most readily available, was used in all subsequent tests. In most cases the adsorbent was added to the juice as a dry powder, but preliminary investigations showed that better results could be obtained if the polyamide was moistened for

TABLE I

Comparison of the activities of various polyamides, wet and dry, in the adsorption of limonin from bitter orange juice sera (100 ml), after 30 minutes gentle stirring

Type of polyamide	Condition*	Amount of polyamide g	Initial limonin content ppm	Final limonin content ppm	Amount of limonin removed ppm	Percentage limonin removed	Final bitterness
nylon 66	dry	5	18·4	9·6	8·8	47·8	slight
polycaprolactam	,,	5	18·4	9·9	8·5	46·2	—
nylon 66	,,	1	18·4	14·4	4·0	21·7	moderate
polycaprolactam	,,	1	18·4	15·6	2·8	15·2	—
nylon 66	wet	4	21·1	11·2	9·9	46·9	slight
,,	dry	4	21·1	11·2	9·9	46·9	slight
,,	wet	1	21·1	15·2	5·9	28·1	moderate
,,	dry	1	21·1	14·1	7·0	32·6	moderate
,,	wet	4	8·4	4·4	4·0	47·6	none
,,	dry	4	8·4	5·1	3·3	39·4	none
polycaprolactam	wet	4	8·4	4·5	3·9	46·4	none
,,	dry	4	8·4	5·1	3·3	39·4	none
polyvinylpyrrolidone	wet	4	8·4	5·3	3·1	36·9	none
,,	dry	4	8·4	4·4	4·0	47·6	none
nylon 66	wet	4	9·8	5·8	4·0	40·8	none
,,	dry	4	9·8	8·0	1·8	18·4	doubtful
polycaprolactam	wet	4	9·8	6·5	3·3	33·7	doubtful
,,	dry	4	9·8	7·3	2·5	25·5	doubtful
polyvinylpyrrolidone	wet	4	9·8	6·1	4·7	48·0	none
,,	dry	4	9·8	6·9	2·9	29·6	doubtful

* 'wet' indicates that the polyamide was made into slurry with water 24 hours before use; all other treatments were with dry powder

24 hours before use. This procedure is known to activate polyamide adsorbents for other purposes,[9] although the present studies showed that its effect could vary from negligible to very marked, apparently depending upon the juice sample (Table I).

As shown by the results, particularly those in Table II, there were quantitative differences in the amounts and percentages of limonin removed from individual juices but for each serum sample the reproducibility of the results lay within the order of accuracy of the determination. Although there is no fixed relationship between the extent of limonin removal and the amount of polyamide used or the duration of the treatment, Table II demonstrates the general result of polyamide treatment.

Table III summarises experiments relating to the effect of time of treatment and ratio of polyamide to juice on the efficiency of limonin adsorption with respect to only one juice for each variable. Similar results were obtained with other juices. Most of the limonin (about 75%) removed in any one treatment is removed in the first 10–20 minutes, and there is an optimum ratio of polyamide to juice, corresponding to about 2 g per 100 ml, beyond which further addition of polyamide has little effect on the amount of limonin absorbed.

The most efficient removal of limonin from juice serum was achieved with a two-step procedure (Table IV); and even a freshly processed Navel juice of abnormally high limonin content (33·7 ppm) yielded a juice of doubtful bitterness

TABLE II

Juice-to-juice variation in efficiency of limonin removal from strongly bitter juice sera by nylon-66 powder (2 g per 100 ml serum) after 30 minutes gentle stirring

Juice	Initial limonin content ppm	Final limonin content ppm	Amount of limonin removed ppm	Percentage limonin removed	Final bitterness
A	19·8	15·6	4·2	21·2	moderate
A	19·8	16·1	3·7	18·7	moderate
B	21·4	17·0	4·4	20·6	strong
C	20·2	14·4	5·8	28·7	moderate
C	20·2	13·6	6·6	32·6	moderate
D	22·3	14·2	8·1	36·2	moderate
E	17·5	10·7	6·8	38·8	slight
E	17·5	9·8	7·7	43·5	slight

TABLE III

Removal of limonin from strongly bitter orange juice sera (100 ml) by various treatments with polyamide

Amount of polyamide g	Duration of treatment min	Initial limonin content ppm	Final limonin content ppm	Amount of limonin removed ppm	Percentage limonin removed	Final bitterness
2	5	23·7	18·9	4·8	20·1	strong
,,	10	,,	15·7	8·0	33·5	moderate
,,	20	,,	14·5	9·2	38·8	,,
,,	30	,,	14·9	8·8	37·0	,,
,,	45	,,	13·7	10·0	42·0	,,
,,	60	,,	12·9	10·8	45·7	slight
0·5	30	21·5	15·8	5·7	26·5	moderate
1·0	,,	,,	14·8	6·7	31·1	,,
2·0	,,	,,	12·6	8·9	41·3	slight
3·0	,,	,,	12·8	8·7	40·4	,,
4·0	,,	,,	12·0	9·5	44·0	,,
5·0	,,	,,	12·3	9·2	42·8	,,

TABLE IV

Comparison of the efficiencies of single and double treatments with polyamide for the removal of limonin from strongly bitter orange juice sera

Juice storage history	Treatment	Amount of polyamide g	Duration of treatment min	Initial limonin content ppm	Residual limonin content ppm	Amount of limonin removed ppm	Percentage limonin removed	Final bitterness
2 years at 0°C	single	4	30	19·2	8·5	10·7	55·7	none
	double—1st step	2	15	19·2	12·8	6·4	30·2	slight
	,, —2nd step	2	15	12·8	4·3	8·5	67·0	none
	,, —overall	2 × 2	2 × 15	19·2	4·3	14·9	77·6	none
1 year at 0°C	single	2	30	20·4	11·8	8·6	40·2	slight
	double—1st step	1	15	20·4	15·6	4·8	23·5	moderate
	,, —2nd step	1	15	15·6	8·5	7·1	45·5	none
	,, —overall	2 × 1	2 × 15	20·4	8·5	11·9	58·4	none
freshly processed	single	2	30	33·7	23·4	10·3	30·5	strong
	double—1st step	1	15	33·7	27·6	6·1	18·1	strong
	,, —2nd step	1	15	27·6	9·6	18·0	65·0	doubtful
	,, —overall	2 × 1	2 × 15	33·7	9·6	24·1	71·5	doubtful

(8·6 ppm) after two fifteen-minute stirrings with 1 g Nylon-66 per 100 ml. Comparison of the amounts of limonin removed in the first and second extraction clearly demonstrates that the second process removes much more limonin than the first.

The pulp particles separated in the initial centrifugation were satisfactorily resuspended in the juice serum simply by stirring for a few minutes. No significant differences in rates of cloud separation were observed between treated and untreated juices.

Determination of the ascorbic acid contents of juices before and after polyamide treatment indicated that up to 30% loss could occur in the operation, but stirring of juices without addition of polyamide reduced the ascorbic acid contents by up to 12%. This secondary loss was negligible in juices stored less than three months and most apparent in juices stored long enough for can corrosion to have occurred; it may thus be ascribed to iron-catalysed atmospheric oxidation of ascorbic acid in the control of which the antioxidant, butylated hydroxyanisole, is ineffective.

To determine the extent of ascorbic acid adsorption by polyamide it was therefore necessary to use as controls stirred juices containing no polyamide. In this way the following percentage losses of ascorbic acid by adsorption were recorded for juices stirred for 30 minutes with the specified amount of polyamide (in g per 100 ml): 0·5, 7%; 1·0, 14%; 1·5, 21%; 2·0, 21%. In a two-step treatment with two one-gram lots of polyamide used for 15 minutes each, the first process removed 12% of the ascorbic acid and the second a further 13%, i.e. 15% of that remaining. In the case of ascorbic acid adsorption, the second process was therefore only slightly more efficient than the first; the difference in the behaviour of limonin in this regard may be due to the much greater solubility of ascorbic acid in water which makes its adsorption by polyamide much less complete than limonin adsorption.

Discussion

The procedure described above for 'de-bittering' orange juice, although only studied on a laboratory scale, has the great advantage over the previous methods in that it does not require chemical modification of the juice, either by pH adjustment or by addition of solvents. The loss of ascorbic acid in the process may be considered a drawback, but it

can be limited to less than 25% by use of freshly processed juice or by control of atmospheric oxidation.

To be successful the polyamide treatment must be applied to heat-pasteurised juices since the adsorption process requires the limonin to be present in solution and not contained, as in the fresh juice, within the pulp particles. Unless the amount of limonin in the centrifuged pulp is low, bitterness would develop in the reconstituted juice on pasteurisation, as it does with fresh juice. In most Navel and in all Valencia juices the limonin content of the processed juice does not exceed the saturation point; consequently the amount of limonin remaining in the pulp would be too small to cause bitterness on reconstitution and re-pasteurisation of the treated juice.

The most efficient adsorption so far obtained involved a two-step treatment, and it is noteworthy that the second step can remove up to three times as much limonin as the first step. These results are consistent with the known heterogeneity of polyamide powders and the preferential adsorption by polyamide of flavonoids, such as hesperidin, which contain phenolic groups for which polyamides have a pronounced affinity.[10] Thus, in a double batch treatment, most if not all of the polyphenolics is removed in the first treatment, so that the second treatment provides polyamide of full activity for limonin adsorption; by contrast, in a single-batch treatment with the same total amount of polyamide, adsorption of polyphenolics leaves a less active polyamide available for limonin removal. The variations in the efficiency of the treatment from juice to juice may be due to variations in the amount of polyphenolics present in juices of different maturities and storage histories.

The commercial significance of the adsorption of limonin from orange juice by polyamide remains to be tested. These results demonstrate that a non-bitter serum can be prepared from highly bitter orange juices by simple and direct treatment with polyamide. It has also been found that the insoluble pulp particles, separated from the juice prior to this treatment, can be readily re-suspended in the serum to give a non-bitter orange drink of satisfactory flavour and appearance. Hence, polyamide treatment, which is now an industrial operation in the preparation of other fruit drinks and beverages[11,12] may be considered to be a feasible process for the commercial production of non-bitter juice from Washington Navel oranges. The greater efficiency of the two-step treatment over the one-step treatment suggests that even better results would be obtained with a column procedure, and investigations are continuing along these lines.

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Received 20 July, 1967

References

1. Higby, R. H., *J. Am. chem. Soc.*, 1938, **60,** 3013
2. Arigoni, D., *et al.*, *Experientia*, 1960, **16,** 41
3. Chandler, B. V., & Kefford, J. K., *Nature, Lond.*, 1966, **210,** 868
4. Pritchett, D. E., U.S.P. 2,816,033 (1957)
5. Swisher, H. E., U.S.P. 2,834,687 (1958)
6. Wang, K., & Wang, I. S. Y., *Nature, Lond.*, 1966, **210,** 1039
7. Neu, R., *Arch. Pharm., Berl.*, 1960, **293,** 169
8. Chandler, B. V., & Harper, K. A., *Aust. J. Chem.*, 1962, **15,** 114
9. Curtis, N. S., & Clark, A. G., *J. Inst. Brew.*, 1960, **66,** 226
10. Horhammer, L., Wagner, H., & Leeb, W., *Naturwissenschaften*, 1957, **44,** 513
11. Elder, M. E., Moore, C., & Tousingnant, W. F., U.S.P. 3,146,107 (1964)
12. Wucherpfennig, K., & Bretthauer, G., *Fruchtsaft-Ind.*, 1962, **7,** 40

KAFFIRCORN MALTING AND BREWING STUDIES XIX.*—Gibberellic acid and amylase formation in kaffircorn

By K. H. DAIBER and L. NOVELLIE

Gibberellic acid had little effect on amylase development in normal kaffircorn. Only immature seeds and very large grains produced more amylase when treated with gibberellic acid, but this effect was much smaller than that found with barley. Addition of gibberellic acid to isolated sorghum endosperms failed to stimulate significant amylase formation. Isolated endosperms also failed to produce amylase after normal contact with their embryos for up to 48 hours of germination. Amylase formation in sorghum appears, therefore, to be preponderantly a function of the embryo.

Introduction

The resting kaffircorn (sorghum) grain has been shown in several investigations[1-3] to contain no α- and no β-amylases. Both α- and β-amylases are formed in the grain only in the course of germination. In a recent publication of this series[3] the appearance of the amylases in the embryos and endosperms of kaffircorn during normal germination was investigated. It was found that during the germination of kaffircorn the concentration of diastatic activity and of α-amylases in the embryo (including the scutellum) is usually higher than in the endosperm. This is contrary to the distribution of amylase found by Briggs[4] in corresponding parts of barley. Furthermore the enzyme distribution in the sorghum grain is not compatible with the modern concept[5] of *de novo* α-amylase formation in the aleurone layer.

Gibberellin growth hormones have greatly assisted in establishing the site and the mode of enzyme formation in barley and other cereals. Recently Netien[6] found gibberellin-like substances in immature seeds of *Sorghum vulgare*. No studies have, however, been reported on the effect of gibberellins on enzyme formation in kaffircorn. In the present study the formation of amylase in kaffircorn and the influence of gibberellic acid on this formation were investigated.

Experimental

The kaffircorn grain used in most of the experiments was of the Short Red variety and was a sample with good malting characteristics. Grain harvested at various stages of maturity came from one experimental plot and was of the Barnard Red variety. The individual heads were classified in the field, divided into base and top part, hand-threshed and stored at 2° until used. The barley was of an undefined 2-row variety from Australia and was used immediately after being dehusked with 50% sulphuric acid.

The kaffircorn was malted at 30° as described previously,[7] and when necessary on a micro-scale (1 to 4 g grain or grain-sections) in wide-mouthed glass funnels on nylon gauze closed by a Petri dish lid. In one experiment kaffircorn and barley sections were malted in Petri dishes on two water-saturated discs of filter paper. The barley was malted at a temperature of 20·5° ±1°.

For the control of moulds, the grain, after a pre-steep of 4 hours, was immersed for 10 minutes in agitated water at 55°, cooled and steeped further. Although this hot-water steep is not fully effective against certain thermophilic fungi and bacteria which occur occasionally on certain kaffircorn samples, good control of moulds was achieved on the grain material used. The treatment had no adverse, and in several cases even a beneficial, effect on viability and enzyme production of the grain.

The gibberellic acid used was of c-grade from California Biochemical Research. It was found active in a modified bioassay with barley endosperms. Gibberellic acid (GA) solutions were prepared immediately before use. Normally the grain was steeped in a once-changed solution of 2 ppm GA for the last 2 hours of steeping. For the application of predetermined amounts of GA to endosperm sections, the volume of water taken up by the endosperms with time was first determined. The content of GA in the steep solution was then so adjusted that the endosperms imbibed the desired amount of hormone in the predetermined volume of water.

In experiments with endosperm material, the embryo was removed from the air dry-grain with a low speed drill of $\frac{1}{16}$ in. diameter. The dissection of moist grain was carried out as described previously,[3] by making a V-shaped incision in the grain and removing the embryo with a small amount of adhering floury endosperm. The fractions were then either dried immediately at 50° or further malted. On a dry weight basis the embryos represented between 17 and 32% of the total grain; the proportion increased with time of germination as was shown previously.[3]

The malted grains or their fractions were dried at 50°, i.e. without damage to enzymes,[7] and were analysed either with or without their roots and shoots. As the roots and shoots have a diluting effect on the amylase concentration[3] the pretreatment is specified in the tables. The diastatic power of the kaffircorn malt milled to pass 40 mesh was always determined on the peptone extract and thus represents the content of the total amylases.[8] Barley malt was extracted in 0·1 M-NaCl, and further assayed as the kaffircorn malt.

* Part XVIII: *J. Sci. Fd Agric.*, 1967, **18**, 415

Results

Effect of gibberellic acid on mature kaffircorn

An orientating survey on the effect of GA on amylase formation in several South African malting varieties showed a response peculiar to large grains. Some of the samples were, therefore, separated into fractions of different kernel size and malted separately. The results in Table I show that GA gave only minor increases in diastatic power, on the average about 7%, except in the case of the very large grains, for which the increase was 10–29%. In contrast, the same treatment applied to barley nearly doubled the amylase formation. The poor response of kaffircorn to added GA could be due to an endogenous hormone content, almost sufficient for maximum amylase formation. The source of the endogenous hormone,[5] i.e. embryo and scutellum, was therefore removed from the endosperm before steeping. The remaining endosperm sections were steeped in GA solutions of a wide range of concentrations and malted for 5 or 7 days. The results in Table II show that GA failed in all cases to stimulate appreciable amylase formation in the kaffircorn endosperm. The maximum diastatic power was found with an application of 0·1 ppm GA, but the amount produced (6·7 Kaffircorn Diastatic Units per gram (K.D.U./g) was only about 8% of that produced by intact grains. In contrast, barley endosperm thus treated produced roughly the same amylase activity as intact barley[9] (see also Table III).

Kirsop & Pollock[10] and later Yomo[11] demonstrated with barley that, if there was a certain degree of contact between the embryo and the endosperm, amylase formation in the endosperm would proceed normally or almost normally. Sufficient contact could be obtained by leaving the embryo in contact with the endosperm for the first 2 or 3 days of malting[10] or cultivating the separated embryo and endosperm in the same solution.[11] The obvious interpretation here is that sufficient endogenous hormone passes from embryo to endosperm to initiate more or less normal enzyme synthesis in the endosperm. In the case of barley, added GA can replace the endogenous hormone, but it cannot in the case of kaffircorn. It was thought that the endogenous hormone of kaffircorn could be of quite a different nature. To examine the possible passage of this hypothetical endogenous hormone from embryo to endosperm, grains of kaffircorn and barley were separated at different times into embryos and endosperms and either malted further under aerobic conditions with or without GA application, or they were dried immediately for an assessment of the amylase content at the time of separation. The results (Table III) show that lengthening the time for which the embryo of kaffircorn remains in contact with the endosperm did not increase the amylase content in the isolated endosperm. When these were separated after 2 days of malting, the amylase content in the endosperm decreased during subsequent malting. Again the addition of GA did not appreciably increase the amylase content in sorghum, although the expected increase was found in a parallel experiment with barley. If there is a hormone present in the sorghum embryo, it does not pass into the endosperm to initiate amylase synthesis under conditions which are successful with barley.

The separated embryos, however, produced large amounts of amylase, whereas according to Briggs[4] the embryo of barley contributes only minor amounts of amylase.

Effect of gibberellic acid on immature kaffircorn

Although mature kaffircorn was only slightly affected by GA (Table I), it was thought possible that its influence

TABLE II

Effect of gibberellic acid in various concentrations on the formation of diastatic power in endosperm sections of kaffircorn

GA imbibed by endosperm (ppm)	Diastatic power, K.D.U./g material	
	malted 5 days	malted 7 days
0 (control)	1·4	1·5
10^{-5}	—	0·6
10^{-4}	—	3·4
10^{-3}	—	4·0
10^{-1}	5·4	6·7
1	5·5	—
10	3·3	—
100	2·6	—
1000	0·8	—
Polished malt without GA	65·8	80·5

TABLE I

Diastatic power of various South African kaffircorn samples malted normally or after treatment with gibberellic acid (GA)

Sample	Grain size mm	Diastatic power, K.D.U./g malt (unpolished)		Value found with GA as % of control
		malted normally	malted with GA	
Barnard Red	unselected	62·1	59·9	96
Barnard Red	,,	71·1	71·1	100
Swazi Red	,,	28·3	29·7	105
Framida	,,	42·9	42·7	100
Short Red	,,	63·4	67·3	106
,, ,,	>3·3	61·4	67·8	110
,, ,,	3·0–3·3	64·6	64·5	100
,, ,,	2·8–3·0	62·7	67·9	108
,, ,,	2·4–2·8	61·7	65·0	105
,, ,,	2·0–2·4	58·7	63·5	108
Short red type	>3·3	56·9	68·8	121
,, ,, ,,	2·8–3·0	59·2	58·9	99
Hybrid	>3·3	58·6	75·4	129
Barley	unselected	89·1	176·6	198

might be greater on immature grain. When grain matures it is believed that the balance between growth inhibitors and promoters changes, the change influencing the metabolic activity of the seed.[12] Immature kaffircorn was malted with and without GA treatment (Table IV) to observe the physiological effect of a change in the hormone equilibrium.

The germination capacity of the immature sorghum was generally poor. Germination increased with increasing maturity to reach a peak at the soft dough stage, declined thereafter to a minimum at the hardening stage, and improved again with further ripening. Ability to produce amylases was also below normal. It was at a minimum at the milkripe and hardening stage, but increased at the soft dough and ripening stages. GA had no effect on germination at the soft dough stage, i.e. when germination capacity was at one of the maxima observed. In general GA had its maximum effect on germination when the percentage germination was poorest, and it had a greater effect on amylase development than on germination, increases in amylase formation being between 26 and 68%, compared with the maximum of 28% in the case of germination. The peak of hormonal effect on amylase was reached at the soft dough stage when its effect on germination was negligible. A minimum effect on amylase development occurred when the hormone's influence on germination was high.

Distribution of amylase within the kaffircorn grain

Evidence already obtained[3] pointed to the embryo as an important contributor to total amylase. The failure of GA to appreciably activate amylase production in the endosperm made it necessary to re-investigate the question of amylase distribution within the grain. To this end, not only were the germs removed from the endosperms, but the endosperm was subdivided into proximal and distal portions.

From the results in Table V, it can be seen that diastatic activity, practically absent in steeped grain, increased rapidly and almost continuously with time of malting. Within the grain the amylase concentration throughout the malting period was by far the highest in the embryo, was lower in the proximal and least in the distal part of the endosperm, i.e. the amylase concentration decreased with increasing distance from the embryo. The relative contribution of the embryo to the amylase activity of the whole grain was more than 75% up to the second day of malting, diminishing on further malting, whilst the contribution of the proximal and distal portions increased. However, only on the last day was the

TABLE III

Development of diastatic power in grain and grain fractions of kaffircorn and barley malted with and without gibberellic acid (GA)

Treatment	Steeped in	Diastatic power, K.D.U./g after malting for different periods				
		Kaffircorn[1]				Barley
		1 day	2 days	7 days		7 days
Intact grain	H_2O	1·3	10·4	61·4		89·1
	GA	—	—	67·8		176·6
Dissected after		Endosperm		Embryo[1]	Endosperm	
6-hour steep	H_2O	0	—	0·2	—	—
	GA	—	—	6·7	—	—
18-hour steep	H_2O	0	—	0	36·9	2·0
	GA	—	—	2·6	—	62·3
1-day malting	H_2O	0·7	—	1·1	21·1	—
	GA	—	—	2·0	—	—
2-day malting	H_2O	—	4·2	0·9	25·8	—
	GA	—	—	0·8	—	—

[1] with roots and shoots

TABLE IV

Effect of gibberellic acid (GA) on kaffircorn grain of different stages of maturity. Malted for 7 days

Stage of maturity	At harvest		Germination capacity			Diastatic power		
	Moisture, %	1000-grain weight, dry weight g	Control	GA	GA as a % of control	K.D.U./g Control	K.D.U./g GA	GA as a % of control
Premilk[1]	14·9	9·3	(28)	(36)	(128)			
Milk ripe	15·2	12·8	61	63	103	21·9	28·4	130
Soft dough	14·3	16·7	78	77	99	39·7	66·6	168
Hard dough	13·9	19·0	57	67	118	30·6	46·8	153
Hardening	14·3	21·5	38	48	126	17·5	22·0	126
Hard	13·1	22·4	55	69	125	26·5	40·3	152
Ripe	12·5	22·1	80	82	103	35·7	47·2	132

[1] Only one, unstable sample

TABLE V

Distribution of diastatic activity in 3 fractions of Short Red kaffircorn grain in the course of germination

Days of germination	Diastatic power, K.D.U./g material				Contribution of fraction to total diastatic power of grain (in %)		
	Whole grain[1]	Embryo[2]	Prox. Endosp.	Distal Endosp.	Embryo[2]	Proc. Endosp.	Distal Endosp.
0, Steeped	0·1	0·4	0·1	0	(75)	(25)	0
0·2	0·1	0·4	0	0	(100)	0	0
1	6·4	23·7	3·2	0·2	79	20	1
1·2	12·3	42·6	7·3	0·7	75	23	2
2	29·1	89·9	14·2	4·5	77	17	6
3	37·1	87·9	27·7	11·8	62	25	13
4	47·6	103·2	38·0	17·0	59	27	14
7	71·4	106·6	70·2	40·7	48	32	20

[1] The diastatic power of the whole grain was calculated from that of the fractions. The diastatic power determined on a separate sample of polished whole grains is not directly comparable, but agreed well with the calculated value

[2] Roots and shoots removed before dissection

total contribution of the endosperm slightly greater than that of the embryo, which represents in weight only about a quarter of the total grain.

Discussion

The amylase concentration in kaffircorn decreases from the germ through the proximal to the distal portion of the endosperm. This concentration gradient is steepest during the early phases of malting, but remains significant to the end of malting and indicates the embryo of kaffircorn as the major contributor to the total amylase of the grain. GA does not stimulate the isolated sorghum endosperm to produce amylase, neither does contact of the embryo for limited periods appear to initiate amylase synthesis in the subsequently isolated endosperm. It appears, therefore, that GA, which showed only an effect on immature and very large grain, does not act directly on the endosperm of kaffircorn as in the case of barley,[5] but possibly indirectly on mechanisms in the embryo.

The methods used in these experiments with sorghum, when applied to barley, gave results consistent with the accepted concept of amylase development in the aleurone layer of barley and wheat endosperms.[5] The mechanisms of amylase formation in sorghum, therefore, appears to be quite unlike that of barley and wheat. The results suggest that in sorghum the amylases are synthesised in the embryo and apparently diffuse into the endosperm. This hypothesis is supported by microscopic evidence (Malherbe & Novellie, unpublished) that modification in sorghum starts very largely from the embryo and not from the aleurone layer.

Acknowledgments

For the supply of immature kaffircorn we wish to thank Mr. Davie and the Oilseeds Development Corporation.

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Received 15 June, 1967

References

1. Kneen, E., *Cereal Chem.*, 1944, **21**, 304
2. Novellie, L., *J. Sci. Fd Agric.*, 1960, **11**, 408
3. Dyer, T. A., & Novellie, L., *J. Sci. Fd Agric.*, 1966, **17**, 449
4. Briggs, D. E., *J. Inst., Brew.*, 1964, **70**, 14
5. Paleg, L. G., *A. Rev. Pl. Physiol.*, 1965, **16**, 291
6. Netien, G., *C. r. Séanc. Soc. Biol.*, 1965, **159**, 1725
7. Novellie, L., *J. Sci. Fd Agric.*, 1962, **13**, 115
8. Novellie, L., *J. Sci. Fd Agric.*, 1959, **10**, 441
9. Paleg, L. G., & Sparrow, D. H. B., *Nature, Lond.*, 1962, **193**, 1102
10. Kirsop, B. H., & Pollock, J. R. A., *J. Inst. Brew.*, 1958, **64**, 227
11. Yomo, H., *Chem. Abstr.*, 1961, **55**, 26145
12. van Overbeek, J., *Science, N.Y.*, 1966, **152**, 721

PHYSICO-CHEMICAL ASPECTS OF THE AVAILABILITY OF PESTICIDES IN SOIL

II.*—Controlled release of pesticides from granular formulations

By C. G. L. FURMIDGE, A. C. HILL and J. M. OSGERBY

The extent to which formulation factors control the release of a toxicant from granular formulations, under the leaching action of rain, has been investigated. Using granules prepared by an agglomeration process and containing the herbicide chlorthiamid, the rate of release can be varied by changing the filler base and by the use of different binding agents. By varying the binding agent a considerable degree of control can be exercised over the release of chlorthiamid.

The release of toxicant is partly by direct leaching from the granule and partly by disintegration of the granule in water which renders the toxicant more accessible.

The extent to which the release of other toxicants may be controlled in a similar way has been examined using 'Bidrin', fenuron, 2,4-D, chlorfenvinphos and *N*-tritylmorpholine. Toxicant release appeared to be governed by the filler/binding agent combination used in the granule and by the water solubility of the toxicant. Where the toxicant solubility is high, control over release is difficult to achieve but where the solubility is below 2000 ppm, a considerable control is possible.

Introduction

The possibility of controlling toxicant release is one of the major advantages of granular formulations over dusts and sprays. The majority of pesticides in use today are compounds of low volatility, so the most important mechanism of release from granules is leaching by rain. In Part I[1] it was pointed out that the many factors which would affect the leaching of a toxicant from granules could be considered under three general headings, climatic, applicational and formulation, the first two of which were discussed in detail. In general, the climatic factors such as temperature, quantity and intensity of rainfall cannot be controlled, although in many regions of the world they can be predicted with a reasonable degree of accuracy. The applicational factors are those that govern the effective coverage of the soil surface by the formulations, dosage rate, granule size, toxicant content and granule density, and these may be used to control toxicant release to a limited extent. The formulation factors, which include variations in the method of preparing the granules and in the constituents used in their preparation, provide the best means of controlling toxicant release.

Granules are normally prepared in one of three ways; by impregnation, by coating or by an agglomeration process. In the impregnation method, the toxicant is dissolved in a suitable solvent which is then absorbed into a preformed granular carrier. Control over release can be achieved by the careful choice of the carrier,[2,3] the solvent[1,5] and the use of additives such as surface-active agents[2,6,7] or water-proofing agents. The coating method, in which the toxicant is stuck on to the outside of a non-absorbent granular carrier, does not provide so much scope for controlled release except in terms of water-proofing the toxicant coating. In the agglomeration method, the granules are built up from an intimate mixture of the powdered ingredients. The physical structure of the resulting granules, and hence their release characteristics, may be affected by many factors including the nature of the powdered filler, the quantity and type of the toxicant incorporated and the inclusion of other materials which can control the adhesion of the individual particles of the granule.

The limited amount of published work on toxicant release from granules has been largely confined to a study of impregnated granules used as larvicides for mosquito control.[2,7] The results were all obtained by measuring the extraction of the toxicant when the granules were immersed in a large volume of water. Thus the leaching conditions of the test were very different from the leaching conditions encountered by granules scattered over a soil surface. These differences are most important if the granules have any tendency to disintegrate in water. In general, impregnated granules do not have this tendency and therefore the results obtained in the larvicide work on the effects of constituents on toxicant release are likely to be applicable to the release of toxicants from soil-applied granules. However, the present work has been concerned with granules prepared by agglomeration and these may well disintegrate in water. Thus it is essential that any test method used on this type of formulation should simulate field conditions of leaching as closely as possible.

Materials

The toxicants used for the release measurements are listed in Table I.

The granules were prepared by a simple pasting and drying technique already described.[1] Unless otherwise indicated they were based on a finely divided kaolinite (particle size less than 2 μ) and were screened to a size range BS 16–25 mesh.

Experimental

The release of toxicants from granules was carried out using the simulated rain equipment described previously.[1]

*Part I: *J. Sci. Fd Agric.*, 1966, **17**, 518

TABLE I

Toxicants used in the granular formulations

Name	Chemical name	Melting point °C	Solubility in water at 20°C, ppm
'Bidrin'	(3-Dimethoxyphosphinyloxy)-*N*,*N*-dimethyl-*cis*-crotonamide	bp 138/0·6 mm	miscible
Fenuron	*N*,*N*-Dimethyl-*N'*-phenylurea	136	2600
Chlorthiamid ('Prefix')	2,6-Dichlorothiobenzamide	151–152	960
2,4-D	2,4-Dichlorophenoxyacetic acid	138–142	450
Chlorfenvinphos ('Birlane')	2-Chloro-1-(2,4-dichlorophenyl)-vinyl diethyl phosphate	bp 167–170/0·5 mm	100
('Frescon')	*N*-Tritylmorpholine	174–175	<0·05

It consisted of a BS No. 200 mesh sieve that was rotated at a constant 6 rpm under a controlled shower of water drops. The shower of drops fell diametrically across the sieve and was formed from a series of nozzles attached to a common manifold; the rate of water flow was controlled by a constant head of water. The water passing through the sieve was collected through a funnel into a graduated cylinder. Using standard 8 inch diameter sieves, each 200 ml fraction collected was equivalent to 0·25 inches of rain (0·65 cm).

A weighed amount of the formulation containing 250 mg of toxicant was scattered uniformly over the sieve surface. The toxicant contents of the sample before leaching and of the washings collected through the sieve were determined.

All measurements were carried out at room temperature (20°).

The extent of breakdown of the granules in water was determined by placing a 10 g sample together with 200 ml of water in a 400 ml screwcapped bottle. The bottle was rotated, end-over-end, for 5 minutes at 30 rpm, after which the contents were separated on a BS 60 mesh sieve. The residue on the sieve was dried and weighed; the ratio of the weight of granules passing through the sieve to the weight initially taken gave the percentage breakdown.

The dry strength of the granules was determined by placing a 20 g sample in a metal cylinder (length 6 inches, internal diameter 1 inch) into which a closely-fitting piston could be inserted. The sample was subjected to a pressure of 140 lb/in.^{2} for 5 minutes. The dry strength was expressed as the percentage of the crushed sample passing through a BS 25 mesh sieve.

Results and Discussion

Control of toxicant release from granules

The rate of release of a toxicant from granules prepared by agglomeration can be varied by changes in the mineral filler used as the granule base and by changes in the materials used to bind the filler/toxicant particles together.

The effect of the filler is illustrated in Fig. 1 which shows the variation of chlorthiamid release from granules containing 20% wt. toxicant and 1% wt. of a vinyl polymer (75% hydrolysed) as binding agent. The results indicate that under simulated rain conditions, the nature of the filler can have some effect on the release of toxicant from agglomerated granules. The effect does not appear to be as pronounced as that reported by Sutherland and Majunkewicz[2] who studied the release of DDT from impregnated granules immersed in static water. Under their conditions of test, attapulgite granules released between 10 and 100 times as much DDT as did vermiculite granules. Diatomaceous earth granules showed an intermediate degree of release, while granules based on organic fillers (corn-cob and tobacco stems) released only slightly more than vermiculite. None of the agglomerated granules used for the results shown in Fig. 1 disintegrated appreciably during the simulated rain test. The two-fold difference in rate shown between the granules giving the fastest and the slowest release of toxicant indicates the extent of the control that can be expected by changing the filler when a very efficient binding agent is used.

When a less efficient binding agent was used (0·1% wt. of sodium polyacrylate) the release of chlorthiamid was very much faster. This is illustrated by the results in Table II which also includes the dry strengths and wet strengths of both series of formulations. Although the dry strength of the granules containing sodium polyacrylate as binding agent tended to be less than that of the granules containing the

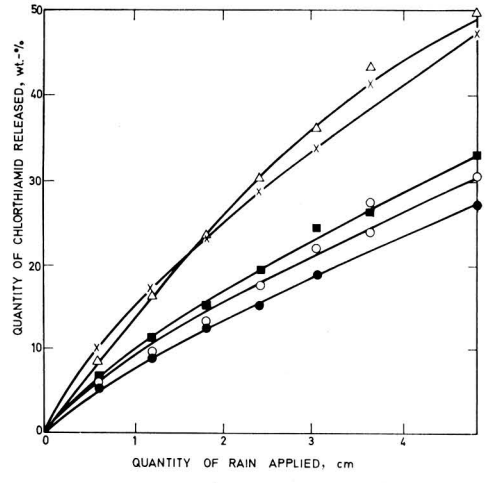

FIG. 1. *Variation of chlorthiamid release using various fillers*

Granules contained 20% wt. chlorthiamid and 1% wt. of a vinyl polymer (75% hydrolysed). Measurements made at 20°C

Fillers: △ — diatomaceous earth
× — talc
■ — ball clay
○ — kaolinite
● — Dolomite

TABLE II

Effect of fillers on the leaching of chlorthiamid from granular formulations containing 20% wt. toxicant

Filler	Binding agent	Toxicant released by 0·65 cm rain wt.-%	Toxicant released by 5 cm rain wt.-%	Dry strength breakdown %	Wet strength breakdown %
Dolomite	1% vinyl polymer (75% hydrolysed)	6·1	27·0	18·3	4·5
Kaolinite	,,	6·2	31·5	16·8	5·0
Ball clay	,,	6·4	31·7	18·0	4·4
Calcite	,,	5·6	37·3	19·8	7·2
Talc	,,	9·1	48·9	29·8	8·1
Diatomaceous earth	,,	8·1	49·8	33·0	9·4
Dolomite	0·1% sodium polyacrylate	100		44·3	99·1
Kaolinite	,,	100		42·5	95·0
Ball clay	,,	100		36·6	89·8
Calcite	,,	100		37·9	94·2
Diatomaceous earth	,,	100		33·3	96·3

vinyl polymer, there is no apparent relationship between dry strength and release of toxicant. However, in all cases where the wet strength was poor, release rate was high and it would appear that the nature of the binding agent has a much more pronounced effect on the release of toxicant, through its effect on the wet strength of the granules, than does the nature of the filler.

Many materials could be used as binding agents in the preparation of agglomerated granules, including gums, resins, plastics and polymeric materials. A survey has been made of possible binding agents for the preparation of 20% wt. chlorthiamid granules based on kaolinite and containing 1% wt. of the binder.

Over forty possible binding agents were tested, and although there was no relationship between toxicant release and the dry strength, the rough correlation noted above between the wet strength and the release of toxicant appeared to be substantiated. Most of the results of the wet strength test fell into two distinct categories, showing over 90% breakdown or under 10% breakdown. In almost every case the granules showing high breakdown in water released more than 50% of their toxicant in 5 cm of simulated rain, while those showing low aqueous breakdown gave less than 50% release.

Fig. 2 shows the quantity of chlorthiamid released plotted against the quantity of simulated rain applied for a representative selection of the binders used. The results indicate that by careful selection of the binding agent a very considerable degree of control can be exercised over the release of chlorthiamid.

It has been shown previously[1] that the release of toxicant increases with the water solubility of the toxicant and that release also tends to increase when liquid, instead of solid, toxicants are used. To determine whether different toxicants would interfere with the way in which the binding agents act, several binders were selected from the chlorthiamid results to provide fast, medium and relatively slow release rates. These were incorporated (1% wt.) in formulations based on kaolinite and containing 10% wt. of various toxicants. Fig. 3 shows the quantities of toxicant leached from a selection of these formulations when subjected to the simulated rain test and in Table III the release of toxicant is compared to the wet strength of the granules.

The results in Fig. 3 show that the relative positions of the release curves using the same range of binders were similar with all the toxicants tested. This suggests that control of toxicant release in these cases is a property of the clay/binder combination and that the properties of the

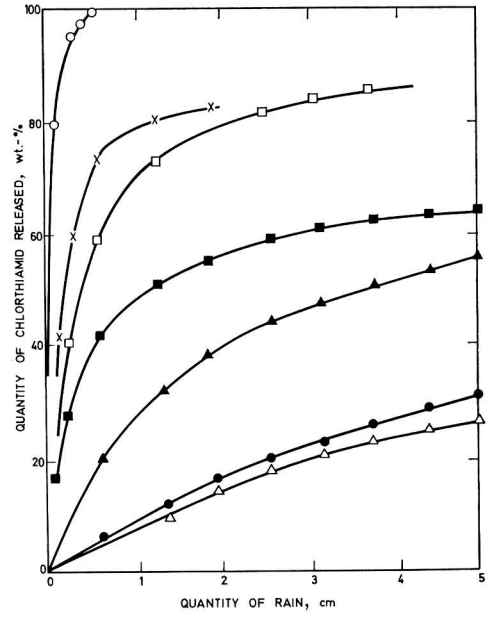

FIG. 2. *Effect of binding agent on the release of chlorthiamid from granules prepared by agglomeration*

Granules were based on kaolinite and contained 20% wt. chlorthiamid and 1% wt. binder. Measurements made at 20°C.

Binding agents: ○ — ○ aluminium sulphate
× — × no binder
□ — □ a colloidal polysaccharide
■ — ■ sodium silicate
▲ — ▲ salt of alginic acid
● — ● vinyl polymer (75% hydrolysed)
△ — △ ester of alginic acid

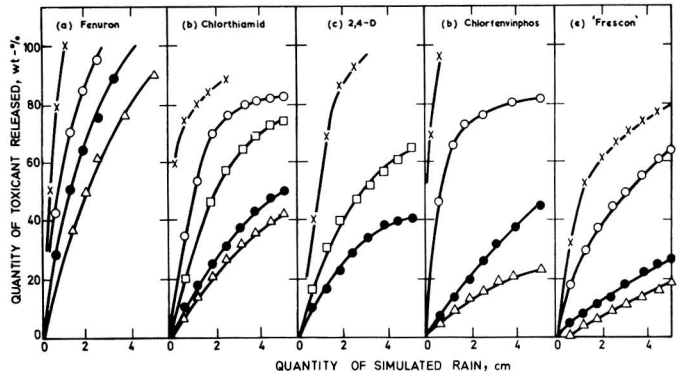

FIG. 3. *The degree of control in the release of various toxicants from granules by varying the binding agent*

Granules were based on kaolinite and contained 20% wt. toxicant and 1% wt. binder. Measurements were made at 20°C

Binding agents: × — × no binder
○ — ○ vinyl polymer (99% hydrolysed)
□ — □ polyterpene resin
● — ● vinyl polymer (75% hydrolysed)
△ — △ ester of alginic acid

TABLE III

Effect of binding agents on the release of toxicant and on the wet strength of granular formulations containing 10% wt. toxicant

Binding agent (1% wt.)	Toxicant released by 2·5 cm rain wt.-%	Wet strength breakdown %
	'Bidrin'	
None	100	100
Vinyl polymer (99% hydrolysed)	100	84·0
Polyterpene resin	100	72·0
Vinyl polymer (75% hydrolysed)	100	70·0
Ester of alginic acid	100	10·8
	Fenuron	
None	100	100
Vinyl polymer (99% hydrolysed)	97	98·0
Polyterpene resin	—	—
Vinyl polymer (75% hydrolysed)	77	50·0
Ester of alginic acid	63	12·0
	Chlorthiamid	
None	90	100
Vinyl polymer (99% hydrolysed)	76	90·0
Polyterpene resin	55	89·0
Vinyl polymer (75% hydrolysed)	32	18·5
Ester of alginic acid	28	6·0
	2,4-D	
None	94	100
Vinyl polymer (99% hydrolysed)	—	—
Polyterpene resin	48	84·0
Vinyl polymer (75% hydrolysed)	32	12·5
Ester of alginic acid	—	—
	Chlorfenvinphos	
None	100	100
Vinyl polymer (99% hydrolysed)	77	90·0
Polyterpene resin	—	—
Vinyl polymer (75% hydrolysed)	26	39·6
Ester of alginic acid	16	5·0
	'Frescon'	
None	66	100
Vinyl polymer (99% hydrolysed)	45	85·0
Polyterpene resin	—	—
Vinyl polymer (75% hydrolysed)	15	43·0
Ester of alginic acid	9	8·0

toxicant merely set the limits within which the release may be controlled. In this connection one of the most important properties is the solubility of the toxicant in water.

Where the solubility was high, as with 'Bidrin' all the toxicant was leached from the granules in the first 0·5 cm of simulated rain with all the binding agents tried. The release of fenuron was somewhat slower than that of 'Bidrin' but even so, all the formulations tested gave a fast release of toxicant. These results suggest that little practical control over toxicant release is possible by this means when the toxicant solubility is in excess of approximately 2,000–3,000 ppm. If control is required with such toxicants, the granules would have to be made hydrophobic or impervious to water.

When the toxicant solubility was reduced below 1,000 ppm a good control over release was found to be possible using this range of binding agents. The results using 'Frescon' are interesting since this compound is virtually insoluble in water. The fact that considerable quantities of toxicant were released indicates that in all cases some breakdown of the granules must have occurred to allow solid particles of the toxicant to be washed through the supporting sieve.

The results in Table III show that with each toxicant, except 'Bidrin', there is a rough correlation between toxicant release and the wet strength of the granules. This correlation applies only to a particular toxicant; there is no correlation between the wet strength of granules and the release of different toxicants.

Conclusions

The release of toxicants from granules prepared by agglomeration can be modified by changes in both the type of filler and the type of binding agent used in the formulation. By careful selection of the binding agent a considerable degree of control can be exercised over the rate at which a toxicant is released from the granules. Such control of toxicant release appears to be governed by the filler/binder combination used and the properties of the toxicant merely set the limits within which the release may be controlled. Thus, using kaolinite as the filler, the relative positions of the

release curves using the same range of binding agents were similar with all the toxicants tested.

The extent to which this type of formulation may be valuable in allowing the toxicant release by rain to be controlled depends to a considerable extent upon the solubility of the toxicant in water. Where the toxicant solubility was high, as with 'Bidrin', no control of the rate of release was found to be possible. Although the differences between the formulations of fenuron were measurable, from the practical viewpoint all the formulations gave fast release. On the other hand, the release of chlorthiamid, 2,4-D, chlorfenvinphos and 'Frescon' could be controlled to a reasonable extent. From these results it may be concluded that control over toxicant release in this type of formulation will be difficult when the toxicant solubility is in excess of approximately 2,000 ppm. Where control is required with such toxicants, the granules would have to be made hydrophobic or impervious to water.

The release of a toxicant by rain is not governed merely by the rate at which it is leached out of the granules but is also governed by the mechanical strength of the wet granules. The release of toxicant through the mechanical breakdown of the wet granules becomes relatively more important as the solubility of the toxicant is decreased. For any given toxicant, the wet strength of the granules provides a very rough correlation with the release of toxicant. However, it is not sufficiently sensitive to be relied upon to predict toxicant release with any certainty and under no circumstances can it be used to correlate the release of different toxicants.

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Received 3 July, 1967

References

1. Furmidge, C. G. L., Hill, A. C., & Osgerby, J. M., *J. Sci. Fd Agric.*, 1966, **17**, 518
2. Sutherland, D. J., & Majunkewicz, M. A., *Mosquito News*, 1963, **23**, 132.
3. Mulla, M. S., & Axelrod, H., *J. econ. Ent.*, 1962, **55**, 227
4. Mulla, M. S., & Axelrod, H., *J. econ. Ent.*, 1960, **53**, 938
5. Mulla, M. S., *Mosquito News*, 1960, **20**, 362
6. Mulla, M. S., *Proc. Calif. Mosquito Control Ass.*, 1962, **30**, 70
7. Mulla, M. S., *Proc. Calif. Mosquito Control Ass.*, 1963, **31**, 94

EFFECT OF LOAF SPECIFIC VOLUME ON THE RATE AND EXTENT OF STALING IN BREAD

By D. W. E. AXFORD, K. H. COLWELL, S. J. CORNFORD and G. A. H. ELTON

The quantitative relationships between loaf specific volume and the rate and extent of staling in bread as measured by changes in crumb elastic modulus have been examined. The results show that loaf specific volume is a major factor in determining both the rate and extent of staling, both of which decrease in a linear manner, over the range studied, as loaf volume increases. Only two factors have been found that have a significant influence on these curves, the basic breadmaking process and the storage temperature. The influence of changes in loaf specific volume on staling characteristics is greater in bread prepared by bulk fermentation than in bread prepared by the Chorleywood Bread Process. Bread made by the Chorleywood Bread Process stales less rapidly than bread made by the conventional bulk fermentation process. The effect of loaf specific volume on the rate of staling is more marked as the storage temperature is lowered.

Introduction

Many papers have been published concerning the effect on the staling rate of bread of factors such as processing methods and the use of additives. In evaluating the results it is important to take account of changes, induced in loaf specific volume, which will directly influence crumb compressibility; Ofelt *et al.*[1] attempted to do so in a study of the effect of various emulsifying agents. However, no systematic study of the effect of changes in loaf specific volume on the rate and extent of staling has been reported. During the past two years, in the course of a detailed investigation based on quantitative findings already published,[2] a large number of results has been obtained which enables the significance of loaf specific volume to be clearly established and the effect of changes in processing and of additives to be more precisely assessed.

Experimental

Basic breadmaking procedures

The breads studied were made both by the Chorleywood Bread Process[3] and by a conventional bulk fermentation process.

Chorleywood Bread Process

The baking formula was equivalent to: 280 lb flour, 6 lb yeast, 5 lb salt, 2 lb fat, 75 ppm ascorbic acid (based on flour weight), and water (level determined by standard rheological tests).

The doughs were mixed in a high-speed mixer[3] to a work input of 5 watt-hour/lb. The doughs were hand-scaled at 1lb, moulded on a Mono Universal moulder, given a 10 minute recovery, remoulded and placed in tins. Final proof to a height of 3·9 inches was at 105°–110°F with sufficient humidity to prevent dough pieces from skinning. Baking was at 430°F for 25 minutes. Mixes were of either 7 lb or 14 lb of flour.

Conventional bulk fermentation process

The baking formula was equivalent to: 280 lb flour, 4 lb yeast, 5 lb salt, and water (level determined by standard rheological tests).

The dough was mixed in a laboratory-scale Artofex mixer for 7 minutes, to a final temperature of 80°F, followed by bulk fermentation for 3 hours at 80°F in closed aluminium containers. Doughs were hand-scaled at 1 lb, moulded on a Mono Universal moulder, given a 10 minute recovery, remoulded and placed in tins. Final proof to a height of 3·9 inches was at 105°–110°F, with sufficient humidity to prevent dough pieces from skinning. Baking was at 430°F for 25 minutes. Mixes were of 7 lb of flour.

Test procedure

As in a previous paper[2] the progress of staling was followed by observing changes in the elastic modulus of the crumb and by calculating the values of the quantities ($E_L - E_0$) and ($E_L - E_t$) where E_0, E_t and E_L are the crumb elastic moduli initially, after time t and finally (limiting modulus).

With either method of preparation the test procedure involved five replicate mixings of any one variation. After being baked, loaves were allowed to cool for two hours, then the crumb modulus of one loaf per mix was measured to give E_0. The remaining loaves were sealed individually in polyethylene bags. Four loaves per mix were stored at 40°F and five loaves at 70°F, unless storage temperature was the variable being studied, in which case the five loaves were stored at the appropriate temperature. Crumb modulus measurements were made after 24, 48 and 72 hours' storage at 70°F, using one loaf per mix on each occasion. This gave respectively E_{24}, E_{48} and E_{72}. The limiting value, E_L, of the modulus was determined on the four loaves at 40°F after 10 days' storage. Tests have shown that after 7 days' storage at 40°F no further measurable firming takes place. 10 days' storage was chosen as being experimentally convenient. Loaf specific volumes were measured on two loaves per mix after 24 hours' storage at 70°F. The results from the five replicate mixings were averaged.

Crumb modulus measurements

The experimental procedure by which the crumb modulus was measured using a cone indenter has been described previously.[2] The calibration curve given earlier has now been modified to cover a wider range of modulus values, and may be represented by:

$$E = 0{\cdot}696y - 1{\cdot}152 \times 10^{-3}y^2 + 1{\cdot}920 \times 10^{-6}y^3,$$

where E is the value of the crumb elastic modulus in kdyne/cm^2 and $y = m/d^2$, m being the applied weight in g and d the indentation depth in cm.

Measurement of loaf specific volume

Loaf specific volumes were obtained by weighing loaves and measuring their volume by pearl barley displacement, according to a procedure already described.[4]

Variables studied during the course of the investigation

These include: changes in ingredients (flour protein content, level of starch damage, addition of malt flour and amylolytic enzymes, addition of fat and emulsifiers); changes in baking conditions; changes in storage conditions; and partial replacement of flour by a mixture of wheat starch and gluten.

Organoleptic assessment of staling

Bread was prepared by the conventional bulk fermentation process. Six mixes were given only 1 hour fermentation time and 6 the normal 3 hours' fermentation time. This produced loaves of different crumb firmness but having similar appearance. After being cooled, the loaves were wrapped in MSAT 300 film and stored at 70°F. Three days later the process was repeated.

On the following day, samples of the loaves, 1 and 4 days' old, were given to the panel.

The panel consisted of 40 members. Each member was given 4 slices of bread, with the top crust removed, one from each fermentation time and 1 day and 4 days old.

The panel member was asked to assess each slice for staleness on a scale from absolutely stale to absolutely fresh. The ratings obtained were given a numerical value on a scale from 1 to 8 and the results from the 40 panel members were averaged for any one variation of sample. Crumb modulus measurements were made using the cone indenter at the same time as the panel assessments were being made. The complete test was repeated on three occasions.

Results

Extent of staling

Figs 1 and 2 show plots of the extent of staling of various breads (measured by the difference between final and initial moduli) against loaf specific volume for Chorleywood Bread Process and bulk-fermented breads respectively. In practice, the limiting value E_L of the crumb modulus is obtained by storage of bread at 40°F for 10 days.

The regression lines and respective correlation coefficients for these two Figs are given in Table I, which shows that the correlation between the extent of staling and loaf specific volume is significant at the 0·1% level for both Chorleywood Bread Process and bulk-fermented breads.

A statistical comparison of the regression coefficients by means of Student's 't' test between the results for Figs 1 and 2 is given in Table II, which shows that the effect on the extent of crumb firming of changes in loaf specific volume is significantly different between breads prepared by the Chorleywood Bread Process and breads prepared by bulk fermentation. A comparison of the gradients of the regression lines of Figs 1

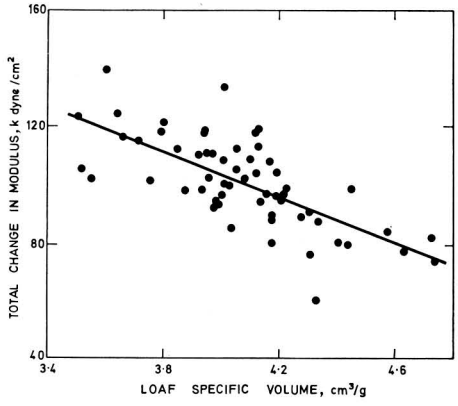

FIG. 1. *Total change in modulus vs. loaf specific volume for Chorleywood Bread Process breads*

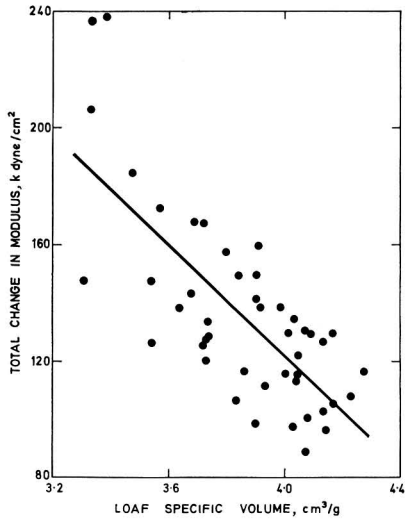

FIG. 2. *Total change in modulus vs. loaf specific volume for bulk fermented breads*

and 2 shows that the effect is greater with bulk-fermented bread than with Chorleywood Bread Process bread. These regression lines should not be used for extrapolation outside the range of specific volumes covered by the experiments. The intersection of the regression lines for the extent of staling of Chorleywood Bread Process and fermented breads is probably due to a linear relationship only applying over a limited range of specific volumes.

Rate of staling

The major physical changes occurring in bread during staling are increasing crumb firmness and opacity, reduction in the water absorption capacity of the crumb, reduction in the proportion of soluble starch, reduction in the enzyme susceptibility of the starch and a change in the *X*-ray diffraction pattern.[5] Parallel changes observed in starch gels during retrogradation have led to the theory that starch retrogradation is the major change in bread during staling. Recent studies of the rate of staling at different storage temperatures[2] have strengthened this theory. These studies have also led to a simple exponential equation to describe the progress of staling of the form:

$$(E_L - E_t)/(E_L - E_o) = \exp(-kt) \quad \ldots . (1)$$

Where E_o and E_L are the moduli corresponding to the initial and final stages of staling, E_t is the modulus at time t, and k is a rate constant the reciprocal of which is equal to the time required for $(E_t - E_o)$ to reach $(1 - 1/e)$, that is 63·2% of its final value. This equation was derived on the assumption that crumb modulus is a linear measure of increasing degree of crystallisation, and that the rate of crystallisation is

TABLE I

Correlation coefficients and equations for the lines shown in the Figures

Fig.	Number of Results	Regression line	Correlation Coefficient	Significance
1	59	$(E_L - E_o) = 261 - 39{\cdot}2 Vs$	0·696	***
2	46	$(E_L - E_o) = 505 - 95{\cdot}9 Vs$	0·738	***
4	59	$(E_{72} - E_o) = 142 - 21{\cdot}5 Vs$	0·620	***
5	46	$(E_{72} - E_o) = 299 - 58{\cdot}7 Vs$	0·712	***
6	19	$(E_{72} - E_o) = 174 - 23{\cdot}3 Vs$	0·717	***
7	19	$(E_{72} - E_o) = 175 - 24{\cdot}7 Vs$	0·821	***
8	19	$(E_{72} - E_o) = 78 - 9{\cdot}9 Vs$	0·687	***
10	59	$E_L = 322 - 47{\cdot}8 Vs$	0·734	***
11	46	$E_L = 595 - 111{\cdot}2 Vs$	0·750	***

*** = Significant at the 0·1% level
E_o = Crumb elastic modulus initially.
E_{72} = Crumb elastic modulus after 72 hours
E_L = Limiting value of crumb elastic modulus, determined after 10 days storage at 40°F
Vs = Loaf specific volume.

described by the Avrami equation.[2] The left-hand side of Equation (1) can be considered to be a measure of the ratio of the amount of material unchanged at time t to the initial amount of material available for change, and it is thus of the form governing a unimolecular transformation. Therefore, the time constant ($1/k$), or indeed the time for any given fraction of material to be converted into the stale form should be independent of the concentration in the solid or condensed phase. However, this concentration will in any case not be affected by change in the specific volume of the bread, and it can be seen from Fig. 3 that the constant $1/k$ is virtually independent of the specific volume, as is shown by a value of 0·105 calculated for the correlation coefficient. The results for breads prepared by the Chorleywood Bread Process and breads prepared by bulk fermentation are differentiated on Fig. 3 by the use of different symbols. There is no apparent effect on the time constant between the two methods of preparation. The mean time constant, calculated for the values plotted in Fig. 3, for the breads prepared by the Chorleywood Bread Process is 3·52 days, and for breads prepared by bulk fermentation 3·76 days, but these are not significantly different. These values differ slightly but not significantly from those given later in Table IV, which are calculated from different experimental results.

Although the percentage of total staling occurring in a given time is independent of specific volume, the actual rates of staling would be expected to be dependent on the specific volume of the bread, provided that the temperature is constant, because the initial and final moduli are functions of specific volume. Figs 4 and 5 show plots of the extent of staling after 72 hours' storage at 70°F against specific volume for Chorleywood Bread Process and bulk-fermented breads respectively. The rate of staling varies with specific volume, and the correlation coefficients are given in Table I; they are significant at the 0·1% level. The regression lines of best fit over the experimental range are also given in Table I.

The results for Figs 4 and 5 have been statistically compared by means of Student's 't' test (Table II). These results show that the effect on the rate of crumb firming of changes in loaf specific volume parallels that of the effect on the extent of crumb firming. There is a significant difference between the effect on the rate of crumb firming of changes in loaf specific volume with breads prepared by the Chorleywood Bread Process and with breads prepared by bulk fermentation. A

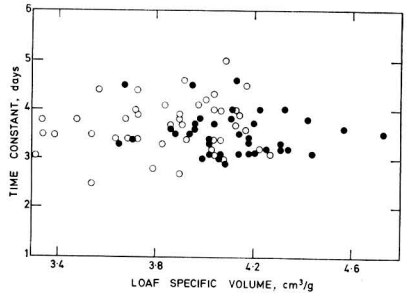

FIG. 3. *Time constant* ($1/k$) *vs. loaf specific volume*
● Chorleywood Bread Process Bread
○ Bulk Fermented Bread

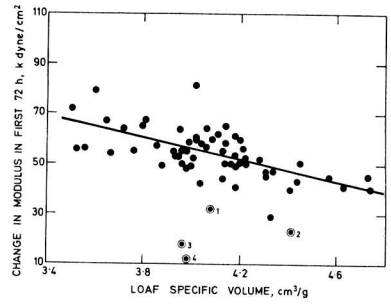

FIG. 4. *Change in modulus in first 72 hours vs. loaf specific volume for Chorleywood Bread Process breads stored at* 70°F
1, 2 Bread Stored at 110°F
3 Bread Stored at 130°F
4 Bread Stored at 150°F

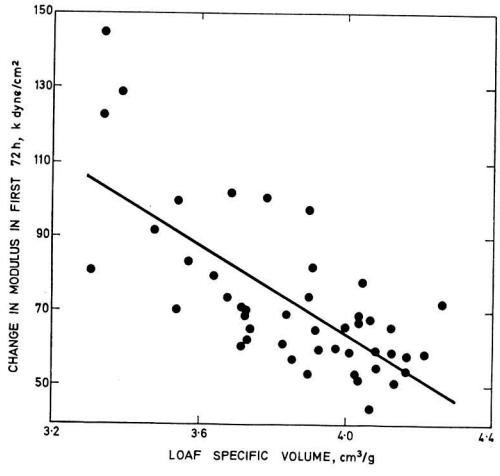

FIG. 5. *Change in modulus in first 72 hours vs. loaf specific volume for bulk fermented breads stored at* 70°F

TABLE II

Significance of differences between slopes of lines

Comparison between	Degrees of Freedom	't' value	Significance
Figs 1 and 2	101	4·38	***
Figs 4 and 5	101	4·34	***
Figs 6 and 7	34	0·046	N.S.
Figs 7 and 4	74	0·25	N.S.
Figs 4 and 8	74	2·08	*

N.S. = not significant
* = significant at the 5% level
*** = significant at the 0·1% level

comparison of the gradients of the regression lines of Figs 4 and 5 shows that the effect is greater with bulk fermented than with Chorleywood Bread Process breads. As for Figs 1 and 2, it would be unjustified to use the regression lines for extrapolation beyond the range of specific volumes studied.

Effect of storage temperature

Some of the tests using the Chorleywood Bread Process were designed to study the effect of storage temperature on the variation of rate of staling with change in loaf specific volume. In these tests loaves from each of five mixes of any one variation were stored, after the initial 2 hour cooling period, at temperatures of 30°, 50°, 70° and 90°F. The subsequent staling was then followed by making crumb modulus measurements after 24, 48 and 72 hours' storage. The results obtained at a storage temperature of 70°F are included in Fig. 4. Figs 6, 7 and 8 are plots of the change in crumb elastic modulus in the first 72 hours versus loaf specific volume at storage temperatures of 30°, 50° and 90°F, respectively.

The correlation coefficients and regression lines of best fit for the data of Figs 6, 7 and 8 are presented in Table I. In Fig. 9 these regression lines together with the regression line of Fig. 4 are plotted without the individual experimental points from which the lines were obtained.

Table II contains the results of a Student's 't' test comparison of the regression coefficients of the data represented by the regression lines in Fig. 9. These show that the gradients of the lines obtained at 30°, 50° and 70°F are not significantly different and that the difference between the lines at 70° and 90°F is significant only at the 5% level.

The tests at four different storage temperatures provide sets of data for the change in crumb elastic modulus at these storage temperatures, and at a given specific volume. This enables a statistical comparison of the intercepts of the lines in Fig. 9 to be carried out using the variance ratio test. The term 'intercept' refers to the hypothetical value of $E_{72}-E_0$ at a loaf specific volume of zero. The results are presented in Table III. Table III shows that the lines at 90°, 70° and 50°F are significantly different in intercept at the 0·1% level but there is no significant difference between the lines at 50° and 30°F.

The time constants at these storage temperatures have been calculated from the data plotted in Fig. 9 on the same basis as given in an earlier publication[2] and are presented in Table IV. Included in Table IV are the time constants previously calculated[2] for bulk-fermented breads. The difference between the time constants for the two methods of bread preparation at any one storage temperature is small and less than the experimental error involved in calculating the time constant.

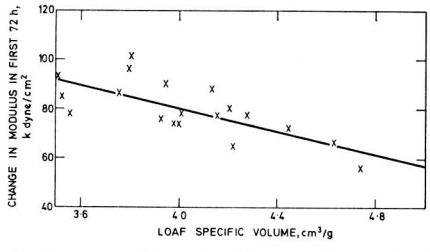

FIG. 6. *Change in modulus in first 72 hours vs. loaf specific volume for Chorleywood Bread Process breads stored at* 30°F

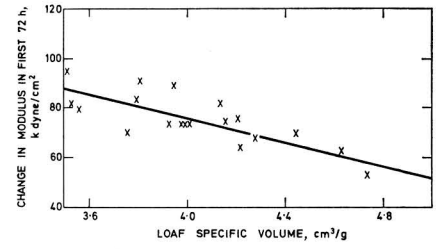

FIG. 7. *Change in modulus in first 72 hours vs. loaf specific volume for Chorleywood Bread Process breads stored at* 50°F

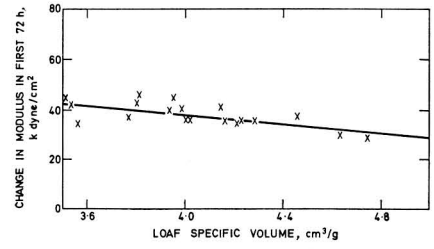

FIG. 8. *Change in modulus in first 72 hours vs. loaf specific volume for Chorleywood Bread Process breads stored at* 90°F

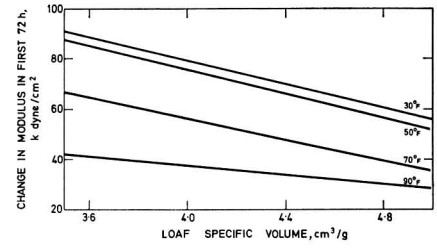

FIG. 9. *Change in modulus in first 72 hours vs. loaf specific volume for Chorleywood Bread Process breads stored at* 30°F, 50°F, 70°F *and* 90°F

TABLE III

Significance of differences between 'intercepts' of lines in Fig. 9

Comparison between	Variance Ratio	Significance
30°F and 50°F	3·16	N.S.
50°F and 70°F	105·3	***
70°F and 90°F	115·3	***

TABLE IV

Time constants (in days) at different storage temperatures

Temperature °F	Chorleywood Bread Process	Bulk Fermented[a]
30	1·44	1·39
50	1·84	1·89
70	3·28	3·68
90	5·02	5·51
110	9·0	—
130	13·5	—
150	23·3	—

[a]Presented in an earlier publication[2]

This implies that the rate process by which staling is occurring is probably the same in both types of bread, although the absolute rates of staling are different. This is related to the experimental observation that the extent of staling ($E_L - E_0$) is also different for the two types of bread. Figs 10 and 11 show the variation of the limiting modulus, E_L, with loaf specific volume for Chorleywood Bread Process and bulk-fermented breads. Examination of these graphs shows that the difference $E_L - E_0$ for Chorleywood Bread Process and fermented breads arises mainly from the difference in the limiting modulus, E_L, for the two types of bread. In general, E_L is less for Chorleywood Bread Process than for fermented bread at a given specific volume. The reason is unknown, but this fact is chiefly responsible for the difference in staling behaviour of the two types of bread.

A limited number of storage tests has been carried out at temperatures higher than 90°F. The results of these tests, on breads prepared by the Chorleywood Bread Process, are included as points on Fig. 4. These points fall outside the pattern shown by the other results in Fig. 4, as would be expected, since the time constant is temperature-dependent. The values of the time constant obtained are given in Table IV. It should be borne in mind that these higher temperature values are based on only very limited experimental results.

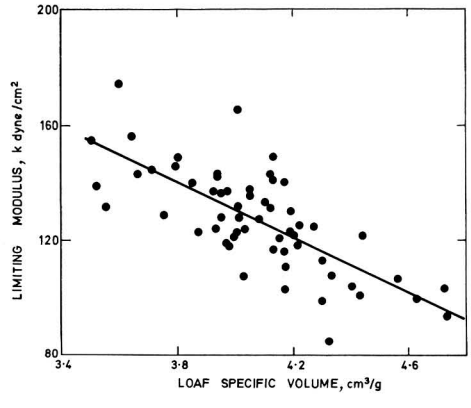

FIG. 10. *Limiting modulus vs. loaf specific volume for Chorleywood Bread Process breads*

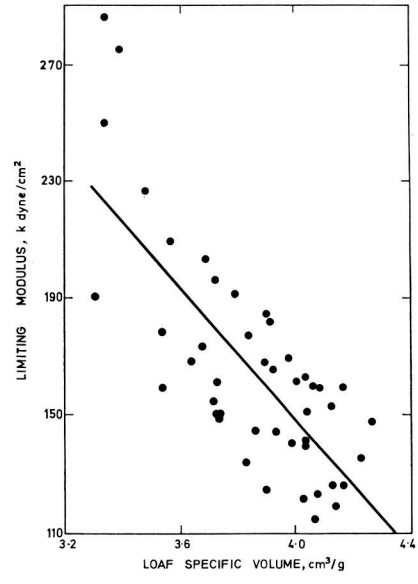

FIG. 11. *Limiting modulus vs. loaf specific volume for bulk fermented breads*

At higher temperatures, the rate of staling is very much reduced though it has not yet been possible to establish experimentally the existence of a transition temperature above which bread does not stale.

Organoleptic assessment of staling

Although it is widely accepted that crumb firmness measurements are highly correlated with organoleptic assessment of staling, it was felt desirable to obtain further evidence on this point. A careful comparison was made of taste panel evaluation of a series of breads and of their crumb elastic moduli. Fig. 12 represents the results of these taste panel experiments. It shows that the panel rating of staleness and the logarithm of the measured firmness of the bread are highly significantly correlated, the calculated correlation coefficient being 0·98.

Discussion

It has long been known that bread from strong flours (i.e. generally of high protein content) stales less rapidly than bread from weak flours (i.e. generally of low protein content), processed under identical conditions. However, bread from strong flours is of higher specific volume than bread from weak flours, and the difference in the rate of staling is largely accounted for by this factor, as results from flours of widely differing protein content fall on the lines shown in Figs 1–9. In the same way, it is believed[6] that properly fermented bread stales less rapidly than bread from doughs which are grossly under-fermented or over-fermented. Again, however, incorrect fermentation normally leads to a diminution in loaf

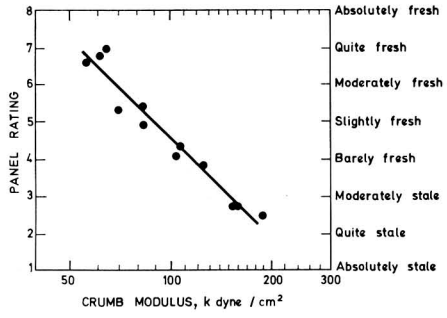

FIG. 12. *Panel rating of staleness vs. logarithm of crumb modulus*

volume and Figs 2 and 5 include results from experiments using a wide range of fermentation conditions.

The conclusion is that for a particular breadmaking process factors which lower the specific volume increase the staling rate, and factors which raise the specific volume lower the staling rate; this has been confirmed for all the factors investgated here.

Since the commercial introduction of the Chorleywood Bread Process in 1962 there have been several observations of a lower rate of staling of breads prepared by this process when compared with breads prepared by bulk fermentation.[7,8] The use of the Chorleywood Bread Process, other things being equal, usually leads to an increase in the specific volume of the resultant bread. The results presented earlier in this paper show that the improved staling characteristics of breads prepared by the Chorleywood Bread Process are an inherent feature of the process and not merely a reflection of an increased specific volume. Thus a specific reduction in both the rate and extent of staling is obtained by the use of the Chorleywood Bread Process, although the fundamental staling mechanism appears to be the same.

The reduction in rate and extent of staling with Chorleywood Bread Process bread is apparently due to the limiting modulus for that type of bread being less than for conventional fermented bread at any given specific volume. No explanation is available for this although the most obvious suggestion would be that the amount of crystallisable starch is less in the case of Chorleywood Bread Process bread. Similarly, the fact that rate and extent of staling vary less rapidly with loaf specific volume for Chorleywood Bread Process bread appears also to be related to the lower rate of change of E_L with specific volume for that type of bread, as can be seen from Figs 10 and 11.

The regression lines in Figs 1, 2, 4 and 5 indicate the general effect of loaf volume on extent of staling and on staling rate. Only when a large number of experiments shows a statistically significant deviation of points from these lines can it be accepted that a specific anti-staling effect arises from the factor under consideration. The only factors in this investigation which have shown an over-riding effect on staling rate, comparable with the magnitude of the effect of specific volume, are the basic breadmaking procedure and storage temperature. This is not to deny that some of the other factors investigated may have a second-order effect, but it is of vital importance that in evaluating an addition or change in process due weight is given to the effect of loaf volume changes which are normally responsible for the major part of any noted effect.

There is a need for a simple technique which follows directly the progress of starch retrogradation during staling of bread. Such a technique would overcome the imperfections of measurement of crumb firmness and circumvent the loaf specific volume effect. *X*-ray diffraction patterns can be used to follow directly the crystallisation changes in the starch, but the technique is slow and complex. Recent work in these laboratories[9] has shown that differential thermal analysis may be a much quicker and simpler technique for studying changes in the starch fraction during staling.

Acknowledgments

The authors wish to thank Mrs. C. E. French and Mr. P. I. Maris for practical assistance. The co-operation of Mr. T. H. Collins and the staff of the Bread Bakery is also gratefully acknowledged.

This work is supported by a grant from the Ministry of Technology.

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Received 13 March, 1967; amended manuscript 7 November, 1967

References

1. Ofelt, C. W., MacMasters, M. M., Lancaster, E. B., & Senti, F. R., *Cereal Chem.*, 1958, **35**, 137
2. Cornford, S. J., Axford, D. W. E., & Elton, G. A. H., *Cereal Chem.*, 1964, **41**, 216
3. Elton, G. A. H., *Proc. R. Aust. Chem. Inst.*, 1965, **32**, 25
4. Cornford, S. J., & Coppock, J. B. M., *Research*, 1950, **3**, 558
5. Bice, C. W., & Geddes, W. F., Chap. 10 in 'Starch and its derivatives' by J. A. Radley, Vol. 2, Chapman and Hall: London, 1953
6. Kent-Jones, D. W., & Mitchell, E. F., 'The Practice and Science of Bread Making'. Northern Publishing Co: Liverpool, 1962, p. 158
7. Chamberlain, N., Collins, T. H., & Elton, G. A. H., *Cereal Sci. Today*, 1965, **10**, 412
8. Collins, T. H., *Milling*, 1966, **146**, 296
9. Axford, D. W. E., & Colwell, K. H., *Chemy. Ind.*, 1967, No. 11, 467

HAGBERG PENETROMETER METHOD FOR α-AMYLASE ACTIVITY IN SPROUTED GRAIN: PREDICTION OF ACTIVITY OF FLOUR BLENDS

By T. A. MITCHELL

A penetrometer method for sprout damage has been applied to New Zealand flours, and a close relationship between the results and crumb doughiness in bread has been demonstrated.

The pressure-sustaining capacity of the flour gels, as calculated from the penetrometer readings, is inversely proportional to the enzyme activity.

A simple relationship exists between penetrometer number and the proportion of flour from sprouted grain in flour blends.

The penetrometer number appears to be primarily determined by the α-amylase activity of the flour from sprouted grains and to be unaffected by variations in the level of 'damaged' starch in the flour. A method is proposed whereby the amounts of sprouted lines of wheat necessary in a grist, to produce flour with a predetermined level of activity, may be calculated.

Introduction

Adverse weather conditions occasionally cause pre-harvest sprouting of some of the New Zealand wheat crop. The high level of flour α-amylase activity which results from sprouting, has effects in bread ranging from a desirable crumb softening to undesirable stickiness and collapse of the loaf interior. Laboratory control of the proportion of sprouted grain in milling grists is essential if maximum use is to be made of this grain consistent with the production of satisfactory bread. The falling-number[1] and penetrometer[2] methods devised by Hagberg for assessing the α-amylase activities of flours, may both be used for this purpose. We have found the falling-number method to be very suitable for rapid determinations on a few samples but prefer the penetrometer method for testing large numbers of samples (say 600 per day). This paper suggests a method for making the necessary computations from results obtained by an adaptation of the penetrometer method.

Experimental

Penetrometer method

The apparatus used was similar to that of Hagberg.[2] Tapered aluminium beakers 9·5 cm high, 5·3 cm base diameter, 7·4 cm top diameter fitted with aluminium caps, were used. These were supported in racks of six in an electrically heated water-bath and, in use, floated immersed to a depth of 8 cm. The bath, which was 32 cm diameter and 26 cm deep, contained 17 litres of water (at boiling point). It was lagged with 2 cm of felt. The penetrometer had a metal cone 6·6 cm high, included angle 40°, mounted on a metal shaft, and the weight of this assembly was 30·0 g. Depth of penetration was determined by observing a mark engraved on the shaft against a vertical scale graduated in millimetres.

Wheat samples were ground to a fine meal by one passage through a laboratory grinder (Regent Maskin AB, Stockholm, Model R, 1,800 rpm.) adjusted so that the burrs just cleared one another (gap 0·005 in. or less). Commercial flours and flours milled on an Allis-Chalmers experimental mill, both of nominal 78% extraction, were used without further preparation.

50 g of meal or flour and 150 ml of distilled water were shaken together vigorously in a capped beaker. A rack of six beakers was suspended in an initially boiling water-bath for forty minutes, during which time the water temperature fell to 87°. The gels which formed were then stirred to break up any lumps and the beakers cooled in water at 15–17°. After one hour the surface skin was gently removed, the surface levelled with a square-ended spatula, and the depth to which the penetrometer sank into the gel, in ten seconds, recorded in millimetres. This will be referred to as the Penetrometer Number (N).

Milling and baking tests

The extent of sprouting damage in commercial flours and wheats has hitherto been assessed in this Institute by test-baking. Wheats were milled to 78% extraction on an Allis-Chalmers experimental mill. The test-baking procedure used 125 g of flour, 1½% yeast, 2% salt and ¾% sugar on flour weight. Doughs were finished at 78°F with 5¾ hours in the oven, and the loaves were cooled overnight before judging. The test-baker assessed doughiness from the feel of the crumb, and recognised six grades of crumb quality relative to sprouting:

1. Sound
2. Slightly doughy
3. Doughy
4. Very doughy
5. Sticky
6. Very sticky

S.K.B. determinations

α-Amylase activity was also determined by the method of Sandstedt, Kneen and Blish (S.K.B.).[3] Because the α-amylase activity of the samples was not as high as that of the malted

grain for which the method is normally used, 0·2% $CaCl_2$ solution was used to make the extracts, which were not diluted.

Results

Experimental comparison of baking and penetrometer tests

Crumb grades of loaves and penetrometer numbers of duplicate gels were determined for seventy commercial flour samples. The results are plotted in Fig. 1 in which crumb grades have been arbitrarily spaced at equal intervals on the ordinate. Though there is a considerable scatter of points within each grade, crumb grade is clearly related to mean penetrometer number within a grade. The relationship appears to be linear but this would only be true, in the unlikely event, that the test-baker's assessments represent equal increments of sprout damage throughout the range.

Our experience has been that any stickiness (crumb grades 5 and 6) renders bread unacceptable to the consumer. This corresponds to penetrometer numbers of 35 and over.

Relationship between enzyme concentration and penetrometer number

A highly active sprouted flour may be regarded as a concentrated source of wheat α-amylase, and a sound flour as a substantially inactive substrate. Blends of a pair of such flours, designated 'active' and 'inactive', were used to establish a relationship between penetrometer number and enzyme concentration. The relation between penetrometer number and percentage of 'active' flour is shown in Fig. 2 (Curve A). Curve B shows the relation between penetrometer number and the logarithm of the percentage of active flour. The equation of this line within the limits $N=11$ and $N=45$ is:

$$\text{Log}_{10}S = 0{\cdot}034N - 0{\cdot}189$$

where S is the percentage of active flour and N the penetrometer number.

These curves represent the relationship between α-amylase concentration and penetrometer number for the particular flours used. If the penetrometer number of any flour depended only on its α-amylase activity the 'equivalent percentage of active flour' might be used as a general index of the level of enzyme activity. To explore this possibility, a series of 78 blends of the 'active' and 'inactive' flours with commercial flours showing widely varying degrees of sprout damage, were prepared, and the penetrometer numbers of the flours and blends determined. By means of the curve A in Fig. 24, predictions of the penetrometer numbers of the blends were made. For 65 of the blends the agreement between the observed and predicted penetrometer numbers was within ± one unit and in no case was the difference greater than three units. A selection of typical results is shown in Table I.

For the 78 commercial flours examined, the penetrometer number was apparently determined by one factor only, presumably the enzyme activity of the flour. Recently in these laboratories, Cawley has shown that for flours there is a close relationship between results of the penetrometer test and S.K.B. α-amylase determinations.

In another experiment the effect of severe milling damage on the penetrometer test was investigated. A commercial flour was passed through the smooth rolls of the Allis-Chalmers mill five times. Damaged starch estimates by the method of Jones,[4] and penetrometer tests were made on the original flour and after each passage through the mill. Table II shows that in spite of the large increase in the level of damaged starch the penetrometer number remained almost constant.

The effect of severe milling damage on the baking test was also checked. In contrast to the penetrometer test there was a marked change in crumb grade. The commercial flour, initially assessed as 'slightly doughy', became 'very sticky' after five passes through the Allis-Chalmers mill.

Application to wheatmeals

Wheats were ground to meals on a Regent mill. Separate portions of each of 33 wheat samples selected from the 1958 harvest, were milled to flours on the Allis-Chalmers mill or

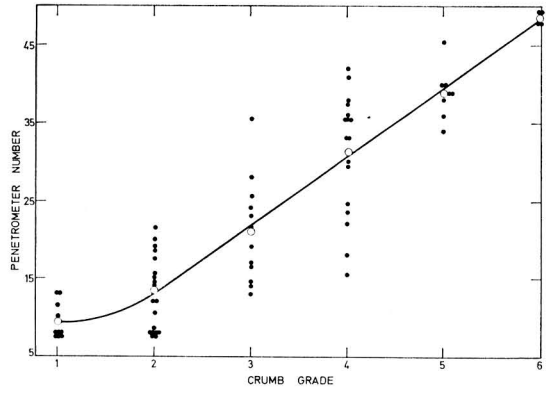

FIG. 1. *Comparison of penetrometer numbers and crumb grades*
● Individual samples ○ Means

ground to meals by one passage through the Regent mill. Penetrometer number determinations on the meals and flours showed a close coincidence between the two sets. A changed relationship between meals and flours was found with 1961 wheats, however, as discussed by Cawley and Mitchell.[5]

Discussion

Methods of estimating amylase activity such as the 'maltose test', which involves autolysis at dough fermentation temperatures, are primarily an indication of the susceptibility of the flour starch to enzyme attack.[6] When the main enzyme attack occurs at an elevated temperature on gelatinised starch, however, as in the Hagberg test, differences in the susceptibility of the raw starch granules may be expected to have little effect on the result. The evidence in the present work is that the Hagberg penetrometer method measures primarily α-amylase activity, and that under the conditions used variations in the extent of milling damage of the starch granules do not cause variations in the susceptibility of the starch to enzyme attack.

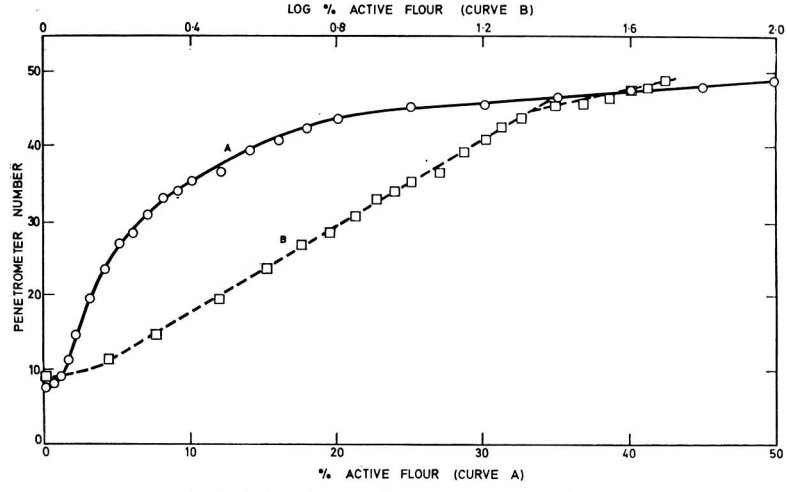

FIG. 2. *Effect of flour activity on penetrometer number*

TABLE I

Observed and predicted penetrometer numbers for blends of 'active' and 'inactive' flours with commercial flours

Penetrometer number of the commercial flour		Commercial flour in blend %	Penetrometer number of blend	
			Observed (Mean of duplicates)	Predicted
Blends with 'active' flour	13	97·5	23·8	24·0
	21	97·5	31·2	29·8
	30	97·5	36·2	33·8
	35·5	97·5	37·5	37·8
	42	97·5	42·8	43·1
	49	97·5	49·0	49·2
	8	95	27·5	27·5
	12	95	29·4	29·5
	14	95	30·9	30·5
Blends with 'Inactive' flour	38	50	30·3	29·5
	40	50	32·5	31·8
	42	50	34·6	34·0
	19	25	9·4	9·5
	24	25	11·0	10·8
	30	25	14·6	14·8
	39	25	22·8	22·5
	49	10	27·5	27·5

TABLE II

Effect of 'damaged' starch on penetrometer number

	'Damaged' starch Granules %	Penetrometer number (Mean of duplicates)
Original flour	3	19·5
After 1 passage through mill	6½	20
After 2 passages through mill	9	19·5
After 3 passages through mill	13	19·5
After 4 passages through mill	18½	18·5
After 5 passages through mill	25	18·5

Sprout units

The equation:

$$\text{Log}_{10}S = 0{\cdot}034N - 0{\cdot}189$$

obtained experimentally for a particular pair of flours, may be simplified to the general expression:

$$\text{Log}_{10}S_0 = 0{\cdot}034N, \text{ where } S_0 = 1{\cdot}55S$$

Then S_0 = Antilog $(0{\cdot}034N)$. We use S_0 as an index of the α-amylase activity of flours and wheats, and term the units in the scale 'Sprout Units'. Prior to February 1963, the actual numerical values of S_0 were reported as sprout units but since that date a more convenient scale in which the numerical values are halved has been used. On the old scale the maximum value reported was 30 sprout units ($N = 43{\cdot}3$) and since 1963 on the new scale it has been 12 sprout units ($N = 40{\cdot}6$). Results of tests on commercial wheat and flour samples are now always reported in sprout units, which have the advantage over penetrometer numbers in that they may be added and averaged when flours or wheats are blended. The upper limit for accurate determinations is about 12 sprout units, (new scale), but with samples of higher activity (including malt flours) a useful result may be obtained by first diluting the sample with an inactive flour.

α-Amylase S.K.B. activities of flours are linearly related to activities expressed in sprout units. For flours, one S.K.B. unit is equivalent to 25 sprout units, (50 sprout units on old scale). For a fuller discussion of this work see Cawley & Mitchell.[5]

Prediction of enzyme activity in grists

In seasons when sprout damage is widespread it is important that millers make the maximum safe use of sprouted grain, and to do this millers require a method by which they can assess the quantities of individual lines necessary in their grists to produce flour of a predetermined level of activity. The work described in this paper suggests that this could be achieved by determining the activities of wheatmeals (in sprout units) and making the necessary calculations. This procedure was adopted during full-scale testing of the 1961 harvest, with the results reported by Cawley & Mitchell.[5]

Penetrometer

The pressure on a gel due to the penetrometer, at any particular depth of penetration, may be calculated from the expression

$$P = \frac{1}{N^2} \cdot \frac{W}{\pi \tan^2 \theta} - N\tfrac{1}{3}\varrho$$

where P is the pressure in g/cm², N is the penetrometer number, W the weight of the penetrometer in grams, θ the angle of the cone, and $\varrho = 1{\cdot}05$ the density of the gel. This value of ϱ is the mean of a series of determinations by a simple displacement method. When penetrometer number was plotted against log 10 pressure an approximately linear relationship within the limits $N = 13$ and 50 was obtained. This suggested that the apparently logarithmic relationship between penetrometer number and percentage of active flour might result from a pseudo-logarithmic relationship between penetrometer number and pressure. Fig. 3 in which pressure is plotted against the reciprocal of the percentage of active flour shows a near linear relationship between these quantities over a range from 2 to 20% of active flour, which corresponds to a range of penetrometer numbers from 15 to 45.

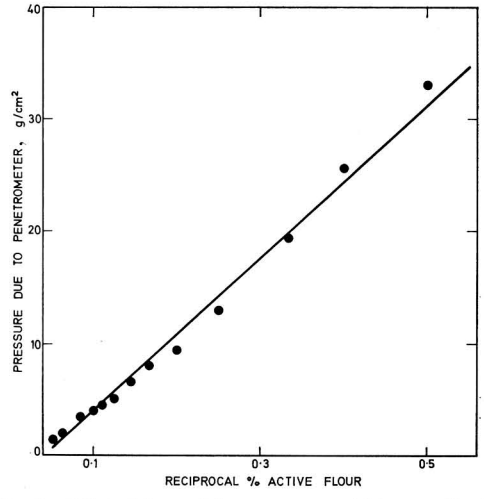

FIG. 3. *Effect of flour activity on pressure-sustaining capacity of flour gels*

Thus the pressure-sustaining capacity of these gels is inversely proportional to the α-amylase activity, and the apparently logarithmic relationship between penetrometer number and enzyme activity results from the pseudo-logarithmic relationship between pressure due to the penetrometer and depth of penetration.

Conclusions

Assessment of the extent of sprout damage in New Zealand flours by a gel-penetrometer method agrees well with subjective assessments of crumb doughiness of test-baked loaves.

In commercial flours the enzyme activity appears to be the main factor determining the penetrometer number.

There is an apparently logarithmic relationship between penetrometer number and α-amylase activity.

The pressure-sustaining capacity of the gels decreases linearly with increase in enzyme activity.

Acknowledgments

The author wishes to acknowledge the advice and assistance of Mr. E. W. Hullett, then director, who suggested this project and helped in devising the system of Sprout Units. The test-baking and loaf judging were carried out by Mr. G. Larson.

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Received 3 July, 1967

References

1. Hagberg, S., *Cereal Chem.*, 1960, **37**, 218
2. Hagberg, S., *Trans. Am. Ass. Cereal Chemists*, 1951, **9**, 53
3. Sandstedt, R. M., Kneen, E., & Blish, M. J., *Cereal Chem.*, 1939, **16**, 712
4. Jones, C. R., *Cereal Chem.*, 1940, **17**, 133
5. Cawley, R. W., & Mitchell, T. A., *J. Sci. Fd Agric.*, 1968, **19**, 106
6. Kneen, E., & Sandstedt, R. M., Ch. 3 of 'Enzymes and their Rôle in Wheat Technology', Edited by J. Ansel Anderson, 1946, p. 97. (Interscience Publishers, Inc.: New York)

INHIBITION OF WHEAT α-AMYLASE BY BRAN PHYTIC ACID

By R. W. CAWLEY and T. A. MITCHELL

A proportion of the wheat samples tested for α-amylase activity by the Hagberg penetrometer method during the 1961 New Zealand harvest gave higher results on flours than on wheatmeals milled from the same wheats. This effect is due to the lower level of calcium available as enzyme cofactor in the meals, caused by combination with phytic acid. Because of this effect and because the internal distribution of α-amylase in wheat grains is variable, testing for sprout damage in milling wheat is best carried out on flours rather than wheatmeals.

Introduction

The weather was wet during the harvest in 1961 over much of the New Zealand wheat growing area. Sprouting was both widespread and severe, and this Institute operated a testing service on wheat meals, using the Hagberg penetrometer method modified as described by Mitchell.[1] Samples from nearly every crop in the South Island were tested, over 10,000 in all. Some 2,000 samples were also milled (78% extraction) and baked and many Hagberg tests were carried out on 78% flours and mixtures of wheatmeal and sound flour, as well as on the wheatmeals themselves.

Testing commenced on 26 January, and by 12 February some 2,000 samples had been tested. The agreement between tests on wheatmeals and 78% extraction flours milled from the same wheat was quite close, as found by Mitchell (*loc. cit.*) in 1958. Samples showing this agreement will be referred to as 'normal'. By 3 March, however, when some 8,000 samples had been tested, it was apparent that about half the samples then coming forward, yielded 78% extraction flours with much higher activities than had been expected from the results of Hagberg tests carried out on the wheatmeals. These samples will be referred to as 'anomalous'.

Experimental

The test method has been described by Mitchell.[1] Results throughout this paper are reported on the old scale of sprout units. The wheat samples were from the 1961 New Zealand harvest, predominantly of the variety Aotea.

Water extract of bran

A mixture of 10 g bran and 150 ml water was subjected to the heating and cooling sequence of a Hagberg test. It was then filtered and the whole filtrate of about 120 ml used to replace an equal quantity of water in a test on a flour from sprouted wheat.

Soxhlet-extracted bran

Ten g bran was extracted 24 hours with water in a Soxhlet apparatus. The damp bran was then included with 40 g of a flour from sprouted wheat in a Hagberg test. To allow for the water absorbed by the bran during the extraction, the increase in weight of the bran was deducted from the quantity of water added in the test.

Results

As the harvest advanced, and unfavourable weather continued the level of sprout damage became higher, until some 20% of the samples from the Canterbury province were giving results above 25 sprout units. At this level the accuracy of the Hagberg test declines, because starch conversion is very nearly complete. In an effort to provide more accurate results, a substantial number of tests were carried out on wheatmeals, diluted with an equal quantity of 78% extraction flour made from unsprouted wheat, as described by Mitchell.[1] In many cases, the wheat activity calculated from the result for the mixture, was much higher than that of the wheatmeal alone. However, the results showed good

agreement with the activities of 78% flours milled from the wheats, particularly with 'anomalous' samples, as shown in the upper section of Table I.

TABLE I

Hagberg and S.K.B. assessments of sprouted flours and meals

Sample	Sprout units			
	Normal sprouting		Anomalous sprouting	
	A	B	C	D
Penetrometer method				
78% flour	2	6	18	17
Wheatmeal	2	6	8	9
Diluted wheatmeal	2	8	17	20
Wheatmeal plus calcium	2	7	24	19
Wheatmeal plus oxalate	0	2	8	8
Wheatmeal incubated	2	6	40	21
S.K.B. method				
78% flour	6		18	
Wheatmeal	11	24	50	102
Bran	27		72	
Pollard	28		130	

Bran Effect

These results suggested that the bran, removed in milling 78% flour, might contain an interfering factor. This was confirmed by the addition of bran from a sound wheat to a 78% flour made from sprouted wheat. Typical results are given in Table II. The effect of bran is not mechanical because bran ground to pass through a 1 mm sieve or even a water extract of bran also depressed the results. Bran from unsprouted wheat, or bran made from sprouted wheat and treated with boiling water to destroy α-amylase, always depressed the apparent activity of 78% flour. Untreated bran from sprouted wheat produced variable results presumably because the depressing effect was opposed by the α-amylase in the added bran. It is known that the aleurone layer and the cells immediately beneath it are sources of α-amylase in sprouted cereals.[2]

TABLE II

Effect of bran and phytic acid on the apparent activity of flour from sprouted wheat

	Sprout units
40 *g Flour*	
Control—no additives	24·4
+10 g coarse bran	16·0
+10 g fine bran	12·5
+10 g Soxhlet extracted bran	22·3
50 *g Flour*	
Control—no additives	22·2
+ water extract of bran	14·7
+0·25 g sodium phytate	15·6
+0·5 g sodium phytate	6·8
+1·0 g sodium phytate	3·2

Calcium and phytic acid experiments

Calcium is known to be a stability factor for α-amylase.[3] Wheat bran contains phytic acid that forms an insoluble calcium phytate. To assess the effect of calcium, Hagberg tests were carried out with additions of calcium chloride or ammonium oxalate. Five ml of a 5% solution of either salt was substituted for 5 ml of the water used to carry out the test. The solutions were adjusted to pH 6·1, the normal pH of meal/water suspensions. The results of these tests are given in Table I. The anomalous wheats show a marked positive response to calcium, but no response to oxalate. This suggests that these wheats have virtually no free calcium. On the other hand, normal wheats show no response to calcium, presumably because sufficient is already present, and a negative response to oxalate. When the calcium additions were systematically varied, the maximum effect was reached with 2 ml of 5% calcium chloride solution per 150 ml water used in the test. Five ml of 5% solution was used throughout these experiments.

Sodium phytate markedly depressed the apparent activity of a flour from selected wheat as shown in Table II.

Phytic acid may be destroyed by an enzyme, phytase, normally present in wheat.[4] Wheatmeal/water suspensions placed in aluminium beakers in the usual way, were incubated overnight at room temperature. Under these conditions complete destruction of phytic acid by phytase could be expected.[4] The suspensions were then thoroughly stirred and cooked in the boiling water bath in the usual way. The results are given in Table I. The anomalous wheats show a marked increase in apparent activity while normal wheats do not.

S.K.B. determinations

The S.K.B. results given in Table I were obtained by the method of Sandstedt, Kneen & Blish.[3] It has already been reported by Mitchell that for 78% extraction flour one S.K.B. unit was equivalent to 50 sprout units. The results here have been expressed in sprout units, using this conversion factor.

It is apparent that the activities of wheatmeals as determined by the S.K.B. method are much higher than would be expected from the results of Hagberg tests, even in the presence of added calcium. This effect is apparently not the result of mechanical reinforcing of the starch gels by bran flakes, first because fine grinding of wheatmeals, to pass through an 8xx silk (i.e. of the same fineness as 78% flour) did not affect the penetrometer result and, secondly, because Soxhlet extracted bran had no effect on the apparent activity of a 78% flour when tested by the penetrometer method.

Discussion

It is well known that the distribution of α-amylase within the sprouted wheat grain is by no means uniform. This is exemplified by the S.K.B. results in Table I. The 78% flour (substantially endosperm) is less active than either the bran (the outer coat of the grain), or the pollard (a mixture of the inner grain coat and outer endosperm, the embryo and some endosperm material).

Further, Meredith[5] showed that different samples of sprouted grain have sharply differing distributions of activity. He took twenty samples of sprouted wheat and removed the germ ends from the grains by hand cutting. The remainder of the grain was then assayed for α-amylase. In some cases removal of the germ ends sharply reduced the α-amylase activity, and in others it was without effect. This variability of distribution pattern may be caused by differing wetting and drying conditions.

The flour produced by milling sprouted wheat often has a lower α-amylase activity than the whole grain, but the relationship between the two activities varies considerably. For this reason it is not possible to predict the activities of flours from the results for wheatmeals. Tests may be carried out on flour such as that obtained from a Brabender Quadrumat Junior mill. Extraction rate does not appear to be critical. Carefully screened semolina from a sprouted wheat was ground to flour, and the extraction rate of this flour was less than 50%. The activity, as determined by the S.K.B. method, was the same as that of 78% flour from the same wheat. It is, therefore, recommended that when the Hagberg penetrometer test is employed on wheatmeals, calcium ions should be added to offset the interfering effect of phytic acid in the bran.

Utilisation

We have found, as did Stewart,[6] that some severely sprouted lines of wheat showed little or no visible signs of sprouting, and that sometimes a visual grading could be misleading. Conversely some lines showing obvious visible sprouting had very low levels of enzyme activity.

Aided by the testing programme millers were able to exclude severely damaged lines from their grists, and to blend wheats to give a fairly steady level of sprout damage.

Bakers were advised to take the usual precautions such as the elimination of malt flour, use of acidifying agent in doughs, baking at the highest practicable temperature, and thorough cooling before slicing. Flour with up to 15 sprout units which corresponds to 0·3 S.K.B. units, could then be used with little difficulty and with few customer complaints.

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Received 3 July, 1967

References

1. Mitchell, *J. Sci. Fd Agric.*, 1968, **19,**
2. Linderström-Lang, K., & Engel, C., *Enzymologia*, 1937, **3,** 138
3. Sandstedt, R. M., Kneen, E., & Blish, M. J., *Cereal Chem.*, 1939, **16,** 712
4. Peers, F. G., *Biochem. J.*, 1953, **53,** 102
5. Meredith, P., *Cereal Chem.*, 1962, **39,** 168
6. Stewart, B. A., *Nature, Lond.*, 1964, **204,** 1088

CHLOROGENIC AND QUINIC ACIDS IN SUNFLOWER MEAL

By B. MILIĆ, S. STOJANOVIĆ, N. VUČUREVIĆ and M. TURČIĆ

Chlorogenic and quinic acids were isolated from the kernel of sunflower seed by column chromatography and identified by paper chromatography. Ultra-violet and infra-red spectra of the above acids were compared with those of pure preparations. The contents of both acids in the kernel and hull of sunflower seeds were determined and the conversion of chlorogenic acid into quinic acid at temperatures of 100° and 135° was followed. During storage of sunflower meal re-synthesis of chlorogenic acid was observed. Effects of isolates of chlorogenic and quinic acids on α-amylase, trypsin and lipase activity were detected.

Introduction

Guenzi *et al.*[1] have studied some phenolic acids in maize, wheat, sorghum and oats residues and their phytotoxicity. Towers *et al.*[2] have shown the distribution of twelve phenolic compounds, phenolic acids and phenolic glucosides in the parts of the species *Gaultheria*.

Although knowledge about the content and the role of phenolic compounds in plants is comprehensive, very little has been done in connexion with the examination of the effect of the temperature on the content of these compounds and their influence on various enzymes. Hasegawa *et al.*[3] have studied the content of chlorogenic acid in potato varieties and reported an increase of this phenolic acid of about 40% in the course of three months, depending upon the temperature of storage and the variety of potato. Rabin & Kein[4] studied the influence of chlorogenic acid on indoleacetic acid oxidase activity and observed that quinic acid, as a component of chlorogenic acid, did not inhibit IAA oxidase whereas caffeic acid was as effective as chlorogenic acid.

Since the amount and distribution of chlorogenic acid and other polyphenolic compounds in sunflower seed are significant from the nutritional aspect, owing to their effect on enzyme systems, as are the changes occurring under different temperatures, the aim of this work was to study the conditions by which these values were limited.

Experimental

Materials

Sunflower seed and sunflower meal were used for these studies. For chlorogenic acid and quinic acid analyses, two samples from 1·0 to 1·5 kg of sunflower kernels, shelled seed and hulls were taken, each representing one replicate. The same samples were analysed after 5 hour temperature treatment at 70°, 100° and 135°. All samples were stored for 8 weeks at 20° and analysed again after storage for 30 and 56 days. Similar examinations were made of the samples of cooled and uncooled sunflower meal after this was dried at 135° following the extraction procedure applied in the technological processing in oil factories. The same samples

were stored at the temperature of 20° for eight weeks and analysed after storage for 30 and 56 days.

Analysis of chlorogenic acid and quinic acid

Extraction

Sunflower seed was ground in a meat grinder and the hull was ground in Condux Mill to pass a 60 mesh screen. Oil extraction with water-free ether was conducted in a Soxhlet extractor for 7 hours. A 100 g air-dried sample was extracted with 350 ml 95% ethanol in the shaker for 20 minutes and filtered through a Büchner funnel. The extraction with 95% ethanol was repeated twice, and finally the residue was washed with 200 ml 95% ethanol. The combined ethanolic extracts were evaporated at 36° under reduced pressure to near dryness.

Isolation

The method used to isolate chlorogenic and quinic acids involves partition chromatography after the method of Hasegawa *et al.*[3] For the preliminary separation of phenolic compounds from ethanol extract a column of silicic acid, 2·5 × 22 cm, was employed, and for the separation of individual phenols a column of silicic acid 1·0 × 22 cm was employed. Silicic acid, prepared by the method of Bulen,[5] was treated with 0·5 N-H_2SO_4 by the method of Hanson & Zucker.[6] The solvent systems for eluation contained cyclohexane, 2-methyl-2-propanol and chloroform:

System A: 2-methyl-2-propanol–chloroform (2 : 3)
System B: cyclohexane–chloroform (1 : 9)
System C: 2-methyl-2-propanol–chloroform (3 : 7)

Each system was saturated with 0·5 N-H_2SO_4 at room temperature.

The concentrated ethanol extract was acidified with 0·5 ml 0·5 N-H_2SO_4, mixed thoroughly with 5 g silicic acid and transferred to the top of the preliminary column, and the phenol compounds were eluated with 250 ml solvent system A. The absorbence of the final eluate must be less than 0·02 at a wavelength of 328 nm in the case of the determination of chlorogenic acid and at 550 nm in the case of the determination of quinic acid. If the absorbence is greater, eluation procedure is continued with the same solvent system until the absorbence is reduced to less than 0·02.

The eluate was brought to dryness at a temperature of 30° under reduced pressure. The residue was dissolved in 0·5 ml 0·5 N-H_2SO_4, mixed well into 1 g silicic acid and transferred to the top of the analytical column. The individual phenol compounds were eluated at a flow rate of approximately 50 ml/hour with the mixture of different proportions of B and C as follows: Eluation was started with 50 ml of B, followed successively by 40 ml of B and 5 ml of C; 40 ml of B and 10 ml of C; 35 ml of B and 15 ml of C; 30 ml of B and 20 ml of C; 40 ml of B and 60 ml of C and finally 30 ml of B and 70 ml of C.

Identification

The eluate was collected in 5-ml fractions by a collector of the Norbert Vogel Type 'Robot-4'. The absorbence of each fraction was measured with a Beckman DU spectrophotometer at the wavelength of 328 millimicrons and at 550 millimicrons in order to determine quinic acid using periodate–2-thiobarbituric acid; an eluation pattern is shown in Fig. 1.

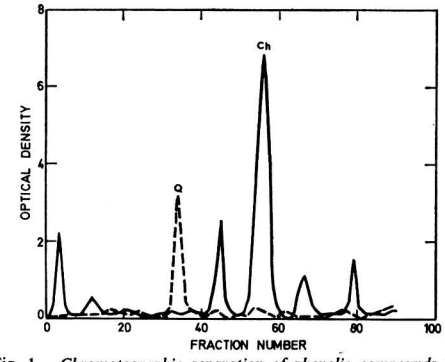

Fig. 1. *Chromatographic separation of phenolic compounds from ethanol extract of sunflower kernel* (shelled seed)
Ch = chlorogenic acid Q = quinic acid
———— at 328 nm
- - - - - - - at 550 nm after periodate–2-thiobarbituric acid procedure

Seven peaks were detected, from which one, located between fractions 30 and 40, was characterised by measurement in visible light (550 nm) and the others under u.v. fluorescence (328 nm). The fractions, belonging to individual peaks, were combined and identified by paper chromatography. For comparison, pure preparations of chlorogenic and quinic acids (Kock-Light Laboratories Ltd., Colnbrook, Bucks.) were used. The determination was carried out by ascending paper chromatography and the chromatograms were developed on Whatman no. 1 filter paper at 25° with the following solvent systems: n-butanol–acetic acid–water (4 : 1 : 5); 5% acetic acid; benzene–acetic acid–H_2O (6 : 7 : 3) upper phase; n-butanol–88% formic acid–H_2O (4 : 1 : 5) upper phase. Spots were detected by u.v. fluorescence or by treatment with the Benedict solution[7] and periodate–2-thiobarbituric acid.[8]

R_f values of the isolates of chlorogenic and quinic acids compared with pure preparations are illustrated in Table I.

TABLE I

R_f values and ultra-violet spectra of isolates and pure acids

Sample	R_f in system 1		R_f in system 2		R_f in system 3	R_f in system 4	Absorption spectra	
							λ min	λ max
Chlorogenic acid		0·60	0·58	0·70	0·83		265	328
Isolate of chlorogenic acid		0·60	0·59	0·70	0·83		265	328
Quinic acid	0·29	0·13		0·58		0·36		
Isolate of quinic acid	0·29	0·13		0·58		0·36		

Spectra of isolated chlorogenic acid, determined with a Beckman DU spectrophotometer were in accordance with u.v. spectra of pure chlorogenic acid (Fig. 2).

The values for λ_{min} and λ_{max} of u.v. absorption spectra of the isolates and pure chlorogenic acid are presented in Table I.

The peak which was tentatively identified as quinic acid by the method of Mesnard & Devaux[8] had no expressed maxima in u.v. light but absorbence decreased from 250 to 310 nm after which none was detected. The pure preparation of quinic acid had the same absorption spectra. In the u.v. range, the fraction containing quinic acid showed no absorbence at 328 nm, and the presence of quinic acid was determined in an aliquot of each fraction with periodate–2-thiobarbituric acid.

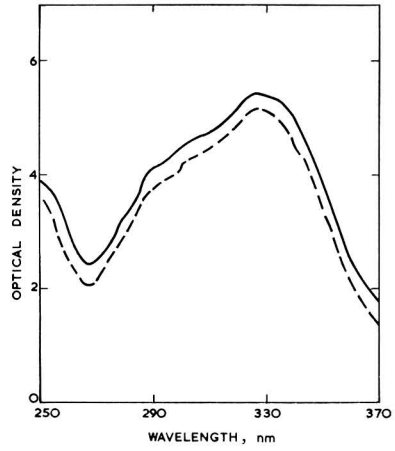

Fig. 2. *Ultra-violet absorption spectra of chlorogenic acid*
———— pure preparation
– – – – – – compound isolated from sunflower kernel (shelled seed)

The combined fractions of chlorogenic acid were brought to dryness, the residue was dissolved in 150 ml water, and any insoluble substances were removed by filtration. The supernatant was treated with 10 ml 10% lead acetate and the pH was adjusted to 9·0 with 0·5 N-NaOH. The yellow precipitate was removed by centrifugation, suspended in little water for washing, followed by re-centrifugation, and washing was repeated twice. The precipitate, after being washed, was suspended in 150 ml water and lead was precipitated by passage of H_2S through the suspension. The PbS precipitate was removed by filtration and the clear solution obtained was evaporated at 35° with applied suction and then freeze-dried. Ten mg of the dried sample were mixed well with 500 mg KBr (i.r. grade) in a small mortar, a pellet was made, and i.r. absorption spectra of the isolated substance were determined in a Perkin Elmer spectrophotometer, Infracord Type 137E (Fig. 3).

Collected fractions of quinic acid were brought to dryness at 36° with a rotary evaporator. The residue was recrystallised from water. The pale yellow powder was mixed well with KBr (i.r. grade), a pellet was made, and i.r. absorption spectra were determined (Fig. 4).

Infra-red spectra of the chlorogenic and quinic acids isolated were in good agreement with those of the pure preparations of both acids.

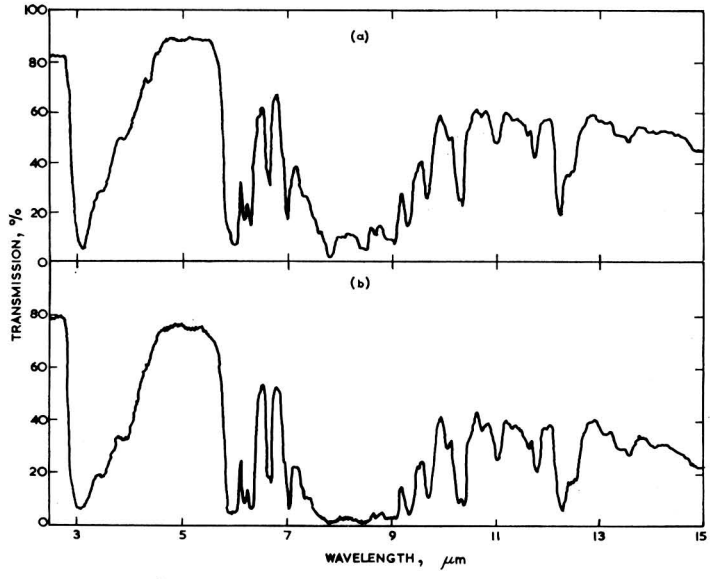

Fig. 3. *Infra-red absorption spectra of chlorogenic acid*
(a) pure preparation
(b) compound isolated from sunflower kernel (shelled seed)

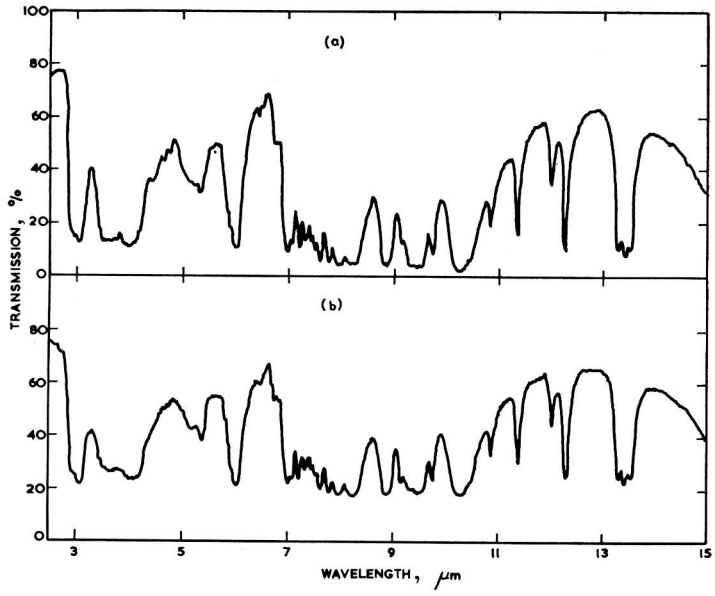

Fig. 4. *Infra-red absorption spectra of quinic acid*

(a) pure preparation
(b) compound isolated from sunflower kernel (shelled seed)

Determination

Combined fractions, after being separated on the analytical column, were made to volume with the solvent systems B and C, respectively, and the determinations of chlorogenic and quinic acids were conducted by the method of Mesnard & Devaux[8] in aliquots corresponding to approximately 40 gamma of acid.

Results

The results related to the content of chlorogenic and quinic acids in the samples analysed are reported in Table II.

The contents of chlorogenic and quinic acids in sunflower kernels were 1·56% and 0·48%, respectively. The temperature treatment at 70° for 5 hours destroyed 12% of the chlorogenic acid. Temperatures of 100° and 135° destroyed about 43% of the chlorogenic acid.

The content of quinic acid was inversely related to that of chlorogenic acid in the samples tested. Treatment at 70° caused a rise of 12·5% in the quinic acid, i.e. about the same as the amount by which the content of chlorogenic acid was reduced. Higher temperatures produced an increase of quinic acid amounting to 44%, a value similar to the decrease of chlorogenic acid in the same samples.

Storage of the control for 56 days and of the sample previously exposed to 70° for 5 hours did not result in any marked changes in the content of chlorogenic acid. However, marked increases of 12·2% and 53·3% for the sample treated at 100°, and 13·6% and 55·6% for the sample treated at 135°c for periods of 30 and 56 days, respectively, were observed.

TABLE II

Contents of chlorogenic and quinic acids expressed as % on dry matter of the sample

Sample	Chlorogenic acid			Quinic acid		
	days			days		
	0	30	56	0	30	56
Sunflower kernel						
Original (untreated)	1·56	1·60	1·66	0·48	0·48	0·46
After 5 h at 70°c	1·37	1·44	1·43	0·54	0·51	0·50
After 5 h at 100°c	0·90	1·01	1·38	0·69	0·61	0·45
After 5 h at 135°c	0·88	1·00	1·37	0·69	0·60	0·46
Sunflower meal						
Uncooled	0·71	0·79	1·10	0·41	0·38	0·25
Cooled	0·72	0·69	0·73	0·43	0·42	0·39

The contents of chlorogenic acid and quinic acid in the sample of cooled sunflower meal remained nearly unchanged during storage for 56 days. However, in the samples of uncooled sunflower meal with the same original levels of these acids, the content of chlorogenic acid increased to 0·79% during storage for 30 days and to 1·10% during storage for 56 days. These rises, of about 12% and 55%, respectively, for the same storage periods produced corresponding decreases in quinic acid, to 0·38% and 0·25%, respectively.

Effect of polyphenol isolate on enzyme activity

Measurements of trypsin activity and determination of the inhibition by the chlorogenic and quinic acids isolated on the casein substrate were conducted by the method described by Beauchene & Mitchell and modified by Ramirez & Mitchell.[9] α-Amino nitrogen, produced by enzyme activity, was expressed in mg per total volume of the reaction mixture. Results relating to the α-amino nitrogen produced after the defined period of incubation, are reported in Table III.

TABLE III
Inhibition of trypsin activity by chlorogenic and quinic acids

Substance added to casein solution	Absorbence at 620 nm	α-amino nitrogen in mg after incubation	Inhibition %
Trypsin	0·680	3·60	0
Trypsin + 2 ml 0·01 M chlorogenic acid	0·480	2·51	30·28
Trypsin + 2 ml 0·01 M quinic acid	0·678	3·59	0·30

The activity of the α-amylase preparation was determined by the modified Wolgemudt method after the procedure described by Hagberg.[10]

Inhibition of lipase activity by chlorogenic and quinic acids isolated was estimated by the method of Damade;[11] refined olive oil was used as the substrate.

In the control samples, distilled water was used instead of the isolates of chlorogenic and quinic acids.

Discussion

R_f values, u.v. and i.r. spectra indicate that the isolates from sunflower meal obtained by partition chromatography on columns of silicic acid correspond to chlorogenic and quinic acids. R_f values of chlorogenic and quinic acids for the solvent system n-butanol–acetic acid–water (4 : 1 : 5) were identical to those cited in the work of Sechet-Sirat *et al.*[12] Ultra-violet spectra of the chlorogenic acid isolated were nearly identical to those of the pure preparation in the range 250–360 nm. Sechet-Sirat *et al.*,[12] Cheng & Hanning[13] and Hasegawa *et al.*[3] reported a very similar absorption curve for chlorogenic acid.

Sechet-Sirat *et al.*[14] have shown the presence of free caffeic acid, existing in the plant, in sunflower seeds. However, this was not produced by hydrolysis of chlorogenic acid under the influence of the reagents employed. The attempt to determine caffeic acid from the remaining unidentified peaks failed, probably owing to conversion of *trans*-caffeic acid to its *cis*-isomer and formation of esculetine. One of the peaks on the paper chromatograph had three spots but it was not identified as caffeic acid.

The results obtained (Table II) show that the polyphenolic acids examined are closely correlated and that temperature treatment affects the degradation of chlorogenic acid into its components. Factors inducing re-synthesis of chlorogenic acid are not of physical nature only. Increased temperatures, apart from degrading chlorogenic to quinic acid, caused the conversion of proteins and polysaccharides to the lower degradation complexes that are suitable substrates[3] for post-synthesis of chlorogenic acid. Although high temperatures contributed to the degradation process, the lower temperatures were unable to provide the substrate for chlorogenic acid synthesis. According to Hasegawa *et al.*,[3] the content of chlorogenic acid remains constant if the sample is stored at a temperature of 15·6°, whereas a considerable increase (accounting for 50–60%) is observed during 2-week storage period at 4·44°. The possible explanation for chlorogenic acid synthesis occurring during cold storage of potatoes is presented in the work of Zucker & Levy[15] who found that glucose, fructose and saccharose were very effective substrates for chlorogenic acid synthesis and the above sugars accumulated in potatoes during cold storage. Since the content of carbohydrates in the sunflower kernel is relatively small, the only possible explanation is in the interaction of proteins and quinic acid. According to Hanson & Zucker[6] L-phenylalanine with quinic acid was found to be an effective substrate for chlorogenic acid synthesis. Degradation of chlorogenic acid by higher temperatures provided the optimum quantity of quinic acid for the interaction with free amino acids, L-phenylalanine (or any other free amino acid) to produce post-synthesis of chlorogenic acid. The level of moisture in the sample is also an important factor. The above data are confirmed by the results of the analysis on the content of chlorogenic and quinic acids in sunflower meal. During cooling of sunflower meal of 12% moisture level at the outlet from the drier system, the moisture content was reduced to 4·5%. The moisture content remained at this level during storage, whereas in uncooled sunflower meal the same level remained as at the outlet from the drier system.

Studies on the effect of the isolates and pure preparations of chlorogenic and quinic acids on enzyme activity showed that neither had any inhibitory effect on α-amylase activity. The percentage inhibition of trypsin digestion of casein by the isolate amounted to 30·28% for chlorogenic acid under the described laboratory conditions, and quinic acid did not exert any inhibitory effect. Inhibition of lipase activity on a substrate of refined olive oil by both acids was determined by the method of Damade,[11] and it accounted for 42·6% and 10·2% for quinic and chlorogenic acids, respectively.

Acknowledgment

The authors would like to thank Mr. Peter Tausik (Koch-Light Laboratories Ltd., Colnbrook, Bucks.) for the pure samples of chlorogenic and quinic acids; Miss Ana Bayer and Mr. Radmilo Matić for technical assistance; and Mr. Tibor Juhas (Agricultural-Industrial Combine, Vrbas, Yugoslavia) for the careful collection of the samples required in the present study. The authors gratefully acknowledge the assistance of Miss Djurdjica Mandrino.

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Received 22 May, 1967; amended manuscript 8 August, 1967

References

1. Guenzi, V. D., & McCalla, T. M., *Agron. J.*, 1966, **58**, 303
2. Towers, G. H. N., Tse Aida, & Gould, A. W., *Phytochemistry*, 1966, **5**, 677
3. Hasegawa, S., Johnson, R. M., & Gould, W. A., *J. agric. Fd Chem.*, 1966, **14**, 165
4. Rabin, R. S., & Kein, R. M., *Archs Biochem. Biophys.*, 1957, **70**, 11
5. Bulen, W. A., Varner, J. E., & Burrell, R. C., *Analyt. Chem.*, 1952, **24**, 187
6. Hanson, K. R., & Zucker, M., *J. biol. Chem.*, 1963, **238**, 1105
7. Gage, T. B., Douglass, C. D., & Wender, C. A., *Analyt. Chem.*, 1951, **23**, 1582
8. Mesnard, P., & Devaux, G., *Bull. Soc. chim. Fr.*, 1964, **1**, 43
9. Ramirez, J. S., & Mitchell, H. L., *J. agric. Fd Chem.*, 1960, **8**, 393
10. Hagberg, S., *Cereal Chem.*, 1961, **38**, 241
11. Damade, R., 'Kliničke laboratorijske metode' (Clinical laboratory methods), 1953 (ed. Oton Stark) (Beograd: Medicinska knjiga)
12. Sechet-Sirat, J., & Tayeau, F., *Bull. Soc. Chim. biol.*, 1959, **41**, 1059.
13. Cheng, R. C., & Hanning, F., *Fd Res.*, 1955, **20**, 506
14. Sechet-Sirat, J., Masquelier, J., & Tayeau, F., *Bull. Soc. Chim. biol.*, 1959, **41**, 1067
15. Zucker, M., & Levy, C. C., *Pl. Physiol.*, 1959, **34**, 108

PHYSIOLOGY OF BENGAL GRAM SEED

III.*—Changes in the phosphorus compounds of the seed parts during ripening of the seed

By B. M. LAL and S. C. VERMA**

Seed parts of Bengal gram have been studied for their total, acid-soluble, inorganic, phytin and phosphatide phosphorus fractions at different stages of seed development.

Except for the phytin phosphorus in the cotyledons and phytin and total phosphorus in the embryo (i.e. excluding the cotyledons), all phosphorus fractions decreased over the period of sampling. Largest decreases occurred in the seed coat which is commensurate with its metabolic necessity.

The cotyledons have been found to contribute largest amounts to the content of the phosphorus fractions in the whole seed, though at early stages of maturity, the seed coat also contributes a fairly large proportion.

Introduction

It has been earlier reported,[1] that the total phosphorus content of the seed parts of Bengal gram (*Cicer arietinum* L.) seed changes considerably during maturity of the seed. There is a large change in the seed coat and the cotyledons, but very little change in the embryo, which at all stages of maturity contains the highest concentration of total phosphorus. However, the cotyledons were found to contribute the greater part of the phosphorus content of the seed, mainly owing to their being the largest fraction of the seed at almost all stages of seed development. More recently[2] it has been shown that the changes in the total phosphorus content of the seed and the pod are a consequence of the changes in the various phosphorus fractions. A significant observation has been in respect of phytin phosphorus which has been found to be present in the pod as well as in the seed, even at the earliest stage of sampling.

The present investigation studies the changes in the phosphorus fractions in the seed parts, with a view to explaining the overall changes in the seed.

Materials and Methods

Details in respect of raising the plants and the method of collecting the samples have been reported earlier[2]. Immediately after harvesting the pods and their separation into the seed and pod covers, the seeds were hand-dissected into

* Part II: *J. Sci. Fd Agric.*, 1966, **17**, 43

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component parts – the cotyledons, seed coat and the embryo. At the last stage of sampling, however, the seeds had to be soaked in water to facilitate the separation of the seed parts.[1,3]

Seed parts were prepared for analysis as described previously[1]. The separation and estimation of various phosphorus fractions was carried out according to the procedures used in earlier work.[2]

Results and Discussion

Results are presented in Figs 1–4 and in Table I.

Fig. 1 shows the changes in the percentage of the seed parts; the results are in agreement with those published earlier[1] in that the cotyledons at all stages of sampling form the biggest fraction of the seed and their proportion increases considerably over the period of sampling. The proportion of the seed coat shows a decline over the same period, though at the first stage its proportion is much higher than was previously reported.[1] This is all the more significant, when it is considered that the variety used in the present investigation had a much lower proportion of seed coat in the mature seed than did the varieties used in the earlier investigations.[1,3] The proportion of the embryo remains almost constant throughout the period of sampling, hence it can be assumed that its growth keeps pace with the rate of growth of the seed.

Distribution of the phosphorus fractions in the seed parts on a moisture-free basis is shown in Figs 2, 3 & 4.

While the concentration of total phosphorus in the cotyledons shows a gradual fall, that of the seed coat registers a very steep fall over the period of sampling and that of the embryo shows an overall increase during the same period. These results are in general agreement with those reported earlier,[1] except that the values in the case of the cotyledons and the seed coat are much higher. It may be noted that the concentration of total phosphorus in the seed coat at day 20 is considerably higher than that of the cotyledons at the same stage. This indicates that in the early stages of seed development the seed coat is metabolically a very active part of the seed.

The acid soluble fraction shows a continuous decline in both the cotyledons and the seed coat, though the rate and magnitude of the decrease in the case of the seed coat is much sharper than in the cotyledons. Again it is evident from Figs 2 & 3 that at day 20 the seed coat contains appreciably higher concentrations of acid-soluble phosphorus than the cotyledons, but at day 43, the concentration of this fraction in the cotyledons is much higher than in the seed coat. It is considered that this is commensurate with the metabolic requirements of these parts at the time of seed germination.

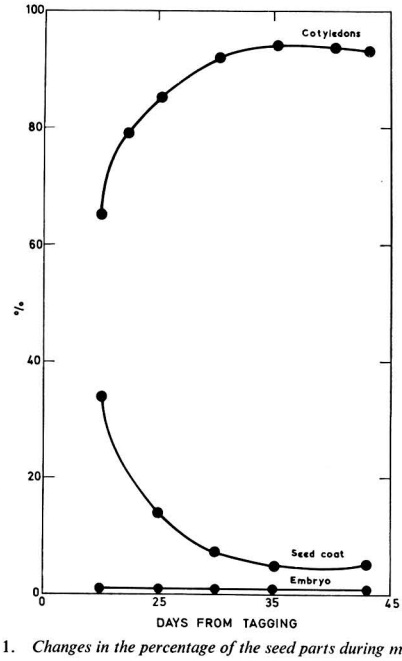

FIG. 1. *Changes in the percentage of the seed parts during maturity*

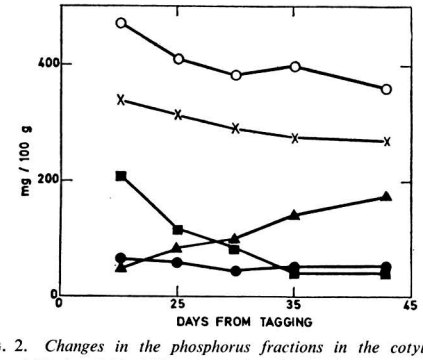

FIG. 2. *Changes in the phosphorus fractions in the cotyledons*
○ Total, × Acid soluble, ■ Inorganic, ▲ Phytin, ● Phosphatide

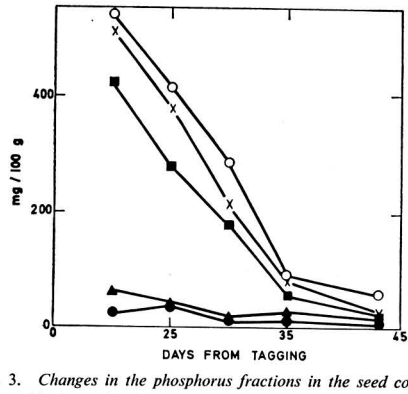

FIG. 3. *Changes in the phosphorus fractions in the seed coat*
○ Total, × Acid soluble, ■ Inorganic, ▲ Phytin, ● Phosphatide

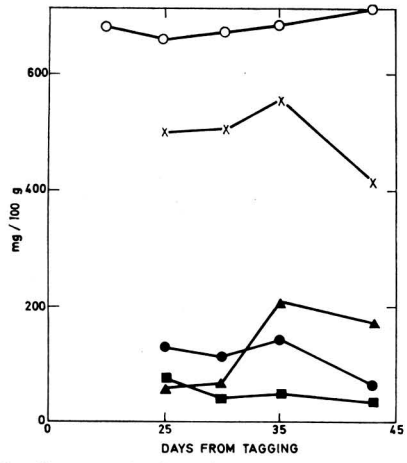

FIG. 4. *Changes in phosphorus fractions in the embryo*
○ Total, × Acid soluble, ■ Inorganic, ▲ Phytin, ● Phosphatide

Fig. 2 shows that there are inverse changes in the concentrations of inorganic phosphates and the phytin phosphorus in the cotyledons at different stages of seed development. These changes are similar to those observed in the whole seed[2] and in wheat endosperm,[4] and lend support to the earlier conclusion[2] that the phytin fraction is synthesised at the cost of inorganic phosphates for which the existence of an active enzyme system in the endosperm as well as the testa and pericarp of wheat has been postulated by Jennings & Morton.[4]

The situation in respect of these two fractions is rather different in the seed coat, where decreases are observed in the concentration of both of them. A similar decrease was observed in the pod covers, though the magnitude of the decrease was much less.[2]

Since the acid-soluble fraction consists of phytin, inorganic phosphates and ester-phosphorus it is desirable to consider these in detail. The phytin and the inorganic phosphate fractions were therefore determined, and an indirect estimate of the third group of compounds in the acid-soluble phosphorus was obtained by the following relationship:[5]

Ester phosphorus = Acid-soluble phosphorus — (phytin phosphorus + inorganic phosphorus).

A consideration of the ester phosphorus concentration present in the cotyledons at day 20 and day 43, showed that a considerable amount of the acid-soluble phosphorus cannot be accounted for by phytin phosphorus and inorganic phosphorus at the initial stage and also at the final stage of sampling (Fig. 2), and is present in the form of ester-phosphorus.

However in the seed coat, while some phosphorus is present in the form of ester phosphorus at day 20 and day 25, at any other stage of sampling almost all the phosphorus is accounted for by inorganic and phytin phosphorus fractions taken together. These indirect estimates lead to the conclusion that while the cotyledons at maturity have a potential for metabolic activity, the seed coat has very little, if any.

Fractionation of the phosphorus compounds in the embryo could not be carried out at day 20, as enough material was not available, but the results for other stages of sampling are presented in Fig. 4. The acid-soluble and the inorganic phosphorus fractions show an overall decrease, while the phytin fraction shows an increase of about threefold. It may be noted that between day 30 and 35 there is a sharp increase in the phytin phosphorus in the embryo, which is reflected in the acid-soluble phosphorus fraction during the same period. In the embryo a fairly large proportion of the phosphorus is not accounted for by inorganic and phytin phosphorus fractions, and this indicates a much higher potential for metabolic activity.

The phosphatide phosphorus fraction shows an overall decline in all the seed parts, the greatest decrease occurring in the seed coat.

The distribution of phosphorus fractions in the seed parts as a percentage of the whole seed has been calculated from the results presented in Figs 1–4 and tabulated in Table I. The table shows that for each of the fractions analysed, the cotyledons at each stage of sampling contribute the largest proportion of the phosphorus and that this proportion shows an increase over the period of sampling. On the other hand, the contribution of the seed coat to the phosphorus content for each of the fractions shows a marked

TABLE I
Distribution of phosphorus fractions in the seed parts as percent of whole seed

Phosphorus fraction	Seed parts	Days for tagging					
		20	25	30	35	43	S.D.
		%	%	%	%	%	
Total	Cotyledons	61·4	84·4	92·7	97·0	96·9	±13·49
	Seed coat	37·0	13·8	5·4	1·1	0·8	±13·53
	Embryo	1·5	1·8	1·9	1·9	2·2	± 0·23
Acid soluble	Cotyledons	N.D.*	82·2	92·9	96·3	98·1	± 6·16
	Seed coat		16·1	5·1	1·4	0·1	± 6·29
	Embryo		1·7	2·0	2·3	1·8	± 0·23
Inorganic	Cotyledons	N.D.	71·4	85·4	92·4	97·5	± 9·81
	Seed coat		28·1	14·0	6·2	1·8	± 9·99
	Embryo		0·5	0·6	1·4	0·7	± 0·45
Phytin	Cotyledons	N.D.	91·5	97·7	97·4	98·5	± 2·78
	Seed coat		7·6	1·6	0·9	0·3	± 2·92
	Embryo		0·9	0·7	1·7	1·2	± 0·38
Phosphatide	Cotyledons	N.D.	88·9	95·4	95·7	97·7	± 3·31
	Seed coat		8·7	1·8	1·0	0·8	± 3·27
	Embryo		2·4	2·8	3·3	1·5	± 0·66

*Not determined

decline over the period of sampling.

Not much variation in the phosphorus contribution of the embryo to the total present in the seed is observed although, except in the case of the phophatide fraction, there is a slight increase in this contribution from day 25 to 43.

The results presented above show that the content of the phosphorus fractions in the whole seed is largely dependent upon the quantities present in the cotyledons, although the seed coat contributes a substantial amount in the early stages of seed development.

Acknowledgments

The authors are grateful to Dr. M. S. Swaminathan, Director of the Institute, for his interest and encouragment during the course of these investigations.

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Received 9 June, 1967; amended manuscript 11 October, 1967

References

1. Verma, S. C. Lal, B. M., & Prakash, V., *J. Sci. Fd Agric.*, 1964, **15**, 25
2. Verma, S. C., & Lal, B. M., *J. Sci. Fd Agric.*, 1966, **17**, 43
3. Lal, B. M., Prakash, V., & Verma, S. C., *Experientia*, 1963, **19**, 154
4. Jennings, A.C., & Morton, R.K., *Aust. J. biol. Sci.*, 1963, **16**, 332
5. Pons Jr., W. A., Stansbury, M. F., & Hoffpauir, C. F., *J. Ass. off. agric. Chem.*, 1953, **36**, 492

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

FEBRUARY, 1968

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Flow of water under transient conditions in unsaturated soils. J. L. Thames (*Diss. Abstr.* B, 1967, **27,** 2573).—The pattern of water movement during vertical infiltration in a sandy loam and a silt loam under transient unsaturated conditions, is examined. Water was allowed to enter air-dry columns of the soils under a small constant suction and its subsequent distribution was determined by γ-radiation attenuation. From multiple regression analysis of the data, the water content was expressed as a function of depth and time, from which were calculated the instantaneous flux and water concn. gradient at given soil water contents. In the early stages of infiltration the relationship between flux and gradient was linear in accordance with the Darcy equation, but later as the gradient became less steep the relationship broke down. This occurred in both soils over a wide range of water contents. The deviation from linearality was similar in both soils. An empirical flow equation, based on the Darcy relationship, fitted the data satisfactorily. A. G. POLLARD.

Steady state flow of water through unsaturated soil in the low capillary potential region. W. N. Stammers (*Diss. Abstr.* B, 1967, **27,** 2554).—The validity of the flux equation for water movement in unsaturated soils is examined as a basis of the theory of unsaturated flow phenomena. Use of the γ-ray attenuation method in measuring the steady state water content profiles in a coarse-textured soil in the low capillary potential region suggests that the water content gradient form of the flux equation is a suitable model for the movement of water in unsaturated coarse-textured soils in the appropriate range of water content. Two steady-state methods for evaluating capillary conductivity and water diffusibility are developed. A. G. POLLARD.

Difficulties of estimation of moisture retention characteristics of soils rich in organic matter. S. Perigaud, J.-M. Servier (*C.r. hebd. Séanc. Acad. Agric.*, 1967, **53,** 932–939).—Available methods fail to give reliable results for the water-retention capacity of these soils as sampled in their natural state. This is due to the great diminution in water-absorbing capacity of the org. matter during drying and to alterations in its capacity with the conditions of hydration. P. S. ARUP.

Transpiration as a function of soil temperature and soil water stress. L. M. Cox and L. Boersma (*Plant Physiol.*, 1967, **42,** 550–556).—*Trifolium repens* was studied. Significant interaction between soil water stress and soil temp. was observed for stomatal closures even in the so-called wet range of soil water stress. Mesophyll resistance increased as soil water stress increased. E. G. BRICKELL.

Emanation of water from underground plant parts. B. Schippers, M. N. Schroth, and D. C. Hildebrand (*Plant & Soil*, 1967, **27,** 81–91).—When the French bean was grown at approx. 100% R.H. and temp. of 28–30°, about 0·12 ml water emanated from an 11 mm length of stem over 24-h. Less water emanated at lower R.H. and temp. Water emanation was more rapid in compacted than in loose soil, and also increased with increasing fineness of texture of soils. A. H. CORNFIELD.

Some aspects of cation-exchange in soils. E. P. Papanicolaou (*Diss. Abstr.* B, 1967, **27,** 2572).—Adsorption of index cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) over a wide range of pH (4–11) by a bentonite and a loam is examined. Data obtained were compared with those determined by isotopic dilution using ^{137}Cs and ^{140}La. All cations except Ca in the loam showed similar adsorption values at similar pH levels and at pH <7·0. With pH >7·0 univalent cations showed similar adsorption values at similar pH but the polyvalent cations gave much higher values. The cation-exchange capacity (*CEC*), afforded a measure of the electrostatic changes of the two materials. The amount of Ca adsorbed exceeded the *CEC* of the loam over the pH range 4–11. The discrepancy is attributed to the presence of Ca compounds sol. in acetates. The high values for adsorbed polyvalent cations at pH >7 is explained by the preferential adsorption of complex ions. No evidence of anion adsorption in the pH range 4–11 or of complex ion adsorption at a pH <7 was obtained. In the *CEC*–pH isotherm for the bentonite, polyvalent cations showed a characteristic supersaturation associated with a negative suspension effect. A. G. POLLARD.

Chromatographic transport through soils. II. Column experiments with strontium and calcium isotopes. M. J. Frissel and P. Poelstra (*Plant & Soil*, 1967, **27,** 20–32).—The rates of transport of labelled Ca^{2+} and Sr^{2+} at varying water fluxes through resin–sand mixtures agreed well with calculated values. There was good agreement between experiments done on clay–sand and resin–sand mixtures. A. H. CORNFIELD.

Preservation unaltered, of mineral nitrogen in tropical soils and soil extracts. J. B. D. Robinson (*Plant & Soil*, 1967, **27,** 53–80).—Addition of various chemicals (toluene, chloroform, etc.) before storage at ambient temp., or storage at sub-zero temp. did not preserve unaltered the NH_4^+ and NO_3^- contents of tropical soils even over a few days. Sub-zero storage affected the subsequent mineralisation of N during incubation. The mineral-N content of soil extracts using 2N-KCl (pH 7·0) was preserved during storage at sub-zero temp., but addition of Cu^{2+} and storage at ambient temp. was ineffective. The mineral-N in acidified 0·5N-K_2SO_4 extracts was not preserved by addition of org. chemicals or Cu^{2+}, or by sub-zero storage. A. H. CORNFIELD.

Effect of soil texture on recovery of applied nitrogen. R. G. Nash and D. D. Johnson (*Agron. J.*, 1967, **59,** 272–275).—Grasses were grown in a calcareous silty clay mixed with varying levels of sand to alter texture to varying degrees down to a sandy loam (70% sand). Soil texture had no effect on the recovery by grasses of N added as NO_3^-. When HN_4^+-N was added (together with N-serve to prevent nitrification) recovery of applied N increased with coarseness of texture. Approx. 75% of the NO_3^--N and 50% of the HN_4^+-N were recovered by the grasses. Applied NO_3^-N was accounted for almost entirely by plant uptake and residual soil N, but only 50–70% of the applied NH_4^+-N could be accounted for in this way. A. H. CORNFIELD.

Influence of organic matter and pH on transformations of nitrites to elemental nitrogen. M. A. Rashid (*Diss. Abstr.* B, 1967, **27,** 2553–2554).—Evidence was obtained of an interaction between NO_2^- and org. matter (dry and green lucerne) added to acid soils (pH 4·6–5·6), free N_2 being the major product. In non-incubated soils addition of dry lucerne with NO_2^- caused the formation of less N_2 than when NO_2^- alone was added. In incubated soils the effect of lucerne was reversed and the increased production of N_2 was intensified by prolonged incubation: it was, however, drastically diminished by the previous destruction of org. matter by H_2O_2. In this respect the action of green lucerne was the reverse of that of dry lucerne. Soil pH was an important factor controlling these changes and alterations in pH during incubation or due to materials added, modified the amount and timing of the evolution of N_2. Addition of aromatic compounds, e.g., cinnamic acid, vanillin and, notably, quinol, increased the production of N_2; some phenolic compounds (pyrocatechuic acid, pyrogallol, phloroglucinol) diminshed the evolution of N_2. A. G. POLLARD.

Phosphorus transformations associated with soil organic matter. P. M. Mehta (*Diss Abstr.* B, 1966, **27,** 1696–1697).—For the determination of org. P in soil, ignition of the sample for 1 h at 400° was preferable to ignition at 240°. Addition of sol. inorg. P to a highly org. soil stimulated the synthesis of org. P; large additions of inorg. P tended to restrict the formation of org. P and increase mineralisation. Addition of energy material to a soil previously treated with sol. inorg. P (200 ppm) had little effect on the synthesis of org. P,

but addition of 2% glucose to another soil previously treated with sol. inorg. P at 50 ppm considerably increased the formation of org. P. Sterilisation of the former soil by steam increased its org. P content more than did that by γ-irradiation or treatment with MeBr. Use of ^{32}P to distinguish between normal soil P and added sol. inorg. P showed that in both soils addition of energy material increased labelled org. P material. In a soil incubated with cellulose and various proportions of sol. inorg. P and N, immobilisation of P occurred during the first week of incubation and was increased by additions of cellulose or inorg. P; with added N mineralisation began after 4 weeks. A. G. POLLARD.

Aluminium complexed with organic matter in various acid soils. E. Lefebvre-Drouet (*C.r. hebd Séanc. Acad. Agric. Fr.*, 1967, **53,** 926–932).—The complexed Al was determined in 40 soils containing 0–40% of org. matter by determining the increase in extractable Al (cf. *Ann. Agron.*, 1966, **17,** 553) found after ashing the soils. The content of complexed Al was proportional to ($r=0·8$) that of the org. matter especially the fulvic and humic acids. P. S. ARUP.

Spectrophotometric determination of aluminium in soil extracts with xylenol orange. D. T. Pritchard (*Analyst, Lond.*, 1967, **92,** 103–106).—The Al is determined in an aliquot of the extract by measuring the extinction at 550 nm of the Al-xylenol orange complex formed at pH 3·8 and 40° in presence of EDTA to mask Fe^{3+} and Fe^{2+}. The working range is 0–60 μg; permissible limits of interfering ions are listed. The method is regarded as more practicable than the lake methods. W. J. BAKER.

Course of behaviour of zinc applied to soil containing different levels of freshly-applied organic matter. A. S. Henry (*Diss. Abstr.* B, 1967, **27,** 2552).—Possible relationships between the inactivation of Zn in soils and components or products of decomposition of humic acids are examined. Ground lucerne and ^{65}Zn were added to soils which were then moistened to approx. field capacity and incubated. ^{65}Zn was determined periodically. Initially >70% of the added Zn occurred in the fulvic acid and water-sol. fractions but subsequently this proportion declined and after 20 weeks >80% of the ^{65}Zn was detected in the insol. soil residue. Similar results were obtained following incubation of the treated soil with ground sugar-beet tops. During the incubation period more Zn was associated with the sand and silt than with the clay fraction of the soil. No evidence was obtained of the transfer of ^{65}Zn from the humic acid to the fulvic acid fraction of the soil. A possible mechanism of these effects is discussed. A. G. POLLARD.

Soil test for evaluating plant-available zinc. J. F. Trierweiler (*Diss. Abstr.* B, 1966, **27,** 1687–1688).—The method described is claimed as simple and readily adaptable for routine procedure. 10 g of sieved (<1 mm) soil is shaken for 30 min. with 20 ml of an extractant consisting of 0·01 M-EDTA and M-$(NH_4)_2CO_3$ at pH 6. The suspension is filtered and Zn is determined in the filtrate with an atomic absorption spectrophotometer. Results obtained gave a clear distinction between Zn-deficient and non-deficient soils and showed high correlation between extractable Zn and various parameters of plant-available Zn. A. G. POLLARD.

Fixation of zinc in calcareous soils and its availability to tomato plants. J. Navrot, B. Jacoby, and S. Ravikovitch (*Plant & Soil*, 1967, **27,** 141–147).—Studies with $^{65}Zn^{2+}$ applied to a rendzina (52% $CaCO_3$) and a loess (21% $CaCO_3$) showed that Zn fixation against movement by leaching with water was very rapid and almost complete in both soils. 82% of the Zn applied to the loess and 89% of that applied to the rendzina was found in the top 0·5 cm of the soil. Zn availability, as measured by Zn uptake by tomatoes and extractability by a no. of reagents, was higher from the rendzina than from the loess. A. H. CORNFIELD.

Alteration of surface soil characteristics by four tree species. D. Challinor (*Diss. Abstr.* B, 1967, **27,** 2560).—Changes in some chemical and physical properties of soil due to 30-year growth of Norway spruce, red pine, red oak and white pine are examined. Attention is given to the extent to which the original nutrient elements of the soil were re-cycled by the different species *via* the annual leaf-fall. The current annual leaf-fall is about the same wt from each species but the surface accumulation is greatest under the pines. Soil under spruce contains most org. matter, this being attributable to the high concn. of roots at the depths sampled. Infiltration rates were highest under white pine and lowest under oak. No relationship was apparent between infiltration rates, pore vol. and/or org. matter content. Differences between species were less marked in respect of chemical than in that of physical factors. Soil acidity increased with depth under all species with a trend towards higher values under spruce. Soil pH was directly related to $[Ca^{2+}]$ in the soil. The % total N and exchangeable K declined with depth under all species, the two values being highest under spruce and lowest under white pine; in both cases the concn. in soil was related to that in the litter and to the level of soil org. matter. The $[Ca^{2+}]$ in the surface 5 in. of soil was similar under all species. A. G. POLLARD.

Toxicity of decomposing crop residues to cotton germination and seedling growth. C. A. Megie, R. W. Pearson, and A. E. Hiltbold (*Agron. J.*, 1967, **59,** 197–199).—Poor germination and reduced growth of cotton seedlings when seed was sown in a sandy loam (pH 6·7) mixed with crop residues was due to the presence of unionised NH_3 in the soil. The accumulation of NH_3 in toxic amounts resulted from increased soil pH and NH_4-N content, which were proportional to the org. N content of the residues. Plant growth decreased with increasing soil NH_3, and levels above about 10 ppm were lethal. A. H. CORNFIELD.

Presence and persistence of phytotoxic substances in wheat, oats, maize and sorghum residues. W. D. Guenzi, T. M. McCalla, and F. A. Norstadt (*Agron. J.*, 1967, **59,** 163–165).—Wheat, oat, maize and sorghum residues, collected at the time of harvest of each crop, contained water-sol. substances that were toxic to wheat seedlings; toxicity increased in the order wheat, oat, maize, and sorghum residues. Wheat and oat residues were essentially free of water-sol. toxic substances 8 weeks after exposure to field conditions. Maize and sorghum residues had higher contents of water-sol. toxic substances than had wheat or oats and required 22–28 weeks of field exposure before the toxic effects disappeared. A. H. CORNFIELD.

Immobilisation and mineralisation of tracer N in forest raw humus. I. Effect of temperature on the interchange of nitrogen after addition of urea, ammonium-, and nitrate-nitrogen. L. N. Overrein (*Plant & Soil*, 1967, **27,** 1–19).—The rate of immobilisation of added urea-N during incubation of forest raw humus increased with temp. (4–20°). When NH_4^+-N was added immobilisation reached a max. after 10 days at 20°, whilst at 4° and 12° immobilisation continued for 90 days. When NO_3^--N was added the turnover of added N was correlated with temp. At 20° part of the immobilised N was again mineralised within 3 days. Part of the immobilised urea-N was later mineralised as NO_3^-, but this did not occur where NH_4^+-N was added. At 4° and 12° micro-organisms utilised NH_4^+-N more rapidly than NO_3^--N. A. H. CORNFIELD.

Value of urea nitrate and urea phosphate as nitrogen fertilisers for grass and barley. J. K. R. Gasser and A. Penny (*J. agric. Sci., Camb.*, 1967, **69,** 139–148).—At low levels similar amounts of N were recovered, in glasshouse pot trials with grass, from urea nitrate (**I**) and NH_4NO_3 (**II**) applied to a sandy loam and a clay loam, although at high applications the early growth was damaged by **I**. In the field, herbage was scorched by **I**, and **I** at 200 lb/acre produced less dry matter than did **II**. **I**, urea phosphate, (**III**), and a **III**–urea mixture were compared with **II** for barley and grass. On a light soil growth was least good with **I**. **III** and the mixture were superior to **II**. Whilst HNO_3, due to its mobility in the soil, was ineffective in absorbing the free NH_3 from the hydrolysis of urea, H_3PO_4 because of its lower mobility allowed the urea to diffuse into a larger vol. of soil before hydrolysing and was still present to absorb the free NH_3 formed. **I** and **III** have good physical forms and **III** has promise for mixed NP and NPK fertilisers. M. LONG.

Coated urea, thiourea, urea-formaldehyde, hexamine, oxamide, glycoluril, and oxidised nitrogen-rich coal as slowly available sources of nitrogen for orchardgrass. J. D. Beaton, W. A. Hubbard, and R. C. Speer (*Agron. J.*, 1967, **59,** 127–133).—The recovery by orchardgrass of N applied in various forms as a surface dressing in pot tests was, for urea coated with dicyclopentadiene copolymer 75%, for NH_4NO_3 74%, for thiourea 69%, for finely-ground oxamide 65%, for oxamide (8–14 mesh) 65%, for urea + thiourea 63%, for hexamine 59%, for glycoluril 49%, for urea-formaldehyde 41%, and for oxidised N-enriched coal (NH_4 salt) 39%. Yields and N uptake in the first harvest were greatest with NH_4NO_3, urea, urea + thiourea and finely ground oxamide. Glycoluril and coated urea produced highest yields and N uptake in the second and third harvest. In later harvests yields and N uptake from urea-formaldehyde and thiourea increased. A. H. CORNFIELD.

Plant Physiology, Nutrition and Biochemistry

Kinetics of the daily rate of photosynthesis at low temperatures for two conifers. R. P. Pharis, H. Hellmers and E. Schuurmans (*Plant Physiol.*, 1967, **42,** 525–531).—*Pinus ponderosa*, Laws, and *Pseudotsuga menziesii*, (Mirb.) France, were studied and photosynthesis at 1200 ft-c was measured. Attainment of max. photo-

synthesis depended on species, current temp., and temp. at which plants had become acclimatised. Fluctuations in the rate of photosynthesis during the day varied with species and temp. and a plant transferred to another temp. acquired a new stable daily photosynthetic pattern, the no. of days required for stabilisation depending upon the previous temp. history. E. G. BRICKELL.

Carbon dioxide metabolism in maize roots. I. Kinetics of carboxylation and decarboxylation. I. P. Ting and W. M. Dugger, jun. **II. Intracellular distribution of enzymes.** J. Danner and I. P. Ting (*Plant Physiol.*, 1967, **42**, 712–718, 719–724).—I. Data indicates that the first product is oxaloacetate followed by malate and aspartate. CO_2 fixation is via P-enolpyruvate carboxylase and malic dehydrogenase and subsequent malate metabolism is by direct decarboxylation, possibly by the malic enzyme.

II. Intracellular location of the three enzymes, P-enolpyruvate carboxylase, malic dehydrogenase, and the malic enzyme is non-particulate. Sol. malic dehydrogenase differs both in structure and function and is apparently involved in CO_2 metabolism.
E. G. BRICKELL.

Involvement of an endogenous rhythm in the photoperiodic response of *Hyocyamus niger*. J. C. S. Hsu and K. C. Hamner (*Plant Physiol.*, 1967, **42**, 725–730).—Plants were grown in a modified White's medium under conditions of variable-cycle length and light interruption. Results confirm the involvement of an endogenous rhythm in flowering response. E. G. BRICKELL.

Action spectrum for an enhancement of endogenous respiration by light in *Chlorella*. W. Kowallik (*Plant Physiol.*, 1967, **42**, 672–676).—An action spectrum for the enhancement of respiration of *Chlorella vulgaris* shows two peaks around λ 450 and 375 mμ pointing to participation of either a *cis*-carotenoid or flavin.
E. G. BRICKELL.

Inhibition of oxidative phosphorylation and respiration by ozone in tobacco mitochondria. T. T. Lee (*Plant Physiol.*, 1967, **42**, 691–696).—The phosphorylative system of tobacco leaf mitochondria is more sensitive to O_3 than is the respiratory system and uncoupling of phosphorylation by O_3 is not necessarily preceded by mitochondrial swelling. E. G. BRICKELL.

Ultrasonic energy as a cleaning agent; its influence on the respiratory activity and leaf anatomy of *Brassica oleraceae* var. Acephala. D. E. Hudson (*Diss. Abstr.* B, 1967, **27**, 2566–2567).—The use of ultrasonic energy for removal of spray residues from collards is examined. The plants were sprayed with Thiodan (1 lb/acre) and samples, taken at intervals of up to 14 days, were washed in an ultrasonic cleansing unit working at 200–450 m.-A. power output for 5, 10 or 20 min., hand-washed samples being used for comparison. Ultrasonic washing at 450 m.-A. for 20 min, reduced the residue from 10·63 to 2 ppm (tolerance level) on the day of application. All other treatments, except hand-washing for 5 min., reduced the residue to the tolerance level 3 days after the application. The residue on unwashed leaves reached the tolerance level only after 7 days. Differences between residues after the various ultrasonic washes were small and in all cases residues were smaller than those remaining after the 5-min. hand-wash. The respiratory activity of leaves was lowered by ultrasonic treatment, except in the case of that at 450 m.-A. for 10 min. which increased it. Decreased respiration is attributed to degassing by cavitation and diminution of gas in intercellular spaces. Increased respiration results from damage to tissues.
A. G. POLLARD.

The transpiration process as a function of environmental parameters. L. M. Cox (*Diss. Abstr.* B, 1967, **27**, 2571).—Relationships between soil moisture stress, soil temp., and transpiration rates in plants are examined, moisture stress being controlled osmotically by solutions of Carbowax 60. Under steady-state conditions, resistance to the transpirational flow was calculated by Fick's first law, resistance being expressed as that in leaves, boundary layers and mesophyll. Interelationships between the parameters are indicated. The degree of stomatal closure increased with rise in soil moisture stress and with fall in temp.
A. G. POLLARD.

Relative permeabilities of plastic films to water and carbon dioxide. J. T. Woolley (*Plant Physiol.*, 1967, **42**, 641–643).—No material was found to have a CO_2 permeability as great as its water permeability. E. G. BRICKELL.

Relation between calcium and strontium transport rates as determined simultaneously in isolated segments of the primary root of *Zea mays*. M. E. Hutchin and B. E. Vaughan (*Plant Physiol.*, 1967, **42**, 644–650).—In simultaneous determinations the ratio of Sr to Ca moved was exactly equal to the ratio of their concn. in nutrient solutions and there was no evidence of discrimination. DNP reduced the transport of Sr and Ca to an equivalent extent (2–9%). E. G. BRICKELL.

Steady-state sodium and rubidium effluxes in *Pisum sativum* roots. B. Etherton (*Plant Physiol.*, 1967, **42**, 685–690).—Fluxes appeared to be from two cellular compartments, a small one with a high flux rate and a larger one with a slow flux rate. Results support the theory that Na is transported actively from *Pisum* roots.
E. G. BRICKELL.

Iron uptake and translocation by tomato plants as influenced by root temperature. J. W. Riekels (*Diss. Abstr.* B, 1966, **27**, 1689).—The plants were grown at controlled temp. in a nutrient medium containing 1 μ mol. Fe/l, provided by Fe^{3+} ethylene diamine di (*o*-hydroxyphenyl acetate). After pretreatment of the plants with various levels of Fe or Mn the absorption and translocation of radioactive Fe was examined in plants the roots of which were maintained at 55, 65, 75 or 85°F. Both intake and translocation of Fe increased with root temp. At the lower temp., Fe chlorosis may be due to the rate of growth exceeding the requisite rate of intake and translocation of Fe. at the higher temp. the rate of uptake may equal or exceed that needed for growth. Increase in the amount of Fe supplied to the plants in the pretreatment lowered the further rate of intake and *vice versa*. The effect of root temp. on Fe intake diminished with increase in the Fe content of the plant. Separate process may exist for the absorption and translocation of Fe. The Fe content of plant tissues was increased by adding Mn to the nutrient, either during the pretreatment or during the normal growth, up to the level, 1 μ mol./l. Higher [Mn] reversed this effect. Fe uptake was depressed more by deficiency than by excess of Mn. A. G. POLLARD.

Uptake and metabolism of iron-59 in chlorosis-susceptible and -resistant grain sorghum. A. A. Abdul Muhai (*Diss. Abstr.* B, 1967, **26**, 2568).—Genetically-related but chlorosis-susceptible and -resistant strains of sorghum were grown in soils known to produce chlorotic and non-chlorotic plants. Differences in the distribution of ^{59}Fe and in nutrient balances in the two strains are examined. No Mendelian relationships or hereditary influence in plant nutrition affecting chlorosis was apparent. A close association of Ca with Fe nutrition in the production of chlorosis was shown whereas variations in Na, K and P in chlorotic plants appear to be after-effects. Distribution of ^{59}Fe between varieties or between soils in relation to the appearance of chlorosis, showed no gross variation in pattern. Uptake of ^{59}Fe by plants was greater from the 'non-chlorotic' soils and greater by susceptible varieties of plants. Deposition of ^{59}Fe in plant tissues was greater in the susceptible varieties. Chlorosis had no effect on the distribution of ^{32}P or ^{45}Ca in the plants. A. G. POLLARD.

Effect of light on the redistribution of labelled strontium, caesium and zinc in germinating soya-bean seed. T. W. Sudia and D. G. Green (*Plant & Soil*, 1967, **27**, 103–112).—In seed obtained from plants treated with labelled Sr, Cs, and Zn, 20–30% of the total Sr in the seed but only about 5% of the total Zn and Cs, was present in the seed coat. During germination all the Zn, but none of the Sr and Cs, moved from the seed coat. There was only slight movement of Sr, but considerable movement of Zn and Cs, from the cotyledon. Exposure to light, compared with dark, increased the movement of Zn and Cs into the leaves and hypocotyl.
A. H. CORNFIELD.

Effect of girdling of *Hibiscus* on root initiation in relation to changes in concn. of chemical constituents adjacent to the girdled zone. L. P. Stoltz and C. E. Hess (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 734–743).—Carbohydrates, particularly starch, accumulated in large amounts above the girdle. Approx. 3 times as much starch accumulated in an easy-to-root red variety of *Hibiscus* than in a difficult-to-root white variety. Amino-acids accumulated in large amounts only in the white variety 5–10 days after girdling and were associated with leaf senescence. No leaf senescence occurred in the red variety. There was no correlation between amino-acid content and the increased rooting response of red variety cuttings.
A. H. CORNFIELD.

Metabolic changes in after-ripening seed of *Prunus cerasus*. L. J. LaCroix and A. S. Jaswal (*Plant Physiol.*, 1967, **42**, 479–480).—During the seventh week of after-ripening, a striking increase in the respiration rate of 25° of embryonic axes occurred, together with a sharp change from the dormant to the non-dormant state of the seed. On the basis of C-6/C-1 ratio these changes may be related to an increased activity of the pentose phosphate cycle.
E. G. BRICKELL.

Metabolic processes in cytoplasmic particles of the avocado fruit IX. Oxidation of pyruvate and malate during the climacteric cycle. C. Lance, G. E. Hobson, R. E. Young and J. B. Biale (*Plant Physiol.*, 1967, **42**, 471–478).—Addition of thiamine pyrophosphate externally to avocado fruit increased pyruvate oxidation both before and after the climacteric period. Results from the incubation of mitochondria from preclimateric fruit with malate-U-^{14}C indicates that the ability to carry out oxidative decarboxylation of α-keto acids improves as the ripening process progresses.
E. G. BRICKELL.

Formation of a soluble amylopectin-like polysaccharide in potato tubers. R. B. Frydman and C. E. Cardini (*Plant Physiol.*, 1967, **42**, 628–630).—Incubation of potato tuber slices or etiolated sprouts with 0·1 M-glucose 1-phosphate led to the accumulation of maltooligosaccharides and a sol. amylopectin-like polysaccharide. Neither 2,4-DNP nor N affected the excretion of the polysaccharide which was a very good primer for phosphorylase yet a poor one for starch synthetase. E. G. BRICKELL.

Formation of amylase in disks of bean hypocotyl. H. H. Clum (*Plant Physiol.*, 1967, **42**, 568–572).—*Phaseolus vulgaris*, L. was studied. Although some amylase activity was present in untreated hypocotyls it was greatly increased when disks were incubated for 4 days in water containing kinetin (5 mg/l). Gibberellic acid and 2,4-D did not increase this activity appreciably.
E. G. BRICKELL.

Control of flowering of *Xanthium pensylvanicum* by red and far-red light. H. B. Reid, P. H. Moore and K. C. Hamner (*Plant Physiol.*, 1967, **42**, 532–540).—In short dark periods, far-red light alone did not greatly affect flowering but was able to overcome the inhibition of flowering caused by red light. In periods >15 h, far-red inhibited flowering and added to rather than overcame the inhibition by red light. E. G. BRICKELL.

***Proserpinaca*: photoperiodic and chemical differential of leaf development and flowering.** G. J. Davis (*Plant Physiol.*, 1967, **42**, 667–668).—Treatment of plants of *Proserpinaca palustris* L. growing on 8- and 14-h photoperiods with gibberellic acid caused stem elongation and inhibited flowering. E. G. BRICKELL.

Flowering responses of *Xanthium pensylvanicum* to long dark periods. P. H. More, H. B. Reid and K. C. Hamner (*Plant Physiol.*, 1967, **42**, 503–509).—Factors such as plant height (age), partial defoliation, and various pretreatments had little effect on the general nature of the flowering response. Max. sensitivity to red light occurred at the 8th h of 24-, 48-, and 71-h dark periods, temp. having little effect on this timing. Max. sensitivity was delayed to the 10th h by a pre-treatment with 8 h of darkness followed by 6 h of light, thus showing a similarity to *Pharbitis*. E. G. BRICKELL.

Partial purification of a legume nodulation factor present in coconut water. A. C. Schaffer and M. Alexander (*Plant Physiol.*, 1967, **42**, 563–567).—An approx. 70-fold purification (on a dry wt. basis) was obtained using activated C but at least 10 different compounds were present in the active fractions. E. G. BRICKELL.

Assay of substances stimulatory to legume nodule formation. A. G. Schaffer and M. Alexander (*Plant Physiol.*, 1967, **42**, 557–562).—Improved methods for the bioassay of stimulatory substances in coconut water, extracts of cotyledons, hypocotyls and leaves of beans, and of horse chestnut fruits, are reported.
E. G. BRICKELL.

Correlative studies on plant growth and metabolism. III. Metabolic changes accompanying inhibition of the longitudinal growth of stem and root by kinetin. D. Banerji and M. M. Laloraya (*Plant Physiol.*, 1967, **42**, 623–627).—Kinetin-induced expansion of lettuce (*Lactuca sativa*) cotyledons and inhibition of root are accompanied by parallel changes in protein N. In seedlings kinetin restricted the mobilisation of N reserves from cotyledons and the induced growth was accompanied by a high protein N/sol. N ratio similar to that of growth in light. E. G. BRICKELL.

Translocation of radioactive kinetin. H. B. Lagerstedt and R. G. Langston (*Plant Physiol.*, 1967, **42**, 611–622).—Radioactivity from kinetin-8-^{14}C moved freely in the vascular system of several types of leaves, usually distal to the point of application. Basipetal as well as acropetal translocation was achieved in tobacco leaves. Root treatments showed that cotton seedlings did not take up radioactive kinetin but that similarly treated tobacco seedlings both absorbed and translocated the isotope readily. E. G. BRICKELL.

Isolation and identification of indole-3-ethanol (tryptophol) from cucumber seedlings. D. L. Rayle and W. K. Purves (*Plant Physiol.*, 1967, **42**, 520–524).—Purification of crude ether extracts of green shoots of *Cucumis sativus* L. by DEAE cellulose, silicic acid, and Mg silicate chromatography followed by gel filtration and preparative thin layer chromatography, is described. Identification of the growth regulator indole-3-ethanol was achieved by mass spectrometry, thin layer and gas chromatography, u.v. and visible spectroscopy, and by physiological characteristics.
E. G. BRICKELL.

Isolation, purification, and characterisation of an endogenous root-promoting factor obtained from basal sections of pear hardwood cuttings. M. S. Fadl and H. T. Hartmann (*Plant Physiol.*, 1967, **42**, 541–549).—Paper chromatographic studies, tests with spray reagents, solubility determinations, biological tests, u.v. spectrum analysis, and i.r. spectroscopy suggest that a rooting factor extracted from basal segments of Old Home pear cuttings, could be a condensation product between exogenous auxin (indolebutyric acid) and a phenolic compound produced by the physiologically active buds. E. G. BRICKELL.

Growth-inhibiting substance and bud dormancy in woody plants. M. Kawase (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 752–757).—A growth-inhibiting substance(s), extracted with CH_3OH was identified in the buds of four woody species by the oat coleoptile elongation method. The concn. of growth-inhibiting substance was at a max. in buds during the winter season, but declined thereafter towards spring, and was absent at bud-break.
A. H. CORNFIELD.

Distribution of an indoleacetic acid-oxidase-inhibitor in the storage root of *Daucus carota*. B. S. Jacobson and S. M. Caplin (*Plant Physiol.*, 1967, **42**, 578–584).—In the phloem a high IAA-oxidase activity was distributed uniformly along the radius but in the xylem a somewhat lower concn. decreased from the cambium. IAA-oxidase inhibitor in the phloem increased exponentially from a very low concn. near the cambium whereas in the xylem an appreciable concn. was present near the cambium, decreasing linearly with distance from the cambium. E. G. BRICKELL.

Kinetics of growth retardant and hormone interactions in affecting cucumber hypocotyl elongation. T. C. Moore (*Plant Physiol.*, 1967, **42**, 677–684).—One μg of GA_3 (gibberellin A_3) applied to the short tip completely nullified the effect of 10 μg of Amo-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride) or 25 μg of B-995 (*N,N*-dimethylaminosuccinamic acid) applied simultaneously; 10 μg of GA_3 counteracted the effect of 10^{-3} M-CCC (2-chloroethyltrimethylammonium chloride) added to the root medium. One μg of IAA countered the effect of 10^{-3} M-CCC in the root medium but did not nullify either Amo-1618 or B-995. Experiments with two growth retardants indicate that Amo-1618 and CCC inhibit a common process, namely GA biosynthesis but B-995 affects a different process.
E. G. BRICKELL.

Possible rôle of hydroxyproline-containing proteins in the cessation of cell elongation. R. Cleland and A. M. Karlsnes (*Plant Physiol.*, 1967, **42**, 669–671).—Hydroxyproline-containing proteins increase markedly in the cell walls of Alaska pea epicotyl maturation and may be a factor in the cessation of cell elongation.
E. G. BRICKELL.

Foliar analysis. I. Sampling techniques for citrus. Lemon trees. O. Carpena, E. Monllor and E. Hellin (*Revta. Agroquím. Technol. Aliment*, 1967, **6**, 459–467).—The results of determinations of N, P, K, Na, Ca, Mg, Fe and Mn in leaf samples covering a range of sizes of sample collected from individual trees or plots of lemon trees (varieties Verna and Primofiore) are reported. Statistical analyses of the data show that 15 leaves are sufficient to constitute a representative sample from one tree, and that samples from 15 or 5 trees per 100 of the variety Verna or 11 or 4 per 100 of Primofiore are sufficient to represent the whole plot with error <10% or 20% respectively ($P = 0·95$). (12 references.) E. C. APLING.

Crops and Cropping

Complete formulation of nutrients for sandy or decalcified soils. G. Escande, L. Genevois, and D. Bertrand (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 903–905).—Under natural conditions rain is the sole source of nutrient for certain soils south of Bordeaux. A formula is given for a dil. nutrient solution for watering such soils for the cultivation of maize. P. S. ARUP.

Chemical fallow in dryland cropping sequences. A. F. Wiese, E. Burnett, and J. E. Box, jun. (*Agron. J.*, 1967, **59**, 175–177).—In continuous crops of dryland sorghum, propazine, [2-chloro-4,6-bis

(isopropylamino)-*s*-triazine], 2·2 kg per hectare eliminated one tillage and one cultivation operation during the crop year. At least one tillage operation was required to control volunteer sorghum before planting. In a wheat–sorghum–fallow cropping sequence propazine applied immediately after wheat eliminated three tillage operations during the fallow period after wheat and one cultivation in the sorghum crop. Diuron [3-(3,4-dichlorophenyl)1, 1-dimethyl-urea], 1·1 kg per hectare applied in March to sorghum stubble eliminated cultivation in cotton crops for 2 of 4 years.
A. H. CORNFIELD.

Increase of protein production by cereals. M. J. Dufrenoy (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 906–907).—Statistical analyses are presented of results obtained by Chrominski and by the author concerning increases in protein yields obtainable by the use of chlorocholine with increased applications of N-fertiliser.
P. S. ARUP.

Polyethylene mulches for sweet maize in northern latitudes. D. H. Dinkel (*Proc. Amer. Soc. hort. Sci.*, 1966, **89**, 497–504).—In Alaska a soil surface covering of clear polyethylene (0·0015 in.) raised soil temp. and advanced germination and growth of sweet maize sufficiently to produce marketable yields. Black polyethylene, black paper, and petroleum mulches were inferior to clear polyethylene. A. H. CORNFIELD.

Effect of germination temperature on surface area and carbon dioxide production of maize seedlings. E. R. Perrier, M. Twersky, D. B. Peters, and V. Gylys (*Agron. J.*, 1967, **59**, 133–136).—Growth rate (at 25° in the dark), as measured by surface area and CO_2 respired, of maize seedlings which had been germinated at 29° was higher than that of seedlings which had been germinated at 25°. The flux of CO_2 declined with time and the rate of decline increased with the temp. of germination. A. H. CORNFIELD.

Manganese and silicon interaction in the *Gramineae*. J. Vlamis and D. E. Williams (*Plant & Soil*, 1967, **27**, 131–140).—In nutrient solution studies dry matter yields increased with level of Mn in the nutrient up to 0·1–0·2 ppm and then declined with further increasing Mn level for oats, barley, rye, wheat, and ryegrass. Rice yield was hardly affected even by 5 ppm Mn in the nutrient. The presence of 10 ppm silicate-Si in the nutrient increased the yields in all species except rice and ryegrass, but had little effect on Mn uptake. The Mn% of the leaves of rice was considerably higher than that of the other species. Root-Mn% was higher than shoot Mn% in all species except rice, where the reverse was true. Toxicity symptoms (necrotic brown spots) which appeared on old leaves of barley, rice, rye and ryegrass were prevented by addition of Si to the nutrient.
A. H. CORNFIELD.

Results obtained by nitrogen fertilisation of maize. L. Soubies and M. Lenain (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 922–926).—The efficacy of NH_4^+-N or urea-N in increasing yields of maize is greatly improved by placement at 15–20 cm below the surface. Machinery for the economic placement of solid or liquid fertilisers in this way has recently become available.
P. S. ARUP.

Relative efficiency of zinc sources for maize. U. C. Shukla and H. D. Morris (*Agron. J.*, 1967, **59**, 200–202).—Maize growth on a sandy soil (which produced Zn deficiency symptoms in maize in the field) in pot tests was hardly affected by Zn added as various sources. In a loamy sand yields were increased up to 2·5-fold. Org. (polyflavonoid) Zn was not as effective as $ZnSO_4$, ZnO or Zn-chelate in increasing yields. Liming reduced plant growth and Zn uptake, especially when no Zn was applied. High P fertilisation reduced plant Zn content, but tended to increase growth when applied with Zn. A. H. CORNFIELD.

Growth and phosphorus uptake by maize as influenced by phosphorus placement. K. P. Garg and L. F. Welch (*Agron. J.*, 1967, **59**, 152–154).—In greenhouse tests with a silt loam (pH 6·2) with $Ca(H_2PO_4)_2$ mixed with the soil, banded beneath the seed or placed in contact with the seed emergence was not affected by any of the treatments. Yields of forage 21 or 28 days after planting were greater where P was placed in contact with the seed than where the two other methods of placement were used. P% in the forage was greater with seed placement than with the two other methods of placement, but the differences decreased with length of harvest (14–21 days after planting). Total uptake of P was greater with seed than with the two other types of placement at all harvest dates. Mixed placement was superior to banding with early harvesting, but the reverse was true with late harvesting.
A. H. CORNFIELD.

Influence of algal growth-promoting substances on growth, yield and protein contents of rice plants. A. C. Shukla and A. B. Gupta (*Nature, Lond.*, 1967, **213**, 744).—Rice seeds were soaked for 24 h in 0·5, 1 and 5% water and ether extract of *Phormidium foveolarum* suspended in water. The growth, crop yield and protein content of plants grown from these seeds were measured. The height of the plant, the no. of tillers, the no. and size of leaves, the total wt. of all ears and the protein content of seeds all increased in comparison with control plants. S. A. BROOKS.

Effect of stage of maturity and postharvest conditions on sugar conversion and chip quality of potato tubers. M. Yamaguchi, H. Timm, M. D. Clegg, and F. D. Howard (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 456–463).—Tubers of two varieties harvested 89 days after planting were higher in sucrose% and glucose% than tubers harvested 103–117 days after planting. Fructose% was <0·02% in tubers from all harvests. Glucose% was higher in White Rose than in Kennebec. In both varieties sucrose% decreased with time of storage at 25° or lower. At 30° there were either no changes or slight increases. Sucrose% increased significantly after 4 days storage at 33°, and after 2 weeks there was a 2·5–4-fold increase. Chips made from these tubers were darker than those from tubers stored at lower temp. A. H. CORNFIELD.

Field experiments on the fertiliser requirements of maincrop potatoes. J. A. Birch, J. R. Devine, M. R. J. Holmes and J. D. Whitear (*J. agric. Sci., Camb.*, 1967, **69**, 13–24).—The N requirement of a potato crop depends on the previous cropping history and on the rainfall of the preceding winter. Yields are usually increased by P fertilisers on soils of low citric-sol. P content. Optimum rates of P application are at least 120 lb of P_2O_5/acre, irrespective of the soil P status. Yield increases by K and the optimum rate of application are both related to the citric-sol. K status. Recommended levels are 50–110 lb of K_2O acre on soils with >200 ppm citric-sol. K_2O, 160–200 lb with 70–200 ppm and about 240 lb with <70 ppm. N increases the yield of large more than that of medium tubers, K only increases the large tuber yield, whilst P increases the medium tuber yield most. Neither nutrient has any marked effect on small tuber yield. K often depresses dry matter content and N sometimes affects texture and flavour, although both these effects are slight compared with those of climate, season and soil
M. LONG.

Grassland systems. W. Holmes and G. Allanson (*J. Br. Grassld Soc.*, 1967, **22**, 77–84).—A review is given of the merits of various intensive grassland systems ranging from improved versions of conventional techniques to highly capitalised mechanical grazing and storage systems. A. H. CORNFIELD.

Nitrate accumulation in forage crops. L. S. Murphy (*Diss. Abstr.* B, 1966, **27**, 1686–1687).—Factors influencing the accumulation of NO_3^- in numerous species and varieties of forage crops are examined. The capacity for accumulating NO_3^- differs widely between species and also between similar varieties. In general the N content of individual forages declines as maturity is approached; it is directly related to the amount of N fertiliser applied and often lowered by use of P and K fertilisers although no consistent relationships are established. In Sudan grass an inverse relationship exists between the P content and rate of application of N fertiliser but in further investigations no association between the NO_3^--N content and that of pyridine nucleotides was found. The concn. of NO_3^- and CN^- in the grass responded similarly to N fertilisers but there was no evidence of a metabolic relationship between the two forms of N. A. G. POLLARD.

***Ophiobolus* patch disease in turfgrass as related to soil fertility level.** R. L. Goss and C. J. Gould (*Agron. J.*, 1967, **59**, 149–151).—A year after application of N, *Ophiobolus* patch disease was more severe where 9·8 kg N than where 2·9 kg N per hectare or no N had been applied. Three years later the disease was most severe where 2·9 kg N had been applied. Application of P or K suppressed the severity of the disease, the effect of P taking longer to develop than that of K. A. H. CORNFIELD.

Growth, water use and nutrient uptake from the subsoil by grass swards. E. A. Garwood and T. E. Williams (*J. agric. Sci., Camb.*, 1967, **69**, 125–130).—No response of perennial ryegrass to surface-applied N occurred when the surface soil was dry and a soil water deficit of 2 in. existed. Injection of N at a depth of 18 in. into moist soil was effective and a marked interaction between N and PK was found. Of the N applied to the subsoil 59–90% was recovered, provided water was available in the horizon concerned. Failure of a sward to regrow when water had been removed from the top horizons was largely due to deficiency of nutrients, especially of N. M. LONG.

Influence of lime sources and rates on Coastal Bermuda-grass production and exchangeable calcium and magnesium. W. E.

Adams, A. W. White, jun. and R. N. Dawson (*Agron. J.*, 1967, **59**, 147–149).—Forage production over 5 years on a sandy loam was increased by adding sufficient limestone to increase soil pH to 4·8, but was not increased further by adding more limestone. Calcitic and dolomitic limestone had very similar effects. Without application of liming materials exchangeable Ca, particularly, and Mg fell to low levels in the upper 30–45 cm soil depths. Depletion of both Ca and Mg was largely prevented by application of 20 tons limestone per ha. A. H. CORNFIELD.

Effect of clipping height on forage distribution and regrowth of Pensacola Bahiagrass. R. L. Stanley, E. R. Beaty and J. D. Powell (*Agron. J.*, 1967, **59**, 185–186).—In the greenhouse application of N (84–356 kg per hectare) to Bahiagrass increased the % of forage which grew above 5 cm in height. Regrowth of forage was higher when clipped at ⩾5 cm in height than when allowed to grow to greater heights. Extent of regrowth increased with level of applied N. A. H. CORNFIELD.

Nitrate accumulation in forage crops. L. S. Murphy and G. E. Smith (*Agron. J.*, 1967, **59**, 171–174).—Of the species studied Sudan-grass, orchard-grass and tall fescue accumulated NO_3^- to the greatest extent, bromegrass, timothy and ladino clover to intermediate extents, whilst lucerne and wheat accumulated little NO_3^-. Increasing N fertilisation increased NO_3^- accumulation, particularly early in the growing season. P fertilisation had little effect on the extent of NO_3^- accumulation. NO_3^- content of most species decreased with advancing maturity. A. H. CORNFIELD.

Evaluation of tall fescue pasture under different fertilisation treatments. R. L. Reid, E. K. Odhuba and G. A. Jung (*Agron. J.*, 1967, **59**, 265–271).—Fertilisers (N, P, K or N + P) had little effect on *ad lib* intake of tall fescue herbage by sheep. Intake declined with advancing maturity in the first growth herbage, but not with regrowth herbage. Dry matter digestibility decreased from first cutting to regrowth, but was not affected by fertiliser treatments. Cutting date had no effect on intake within first growth and regrowth trials, but herbage fertilised with high levels of N or N + P was consumed in greater amount than that fertilised with P or K. The animals showed a marked preference for fescue fertilised with N or N + P. A. H. CORNFIELD.

Tannin content of the herbage of crown vetch *Coronilla varia*. R. E. Burns, P. R. Henson and D. G. Cummins (*Agron. J.*, 1967, **59**, 284–285).—The tannin content of three varieties of crown vetch ranged from 3·2 to 3·8%, about half that found in sericea. The astringent fraction was approx. 30% of that found in sericea. Leuco-cyanidin and leuco-delphinidin were present in equal amounts. It is unlikely that the small amount of tannin present, especially of the astringent fraction, would affect forage quality of crown vetch. A. H. CORNFIELD.

Leys and soil organic matter. II. Accumulation of nitrogen in soils under different leys. C. R. Clement and T. E. Williams. (*J. agric. Sci., Camb.*, 1967, **69**, 133–138).—Under a ryegrass white clover sward the average annual increase of N was 0·005% per year to a depth of 15 cm. Without clover or N fertiliser the increase of N amounted to 0·017% over three years in the top 7·5 cm. Where clover existed in the sward the N increase in 3 years was, in two trials, 0·026% and 0·014% down to 15 cm. Whilst grass species had little effect on N accumulation, where clover was not present, a ryegrass/clover sward showed greater increases of N than did a cocksfoot/clover sward. N applications up to 314 kg/ha per year had no effect on N accumulation. Grazing effects differed from those of cutting and removing pasture. Most of the increase in soil N was found in the top 2 cm of soil. M. LONG.

Effect of sawdust on the germination and seedling growth of several turfgrasses. D. V. Waddington, W. C. Lincoln, jun. and J. Troll (*Agron. J.*, 1967, **59**, 137–139).—Fresh sawdust, particularly that from ash and red oak, was toxic to germination of several turfgrass species. Abnormal seedlings with stunted roots occurred when seed was germinated in water extracts of these sawdusts. N-fertilisation did not overcome the toxic effects. There were differences due to grass species in susceptibility to the toxic effects of sawdust. The toxic effects disappeared in sawdust which had been allowed to weather for 2–7 months. A. H. CORNFIELD.

Comparison of Coastal and common Bermuda-grass in the Piedmont Region. II. Effect of fertilisation and crimson clover on nitrogen, phosphorus and potassium content of the forage. W. E. Adams, M. Stelly, H. D. Morris and C. B. Elkins (*Agron. J.*, 1967, **59**, 281–284).—Common Bermuda-grass forage grown with and without crimson clover contained significantly higher % of N, P, and K than did Coastal Bermuda-grass similarly fertilised. Coastal Bermuda-grass recovered more of the applied N, P, and K than did common Bermuda-grass, presumably because of the higher yields given by the former. The P and K contents of crimson clover grown with the grasses were increased by fertilisation. The K% of crimson clover grown with common Bermuda-grass was greater than that of clover grown with Coastal Bermuda-grass. A. H. CORNFIELD.

Effect of boron on seed production by white clover, *Trifolium repens*. W. C. Johnson and J. I. Wear (*Agron. J.*, 1967, **59**, 205–206).—The addition of B (560 g per hectare) to a coarse-textured soil (water-sol. B content 0·1 ppm) increased seed yields from white clover, but had no effect on forage yields. Higher applications of B did not increase seed yields further. Zn, Cu, Mn, and Mo applied with B did not increase yields above those given by B alone. A. H. CORNFIELD.

Relationship of tillage and fertilisation to the yield of lucerne on Freeman silt loam. E. E. Cary, G. M. Hormer and S. J. Mech (*Agron. J.*, 1967, **59**, 165–168).—Tillage and fertilisation studies were made with a view to improving the performance of lucerne on an acid silt loam developed under coniferous forest. Chlorotic and spindly symptoms often appear late in spring. Liming and fertilisation with P and S was the most effective combination of treatments and results were not improved by adding inorg. N. Subsoiling to a depth of 122 cm combined with some mixing of top and sub-soil improved further the performance of lucerne. A. H. CORNFIELD.

Influence of freezing during dormancy on the cultivated strawberry (*Fragaria Virginiana chiloensis*) cultivar Catskill. B. R. Boyne (*Diss. Abstr.* B, 1966, **27**, 1685–1686).—Under field conditions injury to the crowns of the plants due to freezing appeared unlikely to be caused by high rates of freezing or thawing. Thawing at the rate of 6°F/min. caused some injury but within the range 1–18°F/h did not add injury to that already caused by low-temp. freezing. Freezing at low temp. for long periods or repeated freezing and thawing intensified injury. Plants dug in Nov (New Jersey, U.S.A.) and stored at 33°F were injured at 28°F and killed at 16°F. Injury was shown by the amount of electrolytes leached from crown sections and was reflected in the size of leaves, no. of runners and no. of flowers subsequently produced. Dormant plants removed from storage began to lose their hardiness within 2 days of being placed in a greenhouse at temp. suitable for growth; after 10 days the plants were injured by transfer to temp. of 24°F as much as when taken directly from storage and exposed to 16°F. Field plants, covered with snow during most of the winter, showed no differencies in injury, whether or not they were mulched, whereas in a much milder winter unmulched plants showed more injury than did mulched controls. A. G. POLLARD.

Relationship between quality and composition of six tomato varieties. P. A. Simandle, J. L. Brogdon, J. P. Sweeney, E. O. Mobley and D. W. Davis (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 532–538).—Fruits of two yellow varieties were higher in acidity than were those of four red varieties. All varieties contained galacturonic acid, not previously reported in fresh tomatoes. Varieties rated low by a panel for intensity of natural flavour also had lower amounts of citric acid, fructose, titrable acids, sol. solids, and sol. solids/titrable acids ratios than did varieties rated high. Panel flavour scores were significantly correlated with sol. solids, pH, and sol. solids/titrable acids ratio. Panel texture scores agreed well with firmness as determined by shear-press curve areas. A. H. CORNFIELD.

Changes in the concentration of carotenes of ripening tomatoes. F. I. Meredith and A. E. Purcell (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 544–548).—The concn. of phytoene, phytofluene, ζ-carotene, γ-carotene, and lycopene (fresh wt. basis) increased in tomato fruit through the six maturity stages of the U.S.D.A. classification standards of tomato maturity. The concn. of α-carotene and β-carotene increased up to the pink to light red stages and then declined slightly. A. H. CORNFIELD.

Effects of damage to tomato plants on development and fruit yields. G. E. Wilcox, S. Kristof and R. Baker (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 571–576).—Varying degrees of defoliation and stem damage were made on tomato plants at different stages of growth. Damage done up to 6 weeks after field planting had little effect on yields. Damage applied later reduced yields in proportion to the amount of damage. Injury and breakage of stems did not affect yields as much as did defoliation. Regeneration of damaged plants occurred rapidly, but late damage resulted in a delay in fruit development and insufficient time for the crop to ripen. An

impact to the tomato fruit resulted in bruising of inner and outer tissue, disappearance of lycopene, and disruption of inner cells. A. H. CORNFIELD.

Effects of herbicides on composition of tomato plants. R. B. Taylorson (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 539–543).—Tomato plants were sampled from field plots that had received pre-planting treatments of vernolate (*S*-propyl dipropylthiocarbamate, 1 lb per acre), pebulate (*S*-propyl butylethylthiocarbamate, 2–4 lb), and pre-emergence treatments of diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide, 2·5–10 lb) and amiben (3-amino-2,5-dichlorobenzoic acid, 3 lb). The highest levels of pebulate, diphenamid, and amiben increased reducing and total sugars in roots, stems and leaves. The two thiocarbamate weedicides caused increased N in the tissues. Tissue P% was not affected by any of the treatments. A. H. CORNFIELD.

Sampling variation of acidity and solids in tomatoes. R. L. Lower and A. E. Thompson (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 512–522).—There were highly significant variations due to variety, sampling date and sampling method in pH, titrable acidity, sol. and total solids, sol. solids/acid ratio, fruit wt. and % locular tissue of tomato fruits of two high-acid breeding lines and four standard varieties of tomato collected at different dates. Field-ripened fruit gave essentially the same results as chamber-ripened fruit. A. H. CORNFIELD.

Fibre development in snap bean (*Phaseolus vulgaris*, L., Cv. Wade) as influenced by *N*-dimethylamino succinamic acid sprays and moisture stress. A. E. Nightingale (*Diss. Abstr.* B, 1967, **27**, 2568).—Greenhouse-grown snap bean plants were sprayed with the growth-retardant (*DMAS*) in various concn. at different stages of growth and with different levels of control of soil moisture, from the time when buds appeared on the first inflorescence. The fibre content of the beans was lowered significantly by *DMAS* at 1000 ppm but was not affected by the time of application of the spray or by soil moisture levels. A. G. POLLARD.

Water relations and growth of beans as influenced by nutrient solution temperatures. P. W. Unger and R. E. Danielson (*Agron. J.*, 1967, **59**, 143–146).—When bean plants were grown in nutrient solutions with temp. ranging from 10° to 32·5° plant water stress occurred at 15° and lower temp. Leaf sugar% (dry wt. basis) increased with time for all temp. treatments, but the greatest increase occurred at the lowest temp. Water use efficiency increased with decreasing solution temp., the increases being greater for tops than for roots and greater for dry wt. than for fresh wt. A. H. CORNFIELD.

Magnesium nutrition of spinach. H. E. Hohlt and D. N. Maynard (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 478–482).—Of various plant parts tested mature leaf lamina tissue provided the most accurate estimate of the Mg status of the spinach plant. Increasing Mg% was accompanied by decreasing Ca% and K%. When mature leaf lamina Mg was less than 0·17% (dry basis) a chlorosis developed which subsequently became necrotic. There were differences between spinach cultivars in their ability to accumulate Mg with high Mg supply. High K supply reduced Mg uptake where Mg supply was high. A. H. CORNFIELD.

Rhubarb petiole colour and forced production as influenced by gibberellin, sucrose, and temperature. D. R. Tompkins (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 472–477).—Rhubarb crowns sprayed with 250 ppm gibberellic acid produced higher earlier and total yields of petioles, with colour as good as that of control crowns forced at 13·3°. When forced at 18·3° treated crowns had the same total yield as controls, but during the latter part of the season yields were less and petiole colour poorer than controls. Crowns which had been treated with sucrose (eight applications of 1·2% sucrose over 1 month) and forced at 13·3° or 18·3° produced higher late yields which were better coloured (darker) than controls. A. H. CORNFIELD.

Effects of injection of nutrient solutions into composts on the yield of mushrooms. J. P. San Antonio (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 415–422).—Injection of casein hydrolysate into mushroom composts either before or after casing sometimes increased mushroom yields, whilst injection of vitamins, N-H_2SO_4, and glucose had no effect. A. H. CORNFIELD.

Effects of chemicals on vase-life and spike characteristics of cut snap-dragons. F. E. Larson and J. F. Scholes (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 694–701).—Compared with tap water, vase-life of cut snapdragons was increased 2–3-fold by 300 ppm 8-hydroxyquinoline citrate, 10–50 ppm *N*-dimethylamino succinamic acid (Alar), and 1·5% sucrose. Mean increase of florets was 3·3 times and that of spike length 4·2 times that of water control. Natural floret colour was maintained on spikes treated with these solutions, whereas in water little pigment developed in florets which opened after cutting. A. H. CORNFIELD.

Classification of carnation cultivars according to foliar nutrient content. P. V. Nelson and J. W. Boodley (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 620–625).—Leaf N, P, K, Ca, and Mg levels (dry basis) of 16 carnation cultivars differed significantly when grown under the same cultural conditions. The 16 cultivars could be placed in three groups on the basis of differential nutrient accumulation, indicating that three sets of leaf tissue nutrient standards may be necessary for diagnostic purposes. A. H. CORNFIELD.

Anthocyanins and flavonol glycosides of magnolia flowers. F. J. Francis and J. B. Harborne (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 657–665).—Six anthocyanins and 4 flavonol glycosides were isolated and identified in the flowers of 7 species of magnolia. The presence of anthocyanins of relatively complex structure indicate that the Magnoliaceae is one of the more highly developed families in the primitive angiosperm group. A. H. CORNFIELD.

Hydroponics as a medium for production of tree planting stock. S. A. Wilde and D. E. Spyridakis (*Agron. J.*, 1967, **59**, 275–278).—The technique of raising tree planting stock in nutrient solutions and the results of the field performance of these stocks over 20 years are described. A. H. CORNFIELD.

Effects of nitrogen, phosphorus, and potassium on growth and chemical composition of *Taxus media*. (F. R. Gouin and C. B. Link (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 702–705).—When rooted cuttings of *Taxus media* were grown in spaghnum peat moss–sand mixture + $CaCO_3$ receiving nutrient solutions with varying combinations and levels of N (112–448 ppm), P (25–225 ppm), and K (45–405 ppm) the best growth was obtained with 224 ppm N + 75 ppm P + 135 ppm K. The highest level of N reduced growth more than did the highest levels of P or K. High N reduced plant K% but not plant P%. High P or K supply did not reduce plant content of the two other nutrients. A. H. CORNFIELD.

Leaching of metabolites from cuttings propagated under intermittent mist. G. L. Good and H. B. Tukey, jun. (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 727–733).—Cuttings of several ornamental plants were rooted in quartz sand under an intermittent distilled water mist. Levels of N, P, K, Ca, Mg and sol. carbohydrates were determined in the cuttings before and after rooting. Herbaceous, softwood and semi-hardwood cuttings grew substantially during rooting and lost small amounts of metabolites by leaching during the rooting period. Hardwood cuttings of mature tissue grew less during rooting and lost greater quantities of metabolites by leaching than did softwood cuttings. A. H. CORNFIELD.

Leaf roll-necrosis complex of lilacs in an urban environment. C. R. Hibben and J. T. Walker (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 636–642).—Lilacs in metropolitan New York developed a foliar disorder characterised by leaf roll, intervenal and marginal necrosis, chlorosis, bronzing and early leaf abscission that was sometimes followed by a break in dormancy of terminal buds. The disorder was not due to lack of major or trace elements, fungal or bacterial pathogens, or nematode damage. When cuttings from affected plants were grown in a rural environment no symptoms appeared. Air pollutant(s) possibly O_3, may be responsible, since, when lilacs were grown in city air filtered through activated charcoal, virtually no symptoms appeared. A. H. CORNFIELD.

Effect of phosphorus and nitrogen placement on safflower growth and phosphorus absorption. C. H. E. Werkhoven and F. Massantini (*Agron. J.*, 1967, **59**, 169–171).—Banding of $Ca(H_2PO_4)_2$ (28–56 kg P per hectare) 2·5 cm below the seed was more effective in increasing yields of safflower and utilisation of applied P than was broadcasting. Banding 28 kg P was as effective as broadcasting 56 kg. N placement (112 kg per hectare as NH_4NO_3) was not as important as P placement. A. H. CORNFIELD.

Influence of nitrogen and potassium on the contents of malate, citrate, and malonate in non-nodulating soya-beans. H. E. Pattee and M. R. Teel (*Agron. J.*, 1967, **59**, 187–189).—The malate content decreased when K was applied without N, but increased when it was applied with N. Citrate was reduced by applying K, irrespective of N level. Malonate was decreased in N-deficient plants when K was supplied. The sum of the three acids accounted for about 72% of the total acidity, irrespective of fertiliser treatment. A. H. CORNFIELD.

Variations in germination, microflora and fatty acids of soya-beans stored under different combinations of moisture content, temperature and time. C. E. Dorworth (*Diss. Abstr.* B, 1967, **27**, 2569).—For

routine determination of the moisture content of soya-beans, the two-stage air-oven method gave more satisfactory results than did the Karl Fischer method. In beans stored for 130 days at 9·0–12·6% moisture content no *Aspergillus*, spp. were found, but acidity increased markedly. Samples stored at higher moisture contents (12·6–18·3%) and at various temp. (15–30°) showed an increasing growth of the *A. glaucus* group with advancing time and with increasing water content and/or temp. *Penicillium* spp. also increased during storage, infecting 100% of the seeds stored at 18·3% moisture content. All other fungi decreased almost to zero over the experimental period, at rates which increased with temp. but not with increase in water content. The % germination of the seeds diminished with increase in moisture content, temp. and storage period. The % of palmitic, stearic, oleic and linoleic acid in the beans showed small but significant differences according to storage conditions. Evidence obtained suggests that under certain conditions of storage of the seed, *A. flavus* could invade the resulting seedlings. A. G. POLLARD.

Resistance of *Arachis* to drought. II. Test of relative rapidity of growth. J. Gautreau, P. Prevot, M. Ollagnier and P. Gillier (*C.r. hebd. Séanc. Acad. Agric., Fr.*, 1967, **53**, 908–914).—Further to earlier work (*cf. ibid.*, 1967, **53**, 555) tests were carried out on 46 varieties of 15 to 20-day-old plants of different varieties by exposure to 61° for 1 h in a moisture-saturated atm. Resistance was assessed by the degree of leaf-damage and the capacity for recovery. Field tests were carried out by excluding rain by means of coverings. Three varieties were finally selected as showing max. resistance to drought compatible with high yields. P. S. ARUP.

Effect of boron nutrition on growth and protein and nucleic acid metabolism in groundnut plants. E. W. Stoller (*Diss. Abstr.* B, 1966, **27**, 1697).—In groundnut plants grown in controlled environmental conditions and with similar proportions of B in the substrate, the occurrence of B deficiency was intensified by either long days or high light intensity. Plants showing deficiency symptoms yielded more dry matter per unit B content under short-day conditions. Toxic effects were apparent in plants containing B >100 ppm. Between deficiency and toxicity limits, dry matter production was almost a linear function of light energy; at levels causing deficiency symptoms dry matter was greater in plants receiving less than max. light. The ratios, deoxyribonucleic (**I**): nucleotide and ribonucleic acid (**II**): nucleotide, were greater in terminal buds of deficient plants grown under short than when under long-day conditions. Incorporation of glutamate-C into protein and nucleic acid in excised roots showed the rate of synthesis of nucleic acid to be affected by B deficiency at an earlier stage than was the synthesis of protein. Exposure of excised roots to $^{32}PO_4^{3-}$ for 30 sec. and then to $^{31}PO_4^{3-}$ for 90 min. caused the appearance of radioactivity in inorg., org., nucleotide, **I, II** and lipid P fractions within 1 min. of the initial exposure. The first effect of B deficiency was an increased nucleotide content.
A. G. POLLARD.

Some effects of curing and length of storage on fatty acids in periderm tissue of the sweet potato (*Ipomoea batatas*, Poir). D. M. Alford (*Diss. Abstr.* B, 1967, **27**, 2564–2565).—The periderm tissues of two varieties, Centennial (*C*) and Goldrush (*G*) were examined. The contents of total solids and of total lipids were greater in *C*, as also was the O_2-consumption rate but the R.Q. was much lower. The fatty acid distribution in the tissues of the two varieties was similar, the general (descending) order of concn. being oleate, (**I**), stearate, (**II**), linoleate, (**III**), myristate, (**IV**), laurate, (**V**), palmitate, (**VI**), caprate (**VII**), and linolenate (**VIII**). Only **I, II** and **III** occurred regularly in both species. The *C* tissue contained more of each fatty acid (wt. basis) than did that of *G*, on the basis of % of total fatty acids present **I** and **II** were higher in *G*. After curing and storage for 20 days the shorter-chain acids (**IV, V, VI** and **VII**) increased rapidly: the longer-chain acids also increased but less abruptly. A. G. POLLARD.

Pest Control

Honey bee insecticide loss: an unusual case. R. A. Morse and A. F. Gunnison (*J. econ. Ent.*, 1967, **60**, 1196–1198).—Some loss of adult bees was recorded 3·25 miles from an area sprayed with 1 lb carbaryl/acre. C. M. HARDWICK.

Improvements of mechanical methods for transmission of virus by adsorption of infection-inhibitors on vegetable charcoal. J. Marrou (*C.r. hebd. Séanc. Acad. Agric., Fr.*, 1967, **53**, 972–981).—Several examples are described of the sucessful use of vegetable charcoal (free from alkaline matter) for this purpose without affecting the activity of the virus. The charcoal is added to the leaf-extract containing the virus, and the suspension is applied directly to the leaves of the host plant. (Filtration would entail the adsorption of some of the virus). The charcoal adhering to the leaves of the host plant is finally washed away. P. S. ARUP.

Bactericidal action of electrohydraulic shock. S. E. Gilliland (*Diss. Abstr.* B, 1966, **27**, 1713).—The shock treatment was effected by discharging high-voltage electricity (8–15 kV) across an electrode gap below the surface of aq. suspensions of the organisms. The effect was not due to current or heat flow, to mechanical action of shock waves or to chemical action. The treatment, applied to *E. coli*, caused some metabolic injury; it produced oxidative effects which inactivated substances important in metabolism; it also caused loss of activity in lactic dehydrogenase, trypsin, and in the proteolytic system of *Bacillus subtilis*. In the last-named, free -HS groups and reduced diphosphopyridine nucleotide were oxidised. Destruction of adenosine triphosphate resulted from the treatment and was demonstrated by decreased absorption at 260 mμ; similarly absorbent matter in intra cellular contents of *E. coli* were also destroyed. The bactericidal action of the shock treatment is attributed to non-selective oxidation reactions produced by the discharge under water, oxidation being mediated by free radicals produced in the water. A. G. POLLARD.

Effects of radio frequency electrical fields on the metabolism of the yellow mealworm (*Tenebrio molitor*, L.). A. M. A. Abdel-Gawad Kadoum (*Diss. Abstr.* B, 1967, **27**, 2553).—Effects of exposure of the worms to electric fields of radio-frequency, either continuous at 39 mc and 3·5 kv for 5·5 sec., or pulse-modulated at the same frequency using 6·0 kv at the rate of 30 pps and a pulse width of 5 ms, are examined. During the 5 days following exposure treated larvae lost more wt. than did starved or control larvae and respiratory rates were higher than those of controls. The increased O_2, consumption by treated larvae was maintained throughout the fourth day of treatment; the rate of incorporation of labelled amino-acids into protein was also increased. Death of the irradiated insects may result from a change in normal metabolic activity and/or from physical change in the waxy layer of the epicuticle rather than from general internal heating.
A. G. POLLARD.

Autocidal control of codling moth release of males and females sterilised as adults by γ radiation. M. D. Proverbs, J. R. Newton and D. M. Logan (*J. econ. Ent.*, 1967, **60**, 1302–1306).—In laboratory experiments, the use of 48 krad produced max. sterility without affecting the mating behaviour of *Carpocapsa pomonella*. When released in an abandoned orchard, injury at harvest was reduced from 60% to 1·6% of the crop in the first year and 0·31% in the second year. C. M. HARDWICK.

Eradication of *Anopheles pharoensis*, Theobald, by the sterile-male technique using cobalt-60. VI. Sperm activity in males irradiated with the sterilising dose. A. A. Abdel-Malek, A. O. Tantawy and A. M. Wakid (*J. econ. Ent.*, 1967, **60**, 1300–1302).—Irradiation of *A. pharoensis* males with 12,000 R γ-rays sterilised them but did not reduce the competitiveness of their sperm. When mated to unirradiated females, egg hatch decreased. Viability was not restored by delaying matings for 5 days. (12 references.)
C. M. HARDWICK.

Tepa as a chemosterilant for maize earworm, armyworm and granulate cutworm. J. R. Young and J. W. Snow (*J. econ. Ent.*, 1967, **60**, 1426–1430).—When fed a sucrose solution containing 53 μg tepa, male *Heliothis zea*, *Pseudaletia unipuncta* and *Feltia subterranea* were sterilised. Females needed higher dosages, particularly those of *H. zea*. The dosages were not toxic.
C. M. HARDWICK.

Residues of tepa on chemosterilised codling moths. J. C. Maitlen and L. M. McDonough (*J. econ. Ent.*, 1967, **60**, 1391–1393).—*Carpocapsa pomonella* were sterilised with an aerosol giving 4·5 and 22·8 μg per moth. The residues were analysed by thin-layer chromatography. The log concn. of tepa decreased linearly with time giving a 88 and 97% loss respectively, 72 h after treatment.
C. M. HARDWICK.

Sex pheromone of the autumn armyworm moth; isolation, identification, and synthesis. A. A. Sekul and A. N. Sparks (*J. econ. Ent.*, 1967, **60**, 1270–1272).—The sex pherome of female *Spodoptera frugiperda* was identified by infrared spectrometer as cis-9-tetradecen-1-ol acetate and this was synthesised. C. M. HARDWICK.

Use of *Daphnia magna* for the microbioassay of pesticides. I. Development of standardised techniques for rearing *Daphnia* and preparation of dosage-mortality curves for pesticides. D. E. H.

Frear and J. E. Boyd. **II. Comparison of microbioassay with gas chromatography for analysis of pesticide residues in plant residues.** D. E. H. Frear and N. S. Kawar (*J. econ. Ent.*, 1967, **60**, 1228–1236; 1236–1239).—I. The optimum conditions for rearing *Daphnia* to give a homogenous population by asexual reproduction, are described. A standard test medium is described and dosage mortality curves are given for 15 insecticides.

II. Extracts of plants such as lettuce and radish killed *D. magna* after clean-up procedures. Only extracts of carrot tops had low toxicity. Gas–liquid chromatography gave much more consistent and accurate results for DDT, ethion and lindane residues than did bioassay. C. M. HARDWICK.

Ultra-low-volume technical malathion for suppression of an incipient infestation of Japanese beetle. D. W. Hamilton, W. W. Maines, A. J. Coppinger and H. L. Bruer (*J. econ. Ent.*, 1967, **60**, 1480–1481).—The effectiveness of 8 oz/acre sprays was confirmed by the collection of dead beetles and the no. found in bait traps. C. M. HARDWICK.

Insecticidal activity of hexamethylditin (Pennsalt TD-5032) on six species of Noctuidae. K. Harrendorf and R. E. Klutts. (*J. econ. Ent.*, 1967, **60**, 1471–1472).—The mortality produced in six noctuid larvae, 2 and 48 h after topical application of 0·156–2·5 μg per larvae is given. C. M. HARDWICK.

Effect of inhibitors and inducers of microsomal enzymes on toxicity of carbamate insecticides to mice and insects. B. Meksongsee, R. S. Yang and F. E. Guthrie (*J. econ. Ent.*, 1967, **60**, 1469–1471).—The effect of the inhibitor SKF 525-A [2-(diethylamino)ethyl 2,2-diphenyl valerate hydrochloride] on the toxicity of carbaryl, dimetilan and UC 20047A [exo-5-chloro-6-oxo-endo-2-norbornane-carbonitrile *O*-(methylcarbamoyl)oxime] to white mice is described together with the action of the inducers, phenobarbital and DDT, in white mice and insects. (14 references.) C. M. HARDWICK.

Effect of soil clay and organic matter content on systemic efficacy of two carbamate insecticides. M. A. Abdellatif, H. P. Hermanson and H. T. Reynolds (*J. econ. Ent.*, 1967, **60**, 1445–1450).—Temik and Nia 10242 (2,3-dihydro-2,2-dimethyl 7-benzofuranylmethyl-carbamate) were applied to 5 soil mixtures and their uptake by growing cotton plants estimated by aphid bioassay. Slow uptake was associated with a high clay content. Degradation was higher with increased org. content of the soil. Temik was taken up more rapidly and persisted longer than NIA 10242. The effect of these factors on soil treatment is discussed. (13 references.) C. M. HARDWICK.

Integrating *Phytoseiulus persimilis* releases, chemical application, cultural manipulations, and natural predation for control of two-spotted spider mite in southern California. E. R. Oatman, J. A. McMurtry, H. H. Shorey and V. Voth (*J. econ. Ent.*, 1967, **60**, 1344–1351).—Plots were treated with a pre-planting furrow and a later application of phorate granules or a foliar spray of binapacryl. Mass releases of *P. persimilis* were made weekly and counts were made of prey and predators, throughout the season. Removal of non-functioning leaves resulted in a 55·4% reduction in mites. C. M. HARDWICK.

Systemic insecticides applied to soil for control of tobacco flea beetle on tobacco. C. B. Dominick (*J. econ. Ent.*, 1967, **60**, 1468–1469).—When applied as granules, Nia 10242 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) and Amer. Cyan. 47031 [cyclic ethylene(diethoxyphosphinyl)dithioimidocarbonate] gave better protection against damage by *Epitrix hirtipennis* in newly set and large tobacco than did three other experimental insecticides tested. C. M. HARDWICK.

Biological activity of *N*-methylcarbamate and dimethyl phosphorothionate esters of various phenols and monoterpenoids. C. O. Knowles and B. W. Arthur (*J. econ. Ent.*, 1967, **60**, 1417–1420).—10 *N*-methylcarbamate and 10 dimethyl phosphorothionate esters of various phenols and monoterpenoids were prepared and evaluated against various insects. The esters of the substituted phenols were more active than those of terpenoids. Piperonyl butoxide was synergistic to some compounds. (13 references.) C. M. HARDWICK.

Temperature effect on mortality of confused flour beetles treated with CO_2 or N_2 before fumigation. S. D. Carlson (*J. econ. Ent.*, 1967, **60**, 1248–1250).—Adult *Tribolium confusum* were preconditioned for ½ h at 20°, 30° and 40° with N_2 or CO_2 before fumigation with $CCl_4 : CS_2$ (80 : 20). Conditioning temp. was the most significant factor controlling the effect of subsequent fumigation. Mortality was max. after pre-conditioning at 30°. C. M. HARDWICK.

Cabbage maggot resistance to organochlorine insecticides. F. L. McEwen, H. B. Rinick, jun., A. C. Davis and R. C. Little, jun. (*J. econ. Ent.*, 1967, **60**, 1261–1264).—Organo-chlorine insecticides were tested as in-furrow, drench or as preplanting broadcast application for control of *Hylemya brassicae* in two areas, in 1965–6. High levels of resistance were found to the common chlorinated hydrocarbons. Stauffer N-2790 (*O*-ethyl *S*-phenyl ethylphosphonodithioate) was the most effective compound tested. C. M. HARDWICK.

Carbamate and phosphate resistance in adult granary weevils. V. Kumar and F. O. Morrison (*J. econ. Ent.*, 1967, **60**, 1430–1434).—When selected with Baygon for 14 generations, *Sitophilus granarius* showed 7·5-fold resistance to Baygon but only 1·6-fold to fenthion. There was cross resistance to 2 other carbamates and a 2½-fold increase in resistance to DDT. Piperonyl butoxide acted as a synergist for Baygon against resistant strains. C. M. HARDWICK.

Malathion resistance in the red flour beetle. R. D. Speirs, L. M. Redlinger and H. P. Boles (*J. econ. Ent.*, 1967, **60**, 1373–1374).—*Tribolium confusum* were collected from 10 different storage places and tested for the level of resistance. The most resistant strain was 11·3× more resistant than the least. C. M. HARDWICK.

Azodrin for maize earworm and autumn armyworm control and its persistence in sweet maize. J. R. Young and M. C. Bowman (*J. econ. Ent.*, 1967, **60**, 1282–1284).—Using leaf disks, azodrin was as effective as DDT against third instar *Spodoptera frugiperda*. In field trials, azodrin gave better control than did DDT. Residues, examined were proportional to the rate of application on different parts of the plant. C. M. HARDWICK.

Arrestant-feeding stimulants from maize used in conjunction with an insecticide against larvae of maize earworm and autumn armyworm. K. J. Starks, J. R. Young and W. W. McMillian (*J. econ. Ent.*, 1967, **60**, 1483–1484).—Extracts from maize kernels which contain an arrestant-feeding stimulant, were more attractive than those of the silks or leaves. When these were used in conjunction with an insecticide, the mortality of maize earworm larvae could be increased. C. M. HARDWICK.

Laboratory tests of five new insecticides as protectants for stored rough rice. R. R. Cogburn (*J. econ. Ent.*, 1967, **60**, 1286–1289).—CL 47300 (*O,O*-dimethyl *O*-4-nitro-m-tolyl phosphorothioate) gave high initial mortality of the three test species, and was very effective after 3 months when malathion was less effective. Ciodrin, Bayer 39007 (*o*-isopropoxyphenyl methylcarbamate) bromodan and SD 7438 (*S,S'*-benzylidene bis (*O,O*-dimethyl) phosphorodithioate) were ineffective against at least one species. C. M. HARDWICK.

Field evaluation of insecticides for control of aphids on potatoes. D. D. Pond (*J. econ. Ent.*, 1967, **60**, 1203–1205).—For the 4 years of the experiment, aphid no. were below normal due to weather conditions. The most effective granular insecticide was Temik applied in the furrow at planting time. The most effective spray was oxydemetonmethyl applied three times. C. M. HARDWICK.

Insecticides applied to soil for control of eight species of insects on Irish potatoes in Virginia. R. N. Hofmaster, R. L. Waterfield and J. C. Boyd (*J. econ. Ent.*, 1967, **60**, 1311–1318).—Twenty-five compounds applied in different ways were evaluated over 6 years. The effect on different insects is given. Infestation control often gave striking increases in yield and improved quality. (21 references.) C. M. HARDWICK.

Incidence of black scurf caused by *Rhizoctonia solain*, Kühn, and common scab caused by *Streptomyces scabies*, (Thaxt.), Waksm. and Henriki on potato varieties. M. J. Downes and J. B. Loughnane (*Scient. Proc. R. Dubl. Soc.*, 1966, **2**, no. 2. pp. 7–16).—In trials on eight Irish farms in 1964 and two in 1965 it was found that the varieties King Edward, Arran Consul and Up-to-date were increasingly susceptible, in that order, to black scurf caused by *Rhizoctonia solani*, Kühn. This difference between varieties was consistent in all soils but different soils yielded very different amounts of black scurf; high soil moisture appeared to reduce black scurf levels. At high levels of black scurf the intensity per colonised tuber increased with the no. of tubers colonised but at lower levels the two were independent. Common scab occurred on the varieties in the same order of severity but there was no evidence that the incidence of black scurf is in any way correlated with the incidence of common scab on the tubers of a given variety. S. A. BROOKS.

Residues of aldrin, chlordane, endrin and heptachlor in groundnuts grown in treated soil. L. W. Morgan, D. B. Leuck, E. W. Beck and D. W. Woodham (*J. econ. Ent.*, 1967, **60**, 1289–1291).—

Granules were applied to the soil at 2 lb/acre; aldrin and heptachlor caused relatively high residues in groundnut meats and shells and in whole nuts from chlordane and endrin. Measurable residues of aldrin and heptachlor and their epoxides were found in the following season's crop. C. M. HARDWICK.

Laboratory studies on the residual toxicity of certain insecticides to the mustard aphid, *Lipaphis erysimi*. G. Rout and B. Senapati (*J. econ. Ent.*, 1967, **60**, 1458–1459).—Methyl demeton, menazon and dimethoate had longer residual effects than had phosphamidon, mevinphos and parathion as soil and spray applications. The effectiveness of all treatments was reduced after 12 days and by 20th day only dimethoate was at all effective. C. M. HARDWICK.

Baits and simulated sprays for control of the cribrate weevil. N. F. McCalley (*J. econ. Ent.*, 1967, **60**, 1473–1474).—Of 12 formulations tested, NIA 10242 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) gave 100% mortatilty of *Brachyrhinus cribricollis*, in bait and solution tests. Ca arsenate–metaldehyde bait was promising. C. M. HARDWICK.

Laboratory test to determine the amount of ethylene dibromide required to control the greater wax moth. T. Lehnert and H. Shimanuki (*J. econ. Ent.*, 1967, **60**, 1486–1487).—Ethylene dibromide (0·02 ml/liter) killed all stages of *Galleria mellonella* after 24 h. C. M. HARDWICK.

Variability and changes in susceptibility of field populations of citrus red mites and European red mites to dicofol. L. R. Jeppson and J. Complin (*J. econ. Ent.*, 1967, **60**, 1259–1261).—Big variations in the susceptibility of field-collected samples of mites to dicofol were found over a 3 year period. Variations within samples was greater than between them. Failure of commercial applications was due to the effect of changes in weather on the mites or host plants rather than to the development of genetic resistance. C. M. HARDWICK.

Influence of constituents of the cotton plant on feeding, oviposition and development of the boll weevil. F. G. Maxwell, J. N. Jenkins and W. L. Parrott (*J. econ. Ent.*, 1967, **60**, 1294–1297).—Quercetin, rutin, tannin and gossypol were added to the diets of adult and larval *Anthonomus grandis*. The effect on feeding, weevil wt., and oviposition at different dosage levels is recorded. C. M. HARDWICK.

Relative toxicity of Bayer 77488 and Dursban against stored-product insects. L. L. McDonald and H. B. Gillenwater (*J. econ. Ent.*, 1967, **60**, 1195–1196).—At LD_{95} level, Bayer 77488 (*O,O*-diethyl phosphorothioate *O*-ester with phenylglyoxylonitrile oxime) and Dursban were more effective than malathion when applied dorsally to *Tribolium confusum*, *Lasioderma serricorne*, *Plodia interpunctella* and *Athopenus megatoma*. Bayer 77488 had low mammalion toxicity. C. M. HARDWICK.

Control of the louse *Polyplax serrata* with systemic insecticides administered in silastic rubber implants. C. M. Clifford, C. E. Yunker and M. D. Corwin (*J. econ. Ent.*, 1967, **60**, 1210–1213).—Silicone rubber capsules giving slow release of their contents were implanted in the peritoneal cavity of mice. Those with capsules containing proban (*O,O*-dimethyl *O-p*-sulphamoyl phenyl phosphorothioate) developed no lice in 18 weeks. Ronnel gave some short lived reduction in lice. C. M. HARDWICK.

Field studies on control of lygus bugs and onion thrips infesting safflower. L. J. DePew (*J. econ. Ent.*, 1967, **60**, 1224–1226).—Of 12 sprays tested over 2 years, dimethoate gave good residual control of *Lygus elisus* and *Thrips tabaci*. Phosphamidon gave good initial control. C. M. HARDWICK.

Laboratory evaluation of malathion as a protectant for almonds during storage. G. H. Spitler and P. L. Hartsell (*J. econ. Ent.*, 1967, **60**, 1456–58).—Dusts and sprays were applied by the tumbling method. After 3 days, dust residues contained 44–70% and spray residues 8–11% of the amounts applied. At the highest dosages dusts gave 9–12 months control of all stages of *Oryzaephilus mercator* and *Plodia interpunctella* except the larvae. Sprays were also less effective against immature *P. interpunctella*. There were no off-flavours. C. M. HARDWICK.

Bioassays of Mexican fruit flies to determine residual effectiveness of Mediterranean fruit fly bait sprays in southern Texas. W. G. Hart, S. Ingle, D. Reed and N. Flitters (*J. econ. Ent.*, 1967, **60**, 1264–1265).—Aerial applications of a malathion + protein hydrolysate bait spray remained effective for 9 days on foliage for control of *Ceratitis capitata*. Mortality varied with types of foliage. C. M. HARDWICK.

Effect of antibiotics on reproduction of the black bean aphid, *Aphis fabae*. S. Jayaraj, P. Ehrhardt and H. Schmutterer (*Ann. appl. Biol.*, 1967, **59**, 13–21).—The antiobiotic Terramycin at 0·2% concn. was consistently effective in greatly reducing the populations of *Aphis fabae* on beans when the larvae were sprayed on or off the plant. The treatment did not kill the insects, but their fertility was reduced by over 97% in the first generation and the second generation was totally sterile. Affected insects could not regain their reproductive capacity even when transferred to untreated plants. Surfactants increased the efficiency of control by terramycin when the insects were sprayed off, but not when sprayed on, the plant. Tetracycline, Aureomycin, chloramphenicol, and reverin were not effective at 0·2% concn. when the plants were sprayed before introducing the aphids. A. H. CORNFIELD.

Analysis of parathion on broccoli: comparison of chemical, physical and bioassay methods. P. C. Lippold, A. C. Davis, A. W. Avens and S. D. Gibbs (*J. econ. Ent.*, 1967, **60**, 1364–1367).—Parathion was applied at 0·5 and 0·8 lb/acre and samples were taken 0–7 days after the last application. Gas–liquid chromatography, spectrophotometric analysis and bioassay with *Drosophila* were compared and gave similar results. Bioassay gave the highest values. (17 references.) C. M. HARDWICK.

Effect of atrazine on water use, stomatal action, and transpiration of maize. J. E. Pallas, jun and A. R. Bertrand (*Agron. J.*, 1967, **59**, 139–142).—Application of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 6 lb per acre) immediately after planting maize on a sandy loam had no significant effect on yields, water use efficiency, or stomatal activity either where irrigation was practised or under simulated drought conditions (plastic-covered soil). A. H. CORNFIELD.

Biosynthesis of *N*-glucosyl amiben [*N*-(3-carboxy-2,5-dichlorophenyl)-glucosylamine] in plant tissue. D. S. Frear, C. R. Swanson and R. E. Kadunce (*Weeds*, 1967, **15**, 101–104).—The formation of N-glucosyl amiben (**I**) from application of amiben to soya-bean hypocotyl sections was heat labile and sensitive to freezing and thawing, but was unaffected by anaerobic conditions. The optimum pH for formation of **I** was 6·3 to 7·3. Formation was inhibited by NaF, $HgCl_2$, iodoacetate, iodoacetamide and N-ethylmaleimide, but not by NaCN or NaN_3. A. H. CORNFIELD.

Determination and distribution of toxic levels of arsenic in a silt loam soil. J. T. Arnott and A. L. Leaf (*Weeds*, 1967, **15**, 121–124).—In a silt loam the presence of 4,000 lb of As_2O_3 per acre was moderately toxic and of 8,000 lb per acre very toxic to Monterey pine seedlings. Toxicity increased with increasing soil moisture content. Soil as sol. in water or EtOH was a good indicator of toxic levels of As. When applied on the soil surface and subjected to leaching the bulk of the applied As was retained in the surface 3 in. of soil. A. H. CORNFIELD.

Effect of atrazine on nitrogen metabolism of resistant species. J. V. Gramlich and D. E. Davis (*Weeds*, 1967, **15**, 157–160).—Maize and Johnsongrass (*Sorghum halapense*) grown in a sandy loam treated with atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 2–16 lb per acre) made slightly poorer growth than controls, and at the high atrazine rates, always resulted in lower uptake of N. Atrazine increased the % of both 80% EtOH-sol. N, insol. N, and NO_3 in the plant, but had no effect on NH_3. A. H. CORNFIELD.

Effect of picloram (4-amino-3,5,6-trichloropicolinic acid) on field-grown tobacco. G. C. Klingman and H. Guedez (*Weeds*, 1967, **15**, 142–146).—When picloram was applied before or after planting out tobacco, yields and quality were seriously reduced when more than 5 gal. per acre was applied. Application at up to 2·5 gal per acre after topping did not reduce the value of the crop. In general the more seriously the tobacco was damaged the higher was the nicotine and the lower was the total sugar content. A. H. CORNFIELD.

Control of weeds in side-oats grama, *Bouteloua curtipendula* grown for seed. M. K. McCarty, L. C. Newell, C. J. Scifres and J. E. Congrove (*Weeds*, 1967, **15**, 171–174).—Tests with a no. of herbicides showed that the best control of weeds and highest yield and quality of seed produced by side-oats grama were obtained by pre-emergence application of bromacil (5-bromo-3-*sec*-butyl-6-methyluracil, 2 lb per acre). Other herbicides controlled broad-leaf weeds but not grass weeds. A. H. CORNFIELD.

Effects of relative humidity on absorption and translocation of foliage-applied Dalapon, (2,2-dichloropropionic acid.) R. Prasad, C. L. Foy and A. S. Crafts (*Weeds*, 1967, **15**, 149–156).—Tracer studies with dalapon applied as foliage sprays showed that absorption and translocation were higher at 88% R.H. than at 60% or 28% R.H. imposed after treatment. Droplets dried less rapidly at

high than at low R.H., thus prolonging the period of effective absorption at high R.H. At low R.H. periodic re-wetting of the droplet area enhanced absorption and translocation, but never to the extent obtained at constant high R.H. The results help to explain the greater toxicity of dalapon in regions of high R.H., and its sometimes erratic performance in drier climates.
A. H. CORNFIELD.

Indicator plant aberrations at threshold soil herbicide levels. J. Q. Lynd, C. Rieck, D. Barnes, D. Murray and P. W. Santelman (*Agron. J.*, 1967, **59**, 194–196).—Threshold concn. (concn. at which the plant survived but chlorotic or malformed leaves occurred) to *Cucumis sativa* growing in a sandy soil (pH 5·7) were for Fluometuron [3-(m-trifluoromethylphenyl)1,1-dimethylurea] 1 ppm (soil basis), for Prometryne [2-methylmercapto-4,6-bis-(isopropylamino)-1,3,5-triazine] 1 ppm, for picloram (4-amino-3,5,6,trichloropicolinic acid) 0·01 ppm, and for Pyriclor (2,3,5-trichloro-4-pyridinol) 1 ppm. Threshold concn. of N-serve [2-chloro-6-(trichloromethyl) pyridine] to *Robinia pseudoacacia* was 1 ppm. Symptoms are described and illustrated. A. H. CORNFIELD.

Efficiency of Barban in relation to time of application in controlling wild oats, *Avena fatua*, in spring wheat. G. Friesen (*Weeds*, 1967, **15**, 160–162).—Satisfactory selectivity of Barban (4-chloro-2-butynyl *m*-chlorocarbanilate), 4 oz per acre in controlling wild oats in spring wheat was obtained when it was applied from 4 to 14 days after emergence of wild oats. When applied later the effectiveness of Barban in controlling wild oats declined rapidly and wheat yields were significantly reduced. A. H. CORNFIELD.

Herbicidal interactions of potassium azide and calcium cyanamide. S. R. Colby and R. W. Feeny (*Weeds*, 1967, **15**, 163–167).—Field and greenhouse tests showed that KN_3 and $CaCN_2$ applied pre-emergence were highly synergistic in controlling several plant species, including crabgrass, pigweed, and jimsonweed. In the field the combined treatment reduced weed seed germination over a 6-month period. Broadleef weeds were controlled better than grasses. The presence of $CaCN_2$ reduced the rate of decomposition of KN_3 in soil. A. H. CORNFIELD.

Mode of action of BV207 [1-(3-chloro-4-methylphenyl)-3-methyl-2-pyrrolidinone]. Y. Eshel (*Weeds*, 1967, **15**, 147–149).—BV207 was more toxic to several species when plants were exposed to light than when they were kept in the dark after application. 10^{-4}M-BV207 almost completely inhibited fixation of labelled CO_2 by leaf disks. Root growth was inhibited only slightly by BV207.
A. H. CORNFIELD.

Trifluralin behaviour in soil. I. Toxicity and persistence as related to organic matter. C. E. Bardsley, K. E. Savage and V. O. Childers. (*Agron J.*, 1967, **59**, 159–160).—The incorporation of either the alkali-extractable colloidal fraction of leonardite or activated C in mineral soils of low org. matter resulted in increased toxicity to barley and cucumbers from surface-applied trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) (**I**). Toxicity due to **I** increased with level of org. material (1·5–6·0% humic + ulmic acid) added to the soil. The toxicity persisted for 93 days after application. Subsurface applications of **I** resulted in greater toxicity than did surface applications, but only where org. matter was high. The greater toxicity of **I** where org. matter is high is probably due to greater retention, by adsorption, of the vapour phase of the chemical. A. H. CORNFIELD.

Evaluation of herbicides for weed control in southern peas, *Vigna sinensis*. D. S. Burgis (*Weeds*, 1967, **15**, 180–181).—Of a no. of herbicides tested 4,5,7-trichlorobenzthiadiazole-2,1,3 (4–6 lb per acre) and 4-(methylsulphonyl)-2,6-dinitro-*N*,*N*-dipropylaniline (1–2 lb), applied pre-emergence, gave good weed control without producing phytotoxic effects in southern peas.
A. H. CORNFIELD.

Uptake and adsorption of diquat and paraquat by tomato, sugar beet and cocksfoot. R. C. Brian (*Ann. appl. Biol.*, 1967, **59**, 91–99).—Uptake of diquat and paraquat sprayed on tomato, sugar beet and cocksfoot leaves was rapid in the light, but was increased by darkness, indicating that uptake takes place through the cuticle and not through the stomata. Both materials were rapidly and strongly adsorbed both to leaf tissue and to extraneous matter on the leaf surface. Uptake in the field is so rapid that rain immediately after treatments has little adverse effect. A. H. CORNFIELD.

Root absorption and translocation of atrazine in oats. R. C. Shimabukuro and A. J. Linck (*Weeds*, 1967, **15**, 175–178).—Visible injury symptoms in oat seedlings were not apparent until 4 days after transference to a solution containing 1 ppm atrazine (2-chloro-4-ethylamino-6-iso-propylamino-*s*-triazine) even though absorption and translocation were affected within 1–4 days after exposure to the herbicide. The expected translocation and uniform distribution of ^{14}C-labelled atrazine transported in the xylem was stopped after 2 days exposure to atrazine, whilst absorption was not reduced until 3 days after exposure. Reduced atrazine uptake was correlated with increased carbohydrate concn. in the tissues.
A. H. CORNFIELD.

Loss of 2,4-D in washoff from cultivated fallow land. A. P. Barnett, E. W. Hauser, A. W. White and J. H. Holladay (*Weeds*, 1967, **15**, 133–137).—Wash-off of 2,4-D (in various forms) from a cultivated fallow sandy loam using artificial rainfall increased with rate of 2,4-D applied and was greatest in the early stages of wash-off. The amine salt of 2,4-D was far less susceptible to wash-off than were iso-octyl and butyl ether formulations. Most of the 2,4-D remained in the surface 3-in. of soil. A. H. CORNFIELD.

Control of Eurasian watermilfoil, *Myriophyllum spicatum*. G. E. Smith, T. F. Hall, jun. and R. A. Stanley (*Weeds*, 1967, **15**, 95–98).—The most effective method of controlling Eurasian watermilfoil (a submerged aquatic weed) involved lowering lake levels enough to permit complete drying of stems and root crowns followed by application of 2,4-D-butoxyethanol ester in 20% granular form at 20–40 lb per acre. Control was satisfactory in still, but not in moving water. A. H. CORNFIELD.

Effect of late pre-harvest applications of 2,4-dichlorphenoxyacetic acid on wheat. W. M. Phillips, G. Yip, F. Finney, J. L. Hilton and W. C. Shaw (*Weeds*, 1967, **15**, 107–111).—Application of 2,4-D (dimethylamine salt, isopropyl, or isooctyl ester formulations) at 0·5–4 lb per acre 7–21 days before harvesting winter red wheat had no significant effect on yield, chemical composition, and milling and baking properties of the grain. When treated with 0·5–1 lb 2,4-D per acre (the recommended rate) grain contained 0·04–0·27 ppm 2,4-D. Grain from plots treated 21 days before harvest was lower in 2,4-D than was grain from plots treated 7–14 days before harvest.
A. H. CORNFIELD.

Merion Kentucky bluegrass response to soil residues of pre-emergence herbicides. R. E. Engel and L. M. Callahan (*Weeds*, 1967, **15**, 128–130).—The growth of Kentucky bluegrass in lawn turf soil treated 8 months previously with preemergence herbicides was studied. Where chlordane (80 lb per acre), DCPA (dimethyl 2,3,5,6-tetrachloroterephthalate), 12 lb, DMPA [O-(2,4-di-chlorophenyl)-O-methyl-isopropylphosphoramidothioate,] 15 lb), and polychlorodicyclopentadiene isomers (30–60 lb) had been applied root growth of bluegrass was normal. Where terbutol (2,6-di-t-butyl-*p*-tolylmethyl-carbamate), 10–20 lb and bensulide [*N*-(2-mercaptoethyl)benzenesulphonamide], 15–20 lb had been applied root growth of bluegrass was seriously reduced in soil taken from the 0–2 in. but not in soil taken from the 2–4 in. depth. Topgrowth of bluegrass was reduced somewhat where all materials, particularly polychlorodicyclopentadiene isomers, had been used.
A. H. CORNFIELD.

Control of Virginia chain fern, *Woodwardia virginica*, in cranberry bogs. W. V. Welker, jun. (*Weeds*, 1967, **15**, 179).—Application of Dichlobenil (2,4-dichlorobenzonitrile), 4–8 lb per acre when the weed emerged resulted in excellent control of Virginia chain fern in cranberry bogs. No regrowth occurred in subsequent years.
A. H. CORNFIELD.

Control of *Poa annua*. F. V. Juska and A. A. Hanson (*Weeds*, 1967, **15**, 98–101).—Application of trifluralin (α-α-α-trifluoro-2, 6-dinitro-*N*,*N*-dipropyl-*p*-toluidine, 2 lb per acre) completely controlled annual bluegrass, *Poa annua*. A. H. CORNFIELD.

Control of undesirable species in short-leaf pine, *Pinus echinata*, and native grass. H. M. Elwell (*Weeds*, 1967, **15**, 104–107).—Oak and hickory infestations in short-leaf pine stands were controlled by aerial applications (over three seasons) in May of 2,4,5-trichloro-phenoxyacetic acid-propylene glycol butyl either ester (1·5 lb per acre). Native grass growth improved where undesirable tree species were controlled. A. H. CORNFIELD.

Effect of weed control by herbicides on quality and yield of sweet potatoes. W. V. Welker, jun. (*Weeds*, 1967, **15**, 112–114).—Application of amiben (3-amino-2,5-dichlorobenzoic acid, 2–4 lb per acre), dichlobenil (2,6-dichlorobenzonitrile, 4–6 lb), diphenamid (*N*,*N*-dimethyl-2,2-diphenylacetamide, 2–4 lb), and DCPA (2,3,5,6-tetra-chloroterephthalate, 8–16 lb) 7–10 days before planting out sweet potatoes gave excellent control of annual weeds without affecting yield and quality factors of the crop. A. H. CORNFIELD.

Bio-assay method for determining phytotoxicity, leaching, and adsorption of herbicides in soils. Y. Eshel and G. F. Warren (*Weeds*, 1967, **15**, 115–118).—The method is based on measuring

the extent of primary root growth of cucumber or sorghum 48 h after sowing in sand saturated with various concn. of the test herbicides. The rate of leaching of herbicides was determined by measuring the effect on seedling growth of leachates obtained from soil columns of various heights. The extent of inactivation of herbicides by adsorption by soil was determined by measuring seedling growth in soil diluted with different amounts of sand. Typical results obtained with four herbicides and three soil types are presented. A. H. CORNFIELD.

Effects of atrazine- or simazine- nitrogen fertiliser combinations on maize and on succeeding crops. R. J. Fink (*Diss. Abstr.* B, 1967, **27**, 2566).—Combined applications of atrazine, (**I**), or simazine, (**II**) with N fertiliser to a silt-loam soil sown with maize decreased the height of the plants to extents increasing with the amounts of either herbicide used. Yields of maize forage (dry basis) 5 and 11 weeks after planting were similarly diminished. The average yield of maize stover at maturity and the yield of grain were unaffected by the herbicides except where a 10 lb dressing of **II** was used. In the first two cuttings of forage the %N content increased with the herbicide dressing and at maturity the N content of the stover had increased slightly. The $\%NO_3^-$ in the forage early in the season was increased by the herbicides in absence of N fertiliser but the reverse effect was produced in the mature stover. In his bio-assays, yields of soya-bean and sorghum were reduced by the herbicides, particularly by **II**. Yields of soya-beans in soil treated, in the previous year, with **II** at 2·5 or 5 lb/acre, were not reduced. Combinations of **I** or **II** with N fertiliser modified soil bacterial counts; total fungal counts were unaffected but the species distribution was changed in some cases.
A. G. POLLARD.

Movement and persistence of methanearsonates in soil. R. Dickens (*Diss. Abstr.* B, 1967, **27**, 2565–2566).—Clay minerals having exposed Al_2O_3 sheets and/or surface hydroxyl groups e.g. kaolinite, (**I**), limonite, (**II**), adsorbed more Na_2 methanearsonate, (*DSMA*), than did those having expanded SiO_2-O- sheets vermiculite, (**III**), bentonite, (**IV**). Adsorption of *DSMA* by both **I** and **III** increased with the [*DSMA*] in the equilibrium solution. The most adsorbant fraction in soil was the clay, very little adsorption occurring in sand or silt fractions. Org. matter contributed to the adsorption. Approx. 50% of the *DSMA* was leached from a 9-in. column of a loamy sand by 20 separate 1-in. increments of water applied to the suface; none was leached from a clay loam under these conditions. Soil pH in the range 5·5–6·5 did not affect the movement of the herbicide in the loamy sands. Approx. 50% of the applied *DSMA* remained in the upper 1-in. of the clay soil after the leaching; none was found at > 6 in. depth in the column. Decomposition of *DSMA* in each soil was correlated with the oxidation of the org. C present; the amount decomposed per unit of soil C differed between soils, particularly during the first 10 days of reaction. Addition of readily decomposable org. matter to the loamy sand increased its ability to decompose *DSMA*.
A. G. POLLARD.

Detection of nitrogen-containing herbicides on thin-layer chromatograms. D. C. Whitenberg (*Weeds*, 1967, **15**, 182).—Herbicides containing primary or secondary amino-groups can be detected on thin layers of silica gel G or H after developing the chromatograms with light petroleum: $CHCl_3$: 95% EtOH (7 : 2 : 1 v/v) drying, spraying with 1% t-butyl hypochlorite in cyclohexane, drying, and then spraying with 1% KI and 1% starch solution. Detection limits range from $< 0{\cdot}05$ μg to 1 μg, depending on the herbicide.
A. H. CORNFIELD.

Methylation of herbicides for gas chromatographic determination. E. A. Woolson and C. I. Harris (*Weeds*, 1967, **15**, 168–170).—Methods of methylating several acid herbicides so as to reduce their polarity and render them more easily determinable by electron-capture gas chromatography were studied. Diazomethane was the best methylating agent for the acid herbicides Fenac (2,3,6-trichlorophenylacetic acid), 2,3,6-trichlorobenzoic acid, picloram (4-amino-3,5,6-trichloropicolinic acid), dicamba (2-methoxy-3,6-dichlorobenzoic acid), and 3,6-dichlorosalicylic acid. BF_3-CH_3OH was equally satisfactory when the compound was not sterically hindered. A. H. CORNFIELD.

2-Substituted benzimidazoles. Merck and Co., Inc. (B.P. 1,071,421, 25.5.64. U.S., 23.5.63 and 12.5.64).—Used in controlling undesired fungus growth of the *Aspergillus*, *Penicillium*, *Alternaria* and *Chaetomium* species, the claimed 2-(4′-thiazolyl)benzimidozoles, can be prepared by treatment of the appropriate nitroaniline with a heterocyclic carboxylic acid or its derivative and reducing the NO_2 group of the resulting anilide, followed by cyclisation; alternatively, *o*-phenylenediamines and the heterocyclic carboxylic acid derivatives may be reacted. Thus, Sabouraud's dextrose sugar at pH 5·6 and 50° is poured into a clean, sterile flask containing 2-(4′-thiazolyl)benzimidazole and Me_2-formamide and after agitation, the mixture is poured into Petri dishes to solidify. A water suspension of the spores of *Trichophyton mentagrophytes* is streaked on to the medium and after storage for 3 days at room temp., complete inhibition of the fungus is noted. S. D. HUGGINS.

Organo-phosphorus *s*-triazine derivatives. Imperial Chem. Industries Ltd. (Inventor: A. J. Floyd) (B.P. 1,063,072, 28.9.62. Addn. to B.P. 899,701, J.S.F.A. Abstr. 1964, i–22).—The title compounds are active against aphids, red spider, and *Dysdercus* and have the formula OR′(SR″)PO.S.CH_2R wherein R′ and R″ are alkyl of 1–4 C and R is 4,6-diamino-*s*-triazin-2-yl). In an example, MeI(4·5) is added dropwise to an ice-cooled solution of desmethyl menazon(8·01) in MeOH 60 cc containing dissolved Na(0·69 g), then the product which separates out overnight is recrystallised from 95% aq. MeOH, to give S-4,6-*diamino-s-triazin*-2-*ylmethyl*-O, S-Me_2 *dithiophosphate*, mp 180° (decomp). F. R. BASFORD.

Tobacco and preparation of a naphthalenone derivative. R. J. Reynolds Tobacco Co. (Inventor: D. L. Roberts) (B.P. 1,071,365, 19.8.65).—The addition of 0·001–1·0% by wt. of the claimed 4,4a,5,6-tetrahydro-4,4,7-trimethyl-2(3H)-naphthalenone gives a peppery, spicey odour to tobacco. The additive is prepared from dihydro-α-ionone, by oxidation, contacting with Bu^t chromate to give 5-oxodihydro-α-ionone which is then contracted with an acid or base catalyst. Thus, an aged, cured and shredded burley tobacco is sprayed with a 1% EtOH solution of 4,4a,5,6-tetrahydro-4,4,7-trimethyl-2(3H)-naphthalenone, the EtOH evaporated and the tobacco manufactured into cigarettes which have a pleasing aroma, detectable in the main and side smoke streams when the cigarette is smoked. S. D. HUGGINS.

Sclerin and the production of same. Meiji Seika Kaisha Ltd. (B.P. 1,071,832, 16.6.65. Japan, 8.7.64).—*Sclerotinia libertiana* or a mutant thereof is grown in aq. nutrient medium, then the culture is worked up (solvent extraction, etc.) to effect recovery of sclerin, $C_{13}H_{14}O_2$, mol. wt. 234, m.p. 123°—a plant growth regulant also useful in reducing the phytotoxicity of certain agricultural chemicals (blasticidin, streptomycin, 2,4-D, indoleacetic acid, etc.).
F. R. BASFORD.

Substituted ureas. Farbenfabriken Bayer A.-G. (Inventors: G. Muller, L. Eue and H. Hack) (B.P. 1,071,507, 4.5.66. Ger., 15.5.65).—Herbicidal compositions contain the claimed active *N*-(nortricyclyl-3)-ureas prepared from nortricyclyl isocyanate and the appropriate s- or t-amine. Thus, 1 part by wt. of active compound is mixed with 5 parts by wt. Me_2CO and 1 part of emulsifier, benzyloxy polyglycol ether, the whole then being diluted with water. Seeds of the test plants are sown in normal soil are watered, after 24 h, with the prep. of active compound and after 3 weeks the degree of damage is determined. With, e.g., *N*-(nortricyclyl-3)-*N′,N′*-diethyl urea, m.p. 78°, in concn. of 2·5, 5 and 10 kg/ha plants of *Echinochloa*, *Sinapis*, *Stellaris* and *Chenopodium* are completely dead or not emerged, while those of *Avena fatus* are partially destroyed after germination or only 25% emerged and wheat and beans are not affected at the lower concn. and only show marked damage at the 10 kg/ha concn. S. D. HUGGINS.

New 3,6-distributed pyridazines and their manufacture. CIBA Ltd. (B.P. 1,071,318, 17.5.65. Switz., 11.6.64).—Compounds claimed are antiparasitic and antibacterial agents (especially active against *Schistosoma*) and comprise pyridazines substituted in the 3-position by 2-(5-nitro-2-furyl)vinyl group and in the 6-position by $O{\cdot}R^1{\cdot}O{\cdot}(R^1{\cdot}O)_nR$ wherein R is H or alkyl of 1–5 C; *n* is 1 or 2; and R^1 is alkylene of 2-4-C between the O. In an example a mixture of 6-(3,6,9-trioxadecoxy)-3-methylpyridazine (12·8), 5-nitrofurfural (7·05 g), and Ac_2O (50 c.c.) is heated at 140–150° during 4 h, then evaporated. The residue is dissolved in 2N-HCl, the clarified solution is adjusted to pH7 with saturated aq. $NaHCO_3$, and ppt is recrystallised from acetone–light petroleum, to give 6-(3,6,9-*trioxadecoxy*)-3-[2-(5-*nitrofur*-2-*yl*)*vinyl*]*pyridazine*, m.p. 85–87°.
F. R. BASFORD.

Animal Husbandry

Determination of phosphate and calcium in feeding stuffs. C. B. Stuffins (*Analyst*, *Lond.*, 1967, **92**, 107–111).—The PO_4^{3-} and Ca^{2+} contents are determined in separate aliquots of the Kjeldahl-digestion solution, the PO_4^{3-} spectrophotometrically at 436 nm with vanadate reagent and the Ca^{2+} volumetrically by titration with

$KMnO_4$ after pptn as oxalate. The method eliminates much manipulative work and also the loss of some H_3PO_4 when dry-ashing is used; moreover, protein, Ca and PO_4^{3-} can be determined on the same solution. Values of CaO, however, are slightly lower than those obtained by the dry-ashing procedure for feed-mixtures.
W. J. BAKER.

Use of ruminal ammonia and blood-urea as indices of the nutritive value of protein in some foodstuffs. A. R. Abou Akkada and H. El Sayed Osman (*J. agric. Sci., Camb.*, 1967, **69**, 25–31).—Nine successive trials were carried out with three adult desert rams. The changes in ruminal NH_3 (**I**) and blood-urea (**II**) concn. are considerably higher 3 h after feeding on leguminous forages than on non-legumes. Among legumes lubia hay and fresh lubia produce the highest concn. of both **I** and **II**. Berseem hay reduces **I** and **II** to below the levels found with fresh berseem. Urinary N accounts for most of the N excretion with legumes, whilst faecal N accounts for most with grasses. The highest N retention is found with berseem hay followed by butterfly pea, fresh berseem, fresh lubia and lubia hay. The desert grasses produce a negative N balance. Significant correlations exist between **I** and **II** on the one hand and urinary N on the other for data from all forages and for legumes alone. Correlations between **I** and **II** and N retention are significant only for legumes alone. Blood-urea is a more practical determination than is ruminal NH_3.
M. LONG.

Nutritive value of the diet selected by grazing sheep. III. Comparison of oesophageal fistula and faecal index techniques for the indirect estimation of digestibility. J. P. Langlands (*Anim. Prod.*, 1967, **9**, 325–331).—Regression equations relating intake factor to the dissolved faeces fraction, faecal N and faecal cellulose contents were derived from a continuous digestibility trial with cut herbage as well as with sheep fitted with oesophageal fistulae grazing the same pasture. In the latter the intake factor was calculated from the *in vitro* digestibility of the fistula samples. Relationships so obtained from the two trials were significantly different. Whilst the estimates of digestibility from the grazing trial, derived from various faecal index relationships established, were very variable, estimates derived from the fistula technique agreed reasonably well with those predicted from a general relationship between intake factor and faecal N previously established.
M. LONG.

Ruminal volatile fatty acid studies with dairy cattle. R. O. Kelley (*Diss. Abstr.* B, 1967, **27**, 2556–2557).—Fistulated non-lactating cows were maintained for a week at 1–6°, 18·2° or 37·7°F and R.H. $\simeq$50%, the feed intake being kept at const. level by feeding via cannula if necessary. Concn. of ruminal acetic (**I**), propionic (**II**) and volatile fatty acids (*VFA*) were similar at temp. 1·6–18·2°F. At 37·7°F the mol.% of **I** exceeded and that of **II** was less than at 18·2°F. No significant differences in the vol. of water consumed at 18·2° and at 37·7°F were apparent but there were significant differences in the no. of times the animals drank at the two temp. In further trials with rumen-fistulated cows differing in production of fat-corrected milk (70, 35 and nil lb-4% fat) were fed a concentrate *ad lib* with restricted amounts of hay. Dil. water-sol. *VFA* were added to the rumen ingesta. Rations providing $\simeq$80% total digestible nutrients originating from the concentrate tended to depress milk-fat production. Correlation of milk-fat test values with **I** concn. was closer than that with mol.% **I**. When groups of heifers were fed individually (*a*) a common grain ration, (*b*) a commercial prep., (*c*) $a + b$ or (*d*) 84% of the daily ration, no relationship was apparent between ruminal *VFA* and rate of gain in wt. The *VFA* levels appeared to decline in the interval between feeding and sampling. Rates of gain in wt. were similar in animals fed 1·25 or 1·5 lb (*a*) per 100 lb. live wt but feed conversion was greater at the higher feeding rate.
A. G. POLLARD.

Utilisation of various non-protein nitrogen sources by rumen micro-organisms in a continuous flow, *in vitro*, fermentation system. B. E. Brent (*Diss. Abstr.* B, 1967, **27**, 2551).—The fermentation system described, provides for sampling the fermentation fluid and for the continuous dialysis and removal of the products. Various non-protein sources of N were compared using the same substrate, periodical replacement being made of the fermentation liquor and dialysing solution as samples were withdrawn. Comparison was made of urea (**I**), 1,3-dimethylurea (**II**), biuned (**III**), biurea (**IV**), guanidine hydrochloride, guanylurea sulphate and thiocarbanalide (**V**) with controls having no added N or soya-bean protein (**VI**). Determinations were made of precipitable N in the fermentation liquor and of total N, NH_3 and volatile fatty acids in the clear fermentation liquor and in the dialysate. Of the N sources only **I** was utilised in amounts approaching completion; **II**, **III**, **IV** and **VI** all released more NH_3 than did the control but only **VI** and **II** were hydrolysed to a sufficient extent to be of some value to ruminants. The amounts and proportions of volatile fatty acids obtained were generally similar to those found in intact animals.
A. G. POLLARD.

Fatty acid composition of certain bovine tissues. R. C. Thompson (*Diss. Abstr.*, B, 1967, **27**, 2558–2559).—The subcutaneous, inter- and intra-muscular fats from Hereford steers and from one pair each of Angus and Shorthorn identical twins were examined by gas–liquid chromatography. Of ten fatty acids determined only the 17-C acid and one 18-C branched-chain acid reflected differences between rations. Both acids are regarded as being synthesised by certain rumen bacteria and differences may be attributed to differences in the supply of suitable precursors in the rations. A highly significant relationship was found between the average daily gain in wt. and the linoleic acid content of the fat. Back-fat thickness was correlated, negatively, with both the contents of myristic and palcmetic acid contents, and positively, with the 17-C and 18-C acids (except stearic acid). Rib-eye area/cwt, and estimated cutability, conversely, were positively correlated with the 14-C and 16-C acids and, negatively, with the longer-chained fatty acids. Both liver- and muscle-phospholipids contained considerable amounts of arachidonic and eicosatrienoic muscle phospholipids and all liver fractions showed considerable variation within and between twin pairs. Only the muscle glycerides showed similar patterns within twin pairs.
A. G. POLLARD.

Effects of in-breeding and environment on the reproductive performance of beef cows. E. V. Krehbiel (*Diss. Abstr.*, 1967, **27**, 2557).—Observations over a 12–72 period using over 400 each of Angus and Shorthorn cows are reported. Factors affecting fertility (e.g., line of mating, ages of sire and dam at service) are examined and some differences between the two breeds in this respect are noted. The fertility of all in-bred lines was not significantly different from that of non-inbreds. The viability of calves and the calving interval were influenced by the above fertility factors and also by the time of year at birth. The effects of in-breeding intensity were not always predictable.
A. G. POLLARD.

Effect of Scotch grain distillers' solubles on the milk production of dairy cows. M. E. Castle, A. D. Drysdale and J. N. Watson (*Anim. Prod.*, 1967, **9**, 409–412).—From the results of feeding trials with lactating cows it is concluded that 1 or 2 lb of distillers solubles (**I**) can replace other ingredients in the concentrate ration and maintain the yield and quality of milk. No special value can be attached to **I** with regard to milk production. Trials with higher levels indicate that, given in acceptable form, **I** can form up to 20% of the total daily concentrate ration without ill effect.
M. LONG.

Silage as a feed for pregnant ewes. J. M. Forbes, J. K. Rees and T. G. Boaz (*Anim. Prod.*, 1967, **9**, 399–408).—Silage with low crude protein and high crude fibre contents, low pH and low dry matter was eaten in smaller quantities than other silages and ewes fed on this silage gained less wt. during the latter half of pregnancy. Ewes fed silage outside gave the same results as those housed, whilst ewes fed kale consumed less and gained less wt. when fed outside. Intake of silage was higher with a group of ewes lambing 5 weeks after another group, although lamb wt. were unaffected. Speckledfaced Welsh ewes ate more silage than did Scottish Halfbred and also gained wt. It is recommended that silage for pregnant ewes should have at least 20% dry matter and 14% crude protein and $\gtrsim$32% crude fibre (dry matter basis).
M. LONG.

Growth of lambs grazing tall fescue receiving high and low levels of nitrogen fertiliser. R. C. Grimes (*J. agric. Sci., Camb.*, 1967, **69**, 33–41).—Live-wt. gain, final body composition and intake of digestible org. matter do not differ significantly between fertiliser treatments, although the mol. proportions of acetic acid in the ruminal fluid are higher and those of n-butyric acid are lower on the high N treatment. Water-sol. carbohydrate and cellulose levels are lower and crude protein and NO_3^- levels are higher in the herbage from the high-N treatments. None of the factors measured are significantly related to live-wt. gain. Sol. carbohydrates are significantly positively correlated with ruminal acetic acid, whilst being correlated negatively with both propionic and butyric acids. Volatile fatty acid concn. in the rumen and org. matter digestibility are both correlated with crude protein, sol. carbohydrates plus crude protein and cellulose, but not with sol. carbohydrates alone.
M. LONG.

Copper sulphate in the diet of pigs. G. Berek, L. Urbanyi and T. Lakatos (*Anim. Prod.*, 1967, **9**, 421–424).—In early stages up to 40 kg live-wt. a Cu supplement of 250 ppm in the feed causes a better growth rate and efficiency of feed conversion. In later stages the control group, receiving only normal Cu supplementation,

catch up and tend to overtake the group receiving the high Cu supplement. M. LONG.

Utilisation of poultry diets containing high, low and intermediate levels of protein of identical amino-acid pattern. R. E. Smith (*Poultry Sci.*, 1967, **46**, 730–735).—When fed from 12 weeks of age to 50% production with diets containing 11%, 15% or 19% protein with the same amino-acid pattern, the 11% protein diet delayed sexual maturity but did not reduce body wt. gains. Thereafter in laying pens egg production was similar on all diets, but egg wt. and body wt. increased with protein level. Supplementing the laying diets with lysine and/or methionine did not affect the performance of birds at any protein level. A. H. CORNFIELD.

Water turnover in chickens. T. E. Chapman and A. L. Black (*Poultry Sci.*, 1967, **46**, 761–765).—Tritium-labelled water was used to measure the size of the body water pool and its rate of turnover. The half life of this pool averaged 3·6 days for hens and 7·3 days for cocks. The size of the body water pool in relation to body wt. was not significantly different between the two sexes. A. H. CORNFIELD.

Vitamin B_{12}-formaldehyde complex as a one-carbon unit precursor in the biosynthesis of methionine in turkey poult liver homogenates. B. W. Langer, jun. and F. H. Kratzer (*Poultry Sci.*, 1967, **46**, 749–754).—The reaction product of HCHO and vitamin B_{12} was 4–12 times more effective as a precursor of the methionine methyl group than was either free HCHO or serine alone or supplied with free vitamin B_{12} in a turkey poult liver homogenate system. A. H. CORNFIELD.

Effect of tylosin on the reproductive performance of turkeys and the growth rate of their offspring. R. L. Atkinson, C. F. Hall, J. W. Bradley, J. H. Quisenberry, D. I. Gard and J. E. Wachstetter (*Poultry Sci.*, 1967, **46**, 735–742).—Tylosin at nontherapeutic levels (45 and 180 p.p.m.) in the diet of turkey breeders had no effect on egg production, feed consumption, mortality, fertility or hatchability. Poults hatched from hens fed tylosin were more vigorous at hatching, grew faster and weighed more at 8 and 27 weeks of age, and suffered less mortality than did those receiving the basal diet. Tylosin did not improve the growth rate of poults hatched from hens not receiving tylosin. A. H. CORNFIELD.

Distribution of ^{14}C in the chick following the administration of labelled buquinolate. R. J. Herrett, C. W. Williams, G. M. Klein and J. P. Heotis (*Poultry Sci.*, 1967, **46**, 755–761).—When a single oral dose of labelled buquinolate (ethyl 4-hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate) was given to chicks most of the radioactivity was accounted for as unchanged buquinolate in the excreta. Retention of drug in the tissue was usually < 1 ppm. During a short-term continuous administration of the drug levels of ^{14}C reached a steady state early in medication, with the liver showing the highest retention. When the drug was withdrawn the residues were rapidly depleted. A. H. CORNFIELD.

Effects of spironolactone on laying pullets. W. J. Mueller (*Poultry Sci.*, 1967, **46**, 742–749).—Daily intramuscular injections of spironolactone (0·002–0·009 g/pullet day) had no effect on egg production during the first 5 days of treatment; subsequently this declined in birds receiving 0·004 g or more per day. 0·004 g or more spironolactone interfered with shell formation on the first, and to a smaller extent, on the second day, but had no effect on the third day. The effects of the treatments on the pH of blood and shell gland fluid are also reported. A. H. CORNFIELD.

Relationships between chicken coccidia and certain vitamins, amino-acids and anti-metabolites. I. A. Abou-El-Azm (*Diss. Abstr.* B, 1967, **27**, 2591).—The possible effects of dietary deficiencies of some vitamins and amino-acids on mixed or single species of chicken coccidia are examined using synthetic diets. Basal diets (20% protein) did not cause elimination of oocysts by infected hosts equal to that by hosts receiving commercial diets. The action between nutrition and parasite is direct and a physically weakened host could support the normal multiplication of the parasite, provided the ingredient missing from the host diet was not essential to the life cycle of the parasite. Alteration of the levels of certain vitamins in the diet may affect the quant. character of the parasite. *Eimeria tenella* does not require biotin, which is essential to *E. acervulina*. Egg white and desthiobioten were not coccideostatic under the conditions tested. Desoxypyridoxine (500 mg per kg g diet) suppressed the multiplication of *E. acervulina*, and at 1000 mg/kg reduced the no. of oocysts by 99%. A. G. POLLARD.

Accelerated depletion of DDT residues from commercial laying hens. R. L. Wesley (*Diss. Abstr.* B, 1967, **27**, 2559).—Depletion of DDT residues from hens was examined in relation to the nature of the diet. The source and content of dietary protein influenced depletion rates. Starvation (most effectively for the 48 h immediately after exposure to DDT) hastened depletion; injection of androgen had little effect. Fat-free rations (< 0·08% fat) lowered the DDT content of abdominal fat to $\frac{1}{3}$ of that resulting from addition of 5% of stabilised yellow fat to the ration. Exclusion of fat-sol. vitamins from the fat-free ration lowered the residual DDT in the abdominal fat by 15%; similar exclusion from rations containing added fat had no effect. Comparison was made of a low-protein, (*LP*), cracked maize ration, a high-protein (*HP*) ration (soya-bean meal fortified with vitamins and minerals, a standard (*S*) laying ration, a purified high-protein (*PHP*) ration based on casein, vitamins and minerals, and a mixture (*CSB*) of cellulose and isolated soya-bean protein (5 : 1) with minerals and vitamins to give total protein 20%. Depletion of DDT and its metabolites was most effective with *HP*, least with *S* and intermediate with the other rations. A. G. POLLARD.

Mortality of nasal bots in sheep treated with systemic insecticides. R. E. Pfadt (*J. econ. Ent.*, 1967, **60**, 1420–1422).—Dichlorvos (3%) reduced *Oestrus ovis* in lambs by 92% when squirted into the nostrils from a pressurised bottle. Fenthion was 100% effective as an oral drench but not as a feed mix or pour-on treatment. Bayer 9017 (*O,O*-diethyl *O*-[4-(methylthio)3,5-xylylphosphorothioate] was nearly as effective as fenthion. C. M. HARDWICK.

Effect of ronnel mineral block (Rid-Ezy) on control of cattle grubs and weight gain of beef cattle. F. W. Knapp, N. W. Bradley and W. C. Templeton, jun. (*J. econ. Ent.*, 1967, **60**, 1455–1456).—No *Hypoderma* spp. larvae were found on steers having access to the mineral blocks whereas cattle treated only with ciodrin to control horn flies averaged 20·5 grubs per head. The absence of grubs did not increase wt. gains. C. M. HARDWICK.

Larvicidal activity to flies of manure from chicks administered insecticide-treated feed. M. Sherman, G. H. Komatsu and J. Ikeda (*J. econ. Ent.*, 1967, **60**, 1395–1403).—Insecticides (44) were administered to chicks in their feed. The mortality of larvae of 4 species of diptera, 3 days after placement in manure and subsequent adult emergence were recorded. This was compared with the toxicity of droppings innoculated with the insecticide. 17 of the insecticides caused chick mortality. (14 references.) C. M. HARDWICK.

Control of larvae of the housefly and horn fly in manure of insecticide-fed cattle. R. O. Drummond, T. M. Whetstone and S. E. Ernst (*J. econ. Ent.*, 1967, **60**, 1306–1308).—Cattle were fed 11 experimental insecticides for 10 days and the effect on larvae or eggs of *Musca domestica* or *Haematobia irritans* was noted. (19 references.) C. M. HARDWICK.

***Eimeria necatrix* infections and oleic acid absorption in broilers.** D. E. Turk and J. F. Stephens (*Poultry Sci.*, 1967, **46**, 775–777).—*Eimeria necatrix* infection in 4-week-old broilers resulted in reduced absorption of oleic acid from the intestinal tract into the bloodstream, although the extent of reduction was not as great as for Zn. A. H. CORNFIELD.

Some physiological and biochemical responses of fish to chronic poisoning by cyanide. G. Leduc (*Diss. Abstr.* B, 1967, **27**, 2560).—Growth of young coho salmon, *Oncorhynchus kisutch* (Walbaum) was diminished following exposure to HCN (0·08 mg/l). After 24 days the treated salmon were placed in fresh flowing water at 16° with unrestricted access to earthworms. Subsequently the fish previously exposed to 0·02–0·08 mg HCN/l grew faster than controls. With cichlid fish, *Cichlasoma bimaculatum*, L. exposure to HCN (0·008–0·10 mg/l) increased feed consumption per g of fish but lowered the efficiency of food conversion. With [HCN], 0·008–0·02 mg/l growth was faster, initially, than that of controls; at 0·03 and 0·04 g HCN/l growth rates were unaffected but were markedly lowered after exposure to 0·06–0·10 mg HCN/l. The swimming ability of the fish, as tested against measured rates of flow of water in a tubular chamber, was lowered by previous exposure to HCN the effect of which was more marked at lower than at higher swimming velocity. Changes in body wt. and composition of starving cichlids exposed to HCN suggest an increased loss of energy reserves. The proteolytic activity of intestinal homogenates was increased by exposure to HCN. A. G. POLLARD.

Development and application of a method for determining *OO*-dimethyl *S*-(*N*-methylcarbamoylmethyl)phosphorodithioate (Dimethoate) residues in milk and other agricultural crops. H. F. Enos (*Diss. Abstr.* B, 1966, **27**, 1770–1771).—Dimethoate is extracted from milk by ether-hexane mixture; ether is then removed and the

hexane is extracted with acetonitrile. Residual pigments and lipids are removed by adsorption on a Florisil column. Dimethoate is further separated from interfering substances by paper chromatography and detected as a red spot on spraying the dried chromatogram with a solution of 2,6-dibromo-*N*-chloro-*p*-quinoneimine. A method, previously described for determining diazinon is modified and adapted for determining dimethoate residues on fruits and vegetable matter. The insecticide is extracted from a hexane solution with HBr, followed by hydrolysis and determination of the evolved H_2S as methylene blue. In some cases, a certain lack of specificity in the method may render advisable a knowledge of the previous spray history of the crops.

A. G. POLLARD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Retention of carbon-14 by wheat grown from labelled seed. A. Zeller, K. Roth and H. E. Oberlander (*Naturwissenschaften*, 1967, **54**, 45).—Wheat was grown in constant specific activity $^{14}CO_2$, harvested, sown and grown to full harvest (127 days) (in normal air). The C is not very labile: 7% of the original ^{14}C was found in the straw after harvesting, 1·2% in the grain and up to 10% in the roots and seed residues. K. GRAUPNER.

Effect of (2-chloroethyl)trimethylammonium chloride [CCC] on protein content, protein yield, and some qualitative indexes of winter wheat grain. A. Chrominski (*J. agric. Fd Chem.*, 1967, **15**, 109–112).—As regards protein yields, increases in grain yield obtained with the applications of CCC alone were offset by decreases in the protein content of the grain. Greatly increased protein yields could, however, be obtained by the use of CCC (at ⩾4 kg/ha) together with NH_4NO_3. Undesirable changes in the baking quality of the flour due to the use of CCC alone were very largely remedied by simultaneous applications of NH_4NO_3. (13 references.) P. S. ARUP.

Effect of environment on wheat gliadin. J. W. Lee and J. A. Ronalds (*Nature, Lond.*, 1967, **213**, 844–846).—Four varieties of wheat were grown in a wide range of environmental conditions; proteins extracted with acetic acid from flours milled from each of the wheat samples were subjected to chromatography on carboxymethyl cellulose and to starch gel electrophoresis. Comparison of the starch gel and chromatographic profiles showed that varietal differences have a very much greater effect than environmental factors on the distribution of gliadin components. (10 references.) S. A. BROOKS.

Sugars and confectionery

Preparation of substituted phenyl α-D-galactosides. P. M. Dey (*Chemy Ind.*, 1967, 1637).—The method is based on that described by Montgomery *et al.*, (*J. Am. chem. Soc.*, 1942, **64**, 690) and yields are higher than those obtained by other methods. Thus, phenol α-D-galactoside tetra-acetate (**I**) is first prepared by reaction between anhyd. $ZnCl_2$ (in AcOH)-acetic anhydride (19 : 1) and the melt of α-D-galactoside penta-acetate (prepared by the method of Hudson and Parker, *ibid.*, 1915, **37**, 1589) with the appropriate phenol. The dark-red product is dissolved in ethyl ether, the $ZnCl_2$ and excess phenol removed with water and NaOH, and **I** is crystallised from MeOH. Deacetylation of **I** with 0·1 M-NaOH-abs. MeOH yields phenyl α-D-galacto-pyranoside. W. J. BAKER.

Occurrence of isorhoeagenine-glycoside. A. Němečková, A. D. Cross and F. Sântavý (*Naturwissenschaften*, 1967, **54**, 45).—Hydrolysis of the alkaloid R-C (I) of *Papaver rhoeas* L. yields α-glucose and rhoeagenine: it is shown that **I** is a glycoside of isorhoeagenine. Formula is given. K. GRAUPNER.

Synthesis of polysaccharides. T. Goda (B.P. 1,071,912, 12.5.64).—Gaseous CO_2 and water vapour are activated by irradiation with radiations having λλ of 80–40 Å and 180–220 mμ, respectively, and then mixed with a catalyst, e.g., pulverised porous Al silicate that has been treated with radiation of λ 370–140 mμ. This reaction mixture is irradiated with radiations of λ 370–150 mμ and the product of this step is irradiated with successive radiations having λλ of 470–370 mμ and 120–60 mμ to form carbohydrate-like substances which are further irradiated with radiations having λλ of 470–20 mμ and 575–647 mμ. The radiation for each step is obtained by applying a high voltage to a pure quartz tube fitted in the synthesis chamber for that step. J. M. JACOBS.

Amyloglucosidase. H. E. Bode (B.P. 1,071,475, 2.11.64. U.S., 18.11.63).—Used in the hydrolysis of starch dextrins or maltose to dextrose, the title enzyme is obtained by fermenting a carbohydrate-containing nutrient medium, also containing protein, with a culture of *Aspergillus foetidus*, or one of its mutants, at 20–35 and 15–30 psig for 72–96 h. Thus, water containing maize steep liquor concentrate, adjusted to pH 6·0 with NaOH, is added to ground yellow maize and a commercial bacterial amylase suspended in water and anti-foam agent, the fermentor is closed, heated to 15 psig for 30 min. to sterilise the mash, cooled to 32° and then injected with a seed culture of a mutant of *Aspergillus foetidus*. The fermentation is maintained at 32° and 15 psig and aeration at 60 C.F.M. until max. amyloglucosidase activity is reached in 86 h. The mycelium is filtered off, the filtrate evaporated under vacuum at <35° and a liquid concentrate containing 30 units of amyloglucosidase/ml, which gives a 2·3% maltose conversion to unfermentable sugars in a transglucosidase activity determination. S. D. HUGGINS.

Fermentation and Alcoholic Beverages

Aflatoxin B_1 and histamine in wine. P. L. Schuller, Th. Ockhuizen, J. Werringloer and P. Marquardt (*Arzneimittel-Forsch.*, 1967, **17**, 888–890).—A tlc method is described for the determination of aflatoxin B_1 in wine and histamine is estimated biologically by its action of lowering the blood pressure in the cat. Out of 33 wine samples examined only two showed traces (<1 μ g/l) of aflatoxin B_1 although these were not examined biologically. The histamine content of the samples examined varied, two samples had 10μg/1, 2 had traces only; in 19 other samples histamine was not detected. (25 references.) G. R. WHALLEY.

Potable alcoholic liquids. The A.P.V. Co. Ltd. (Inventor: M. G. Royston) (B.P. 1,071,428, 9.9.64).—The continuous fermentation for beer production uses a hopped wort concentrate as feed of 8–15% by wt. of solids and the yeast cell population is dynamically stabilised by pulsing. S. D. HUGGINS.

Fruits, Vegetables, etc.

Identification and organoleptic evaluation of compounds in Delicious apple essence. R. A. Flath, D. R. Black, D. G. Guadagni, W. H. Mcfadden and T. H. Schultz (*J. agric. Fd Chem.*, 1967, **15**, 29–35).—High-resolution GLC revealed the presence of 56 compounds that could be identified by their retention time and by mass spectrometry. The main components judged to be responsible for apple aroma were Et 2-methyl butyrate, hexanal, and 2-hexenal. (36 references.) P. S. ARUP.

Storage of apples for use in production of fruit juice. M. Y. Guinot (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 442–448).—Successful storage over 2 months was not possible without previous elimination of all unsound fruits. Storage in silos (deep or shallow) proved unsatisfactory. Storage in crates under shelter gave satisfactory results that were as good as those obtained with storage in a controlled atm. P. S. ARUP.

Volatile components of apricot. C. S. Tang and W. G. Jennings (*J. agric. Fd Chem.*, 1967, **15**, 24–26).—These components, prepared by several methods, were found by GLC to contain myrcene, limonene, *p*-cymene, terpinolene, trans-2-hexenol, α-terpineol, geraniol, geranial, 2-methylbutyric and acetic acids, linalool, the *cis*- and *trans*-isomers of an epoxydihydrolinalool, γ-octalactone, and γ-decalactone. (16 references.) P. S. ARUP.

Cold storage of Muscat de Hambourg grapes packed in polythene and in presence of continuous and controlled emission of sulphur dioxide. A. Paulin (*Fruits, Paris*, 1966, **21**, 589–596).—The previous experiment (cf. *ibid.*, 127) was repeated on a larger scale with equally satisfactory results. In this case four or five sachets containing aq. $K_2S_2O_5$ were placed in each bag of 3 kg of grapes. P. S. ARUP.

Storage and technology of ripening of banana. Precise reproducible definition of quality of mature banana ripened in its bunch in storage. A. Tsalpatouros (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 438–442).—A method is proposed for the assessment of quality by means of a graph (rectilinear) drawn with the % of fruits at a certain stage of yellowing as ordinates and with the times of observation (at 6-h intervals) as abscissae. The angle formed by the graph with the zero ordinate (representing 6 h before the first observation) is suggested as an index of quality and as a guide for fixing the most suitable time for retailing. P. S. ARUP.

Action of various volatile products emitted by bananas during ripening on growth of *Gloeosporium Musarum*. S. Razakamanantsoa (*Fruits, Paris*, 1966, **21**, 597–604).—Among these substances (cf. McCarthy *et al.*, *J. Sci. Fd Agric.*, 1964, **15**, 1260) isoamyl butyrate and isoamyl isovalerianate inhibited the growth of the fungus on a nutrient medium and on the fruit. The yellowing of the fruit was retarded by the former compound but promoted by the latter. P. S. ARUP.

Volatile compounds of roasted groundnuts. Major monocarbonyls and some non-carbonyl components. M. E. Mason, Bobby Johnson and M. C. Hamming (*J. agric. Fd Chem.*, 1967, **15**, 66–72).—Condensates were prepared by high-vac. distillation and low-temp. trapping. TLC separation of the DNPH deriv. of the carbonyl compounds and GLC combined with mass spectrometry of the separated and regenerated carbonyl compounds revealed the presence of acetaldehyde, isobutyraldehyde, **(I)**, benzaldehyde, and phenylacetaldehyde **(II)**, (tentatively) 2- and 3-methylbutanol **(III** and **IV)**, and 3-methyl-2-butanone. The observed presence of EtOAc, toluene, and *N,N*-dimethylformamide in the regenerated carbonyl compounds remains unexplained. **I, III,** and **IV** were major components; the importance of **II** as a flavouring agent was noted. P. S. ARUP.

Storage of bananas. United Fruit Co. (B.P. 1,071,586, 11.2.55. U.S., 23.2.65).—Bananas are stored, for delayed ripening, in a sealed container the atm. of which has a reduced O_2 concn. and increased CO_2 concn. Thus, the total vol. of normal atm. air (5830 cc.) in a sealed polyethylene bag containing mature green bananas, is reduced to 5020 cc. (equiv. 110·3 cc. O_2/kg banana content), the mouths sealed with a tight twist held by elastic bands and this, and a control, left at 60°F and the O_2 and CO_2 content determined after 4, 8, 12 and 24 h (3% O_2 after 24 h and 3–4% CO_2). After 6 days the samples are unsealed and the bananas ripened in air containing 0·05–0·1% C_2H_4 at 64°F for the first 24 h, followed by ventilation at 58–60°F. After 6–7 days, the bananas are in a commercially excellent fully ripened condition with a good flavour, aroma and texture. S. D. HUGGINS.

Tea, coffee, cocoa

Chemistry of tea. Volatile constituents. H. A. Bondarovich, A. S. Giammarino, J. A. Renner, F. W. Shephard, A. J. Shingler and M. A. Gianturco (*J. agric. Fd Chem.*, 1967, **15**, 36–47).—Recent advances in the knowledge of tea polyphenols are reviewed. By means of preparative followed by analytical GLC, 83 constituents of the volatile fraction of tea were identified. GLC data are tabulated for these compounds. (35 references.) P. S. ARUP.

New developments in coffee aroma research. F. Gautsch, M. Winter, Y. Flament, B. Willhalm and M. Stoll (*J. agric. Fd Chem.*, 1967, **15**, 15–23).—Starting from a mol.-distillate of expelled coffee oil, a large no. of constituents were obtained from GLC analysis of fractions of the oil obtained by distillation liquid–liquid partition, and column chromatography. Descriptions are given of the identification of 22 constituents (including terpenoids and deriv. of furan and Furan-S) by u.v., i.r., and mass spectrometry, supplemented by synethic work; these compounds include 20 new constituents. (17 references.) P. S. ARUP.

Milk, Dairy Products, Eggs

Milk production response of dairy cows fed high-moisture grass silage. 1. Effect of varying levels of hay, and concentrate. F. R. Murdock and A. S. Hodgson (*J. Dairy Sci.*, 1967, **50**, 57–61).—Feeding a higher level of concentrate (0·6 kg/kg 4% FCM over 8·16 kg) to lactating cows, offered high-moisture grass silage *ad libitum*, resulted in the production of significantly more 4% FCM and higher % of milk protein and solids-not-fat than when the cows were fed a lower level of concentrate (0·3 kg/kg 4% FCM over 8·16 kg). Milk production and composition was not significantly affected by varying the level of hay fed between 0·5 and 1·0 kg/100 kg body wt. Increasing the concentrate level was more effective than increasing the hay level for maintaining high dry matter intake and for meeting TDN requirements. (17 references.) M. O'LEARY.

Combined effects of type of forage fed, of concentrate ingredients, and of pelleting concentrates on rumen fermentations, milk yield, and milk composition of dairy cattle. G. E. Hawkins and J. A. Little (*J. Dairy Sci.*, 1967, **50**, 62–67).—Feeding trials showed that animals grazed on oat pastures had a higher concn. and molar % of volatile fatty acids in rumen fluid than had those fed maize silage. A similar pattern was revealed with cows fed maize concentrates and cows fed maize distillers dried grains with solubles (CDDGS). Pelleting of maize concentrates decreased energy per kilogram of milk and milk fat % and increased milk yield. This effect was magnified with cows on oat pasture compared with maize silage. It is considered that the effects of grazing oat pasture and of feeding pelleted maize on the molar % of VFA in the rumen fluid, on milk fat %, on energy per g of milk, and on milk yield were additive. Cows fed CDDGS concentrate produced more milk energy than those fed maize concentrate. (22 references.) M. O'LEARY.

Comparison of the accuracy of the USDA lactometer method with the Mojonnier method for determining total solids of milk. H. A. Morris, S. T. Coulter and C. E. Gates (*J. Dairy Sci.*, 1966, **50**, 96–97).—Herd samples of milk (2,076) were analysed for total solids by both the Mojonnier and USDA lactometer methods. A comparison of the results obtained by the two methods revealed a correlation coeff. of 0·989. A regression equation $Y = 0·2971 + 0·988$ X was formulated, Y being the Mojonnier reading and X the lactometer reading. M. O'LEARY.

Effect of oxytocin injections on mastitis-screening tests and milk composition. R. P. Natzke and L. H. Schultz (*J. Dairy Sci.*, 1967, **50**, 43–46).—Residual milks obtained following injection with 10 i.u. of oxytocin were significantly higher in fat, protein, % chloride, mastitis screening test reactions, and leucocyte concn. than normal milk. No significant difference in lactose content, calculated on a fat free basis, was detected. Injection of oxytocin prior to milking for five consecutive milkings resulted in a significant reduction in % fat and protein and in leucocyte concn. of milk obtained during the post-treatment period. Both milk production and % chloride increased significantly. A further experiment indicated that whereas the reduction in leucocyte concn. occurred only in the residual milk the decrease in fat % and the increase in milk yield occurred in both the normal and residual fractions. (11 references.) M. O'LEARY.

Investigations on the use of the Milko-tester for routine estimation of fat content in milk. M. F. Murphy and T. C. A. McGann (*J. Dairy Res.*, 1967, **34**, 65–72).—Comparison of Milko-tester Mark II and Gerber readings on 894 samples of herd milk showed that the equation G = M had a standard error of estimate of ±0·071, compared with a standard error of 0·070 for the regression equation G = 1·02M–0·075. With 1133 samples of individual milks the equation G = M had a standard error of ±0·107 whereas the regression equation G = 1·03 M–0·186 had an accuracy of ±0·070. The equation G = M–0·077 had a standard error of ±0·071. M. O'LEARY.

Fatty acid composition of bovine milk fat as influenced by intravenous infusion of propionate or glucose. L. J. Fisher, J. M. Elliot and D. A. Corse (*J. Dairy Sci.*, 1966, **50**, 53–56).—Experiments with six Jersey cows showed that intravenous infusion of propionate caused decrease in synthesis of all major milk fatty acids except 16 : 0, whereas glucose increased the secretion of 10 : 0 and 12 : 0, decreased that of 18 : 0, 18 : 1, and 18 : 2, and had little effect on 14 : 0 and 16 : 0. A comparison of these results with changes in the fatty acid pattern associated with ration induced milk fat depression indicated the latter to be inadequately explained by the glucogenic effect of propionate. (13 references.) M. O'LEARY.

Oxidation and utilisation of palmitate, stearate, oleate, and acetate by mammary gland of the fed goat in relation to their overall metabolism, and rôle of plasma phospholipids and neutral lipids in milk-fat synthesis. E. F. Annison, J. L. Linzell, S. Fakakerley and B. W. Nichols (*Biochem. J.*, 1967, **102**, 637–647).—During infusion of [^{14}C] stearate, [^{14}C] oleate, [^{14}C] palmitate, and [^{14}C] acetate into fed lactating goats the rates of entry of these acids into the circulation were 0·18, 0·28, 0·42, and 4·2 mg/min./kg body wt., respectively. Acetate accounted for 23% of the total CO_2 produced by the goat and contributed to oxidative metabolism of mammary gland to approx. the same extent. The corresponding values for each of the long chain acids were < 1%. There were no significant arteriovenous differences of phospholipids, sterol, or sterol esters. There were large arteriovenous differences of plasma triglycerides, and their fatty acid composition showed marked changes across the gland; the proportions of palmitate and stearate decreased and of oleate increased. There was a substantial uptake of plasma free fatty acids (*FFA*) and a roughly equivalent release from mammary tissue. The *FFA* of milk were similar in composition and radioactivity to milk triglyceride fatty acids and were quite unlike plasma *FFA*. Large amounts of oleic acid were formed from stearic acid. The results show that the

milk C_4–C_{14} fatty acids are formed largely from blood acetate, that palmitate is derived partly from acetate and partly from plasma triglyceride which is almost the sole precursor of oleate and stearate. J. N. ASHLEY.

DDT contamination in milk following a single feeding exposure. D. G. Crosby, T. E. Archer and R. C. Laben (*J. Dairy Sci.*, 1967, **50**, 40–41).—The DDT level in blood and milk showed a very large but transient increase when a single dose of 14 mg/kg of the insecticide was administered to dairy cows. 12 h after administration of the dose DDT content of the milk fat reached 300 ppm. Over the following 36 h it declined rapidly to 20 ppm and thereafter declined gradually to a new-plateau of 6 ppm after 3 weeks. M. O'LEARY.

Factors affecting reliability of catalase test results. I. Sanitisers. H. E. Randolph, J. L. Bucy and T. R. Freeman (*J. Dairy Sci.*, 1967, **50**, 32–34).—Chlorine sanitisers, in concn. of 5 to 50 ppm, significantly affected the amount of O_2 produced in the catalase test. The effect increased with increase in Cl concn and with length of storage of the sample at 4·5° after addition of sanitiser. Iodophos, quaternary ammonium compounds, an acid sanitiser, and a sanitiser containing both KI and chloramine-T did not influence test results. M. O'LEARY.

Evaluating susceptibility of milk to oxidised flavour. W. L. Dunkley and A. A. Franke (*J. Dairy Sci.*, 1967, **50**, 1–9).—Descriptions are given of various procedures for evaluating the susceptibility of milk to oxidised flavour. Procedures described include the thiobarbituric acid (TBA) test, flavour scoring, prevention of Cu contamination, and heat treatment of milk samples. The interpretation of the results of the various tests is discussed. (18 references.) M. O'LEARY.

Compound responsible for metallic flavour in dairy products. II. Theoretical consideration of the mechanism of formation of oct-1-en-3-one. R. A. Wilkinson and W. Stork (*J. Dairy Res.*, 1967, **34**, 89–102).—The metallic flavour component, oct-1-en-3-one, is considered to be derived from linolenic and/or arachidonic acid. Possible mechanisms for its formation in a vacuum packed butterfat–antioxidant–synergist mixture, and in open packed worked cream and butter are discussed. (38 references.) M. O'LEARY.

Preparation and evaluation of butter culture flavour concentrates. R. C. Lindsay, E. A. Day and L. A. Sather (*J. Dairy Sci.*, 1967, **50**, 25–31).—A description is given of the prep. of synthetic culture concentrates for butter, Cottage cheese, sour cream, and buttermilk, using the following flavour compounds: diacetyl, acetaldehyde, dimethyl sulphide, AcOH, and lactic acid. Flavour panel evaluations showed that artificially flavoured products were as acceptable as good quality naturally flavoured products. (24 references.) M. O'LEARY.

Efficiency of laboratory steam deodorisation in quantitative recovery of aliphatic δ-lactones from butter-oil. P. S. Dimick and N. J. Walker (*J. Dairy Sci.*, 1967, **50**, 97–99).—A description is given of a laboratory procedure for the recovery of aliphatic δ-lactones from butter-oil. Five h deodorisation with at least 100 ml per hour of steam, measured as aq. distillate, was shown yielded the max. amount of δ-C8, δ-C10, δ-C12, and δ-C14. (11 references.) M. O'LEARY.

Effect of concentrating milk on the fat retention property of the cheese curd. L. A. Mabbitt and G. C. Cheeseman (*J. Dairy Res.*, 1967, **34**, 73–83).—Laboratory experiments showed that concentration of milk to ½ of its vol. resulted in a considerable increase in fat retention by rennet curd formed under agitation. Changes were shown to occur in the composition of the milk fat globule membrane due to incorporation of milk protein and it is suggested that casein, bound to the globule during concentration of the milk, forms links with casein of the coagulated micelles in the curd. (10 references.) M. O'LEARY.

Major free fatty acids of Feta cheese. C. Efthymiou (*J. Dairy Sci.*, 1967, **50**, 20–24).—The dominant free fatty acid of Feta cheese was AcOH ranging from 25 to 46% in Teleme and mild flavoured varieties and from 5 to 12% in sharp flavoured varieties. The major free fatty acids occurred in concn. approximating the ratios of their esterified counterparts in milk fat, with the exception of palmitic and stearic which were present in lower concn. (12 references.) M. O'LEARY.

Brick cheese : pH, moisture, and quality control. W. V. Price and H. J. Buyens (*J. Dairy Sci.*, 1967, **50**, 12–19).—The moisture content of Brick cheese, 8 weeks after making, was increased by using less wash water, longer knives and short exposures to water during manufacture. pH of the cheese was lowered by similar treatment. Prolonging the washing decreased cheese quality, especially where large vol. of water were used on small curd particles. (12 references.) M. O'LEARY.

Evidence for kynurenine in milk. O. W. Parks, D. P. Schwartz, K. Nelson and C. Allen (*J. Dairy Sci.*, 1967, **50**, 10–11).—By means of alkaline degradation studies on the basic fraction of milk serum, obtained by negative pressure dialysis and ion-exchange extraction, kynurenine was present in concn. of about one μM per litre of raw milk serum. Kynurenine concn. decreased on heating of the milk to temp. exceeding 93·3° for 15 sec. as kynurenine is a potential precursor to α-aminoacetophenone, an off-flavour component of stale dry and concentrated milk. M. O'LEARY.

Edible Oils and Fats

Determination of the penetration of oils into fried foods by fluorescence. M. Monteoliva, J. D. Pérez Soler, C. Ibáñez and G. Varela (*Grasas Aceit.*, 1967, **18**, 209–213).—The penetration of oil into frying potatoes is measured by examination of a section 3 mm. thick in a microscope illuminated by reflection with u.v. light. Differences between various oils were much more clearly shown up than by a Soxhlet extraction method. Soya and groundnut oils penetrated further than olive or cottonseed oils. L. A. O'NEILL.

Stability of oils and fatty foods. VI. Antioxidant activity of rice bran antioxidants, gossypol, α-tocopherol and some industrial inhibitors. M. H. Chahine and A. S. Radwan (*Grasas Aceit.*, 1967, **18**, 216–221).—The stability action of six antioxidants at various levels for rice bran methyl esters at 60° was compared by measurement of peroxide values. There was a direct relationship between antioxidant concn. and stability time. At the 0·1% level, butyl hydroxy toluene and santoquin were the strongest inhibitors. Rice bran antioxidants were less effective, but showed a synergistic action with α-tocopherol. L. A. O'NEILL.

Heated fats II. Chemical and physical studies on frying cottonseed oil. M. H. Chahine, M. S. Mameesh and N. M. El-Hawwary (*Grasas Aceit.*, 1967, **18**, 149–155).—The physical and chemical changes in replenished cottonseed oil (*a*) heated for 204 h at 200° in presence of air and (*b*) used for frying taamiah (an Egyptian dish containing beans, spices etc.) for 204 h at 200°, were compared. The general behaviour was similar to that normally found for thermally oxidised oils, the total O content rising from 11·4 to 13–14%, but there were some differences between the two conditions. Under (*a*) the content of unconjugated diene acids fell steadily to 9% and the conjugated diene content rose to 5%, whereas under (*b*) the unconjugated dienes after falling to 16% started to rise, and the conjugated dienes after rising to ~3% remained constant. (In English). L. A. O'NEILL.

Heated fats III. Toxicity of cottonseed oil used in frying of broad bean cubes (taamiah). M. S. Mameesh, F. H. Abdin, M. H. Chahine and N. M. El-Hawwary (*Grasas Aceit.*, 1967, **18**, 213–215).—The oil samples taken after various intermittent heating periods up to a total of 204 h at 195°, were incorporated into the diet of rats, fed for 17 weeks. All the rats survived, and the pathological symptoms were comparatively mild, in contrast with those found when rats were fed laboratory-heated fats. L. A. O'NEILL.

Fatty acid composition of olive oils from Puglia II. Oil from olives of the 'Cima di Mola' variety. A. Cucurachi (*Riv. ital. Sostanze grasse*, 1967, **44**, 260–266).—Gas chromatographic analysis of 158 samples of oil, harvested from 1956–66, gave the following average % composition: C_{16} 13·8, $C_{16:1}$ 1·8, C_{18} 2·1, $C_{18:1}$ 69·6, $C_{18:2}$ 11·4, $C_{18:3}$ 0·6, C_{20} 0·3, $C_{20:1}$ 0·2. The I val. tended to vary from year to year, averages ranging from 83·7 to 86·6. L. A. O'NEILL.

Molecular distillation as a method of separation in the vegetable oil industry. J. Hollo and E. Kurucz (*Riv. ital. Sostanze grasse*, 1967, **44**, 249–259).—Applications of the process are surveyed and experiments with sunflower oil reported. Oil from various sources and of low, medium and high acidity has been molecularly distilled over a temp. range 90°–250° and the chemical characteristics of the various distillate and residue fractions measured. (23 references.) L. A. O'NEILL.

Extraction of oil from a cake of ground olives. Charles L. Lang and Albert L. Petit (B.P. 1,071,830, 21.12.64. Fr., 3.1.64).—In a process for extracting oil from olives, by sorting, stripping, washing and crushing the olives and the cake from the crushing operation is

homogenised and then centrifuged, the oil included in the water used for the washing operation is recovered as well as the oil included in the aq. juices separated by centrifugation and in the discharges from the centrifugal machine. E. ENOS JONES.

Meat and Poultry

Ascorbate and tripolyphosphate in cured, cooked, frozen pork. M. W. Zipser and B. M. Watts (*J. agric. Fd Chem.*, 1967, **15**, 80–82).—Further to previous work (cf. *ibid.*, 1964, **12**, 109) the results of storage experiments indicated the desirability of increasing the % of Na ascorbate (used together with tripolyphosphate) from 0·54 to 1·08. The loss of ascorbic acid during storage was serious only in samples containing both NaCl and $NaNO_2$. Tripolyphosphate was effective only in its capacity to increase the effectiveness of ascorbate. P. S. ARUP.

Spices, Flavours, etc.

Olfaction, musk odour, and molecular properties. E. T. Theimer and J. T. Davies (*J. agric. Fd Chem.*, 1967, **15**, 6–14).—An account is given of the theory of olfaction. From a study of >50 compounds possessing musk odour, relationships are deduced between musk odour intensity, adsorption and desorption rates governing the penetration of the compound to the mucus membrane, and the mol. dimensions of the compound. (28 references.). P. S. ARUP.

Aspects of functional groups and flavour. K. Kulka (*J. agric. Fd Chem.*, 1967, **15**, 48–57).—Dependence of flavour on chemical constitution is demonstrated by a no. of examples. (35 references.) P. S. ARUP.

TLC-spectrophotometric analysis for neutral fraction flavones in orange peel juice. L. J. Swift (*J. agric. Fd Chem.*, 1967, **15**, 99–101).—Flavones separated and determined by these methods in the neutral fraction (cf. *ibid.*, 1965, **13**, 282) were tangeretin, tetra-*O*-methyl-scutellarein, 3,5,6,7,8,3′,4′-heptamethoxyflavone, nobiletin, and sinensitin (5,6,7,3′,4′-pentamethoxyflavone). P. S. ARUP.

Solubilised fumaric acid compositions. Allied Chemical Corp. (B.P. 1,071,501, 12.7.65. U.S., 10.7.64).—Used to replace citric and tartaric acids in instant beverage compositions, the claimed compositions comprise fumaric acid particles of ⩾10 microns and a water-sol. stable surfactant, e.g. 0·1–1% lauryl Na sulphate, dimethyldecyne diol. Thus, 100 parts fumaric acid in 1500 parts water (95–99°) are poured rapidly into 1000 parts ice and the resultant crystalline slurry is agitated, cooled to 20° and filtered. The cake is dried at 50° *in vacuo* to a moisture content of <1% and the dried product passed through a fine screen to <10 microns in size. If added to water (5°) solution occurs in 5 min., but a small amount of scum remains which can be avoided if 1·5 parts of a mixture of tetramethyldecyne diol and an alkyl Ph ether are added to the original water at approx. 95°. S. D. HUGGINS.

Pesticides in Food

Use of paper partition and thin-layer chromatography for identification of active ingredient in Dursban insecticide and its possible metabolites. Grant N. Smith and Faye S. Fischer (*J. agric. Fd Chem.*, 1967, **15**, 182–186).—Values of R_F obtained by paper chromatography with eight (descending) solvent systems, and by TLC with three systems are tabulated for Dursban and eight possible metabolites. Development in darkness and comparison with R_F reference standards on the same chromatogram are recommended. Descriptions are presented of suitable revealing agents for visual inspection, and revealing techniques employing u.v. light and (for radioactive standard compounds) autoradiography. (15 references.) P. S. ARUP.

Extraction and gas chromatographic analysis of chlorinated insecticides from animal tissues. P. W. Sachsenbrecker and D. J. Ecobichon (*J. agric. Fd Chem.*, 1967, **15**, 168–170).—Extraction of seven of these insecticides from various poultry tissues is carried out by blending with a mixture of $COMe_2$ and MeCN (with Na_2SO_4), and the extract (diluted to contain a 2·5 : 1 ratio of MeCN to H_2O) is cleaned by passage through a column of polyethylene-coated Al_2O_3, followed by solvent partition between hexane and MeCN. A GLC process is described for the detection and determination of the insecticides in comparison with standards. Recoveries were for six of the insecticides, 84·4–100·3%, and for *p,p*-DDD 78·7–83·2%. Min. detectable amounts were 0·002–0·006 ppm. P. S. ARUP.

Determination of 2,4-D in animal tissues. Donald E. Clark, Fred C. Wright and Lawanda M. Hunt (*J. agric. Fd Chem.*, 1967, **15**, 171–173).—The process described includes extraction of the tissue with hot EtOH, submission of the matter thus obtained (less the fat) to papain digestion, extraction of the 2,4-D from the mixture with Et_2O, and its hydrolysis to 2,4-dichlorophenol (**I**) by heating with pyridine hydrochloride. The **I**, purified by steam-distillation, is submitted to GLC with electron-capture detection on a column of Chromoport XXX supporting a mixture of HI-EFF and H_3PO_4. Recoveries at the 0·05 ppm level were 68–92% for 5 tissues. Small blank values were obtained from a natural constituent having the same retention time as **I**. P. S. ARUP.

Fluorimetric method for determining residues of terephthalic acid in chicken tissues. P. A. Giang, M. S. Schechter and L. Weissbecker (*J. agric. Fd Chem.*, 1967, **15**, 95–98).—Terephthalic acid (**I**) is extracted by blending the tissue with conc. aq. NH_3 plus MeOH; interfering matter is removed by pptn. together with $Ba(OH)_2$ from aq. $BaCl_2$ and KOH, and by extraction of the alkaline filtrate with $CHCl_3$ followed by Et_2O. The **I**, extracted from the acidified solution with Et_2O plus C_6H_6 is nitrated, and the nitro-**I** is reduced to amino-**I**, the fluorescence of which is used as a measure of **I**. The method is sensitive to 0·1 ppm of **I**. P. S. ARUP.

Method of determining Bromacil in soils and plant tissues. V. A. Jolliffe, B. E. Day, L. S. Jordan and J. D. Mann (*J. agric. Fd Chem.*, 1967, **15**, 174–177).—Bromacil (**I**) is extracted from soils or plant tissues by shaking with aq. $(NH_4)_2SO_4$ plus NaOH during 5 or 30 min., respectively. The alkaline solutions are extracted with hexane, and the **I** is extracted, after acidification, into EtOAc. After evaporation of the solvent the residue is dissolved in $COMe_2$ for GLC analysis with electron-capture detection. Samples and standards are injected alternately. The peak-height *vs* **I** content graph is rectilinear for amounts of **I** up to 7 ng. The min. detectable amounts were (ppm) 0·01 for soils and 0·1 for crop residues. Recoveries of **I** were 90–110% from soils and 60–80% from crop residues. Losses of **I** from moist soils were 31–65% after 6 months' storage. P. S. ARUP.

Determination of ethyl-*p*-nitrophenylthionobenzene phosphonate (EPN) residues by electron-capture gas chromatography. J. J. Kirkland and H. L. Pease (*J. agric. Fd Chem.*, 1967, **15**, 187–191).—Extraction is accomplished by shaking for 2–3 min. samples of coarsely diced fruits or vegetables with three successive portions of benzene. Aliquots of the combined extracts are used directly for GLC with electron-capture detection in the pulsed potential mode. Recoveries from eight fruits or vegetables averaged 104% over a range of 0·2–2·1 ppm without interference from eight S- and P-containing pesticides, and with background interference of <0·01 ppm. (14 references.) P. S. ARUP.

Catalytic hydrolysis of organic phosphate pesticides by copper II. M. M. Mortland and K. V. Raman (*J. agric. Fd Chem.*, 1967, **15**, 163–167).—Dursban, Diazinon, Ronnel, and Zytron were shown by means of u.v. spectrometry to undergo catalytic hydrolysis when treated in aq. MeOH solution at pH 5–6 with $CuCl_2$ (0·004 or 0·008 μmole per 50 ml). Similar effects were obtained with Cu-montmorillonite clays. Chlorides of six other metals had no catalytic effects. Possible catalytic mechanisms are suggested. P. S. ARUP.

Detector cell for measuring picogram quantities of organophosphorus insecticides, pyrethrin synergists, and other compounds by gas chromatography. W. N. Bruce (*J. agric. Fd Chem.*, 1967, **15**, 178–181).—The construction and operation are described in detail of a cell, the performance of which excels that of the usual electron-capture cells. P. S. ARUP.

Multiple insecticide residue determination using column chromatography, chemical conversion, and gas–liquid chromatography. W. W. Sans (*J. agric. Fd Chem.*, 1967, **15**, 192–198).—In order to simplify the GLC analysis of multiple residues, a process has been devised by which the insecticides are fractionated into groups on a Florisil column with the use of four different solvent systems, for separate GLC analysis; two columns containing Dow 11 and QF-1 as liquid stationary phases, respectively, are recommended. For specific identification in doubtful cases, the insecticides from the Florisil fractions are converted into their oxidation products by treatment with a mixture of CrO_3 in AcOH or dehydrochlorinated by treatment with KOH in EtOH; these products are examined by GLC. The oxidation procedure destroys some natural interfering

compounds. Retention times on the two columns are tabulated for 32 insecticides. (17 references.) P. S. ARUP.

Anaerobic degradation of selected chlorinated hydrocarbon pesticides. D. W. Hill and P. L. McCarty (*J. Wat. Pollut. Control Fed.*, 1967, **39**, 1259–1276).—Many chlorinated hydrocarbon pesticides can be degraded under suitable biologically active, anaerobic conditions and, in most cases, more rapidly than under corresponding aerobic conditions. The pesticides studied and their extractable degradation products can be ranked in the following approx. order of increasing persistence under anaerobic conditions: lindane, heptachlor, endrin, DDT, DDD, aldrin, heptachlor epoxide and dieldrin. The anaerobic degradation of lindane is greatly increased by increases in temp. and biological activity. Adsorption of chlorinated hydrocarbon pesticides is greater on algae than on bentonite clay and is inversely related to the solubilities of the pesticides. J. M. JACOBS.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Fractionation and characterisation of alcohol extractables associated with soya-bean proteins. Non-protein components. A. M. Nash, A. C. Eldridge and W. J. Wolf (*J. agric. Fd Chem.*, 1967, **15**, 102–108).—The proteins isolated by isoelectric pptn. from an aq. extract of defatted soya-meal were separated by column-chromatography on diethylaminoethyl cellulose and preparative TLC on silicic acid. The results showed that in addition to phosphatidyl choline and phosphatidyl ethanolamine, the known soya-bean constituents saponins, sitosteryl glycoside, and genistein are also associated with the proteins. (31 references.) P. S. ARUP.

Enzymic modification of extractability of protein from soya-beans, *Glycine max*. K. M. Abdo and K. W. King (*J. agric. Fd Chem.*, 1967, **15**, 83–87).—Processes are described for the prep. of enzymic material from *Pestalotiopsis Westerdijkii* and the use of the material for rendering extractable the protein remaining in the residue after the prep. of soya-bean milk by the usual method. The yield of proteins from the beans is thus increased from 74% to 95%; the nutritional value of the total extracted proteins is improved; about 50% of the crude fibre is solubilised. (22 references.) P. S. ARUP.

Leaf protein concentrate prepared by spray-drying. G. H. Hartman, jun., W. R. Akeson and M. A. Stahmann (*J. agric. Fd Chem.*, 1967, **15**, 74–79).—The powder obtained by spray-drying the press-juice of fresh lucerne and pea vines was, in comparison with commercial dried lucerne products equal or superior as regards the biological value of the protein, and vitamin and growth-factor content. The powder was suitable as feed for non-ruminant animals. A product suitable for human consumption was prepared by removal of chlorophyll with 95% EtOH. (41 references.) P. S. ARUP.

Sodium-plus-potassium ion-activated adenosine triphosphatase of cerebral microsomal fractions: treatment of disrupting agents. J. R. Cooper and H. McIlwain (*Biochem. J.*, 1967, **102**, 675–683).—Na^+ plus K^+ stimulated ATPase of ox brain microsomal prep. is inactivated or loses some activity when treated with 2–8 M-urea, and turbidity of the suspensions is diminished. Low concn. (approx. 2·5 mM) of Na ATP in presence of urea partially or completely protects ATPase but does not affect turbidity. With 3 M urea and 2·5 mM NaATP the activity is increased by 40%. The protective effect is sp. to NaATP. Turbidity of suspensions is decreased by ultrasonic irradiation and up to 55% of the protein and ATPase activity are no longer deposited by centrifuging at $4·5 \times 10^6$ g-min. (these changes are unaffected by NaATP). Pptn. of the supernatant with $(NH_4)_2SO_4$ then gives a 3–5 fold enrichment of (Na^+K^+) ATPase activity. $NaBH_4$ and Me_2SO also decrease turbidity of the suspension but do not enrich the ATPase. The results are discussed in relation to the mechanism of (Na^+K^+) ATPase. J. N. ASHLEY.

Biochemical studies with new cytotoxic immunosuppressive agent, 3-acetyl-5-(4-fluorobenzylidene)2,5-dihydro-4-hydroxy-2-oxothiophen (I.C.I. 47776). T. J. Franklin and B. Higginson (*Biochem. J.*, 1967, **102**, 705–711).—The compound strongly inhibits protein and nucleic acid synthesis and, to a less extent, respiration in lymph-node cells and Landschütz ascites tumour cells *in vitro*. Activity *in vitro* decreases with increase of pH of the medium and is inversely α concn. of serum in the medium. The compound has no effect on incorporation of leucine by a cell-free prep. of ascites cells containing ATP and phosphoenolpyruvate. It stimulates glycolysis in suspensions of ascites cells in presence of glucose but has no effect on glycolysis in suspensions of rat lymph-node cells. The drug markedly decreases ATP concn. in ascites only in absence of glucose. Inhibition of protein synthesis is only partially reversed by glucose. I.C.I. 47776 probably inhibits synthesis of protein and nucleic acid indirectly by acting as a mitochondrial poison. The relation of the *in vitro* activity to the cytotoxic and immunosuppressive action *in vivo* is discussed. J. N. ASHLEY.

Properties of ribosomal proteins from two mammalian sources. P. Cohn (*Biochem. J.*, 1967, **102**, 735–741).—Ribosomal protein fractions are prep. from rabbit reticulocytes and rat liver by extraction of the ribosomes with 0·2 N-HCl or guanidine hydrochloride and subsequent dialysis. Treatment with 0·2 N-HCl for 2·5 h or less dissolved 46–54% of the proteins, which are richer in arginine and lysine and in *N*-terminal alanine groups, and poorer in aspartic acid and glutamic acid and in *N*-terminal glycine groups than are the acid-insol. proteins. Protein fractions from guanidine hydrochloride extracts of rat liver ribosomes are usually more basic than are those from rabbit reticulocytes. Ratios of lysine: arginine of fractions from guanidine extracts are appreciably higher for proteins from rabbit reticulocytes than from rat liver. J. N. ASHLEY.

Amino-acid sequences of peptides from tryptic digest of urea-soluble protein fraction (U.S.3) from oxidised wool. M. C. Corfield, J. C. Fletcher and A. Robson (*Biochem. J.*, 1967, **102**, 801–814).—A tryptic digest of the U.S.3 fraction is separated into 32 peptide fractions by cation-exchange resin chromatography. Most of the fractions are resolved into their component peptides by cation-exchange resin chromatography, paper chromatography, and paper electrophoresis. The amino-acid compositions of 58 of the peptides present in the largest amounts in the digest are determined, and the amino-acid sequences of 38 of these are elucidated and those of six others are partially determined. The results show that the parent protein in wool from which U.S.3 is derived has min. mol. wt. 74000. The structures of wool proteins are discussed in the light of these results. J. N. ASHLEY.

Procedure for quantitative analysis of sulphur amino-acids of rat tissues. M. K. Gaitonde and G. E. Gaull (*Biochem. J.*, 1967, **102**, 959–975).—The method involves the passage of an extract of the tissue through a serial assembly of ion-exchange resins in the order: Dowex 2 (Cl^- form), Dowex 1 (CO_3^{2-} form), Amberlite CG-50 (H^+ form) and Zeo-Karb 225 (H^+ form). Groups of S amino-acids are eluted separately from each column, and individual S compounds are obtained by one- or two-dimensional chromatography. The recovery of S compounds is 91–106%. J. N. ASHLEY.

Stabilisation of vitamin A in the Red Sea shark liver oil. M. H. Chahine, M. S. Mameesh and F. A. El-Shobaki (*Grasas Aceit.*, 1967, **18**, 159–162).—The times required for 20% and 50% loss of vitamin A on heating shark liver oil at 50° in absence and presence of various antioxidants and synergists have been compared. Strong inhibitory effects were shown by NDGA, THBP and to a lesser extent EMQ at 0·04% concn. in presence of syngergists (0·03%), of which ascorbyl palmitate was more effective than citric acid. (In English.) L. A. O'NEILL.

Metabolism of vitamin A. J. Ganguly (*J. scient. ind. Res.*, 1966, **25**, 498–500).—A report on vitamin A covering absorption and storage of vitamin A in the liver, production of vitamin A acid, and the metabolic functions of the vitamin is given. Also considered are uses of vitamin A in protein malnutrition. Mention is made of the development of the Wakil-Ganguly scheme of fatty acid biosynthesis. (68 references.) J. LAMBORN.

Content of tocopherols in oils from different varieties of hazel nuts. J. Baraud, R. Bernhard, C. Cassagne and L. Genevois (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 433–438).—The α-tocopherol content of the oils (mg/kg), determined by the method of Flanzy and Dubois, ranged from 335 to 424 for the variety *Corylus avellana* (five samples), from 600 to 683 for *C. maxima* (four samples), and from 602 to 690 for two hybrid varieties. The oil is of particular value in view of its unusually high content of tocopherol. P. S. ARUP.

Intermittent administration of tocopherol to cows as an approach to increasing oxidative stability of milk. W. L. Dunkley, H. H. Franke, M. Ronning and J. Robb (*J. Dairy Sci.*, 1967, **50**, 100–102).—Experiments, in which up to 6 g of *dl*-α-tocopheryl acetate was administered intramuscularly or orally to cows, indicated no significant increase in the oxidative stability of the resultant milk. (22 references.) M. O'LEARY.

Unclassified

Quantitative determination of glycyrrhizinic acid in liquorice root and extract with acetic anhydride–sulphuric acid. C. H. Brieskorn and W. Wallenstätter (*Arch. Pharm. Berl.*, 1967, **300**, 717–725).—The glycyrrhizinic acid content of liquorice products is obtained by reduction to desoxyglycyrrhetinic acid with PtO/H_2 in glacial AcOH after an initial extraction with $CHCl_3$ and hydrolysis with 6 N H_2SO_4 solution. The resultant acid is then determined photometrically using Huang's modification of the Liebermann–Burchard reaction (T. C. Huang *et al.*, *Anal. Chem.*, 1967, **33**, 1405). The method is not affected by the presence of flavones and chalcones; and the triterpenes and steroids are eliminated by the initial $CHCl_3$ extraction procedure. The glycyrrhizinic acid content is obtained by reference to a standard desoxyglycyrrhetinic acid curve. The glycyrrhizinic acid content of liquorice root is shown to be 2·5 to 4·2% and 4 to 10% in the extracts. G. R. WHALLEY.

New adducts of sulphur dioxide with carbonyls and amino-acids. D. L. Ingles (*Chemy Ind.*, 1967, 1492–1493).—Evidence is given for the formation of a bisulphite adduct of the Schiff base formed by interaction of a carbonyl compound and an amino-acid. Cryst. adducts prepared by reaction of SO_2 in a suspension of glycine and glyoxal, CH_2O and diacetyl, respectively, are strong acids containing bound-SO_2 only. Their i.r. spectra suggest a betaine structure involving amino- and SO_3H groups. Other carbonyls and amino-acids behave similarly but the cryst. adducts cannot always be isolated. Adducts of this type probably exist in foods preserved with SO_2 and may account for part of the bound-sulphite present. (Cf. *J. Sci. Fd Agric.*, 1964, **15**, 176.) J.A.C. ABSTR.

Quantitative determination of neomycin components by paper chromatography. M. K. Majumdar and S. K. Majumdar (*Analyt. Chem.*, 1967, **39**, 215–217).—The neomycins in fermentation broth or similar mixture are adsorbed on Amberlite IRC-50 (NH_4^+ form) and eluted with N-aq. NH_3. The eluate is spotted on Whatman 4 paper, which is then immersed for 12 h in MeOH–Ac_2O–CCl_4 (3 : 2 : 95), and the acetyl neomycins are separated by developing with 1-butanol–water–piperidine (42 : 8 : 1). After complete removal of piperidine and conversion into chloro-deriv. by immersion in Cl_2-CCl_4 reagent, the neomycin spots are cut from the Cl_2-free paper with the guidance of parallel paper-strips (initially sprayed with starch-KI-pyridine reagent). The spots are then extracted for ~30 min. with starch-KI-HCl reagent and the extinctions of the coloured solutions measured at 570 nm. Neomycins B and C and neamine, each in concn. of 4–12 μg, can be determined to within ±4% by reference to one standard graph; ~1 μg of neomycin base can be determined to within ±7%. Interferences and certain quant. aspects are discussed. The method should be applicable to the estimation of other amino-sugar antibiotic complexes and to amino-sugars which are negative to the Morgan–Elson test. W. J. BAKER.

3.—SANITATION, WATER, etc.

Penetration, metabolism, and synergistic activity with carbaryl of simple derivatives of 1,3-benzodioxide in housefly. C. F. Wilkinson (*J. agric. Fd Chem.*, 1967, **15**, 139–147).—Evaluation of the synergistic effects of ring-substituted deriv. (23) of 1,3,benzodioxole showed 5,6-methoxy- and nitro-substituted deriv. to be particularly effective synergists, and much superior to Sesamex. Information as to the penetration of the synergists into flies was obtained by means of electron-capture gas chromatography. (30 references.) P. S. ARUP.

Resistance levels in diazinon-pressured and non-pressured poly-resistant houseflies. A. J. Forgash and E. J. Hansens (*J. econ. Ent.*, 1967, **60**, 1241–1247).—Four wild strains were selected with diazinon, and resistance was stabilised below the peak. Cross resistance was found to ronnel, isolan, dimetilan, malathion, pyrethrum, lethane 384, dimethoate, naled and DDT. The repression of polyresistance was followed. It declined for 95 generations but tended to stop above that of the reference strain. (12 references.) C. M. HARDWICK.

Sterility in houseflies offered a choice of untreated diets and diets treated with chemosterilants. R. L. Fye and G. C. LaBrecque (*J. econ. Ent.*, 1967, **60**, 1284–1286).—Chemosterilants (22) were offered to flies in a sugar syrup together with untreated food. 17 of these compounds produced complete sterility in some or all tests and the rest gave 76–99% sterility. C. M. HARDWICK.

Effect of dietary biotin on reproduction of the housefly. C. A. Benschoter (*J. econ. Ent.*, 1967, **60**, 1326–1328).—Addition to the diet of 0·25–2·0% biotin caused negative correlations of egg fecundity and hatchability with dosage. Continuous feeding at 2% was necessary to produce female sterility but male flies were only slightly affected by it. The degree of sexual maturity did not affect sterilisation. Any repellency apparent seems due to the biotin solvent, NaOH. C. M. HARDWICK.

Penetration, excretion and metabolism of carbaryl in susceptible and resistant German cockroaches. T-Y. Ku and J. L. Bishop (*J. econ. Ent.*, 1967, **60**, 1328–1332).—When carbaryl was applied topically, susceptible and resistant *Blattella germanica* converted it into 1-naphthol and its conjugates. Other trace metabolites were unidentified. Resistant roaches absorbed and retained less, and excreted more carbaryl. The main mechanism of resistance is probably rapid hydrolysis. (14 references.) C. M. HARDWICK.

Infertility induced in male houseflies by sterilant-bearing females. D. W. Meiffert, P. B. Morgan and G. C. LaBrecque (*J. econ. Ent.*, 1967, **60**, 1336–1338).—Male houseflies, exposed for 24 h to females treated directly or on pads with *N,N'*-tetramethylenebis(1-aziridine carboxamide) were 99% sterile for the 15 day test period. At 25% concn. the pads were still as effective after 7 days. C. M. HARDWICK.

Differential susceptibility and resistance to insecticides of coexisting populations of *Musca domestica, Fannia canicularis, F. femoralis* and *Ophyra leucostoma*. G. P. Georghiou (*J. econ. Ent.*, 1967, **60**, 1338–1344).—No large interspecific differences were found when base line data were obtained from little treated areas. The development of resistance to 2-organochlorine, 10 organo-phosphorus and 1 carbamate insecticides in the 4 species showed that *M. domestica* became more resistant than *F. canicularis* and in turn than *F. femoralis*; little resistance appeared in *O. leucostoma*. (14 references.) C. M. HARDWICK.

Oxidative phosphorylation and sensitivity to uncouplers of housefly mitochondria: influence of isolation medium. J. Ilivicky, W. Chefurka and J. E. Casida (*J. econ. Ent.*, 1967, **60**, 1404–1407).—The medium developed contained sucrose, EDTA, citrate, α-keto-glutarate and succinate and was efficient for oxidative phosphorylation, adequate respiratory control and max. sensitivity to uncouplers. (35 references.) C. M. HARDWICK.

Parathion resistance in housefly populations in the Savannah, Georgia, area. W. Mathis, A. D. Flynn and H. F. Schoof (*J. econ. Ent.*, 1967, **60**, 1407–1409).—Flies were collected from three dairies, two farms and a dump and tested for their resistance to parathion-diazinon impregnated cords. Mortalities varied from 21–50% for a 60 min. exposure while a susceptible laboratory strain had 100% mortality. One strain was also tested by immersion in parathion or diazinon solution. C. M. HARDWICK.

Temperature effects on nerve activity in DDT-treated American cockroaches. J. L. Eaton and J. G. Sternburg (*J. econ. Ent.*, 1967, **60**, 1358–1364).—In sensory nerves there was a direct relationship between changing temp. and frequency of DDT-induced trains. The central nervous system showed a negative temp. coeff., through the intoxicated stage. At the prostrate stage, frequency is reduced. In experiments with the six abdominal and thoracic ganglia, it was shown that synaptic transmission may be impaired in different stages of poisoning. The relationship of these factors is discussed. (19 references.) C. M. HARDWICK.

Influence of repellency on the efficacy of blatticides. II. Laboratory experiments with German cockroaches. W. Ebeling, D. A. Reierson and R. E. Wagner (*J. econ. Ent.*, 1967, **60**, 1375–1390).—Using simulated field conditions, *Blattella germanica* were given the opportunity to avoid the toxicant. Among the factors investigated were the addition of adjuvants, toxicants and attractants to boric acid, R.H., light intensity, proximity of food and water, and the effect of substrate on inorg. compounds with or without detergents. The interaction of period of contact and different formulations is discussed. C. M. HARDWICK.

4.—APPARATUS AND UNCLASSIFIED

Automation of the A.O.A.C. photometric phosphomolybdovanadate method for [determining] direct available P_2O_5. C. W. Gehrke, J. H. Baumgartner and J. P. Ussary (*J. Ass. off. analyt. Chem.*, 1966, **49**, 1213–1218).—An automatic procedure, using the Technicon AutoAnalyser, is described. P_2O_5 in the range 2·0–4·5 mg per 100 ml can be determined at a rate of 40 analyses per h, average recoveries of P_2O_5- from KH_2PO_4 being 99·8%, compared with 100·3% by the quinolinium method. A. A. ELDRIDGE.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

FEBRUARY, 1968

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

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Printed in England by Staples Printers Limited at their Kettering, Northants, establishment