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CALCULATION AND PRESENTATION OF PORK MUSCLE COLOUR FROM REFLECTANCE SPECTRA

By R. J. ELLIOTT

Pale and dark *longissimus dorsi* muscle samples with the surface pigments predominantly in the reduced, oxygenated, or oxidised form, were used to determine the effects of the shape and position of the reflection spectra on four mathematical techniques for expressing colour in C.I.E. terminology.

No significant differences in the computation of tristimulus values were obtained by the weighted ordinate method, using 5 and 10 nm intervals, and the thirty and ten point selected ordinate method. The latter method provides a quick and convenient method of calculation. A chart has been constructed for the direct conversion of x and y co-ordinates to dominant wavelength (λ_d) and percentage purity values, the x/y calculation for λ_d being read to less than 1 nm and for purity to less than 1%.

A nomogram to convert C.I.E. brightness (Y tristimulus value) to Munsell value has been produced for the 10–40 Y range; it has an error of less than 3%. A series of four charts has been prepared, with which muscle colour, calculated in terms of C.I.E., can be defined in Munsell notation. The conversion error is maintained below visually perceptible steps. These charts extend the usefulness of reflection spectrophotometers in measuring the colour of pork.

Introduction

The colour of an object is the result of a subjective assessment, which can be defined as *object* + *illumination* + *observer* = *coloured object*. With objective measurement, the observer is replaced by a colour meter, permitting easier standardisation and calibration than is possible with observers.

An advantage of the instrumental method is that meat colour can be measured against a stable primary standard, such as magnesium oxide. To standardise visual measurements, a range of colour standards similar to the test material is required. The translucent and textural properties of muscle make it difficult to prepare suitable artificial standards. This may lead to the use of 'memory' standards, which are not definable and can vary within and between observers.

In 1931 the Commission Internationale de l'Eclairage (C.I.E.)[1] adopted tables defining the colour-matching characteristics of a 'standard' observer, based on independent work by Wright & Guild.[1,2] Subsequent developments in colour science have been correlated with the C.I.E. chromaticity diagram. The Munsell system was later re-notated in terms of C.I.E. co-ordinates.[2,3,4] Several textbooks have been published describing the theory and measurement of colour,[1,2,3,5,6] and method as applied to foods.[7] Colour is quantitatively specified by dominant wavelength or hue. The scale ranges from red (700 nm) to blue-violet (400 nm). The scale for purity or chroma ranges from the fully saturated hue to that diluted with a neutral colour, e.g. grey. Brightness or value for opaque and translucent material changes from white through a range of greys to black. Dominant wavelength, purity and brightness can be represented in a three-dimensional space, with variations in brightness shown as distances along a vertical axis, variations in purity as radial distances from the vertical axis, and variations in dominant wavelength as angular distances around the vertical axis.

The three methods of expressing colour examined in this paper are:

The C.I.E. method, where the colour is defined by the chromaticity co-ordinates, x and y, and the Y tristimulus value (brightness).

The dominant wavelength (λ_d) and excitation purity (p_e) with the Y tristimulus value.

The Munsell notation, which specifies colour as hue, value and chroma, and is usually abbreviated to $H/V/C$.

Colour quantities using the systems described were specified under conditions of C.I.E. source C illuminant, which has a spectrum distribution similar to sunlight plus skylight.

Experimental and Results

The muscle used for analysis was the *longissimus dorsi*. Samples were taken between the 4th thoracic and 5th lumbar vertebrae 20 h *post mortem*. The muscle reflection spectra were obtained on a 4 mm thick sample with a white rear reflector. The top surface of the sample was covered with a microscope slip glass. The spectrophotometric procedure has been described elsewhere.[8]

Twenty muscles were selected to provide a range of lightness from pale to dark. Each sample was measured on the freshly cut surface, where the pigments were predominantly in the reduced form and then after 3 h in a disiccator over acid hydrogen peroxide, where the pigments were predominantly oxygenated. Finally the sample was lightly sprayed with potassium ferricyanide and stored under reduced atmospheric pressure for 3 h, by which time the surface pigments were predominantly oxidised.

Conversion of the reflection spectra to C.I.E. colour space

Four methods of calculation were examined:

(A) The 1931 C.I.E. system using the distribution coefficients[1,2,3,7] weighted by energy values of illuminant C at wavelength intervals of 5 nm in the range 400 to 700 nm.

(B) As in (A) except that wavelength intervals of 10 nm were used.

(C) The thirty selected ordinates procedure for illuminant C[2,3,7,6].

(D) As in (C), using ten selected ordinates.

The data from (A) and (B) were processed with an I.B.M. '1200' series computer; in (C) and (D) computation is principally addition. The C.I.E. colour data obtained by the four methods of calculation were subjected to regression analysis.

The colour range of the muscle samples examined, as defined by method (A), were for Y, 11·88–30·47; x, 0·3330–0·3792 and y, 0·3090–0·3777.

When all the reflection spectra had been converted to C.I.E. terminology by the four methods of calculation, the

mean Y, x and y values of the samples were very similar.

Method of calculation	Y	x	y
A	20·0376	0·3557	0·3304
B	20·0573	0·3560	0·3307
C	20·1491	0·3559	0·3316
D	20·1767	0·3554	0·3320

Regression analysis of the data produced highly significant differences, $P < 0{\cdot}001$, between the regression coefficients of each parameter and zero. This is reflected in the very high correlation coefficients (r) obtained.

	r		r		r
Y_A	Y_A 0·9999 Y_C 0·9993 Y_D 0·9988	x_A	x_B 0·9853 x_C 0·9749 x_D 0·9640	y_A	y_B 0·9990 y_C 0·9898 y_D 0·9768

The regression equations with the standard error of the regression coefficient (S_b) are tabulated below:

		S_b
Y_A	$= 1{\cdot}0011\ Y_B - 0{\cdot}0223$	$\pm\ 0{\cdot}00107$
	$= 1{\cdot}0018\ Y_C - 0{\cdot}0466$	$\pm\ 0{\cdot}00503$
	$= 1{\cdot}0116\ Y_D - 0{\cdot}2381$	$\pm\ 0{\cdot}00665$
		S_b
x_A	$= 0{\cdot}9598\ x_B + 0{\cdot}0217$	$\pm\ 0{\cdot}02172$
	$= 1{\cdot}0476\ x_C - 0{\cdot}0061$	$\pm\ 0{\cdot}03103$
	$= 1{\cdot}0563\ x_D - 0{\cdot}0062$	$\pm\ 0{\cdot}03754$
y_A	$= 1{\cdot}0185\ y_B - 0{\cdot}0044$	$\pm\ 0{\cdot}00618$
	$= 1{\cdot}0594\ y_C - 0{\cdot}0143$	$\pm\ 0{\cdot}02004$
	$= 1{\cdot}0327\ y_D - 0{\cdot}0027$	$\pm\ 0{\cdot}02937$

A further comparison was carried out between the selected ordinate methods (C and D):

	S_b	r
$Y_C = 1{\cdot}0047\ Y_D - 0{\cdot}1942$	$(\pm\ 0{\cdot}00387)$	$Y_C Y_D = 0{\cdot}9996$
$x_C = 1{\cdot}0219\ x_D + 0{\cdot}0007$	$(\pm\ 0{\cdot}02246)$	$x_C x_D = 0{\cdot}9862$
$y_C = 1{\cdot}0263\ y_D + 0{\cdot}0113$	$(\pm\ 0{\cdot}02138)$	$y_C y_D = 0{\cdot}9865$

Preparation of a C.I.E. colour space chart for pork muscle, incorporating dominant wavelength and purity values

The C.I.E. co-ordinates of pig *longissimus dorsi* are confined to the square area defined by 0·30 to 0·40 of x and y. A triangle was prepared, using the chromaticity co-ordinates at 35% purity, of the spectrum colours of 555 and 650 nm and illuminant C co-ordinates on the colour space chart. The 35% p_e values of the spectrum colours were calculated using the formula: $y_a = p_e(y_d - y_w) + y_w$ where y_a = the spectrum colour co-ordinate at 35% purity, y_w = illuminant C co-ordinate, y_d = the spectrum colour co-ordinate at 100% purity[3] and p_e = excitation purity of 35%. The calculated p_e values were plotted on the chart and drawn to the illuminant C position. The p_e values 0 to 35 at 5% intervals were calculated for wavelengths 555, 570, 580, 590, 600 and 630 nm; the matching purity points were joined to form the grid shown in Fig. 1. Purity intervals of 1% were obtained by linearly subdividing the 5% areas. Similarly λ_d at 1 nm intervals were provided by subdividing each 5 nm. From this chromaticity diagram λ_d can be determined to $\pm$ 1 nm and p_e to $\pm$ 1%.

Method of using the dominant wavelength/purity value chart

To convert x and y co-ordinates obtained by the C.I.E. calculation, their polar position on the chart is determined from the vertical and horizontal x and y scales. The nearest overlying λ_d and p_e lines can be found. The complete colour specification is presented as Y, λ_d and p_e.

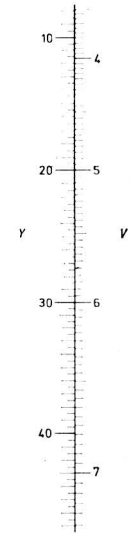
Preparation of charts for the conversion of C.I.E. value to Munsell notation

The C.I.E. Y tristimulus quantity has been converted to Munsell V value using equations or tables. In the range of pork *longissimus dorsi* brightness values a linear log relationship was found between Y and V within 10–45 Y (4–7 V). Between 20 and 40 Y the error in conversion is less than 1%, when compared with Nickerson's table.[5] Below 20 Y the error increased progressively to 3% at 10 Y. In Table I two conversion equations and a table devised from one of them have been compared with a nomogram over the pork brightness range.

Hue and chroma charts at V of 4, 5, 6 and 7 were prepared from the C.I.E. equivalents of Munsell re-notation data compiled by Newhall, Nickerson & Judd.[4] Further interpolation was obtained by subdividing to half steps of chroma along each line of constant hue. This increased the accuracy of converting x and y readings to hue and chroma. The completed charts are shown in Figs 2, 3, 4 and 5.

Method of using the C.I.E. co-ordinates/Munsell notation charts

The C.I.E. Y tristimulus quantity can be directly converted to Munsell V using the Y/V nomogram. When V is exactly 4, 5, 6 or 7, the x, y position on the appropriate chart at constant hue and chroma is plotted, and the nearest overlying chroma and hue lines are identified. When V is part decimal,



Nomogram for conversion of C.I.E. Y *to Munsell* V (*10–45* Y: *4–7* V)

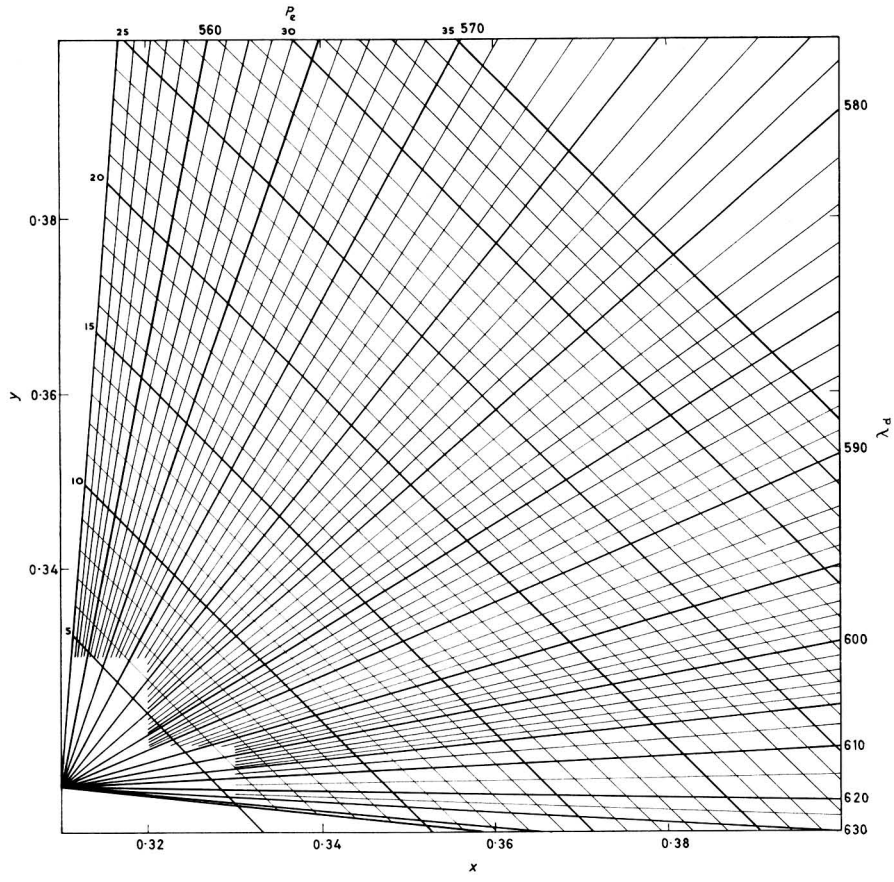


FIG. 1. *Chart for the conversion of C.I.E.* x *and* y *data to dominant wavelength* (λ_d) *and percentage purity* (p_e)

TABLE I

Comparison of estimates of the C.I.E. *Y* tristimulus value calculated for Munsell readings in the range of pork muscle samples

Munsell *V* reading	Converted to C.I.E. *Y* by			
	(1) Equation of Newhall *et al.*[4]*	(2) Ladd & Pinney[9] $V=2{\cdot}468Y^{\frac{1}{3}}-1{\cdot}636$	(3) Nickerson[5] table derived from (1)	(4) Nomogram derived from (3)
4	12·0	11·9	12·0	11·7
5	19·8	19·4	19·77	20·0
6	30·0	29·6	30·05	30·0
7	43·1	42·8	43·06	43·0

* $Y = 1{\cdot}2219V - 0{\cdot}23111V^2 + 0{\cdot}23951V^3 - 0{\cdot}021009V^4 + 0{\cdot}0008404V^5$

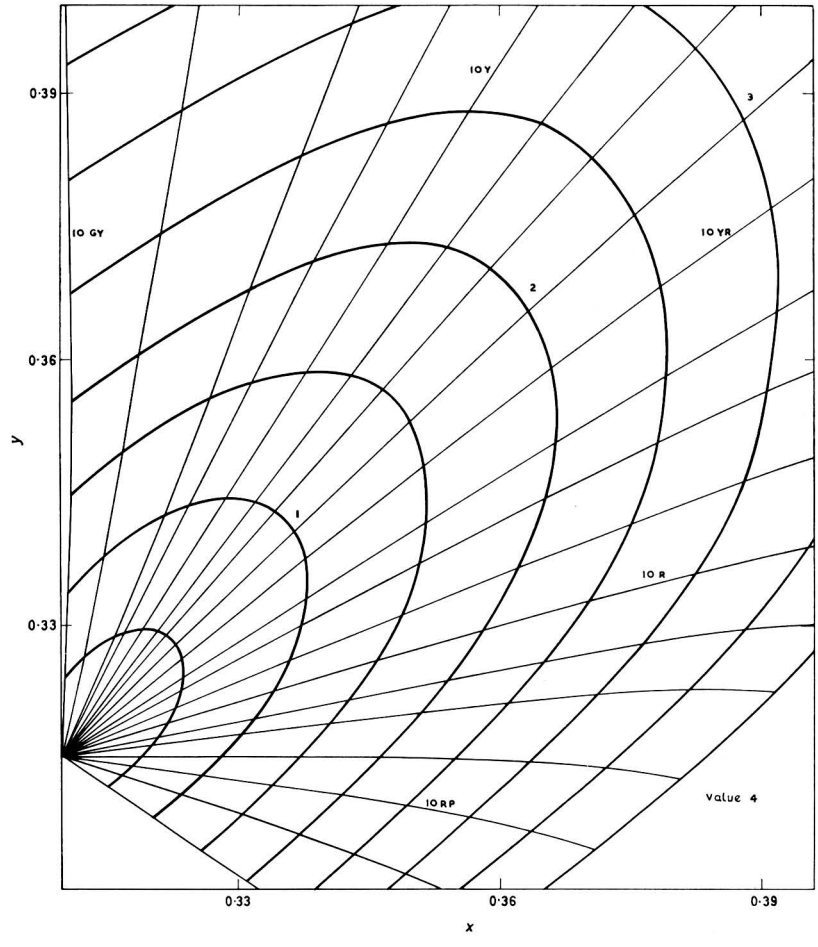


FIG. 2. *Chart for the conversion of C.I.E.* x *and* y *data to Munsell 'hue' and 'chroma' at Munsell 'value' 4*

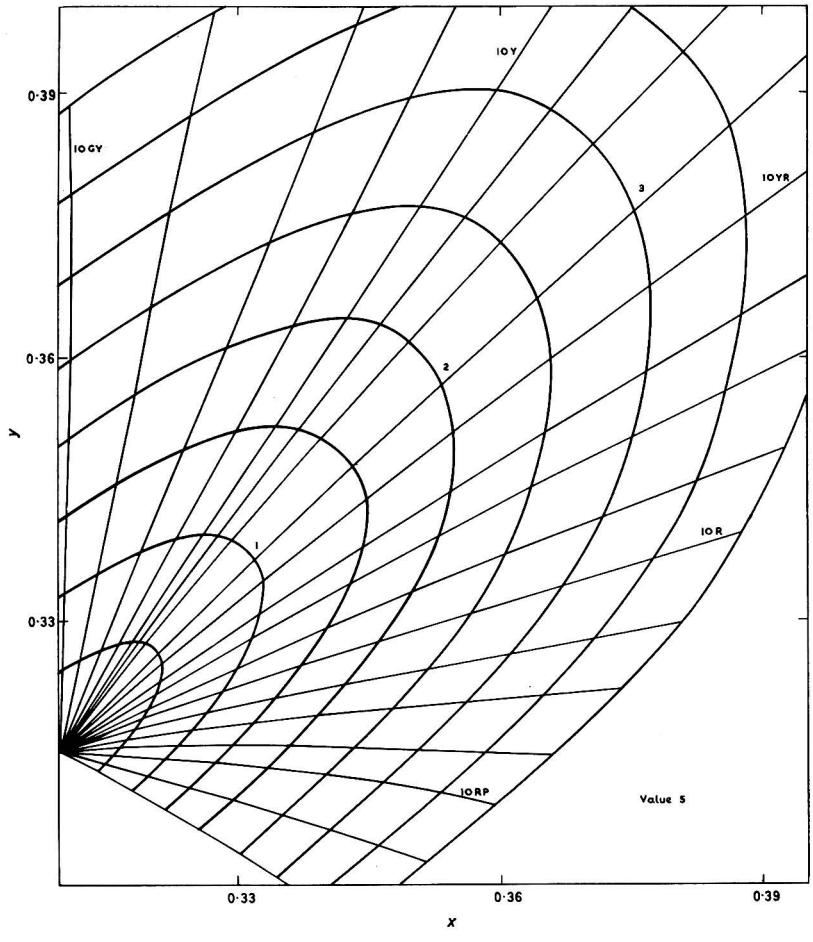


FIG. 3. *Chart for the conversion of C.I.E.* x *and* y *data to Munsell 'hue' and 'chroma' at Munsell 'value' 5*

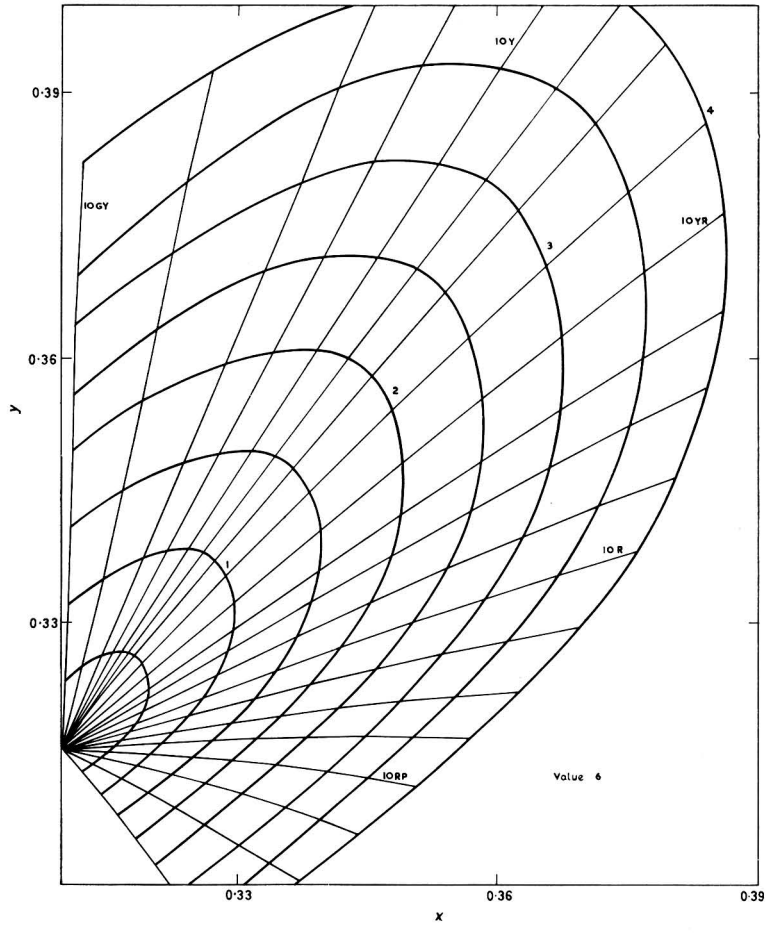


FIG. 4. *Chart for the conversion of C.I.E.* x *and* y *data to Munsell 'hue' and 'chroma' at Munsell 'value' 6*

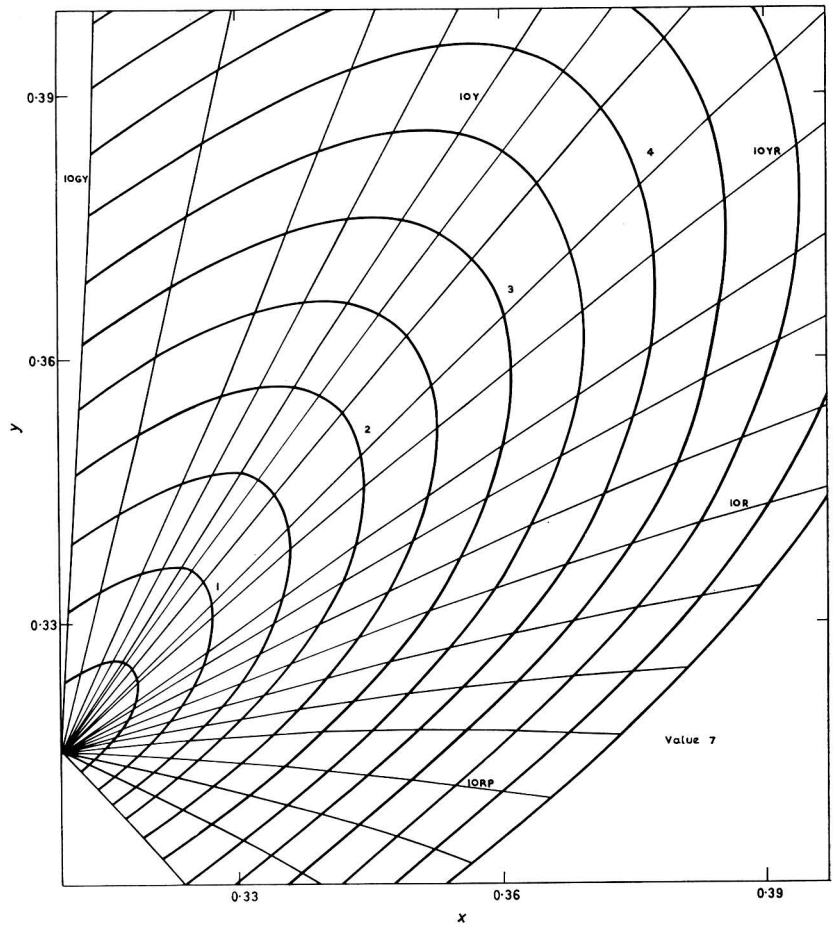


FIG. 5. *Chart for the conversion of C.I.E.* x *and* y *data to Munsell 'hue' and 'chroma' at Munsell 'value' 7*

the charts of the next higher and lower integral value are used. By substituting the two sets of hue and chroma quantities obtained, the proximate hue and chroma figures can be obtained from the equations.

$$H_p = H_l + (V_l(H_h - H_l)) \quad .. \quad .. \quad (1)$$

$$C_p = C_l + (V_l(C_h - C_l)) \quad .. \quad .. \quad (2)$$

where H_p = the proximate hue, H_l = the lower hue reading, H_h = the higher hue reading, and C_p = the proximate chroma, C_l = the lower chroma reading, C_h = the higher chroma reading.

Conclusions

When the variation of colour within a muscle, the method of sample preparation, the accurate reading of percentage reflectance and wavelength are considered with the differences in the methods of colour calculation, it would appear that the simplest of the described systems, the ten-point selected ordinate method, would be satisfactory in specifying meat colour. Where small colour differences are important, the use of the appropriate regression equation would reduce the variance with the 5 nm weighted ordinate method. This, however, should not be necessary with the brightness value.

The ability to transform Y, x and y to Y, λ_d and p_e or $H/V/C$ enables comparisons to be made with published work which is expressed in C.I.E. or Munsell or related systems. However, care should be exercised, as some instrumental metamerism probably exists.[2]

It is also desirable to demonstrate a correlation between subjective and objective grading. When the instrument grading can be specified in several forms, the possibility of finding a linear function with visual ranking is improved. It also provides more information for determining perhaps the most difficult problem in colour grading—the just-perceptible colour difference.

From a review of the literature on meat colour it is apparent that various aspects of colour are required. Muscle colour is influenced principally by changes in brightness, associated with breed, feeding, *ante-mortem* stress and *post-mortem* glycolysis, whereas light, heat, irradiation and packaging affect hue and chroma. Instrumental colour analysis allows each parameter of colour to be specified individually, whereas visual grading provides a composite colour rating.

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References

1. Wright, W. D., 'The measurement of colour', 3rd ed. (London: Hilger & Watts, Ltd.), 1964
2. Judd, D. B., & Wyszecki, G., 'Colour in business, science and industry', 2nd ed. (New York: J. Wiley & Sons), 1963
3. Wyszecki, G., & Stiles, W. S., 'Colour science, concepts and methods, quantitative data and formulas', 1st ed. (New York: J. Wiley & Sons) 1967.
4. Newhall, S. M., Nickerson, D., & Judd, D. B., *J. Opt. Soc. Am.*, 1943, **33**, 385
5. Nickerson, D., 'Colour measurement and its application to the grading of agricultural products', U.S. Dept. of Agric., Misc. Publ. 580, 1946
6. Hardy, A. C., 'Handbook of colorimetry', 1st ed. (Cambridge, Mass: The Technology Press), 1936
7. MacKinney, G., & Little, A. C., 'Color of foods', 1st ed. (Connecticut: AVI Publishing Co. Ltd.) 1962
8. Elliott, R. J., *J. Sci. Fd Agric.*, 1967, **18**, 332
9. Ladd, J. H., & Pinney, J. E., *Proc. Instn Rad. Engrs*, 1955, **43**, 1137

CHEMICAL COMPOSITION OF SOIL SOLUTION*

By S. LARSEN and A. E. WIDDOWSON

Soil solutions were obtained by a liquid displacement technique and their principal ionic constituents were determined. Increases in ionic concentration of the soil solution were observed over a period of 53 days and were due almost entirely to calcium, magnesium and nitrate ions. These increases are assumed to be caused by nitrification.

Introduction

Not many complete analyses of the inorganic constituents in soil solutions have been published. Some examples are, however, given in Table I. A series of results which were obtained in this laboratory are reported together with the methods which were employed. The cause of disequilibrium between soil solution and soil solids is also examined.

Experimental

Three air-dry non-calcareous soils (Table II) were passed through a ¼ in mesh and bulk amounts of 3 kg brought to two moisture levels in a large soil mixer used for preparing pot experiments. The lower moisture level was 40% of the water-holding capacity of the soil and the upper level was dependent on how much water could be added to the soil without causing major structural deterioration. The soils were stored in an incubator at 20° with precautions against moisture loss.

* Presented at a meeting of the Agricultural Group, 25 January, 1966

At intervals over a period of 53 days the soil solutions were separated by the liquid displacement method devised by Burd & Martin[1] and modified by Moss,[2] as follows. A 600 g aliquot of moist soil was mixed with 200 g of acid-washed sand, the mixture added to the displacement column (Fig. 1) in approximately 20 g samples and lightly tamped down after each addition. The remainder of the column was filled with 50% glycerol. The weight of the glycerol, together with compressed air when necessary, forced the soil solution down the column. It was collected in 10 ml aliquots, until the appearance of glycerol from the bottom of the column was detected by a fall in conductivity of the first aliquot containing it. The uncontaminated aliquots were bulked. The amount of soil solution collected was approximately 50% of the water present in the soil.

Conductivity and pH of the soil solutions were recorded, and chemical analyses were carried out according to the methods described in Table III. Phosphorus was not determined as the concentration of this element was below the limit of the most sensitive colorimetric method available.

TABLE I

Chemical composition of soil solutions, mequiv/l of soil solution

Soil Details	Moisture content	pH	Conductivity m mhos	Ca^{2+}	Mg^{2+}	K^+	Na^+	NH_4^+	Total cation	NO_3^-	Cl^-	HCO_3^-	SO_4^{2-}	Total anion
Sandy Loam[4]	24·0%	7·19	2·61	21·0	1·2	0·7	1·8	0·4	25·1	15·6	2·2	1·1	7·0	25·9
,, ,,	11·8%	7·52	4·96	42·2	3·6	0·8	3·0	0·4	50·0	32·0	4·6	3·5	11·8	51·9
Sandy Loam	27·9%	6·72	0·34	1·0	0·7	0·1	1·3	0·5	3·6	0·5	1·0	0·8	0·7	3·0
,, ,,	14·2%	7·41	0·70	2·0	0·9	0·2	2·8	0·1	6·0	1·2	2·9	1·0	1·4	6·5
Sandy Loam	29·6%	5·04	5·20	50·0	13·1	2·0	4·2	2·8	72·1	34·5	2·3	1·2	37·0	75·0
,, ,,	16·3%	6·99	8·90	83·1	16·6	2·6	7·4	4·0	113·7	65·0	5·6	4·2	37·8	112·6
Sandy Loam	30·3%	5·82	1·30	8·1	1·7	1·1	0·8	0·4	12·1	8·0	0·3	1·2	1·0	10·5
,, ,,	19·0%	7·66	1·96	12·8	3·6	1·1	1·2	0·4	19·1	13·2	1·0	1·9	2·8	18·9
Sandy Loam	31·6%	6·11	1·75	10·6	2·5	2·7	0·6	0·4	16·8	9·6	1·6	1·7	3·6	16·5
,, ,,	19·1%	6·89	2·74	17·4	3·9	3·7	1·0	0·5	26·5	16·9	1·9	2·3	6·0	27·1
Loam ,,	31·1%	7·56	2·67	9·2	2·5	6·7	8·4	0·5	27·3	10·0	2·5	4·1	4·8	21·4
,,	25·6%	8·12	3·36	11·7	3·7	7·3	9·2	0·4	32·3	12·4	3·2	6·1	7·6	29·3
Loam	40·6%	7·65	1·05	6·4	1·7	0·3	1·4	0·4	10·2	4·4	1·9	3·4	1·7	11·4
,,	32·8%	7·52	1·68	11·1	2·2	0·3	2·3	0·2	16·1	8·8	2·3	3·8	2·8	17·7
								ppm Fe Al Mn						
Acid Soil[5] (pH 4·3)	20%	4·2		1·0	1·4	0·4	0·4	0·1 6·4 16·0	3·2	3·8	0·2		0·8	4·8
Non-Saline[6]	Saturated		1·50	3·6	1·1	0·1	7·2		12·0	0·0	4·8	3·4	4·9	13·1
,,	,,		0·98	3·9	0·7	0·1	5·8		10·5	0·0	1·8	4·5	0·9	7·2
,,	,,		1·24	1·6	0·8	1·1	8·3		11·8	0·0	1·8	7·9	0·3	10·0
,,	,,		1·12	5·9	2·4	0·3	3·4		12·0	0·3	1·7	6·2	3·8	11·6
,,	,,		1·32	5·6	2·1	0·3	5·0		13·0	0·3	5·1	5·5	3·8	14·4
,,	,,		2·73	15·0	7·9	0·9	8·1		31·9	0·3	7·3	8·9	15·6	31·8
Clay Loam[7]	32·5%	8·22	5·73	28·4	33·9	6·3	14·6		82·2	0	11·3	8·6	62·3	82·0
(pH 7·8)	20·0%	8·26	9·40	43·5	48·0	9·6	21·7		114·8	(31·2)	20·1	7·2	56·3	83·6
,,	14·2%	8·15		47·6	65·7	11·7	30·4		155·4	(56·4)	30·3	10·4	49·3	99·0
Clay	28·9%			23·6	6·0	1·0	22·7		49·3		15·2	3·8	26·1	45·1
Loam	13·6%			13·6	6·8	0·2	1·3		21·9	(12·1)	3·1	5·4	1·3	21·9
Sandy Loam	10·4%			18·2	4·0	1·7	3·3		27·2	17·6	3·3	3·5	2·9	27·3
Californian Soil[8]														
April 30, 1923	18·7%			6·8	3·7	1·1	2·4		14·0	2·3		1·8	7·1	11·2
Sept. 4, 1923	18·7%			4·0	1·9	0·6	1·1		7·6	0·2		2·9	4·4	7·5
April 28, 1924	18·7%			8·5	4·5	1·3	2·4		16·7	2·7		2·3	9·6	14·6

TABLE II

Some details of the experimental soils

Soil type	Chemical properties			Physical properties			
	pH	Ammonium acetate Extractable K ppm	Organic C %	Coarse sand %	Fine sand %	Silt %	Clay %
1. Clay (British)	5·7	73	1·6	10·0	24·6	19·5	42·1
2. Medium Loam (British)	6·5	166	1·4	37·8	28·4	12·3	20·6
3. Red Clay (Rhodesian)	5·5	207	1·5	21·2	32·7	5·7	40·4

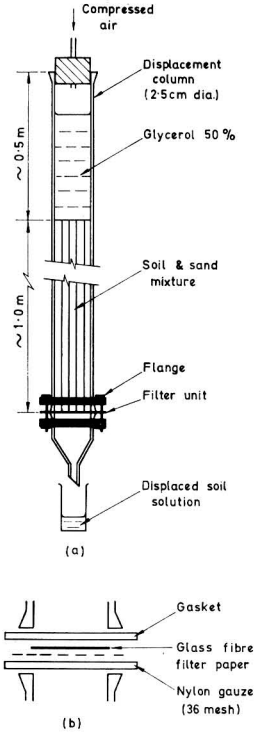

FIG. 1. *Displacement apparatus*
(a) General view; (b) detail of filter unit

Results

The most noticeable feature of the results (Table IV) is the increase in total cation and anion status of the soil solutions at both moisture levels over the experimental period. There was however no essential difference in ionic status between the two water regimes. The increases in soil solution concentrations were due almost entirely to increases in calcium, magnesium and nitrate concentrations, probably brought about by the release of calcium and magnesium into solution following the formation of nitric acid by bacterial activity.

The potassium, sodium, chloride, bicarbonate and sulphate concentrations all remained fairly constant and had little effect on the changes in total cation and anion concentrations. Both conductivity and pH measurements reflected changes in the total ionic concentration.

TABLE III

Analytical procedures

Ion	Method
Ca^{2+} and Mg^{2+}	EDTA titration using Eriochrome Black T[9]
Ca^{2+}	Turbidimetric determination of calcium oxalate precipitate[10]
Mg^{2+}	By difference
K^+	Flame photometry
Na^+	Flame photometry
NO_3^-	Colorimetric determination of the $FeSO_4$. NO complex[11]
Cl^-	$AgNO_3$ titration using potassium chromate[12]
HCO_3^-	H_2SO_4 titration using bromophenol blue[12]
SO_4^{2-}	Precipitation of $BaSO_4$ and back-titrating excess Ba^{2+} with EDTA[13]
$Si(OH)_4$	Colorimetric determination of the silicomolybdic complex.[14] Silicate was assumed to be present as the undissociated acid $Si(OH)_4$[15]

A satisfactory indication of the validity of the analytical methods was obtained from the total-cation : total-anion ratio which was near unity for soils 1 and 2. The persistent deviation from unity in soil 3 may have been due to the presence of significant quantities of Al^{3+}, Fe^{3+} or Mn^{2+} ions, which were not determined.

For soils 1 and 2 (British), the soil solution concentration increased rapidly in the first 25 days after which the rate of release of ions into solution decreased, suggesting a possible approach to equilibrium values. Soil 3 (Rhodesian) provided soil solution concentrations which increased more rapidly as the experimental period progressed, possibly because the microbial population of this soil required more time to become fully active.

These data support those of Moss[2] in showing that soil solutions may take a very long time to attain equilibrium. The principal reason for this delay is probably due to the activity of soil micro-organisms. The rewetting of dry soil is known to bring about a mineralisation of nitrogen resulting initially in the formation of ammonium ions.[3] Although this cation was not determined, the small increase in pH observed, in most cases from 2 to 9 days, probably corresponds to such a mineralisation before the process of nitrification became fully developed.

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TABLE IV

Effect of time on chemical composition of soil solution from three soils

Soil No.	Storage time in days	Conductivity m mhos	pH	mequiv/l										Cation / Anion
				$Ca^{2+}+Mg^{2+}$	K^+	Na^+	Cation total	NO_3^-	Cl^-	HCO_3^-	SO_4^{2-}	Anion total	$Si(OH)_4$	
1. 40% w.h.c. 18·3% H_2O	2	2·78	5·8	47·8	1·7	5·6	55·1	30·4	3·8	1·8	17·0	53·0	0·6	1·04
	9	3·62	6·0	55·2	2·0	6·0	63·2	34·9	3·8	1·7	17·7	58·1	0·7	1·09
	15	3·75	5·7	58·2	1·9	6·3	66·4	43·1	3·8	2·0	15·4	64·3	0·8	1·03
	25	4·16	5·5	63·2	1·9	6·4	71·5	50·5	3·9	2·0	15·3	71·7	0·8	1·00
	39	4·16	5·3	65·6	1·8	6·4	73·8	51·4	3·7	1·9	14·4	71·4	0·9	1·03
	53	4·40	5·5	68·9	1·8	6·6	77·3	53·1	3·5	1·9	14·3	72·8	0·8	1·06
1. 60% w.h.c. 24·0% H_2O	2	2·20	5·9	37·9	1·5	4·3	43·7	22·1	2·6	1·6	14·4	40·7	0·6	1·07
	9	2·90	6·3	44·4	1·8	4·7	50·9	27·6	2·6	1·6	15·6	47·4	0·7	1·07
	15	3·14	5·8	48·8	1·6	4·8	55·2	36·5	2·7	1·6	14·0	54·8	0·8	1·01
	25	3·34	5·6	50·2	1·7	4·9	56·8	38·7	2·7	1·8	14·0	57·2	0·8	0·99
	39	3·35	5·5	52·4	1·6	4·8	58·8	40·1	2·7	1·8	13·5	58·1	0·9	1·01
	53	3·51	5·6	53·2	1·6	5·2	60·0	41·6	2·5	1·9	13·0	59·0	0·7	1·02
2. 40% w.h.c. 19·0% H_2O	2	5·16	6·2	97·6	2·6	5·5	105·7	74·1	5·0	3·0	18·0	100·1	0·6	1·06
	9	6·27	6·3	107·1	2·9	5·9	115·9	77·6	5·0	1·8	18·9	103·3	0·8	1·12
	15	6·70	6·0	112·9	2·9	5·9	121·7	92·1	5·0	2·3	18·7	118·1	1·0	1·03
	25	7·27	5·8	123·2	2·7	6·2	132·1	105·4	4·9	2·2	19·4	131·9	0·9	1·00
	39	7·30	5·6	127·9	2·7	6·1	136·7	105·4	4·9	2·2	19·5	132·0	1·0	1·04
	53	7·68	5·7	131·9	2·6	7·0	141·5	106·0	4·5	2·1	21·4	134·0	0·9	1·06
2. 46% w.h.c. 20·8% H_2O	2	5·00	6·6	93·6	2·5	5·2	101·3	66·7	4·5	2·8	22·8	96·8	0·7	1·05
	9	6·00	6·6	100·0	2·9	5·9	108·8	68·9	4·6	1·8	18·1	93·4	0·8	1·16
	15	6·32	6·2	107·4	2·8	5·6	115·8	85·7	4·7	2·2	17·9	110·5	0·9	1·05
	25	6·93	6·0	115·1	2·6	5·7	123·4	94·7	4·6	2·2	18·1	119·6	0·9	1·03
	39	6·96	5·8	122·0	2·6	5·8	130·4	98·1	4·5	2·2	17·9	122·7	1·0	1·06
	53	7·05	5·9	124·4	2·6	6·7	133·7	97·6	4·0	2·0	21·0	124·6	0·8	1·07
3. 40% w.h.c. 18·4% H_2O	2	1·10	5·7	11·9	0·9	0·8	13·6	10·9	2·9	1·4	0·0	15·2	0·3	0·89
	9	1·20	5·6	11·4	1·0	0·8	13·2	10·7	3·0	0·8	0·0	14·5	0·3	0·91
	15	1·23	5·6	11·1	1·1	0·9	13·1	13·4	3·0	1·2	0·0	14·9	0·3	0·88
	25	1·38	5·4	12·6	1·2	1·0	14·8	14·4	3·0	1·0	0·0	18·4	0·3	0·80
	39	1·63	5·2	17·0	1·3	1·2	19·5	18·8	2·8	0·9	0·0	22·5	0·3	0·87
	53	2·03	5·1	23·5	1·5	1·6	26·6	24·3	2·7	1·0	Trace	28·0	0·3	0·95
3. 49% w.h.c. 21·2% H_2O	2	0·88	5·8	9·5	0·8	0·7	11·0	8·3	2·6	1·1	0·0	12·0	0·3	0·92
	9	1·00	5·9	8·9	0·9	0·7	10·5	8·3	2·6	0·8	0·0	11·7	0·2	0·90
	15	1·01	5·9	9·2	0·9	0·7	10·8	10·0	2·5	1·0	0·0	13·5	0·3	0·80
	25	1·20	5·6	10·5	1·0	0·9	12·4	12·2	2·5	1·0	0·0	15·7	0·3	0·79
	39	1·51	5·2	15·1	1·2	1·2	17·5	17·6	2·5	1·0	0·0	21·1	0·3	0·83
	53	1·96	5·4	23·0	1·5	1·5	26·0	24·1	2·5	0·8	Trace	27·4	0·3	0·95

For soils 1 and 2, Mg content was approximately 10% and for soil 3 approximately 50% of the Ca + Mg total
w.h.c.: water holding capacity

References

1. Burd, J. S., & Martin, J. C., *J. agric. Sci.*, 1923, **13,** 265
2. Moss, P., *Pl. Soil*, 1963, **18,** 99
3. Birch, H. F., *Pl. Soil*, 1960, **12,** 81
4. Eaton, F. M., Harding, R. B., & Ganje, T. J., *Soil Sci.*, 1960, **90,** 253
5. Vlamis, J., *Soil Sci.*, 1953, **75,** 383
6. Campbell, R. B., Bower, C. A., & Richards, L. A., *Proc. Am. Soc. Soil Sci.*, 1948, **13,** 66
7. Reitemeier, R. F., & Richards, L. A., *Soil Sci.*, 1944, **57,** 119
8. Burd, J. S., & Martin, J. C., *Soil Sci.*, 1924, **18,** 151
9. Tinker, P. B. H., *Analyst, Lond.*, 1959, **84,** 743
10. Hunter, J. G., & Hall, A., *Analyst, Lond.*, 1953, **78,** 106
11. Swann, M. H., & Adams, M. L., *Analyt. Chem.*, 1956, **28,** 1630
12. Jackson, M. L., 'Soil chemical analysis', 1958, (Englewood Cliffs, N. J.: Prentice-Hall, Inc.)
13. Asghar, A. G., Qayyum, M. A., & Rana, G. N., *Soil Sci.*, 1957, **83,** 239
14. Armstrong, F. A. J., *J. mar. biol. Ass. U.K.*, 1951, **30,** 149
15. McKeague, J. A., & Cline, M. G., *Can. J. Soil Sci.*, 1963, **43,** 70

EXAMINATION OF THE PROTEIN EXTRACTABILITY METHOD FOR DETERMINING COLD-STORAGE PROTEIN DENATURATION IN COD

By W. P. COWIE and I. M. MACKIE

The protein extractable in 5% sodium chloride solution has been determined for cod fillets stored for various times at −7, −14 and −29° using an Ultra Turrax homogeniser. It was found that extractable protein values obtained by Ultra Turrax homogenisation were consistently higher than published values obtained by Marsh Snow homogenisation. The differences between them are related to ultracentrifuge analyses of both types of extracts.

Introduction

The extractability of the proteins of frozen stored cod muscle in cold neutral 5% sodium chloride solution has been used as a measure of cold-storage protein denaturation.[1,2,3] This denaturation has been defined[2] as 'a change in the protein, such that it is no longer soluble or extractable by salt solutions under conditions in which the native protein is soluble or extractable'. To measure the extractability of the proteins from frozen fish, Dyer[4] used a blending technique[5] in which the fish muscle was homogenised in the salt solution using a Waring Blendor. He found that extractability values depended on the duration and temperature of frozen storage of the fish and he also indicated that the fall in protein extractability paralleled the observed increase in toughness as assessed organoleptically. Love,[3] in his work on the measurement of protein extractability from cod stored at different temperatures, used a Marsh Snow homogeniser to macerate the fish muscle in the salt solution. Japanese workers Shimizu & Simidu[6] used a different type of homogeniser again. While most workers used ratios of muscle to extractant of 1 : 20, there is considerable variation in the details of the experimental methods. This paper illustrates how the value for protein extractable in 5% sodium chloride solution is influenced by the type of homogeniser and duration of storage of homogenate, all other conditions being the same. The ultracentrifuge was used to examine the nature of the extracted proteins and also to provide an accurate measure of their concentration.

Experimental

Materials

Cod (*Gadus morhua*) caught in the North Sea near Aberdeen was used in this work.

For the *rigor* fillets, a live cod was taken from the aquarium, killed and stored in ice until *rigor* had set in, then filleted. To obtain the frozen fillets, medium-sized cod were treated as follows. After having been caught at sea the fish were gutted and kept in ice for 1–2 days before filleting. The skinned fillets were sealed in polythene pouches and blast-frozen to −29°. The frozen fillets, which were from different batches of cod, were then transferred to cabinets at −7, −14 or −29°. These fillets were those used in previous work.[7,8]

Apparatus

The Marsh Snow homogeniser used was made according to the design of Marsh & Snow.[9] The Ultra Turrax homogeniser (Type TP 18/2N) was purchased from the Scottish agents for the manufacturers Janke & Kunkel, KG./Staufen 1, Brunswick, Germany.

Methods

Extractable protein estimation

The extractable protein content of each fillet was determined in quadruplicate by two different methods. A modification of the method of Ironside & Love[10] was used and has been fully described elsewhere.[7] The other method used was developed in the Unilever Research Laboratory, Aberdeen. 1 g of frozen cod muscle free of connective tissue was weighed into a 100 ml polypropylene measuring cylinder containing 100 ml of chilled neutral 5% sodium chloride solution. The mixture was homogenised for 15 seconds in an Ultra Turrax homogeniser, the shaft of which had been fitted with a rubber ring which acted as a baffle to prevent frothing of the protein solution. The resulting macerate was then centrifuged for 30 min at 20,000 *g* at 0°. The clear solution was analysed for nitrogen by the Kjeldahl method and the percentage extractable protein was calculated as previously described.[7]

Ultracentrifuge methods

A Spinco Model E Analytical Ultracentrifuge fitted with Schlieren optics was used. Sedimentation velocity determinations were carried out at 59,780 and 42,040 rev/min in the temperature range 0–5°. The concentration of the protein in solution was calculated from the area under the peaks in the Schlieren diagrams as previously described[11] with the assumption that the specific refractive index increment of the proteins in these extracts is the same as that of cod myosin.[12] At the lower speed a double sector cell was used to obtain a solvent base line.

Results

Extractable protein measured by two methods in fillets stored at −7, −14 and −29°

In cod stored at −7 and −14° protein extractability

measured by the Marsh Snow method fell much more sharply with time of storage than did the protein extractability measured using the Ultra Turrax homogeniser. From Fig. 1, it is seen that protein extractability by the Marsh Snow method[8] had reached a minimum of 25% in cod stored 20 weeks at −7° while the extractability measured using the Ultra Turrax had fallen to only 40% and continued to decline after this period. Similarly in the case of fillets stored at −14° for 34 weeks (Fig. 2), the extractable protein content had fallen to less than 40% by the Marsh Snow method[8] while it dropped to only 60% using the Ultra Turrax method. In Figs 1 and 2, the curves were fitted to the points by inspection.

Fig. 3 shows how the percentage extractable protein as measured using both the Marsh Snow[7] and Ultra Turrax homogenisers varies with time of frozen storage of the cod fillets at −29°. With the Ultra Turrax method, the amount of extractable protein stays virtually constant at about 80% up to 49 months of storage, then falls gradually to about 70% after 82 months.

On the other hand, the Marsh Snow method showed a steady fall in protein solubility from an initial 75% to a final 50% after 82 months of storage of the fillets at −29°.

Ultracentrifuge analyses of Marsh Snow and Ultra Turrax extracts

Homogenates of about 2·0 g of muscle from *rigor* cod, and from cod stored for 5 weeks at −7 and −14° respectively were prepared. In this experiment the ratio of muscle to extractant was increased by two to give a more suitable concentration for analysis by sedimentation velocity runs in the ultracentrifuge. Otherwise extraction conditions were as described above. Aliquots of the Marsh Snow and Ultra Turrax homogenates of the above fish were centrifuged immediately, analysed for protein nitrogen by the Kjeldahl method and examined by sedimentation velocity runs. The residues of the homogenates were allowed to stand for 24 hours at 0° then further aliquots were centrifuged as before. The resulting supernatants were then analysed for nitrogen and examined in the ultracentrifuge as above. Diagrams of some of the extracts are shown in Fig. 4. When the *rigor* extracts are compared (lower diagrams of both (a) and (b)) it is clear from the area under the peaks that the Ultra Turrax extract contains more protein than the Marsh Snow one. In both diagrams the small rapidly sedimenting peak I corresponding to actomyosin is discernable in the first exposure, and by the time of the second exposure the myosin peak II has clearly separated from the slowly sedimenting components of the sarcoplasmic proteins and presumably G-actin (peak III).

The diagrams for the frozen stored cod, as expected, show less protein than the *rigor* extracts. The area for the Marsh Snow extract of the cod stored at −14° is greater than that for the cod stored at −7°, and increases further if the homogenates are stored for 24 hours when it approaches the area for the Ultra Turrax extract of the same fish. The diagrams for the frozen stored cod are similar to each other but differ markedly from those of the *rigor* cod. The actomyosin peak is not visible (Exposure 1) and the myosin peak is very much reduced in area. Peak III corresponding to the sarcoplasmic proteins is similar both in size and appearance to that in the *rigor* extracts. Attempts were not made to measure the area of these small peaks in Exposure 2 because

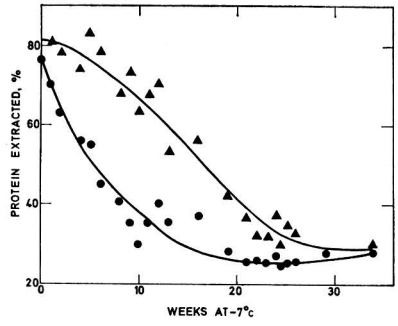

FIG. 1. *Change in protein extractability with time of storage of cod fillets at −7°C*
▲ Ultra Turrax ● Marsh Snow

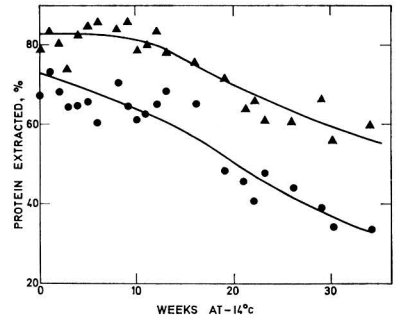

FIG. 2. *Change in protein extractability with time of storage of cod fillets at −14°C*
▲ Ultra Turrax ● Marsh Snow

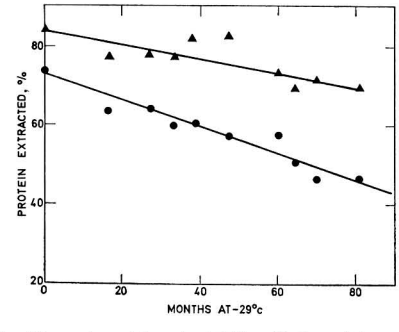

FIG. 3. *Change in protein extractability with time of storage of cod fillets at −29°C*
▲ Ultra Turrax ● Marsh Snow

of the considerable error involved in doing so. The protein concentration corresponding to the area of the single larger peak in Exposure 1 (i.e. before it separates into the components) was considered to give a better estimate of the dissolved protein. Even so this value must be less than the value for the total protein as determined by the Kjeldahl method since material of the molecular weight of actomyosin and above has already sedimented from the boundary. The protein contents of the extracts obtained by area measurements and by the Kjeldahl method are given in Table I. Both show that, for all the fish examined, better immediate extraction is obtained when the Ultra Turrax homogeniser is used. For the unfrozen fish the Marsh Snow figure is lower at 0 hours but if the homogenate is allowed to stand for 24 hours the value for the extractable protein increases to that obtained with the Ultra Turrax homogeniser. In the frozen stored material the value for the amount of protein in the Marsh Snow extracts is again initially less than that in the Ultra Turrax extracts. After the homogenates had been allowed to stand for 24 hours the discrepancy almost disappeared in the case of the $-14°$ stored cod but not in the case of the $-7°$ stored cod which had suffered more cold-storage denaturation.

In a similar experiment, muscle from a *pre-rigor* cod and from a cod held for $5\frac{1}{2}$ months at $-14°$ was used. In this experiment the amount of muscle in the homogenates was reduced to 1·0 g so that the extraction conditions would be the same as for the extractable protein measurements. Aliquots of the macerates were centrifuged after standing at 0° for 0, 24 and 48 hours, and the resulting supernatant solutions were analysed for protein by the Kjeldahl method and by ultracentrifuge analysis in a double sector cell. Because of the low concentration of protein in solution it was necessary to determine the area of the sedimenting peak soon after full speed (Figure 5, Exposure 1) and before it separated into its components (Figure 5, Exposures 2 and 3). The results of this experiment are given in Table II. These confirm those of Table I and show that, under the conditions used for determining protein extractability from fish, the value obtained is dependent on the type of homogeniser used and duration of storage of the homogenate. The frozen stored cod in this experiment suffered more cold-storage denaturation than the $-14°$ stored cod examined in the previous experiment and it can be seen that the discrepancy between the protein contents of the Marsh Snow and Ultra Turrax extracts is greater even after allowing the homogenates to stand for 48 hours.

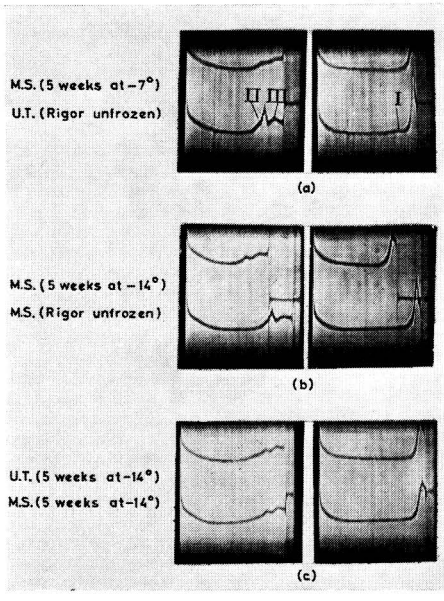

FIG. 4. *Sedimentation diagrams of extracts at 59,780 revs/min and phase plate angle 50°*

Upper diagrams of each exposure are obtained with a 1° positive wedge window. Diagrams of (a) and (b) are of extracts from homogenates of 0 hour storage and (c) are of extracts from 24 hour stored homogenates. Exposures 1 and 2 of each pair of diagrams are taken 5 and 43 min respectively after reaching full speed (Exposure 1 is to the right in each case). Sedimentation from right to left. Weight of fish muscle sample 2·0 g. M.S. = Marsh Snow. U.T. = Ultra Turrax

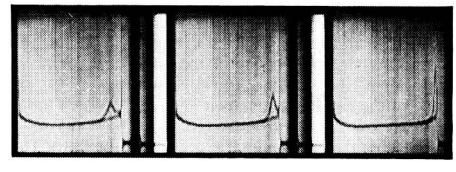

FIG. 5. *Sedimentation diagrams of an Ultra Turrax extract (24 hours stored homogenate) at 42,040 revs/min in a double sector cell and phase plate angle 50°*

Time in minutes after reaching full speed 5, 21 and 37 respectively from right to left. Sedimentation from right to left. Weight of fish muscle sample 1·0 g

TABLE I

Protein contents of cod muscle extracts by Kjeldahl and ultracentrifuge analysis

Fish used	Homogenate standing time, h	Marsh Snow			Ultra Turrax		
		Sample wt, g	% Protein in soln.		Sample wt, g	% Protein in soln.	
			Kjeldahl	Area		Kjeldahl	Area
Unfrozen rigor cod	0	2·11	0·21	0·18	1·98	0·30	0·21
	24		0·31	0·26		0·30	0·27
Cod stored 5 weeks at −7°c	0	2·03	0·13	0·10	2·18	0·20	0·16
	24		0·18	—		0·22	—
Cod stored 5 weeks at −14°c	0	2·08	0·18	0·15	2·06	0·27	0·20
	24		0·26	0·19		0·28	0·22

TABLE II

Protein contents of cod muscle extracts by Kjeldahl and ultracentrifuge analysis

Fish used	Homogenate standing time, h	Marsh Snow			Ultra Turrax		
		Sample wt, g	% Protein in soln.		Sample wt, g	% Protein in soln.	
			Kjeldahl	Area		Kjeldahl	Area
Pre-rigor unfrozen cod	0	1·01	0·11	0·08	1·06	0·12	0·11
	24		0·14	0·12		0·13	0·13
	48		0·14	0·14		0·14	0·13
Cod stored 4½ months at −14°C	0	1·06	0·06	0·04	1·18	0·13	0·08
	24		0·08	0·06		0·13	0·10
	48		0·09	0·07		0·13	0·09

Examination of the homogenates using the light microscope

In Fig. 6 are shown the phase contrast micrographs of typical cell debris in homogenates of unfrozen cod and cod stored for 6 months at −14° as obtained with the Marsh Snow and Ultra Turrax homogenisers respectively.

A comparison of the micrographs of the homogenates of the frozen stored cod shows that the Ultra Turrax homogeniser breaks down the cells much further than does the Marsh Snow homogeniser. In homogenates of the unfrozen muscle on the other hand, the particle size obtained with the Ultra Turrax homogeniser is only slightly less than that obtained with the Marsh Snow homogeniser.

Discussion

The foregoing results show that the value obtained for the amount of protein extractable from frozen stored cod muscle depends very much on the type of homogeniser used. The protein extractability values obtained using the Marsh Snow homogeniser for −14 and −29° stored cod fillets agree well with those of Love,[2] only the results for the 0 weeks samples are somewhat lower. When the protein extractability curves are examined for cod stored at −7, −14 and −29° it is seen that the Ultra Turrax method invariably gives higher results than the Marsh Snow method. Only when the proteins have become completely denatured (e.g. after 36 weeks at −7°) do the results obtained by the two methods agree. This discrepancy would indicate that the Ultra Turrax homogeniser is either solubilising high-molecular-weight 'denatured' protein which is insoluble as determined by the Marsh Snow method or it is creating conditions which allow for greater solubilisation of the undenatured proteins.

When the extracts are analysed by sedimentation velocity determinations in the ultracentrifuge, the latter possibility seems to be the more likely explanation. If there was proportionally more polydisperse high-molecular-weight protein in extracts obtained with the Ultra Turrax homogeniser the bulk of these proteins would be centrifuged out of solution after ½ hour at 20,000 *g* during the final step in the preparation of the Ultra Turrax extract. Also, the areas of the peaks corresponding to the proteins which are in solution at 250,000 *g* as observed in sedimentation velocity runs would be expected to be the same as those from a Marsh

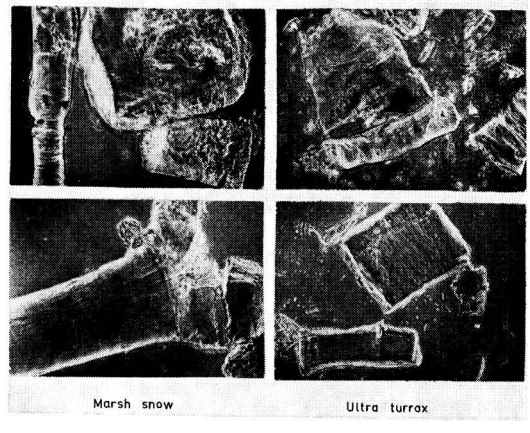

FIG. 6. *Phase contrast micrographs (Magnification × 45) of homogenates of unfrozen rigor cod and cod stored for 6 months at −14°C*

Upper, unfrozen cod Lower, frozen stored cod

Snow extract. In fact the areas of the peaks are always greater in Ultra Turrax extracts than in the corresponding Marsh Snow extracts, and in all cases examined, the total peak areas for the Marsh Snow and Ultra Turrax extracts are in approximately the same ratio as the protein concentrations of extracts as determined from Kjeldahl nitrogen analyses. The results obtained therefore indicate that the greater degree of subdivision of the cells obtained with the Ultra Turrax homogeniser allows for greater solubilisation of the undenatured proteins.

The difference in residual fibre length in the homogenates is obvious from the micrographs in Fig. 6 particularly for those from the frozen stored cod. The Ultra Turrax homogeniser is certainly capable of breaking up the fibres to a much finer state of division as it operates at high speeds (20,000 revs/min) and it has a cutting action which the much slower Marsh Snow homogeniser does not have. The latter homogeniser is a paddle stirrer type with a top speed of 2,200 revs/min. Hamoir[13] pointed out the need for maximum sub-division of fibres if maximum extractability of the muscle proteins was to be achieved.

Storage of homogenates before centrifugation can also affect the value for extractable protein. In unfrozen cod and cod stored for short periods in the cold store, the initial discrepancy between the two methods disappears if the homogenates are allowed sufficient time for equilibrium to be established (usually 24 hours). This would indicate that there is a greater permeability in such cells compared with those of frozen stored cod, and this allows salt to diffuse in and proteins to diffuse out. Also, the cells are easily broken up. In long-term stored frozen fish on the other hand, the cells are more difficult to break up because of the unknown chemical changes which take place during frozen storage and which are thought to be responsible for the toughening phenomenon. In this altered environment and when the percentage of soluble undenatured protein is already less than that of unfrozen cod it is probably all the more important to have as high a degree of sub-division of the cells as possible. This suggestion is supported by the fact that even after the Marsh Snow homogenates of considerably denatured frozen stored cod (e.g. 5 weeks at $-7°$ or 47 months at $-14°$) had been allowed to stand for 48 hours the values for extractable protein were never as high as the corresponding Ultra Turrax values.

This work demonstrates that care is needed to use changes in protein extractability as a measure of cold-storage protein denaturation. The rate of change of protein extractability measured by any one extraction procedure will be meaningful as a guide to storage history, but when results obtained by different workers are compared it is essential to ensure that identical extraction procedures have been followed.

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References

1. Connell, J. J., 'Symposium on foods: proteins and their reactions', (eds. Schultz, H. W., & Anglemier, A. F.) (Avi Publishing Co.: Westport, Connecticut) 1964, pp. 255–293
2. Dyer, W. J., & Dingle, J. R., 'Fish as food', Vol. 1, (ed. Borgstrom, G.) (Academic Press: New York) 1961, pp. 275–321
3. Love, R. M., *J. Sci. Fd Agric.*, 1962, **13**, 269
4. Dyer, W. J., *Fd Res.*, 1951, **16**, 522
5. Dyer, W. J., French, H. V., & Snow, J. M., *J. Fish. Res. Bd. Can.*, 1950, **7**, 585
6. Shimizu, Y., & Simidu, W., *Mem. Coll. Agric. Kyoto Univ.: Fisheries Series Special No.*, June 1958
7. Cowie, W. P., & Little, W. T., *J. Fd Technol.*, 1966, **1** (4), 335
8. Cowie, W. P., & Little, W. T., *J. Fd Technol.*, 1967, **2**, 217
9. Marsh, B. B., & Snow, A., *J. Sci. Fd Agric.*, 1950, **1**, 190
10. Ironside, J. I. M., & Love, R. M., *J. Sci. Fd Agric.*, 1958, **9**, 597
11. Mackie, I. M., *Biochim. biophys. Acta.*, 1966, **115**, 160
12. Connell, J. J., *Biochim. biophys. Acta*, 1963, **74**, 374
13. Hamoir, G., 'Biochemical Society Symposia No. 6. The biochemistry of fish', (Ed. Williams, R. T.) (University Press: Cambridge) 1950, pp. 8–27

PESTICIDE RESIDUES IN FOODSTUFFS IN GREAT BRITAIN

IX.*—Aldrin, dieldrin and other organochlorine pesticide residues in potatoes and carrots

By D. F. LEE

Potatoes from 186 commercial crops from fields treated with aldrin or dieldrin in the year of cropping contained mean residues of aldrin plus dieldrin of 0·01 ppm in the flesh and 0·11 ppm in the skin. Seventy crops from fields that had been treated with aldrin or dieldrin in years previous to the year of cropping and forty-four crops from reputedly untreated fields contained mean residues of aldrin/dieldrin of less than 0·01 ppm in the flesh and 0·02 ppm in the skin. Alpha- and/or gamma-BHC were detected in trace or greater amounts in 73% and pp′ DDE in 10% of the samples.

Carrots from 97 commercial crops from fields treated with aldrin or dieldrin in the year of cropping contained mean residues of aldrin plus dieldrin of less than 0·01 ppm in the flesh and 0·21 ppm in the skin. Fifty crops from fields that had been treated with aldrin or dieldrin in years previous to the year of cropping contained mean residues of aldrin/dieldrin of less than 0·01 ppm in the flesh and 0·07 ppm in the skin. Six crops from reputedly untreated fields contained a mean residue of less than 0·01 ppm dieldrin in the skin only. Alpha- and gamma-BHC were found in the majority of the samples (mean total residue of less than 0·01 ppm in the flesh and 0·03 ppm in the skin) but pp′ DDE and/or pp′ DDT were detected in only 12% of the samples.

Introduction

In Great Britain aldrin and dieldrin are used on potatoes to control damage by wireworms (larvae of *Agriotes* spp.) and were used on carrots to control damage caused by larvae of the carrot fly (*Psila rosae*). Carrot seed is dressed with dieldrin,† gamma-BHC or DDT to control carrot fly, and DDT may be used to control the willow-carrot aphid (*Cavariella aegopodii*), the vector of carrot motley dwarf virus.

The studies reported here were carried out on the recommendation of the Panel on Residues of Pesticides in Foodstuffs.[1] Samples were taken from commercial crops grown in soils that had been treated with aldrin or dieldrin in the year of growth and, because these pesticides persist in the soil, from crops grown in soils that had been treated in previous years. A small number of samples from fields that, reputedly, had never been treated with aldrin or dieldrin was also examined. This procedure does not constitute random sampling of crops, and the results cannot be interpreted as reflecting the general level of residues in all the potatoes or carrots marketed for human consumption. It does, however, indicate the 'worst possible situation' that could arise from good agricultural practice, if all potato and carrot fields were treated with aldrin or dieldrin, which they are not.

Aldrin and dieldrin became available for commercial use on potatoes in 1955. In 1963 and 1964 approximately 20% of the maincrop acreage was treated with aldrin or dieldrin, the principal formulations used being aldrinated compound fertilisers (unpublished data from Potato Marketing Boards' annual crop check survey). Following the report of the Advisory Committee on Pesticides and Other Toxic Chemicals in 1964,[2] aldrinated fertilisers were withdrawn from the market at the end of that year but the use of dust or spray formulations of aldrin and dieldrin on potatoes was permitted to continue.** About 9% of the main-crop potato acreage was treated in 1965 and 5% in 1966.

Reliable information on the extent of use of pesticides on carrots in Great Britain in the years up to 1966 has been difficult to obtain. Strickland[3] has estimated that most of the carrot crop in 1962–63 was treated with dieldrin and, although official approval of the use of aldrin/dieldrin as field treatments and seed dressings for carrots was withdrawn at the end of 1964,[2] some 12% of the carrot crop was treated in 1966 against official advice (Strickland, A. H., unpublished data). BHC has not been extensively used on carrots because of the risk of tainting but in 1966 about 30% of the crop was grown from BHC-dressed seed.

Experimental

Samples

Potatoes

The Potato Marketing Board annually check-weigh samples from some 2,000 potato crops to obtain a pre-harvest estimate of ware tuber production. So far as is possible, the same farms are visited each year, and they are selected so as to be representative of each region of Great Britain in respect of soil type and acreage grown. Information is also collected on various aspects of the cultivation of the crop including the use and the method of application of pesticides. The latter covers the use of aldrin and dieldrin, in the year the crop was grown and in previous years.

Through the co-operation of the Potato Marketing Board samples were obtained in 1963, 1964 and 1966 from fields examined for the purposes described above. Each sample weighed approximately 3 kg and the following aldrin/dieldrin treatment categories were covered:

* Part VIII: *J. Sci. Fd Agric.*, 1968, **19**, 609

† Official approval for the use of dieldrin as a carrot seed dressing was withdrawn with effect from 1 January 1965, following the Government's acceptance of recommendations by the Advisory Committee on Pesticides and Other Toxic Chemicals

** The maximum permitted rates of use on potatoes are: aldrin 48 oz/acre in spray formulation and 67 oz/acre in dust formulation; dieldrin 44 oz/acre in spray formulation

- A_1 aldrinated fertiliser used in the year of sampling on a field that had never before been treated with aldrin or dieldrin;
- A_2 aldrin or dieldrin, in any formulation other than aldrinated fertiliser, used on a field that had never before been treated with these compounds;
- B_1 aldrinated fertiliser used in the year of sampling on a field that had been treated with aldrin/dieldrin previously;
- B_2 aldrin or dieldrin, in any formulation other than aldrinated fertiliser, used in the year of sampling on a field that had been treated with aldrin/dieldrin previously;
- C no aldrin/dieldrin used on the field in the year of sampling but the field had been treated in previous year(s);
- D aldrin/dieldrin had never been used on the field.

Carrots

There is no organisation dealing with the carrot crop in the way that the Potato Marketing Board surveys potatoes. Most of the carrot samples were obtained with the help of officers of the National Agricultural Advisory Service but a few samples were obtained from crops grown under contract for a processing firm. Although an attempt was made to get samples that could be assigned to the same treatment categories used for the potato samples, in many cases the only information available, was that the crop had, or had not, been treated. It was, therefore, neither possible to distinguish between categories A and B nor between the use of aldrinated fertiliser and other formulations. Samples were taken in 1964, 1965 and 1966.

Samples were taken immediately before the crop was harvested and consisted of at least 18 sound roots (total weight about 3 kg) taken along a diagonal traverse of the field.

Untreated potatoes and carrots for blank and recovery determinations were specially grown at these laboratories in soil which had never been treated with organochlorine pesticides.

Preparation and storage of samples before analysis

On receipt 12 sound potato tubers or 18 sound carrot roots were selected from each field sample, scrubbed under running water until free from adhering soil and air-dried. Each tuber or root was halved longitudinally, one half was put aside as a reserve, and the peel was removed from the other half to a depth of about 1 mm with a domestic hand vegetable peeler. The peel fraction averaged some 15% of the whole tuber or root. The peel from the half-tubers or roots was combined, finely chopped and thoroughly mixed. The flesh fraction of the potatoes was macerated and blended without the addition of any liquid, and that of the carrots was finely minced and thoroughly mixed. If the samples were not to be analysed immediately, 200–400 g subsamples of the blended fractions were put into screw-capped bottles and kept at −20° until required. Stored samples were thawed and re-blended before analysis.

Method of analysis

50 g of the blended fractions were macerated with 50 ml cetone and 50 g powdered anhydrous sodium sulphate for ten minutes. 50 ml hexane were added and the mixture was macerated for a further five minutes. The acetone–hexane extract was decanted from the plant material–sodium sulphate slurry. The acetone was removed by washing with water, and the hexane solution was dried over anhydrous sodium sulphate. An aliquot of the dried solution was cleaned up on a 'Florisil'–powdered charcoal column using benzene–hexane mixtures to elute the pesticides. The 'Florisil' was de-activated by storage over a saturated solution of magnesium nitrate. The 'Florisil'–charcoal and benzene–hexane ratios were varied from year to year depending on the activity of the particular batches of absorbants in use but were generally of the order of 3 : 1 'Florisil' charcoal and 1 : 1 benzene–hexane. The eluate from the column after correction to a standard volume was examined for the organochlorine pesticides by gas–liquid chromatography using silicone and Apiezon columns with electron-capture detectors after the method of de Faubert Maunder *et al.*[4] Recoveries of pesticides added in hexane at the 0·1 ppm level at the initial maceration stage were better than 90%. Peaks in the gas chromatograms due to amounts of pesticides equivalent to 0·001 ppm in the samples were readily detected. When a peak assigned to a pesticide representing less than 0·01 ppm but more than 0·001 ppm appeared in the sample chromatograms the amount of pesticide present was recorded as 'trace'. Where residues in excess of 0·1 ppm were indicated by gas chromatography, the hexane extracts, after a clean-up by a dimethylformamide partition technique and concentration, were examined qualitatively by thin-layer chromatography on silica gel plates. In all cases this confirmed the presence of the pesticides.

Results

No residues were detected in the specially grown untreated crops and no major differences were detected in the distribution of residue levels within the various treatment categories in each of the years in which samples were taken.

Potatoes

The ranges and mean levels of residues found are given in Table I. The distributions of the levels of alpha- and gamma-BHC, aldrin, and dieldrin in the flesh and skin fractions are given in Tables II and III and the distribution of total aldrin

TABLE I

Ranges and mean level of organochlorine pesticide residues in potatoes

Sample category (see text)	Number of samples	Total BHC isomers				Aldrin				Dieldrin				pp′ DDE			
		Flesh		Skin		Flesh		Skin		Flesh		Skin		Flesh		Skin	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
A_1	30	0–Tr	—	0–0·01	—	0–Tr	—	0–0·12	0·03	0–0·10	0·02	0·0·26	0·09	—	—	—	—
A_2	91	0–0·2	—	0–0·04	<0·01	0–0·04	—	0–0·25	0·02	0–0·10	<0·01	0–0·50	0·07	—	—	0–0·01	—
B_1	37	0–Tr	—	0–0·23	<0·01	0–Tr	—	0–0·23	0·04	0–0·04	0·01	0–0·69	0·09	—	—	0–Tr	—
B_2	28	0–Tr	—	0–0·03	<0·01	0–Tr	—	0–0·46	0·04	0–0·04	0·01	0–0·49	0·12	—	—	—	—
C	70	0–Tr	—	0–0·01	<0·01	—	—	0–0·04	⩽0·01	0–0·01	⩽0·01	0–0·16	0·02	—	—	0–Tr	—
D	44	0–Tr	—	0–0·03	<0·01	—	—	0–0·12	⩽0·01	0–0·03	⩽0·01	0–0·50	0·02	—	—	0–0·03	—

TABLE II

Distribution of the levels of BHC, aldrin and dieldrin residues found in the flesh fraction of potatoes

Sample type	A_1				A_2				A_1 & A_2				B_1				B_2				B_1 & B_2				C				D			
No. of samples	30				91				121				37				28				65				70				44			
Pesticides	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die
% at the level of:																																
Not detected	93	7	93	10	91	88	95	44	92	66	94	35	92	3	92	18	75	71	93	32	85	32	92	23	89	51	100	74	95	10	100	70
Trace (less than 0·01 ppm)	7	93	7	17	9	10	1	41	8	32	3	35	8	97	8	24	25	29	7	21	15	67	8	23	11	49		21	5	90		20
0·01 ppm				33		1	1	10		1	1	16				22				18				20				5				7
0·02 ppm				13		1	1	1		1	1	14				11				11				18								2
0·03 ppm				17			1	2			1	6				5				11				8								
0·04 ppm				3			1				1	1				9				7				8								
0·05 ppm								1				1																				
0·10 ppm				7				1				2																				

TABLE III

Distribution of BHC, aldrin, dieldrin and pp′ DDE residues found in the skin fraction of potatoes

Sample type	A_1				A_2				A_1 & A_2				B_1				B_2				B_1 & B_2				C				D			
No. of samples	30				91				121				37				28				65				70				44			
Pesticides	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die	α-BHC	γ-BHC	Ald	Die
% at the level of:																																
Not detected	70		13		76	72	21	5	74	54	19	4	68		19	5	64	64	21	4	66	29	20	5	63	49	81	23	50	7	75	36
Trace (less than 0·01 ppm)	27	87	10	7	18	16	27	3	20	33	23	4	32	84	19	5	36	29	29	4	34	58	23	5	34	43	12	19	50	59	16	25
0·01–0·09 ppm	3	13	70	53	6	12	47	72	6	13	53	67		16	48	46		7	43	36		13	45	40	3	8	7	57		34	7	36
–0·19 ppm			7	33			4	11			4	17			11	33			4	43			8	37				1			2	
–0·29 ppm				7			1	8			1	7			3	8				7			2	7								
–0·39 ppm																				3				2								
–0·49 ppm								1				1							3	3			2	2								3
–0·59 ppm																																
–0·69 ppm																3								2								

pp′ DDE was detected in trace amounts in two samples in category C and at 0·02–0·03 ppm in two samples in category D

plus dieldrin in the skin fraction in Table IV.

Aldrin and dieldrin residues

Residues were generally at a low level and it was not possible to establish an exact relationship between the skin and flesh residues, even in samples from treated crops.

Except in two samples residues of aldrin/dieldrin in the skin greater than 0·1 ppm were only found in crops treated in the year of growth (categories A and B). It is considered that incorrect information on treatment was supplied with the anomalous samples.

The flesh residues were always lower, usually of the order of 5 to 10% of those in the skin.

TABLE IV

Distribution of the levels of aldrin plus dieldrin residues found in the skin fraction of potatoes

Sample category (see text)	A_1	A_2	A_1 & A_2	B_1	B_2	B_1 & B_2	C	D
No. of samples	30	91	121	37	28	65	70	44
% in the range								
0–0·1 ppm	53	77	71	54	43	49	98	98
–0·2 ppm	30	13	17	16	32	23	—	—
–0·3 ppm	10	2	4	24	14	20	2	—
–0·4 ppm	7	6	6	3	4	3	—	—
–0·5 ppm	—	—	—	—	—	—	—	—
–0·6 ppm	—	1	1	—	—	—	—	—
–0·7 ppm	—	1	1	—	4	2	—	2
–0·8 ppm	—	—	—	—	—	—	—	—
–0·9 ppm	—	—	—	3	3	3	—	—

Although there appeared to be no difference between the distributions of residue levels in categories C and D, there were indications from categories A and B that treatments in earlier years increased residues resulting from applications in the year of growth.

There were no differences in residue levels that could be ascribed to the kind of pesticide formulations used.

BHC and DDT residues

When residues of these were detected in the skin fractions, the levels were below 0·05 ppm. Residues in the flesh when detected were always less than 0·1 ppm.

Carrots

The ranges and mean levels of residues found are given in Table V. The distributions of the levels of alpha- and gamma-BHC, aldrin and dieldrin in the flesh and skin fractions are given in Tables VI and VII, and the distribution of total aldrin plus dieldrin in the skin fraction in Table VIII.

Aldrin and dieldrin residues

The total residues of these pesticides in the skin were always higher than those in the flesh fraction but, as in the case of potatoes, a constant ratio could not be demonstrated.

BHC and DDT residues

Residues of alpha- and gamma-BHC were apparently found in a large proportion of the samples, but levels reported at below 0·1 ppm must be viewed with caution, because at these levels confirmation by thin-layer chromatography was not usually attempted. When samples had been stored for some

TABLE V

Ranges and mean levels of organochlorine pesticide residues in carrots

Crop treatment	Number of samples	Total BHC isomers				Aldrin				Dieldrin				Total DDE/DDT			
		Flesh		Skin		Flesh		Skin		Flesh		Skin		Flesh		Skin	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Aldrin/dieldrin treated	97	0–0·13	<0·01	0–0·88	0·05	0–0·14	<0·01	0–0·68	0·02	0–0·04	<0·01	Tr–1·39	0·19	0–0·01	—	0–2·5	—
Untreated from aldrin/dieldrin treated soil	50	0–0·02	⩽0·01	0–0·28	0·03	0–0·05	⩽0·01	0–0·07	⩽0·01	0–0·02	⩽0·01	0–0·65	0·07	0–Tr	—	0–1·04	—
Untreated from untreated soil	6	Tr–0·02	<0·01	0–0·28	0·09	—	—	—	—	0–Tr	—	0–0·04	<0·01	—	—	0–Tr	

TABLE VI

Distribution of BHC, aldrin, dieldrin and DDT residues found in the flesh fraction of carrots

Sample type	Aldrin/dieldrin treated*				Untreated from fields treated with aldrin/dieldrin in previous years†				Untreated from untreated fields			
No. of samples	97				50				6			
Pesticides	α-BHC	γ-BHC	Aldrin	Dieldrin	α-BHC	γ-BHC	Aldrin	Dieldrin	α-BHC	γ-BHC	Aldrin	Dieldrin
% at the level of:												
Not detected	27	12	84	23	80	56	90	72			100	33
Trace (less than 0·01 ppm)	71	62	12	55	10	28	4	24	100	50		67
0·01 ppm	2	10		9		12	2			17		
0·02 ppm		8	2	8		4		4		33		
0·03 ppm		3	1	4			2					
0·04 ppm		1		1								
0·05 ppm							2					
0·06 ppm		1										
0·13 ppm		1										
0·14 ppm			1									

* pp′ DDE/DDT was detected at the trace level in 6 samples and at 0·01 ppm in 1 sample
† pp′ DDE/DDT was detected at the trace level in 2 samples

TABLE VII

Distribution of BHC, aldrin, dieldrin and DDT residues found in the skin fraction of carrots

Sample type	Aldrin/dieldrin treated*				Untreated from fields treated with aldrin/dieldrin in previous years†				Untreated from untreated fields‡			
No. of samples	97				50				6			
Pesticides	α-BHC	γ-BHC	Aldrin	Dieldrin	α-BHC	γ-BHC	Aldrin	Dieldrin	α-BHC	γ-BHC	Aldrin	Dieldrin
% at the level of:												
Not detected	12	5	63		70	32	88	24			100	
Trace (less than 0·01 ppm)	80	47	24	3	28	14	6	22	100	50		67
0·01–0·09 ppm	8	35	6	40	2	44	6	38		17		33
–0·19 ppm		6	3	21		6		4		17		
–0·29 ppm		1	3	19		4		2		17		
–0·39 ppm		2		3				4				
–0·49 ppm		1		9				2				
–0·59 ppm		1		3				2				
–0·69 ppm		1	6					2				
–0·79 ppm												
–0·89 ppm		1										
–0·99 ppm												
–1·09 ppm												
–1·19 ppm				1								
–1·39 ppm				1								

* In 8 samples pp′ DDE/DDT was detected in trace amounts, in 3 samples at 0·01 ppm and in single samples at 0·73 ppm and 2·5 ppm
† pp′ DDE/DDT was detected in trace amounts in 5 samples, at 0·02 ppm in 2, at 0·1 ppm in one and at 1·04 ppm in another sample
‡ pp′ DDE/DDT was detected in one sample

TABLE VIII

Distribution of aldrin plus dieldrin residue levels in the skin fraction of carrots

	Treated in season of growth	No treatment but soil treated in previous years
Number of samples	97	50
% of the samples in the range		
0–0·1 ppm	42	80
–0·2 ppm	23	10
–0·3 ppm	15	—
–0·4 ppm	7	4
–0·5 ppm	6	2
–0·6 ppm	1	2
–0·7 ppm	1	2
–0·8 ppm	1	—
–0·9 ppm	—	—
–1·0 ppm	—	—
–1·1 ppm	—	—
–1·2 ppm	1	—
–1·3 ppm	1	—
–1·4 ppm	1	—

time, small peaks sometimes occurred in the gas chromatograms which, on further investigation, were shown not to be due to alpha- or gamma-BHC. The identity of these compounds was not established.

Residues of pp′ DDE or pp′ DDT were detected in 12% of the samples and were usually below 0·1 ppm but of the three samples in which higher levels were detected two were from fields that had been treated with DDT in several previous years.

Discussion

The skin is usually discarded when potatoes and carrots are being prepared for human consumption. Consequently the residues in the flesh are the more relevant in assessing the exposure of the consumer.

In Great Britain maincrop potatoes are usually grown in a four-year, or longer, rotation whereas carrots are frequently grown by specialist growers in a rotation as short as two years. This short rotation coupled with the greater frequency of treatment of the carrot crops with dieldrin in the years preceding this study could be a major reason for the relatively higher residues found in carrot samples, compared with similar potato samples, from untreated crops grown on land treated in previous years. Also, under identical soil residue conditions carrots have been reported to absorb larger quantities of the organochlorine insecticides than do potatoes.[5]

The maximum and mean levels of residues found in samples from crops grown on land treated in previous years were lower than the maximum and mean residues found in samples from crops treated in the year of growth. Therefore, if the trend of decreasing use of aldrin and dieldrin on potatoes and carrots continues, any residues in the crops will be those arising from residues in the soil from usage in previous years. This study has indicated that such residues in potatoes and carrots are very low and are likely to become even lower as the residues in the soil decay.

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References

1. Lee, D. F., *J. Sci. Fd Agric.*, 1966, **17**, 561
2. 'Review of the persistent organochlorine pesticides', 1964, (London: H.M.S.O.)
3. Strickland, A. H., *Ann. appl. Biol.*, 1965, **55**, 319
4. de Faubert Maunder, *et al.*, *Analyst, Lond.*, 1964, **89**, 157
5. Lichtenstein, E. P., *J. agric. Fd Chem.*, 1960, **8**, 448

NEW TROPICAL SEED OILS

II.*—Component acids of leguminous and other seed oils

By F. D. GUNSTONE, GILLIAN M. TAYLOR, J. A. CORNELIUS and T. W. HAMMONDS

The component acids of seed oils from over fifty legumes and over fifty other species are reported, and their potential value is discussed.

Introduction

A preliminary study of a wide range of seed oils, derived mainly from tropical areas, was undertaken in the hope of finding some of commercial value. As a by-product of this activity it was expected that fatty acids of novel structure which would be of academic interest and might have practical value also, might be discovered. Reports on such acids have already appeared.[1,2] A similar but larger investigation is being carried out by American workers, mainly at Peoria (Illinois), and fourteen papers in the series 'Search for New Industrial Oils' have been published.[3]

The composition of 111 seed oils, most of them not previously described, is reported here. These came from 33 different families, with the Leguminosae most widely represented (52 species).

Experimental and Results

Where possible the seed was removed from its outer shell before being crushed in a grinding machine or mortar. The ground seed was extracted exhaustively with petrol (b.p. 40–60°) in a Soxhlet apparatus and the extracted oil was neutralised by passing it (~ 1 g), in chloroform solution, through a short column of alumina (~ 10 g). Methyl esters were prepared by transesterification with sodium and methanol[4] and then examined by chromatographic and spectroscopic procedures. The ultra-violet and infra-red spectra were recorded for all compounds and the n.m.r. spectra in some cases. The esters were examined qualitatively by thin-layer chromatography with silica alone and with silica impregnated with silver nitrate. Quantitative examination was undertaken by gas–liquid chromatography (g.l.c.) using packed columns coated with diethylene glycol succinate and with Apiezon L.

Esters were tentatively identified from their behaviour on these two types of columns. Sometimes saturated, mono- and poly-ethenoid esters were separated by silver ion chromatography and re-examined by g.l.c. Occasionally individual esters were isolated by preparative g.l.c. and/or silver ion chromatography and then identified by von Rudloff oxidation[5] and by n.m.r. spectroscopy.

The results are summarised in Table I.

Discussion

Many of the seeds examined contained only a small amount of oil, and the seeds tested can be divided as shown in Table II. Seed oils obtained in less than 5% yield were not examined further or included in the following discussion.

Oils which had over 60% of linoleic and/or linolenic acid may be of value as drying oils. These are listed in Table III. The most interesting result was that for *Lagenaria mascarena*, a species not reported by Eckey[6] or Hilditch.[7]

Some of the oils resembled cottonseed oil with about 25–35% of saturated acids (mainly palmitic), appreciable amounts of linoleic acid (45–55%), and smaller amounts of oleic acid (15–25%). Those in Table IV came close to this as did several others with less than 10% of oil in the seed.

Other important oils, such as groundnut oil and olive oil, have less than 20% each of saturated acids and of linoleic acid and a high content of oleic acid (> 60%). Species producing oils of this type included several with a high oil content (Table V).

Finally there is an interest in, and a demand for, solid fats similar to cocoa butter, with about 60% of saturated acids, since such fats are rich in glycerides of type 1-saturated 2-unsaturated 3-saturated. Three of the oils approached this composition. In the last of these (Table VI) the saturated acid was predominantly palmitic.

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References

1. Gunstone, F. D., Kilcast, D., Powell, R. G., & Taylor, G. M., *Chem. Commun.*, 1967, 295
2. Gunstone, F. D., & Subbarao, R., *Chem. Phys. Lipids*, 1967, **1**, 349
3. Hagemann, J. M., Earle, F. R., Wolff, I. A., & Barclay, A. S., *Lipids*, 1967, **2**, 371
4. Gauglitz, E. J., & Lehman, L. W., *J. Am. Oil Chem. Soc.*, 1963, **40**, 197
5. von Rudloff, E., *Can. J. Chem.*, 1956, **34**, 1413
6. Eckey, E. W., 'Vegetable fats and oils', 1954, (New York: Reinhold)
7. Hilditch, T. P., & Williams, P. N., 'The chemical constitution of natural fats', 1964, 4th ed., (London: Chapman Hall)

* Part I: *Chem. Phys. Lipids*, 1967, **1**, 349

TABLE I

Acid composition of seed oils

No.	Name	Source (see footnotes)	Oil, %	Component esters, wt. %								
				16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3*	20 : 0	22 : 0	others
	Liliaceae											
1	*Aloe globuligemma*	R	24	11	—	4	16	69	—	—	—	
	Iridaceae											
2	*Morea iridioides*	HK	6	14	—	8	27	25	—	2	—	pre 16:0 (unidentified) 24%
	Casuarinaceae											
3	*Casuarina equisetifolia*	F	1	10	2	4	22	27		7	—	12:0 1%, 14:0 1%, post 20 : 0 (unidentified) 20%
	Ulmaceae											
4	*Celtis sinensis*	HK	10	7	—	4	7	80	2	—	—	
	Ranunculaceae											
5	*Clematis uncinata*	HK	17	14	—	3	10	72	1	—	—	
	Menispermaceae											
6	*Cocculus macrocarpus*	I	2	16	1	9	33	38	3	—	—	
7	*Stephania hernandifolia*	I	15	10	—	4	27	58	1	—	—	
8	*Tinospora cordifolia*	I	25	2	1	8	74	15	—	—	—	
	Capparidaceae											
9	*Cadaba kirkii*	R	20	29	2	5	14	42	1	1	—	14:0 6%
10	*Cladostemon kirkii*	R	35	8	—	5	55	29	—	3	—	
	Rosaceae											
11	*Pygeum africanum*	R	46	20	1	12	32	20	—	1	—	18:3 (conjugated) 14%
	Leguminosae											
12	*Acacia albida*	Z	5	19	1	3	23	51	1	1	1	
13	*A. macrothyrsa*	R	9	14	2	5	21	50	1	1	2	14:0 1%, epoxy ester (?) 3%
14	*A. macrothyrsa*	R	3	10	1	2	27	53	3	2	1	18:1 epoxy ester (?) 1%
15	*A. polyacantha*	R	5	15	1	11	26	41	—	3	1	hydroxy dienoic ester (?) 2%
16	*A. schweinfurthii*	R	1	27	2	8	23	36	2	2	—	
17	*A. tortilis*	R	2	12	—	3	19	60	2	2	2	
18	*Adenodolichos paniculatus*	N	1	18	1	4	18	46	11	1	1	
19	*Aeschynomene indica*	HK	7	19	—	4	19	51	4	2	1	
20	*Albizia amara*	R	11	17	1	7	30	40	1	3	1	
21	*A. harveyi*	R	11	19	2	3	18	52	3	3	—	
22	*A. versicolor*	Z	13	20	1	7	15	53	1	2	1	
23	*A. versicolor*	R	14	23	1	4	14	54	1	2	1	
24	*Alysicarpus vaginalis*	N	3	14	—	6	9	48	21	1	1	
25	*Amblygonocarpus andogensis*	R	12	4	—	4	22	57	2	3	5	20:1 1%, 24:0 2%
26	*A. andogensis*	Z	11	5	—	4	26	57	1	2	3	20:1 1%, 24:0 1%
27	*Baikiaea plurijuga*	R	9	20	—	4	21	46	1	2	2	24:0 3%, 24:1 1%
28	*Bauhinia petersiana*	R	15	16	—	11	21	52	—	—	—	
29	*Brachystegia microphylla*	R	7	18	—	6	24	38	2	2	4	epoxy ester (?) 3%, 24:0 3%
30	*Canavalia ensiformis*	N	1	17	2	2	49	18	7	1	1	pre 16:0 1%, 24:0 2%
31	*Cassia abbreviata*	R	11	22	—	5	16	54	1	—	1	24:0 1%
32	*C. singueana*	R	3	22	1	6	19	46	3	2	—	unidentified 1%
33	*Colophospermum mopane*	R	10	12	—	2	28	45	1	1	5	24:0 4%, unidentified 2%
34	*Elephantorrhiza goetzii*	R	28	14	—	10	63	9	1	3	—	
35	*Erythrina abyssinica*	T	15	15	—	4	31	39	3	2	6	
36	*E. indica*	I	14	12	—	7	44	11	—	4	16	20:1 6%
37	*E. lithosperma*	F	12	17	—	7	21	35	—	—	—	C_{20}–C_{24} esters 20%
38	*E. senegalensis*	N	12	9	—	2	34	44	1	1	4	20:1 4%, 24:0 1%
39	*Gleditsia fera*	HK	5	12	—	2	11	74	1	—	—	
40	*Guibourtia coleosperma*	R	5	6	—	2	18	59	3	2	5	24:0 5%
41	*Isoberlinia angolensis*	Z	3	35	—	4	9	46	3	1	1	24:0 1%
42	*Julbernardia globiflora*	R	8	36	1	8	14	36	1	1	—	unidentified 3%
43	*Lespedeza formosa*	HK	10	10	—	2	15	55	16	1	1	
44	*Lonchocarpus capassa*	R	17	13	—	6	46	6	20	3	4	20:1 1%, 24:0 1%
45	*Mundulea sericea*	R	6	19	—	5	23	33	17	—	3	
46	*Ormosia dasycarpa*	D	5	16	—	12	29	33	1	2	4	20:1 1%, 24:0 2%
47	*O. semicastrata*	HK	6	14	—	5	32	46	1	1	1	
48	*Ostryoderris stuhlmannii*	R	31	17	—	10	24	44	1	2	2	
49	*Piliostigma thonningii*	Z	2	12	—	6	17	63	1	1	—	
49a	*Poinciania regia*	N	8	15	—	10	9	64	1	1	—	
50	*Pongamia glabra*	I	30	12	—	9	46	22	6	1	3	20:1 1%
51	*Pseudarthria hookeri*	Z	5	22	—	3	11	46	15	1	2	
52	*Pseudocadia zambesiaca*	R	17	16	2	6	60	5	6	2	2	24:0 1%
53	*Pterocarpus rotundifolius*	R	12	18	—	12	13	50	2	3	2	
54	*Pterolobium stellatum*	R	10	15	1	5	23	56	—	—	—	
55	*Schotia brachypetala*	R	2	18	1	5	13	62	1	—	—	
56	*Tephrosia purpurea*	C	7	16	—	7	25	24	24	2	2	
57	*T. vogelii*	R	7	15	—	5	23	36	8	3	7	20:1 1%, 24:0 2%
58	*Tetrapleura tetraptera*	T	8	2	—	2	12	45	2	2	20	24:0 8%, unidentified (oxygenated) 7%
59	*Trachylobium verrucosum*	S	7	9	—	3	22	45	—	1	7	20:1 1%, 24:0 11%, 24:1 1%
60	*Vigna multiflora*	N	6	19	1	9	26	33	7	3	1	20:1 1%
61	*V. unguiculata*	N	1	28	—	6	5	32	23	2	4	
62	*Voandzeia subterranea*	N	5	23	—	7	21	42	3	1	3	
	Rutaceae											
63	*Evodia meliaefolia*	HK	23	8	11	3	22	25	31	—	—	
64	*Zanthoxylum avicennae*	HK	36	15	2	2	38	18	25	—	—	
	Simaroubaceae											
65	*Kirkia acuminata*	R	4	10	—	6	15	67	2	—	—	
66	*Simaruba glauca*	K	71	11	—	23	61	4	1	—	—	
67	*S. glauca*	K	65	11	—	27	57	4	1	—	—	
68	*S. glauca*	K	62	10	—	25	60	4	1	—	—	
	Meliaceae											
69	*Azadirachta indica*	N	47	18	—	18	40	21	1	2	—	
70	*Entandrophragma angolense*	N	64	4	15	15	48	13	—	1	—	16:2 (9*c*, 12*c*) 4%
71	*Khaya grandifoliola*	N	41	10	—	11	61	16	1	1	—	
	Euphorbiaceae											
72	*Bridelia cathartica*	R	2	10	—	8	23	15	44	—	—	
73	*B. cathartica*	R	13	9	—	8	19	25	39	—		
74	*Croton sylvaticus*	R	17	9	—	3	19	40	15	2	—	20:1 11%, 20:2 1%
75	*Phyllanthus engleri*	Z	40	11	—	19	18	12	40	—	—	
76	*Securinega virosa*	R	13	10	1	8	13	22	46	—	—	
	Anacardiaceae											
77	*Semecarpus indica*	I	47	11	—	11	48	21	—	5	2	20:1 2%
	Aquifoliaceae											

TABLE I (*continued*)

No.	Name	Source (see footnotes)	Oil %	Component esters wt. % 16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3*	20 : 0	22 : 0	others
78	*Ilex pubescens* Rhamnaceae	HK	19	10	—	6	28	55	—	—	—	unidentified 1%
79	*Berchemia discolor*	R	11	13	—	13	52	17	1	4	—	
80	*Paliurus ramosissimus*	HK	16	9	—	3	45	37	3	2	1	
81	*Ziziphus abyssinica*	R	13	12	—	10	43	29	—	2	1	20:1 3%
82	*Z. mucronata*	N	17	11	—	10	51	25	—	2	—	20:1 1%
83	*Z. mucronata* Malvaceae	R	16	9	—	10	52	26	1	2	—	
84	*Hibiscus mutabilis* Sterculiaceae	HK	9	29	—	2	14	47	—	—	—	pre 16:0 2%, epoxy ester (?) 3%, unidentified 3%
85	*Helicteres isora* Ochnaceae	I	14	11	—	6	15	68	—	—	—	
86	*Brackenridgea zanguebarica* Pentaphylacaceae	R	18	23	—	11	27	38	1	—	—	
86a	*Pentaphylax euryoides* Guttiferae	HK	6	8	—	4	20	66	1	1	—	
87	*Garcinia indica*	I	35	2	—	57	40	1	—	—	—	
88	*G. oblongifolia* Hypericaceae	HK	35	2	—	24	71	2	1	—	—	
89	*Harungana madagascariensis* Caricaceae	Z	7	9	—	2	14	71	2	1	1	
90	*Carica papaya* Lythraceae	I	23	17	1	8	68	6	—	—	—	
91	*Lagerstroemia indica* Combretaceae	HK	6	8	—	2	6	81	2	1	—	
92	*Combretum elaeagnoides* Cornaceae	R	7	6	—	2	17	8	—	1	—	10:0 2%, 12:0 52%, 14:0 11%, 20:1 1%
93	*Alangium lamarckii* Sapotaceae	I	1	4	—	8	32	36	—	—	—	C_{12} and C_{14} acids 20%
94	*Butyrospermum paradoxum*	N	42	3	—	37	42	12	2	4	—	
95	*B. parkii*	X	46	4	—	39	36	13	2	6	—	
96	*Madhuca latifolia*	X	40	21	—	25	38	15	1	—	—	
97	*M. longifolia* Loganiaceae	X	40	22	—	38	37	2	—	1	—	
98	*Strychnos cocculoides*	R	2	14	1	4	47	27	2	—	—	24:0 5%
99	*S. mellodora* Convolvulaceae	R	1	24	—	2	45	29	—	—	—	
100	*Ipomea hederacea* Verbenaceae	HK	8	28	1	7	15	42	4	3	—	
101	*Tectona grandis* Solanaceae	I	34	12	—	9	17	58	1	3	—	
102	*Capsicum frutescens* Pedaliaceae	HK	12	17	—	4	15	64	—	—	—	
103	*Ceratotheca triloba*	R	18	7	—	5	26	60	1	—	—	pre 16:0 1%
104	*Sesamum alatum* Cucurbitaceae	N	7	8	1	6	39	45	—	1	—	
105	*Lagenaria mascarena* Compositae	R	49	11	—	5	5	78	1	—	—	
106	*Bidens pilosa*	Z	5	31	—	5	13	49	—	—	—	14:0 2%
107	*Elephantopus scaber*	C	9	51	—	5	4	38	—	—	—	14:0 2%
108	*Tridax procumbens*	Z	6	14	—	17	12	52	—	3	—	hydroxy dienoic ester (?) 2%
109	*Vernonia ampla*	R	1	14	7	3	17	23	—	—	—	pre 16:0 5%, epoxy ester (?) 22%, unidentified 9%

* Where this value is low it indicates the amount of 18 : 3 and/or 20 : 1

Sources
C = Ceylon; D = Dominica; F = Fiji; HK = Hong Kong; I = India; K = Kenya; N = Nigeria; R = Rhodesia; S = Singapore; T = Tanzania; X = Commercial sample of unknown origin; Z = Zambia

Common names of species listed in Table I

3 Swamp oak, bull oak
8 Giloe
11 Red stinkwood, bitter almond, bitter amandel
12 Ana tree, apple ring acacia, whitethorn, winterthorn
17 Umbrella thorn
24 Alyce clover
27 Rhodesian teak, Zambesi redwood
28 Camel's foot
30 Jack bean, Chickasaw lima, horse bean, swordbean, cut eye, one-eye bean, overlook
33 Ironwood, red angola copal, Rhodesian ironwood, Rhodesian mahogany, turpentine tree
35 Lucky bean, Rhodesian kaffir boom
36 Indian coralbean
40 Bastard mopane, bastard teak
44 Lance tree, rain tree
49 Camel's foot leaf tree, camel foot, redhead, Rhodesian bauhinia, wild bauhinia
49a Flamboyant, gold mohur, flame tree
50 Indian beech
52 Nyala tree
55 African walnut, Hottentot's bean (tree), South African greenheart, Boer bean, tree fushia
56 Ash vetch
57 Fish bean, fish poison
59 Bombay copal, copal wood, gum copal (tree), East African copal
61 Cowpea, Chinese longbean
62 Bambara groundnut, Congo goober, earth pea, haricot pistache, Madagascar or stone groundnut
65 Bastard marula, white seringa
66–68 Aceituno
69 Neem, nim lilas d'Inde
71 Benin mahogany, broad-leaved mahogany, big-leaf mahogany, Khaya mahogany
74 Mt. Silinda linden
79 Bird plum
82–83 Buffalo thorn, Cape thorn, shiny leaf, wait-a-bit
84 Chameleon rose
85 East Indian screw tree
90 Pawpaw, melon tree
91 Cape myrtle, crape myrtle, crepe tree, Indian lilac, pride of India
95 Shea butter tree
96 Mowra, mahwa, Indian butter tree
97 Illipé
98 Kaffir orange, wild orange
101 Teak
102 Cayenne pepper, red pepper, chilli bean
103 Rhodesia foxglove, wild foxglove
106 Beggar sticks, beggar tick, beggar ticks, black fellows, black jack, bur marigold, cobblers pegs
107 Prickly leaved elephant's foot

TABLE II

Oil yield of some seed samples, %

Yield	Samples
1–4	19
5–9	31
10–19	34
20–29	6
30–39	7
40–49	10
> 50	4

TABLE III

Seed samples with oils containing 60% linoleic and/or linolenic acid

No.	Name	oil, %	polyethenoid acids, %
91	*Lagerstroemia indica*	6	83
4	*Celtis sinensis*	10	82
105	*Lagenaria mascarena*	49	79
39	*Gleditsa fera*	5	75
89	*Harungana madagascariensis*	7	73
5	*Clematis uncinata*	17	73
43	*Lespedeza formosa*	10	71
1	*Aloe globuligemma*	24	69
76	*Securinega virosa*	13	68
85	*Helicteres isora*	14	68
110	*Pentaphylax euryoides*	6	67
111	*Poinciania regia*	8	65
73	*Bridelia cathartica*	13	64
102	*Capsicum frutescens*	12	64
40	*Guibourtia coleosperma*	5	62
51	*Pseudarthria hookeri*	5	61
103	*Ceratotheca triloba*	18	61

TABLE IV

Acid content of some seed oils resembling cottonseed oil, %

No.	Name	oil	sat.	mono-ethenoid	poly-ethenoid
9	*Cadaba kirkii*	20	41	16	43
22	*Albizia versicolor*	13	30	16	54
23	*A. versicolor*	14	30	15	55
31	*Cassia abbreviata*	11	29	16	55
87	*Brackenridgea zanguebarica*	18	34	27	39

TABLE V

Acid content of some seed oils resembling groundnut and olive oils, %

No.	Name	oil	sat.	mono-ethenoid	poly-ethenoid
10	*Cladostemon kirkii*	35	16	55	29
34	*Elephantorrhiza goetzii*	28	27	63	10
52	*Pseudocadia zambesiaca*	17	27	62	11
66	*Simaruba glauca*	71	34	61	5
71	*Khaya grandifoliola*	41	21	61	17
90	*Carica papaya*	23	25	69	6
88	*Garcinia oblongifolia*	35	26	71	3

TABLE VI

Acid content of some seed oils resembling cocoa butter, %

No.	Name	oil	sat.	mono-ethenoid	poly-ethenoid
96	*Madhuca latifolia*	40	46	38	16
97	*M. longifolia*	40	61	37	2
107	*Elephantopus scaber*	49	58	4	38

ANTIOXIDANTS IN OATS : GLYCERYL ESTERS OF CAFFEIC AND FERULIC ACIDS

By D. G. H. DANIELS and H. F. MARTIN

Of the antioxidants extracted from oats, 36% (by weight) consists of six compounds which are more strongly adsorbed on to silicic acid than those previously described,[1,2] One of these has been isolated by column chromatography. On hydrolysis it yields caffeic acid (2 moles), ferulic acid (1 mole), glycerol (1 mole) and long-chain ω-hydroxyacid (1 mole). The ω-hydroxyacid fraction contains the homologues, C_{22} (5%), C_{26} (64%), and C_{28} (31%).

The probable structures of the compounds are discussed.

Introduction

In previous work[1] 24 phenolic antioxidants have been detected in extracts from oats by two-dimensional thin-layer chromatography (t.l.c.). Eight of these were identified as esters of caffeic or ferulic acids and long-chain mono-alcohols, diols or ω-hydroxyacids. These esters comprise the main components numbered **I–V** in an earlier publication,[2] together with three minor components separated from them by improvements in technique.

As shown in Fig. 2, of a previous paper,[2] a second group of antioxidants, **VI** and **VII**, is quantitatively as important as the first group. This second group is resolved into 5 spots, labelled 20–24, on two-dimensional t.l.c.[1] Preliminary examination has indicated that these antioxidants are probably more complex than those hitherto described. The present work is concerned with the isolation and analysis of the substance corresponding to spot 23.

Experimental

The same procedure as previously described[1] was used apart from the variations noted. For t.l.c. solvent systems (a), (b) and (e) were used, and also system (f), chloroform–ethanol–acetic acid, 96 : 4 : 2. System (f) resolved spot 24 into two, labelled 24a and 24b.

Preliminary separation of antioxidants into groups

The experiment summarised in Fig. 2 of the earlier paper[2] is illustrative of numerous early separations with low resolution. In this experiment diethyl ether–light petroleum (b.p. 60–80°, 50 : 50, v/v) eluted 81 mg (50%) consisting of antioxidants 1–19, and diethyl ether–light petroleum (75 : 25, v/v) eluted 75 mg (46%) consisting of antioxidants 20–24. In another experiment the two groups were hydrolysed. The first yielded caffeic and ferulic acids and a solid, m.p. 100–105°, in 54% yield; glycerol was absent from the water-solubles. The second group also yielded caffeic and ferulic acids and a solid, m.p. 97–100°, in 48% yield; glycerol was detected in the water-solubles. A yield of 9·8% of glycerol in this hydrolysate may be calculated from that reported for the antioxidant mixture, 4·5%.[2]

Extraction and purification of antioxidant 23

The former procedure[1] was followed repeatedly to obtain several g of the 'Precipitate containing antioxidants 15–24', shown in Fig. 1 of that paper. Subsequent operations are set out in Fig. 1 of the present paper.

This material (540 mg) was chromatographed on a column of silicic acid (60 cm × 1·4 cm in diameter, Mallinckrodt SilicAR CC7 100–200 mesh, activated at 120° overnight and packed by Lovern's method[3]). The antioxidants were eluted with diethyl ether–light petroleum (b.p. 60–80°, 50 : 50, v/v) in 8 fractions varying in volume from 500 ml to 2 l. The purpose of this step was to separate antioxidants 19 and 23. These compounds are not resolved on a column run in chloroform, which is the next step. After analysis by t.l.c. (solvent mixtures (e) and (f)), fractions were treated as described below.

Fractions 1 and 2 were discarded as they contained no antioxidant 23; fraction 3, containing some 19 as well as 23, was recycled with fresh starting material in subsequent preparations; fractions 4–8 (free from 19) were chromatographed on another column of CC7 silicic acid, activated at 120° overnight and packed as a slurry in chloroform. The eluents were chloroform followed by ethyl acetate–chloroform (10 : 90, v/v). 40 ml fractions were collected and bulked as shown in Fig. 1 (again after t.l.c. analysis). Fractions 34–60, which contained antioxidant 23 and a trace of impurity, were combined. The chloroform was removed using a rotary vacuum evaporator at below 10°, and the residue was dissolved in diethyl ether. Addition of light petroleum (b.p. below 40°) to this solution precipitated antioxidant 23 in a pure state as shown by t.l.c. (solvent mixtures (e) and (f)), yielding 24·8 mg. The procedure was repeated until approximately 100 mg was obtained.

Hydrolysis and determination of products

Long-chain compounds

A known weight (~ 15 mg) of antioxidant was hydrolysed in a sealed tube.[1] The insoluble products were filtered, washed with water, dried and weighed. They were extracted with hot light petroleum (b.p. 60–80°) and the dark-coloured residue was weighed.

Caffeic and ferulic acids

The aqueous filtrate and washings from the above hydrolysis were extracted with ethyl acetate (3 × 10 ml), the combined extracts evaporated to dryness under reduced pressure below 50° and re-dissolved in diethyl ether–chloroform (20 : 80 v/v, 50 ml). Ferulic and caffeic acids were determined spectrophotometrically following chromatography on a silicic acid column (30 cm × 1 cm diameter, Mallinckrodt SilicAR CC4, 100–200 mesh, activated at 100° overnight and packed in diethyl ether–chloroform, 20 : 80, v/v). A 5 ml sample was applied to the column; ferulic acid was eluted with this mixture (90 ml) and caffeic acid with

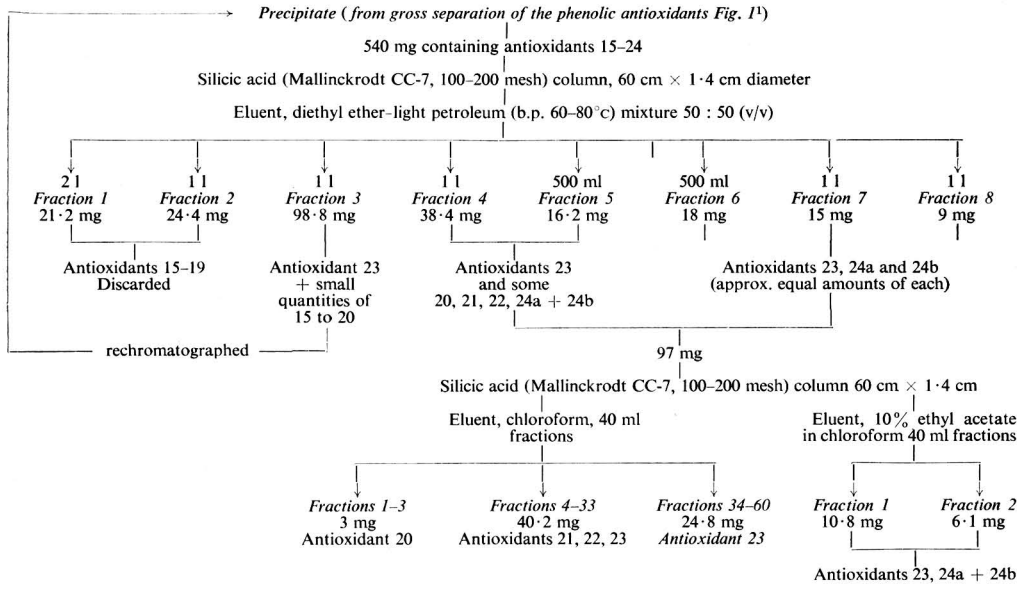

FIG. 1. *Isolation of antioxidant 23*

diethyl ether–chloroform (40 : 60, v/v, 90 ml). The eluates were diluted to 100 ml.

Glycerol

The presence of glycerol in a de-ionised hydrolysate[2] was demonstrated by t.l.c.[4] Lambert & Neish's colorimetric method[5] was employed for the determination of glycerol, after precipitating the organic acids as lead salts in a crude hydrolysate. Antioxidant samples (1·5 mg) were hydrolysed with potassium hydroxide (20% w/v, 0·25 ml). The alkaline hydrolysate was washed into a centrifuge tube, lead acetate solution (10% w/v, 1·7 ml) was added and the solution diluted to 10 ml with water and centrifuged. Excess lead in a 5 ml portion of the supernatant was precipitated by adding sulphuric acid (1·0 N, 5 ml) and the lead sulphate was centrifuged off. Two aliquots (4 ml) of this supernatant were pipetted into 20 ml flasks for oxidation.[5] For colour development, 1 ml samples of the oxidised solutions were mixed with 6 ml of chromotropic acid reagent. An EEL (Evans Electroselenium Ltd.) Colorimeter (626 yellow filter) was used. To prepare a standard curve (straight line), glycerol solutions containing alkali and phenolic acids in similar concentration to the hydrolysates were subjected to the same procedure.

Partial Hydrolysis

Partial hydrolyses were also carried out in evacuated sealed tubes. Various concentrations of alkali (0·1–4·0 N aqueous sodium hydroxide) were used at a constant temperature of 55° for reaction times of 5–100 minutes. The reaction mixtures were neutralised, extracted with ethyl acetate and the extracts examined by t.l.c. (solvent systems (a) and (b)).

Results

Properties of antioxidant 23

The substance is a low-melting gum. The u.v. and i.r. spectra resemble those of other oat antioxidants. The antioxidant activity[6] is 31 units/mg (the activities of other oat antioxidants are listed in a previous paper[1]). The results of chemical analyses are summarised in Table I.

TABLE I

Chemical analyses of antioxidant 23

	Found	$C_{57.33}H_{79.33}O_{14}$ requires
	1038 (Rast) 1077 (Vapour pressure of chloroform solution)	997
	C, 68·8; H, 8·1%	C, 69·5; H, 8·0%
Caffeoyl groups (2)	33·2	32·7
Feruloyl group (1)	18·1	17·8
Hydroxy acid released on hydrolysis	41·5	42·3
Glycerol released on hydrolysis	9·2	9·2

Hydrolysis

Long-chain products

Complete hydrolysis of antioxidant 23 yielded 43·5% of water-insoluble residue, of which 95·4% was soluble in hot light petroleum. This fraction had m.p. in the range 98·2–

99·6°, and its i.r. spectrum was identical with those of 26-hydroxyhexacosanoic acid and the mixed acids derived from antioxidant 11. Its acetyl methyl derivative gave three peaks on gas chromatography corresponding to the derivatives of authentic ω-hydroxy acids: C_{22}, retention time 14·9 min, 5·2% (standard deviation (s.d.) 0·9); C_{26}, 45·3 min, 64·3% (s.d. 3·6); C_{28}, 74·7 min, 30·5% (s.d. 3·5).

Caffeic and ferulic acids

The recovery of ferulic acid from a column of CC4 silicic acid was quantitative whereas that of caffeic acid varied from 90–95%. An overall recovery of 80% of caffeic acid was obtained when two samples of antioxidant, one with added caffeic acid, were subjected to hydrolysis, extraction and chromatography side by side (found: caffeoyl group, 33·2% (single determination, corrected for recovery); feruloyl group, 18·1% (duplicate determination)). The ratio caffeoyl group; feruloyl group is 1·99 : 1. Assuming $E^{1\%}_{1cm}$ (caffeoyl) = 1120 and $E^{1\%}_{1cm}$ (feruloyl) = 1110 at 331 nm for ethanolic solutions of esters, the calculated value of $E^{1\%}_{1cm}$ for the intact antioxidant is 574 (measured directly, 564).

Glycerol

The yield of glycerol in a hydrolysate was 9·16%. Hydrolysis in the presence of added glycerol gave 99% recovery.

Partial hydrolysis

After treatment with 0·1 N sodium hydroxide at 55° for 100 min, much unreacted antioxidant remained and the only detectable hydrolysis products were caffeic and ferulic acids. With 0·5 N, 1·0 N and 4·0 N alkali, other conditions being unchanged, two additional phenolic products were present. Treatment with 1·0 N sodium hydroxide at 55° for periods of 5–40 min showed that the free acids and the additional products were released simultaneously. Comparison with known substances (R_f in t.l.c., solvent systems (a) and (b), and colour reactions with diazotised *p*-nitroaniline) suggested that these products were 26-*O*-feruloyl-26-hydroxyhexacosanoic acid[1] (higher R_f, pink colour reaction) and 26-*O*-caffeoyl-26-hydroxyhexacosanoic acid (lower R_f, brown colour reaction). Phenolic material of lower R_f than antioxidant 23 appeared in early stages of the hydrolysis and then disappeared.

Proposed structure for antioxidant 23

On complete hydrolysis, antioxidant 23 yields caffeic acid, ferulic acid, glycerol and long-chain ω-hydroxy acid, in the molar ratio of 2 : 1 : 1 : 1. As in other members of the oat antioxidant family, the long-chain part is mainly a mixture of the C_{26} and C_{28} acids in the approximate ratio of 2 : 1, but a trace of the C_{22} acid is also present in this case.

These findings lead to the partial structure

$$\left.\begin{array}{l}CH_2O-\\ |\\ CHO-\\ |\\ CH_2O-\end{array}\right\} \quad \left\{\begin{array}{l}-R_1\\ \\ -R_2\\ \\ -CO(CH_2)_nOR_3\end{array}\right.$$

where n = 25 or 27 (trace, $n = 21$)

R_1 = caffeoyl = $-COCH{:}CHC_6H_3(OH)_2$

$R_2 \neq R_3$

R_2, R_3 = caffeoyl or feruloyl.

Table I shows fair agreement between the experimental results and those calculated for the mixture $(C_{57}H_{78}O_{14})_2 + C_{59}H_{82}O_{14}$.

Unfortunately, the experiments on partial hydrolysis do not define the structure of antioxidant 23 more exactly. *O*-caffeoyl- and *O*-feruloyl- fragments are released, and survive in the reaction mixture after the disappearance of the antioxidant and the appearance of considerable amounts of free caffeic and ferulic acids. It may be concluded that ester linkages attached to glycerol are more labile than those at the ω-position of the long-chain hydroxy acids. The presence of both these fragments in hydrolysates requires some explanation. It seems probable that both are present in the antioxidant as isolated, i.e. antioxidant 23 contains various position isomers in addition to the homologues of the long-chain portion, so that the above partial structure is the best that can be written. The difficulty of separating isomeric glycerides is well-known in the lipid field. The alternative explanation that it is a single ester which undergoes transesterification to give the observed products, seems less likely.

Discussion

In confirmation of the early results,[2] approximately 36% of the starting material was recovered as fractions rich in antioxidants 20–24 (**VI** and **VII**) after chromatography on the first column (Fig. 1). Antioxidant 23 was selected for further study, as it appeared to be a typical member of the group and reasonably abundant. Although it was obtained in a pure state as judged by two-dimensional t.l.c., the degradative evidence shows that it probably contains isomers, in which the positions of caffeoyl and feruloyl residues have been interchanged.

It seems probable that other members of the 20–24 group have the same basic make-up as antioxidant 23, but with different proportions of caffeoyl and feruloyl groups. In particular substance 20, which gives the characteristic pink coloration with diazotised *p*-nitroaniline, is probably the triferuloyl analogue, and substance 21 may be the monocaffeoyldiferuloyl analogue of antioxidant 23.

Antioxidant 23 has the second highest activity of all those isolated from oats; the high activity parallels the high proportion of caffeic acid in the molecule.

Acknowledgment

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References

1. Daniels, D. G. H., & Martin, H. F., *J. Sci. Fd Agric.*, 1967, **18**, 589
2. Daniels, D. G. H., King, H. G. C., & Martin, H. F., *J. Sci. Fd Agric.*, 1963, **14**, 385
3. Lovern, J. A., Olley, J., & Watson, H. A., *J. Sci. Fd Agric.*, 1959, **10**, 327
4. Wright, J., *Chemy Ind.*, 1963, p. 1125
5. Lambert, M., & Neish, A. C., *Can. J. Res.*, 1950, **28B**, 83
6. Daniels, D. G. H., & Martin, H. F., *Nature, Lond.*, 1961, **191**, 1302

CAROTENE BLEACHING ACTIVITY OF TOMATO EXTRACTS

By J. A. BLAIN, J. D. E. PATTERSON and M. PEARCE

A water-soluble factor (or factors) in tomato which bleaches β-carotene in the presence of unsaturated fats has been studied. It behaves like haematin and not as a true lipoxidase. Inhibition of this factor and of haematin-catalysed oxidation of linoleate by water-soluble constituents of the juice has also been demonstrated.

Introduction

Studies on the enzyme-catalysed bleaching of carotene have been concerned mostly with lipoxidase (linoleate–oxygen oxidoreductase, Enzyme Commission 1.13.1.13) which destroys carotenoids in the presence of fatty acids containing the pentadiene group. This enzyme is the subject of a review by Tappel[1] in which he limits the sources adequately characterised as containing true lipoxidase to cereal grains and legumes.

Although lipoxidase has not been proved to occur in green leaves, a carotene-destroying enzyme or enzymes from leaves has been described by Walsh & Hauge,[2] Booth[3] and Friend & Mayer.[4] The activity of such enzymes in the absence of lipid material has not yet been demonstrated.

The destruction of carotene in the presence of oxidised linoleate by haematins such as cytochrome *c* has also been studied[5] and must be considered as a possible mechanism when such activity is detected in extracts of vegetable material.

The term carotene oxidase system is thus probably a useful one to retain until it is possible to be more specific.

The work reported in this paper originated in observations on the carotene oxidase activity of tomato extracts. In the course of characterising this some methods which are possibly of more general application have been found.

Experimental

Materials

Linoleic acid and methyl linoleate from the Hormel Institute were stored *in vacuo* at −22° and only freshly prepared solutions were used. They were dissolved separately in ethanol to give a concentration of 5 mg/ml for methyl linoleate and 2 mg/ml for linoleic acid. β-Carotene was dissolved in acetone under reflux to give a fresh solution at a concentration of 10 mg/60 ml.

Ethanol used in the thiocyanate assay was refluxed for 0·5 h over aluminium foil and potassium hydroxide and redistilled, to avoid high blanks.

Soya extracts were prepared by stirring 0·5 g of finely ground de-fatted soya flour with 40 ml of water for 20 min, followed by centrifugation.

McIlvaine's[9] citric acid/phosphate buffers were used.

Agar-Agar (Kobe powder) was dissolved by boiling in buffer (pH 5·5) to give a concentration of 1·5% w/v.

Horse heart cytochrome *c*, (Type III); peroxidase, (Type I) and recrystallised bovine haemoglobin were obtained from the Sigma Chemical Co.

Quercetin and quercitrin samples (grade, Puriss.) were obtained from Fluka AG, Buchs, Switzerland.

Methods

A single source of tomatoes was unavailable so different local varieties were used. In general, no notable differences between different batches of ripe firm tomatoes were observed.

Extracts were usually prepared by homogenising the whole tomato in a blender with 2 volumes of water and centrifuging to obtain a clear superantant. Four subsequent serial dilutions with an equal volume of water were made, and the series were examined simultaneously on cup-plates as described below. Extracts of tomato skin were made by grinding with sand and were treated in a similar fashion.

The cup-plate diffusion technique used to examine tomato extracts was modified slightly from that described by Blain & Todd.[6] To the boiled agar solution, cooled to 50°, 2 ml methyl linoleate solution and 8 ml β-carotene solution were added, followed by other additions when required. The plates were left to gel for 30 min after being poured into Petri dishes, and cups of about 0·2 ml capacity were cut with a cork-borer. Extracts were pipetted into the cups, and the dishes were sealed with plasticine and incubated at 25°, for 20 h. Exposure to light during manipulation and storage was minimised.

The antioxidant properties of fractions from tomato were examined using a system in which linoleic acid was oxidised by cytochrome *c*. The linoleate hydroperoxide formed was estimated by the following modification of the thiocyanate assay of Koch *et al.*[7]

To 25 ml buffer (pH 6·5) kept stirred at 25° were added 2 ml of linoleic acid solution and 1 ml solution containing 0·04 mg/ml cytochrome *c*. Where larger columes of tomato extracts were added for testing the volume of buffer was reduced correspondingly. At intervals 1 ml samples were removed and pipetted into 12·5 ml of redistilled ethanol for assay. Samples were sheltered from strong light during the estimation.

The liquid carotene-bleaching system was that of Blain *et al.*[8] Oxidised linoleic acid was prepared by spreading a weighed sample on a slide, keeping it at 50° for 24 hours and washing it with ethanol. The reaction was carried out in 25 ml of buffer (pH 5·5) containing 3 ml tomato extract or 1 ml of 10^{-5} M haemoglobin solution.

Results

Extracts of tomato skin and dilutions of tomato juice when examined by the cup-plate technique both produced well defined zones of carotene bleaching within 20 h. If the extracts were subjected to prior ultra-filtration through cellophane membrane, the ultra-filtrate failed to give bleached

zones while the residual material was not diminished in activity. Extracts were heated at various temperatures for 3 minute periods before assay and it was found that major inactivation occurred between temperatures of 65° and 70°, although barely perceptible bleaching activity persisted in samples heated above 90°.

Since the active factor was thus apparently both macromolecular and heat-labile, a carotene oxidase system was presumed to be present.

It was observed that bleached zones were visible only after a period exceeding ten hours at all concentrations examined. In this they resembled haematins and differed from soya extracts which, over a wide range of dilutions, give zones within 2 hours.[6]

Oleic acid, which is not a substrate for lipoxidase but which may act as a substrate for haematins, was substituted for linoleate in the plates. As indicated in Fig. 1 this was equally effective in promoting carotene bleaching. Where log enzyme concentration is plotted against a projection of zone diameter[9] the most concentrated tomato extract has been assigned an arbitrary concentration value of one thousand. To test the possibility of direct carotenase action, caprylic acid was substituted for linoleate on the assumption that this would produce a similar dispersion of the carotene without giving rise to fatty peroxides as might oleate and linoleate by autoxidation. No bleaching resulted.

From these observations it seemed likely that the unsaturated lipid oxidase activity of tomato extracts was similar to that of haematins.

To confirm that the carotene bleaching was associated with pre-formed hydroperoxides, a liquid system was used to compare the carotene-bleaching action of tomato extracts in the presence of linoleic acid with their action in the presence of linoleic acid, which had been partly peroxidised by being heated for 20 h at 50°. Haemoglobin activity was compared on the same system. The results, shown in Fig. 2, confirm that tomato extracts destroy carotene mainly by a coupled reaction with oxidised linoleate.

Inhibition by ascorbic acid and quercetin

In the course of this work it was observed that inclusion of 4×10^{-3} M ascorbic acid in the gel would produce a long induction period in the formation of zones when haematins, such as catalase, peroxidase and cytochrome *c*, were used as catalysts but not when soya extracts were used. It seemed possible that other antioxidants would behave in a fashion similar to ascorbic acid in this respect.

A comparison was made of the bleaching effects of soya extract, tomato extract and peroxidase on carotene/linoleate gels in the presence and absence of the antioxidants. The antioxidants were ascorbic acid, 4×10^{-3} M, and quercetin, 3×10^{-5} M. Results shown in Fig. 3 indicate that the extract of tomato again resembles the haematin and differs from the soya extract in being readily inhibited by the antioxidants.

Intrinsic inhibitory material in tomato extracts

It was found that when undiluted tomato juice was used on the plates, bleached zones were slow to appear. If a series of dilutions were prepared, then after a period of 20 h no zones could be observed for the undiluted juice, while at sufficient dilution a bleached zone with an inner unbleached zone was manifest. Such inner zones can be seen in Fig. 3.

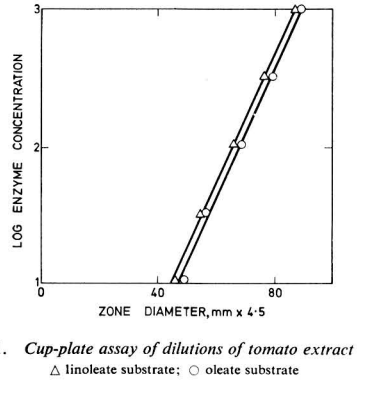

FIG. 1. *Cup-plate assay of dilutions of tomato extract*
△ linoleate substrate; ○ oleate substrate

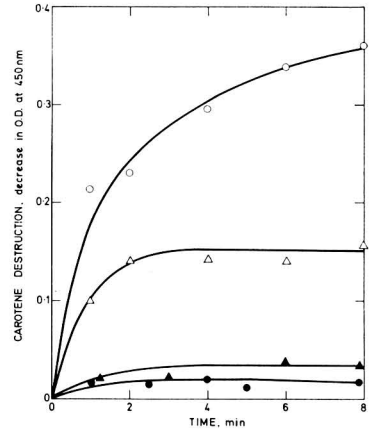

FIG. 2. *Comparison of effects of linoleic acid and oxidised linoleic acid* (7×10^{-4}M) *on rates of carotene destruction by tomato extract and by haemoglobin* ($2{\cdot}5 \times 10^{-6}$M)
○ Haemoglobin and oxidised linoleate; △ Tomato extract and oxidised linoleate;
● Haemoglobin and linoleate; ▲ Tomato extract and linoleate

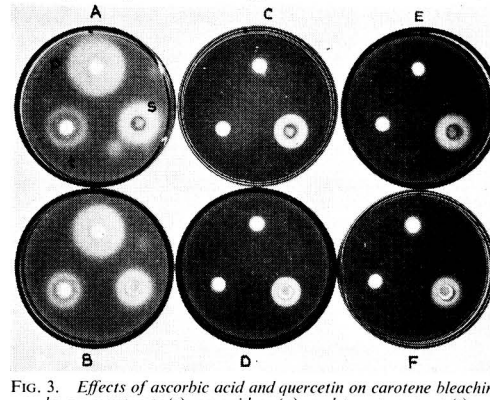

FIG. 3. *Effects of ascorbic acid and quercetin on carotene bleaching by soya extracts* (s), *peroxidase* (p), *and tomato extract* (t)
A and B Control; C and D with ascorbic acid; E and F with quercetin.
In A, C and E the concentrations of extracts are five times those in B, D and F

At sufficient dilution bleaching occurred without the appearance of any unbleached zone. Unbleached zones were observed with extracts of both skin and pulp, the former giving both larger bleached zones and larger inhibited zones.

To ascertain whether these central areas were in fact unbleached carotene, rather than some other pigmentation which had developed, a number were cut out and homogenised with chloroform–methanol (2 : 1 v/v). Water was added, the lower chloroform layer was dried over anhydrous sodium sulphate, and its absorption spectrum was compared with that of extracts made in a similar fashion from the unbleached areas of the plate. Their spectra corresponded. In confirmation it was also found that the unbleached zones could be bleached by oxidation by prior addition of crude lipoxidase in the form of soya extract to the tomato extract before incubation of the plates.

Tomato extracts of a concentration high enough to give inhibitory zones were dialysed overnight and compared with undialysed extracts kept under similar conditions, volume changes due to dialysis being compensated. Dialysis greatly diminished the inhibitory effect.

Inhibition of peroxide formation

A further examination of the inhibitory effect was made using a liquid system in which the formation of hydroperoxide from linoleate was catalysed by cytochrome *c* and measured by a thiocyanate method with and without the addition of tomato extract. As can be seen in Fig. 4 total inhibition over the period studied is effected by addition of 5 ml tomato juice while the addition of 3 ml gives an induction period typical of the effects of antioxidants on fat oxidation.

When haemoglobin, equimolar to the cytochrome *c*, was used as catalyst similar results were obtained.

Separation of enzymic and inhibitory factors

When tomato juice was saturated with ammonium sulphate and the resulting precipitate was removed by centrifugation, the supernatant liquid tested on the cup-plate assay gave greatly diminished bleached zones, still with central inhibitory zones. The precipitate, however, was markedly yellow in colour and its solution failed to give bleached zones. It was considered that part of the inhibitory fraction had remained with the precipitate, and the following separation procedure was used.

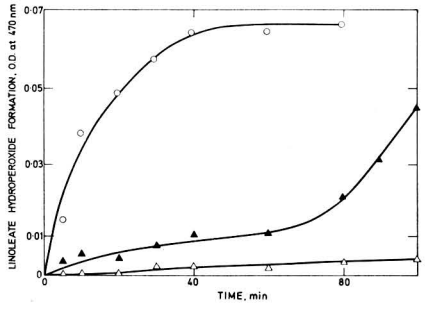

FIG. 4. *Effect of tomato juice on the cytochrome-catalysed oxidation of linoleic acid*

○ Control; ▲ 3 ml tomato juice; △ 5 ml tomato juice

2 kg of tomatoes were homogenised in a blender; the juice was extracted by a hydraulic press, and was strained through cotton wool, saturated with ammonium sulphate and centrifuged to remove the precipitate. This was re-dispersed, using a Potter-Elvehjem homogeniser, in 10 ml water and centrifuged. The yellow colour remained with the undissolved material which was then re-suspended in 10 ml buffer at pH 5·5. Further centrifugation gave a strongly yellow supernatant. A 5 ml sample of this was applied to a 50 × 2·5 cm column of G75 Sephadex previously equilibrated for 72 h with phosphate–citrate buffer (pH 5·5), and diluted with water 1 : 4. The column was eluted with this buffer at the rate of 40 ml/h and 5 ml fractions were collected. The optical density of each fraction at 280 nm in 1 cm cells was determined. All the fractions were assayed on the cup-plate system. Results are shown in Fig. 5. It can be seen that only fractions associated with the first small peak caused bleaching. These had no inner inhibitory zone. The yellow colour was associated with the second peak.

When fractions 11 and 12 from the first peak were mixed and combined with equal volumes of various fractions from the second peak it was found that the latter were inhibitory and caused inner zones or the complete elimination of zones.

Concentration of inhibitory factor

On the supposition that the inhibitory material might be polyphenolic or flavonoid the following procedure was adopted to obtain a concentrate. 1750 g of tomatoes were homogenised with 2 volumes of water and centrifuged to obtain a clear supernatant. The residue was boiled for 5 minutes with twice its volume of water and again centrifuged. Washings were combined to give a total volume of 5 l. To this was added 250 ml concentrated hydrochloric acid and the mixture was boiled for 30 min and allowed to cool. Such a procedure would cause hydrolysis of flavonoid glycosides.[14] Extraction was carried out with three successive 300 ml portions of ethyl acetate. The combined ethyl acetate extracts were washed twice with water, dried over anhydrous sodium sulphate and reduced under vacuum to 5 ml on a rotary evaporator. The concentrated extract was streaked on several large sheets of Whatman 3 MM paper and chromatographed with water using the descending technique. The papers were dried in a stream of nitrogen. A 5 cm band was cut off to include all the material remaining at the origin. The rest of the paper was divided to give fractions within 10 cm of the solvent front, and a middle fraction.

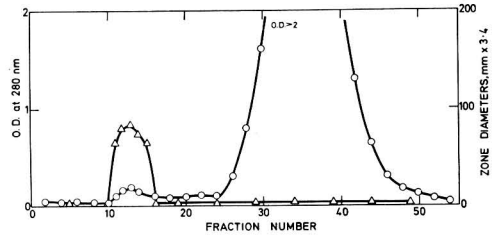

FIG. 5. *Separation on Sephadex of catalytic and inhibitory factors in ammonium sulphate precipitate from tomato juice*

○ Optical density at 280 nm; △ Zone diameters in cup plate assay

Each of the three fractions—front, middle and origin—was washed 3 times with 200 ml of ethanol and in each case the combined washings were reduced to a volume of 10 ml by evaporation under reduced pressure.

Samples from each fraction were then tested as antioxidants using the cytochrome/linoleate assay system to find the concentration required to give an induction period. Results are shown in Fig. 6. It can be seen that each of the fractions is capable of producing an induction period but even at a dilution of the order of one-tenth of those used for the other fractions, that from the origin is superior. For this the quantity, 0·005 ml, which gives an induction period of over 40 min can be calculated to come from between 2 and 3 ml of extracted juice, but despite the removal of other constituents appears to be more effective in inhibition. This might be attributed to its removal from pro-oxidant factors or alternatively to production by hydrolysis of aglycone material which is more effective than its parent glycoside.

To test this latter possibility a comparison was made on the assay system of the antioxidant effects of quercetin against those of its glycoside quercitrin. Results shown in Fig. 7 indicate the superiority of the aglycone.

A further ethyl acetate extract of tomato juice was made as described above. Its antioxidant potency was compared with a parallel preparation which differed only in omission of the heating stage.

A quantity of 0·005 ml of the extract from the heated sample gave an induction period of 50 min. It required 25 times this volume of extract from the unheated sample to produce a comparable induction period. It would appear that the ethyl acetate-soluble material which is the major antioxidant factor is therefore liberated by hydrolysis from its original state of combination.

Discussion

It can be concluded from the data presented that tomatoes contain a carotene-oxidising system which might readily fail to be detected because of the inhibitory material which is present. The concentration of this material was such that it could cause marked inhibition of the cytochrome-catalysed oxidation of linoleate even when diluted by the reaction mixture to a fifth of its natural potency.

The characteristics of the carotene oxidase system were unlike those shown by crude soya lipoxidase in that the former bleached carotene in the presence of oxidised and not fresh linoleate, and was not specific to the pentadiene group. When compared with soya extracts and haematins by the cup-plate method, it showed an induction period comparable to that observed for haematins. Again, being inhibited by ascorbic acid and quercetin, the bleaching factor in tomatoes differed from crude soya extracts and resembled haematins.

Since, in liquid systems, destruction of carotene depended on the presence of oxidised linoleate and in the gel systems, either linoleate or oleate but not caprylate were effective for bleaching, it could be taken that enzymic activity was of a 'lipoperoxidase' nature[5] in coupling destruction of fatty hydroperoxide with carotene oxidation.

There would seem to be no necessity to postulate a specific enzyme for the effects observed since the behaviour of the catalytic factor in the juice parallels that of the haematins so closely. No single haematin may be responsible since there is persistent, though greatly diminished, activity after heating to 90°, while most activity is lost below 75°. This would conform to the effects which might be expected from destruction of, for example, peroxidase and catalase at the lower temperature and survival of cytochrome *c* at the higher temperature.

Tomatoes have been included in studies made on the carotene-oxidising and lipid-oxidising activities of vegetable materials by Booth[3] and by Rhee & Watts[10] respectively.

Rhee & Watts have listed the comparative lipid-oxidising activites of over 30 seed, vegetable and fruit extracts, using filtered extracts of the source material on linoleate substrate and estimating fat oxidation by spectrophotometric estimation of conjugated diene. They pointed out that the possible antioxidant activity of their extracts might detract from a true comparison of enzyme activities. Tomatoes and other fruits appeared to have a comparatively low activity.

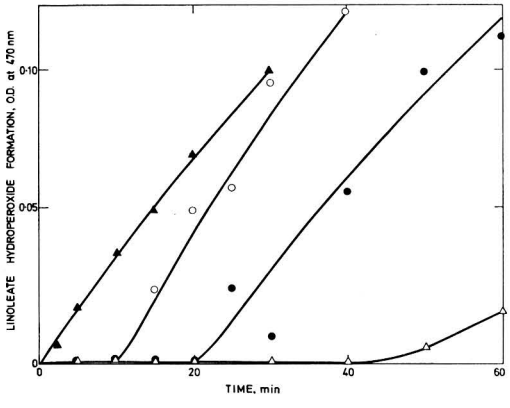

FIG. 6. *Relative antioxidant activities of fractions obtained by paper chromatography of ethyl acetate extract*
▲ Control; ○ Solvent front fraction, 0·06 ml; ● Intermediate fraction, 0·04 ml
△ Origin fraction 0·005 ml

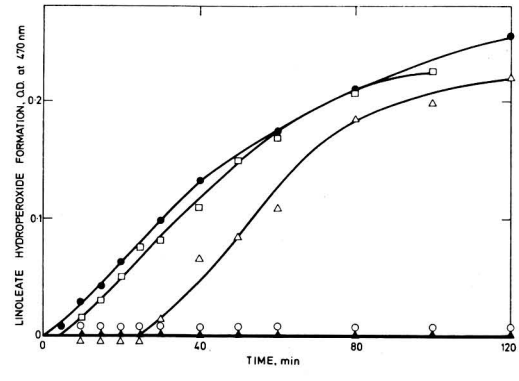

FIG. 7. *Comparison on a molar basis of quercetin and quercitrin antioxidants in the cytochrome-catalysed oxidation of linoleic acid*
● Control; □ Quercitrin, 10^{-5} M; △ Quercetin, 3×10^{-6} M;
○ Quercetin, 10^{-5} M; ▲ Quercitrin, 3×10^{-5} M

It is unfortunate that Rhee & Watts have described the pro-oxidant factors in both seed and vegetable extracts as lipoxidases. Of the eight sources which Tappel regarded as having been characterised as lipoxidases, seven were seeds,[1] and there seems to be little basis for attributing the unsaturated fat oxidase activity of fruit juices to this enzyme.

The distinction may be of practical importance in the processing of vegetable materials since haematins differ from lipoxidases in that they do not readily oxidise peroxide-free substrates, are not specific to the pentadiene group, respond in a different fashion to antioxidants,[11,12] and are likely to be active in lipid oxidation at low moisture levels.[13] The temperatures at which they are denatured will vary considerably.

Booth[3] has included green tomato in a study in which he ground plant tissues with quartz powder and measured loss of intrinsic carotene by enzyme activity. In the sample studied, the green tomato had 2 ppm carotene of which 16–41% was lost one hour after pulping. Carotene destruction of a similar order was found in many of the leaf and vegetable tissues which he examined by this method. Unlike the observations in this paper on centrifuged water-soluble material, this work was carried out on pulp and would include catalysis which might be due to particulate material. Booth has suggested that in higher plants the carotene-destroying enzyme is likely to be in the chloroplasts. Friend & Mayer[4] reported that isolated chloroplasts from sugar-beet leaves contain an enzyme which will oxidise carotene. They found that this could be inhibited by cyanide and ascorbic acid and stimulated by oxidised linoleate but not fresh linoleate. Again it is attractive to associate enzyme activity having these characteristics with the haematins known to be present.

The identities of the inhibitory factors have not yet been determined, and a more detailed study of these is at present being carried out. However, the work of Pratt & Watts[14] and others has established that the antioxidant activity of hot-water extracts of a variety of plant tissues is probably due at least in part to the flavonoid constituents. In one study these workers found water extracts of tomato peel to have some antioxidant activity and in another study to have none. They state that in the second case flavonoids were very low, and the material was not investigated further. Wu & Burrell[15] had previously been able to establish the presence of quercitrin, dutin and naringinin in tomato peel but not in flesh.

In the present work it appeared that the major antioxidant of the water-soluble fraction was extractable into ethyl acetate only after hydrolysis, and when subsequently chromatographed with water on paper remained at the origin. This is the fraction which would be expected to include any flavonoid aglycones. The total potency of the fraction was greater than that of the juice before treatment and extraction, which might be attributed to hydrolysis of glycosides and subsequent superior function of aglycones as antioxidants. The superiority of quercetin over equimolar quercetin in the thiocyanate assay accords with this supposition.

The central zoning of the antioxidant effects on carotene plates would be consistent with the effects of flavonoids since some are known to polymerise and complex with protein under oxidative conditions. Only a more detailed examination of the antioxidant fractions can clarify this point.

Acknowledgment

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References

1. Tappel, A. L., 'Autoxidation and antioxidants', (Lundberg, W. O., Ed.), 1961, Vol. 1, p. 328 (New York: Interscience)
2. Walsh, K. A., & Hauge, S. M., *J. agric. Fd Chem.*, 1953, **1**, 1001
3. Booth, V. H., *J. Sci. Fd Agric.*, 1960, **11**, 8
4. Friend, J., & Mayer, A. M., *Biochim. biophys. Acta*, 1960, **41**, 422
5. Blain, J. A., & Styles, E. C. C., *Nature, Lond.*, 1959, **184**, 1141
6. Blain, J. A., & Todd, J. P., *J. Sci. Fd Agric.*, 1958, **9**, 235
7. Koch, R. B., Stern, B., & Ferrari, C. G., *Archs Biochem. Biophys.*, 1958, **78**, 165
8. Blain, J. A., Hawthorn, J., & Todd, J. P., *J. Sci. Fd Agric.*, 1953, **4**, 580
9. McIlvaine, T. C., *J. biol. Chem.*, 1921, **49**, 183
10. Rhee, K. A., & Watts, B. M., *J. Fd Sci.*, 1966, **31**, 664
11. Blain, J. A., & Shearer, G., *Proc. Nutr. Soc.*, 1963, **22**, 162
12. Shearer, G., & Blain, J. A., *J. Sci. Fd Agric.*, 1966, **17**, 533
13. Stuckey, B. N., 'Symposium on Foods: Lipids and their oxidation', (Shultz, H. W., Day, E. A., & Sinnhuber, R. O., Eds.), 1962, p. 139 (Westport, Conn.: Avi Publishing Company)
14. Pratt, D. E., & Watts, B. M., *J. Fd Sci.*, 1964, **29**, 27
15. Wu, M., & Burrell, R. C., *Archs. Biochem. Biophys.*, 1958, **74**, 114

PERSISTENCE, PENETRATION, AND BREAKDOWN OF CHLORTHIAMID AND DICHLOBENIL HERBICIDES IN FIELD SOILS OF DIFFERENT TYPES

By K. I. BEYNON and A. N. WRIGHT

The persistence, penetration, and breakdown of the herbicides chlorthiamid ('Prefix', 2,6-dichlorothiobenzamide) and dichlobenil ('Casoron', 2,6-dichlorobenzonitrile) have been studied in the field in clay, medium loam, sandy loam, and peat using applications of unincorporated granules and wettable powders at 9 kg/ha (active material).

Conversion of chlorthiamid to dichlobenil was rapid in the soil. After 4 weeks, less than 3% remained of chlorthiamid that was applied to clay, medium loam, and sandy loam and less than 17% remained in peat.

The initial half-life of dichlobenil residues (following dichlobenil application) and 'total nitrile' residues (chlorthiamid + dichlobenil residues following chlorthiamid application) under wet conditions was near 2 weeks in clay, medium loam, and sandy loam, and ranged from 3–20 weeks in peat for different applications. There was very little penetration of chlorthiamid or dichlobenil below the 4 in depth of the soil within 32 weeks of the single application of herbicides except in peat.

2,6-Dichlorobenzamide was formed in all four soil types after application of either chlorthiamid or dichlobenil. At 8–10 weeks after application the benzamide residues in the soil were generally greater than those of the dichlobenil, except in peat. In clay, which was studied in most detail, the benzamide penetrated to greater depths than did the dichlobenil or chlorthiamid.

Introduction

Chlorthiamid ('Prefix', 2,6-dichlorothiobenzamide) and dichlobenil ('Casoron', 2,6-dichlorobenzonitrile) are promising herbicides both for selective use with crops and, at much higher dosage levels, for total weed control. It has been shown[1] that in the field chlorthiamid is converted to dichlobenil in the soil. Studies in the laboratory[2] have shown that soils treated with 10 ppm of ^{14}C-chlorthiamid contained no detectable chlorthiamid after six months storage at 22°. The major breakdown products were 2,6-dichlorobenzamide (3–8 ppm) and dichlobenil (0·8–3·7 ppm). 2,6-Dichlorobenzamide is of very weak herbicidal activity relative to chlorthiamid and is of low mammalian toxicity (acute oral toxicity to rats, LD_{50} 800–1000 mg/kg). It was still considered important, however, to investigate its formation in soils under field conditions.

In the present work chlorthiamid and dichlobenil were applied to peat, sandy loam, medium loam, and clay loam and the soils were sampled at intervals up to 32 weeks after application. The soils were analysed for residues of dichlobenil and the benzamide and the results of this work are summarised here. Some preliminary results of the analysis of these soils treated with chlorthiamid have already been reported[1] for samples taken up to 16 weeks after application but values for the benzamide residues were not included nor values for the residues in soil treated with dichlobenil.

Experimental

Field trial layout and sampling

The field trials all took place in East Anglia in 1965 under relatively wet conditions. There were five plots (20 ft × 20 ft) on each site. The soil was freshly ploughed and raked and the surface was made even before application. Chlorthiamid and dichlobenil both as granules and wettable powder were applied without incorporation on 30th and 31st March, 1965 at the rate of 9 kg/ha (a.m.). Particular care was taken to achieve a uniform distribution of the material because of the sampling procedure that was to be used.

Treated and untreated soils were sampled immediately after application and at 4, 8, 16 and 32 weeks thereafter. A hole 1 foot deep and nearly 1 foot square in area was dug so that one face was vertical. Samples 4 in × 4 in × 4 in in size were then taken from the vertical face corresponding to the 0–4 in, 4–8 in and 8–12 in depth. Five to six soil samples, each at three depths, were taken from each plot at each sampling time. These samples were taken in a line parallel to one side of the plot, starting at one end of the plot at the first sampling time and working along the plot at successive samplings. This procedure ensured that no disturbance of unsampled soil either by the digging operation or by walking occurred during the sampling and that the samples were taken from the depths indicated without contamination with soil from other depths.

The soil samples from the same depth from the same treatment at the same sampling time were bulked. The samples were received at the laboratory within 24 hours of sampling and were stored at −10° for up to 1 month whilst awaiting extraction. Chlorthiamid, dichlobenil, and 2,6-dichlorobenzamide have been shown to be stable in soils when stored under these conditions.

Analysis of the soils for residues of chlorthiamid and dichlobenil

Soils treated with chlorthiamid

The soils sampled immediately after application and 4 weeks thereafter were analysed for residues of both chlorthiamid and dichlobenil using the procedure described previously.[3] This method involved extraction of the soil with dimethylsulphoxide followed by separation of the chlorthiamid and dichlobenil by a partition procedure. The separated chlorthiamid was then converted to dichlobenil by oxidation and

the dichlobenil was analysed by gas–liquid chromatography. After 4 weeks so little unchanged chlorthiamid remained that the soils sampled at 8, 16 and 32 weeks were analysed by the simpler procedure[3] in which the chlorthiamid and dichlobenil were extracted with 20% (v/v) acetone-hexane. In this procedure the residues of chlorthiamid and dichlobenil were not distinguishable and the 'total nitrile' residues of chlorthiamid and dichlobenil were determined together and are reported as ppm of dichlobenil.

Soils treated with dichlobenil

The soils were extracted with 20% (v/v) acetone–hexane and the extracts were analysed for dichlobenil residues by gas–liquid chromatography as described previously.[3]

Analysis of the soils for residues of 2,6-dichlorobenzamide

Soils were mixed thoroughly and a sub-sample (250 g) was ground with anhydrous sodium sulphate (125 g). The sample was set aside for 1 hour in a stoppered bottle and then tumbled end-over-end for 2 hours with ethyl acetate (500 ml). The mixture was filtered through Whatman No. 1 paper. Naturally occurring co-extractives that might interfere in the analysis were removed from the extract by column chromatography. Alumina H (Peter Spence and Co., 1 g) was added to a 0·8 cm internal diameter glass column (fitted with a grease-free tap) containing hexane and the hexane was allowed to drain from the column down to the upper surface of the alumina. An aliquot of the extract (1 ml) was pipetted on to the column and was allowed to penetrate into the alumina. The column was eluted at an approximate rate of 1 drop every 1–3 seconds using 15% v/v ethanol in hexane as eluant. The fraction containing the benzamide (generally the 1–5 ml fraction of eluate) was collected and was concentrated when necessary to a smaller volume.

The eluate was analysed by gas–liquid chromatography with electron capture detection using either of the column conditions A or B summarised below:

	A	B
Columns	Copper, Kunifer 30 or stainless steel 4 ft long and 0·125 in internal diameter	Copper, Kunifer 30 or stainless steel 2 ft long and 0·095 in internal diameter
Packing	3%(w) phenyldiethanolamine succinate on 100/120 mesh Celite	2%(w) Epikote 1001 on 100/200 mesh Gas Chrom Q
Operating temperature:	188°C	163°C
Carrier gas:	Nitrogen at 150 ml/min	Nitrogen at 150 ml/min

Using these conditions 0·05 ng of the benzamide could be detected without difficulty.

The suitability of ethyl acetate as extraction was established after preliminary studies of the extraction of soils containing field-bound residues of the benzamide using solvents such as hexane, acetone-hexane 20% (v/v), 50% (v/v) and 70% (v/v), and acetone.

Untreated control soils were also extracted and analysed and recovery experiments were carried out by adding known amounts of dichlobenil, chlorthiamid and 2,6-dichlorobenzamide at the extraction stage. Single samples only were extracted because of the large number of samples involved.

Results

Mechanical analysis of the soils

The properties of the soils are summarised in Table I.

Recovery experiments and blank values

The recoveries of chlorthiamid, dichlobenil and 2,6-dichlorobenzamide are summarised in Table II. In medium loam, sandy loam and clay, blank values with untreated soils were less than 0·02 ppm, and generally less than 0·01 ppm for the analysis of all three compounds. In peat, blank values were generally less than 0·05 ppm and often less than 0·01 ppm.

TABLE I

The mechanical analysis of the soils from the field trials
The soils were air-dried at 40°C before the mechanical analysis

Soil	Depth, in	Composition of soil (air-dried weight, %)							Difference of total of soil components from 100%	pH	Moisture content (% dry weight)†
		Clay	Silt	Coarse sand	Fine sand	Organic matter	Loss by solution**	Moisture*			
Peat	0–4	16·5	6·2	1·1	3·2	59·5	7·2	13·5	+ 7·2	6·42	65–135
	4–8	16·8	4·5	1·0	4·5	55·1	7·3	13·5	+ 2·7	6·14	
	8–12	13·8	3·4	0·8	3·0	62·5	7·0	16·2	+ 6·7	5·98	
Sandy loam	0–4	11·2	5·8	32·7	43·6	1·6	0·4	1·1	− 3·6	7·88	8–19
	4–8	9·9	4·4	36·9	39·5	1·6	0·1	1·0	− 6·6	7·73	
	8–12	9·8	5·1	38·1	31·7	1·6	0·6	1·0	−12·1	8·05	
Clay loam	0–4	21·8	7·8	21·8	33·3	2·4	1·1	2·2	− 9·6	7·85	17–25
	4–8	10·0	13·1	25·7	35·4	2·3	1·3	2·3	− 9·9	8·05	
	8–12	9·9	14·7	19·5	43·2	1·6	1·2	1·3	− 8·6	7·95	
Medium loam	0–4	9·6	13·8	21·2	38·5	2·7	1·2	2·0	−11·0	7·96	12–23
	4–8	21·3	8·1	26·7	36·9	2·5	1·4	2·2	−10·9	7·92	
	8–12	7·2	13·1	24·8	42·8	2·6	1·2	2·1	− 6·2	8·00	

* After air-drying † As sampled originally

** The filtrate from the peroxide–HCl treatment (for organic matter analysis) contained calcium chloride formed from the calcium carbonate in the soil, the chlorides of the exchangeable bases and chlorides of aluminium, iron etc., produced by breakdown of a small part of the clay fraction. These components were determined and their total concentration is included under this heading

TABLE II

Recoveries of chlorthiamid, dichlobenil and 2,6-dichlorobenzamide from soils

Sample	Mean recovery of compound (corrected for blank value)		
	Chlorthiamid 0·5–5 ppm level, %	Dichlobenil 0·5–5 ppm level, %	2,6-Dichlorobenzamide 0·5–1 ppm level, %
Peat	72	90	85
Sandy loam	78	94	81
Medium loam	81	91	91
Clay	80	92	83

Analysis of the soils for residues of chlorthiamid, dichlobenil, and 2,6-dichlorobenzamide

The analysis of the soils for residues is summarised in Figs 1–4. The residues shown in these figures have been corrected for blank values obtained with untreated controls but not for percentage recovery.

At four weeks after application little of the chlorthiamid that was originally applied remained unchanged in the soils apart from peat. Soils sampled after this time were analysed by the 'total nitrile' procedure in which residues of dichlobenil and chlorthiamid were determined together but not distinguished. In Figs 1–4 'total nitrile' values are shown together with chlorthiamid contents of these soils at 0 and 4 weeks after application of chlorthiamid. Residues of unchanged chlorthiamid could not be detected in the 4–8 in and 8–12 in layers of soil, other than peat, at 4 weeks after application.

Discussion

Chlorthiamid residues

The conversion of chlorthiamid to dichlobenil was rapid in all four soil types. The high dichlobenil contents at zero time (indicated in Figs 1–4 by the difference between the 'total nitrile' residue and the free chlorthiamid residue) reflects the conversion that had occurred during the period that elapsed whilst the samples were being transported to the laboratory. At four weeks after application only a very small proportion ($< 3\%$) of the original chlorthiamid remained unchanged in sandy loam, medium loam, and clay. The rate of conversion of chlorthiamid to dichlobenil was slower in peat than in the other soils. Since dichlobenil is a herbicide the persistence of the herbicidal activity after application of chlorthiamid is best considered in terms of 'total nitrile' persistence and is discussed below.

In sandy loam, medium loam, and clay unchanged chlorthiamid could not be detected below the 4 in depth of soil. In peat residues of up to 0·18 ppm of unchanged chlorthiamid could be detected in the 4–12 in soil depth of 4 weeks after chlorthiamid application.

'Total nitrile' and dichlobenil residues

The persistence of 'total nitrile' residues (after chlorthiamid application) and of dichlobenil residues (after dichlobenil application) is summarised in Table III.

Excluding peat the average initial half-life of the 'total nitrile' residues (1·9 weeks) was similar to that of the dichlobenil residues (1·6 weeks). 'Total nitrile' residues following chlorthiamid applications and dichlobenil residues following dichlobenil applications will therefore be considered together.

TABLE III

Persistence of 'total nitrile' and dichlobenil residues in 0–4 in. soil layer

Soil type	Application at 9 kg/ha	Time (weeks) for 50% of initial residue* to disappear	Time (weeks) for 75% of initial residue* to disappear
Clay loam	Chlorthiamid Gran	1–2	3
	Chlorthiamid WP	1	3–4
	Dichlobenil Gran	1–2	3–4
	Dichlobenil WP	1	2
Medium loam	Chlorthiamid Gran	2	4
	Chlorthiamid WP	1–2	3
	Dichlobenil Gran	3–4	5
	Dichlobenil WP	1	2
Sandy loam	Chlorthiamid Gran	4	8
	Chlorthiamid WP	1–2	3
	Dichlobenil Gran	1–2	2–4
	Dichlobenil WP	1	2
Peat	Chlorthiamid Gran	5	15
	Chlorthiamid WP	3	9
	Dichlobenil Gran	~20	>32
	Dichlobenil WP	~16	>32

*As 'total nitrile' for chlorthiamid applications and as dichlobenil for dichlobenil applications

In general terms the initial half-life in soils was in the order: peat > sandy loam and medium loam > clay. The mean initial half-life in sandy loam, medium loam and clay was nearly 2 weeks and in peat the initial half life ranged between 3 and 20 weeks for different applications. This persistence under the wet conditions that prevailed in 1965 was less than that observed[1] in other trials in the dry conditions of 1964.

The mean initial half-life of the residues ('total nitrile' or dichlobenil) after granular applications (5 weeks) was a little greater than that for the wettable powder applications (3 weeks). In sandy loam, medium loam and clay there was very little difference between the persistence of the 'total nitrile' residues following chlorthiamid applications and the persistence of the dichlobenil residues following dichlobenil applications. In peat the initial half-life of dichlobenil applications appeared to be considerably greater than that of the chlorthiamid applications. However, this difference may not be as large as it first appears. The chlorthiamid applications produced higher initial residues (as 'total nitrile') in peat than the dichlobenil applications. The 'total nitrile' residues, after chlorthiamid application fell off relatively rapidly in the first 8 weeks and after this time the residue levels of 'total nitrile' and dichlobenil respectively, from the two treatments, were in fact similar up to 32 weeks.

In sandy loam, medium loam and clay there was very little penetration of dichlobenil below the 0–4 in depth since in these soil types the compound was insufficiently persistent for significant leaching to occur under the trial conditions. In peat there was significant penetration of dichlobenil residues to at least 12 in depth. It would be expected that movement of dichlobenil would be least in peaty soils because of the strong absorptive properties of such soils. However, this strong absorption also results in increased persistence of the residues and given sufficient time it appears that rain-water can leach dichlobenil residues even into peaty soils.

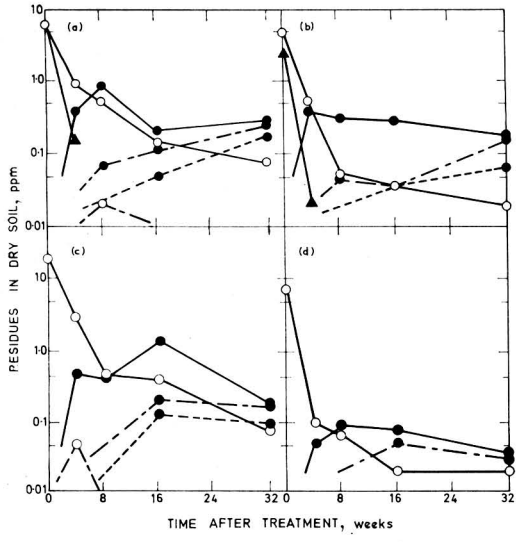

FIG. 1. *Persistence of chlorthiamid and dichlobenil in clay loam (East Anglian trial, 1965)*

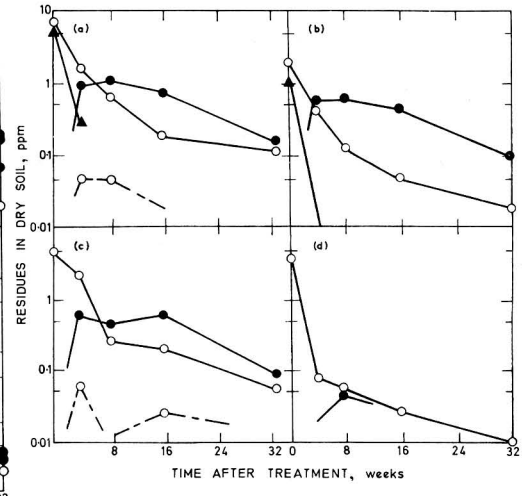

FIG. 2. *Persistence of chlorthiamid and dichlobenil in medium loam (East Anglia trial, 1965)*

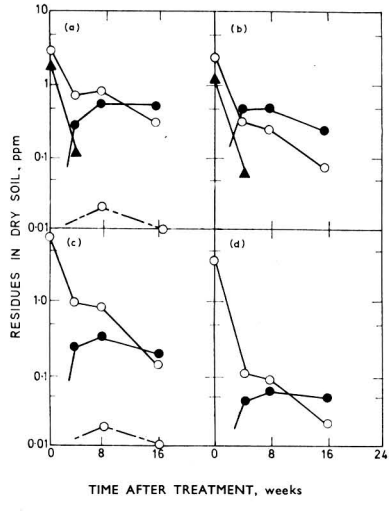

FIG. 3. *Persistence of chlorthiamid and dichlobenil in sandy loam (East Anglian trial, 1965)*

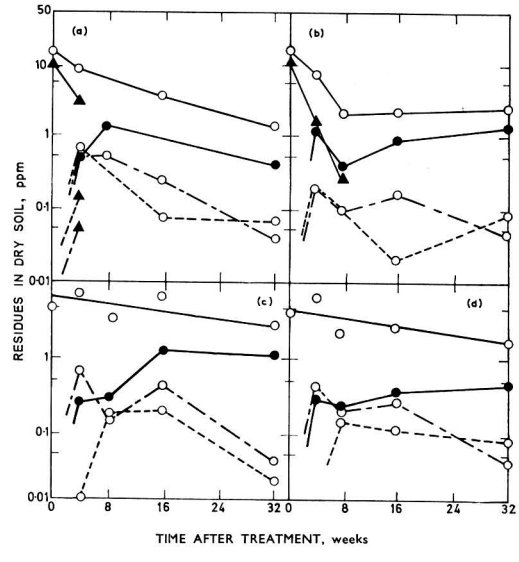

FIG. 4. *Persistence of chlorthiamid and dichlobenil in peat (East Anglian trial, 1965)*

(a) Chlorthiamid granules 9 kg/ha: 'total nitrile' not detectable in 8–12 in layer (Figs 1–3). Benzamide not determined in 4–8 in, 8–12 in layer (Figs 2–4).
(b) chlorthiamid wettable powder 9 kg/ha: 'total nitrile' not detectable (Figs 1–3). Benzamide not determined in 4–8 in, 8–12 in layer (Figs 2–4).
(c) dichlobenil granules 9 kg/ha: 'total nitrile' not detectable in 8–12 in layer (Figs 1–3). Benzamide not determined in 4–8 in, 8–12 in layer (Figs 2–4).
(d) dichlobenil wettable powder 9 kg/ha: 'total nitrile' not detectable (Figs 1–3). Benzamide not determined in 4–8 in, 8–12 in layer (Figs 1–4).

○ 'total nitrile' residues (dichlobenil + chlorthiamid expressed as ppm dichlobenil) after chlorthiamid treatments or dichlobenil residues after dichlobenil treatment; ● 2,6-dichlorobenzamide residues; ▲ chlorthiamid residues at 0 and 4 weeks after application; —— 0–4 in layer; – – – – 4–8 in layer; – – – 8–12 in layer

2,6-Dichlorobenzamide residues

It is evident that the benzamide is formed in the field in all four soil types after application of either chlorthiamid or dichlobenil. From the present experiments it is not possible to determine whether chlorthiamid can react to form the benzamide directly or whether it first decomposes to form dichlobenil which then forms the benzamide. The second possibility must occur to some extent in chlorthiamid-treated soils and may be the main route, if not the only route, to the benzamide in such soils.

In sandy loam, medium loam and clay the benzamide residues in the 0–4 in layer of soil were generally greater than the corresponding 'total nitrile' or dichlobenil residues from 4–12 weeks and onwards after application. In peat, the benzamide residues were present in the 0–4 in layer but at no time did they exceed in concentration the corresponding 'total nitrile' or dichlobenil residues. This reflects the greater persistence of the dichlobenil residues in peat. It is not possible to determine the persistence of the benzamide from these results since the compound was being formed at the same time as it was being dissipated.

A feature of the results is the penetration of the benzamide below the 4 in depth. This penetration was studied in clay only. Unlike the dichlobenil the benzamide residues penetrated to at least 12 in depth. At 32 weeks after treatment of clay with either dichlobenil or chlorthiamid the benzamide residues at 0–4 in, 4–8 in and 8–12 in depth all exceeded those of the corresponding 'total nitrile' or dichlobenil residues in the 0–4 in layer. The benzamide residues in clay loam at 32 weeks after treatment were equivalent to 0·07–0·4 lb/acre and in these terms were 3–20 times greater than the 'total nitrile' or dichlorbenil residues at the same time although the depth profile of the residues of the compounds was different. However, in laboratory screening tests the herbicidal activity of the benzamide has been found to be, in general terms, only one fiftieth the activity of dichlobenil although the spectrum of activity is different. Thus the benzamide remaining in the soil after 32 weeks will be less effective herbicidally than the nitrile that remains although the total quantity of benzamide may be larger.

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References

1. Beynon, K. I., Davies, L., & Elgar, K., *J. Sci. Fd Agric.*, 1966, **17**, 156
2. Beynon, K. I., & Wright, A. N., *J. Sci. Fd Agric.*, 1968, **19**, 723
3. Beynon, K. I., Davies, L., Elgar, K., & Wright, A. N., *J. Sci. Fd Agric.*, 1966, **17**, 151

BREAKDOWN OF THE HERBICIDE ^{14}C-CHLORTHIAMID
I.—Laboratory studies of the breakdown in soils

By K. I. BEYNON and A. N. WRIGHT

Chlorthiamid ('Prefix', 2,6-dichlorothiobenzamide) with a ^{14}C label was added at 10 ppm (on a wet weight basis, equivalent to nearly 20 lb/acre) to moist clay, loam, peat, sand, and brick earth in tightly stoppered bottles. After six months at room temperature (22°) all of the chlorthiamid had broken down. The major products were 2,6-dichlorobenzamide (3–8 ppm), and dichlobenil ('Casoron', 2,6-dichlorobenzonitrile, 0·8–3·7 ppm) together with traces (0·6 to < 0·01 ppm) of two unidentified compounds. No 2,6-dichlorobenzoic acid could be detected in the soils.

Whilst dichlobenil is an active herbicide the benzamide is only weakly active.

Introduction

Chlorthiamid ('Prefix', 2,6-dichlorothiobenzamide) shows great promise as a selective and total herbicide. It has been shown[1] that chlorthiamid decomposes after application to the soil to form dichlobenil ('Casoron', 2,6-dichlorobenzonitrile), and the analysis of soils and crops for residues of chlorthiamid and dichlobenil has been described previously.[1,2] Residues of chlorthiamid and dichlobenil could not be detected in a wide range of crops (other than rice) harvested at 2–6 months after soil applications of chlorthiamid at 1–16 lb/acre. In rice grains, where the plants can be in intimate contact with the herbicide, the total residues of chlorthiamid plus dichlobenil did not exceed 0·05 ppm.

The breakdown of chlorthiamid, apart from the formation of dichlobenil, was not studied in previous work and the authors have examined the breakdown of ^{14}C-chlorthiamid in soils and in crops grown in treated soils. In the studies reported here soils of different types were treated with chlorthiamid and examined for breakdown products after storage for six months.

Experimental

^{14}C-chlorthiamid

The chlorthiamid employed was labelled with ^{14}C in the extranuclear carbon atom and different samples had specific activities in the range 9·7–11 nc/μg. The material was stored in acetone at −10°. The only radio-impurity (above 0·1%) was dichlobenil as indicated by thin-layer chromatography on silica gel G using 5% (v) ethanol in benzene as developing solvent.

Unlabelled marker compounds

Samples of unlabelled dichlobenil and chlorthiamid of over 99% purity were available as analytical standards as were the following unlabelled marker compounds: 2,6-dichlorobenzamide, 2,6-dichlorobenzoic acid, 2,6-dichlorobenzonitrile oxide, 2,6-dichlorobenzaldoxime, and 2,6-dichlorobenzylamine.

The following compounds were supplied by Tunstall Laboratory, who have described[3] their preparation and properties:

4-hydroxy-2,6-dichlorobenzonitrile
3-hydroxy-2,6-dichlorobenzonitrile
3-hydroxy-2,6-dichlorobenzoic acid
3-hydroxy-2,6-dichlorobenzaldehyde
3-hydroxy-2,6-dichlorothiobenzamide

3-Hydroxy-2,6-dichlorobenzamide was prepared by the oxidation of the corresponding benzonitrile with alkaline hydrogen peroxide in absolute ethanol, after the method of Noller.[4]

Soil treatments

Unsterilised samples of soils were freshly collected as follows:

	pH	Moisture (% dry wt)	Organic matter (% dry wt)
Clay (East Anglia)	8·0	21	2
Medium Loam (East Anglia)	8·0	15	3
Peat (East Anglia)	6·2	89	60
Sandy Loam (East Anglia)	7·8	14	2
Brickearth (Kent)	—	18	—

Each soil (200 g fresh wet weight) was treated with 2 mg of ^{14}C-chlorthiamid in 2 ml acetone and stored in a loosely capped 16 oz clear-glass bottle in full daylight for 6 months at room temperature (22° ± 2°). There was a loss of water of only 3–5 g during this period.

Extraction of soils

Samples of soil were extracted by end-over-end tumbling with acetone as described previously[5] and the dried solids were then extracted further using 2 N hydrochloric acid for 12 hours.

Before radiocounting, the acetone extracts (up to 500 ml) of the soils were concentrated by rotary evaporation at reduced pressure at room temperature. The remaining aqueous solution (5 ml) was shaken with benzene (10 ml) and the benzene and aqueous phases were examined separately. It was shown by radiocounting that no radioactive components were lost during this procedure.

The extracts were subjected to thin-layer chromatography and the radioactive components were located by radioscanning of the chromatograms. Components were desorbed from the relevant portion of the layers using 10% (v) ethanol–acetone. The components were examined further by thin-layer chromatography, electrophoresis and radio gas–liquid chromatography and their behaviour was compared with that of unlabelled standards. The chromatographic, radioscanning and radiocounting techniques have been described previously.[5]

Results

The retention values (R_f) of some of the potential breakdown products of chlorthiamid are summarised in Table I.

TABLE I

R_f values of some potential breakdown products of chlorthiamid obtained by thin-layer chromatography

5 μl of hexane or acetone solutions of the compounds were applied to 300 μ layers of silica gel GF_{254} and were developed to 15 cm with ethanol in benzene

Compound	R_f value of compound
3-hydroxy-2,6-dichlorobenzoic acid	<0·1
3-hydroxy-2,6-dichlorobenzamide	0·1
2,6-dichlorobenzoic acid	0·1
3-hydroxy-2,6-dichlorothiobenzamide	0·17
4-hydroxy-2,6-dichlorobenzonitrile	0·22
2,6-dichlorobenzamide	0·25
3-hydroxy-2,6-dichlorobenzonitrile	0·27
3-hydroxy-2,6-dichlorobenzaldehyde	0·35
Chlorthiamid	0·50
2,6-dichlorobenzaldoxime	0·65
Dichlobenil	0·75
2,6-dichlorobenzaldehyde	0·78
2,6-dichlorobenzylamine	0·80
2,6-dichlorobenzonitrile oxide	0·85

No attempt was made to obtain constant R_f values from plate to plate for given compounds in this table and in Tables II–V. However, it is still valid to compare the R_f values of the different compounds when they are run on the same plate at the same time. Furthermore, even the relative R_f values vary from plate to plate (to within 10%) depending on whether or not the compound was run in the presence or absence of plant or soil extractives. For this reason evidence for identification by thin-layer chromatography was based mainly on co-chromatography of the radiocomponent and the unlabelled standard.

The soils treated with 10 ppm (wet weight basis) of ^{14}C-chlorthiamid were extracted after six months storage at 22°. Radioscans of the acetone extracts were obtained and a representative scan is shown in Fig. 1.

Four radiocomponents (A, B, C, D) were detected in the acetone extracts of the soils and these components were examined further after they had been desorbed from the thin-layer plate.

The behaviour of component A on thin-layer chromatography (t.l.c.) is summarised in Table II. Whilst component A and 3-hydroxy-2,6-dichlorobenzoic acid had the same R_f values in three solvent systems this was not so in a fourth system (butanol–ammonia).

High voltage electrophoresis of component A showed that it was acid. Using Locarte equipment operated at 6 kV and 25mA for 22 min, component A ran to 13·8 cm from the origin at pH 10, to 14 cm at pH 6·5, and to 18 cm at pH 2·0.

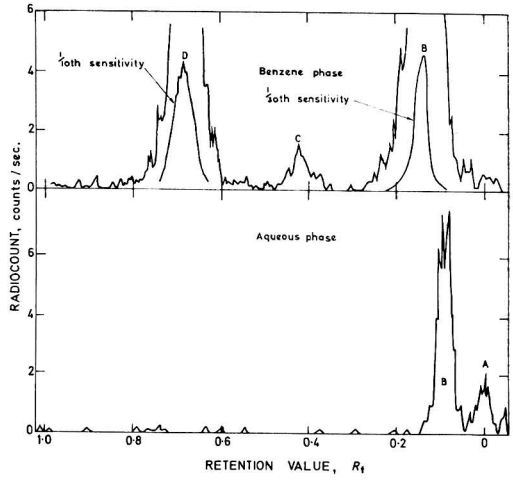

FIG. 1. *Radioscan of thin-layer chromatograms of extract of clay 6 months after treatment with ^{14}C-Prefix at 10 ppm on a wet weight basis*

Components A, B, C, D referred to in text

The acetone extract was concentrated by rotary evaporation and the remaining aqueous phase was shaken with benzene. The benzene and aqueous phase were examined separately using t.l.c. with 5% (v) ethanol in benzene as eluant and silica gel GF_{254} as the absorbent

Under the same conditions 2,6-dichlorobenzoic acid ran to 15 cm, 15 cm, and 11 cm respectively.

The R_f value of component A on t.l.c. (silica gel – 60% (v) ethanol in chloroform) was not significantly different before and after treatment with 10 N hydrochloric acid (20 h at 22°) nor after treatment with diazomethane (20 h at room temperature). Under the same reaction conditions 2,6-dichlorobenzoic acid and 3-hydroxy-2,6-dichlorobenzonitrile reacted completely with diazomethane.

Component A did not appear to be any of the compounds listed in Table I.

The behaviour of component B on t.l.c. is summarised in Table III. After gas–liquid chromatography component B gave a response at the same retention time with electron capture and radiochemical detectors. This response was at the same retention time as the electron capture response of an unlabelled standard of the 2,6-dichlorobenzamide.

TABLE II

Retention values (R_f) of component A in various t.l.c. systems

Solvent system	R_f of Component A	R_f value of standards		
		3-hydroxy-2,6-dichlorobenzoic acid	2,6-dichlorobenzoic acid	3-hydroxy-2,6-dichlorobenzamide
5% (v) acetic acid in ethanol–benzene (1 : 1 v/v)	0·65	0·65	—	—
60% (v) acetone in benzene	0·05–0·10	< 0·15 (streak)	—	0·67
60% (v) ethanol in chloroform	0·80	0·80	0·85	—
5% (v) 0·88 ammonia in n-butanol	0·60	0·25	—	—
2% (v) acetic acid ethyl acetate–hexane (1 : 1 v/v)	0·07	—	0·95	—

Component B is considered to be 2-6-dichlorobenzamide.

The behaviour of component C in various t.l.c. systems is summarised in Table IV. In high voltage electrophoresis (pH 10, 6 kV, 21 mA, 22 min) component C ran to −1 cm from the origin whereas 2,6-dichlorobenzoic acid ran to +15 cm. The movement of component C was probably due only to electro-osmosis and can be disregarded. Using gas–liquid chromatography an electron capture response was obtained from component C, with an associated response on a radiodetector, at a retention time greater than that of 2,6-dichlorobenzamide. The molar response of the electron capture detector to component C was 1·1–1·4 times greater than that of the benzamide (assuming one ^{14}C atom/molecule for component C).

Component C did not appear to be any of the compounds listed in Table I.

The retention values of component D using t.l.c. are summarised in Table V. The t.l.c. data indicate that component D is dichlobenil.

The residues of the different radiocomponents in the soils are summarised in Table V. Unchanged chlorthiamid could not be detected at the 0·01 ppm level.

Discussion

After six months the chlorthiamid in all of the soils had broken down to give dichlobenil and 2,6-dichlorobenzamide as the major products. No unchanged chlorthiamid could be detected in the soils at the 0·01 ppm level. No 2,6-dichlorobenzoic acid was detected in the soils.

In most of the soils the benzamide was present in larger amounts than the dichlobenil both on a weight basis and in terms of molar conversion of the chlorthiamid. In sand as much as 80% of the original chlorthiamid had been converted to benzamide and in peat nearly half of the original chlorthiamid was present as benzamide after six months. There is some indication that a larger proportion of dichlobenil is

TABLE III

Retention values (R_f) of component B in various t.l.c. systems

Solvent system	R_f of component B	R_f values of standards		
		2,6-dichloro-benzamide	4-hydroxy-2,6-dichlorobenzonitrile	3-hydroxy-2,6-dichlorobenzonitrile
10% (v) ethanol in benzene	0·35	0·40	—	—
20% (v) acetone in hexane	0·03	0·03	—	—
20% (v) ethanol in benzene	0·50	0·50	—	—
50% (v) acetone in hexane	0·45	0·47	0·45	0·60
5% (v) ethanol in benzene	0·08–0·17*	0·08–0·17*	0·12	0·14
Technical chloroform (containing 2% (v) ethanol)	0·20–0·34*	0·22–0·32*	0·15	0·25

* R_f value of upper and lower edges of spot on co-chromatography of standard with radiocomponent

TABLE IV

Retention values (R_f) of component C in various t.l.c. systems

Solvent system	R_f of component C	R_f values of standards		
		Chlorthiamid	2,6-dichloro-benzaldoxime	3-hydroxy-2,6-dichlorobenzaldehyde
5% (v) ethanol in benzene	0·42	0·50	0·65	0·35
50% (v) benzene in hexane	0·05	0·05	—	—
5% (v) ethanol in chloroform	0·55–0·65*	0·45–0·55*	—	—
Technical chloroform (containing 2% (v) ethanol)	0·47	0·36	0·50	0·60
20% (v) acetone in hexane	0·10	0·09	—	—
30% (v) ether in hexane	0·0	0·17	0·38	—

* R_f value of upper and lower edges of spot on co-chromatography of standard with radiocomponent

TABLE V

Retention values (R_f) of component D in various t.l.c. systems

Solvent system	R_f of component D	R_f values of standards		
		Dichlobenil	2,6-dichloro-benzaldehyde	2,6-dichloro-benzylamine
5% (v) ethanol in benzene	0·75	0·75	0·78	0·80
10%(v) ethanol in benzene	0·73	0·70	—	—
50% (v) acetone in hexane	0·48	0·47	—	—
Benzene	0·53–0·63*	0·50–0·65*	—	—
Technical chloroform (containing 2% (v) ethanol)	0·68–0·75*	0·68–0·75	0·90	0·90

* R_f value of upper and lower edges of spot on co-chromatography of standard with radiocomponent

TABLE VI

Analysis of soils 6 months after treatment with ^{14}C-chlorthiamid at 10 ppm (wet weight basis)

Sample	Residues (ppm) of ^{14}C-compounds in wet soils				
	Clay	Loam	Peat	Sand	Brick earth
Extract in acetone:					
Component A, unknown	~0·08*	<0·01*	<0·01*	~0·03*	~0·02*
Component B, 2,6-dichlorobenzamide	5·6	6·8	3·4	7·0	6·2
Component C, unknown	0·6*	~0·2*	0·2*	<0·01*	~0·01*
Component D, dichlobenil	1·9	1·8	3·7	0·80	1·7
Total in acetone extract (as chlorthiamid equivalent)	8·5*	9·6*	8·3*	8·7*	8·8*
Extract in 2 N hydrochloric acid:					
Component B, 2,6-dichlorobenzamide	0·6	0·3	Trace (<0·01)	0·5	0·6
Component A, unknown	Trace (<0·01)	Trace (<0·01)	<0·01	<0·01	<0·01
Total in acid extract (as chlorthiamid equivalent)	0·7*	0·34*	0·16*	0·57*	0·70*
Total activity recovered, w%	92	99	85	93	95

* As ppm equivalent of chlorthiamid; other concentrations are in terms of the compound named

found in soils with a high organic matter content. Whilst dichlobenil is an active herbicide, 2,6-dichlorobenzamide is only a weak herbicide and dosage rates of 50 times greater than that of chlorthiamid or dichlobenil are required on average to produce the same herbicidal effect.

Two other breakdown products were also detected in the soils but were not identified. The concentrations of these components in the soils, apart from clay, did not exceed 0·2 ppm.

These experiments at relatively high dosage levels (equivalent to nearly 20 lb/acre) have served to reveal the possible breakdown products of chlorthiamid. However, it should be emphasised that lower concentrations of these breakdown products will be present in field-treated soils where leaching and volatilisation of compounds can occur. Field-treated soils have been analysed for possible residues of 2,6-dichlorobenzamide and such studies will be reported in a subsequent paper.

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References

1. Beynon, K. I., Davies, L., & Elgar, K., *J. Sci. Fd Agric.*, 1966, **17**, 156
2. Beynon, K. I., Davies, L., Elgar, K., & Wright, A. N., *J. Sci. Fd Agric.*, 1966, **17**, 151
3. Griffiths, M. H., Moss, J. A., Rose, J. A., & Hathway, D. E., *Biochem. J.*, 1966, **98**, 770
4. Noller, C. R., 'Organic Syntheses', 1959, Collective Volume II, p. 586 (New York: J. Wiley and Sons)
5. Beynon, K. I., & Wright, A. N., *J. Sci. Fd Agric.*, 1967, **18**, 143

BREAKDOWN OF THE HERBICIDE ^{14}C-CHLORTHIAMID

II.*—Glasshouse studies of breakdown in soils and in crops grown in the soils

By K. I. BEYNON and A. N. WRIGHT

Plants were grown to harvest in a glasshouse in soils treated once with ^{14}C-chlorthiamid (2,6-dichlorothiobenzamide, 'Prefix') at dosage levels (1–3 lb/acre active material) near those recommended for commercial field use.

Rice plants (stems and leaves) grown in soil treated at 1 lb/acre contained dichlobenil (2,6-dichlorobenzonitrile, 'Casoron', 0·03 ppm) and 2,6-dichlorobenzamide (0·06 ppm) and the grain itself showed a total residue of ^{14}C-components of only 0·06 ppm (expressed as equivalent weight of chlorthiamid) at harvest.

Winter wheat plants (stalks and leaves) grown in medium loam treated at 1·2 lb/acre contained residues of 2,6-dichlorobenzamide and free and conjugated 3-hydroxy-2,6-dichlorobenzamide, together with traces of sugar conjugates of 3-hydroxy- and 4-hydroxy-2,6-dichlorobenzonitrile.

The fruit of apple grown in a glasshouse in soil treated once at 1·5 lb/acre, showed no detectable residue (< 0·004 ppm) in spite of total residues of up to 2·4 ppm in the leaves. The latter probably resulted from sublimation of dichlobenil from the soil. Such a mode of uptake would be unlikely to lead to as much residue in the leaves under field conditions. Traces of dichlobenil (0·005 ppm), 2,6-dichlorobenzamide (0·02 ppm) with larger amounts of glycosides of 3-hydroxy-2,6-dichlorobenzamide (1·3 ppm) and 3-hydroxy- and 4-hydroxy-2,6-dichlorobenzonitriles (0·6 ppm and 0·5 ppm respectively) were identified in the leaves at harvest.

All of the breakdown products of chlorthiamid that have been identified are of low acute oral mammalian toxicity.

Introduction

When the herbicide chlorthiamid ('Prefix', 2,6-dichlorothiobenzamide) was added at a concentration of 10 ppm (equivalent to about 20 lb/acre) to soils of different types and stored in closed, but not sealed, containers, the chlorthiamid had decomposed completely after 6 months.[1] The major breakdown products were 2,6-dichlorobenzamide (3–8 ppm), and dichlobenil (0·8–3·7 ppm) together with traces (0·6–< 0·01 ppm) of two unidentified components.

Further experiments have now been carried out in which rice, wheat, and apples were grown in a glasshouse in soil that had been treated with ^{14}C-chlorthiamid and the results of these studies are reported in this paper.

Experimental

Materials

The sources of the ^{14}C-chlorthiamid and the unlabelled marker compounds have been described previously.[1] The ^{14}C-2,6-dichlorobenzamide and the β-D-glucopyranoside of 3-hydroxy-2,6-dichlorobenzamide were synthesised at these laboratories.

Plant growth

The plants were grown in a cubicle of a heated Hartley glasshouse provided with reasonable ventilation and with extra illumination during winter months. The apple trees were grown in a small glasshouse with a permanent through draught. Some heat was used in early spring.

Apples (Ellison's Orange)

Two-year-old Cordons were grown in tubs 14 in × 14 in × 14 in filled with soil over a 1 in layer of stones.

Rice

Seedlings (6–9 in with three leaves) were transplanted into buckets of soil and the buckets were filled with water to 2 in above soil level.

Winter wheat (Rothwell Perdix)

This was sown directly into boxes containing 6–8 in of soil over a 1 in layer of stones. The plants were thinned to 2 in centres before treatment.

Soil and plant treatment

^{14}C-chlorthiamid was applied in aqueous acetone (about 200 μg/ml) to the surface of the soil using a long-stemmed tap funnel to prevent contact with the plants. The volumes of solution applied (50–200 ml) allowed an even cover of the surface. The applications corresponded to 1–3 lb/acre (active material).

A few additional experiments were carried out using ^{14}C-2,6-dichlorobenzamide in an attempt to prepare larger quantities of glycosidic metabolites to facilitate their identification. ^{14}C-2,6-dichlorobenzamide was applied to the foliage of apple in 30 μl of acetone per leaf to give a residue of 28 ppm and the application was repeated after 14 days.

In all of these studies the results refer to crops grown in soil treated with ^{14}C-chlorthiamid except where stated otherwise.

Extraction of crops and soils

Plants were harvested at maturity and soil samples were also taken. The samples were stored at −10° for up to two months until extraction. Chlorthiamid is stable under these storage conditions.

* Part I: Preceding paper

Crops

Rice grain was milled in a coffee grinder, and apple shoots and trunks were cut into strips with secateurs and macerated with acetone as described previously.[2] Wheat straw was cut into 1 cm lengths which were tumbled end-over-end with acetone for 20 h. The other crop samples were extracted by maceration with acetone as before.[2] The overall extraction ratio (g/ml) was generally between 1 : 4 and 1 : 10.

The solids remaining after extraction were dried in a desiccator and were then examined by oxygen flask combustion.[2,3] If this procedure indicated the presence of significant unextractable radioactivity the solids were re-extracted with 2 N-HCl or water or were hydrolysed with an enzyme such as β-glucosidase (1% by wt. in aqueous solution) at about pH 6 at 35° for 48 h.

Polar components in the extracts from apple leaves treated with 2,6-dichlorobenzamide were examined by: (i) hydrolysis with 2 N hydrochloric acid for 60 min at 90°; (ii) oxidation with 0·4 M aqueous sodium periodate at 40° for 20 min: and (iii) formation of the trimethylsilyl derivatives in pyridine by the method of Sweeley *et al.*[4]

Soils

Soils were extracted with acetone (overall ratio of 1 g soil: 2 ml solvent) and the remaining solids were extracted further with 2 N-HCl for 12 h as described previously.[2]

Before the radioactivity count, the acetone extracts (up to 500 ml) of plants and soils were concentrated,[2] the aqueous residue (about 50 ml) was shaken with benzene (about 100 ml), and the benzene and aqueous phases were examined separately.

The extracts were subjected to paper and thin-layer chromatography and the radioactive components were located by radio-scanning of the layer. Components were desorbed from the relevant portion of the layers using 10% (v) ethanol–acetone or 5% (v) ethanol in benzene. The components were examined further by thin-layer chromatography (t.l.c.) and radioactive gas liquid chromatography and their behaviour was compared with that of unlabelled standards. The chromatographic, radio-scanning and radiocounting techniques have been described previously.[1,2]

Results

The extracts of crops grown in soil treated with ^{14}C-chlorthiamid were first examined by t.l.c. using 5% (v) ethanol in benzene as solvent on 300 μ layers of silica gel (Merck GF_{254}). Typical radio-scans of the thin-layer chromatograms of the extracts are shown in Fig. 1.

Identification of breakdown products

The radioactive components in the extracts of crops grown in soil treated with chlorthiamid were examined further and the following evidence for their identity was obtained.

Dichlobenil

Dichlobenil was identified in the soils, rice stems and leaves, and apple leaves. The t.l.c. behaviour of the ^{14}C-component is summarised in Table I.

In addition the ^{14}C-component showed a peak on a gas-liquid chromatograph[5] fitted with an electron capture detector, at a retention time and with a response identical with that of the standard dichlobenil.

2,6-Dichlorobenzamide

The benzamide was identified in the soils, rice stems and leaves, wheat grain, straw and leaves and in apple leaves. The t.l.c. behaviour of the ^{14}C-component is summarised in Table II.

In addition, the extracts showed a response on a gas-liquid chromatograph using both electron capture and radioactivity detectors at a retention time identical with that of authentic 2,6-dichlorobenzamide.

3-Hydroxy-2,6-dichlorobenzamide

3-Hydroxy-2,6-dichlorobenzamide was identified in the extracts of winter wheat (grain, straw and leaf). It was also identified in aqueous extracts when the residues of wheat straw and leaf and apple leaves after acetone extraction were treated with β-glucosidase. The t.l.c. behaviour of the ^{14}C-component is summarised in Table III.

In addition, after diazomethylation, the ^{14}C-component in the extract of winter wheat showed a response on a gas-liquid chromatograph using both electron-capture and radioactivity detectors at a retention time identical with the electron-capture response of the unlabelled diazomethylated standard of the 3-hydroxy-2,6-dichlorobenzamide.

The 3-hydroxy-2,6-dichlorobenzamide identified after β-glucosidase treatment was probably present in the original samples as a glycoside. To obtain further evidence of the

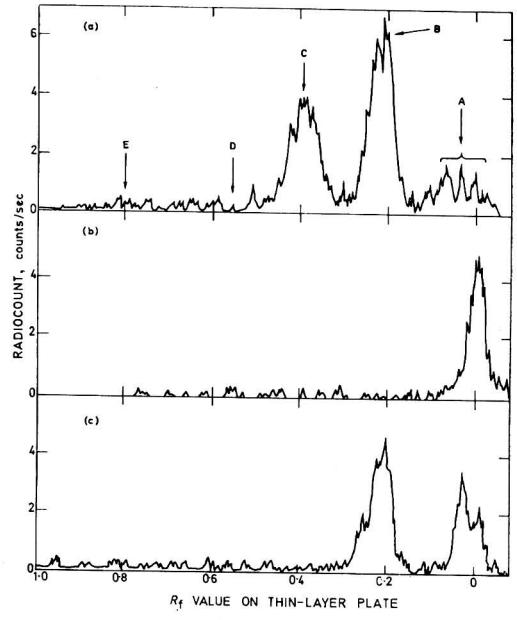

FIG. 1. *Radioscans of thin-layer chromatograms of extracts of plants grown in soil treated with ^{14}C-chlorthiamid*

Developing solvent—10% (v) ethanol in benzene
(a) Leaf from winter wheat. Benzene partition layer of concentrated acetone extract. A = conjugates; B = 3-hydroxy-2,6-dichlorobenzamide; C = 2,6-dichlorobenzamide; D = chlorthiamid; E = dichlobenil
(b) Apple leaf. Acetone extract of old leaves at harvest
(c) Apple leaf. Acetone extract of old leaves, (b) after partial hydrolysis with HCl

structure of the glycosidic components further quantities of the materials were prepared by treating apple leaves with ^{14}C-2,6-dichlorobenzamide itself. At 25 days after the second application of ^{14}C-2,6-dichlorobenzamide at 28 ppm (56 ppm total) the leaves were extracted with methanol. Of the total activity applied to the leaves, 9·8% was present as unchanged 2,6-dichlorobenzamide, 6·8% was present as extractable glycoside components, 1·1% was not extractable with methanol.

TABLE I

Retention values (R_f) of dichlobenil in various t.l.c. systems
300 μ layers of silica gel GF_{254} as adsorbent

Solvent system vol.-%	R_f of ^{14}C-component	R_f of dichlobenil
10% ethanol in benzene	0·75	0·76
5% ethanol in benzene	0·74	0·71
50% acetone in benzene	0·45	0·46
Benzene	0·57	0·57
Hexane	0·07	0·07

TABLE II

Retention values (R_f) of 2,6-dichlorobenzamide in various t.l.c. systems
300 μ layers of silica gel GF_{254} as adsorbent

Solvent system vol.-%	R_f of ^{14}C-activity	R_f of 2,6-dichlorobenzamide
5% ethanol in benzene	0·21	0·19
Acetone	0·90	0·92
30% acetone in hexane	0·30	0·30
5% aniline + 2% acetone in benzene	0·15	0·13
60% acetone in benzene	0·75	0·75
10% ethanol in benzene	0·40	0·40
50% acetone in hexane	0·50	0·50

The remainder of the applied ^{14}C-activity was no longer present on the leaves. No 2,6-dichlorobenzoic acid or free or conjugated 3- or 4-hydroxy-2,6-dichlorobenzamide could be detected in the extracts when at least 0·05 ppm of each component would have been detectable.

The glycosidic material was shown by t.l.c. to contain two components, **I** and **II**, of which **I** was 58% of the total glycosidic material. The chromatographic properties of these components are shown in Table IV.

TABLE III

Retention values (R_f) of 3-hydroxy-2,6-dichlorobenzamide in t.l.c. systems
300 μ layers of silica gel GF_{254} as adsorbent

Solvent system vol.-%	R_f of ^{14}C-activity*†	R_f of 3-hydroxy-2,6-dichlorobenzamide*
5% ethanol in benzene	0·05	0·05
10% ethanol in benzene	0·27–0·31	0·27–0·33
50% acetone in hexane	0·73–0·79	0·72–0·78
60% acetone in benzene	0·64–0·69	0·63–0·70
60% ether in hexane	0·03–0·10	0·02–0·11
10% ethanol in chloroform	0·06–0·17	streak to 0·19
40% ethyl acetate in benzene	0·27–0·35	0·27–0·35
After reaction with diazomethane of extract and standard:		
10% ethanol in benzene	0·38–0·43	0·37–0·42
50% acetone in hexane	0·82–0·88	0·80–0·86

* In each case the ^{14}C-activity and unlabelled standard were co-chromatographed and the R_f values of the upper and lower edges of the respective ^{14}C-spot and unlabelled standard spot are quoted in most cases

† As the free hydroxy compound after enzymic hydrolysis in some cases

TABLE IV

Retention values (R_f) of the glycosides of 3-hydroxy-2,6-dichlorobenzamide extracted from apple leaf treated with ^{14}C-2,6-dichlorobenzamide

Absorbent	Solvent system (volume composition)	Retention value (R_f) of component I	II	Synthetic β-D-glucoside of 3-hydroxy-2,3-dichlorobenzamide
Whatman No. 3 paper	Methyl cyanide: water: ammonia (80 : 18 : 2)	0·1	0·3	0·1
Whatman No. 3 paper*	Ethyl acetate: isopropanol: water (276 : 100 : 50)	0·27	0·79	0·27
Silica gel GF_{254}	Ethyl acetate: isopropanol: water (70 : 20 : 10)	0·38	0·75	0·38
Silica gel GF_{254}**	Ethyl acetate: isopropanol: water (70 : 20 : 10)	0·35	0·70	0·35
Silica gel GF_{254}	Ethyl acetate: water: formic acid: xylene (70 : 4 : 4 : 2)	0·22	0·50	0·22
Silica gel GF_{254}	n-Butanol: acetone: water: chloroform (20 : 60 : 5 : 15)	0·52	—	0·52
	After oxidation with sodium periodate (R_f value before oxidation in parenthesis)			
Silica gel GF_{254}	Acetone: toluene (30 : 70)	0·15 (0·0)	0·11 (0·0)	0·15 (0·0)
Silica gel GF_{254}	Ethanol: chloroform (20 : 80)	0·45 (0·0)	—	0·45 (0·0)
Silica gel GF_{254}	Ethyl acetate: water: formic acid: xylene (70 : 4 : 4 : 2)	0·66 (0·22)	0·80 (0·50)	0·60 (0·22)
	After conversion to trimethyl silyl derivative (R_f value before silylation in parenthesis)			
Silica gel GF_{254}	Acetone: toluene (30 : 70)	0·75 (0·0)	0·38 (0·0)	0·75 (0·0)
Silica gel GF_{254}	Ethanol: chloroform (7 : 93)	0·65 (0·0)	0·20 (0·0)	0·65 (0·0)

* Paper impregnated by dipping in 0·01M sodium acetate
** Silica gel containing 2% by wt. of boric acid

Both components I and II were hydrolysed by acid to form 3-hydroxy-2,6-dichlorobenzamide.

The results indicate that component I is the β-D-glucoside of 3-hydroxy-2,6-dichlorobenzamide and the component II is a glycoside of the same algycone with a sugar other than β-D-glucose.

3-Hydroxy-2,6-dichlorobenzonitrile

3-Hydroxy-2,6-dichlorobenzonitrile was identified in apple leaf and wheat leaf only, after enzymic hydrolysis, by t.l.c. as summarised in Table V. The component was probably present in the original leaf as a glycoside.

4-Hydroxy-2,6-dichlorobenzonitrile

4-Hydroxy-2,6-dichlorobenzonitrile was identified in apple leaf and wheat leaf, after enzymic hydrolysis, by t.l.c. as summarised in Table VI. The component was probably present in the original leaves as a glycoside.

Rice

The distribution of the radioactivity in the rice and soil is summarised in Table VII.

Winter wheat

The distribution of ^{14}C-activity in winter wheat and the soil at harvest is summarised in Table VIII.

Apples

The distribution of ^{14}C-activity in apple trees and soils is summarised in Table IX. The apple trees grown in soil treated at 3·0 lb/acre bore no fruit. However, it should be borne in mind that the trees were still young and that in normal growing practice fruiting cannot be ensured in the first year after transplanting.

Discussion

Residues in rice

The total residue of ^{14}C-components in rice grain at harvest, 4 months after post-emergent treatment of the soil at 1 lb/acre with ^{14}C-chlorthiamid, was small (0·06 ppm) but significant. Since it was not extracted in acetone (Table VII) it was unlikely to be either dichlobenil or 2,6-dichlorobenzamide, but was likely to be mainly plant conjugates of phenolic compounds that occur in apple and wheat. However, the amount was too small to study in detail.

The rest of the plant at harvest contained 0·09 ppm of acetone-soluble activity, with very small amounts of unextracted activity. The components present in the acetone extracts were dichlobenil and 2,6-dichlorobenzamide, with no evidence of any unchanged chlorthiamid.

The soil below the rice at harvest contained extractable residues of 0·10 ppm or less, mainly of dichlobenil, with traces of 2,6-dichlorobenzamide. This suggests a considerable depletion of herbicide, probably by volatilisation of dichlobenil.

Residues in winter wheat

At harvest the total residue in the grain of winter wheat grown on soil treated at 1·2 lb/acre was 0·11 ppm for plants grown on compost and 0·16 ppm for plants grown on loam (Table VIII). Most of this residue was due to 3-hydroxy-2,6-dichlorobenzamide, with smaller amounts of what was considered to be 2,6-dichlorobenzamide. After thorough extraction of these components, the grain contained very little unextracted activity.

The stalk contained larger residues totalling 0·6 ppm (equivalent to chlorthiamid) when grown on compost and 0·4 ppm when grown on loam. These residues were composed of 2,6-dichlorobenzamide and 3-hydroxy-2,6-dichlorobenzamide, together with 3-hydroxy-2,6-dichlorobenzamide bound to a plant substance, probably a sugar.

The leaves of both samples contained residues totalling 2 ppm (equivalent to chlorthiamid) for the crop grown on compost and 2·7 ppm on loam. The acetone extract of the sample grown on loam contained 2,6-dichlorobenzamide (0·30 ppm) and 3-hydroxy-2,6-dichlorobenzamide (0·45 ppm). The activity remaining in the plant after acetone extraction was released on treatment with β-glucosidase, with the formation of 3-hydroxy-2,6-dichlorobenzamide as the major identifiable component. There was some evidence of the presence of trace amounts of 3-hydroxy- and 4-hydroxy-2,6-dichlorobenzonitriles after the enzymic hydrolysis. Repeated treatment with β-glucosidase of the plant material remaining after extraction with acetone released only further 3-hydroxy-2,6-dichlorobenzamide. This suggests that it was the only conjugate present after the first treatment with enzyme, but nonetheless, it was found impossible to produce a completely inactive plant sample solely by repeated enzyme treatment. About 0·2 ppm of residual activity was always left.

The control (untreated) wheat showed some activity due to volatilisation of ^{14}C-compounds from the treated soils but much less than the treated wheat grown next to it.

It is considered that the high residues of metabolites in the leaves and stalks were probably due to volatilisation of ^{14}C-activity, possibly as dichlobenil, from the soil to plant. In comparison, the residues in the grain were much lower. As indicated above, some volatilisation of ^{14}C-activity from the treated samples to the untreated control samples nearby also occurred, but to a much smaller extent.

Residues in apple

The fruit of apple trees grown in soil treated with ^{14}C-chlorthiamid at 1·5 lb/acre and sampled at harvest 116 days after treatment, was mature and averaged 450 g in weight. The fruit contained no significant residue of chlorthiamid or its metabolites (Table IX). The method of counting radioactivity would have detected < 0·01 ppm of residue if present.

The absence of chlorthiamid and its breakdown products in apple fruit is particularly significant when compared with the incorporation of residues into the leaves and other parts of the tree (Table IX). Total ^{14}C-residues of 2·4 ppm were present in old leaves which had been on the tree throughout the 116 days to harvest, and residues were also present in the woody parts (0·39 ppm) and in the roots (0·45 ppm). The control trees, grown in untreated soil next to the treated trees, contained residues of ^{14}C-activity which must have evaporated from the treated soil, probably as dichlobenil since chlorthiamid and 2,6-dichlorobenzamide are less volatile.* This suggests that much of the residues in the treated leaves is also due to volatilisation from the soil.

* The vapour pressures at 20°, in mm of mercury, are approximately: chlorthiamid, 3×10^{-7}; dichlobenil, 5×10^{-4}; 2,6-dichlorobenzamide, $1{\cdot}8 \times 10^{-7}$

TABLE V

Retention values (R_f) of 3-hydroxy-2,6-dichlorobenzonitrile in various t.l.c. systems

300 μ layers of silica gel GF_{254} as adsorbent

Solvent system vol.-%	R_f of extract*	R_f of 3-hydroxy-2,6-dichlorobenzonitrile*
50% acetone in hexane	0·60	0·58
10% ethanol in chloroform	0·35–0·52	0·35–0·52
30% ethyl acetate + 30% benzene in hexane	0·63–0·79	0·63–0·79
60% acetone in benzene	0·40–0·50	0·41–0·49
60% ether in hexane	0·85–0·92	0·82–0·94
10% ethanol in benzene	0·60	0·58

* See footnote to Table III

TABLE VI

Retention values (R_f) of 4-hydroxy-2,6-dichlorobenzonitrile in various t.l.c. systems

300 μ layers of silica gel GF_{254} as adsorbent

Solvent system vol.-%	R_f of extract*	R_f of 4-hydroxy-2,6-dichlorobenzonitrile*
50% acetone in hexane	<0·32 (streaks)	<0·35 (streaks)
10% ethanol in benzene	0·27	0·27
30% ethyl acetate + 30% benzene in hexane	0·59–0·66	0·60–0·67
60% acetone in benzene	0·67–0·78	0·68–0·80
60% ether in hexane	<0·08 (streaks)	<0·15 (streaks)

* See footnote to Table III

TABLE VIII

Residues of ^{14}C-chlorthiamid and breakdown products in winter wheat and soils

Sampled at harvest, about 8 months after treatment.
Variety: Rothwell Perdix

	Sample	^{14}C-Residues in sample in equivalent ppm of chlorthiamid†			
		Dosage level for John Innes No. 2 compost		Dosage level for medium loam	
		1·2 lb/acre	Untreated Control	1·2 lb/acre	Untreated Control
Grain	Acetone extractable:				
	2,6-Dichlorobenzamide	**	<0·002	0·06	<0·002
	3-Hydroxy-2,6-dichlorobenzamide	**	<0·002	0·1	<0·002
	Unextractable in acetone	**	<0·01	0·005	<0·002
	Total	0·11	0·01	0·16	<0·01
Stalk	Acetone extractable:				
	2,6-Dichlorobenzamide 3-Hydroxy-2,6-dichlorobenzamide	0·25	0·004	0·08 0·18	<0·002
	Unextractable in acetone				
	*3-Hydroxy-2,6-dichlorobenzamide	0·37	0·23	0·13	0·03
	Total	0·62	0·23	0·39	0·03
Leaves	Acetone extractable:				
	2,6-Dichlorobenzamide 3-Hydroxy-2,6-dichlorobenzamide	0·27 0·27	0·06	0·30 0·45	0·09
	Unextractable in acetone				
	*3-Hydroxy-2,6-dichlorobenzamide	1·2		1·9	
	*3-Hydroxy-2,6-dichlorobenzonitrile	0·1	0·12	~0·05	<0·04
	*4-Hydroxy-2,6-dichlorobenzonitrile	0·1		~0·05	
	Total	2·0	0·18	2·7	0·13
Soil	Acetone extractable:				
	Dichlobenil (2,6-dichlorobenzonitrile)	0·01	<0·002	0·02	<0·002
	2,6-Dichlorobenzamide	0·02	<0·002	0·02	<0·002
	Total	0·03	<0·004	0·04	<0·004

* As glycoside conjugate
** Not examined
† Unchanged chlorthiamid could not be detected at the 0·002 ppm level

TABLE VII

Residues of ^{14}C-chlorthiamid and breakdown products in rice and soils

Plants sampled at harvest, 4·5 months after treatment with ^{14}C-chlorthiamid at 1·0 lb/acre
Soil type: John Innes No. 2 compost

	Sample	^{14}C-residues in sample as equivalent ppm of chlorthiamid*
Grain	Acetone extractable	0·01
	Unextractable	0·05
	Total	0·06
Stems and leaves	Acetone extractable as:	
	Dichlobenil	0·03
	2,6-dichlorobenzamide	0·06
	Other unidentified components	<0·005
	Unextractable	0·004
	Total	0·09
Moist soil	Acetone extractable as:	
	Dichlobenil	0·085
	2,6-dichlorobenzamide	0·005
	2N hydrochloric acid extract	0·006
	Total	0·10

Control values for untreated samples were less than 0·004 ppm for all samples other than the stems and leaves for which the control plant had a blank value of < 0·01 ppm
* Unchanged chlorthiamid could not be detected at the 0·002 ppm level

TABLE IX

Residues of ^{14}C-chlorthiamid and breakdown products in apples and soils at harvest

Sampled at harvest at 116 days (4 months) after treatment.
Ellison's Orange cordons in John Innes No. 2 compost

	Sample	^{14}C-residue in sample in equivalent ppm of chlorthiamid at corresponding dosage level†		
		3 lb/acre	1·5 lb/acre	Untreated Control
Fruit	Extractable in acetone	—	<0·002	<0·002
	Unextractable in acetone	—	<0·002	<0·002
	Total	—	<0·004	<0·004
Old leaf*	Extractable in acetone:			
	Dichlobenil	0·005	—	
	2,6-Dichlorobenzamide	0·02	—	
	Glycoside of 3-hydroxy-2,6-dichlorobenzonitrile	0·30	—	0·36
	Glycoside of 4-hydroxy-2,6-dichlorobenzonitrile	0·20	—	
	Glycoside of 3-hydroxy-2,6-dichlorobenzamide	0·50	—	
	Unextractable in acetone:			
	Glycoside of 3-hydroxy-2,6-dichlorobenzonitrile	0·30	—	
	Glycoside of 4-hydroxy-2,6-dichlorobenzonitrile	0·30	—	0·64
	Glycoside of 3-hydroxy-2,6-dichlorobenzamide	0·80	—	
	Total	2·4	—	1·0
Young leaf	Extractable in acetone	0·05	—	0·02
	Unextractable in acetone	0·05	—	0·03
	Total	0·10	—	0·05
Shoots and trunk	Extractable in acetone	0·05	—	<0·01
	Unextractable in acetone	0·34	—	0·06
	Total	0·39	—	0·07
Root	Extractable in acetone	0·20	—	<0·01
	Unextractable in acetone	0·25	—	0·11
	Total	0·45	—	0·12
Soil	Extractable in acetone			
	Dichlobenil	0·40	—	<0·002
	2,6-Dichlorobenzamide	0·30	—	<0·002
	Total	0·70	—	<0·006

* Present on trees at time of treatment
† Unchanged chlorthiamid could not be detected at the 0·002 ppm level

Some downward translocation probably occurred in the trees since the treated trees were consistently higher in ^{14}C-residue level than the controls, and also because the residues found in the roots of the control tree could only have occurred by downward translocation from the trunk or leaves. The untreated soil showed no residue (< 0·01 ppm).

Volatilisation will occur from field treated soils, but the vapour of dichlobenil will be dissipated by movement of the air and it is therefore likely that leaf residues in field-treated apple trees will be much lower than those reported here.

In the present glasshouse experiments, residues of dichlobenil itself in the leaves are very small. The herbicide has, in fact, been converted to phenolic compounds which were then bound to plant substances, including glucose. Table IX shows that the glucosides of the phenols are incompletely extracted by acetone. Both the extracted and unextracted ^{14}C-components were hydrolysed with acid or enzyme to give the same three phenols: 3-hydroxy-2,6-dichlorobenzamide, 3-hydroxy-2,6-dichlorobenzonitrile and 4-hydroxy-2,6-dichlorobenzonitrile.

The metabolites of chlorthiamid in the leaves of the control tree were similar to those from the treated trees.

The soils at harvest contained residues of dichlobenil and 2,6-dichlorobenzamide (0·30 ppm) but no chlorthiamid (Table IX), the initial treatment having corresponded to about 4 ppm.

Work is in hand on the analysis of crops, grown in the field in chlorthiamid treated soils, for possible residues of the benzamide and the conjugated hydroxybenzamide, the results of which will be reported in a later paper. The metabolism of dichlobenil in wheat and rice seedlings has been studied simultaneously and independently by Verloop & Nimmo.[6] The plants were grown in nutrient solutions containing ^{14}C-dichlobenil and the major breakdown product in the leaves was 3-hydroxy-2,6-dichlorobenzonitrile and its conjugates. 2,6-dichlorobenzamide was absent in the plants and is probably formed from dichlobenil only in the soil.

Conclusions

Soils treated with chlorthiamid contained dichlobenil and 2,6-dichlorobenzamide and the residues of the chlorthiamid had decayed by harvest as found previously with the more detailed studies of chlorthiamid in soils.[1] It is significant that the different crops contained different breakdown products. Unchanged chlorthiamid could not be detected in any of the samples at the 0·01 ppm level.

Rice plants contained no detectable phenolic derivatives of the aromatic nucleus, and apparently did not conjugate the herbicide.

Winter wheat contained only 2,6-dichlorobenzamide with 3-hydroxy-2,6-dichlorobenzamide and conjugates of it. Since the soil contained dichlobenil and the benzamide it is not possible to say from the present work whether the benzamide was formed in the plant or not, although the work of Verloop & Nimmo[6] would suggest that it is only formed in the soil.

Apple tree leaf alone showed all three phenols—as conjugates—in similar amounts. This could be due either to faster conversion of the 2,6-dichlorobenzonitrile to phenolics or to a slower conversion to amide.

Studies of the metabolism of chlorthiamid in mammals,[7] showed that the major product eliminated was 3-hydroxy-2,6-dichlorobenzonitrile together with lesser amounts of 4-hydroxy-2,6-dichlorobenzonitrile, 2,6-dichlorobenzoic acid and 3-hydroxy- and 4-hydroxy-2,6-dichlorobenzoic acids and their conjugates. Plants growing in treated soils therefore appear to differ in not giving rise to benzoic acids and in giving 3-hydroxy-2,6-dichlorobenzamide which is not a product of mammalian metabolism.

Although this work shows that finite residues of metabolites of chlorthiamid may occur under glasshouse conditions, it should be emphasised that the residues found in the edible portions of apple, rice and wheat were small and are likely to be even smaller under field conditions. Furthermore chlorthiamid, dichlobenil, 2,6-dichlorobenzamide and the three phenolic derivatives are not of high mammalian toxicity since the Tunstall Laboratory have shown that the LD_{50} for acute oral toxicity to rats is 400–800 mg/kg for the 4-hydroxy-2,6-dichlorobenzonitrile and over 800 mg/kg for the other compounds.

Acknowledgments

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References

1. Beynon, K. I., & Wright, A. N., *J. Sci. Fd Agric.*, 1968, **19**, 723
2. Beynon, K. I., & Wright, A. N., *J. Sci. Fd Agric.*, 1967, **18**, 143
3. Dobbs, H. E., *Analyt. Chem.*, 1963, **35**, 783
4. Sweeley, C. C., Bentley, R., Makita, M., & Wells, W. W., *J. Am. chem. Soc.*, 1963, **85**, 2497
5. Beynon, K. I., Davies, L., Elgar, K., & Wright, A. N., *J. Sci. Fd Agric.*, 1966, **17**, 151
6. Verloop, A., & Nimmo, W. B., *Weed Res.*, in press
7. Griffiths, M. H., Moss, J. A., & Hathway, D. E., *Biochem. J.*, 1966, **98**, 770

MELANISM AND ASSOCIATED SYMPTOMS IN WHEAT GROWN ON COPPER-RESPONSIVE CHALKLAND SOILS

By L. J. HOOPER and D. B. DAVIES*

Copper applications increased yields and reduced 'blackening' symptoms of wheat grown on chalk rendzina soils (Icknield Series—organic phase) in southern England. The 'blackening' syndrome is recognised by an overall dark olive-green colour of the plant and internodal melanism after ear emergence followed by 'rat-tailing' of ears due to pinched or absent grains.

The symptoms, which are aggravated by nitrogen applications, wet warm summers, and prior cropping with kale, have not previously been associated with inadequate copper nutrition. When Cu, Mg, Mn, Mo and Zn were applied to spring wheat grown in pots the only significant effects were from Cu and to a much smaller extent Mo. Soils where blackening occurred contained 3–7 ppm total Cu and 0·4–1·5 ppm extractable Cu (0·5 M-EDTA). Yield increases of up to 200% were obtained both by foliar sprays of copper oxychloride (1 lb Cu/acre) or copper sulphate (0·2 lb Cu/acre) and by soil applications of copper sulphate (14 lb Cu/acre). Attempts to induce melanism in wheat plants grown in sand and water cultures with inadequate copper supply, high nitrogen levels, and small quantities of three amino-acid melanin precursors were unsuccessful, although roots developed a dark pigmentation in certain of the treatments.

Introduction

The occurrence of clearly defined dark patches in wheat crops growing on certain chalk soils in southern England was first noted by farmers in the mid-nineteen fifties and 'blackening' was the term used to describe the condition (Fig. 1). Invariably within the blackened areas, the yield of the wheat grain was severely reduced and the plants showed a number of symptoms, one of which was formation of a dark pigment—melanism. Blackening had not previously been described in Britain, but similar disorders had been reported from Holland[1]. In this reference workers considered a pathogen to be involved, mainly *Septoria nodorum* Berk, but the blackening encountered in southern England has not been associated with infections of this fungus.[2] The absence of a fungal association prompted investigation into alternative causes.

Symptoms of blackening

A complete description of visual symptoms is of value in comparing crop disorders in different areas, and a detailed account of the blackening symptoms, as they have been observed in the field with spring wheat varieties Atle and Atson, are given.

During early growth the plants appear normal or slightly darker than normal but no real visual differences occur until after ear emergence. About mid-July the ears are curved, unlike the upright habit of ears in normal plants. Soon afterwards continuous or discrete areas of dark pigment are discernable, immediately below the ear, on the top internode. Pigment formation is progressive, extending down the internode, and appearing on the peduncle and on the glumes, and finally on the lower internodes. The spread varies from plant to plant and from tiller to tiller and seems to be encouraged by warm moist conditions.

Concurrent with pigment formation the other symptoms of blackening become evident. Affected plants assume an overall dark olive-green colour contrasting with the normal green of healthy plants. Grain formation is severely affected, grains being either absent altogether or at best small and wizened, so that ears remain thin and curved, a condition sometimes referred to as 'rat-tailed'. (Fig. 2.)

With the onset of ripening, when healthy plants turn a normal straw colour, severely affected plants gradually turn darker eventually dying-off, and fields appear from a distance to be black in the affected areas (Fig. 1). The strength of the straw is impaired and because of this the affected crop often curves over and the straw fractures.

Yields of grain from affected areas are much reduced; as little as one fifth of the normal may be harvested, and grain size is very poor. Microscopic examination of tissue with melanism shows dark pigment in the chlorenchyma cells of the outer stem, similar to the description of pigment in wheat plants exhibiting 'pseudo-black chaff'.[3]

Experimental

Investigations into causes of blackening

Blackening almost invariably occurs on one soil type, the shallow organic silty loams over Middle or Upper Chalk (see Appendix for profile description). This soil, one of the phases of the Icknield Soil Series, can be unsaturated with bases in its natural state, but under cultivation its free calcium carbonate content rises as the soft parent material is brought into the plough layer. The soil is of high organic matter (10–20%), recently reclaimed from chalk downland scrub, and it was partly the organic nature of the soil and partly the knowledge that pure carbonate rocks are frequently low in available trace elements,[4] which suggested that trace element supply might be limiting.

Observations of the patchy distribution of blackening in fields showed that the affected areas were on soil of somewhat

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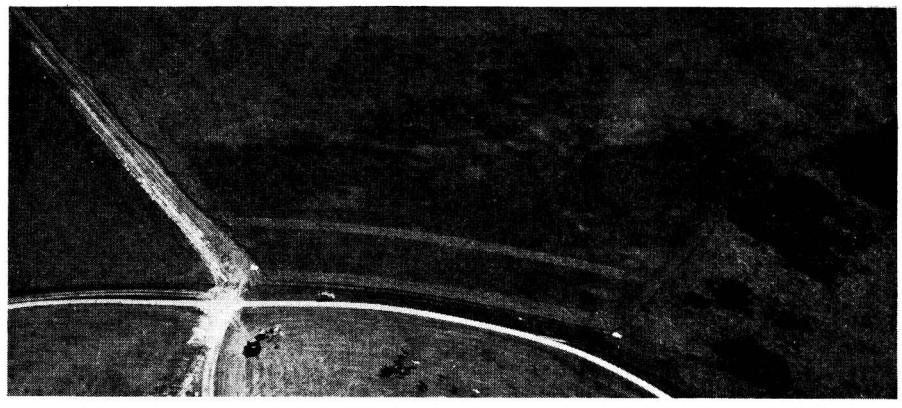

FIG. 1. *Blackening in a field of spring wheat growing on chalk downland*
(Two lighter strips beyond the road have been treated)

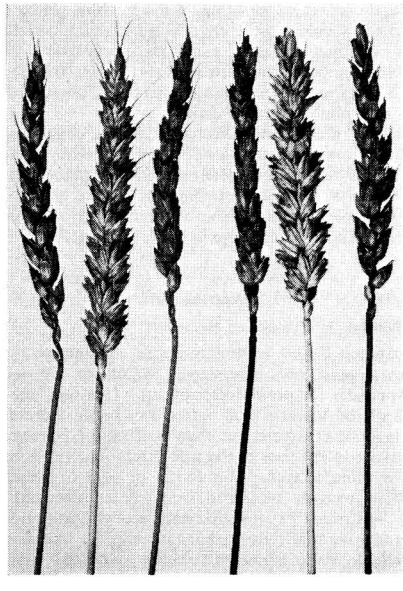

FIG. 2. *Blackening symptoms on two varieties of spring wheat* (Left, JUFY, Right ATLE). The central head of each is normal

higher organic matter content with rather darker colour. Analysis of soil and plant from affected patches and adjacent normal areas showed low total soil copper in both (3–5 ppm), whereas the affected plants contained a lower concentration of copper (3 ppm vs. 4·5 ppm) and a higher nitrate level (220 ppm vs. 80 ppm nitrate-N), than normal plants.

Pot Work 1960

A diagnostic spray of five elements individually and in combination, (copper, magnesium, magnanese, molybdenum and zinc) was applied to spring wheat in pots containing soil with a history of blackening.

All individual spray treatments reduced the 'severe melanism' (greater than 2 in. continuous pigmentation) compared with the control, but the best treatment was the copper sulphate spray which reduced the 'total melanism' from 62% to 26% and 'severe melanism' from 45% to 9%. The only other treatment which gave comparable results was the mixture of copper and molybdenum. These results were sufficiently conclusive to suggest treatments for field work in 1961.

Field work 1961

The work carried out in 1960 suggested that the treatments most likely to be beneficial in the field control of melanism, and perhaps also of the other symptoms of blackening would be those of copper and molybdenum. As other workers had considered plant pathogens to be involved, it was thought appropriate to include soil treatments, thus minimising the fungicidal action of the treatments on aerial parts of the plant.

Field observations had indicated that the disorder was more prevalent when the weather during grain formation was moist and warm; an effect possibly associated with late nitrification of soil nitrogen. This effect, together with the high nitrate content of the straw at ripening, suggested that a nitrogenous fertiliser allowing a slow release of nitrogen would yield useful information when compared with more rapidly available nitrogen forms.

Treatments

The following copper sulphate, sodium molybdate, and organic or mineral nitrogen treatments were applied.

Cu_0 —No copper
Cu soil —Soil treatment: 40 lb $CuSO_4.5H_2O$ broadcast on seedbed
Cu spray —Spray treatment: 12 oz $CuSO_4.5H_2O$/100 gallons/acre (200 ppm Cu) sprayed at end of tillering
Mo_0 —No molybdenum
Mo soil —Soil treatment 4 lb $Na_2MoO_4.2H_2O$/acre broadcast on seedbed
Mo spray—Spray treatment 4 oz $Na_2MoO_4.2H_2O$/100 gallons/acre (100 ppm Mo) sprayed at end of tillering
N_0 —No nitrogen
N org. —73 lb N/acre as 13% coarse grist hoof and horn
N min. —73 lb N/acre as 21% ammonium nitrate-lime fertiliser

The layout was a 3 × 3 × 3 factorial design in 3 blocks, with second order interations confounded for residuals. Spring wheat variety Atle, was grown in plots of 1/40 acre and harvested by self-propelled combine harvester.

The site was at 620 ft o.d. on undulating chalk downland reclaimed from scrub 11 years previously. The soil was a flinty organic loam over soft white chalk at 10 in. The EDTA-extractable copper content (see Appendix) of the 0–6 in sample was 0·6 ppm Cu.

Assessments

Plots were scored by eye before harvest for various blackening symptoms. 100 tillers, taken at random were assessed for degree of melanism, ear formation and depth of green colour according to the scheme detailed below:

Melanism symptoms

1—No pigmentation (normal)
2—Slight pigmentation
3—less than 3 in. continuous pigmentation on upper internode
4—more than 3 in. continuous pigmentation on upper internode

Ear formation

1—Ears full and upright (normal)
2—Ears showing intermediate 'rat-tailing'
3—Ears showing severe 'rat-tailing'

Colour of plant

1—Whole plant light green (normal)
2—Whole plant olive green
3—Whole plant dark olive-green.

Results

Results of both yield data and visual assessments are shown in Tables I and II.

TABLE I

Yield of grain; cwt/acre at 85% dry matter

	Cu_0	Cu Soil	Cu Spray	Mean
N_0	22·0	26·7	26·6	25·1
N org.	19·4	28·0	29·2	25·5
N min.	15·8	24·1	29·6	23·2
	Mo_0	Mo Soil	Mo Spray	Mean
Cu_0	20·6	19·0	17·6	19·1
Cu soil	25·0	30·4	23·5	26·3
Cu spray	29·2	27·9	28·2	28·5
	N_0	N org.	N min.	Mean
Mo_0	23·7	26·9	24·2	24·9
Mo soil	26·1	27·6	23·6	25·8
Mo spray	25·6	22·1	21·7	23·1

S. E. for comparison within table 2·3 cwt/acre
S. E. for comparison of means 1·3 cwt/acre

TABLE II

Visual scores of blackening symptoms

Treatment	Melanism	Ear formation	Colour of plant
N_0	1·8	1·8	1·4
N org.	2·1	1·8	1·6
N min.	2·4	2·3	2·4
Cu_0	2·3	2·3	2·1
Cu soil	2·1	1·9	1·7
Cu spray	2·1	1·7	1·6
Mo_0	2·1	2·1	1·9
Mo soil	2·1	2·0	1·8
Mo spray	2·2	2·1	1·7

Discussion

It will be seen in Table I that at Cu_0 the effect of the nitrogen in the form of hoof and horn (N org.) appeared to be intermediate between the effect of treatments N_0 and N min. on Cu_0. This suggests either incomplete nitrification of the coarse material or that the later release of mineral nitrogen has less effect on the disorder than the same rate of nitrogen applied in mineral form. Because of this intermediate effect of the hoof and horn at Cu_0, discussion will deal only with the mineral nitrogen treatment (N min.).

Where no copper was applied, mineral nitrogen significantly depressed the yield by 6·2 cwt/acre whereas in the presence of sprayed copper, it increased the yield by 3·0 cwt/per acre. At the typical nitrogen rate for the area of about 73 lb N/acre the response to copper was 13·8 per acre. The agronomic significance of the nitrogen-copper antagonism has become more important as the yield potential of modern wheat varieties has increased. In order to realise this potential more nitrogen is required, but where the blackening disorder is prevalent higher nitrogen use could reduce yields.

Copper applied to the soil was as effective as the foliar spray, where no fertiliser nitrogen was used, but less effective where mineral nitrogen was applied. Although soil applications may be less efficient than spray treatments, the positive response suggests that copper is playing a nutritional rôle in reducing blackening, rather than having a fungicidal action.

None of the effects of molybdenum were statistically significant, but there appeared to be a yield depression due to molybdenum, both as a soil application and a foliar spray, in the absence of copper. The soil application of molybdenum appeared to enhance the effect of the soil copper applications.

The three symptoms of blackening were all intensified by the application of mineral nitrogen (Table II); they were all reduced by both soil and spray applications of copper, the latter being slightly more effective. Of the three symptoms, melanism was the least affected by copper treatments. The spray control of blackening symptoms in the crop immediately before harvest is shown in Fig. 3.

The 1,000-grain weights shown in Table III were determined on samples taken from the combine harvester and show only very small differences between the lowest yield and the highest yield treatments (i.e. N min. Cu_0 and N min. Cu spray). The results are deceptive, for some of the very small and shrivelled grains are lost during the threshing process in the combine harvester and results are not representative of the complete grain sample. Comparison of grain weights on hand-threshed samples from another blackening trial showed an increase of 5 g/1,000 grains from copper treatments. The total increase in yield from copper treatments can therefore be attributed to a reduction in 'blindness' in ears and a small increase in mean grain size. The copper content of the grain is shown in Table IV. The grain weights in Table III (circa 31 g/1000 grains) are typical of the grain produced from these copper-deficient soils even when copper sprays are used, and compare unfavourably with plump wheat, which weighs about 55–60 g/1000 grains. Straw on plots not receiving copper showed a greater tendency to be weak than on those receiving copper, an observation which is in agreement with work in Russia using oats and rye.[5,6] This 'pinched grain' problem is apparently not related to other micronutrient effects, for the authors have shown over a three year period that various treatment combinations of copper with molybdenum, manganese, boron, zinc, cobalt, iron and magnesium have not improved grain size when compared with treatments of copper alone.

TABLE III

Grain size—1000-grain weight, g
(Only the N × Cu table is shown)

	Cu_0	Cu soil	Cu spray	Mean
N_0	32·0	31·5	32·0	31·8
N org.	30·0	31·5	32·0	31·3
N min.	30·5	30·5	31·5	30·8
Mean	30·8	31·2	31·8	

TABLE IV

Copper content of grains, ppm

Cu_0	Cu soil	Cu spray
3·9	4·1	4·0

FIG. 3. *Atle wheat in 1961 field trial, showing sprayed plot (foreground) between two unsprayed plots which were exhibiting well-developed blackening symptoms*

Field work 1962

The results in 1961 led to a more detailed field study in 1962 when rates of nitrogen and copper were investigated on a similar site also using spring wheat, variety Atle.

Treatments

Cu_0—No copper
Cu_1—1½ lb copper oxychloride (12 oz Cu) in 20 gal water/acre sprayed at end of tillering.
Cu_2—3 lb copper oxychloride (24 oz Cu) in 20 gal/acre sprayed at end of tillering.
N_1 —22 lb N per acre as ammonium nitrate–lime fertiliser applied to seedbed.
N_2 —44 lb N per acre as ammonium nitrate–lime fertiliser applied to seedbed.
N_3 —66 lb N per acre as ammonium nitrate–lime fertiliser applied to seedbed.

The layout was a 3 × 3 factorial design replicated in four randomised blocks. Plot area was 1/40 acre. A self-propelled combine harvester was used to harvest individual plots.

Results

Results shown in Tables V and VI are the means of the four replicates.

Discussion

The response to copper and nitrogen were both significant, there being no additional response to the higher level of copper oxychloride application. In spite of the small response to copper the interaction between the copper and nitrogen responses was significant thus confirming the 1961 results. The interaction is shown by comparing the lack of response to nitrogen without copper with the 4·2 cwt response in the presence of copper (Table V). Although the overall response to copper was much smaller than in 1961 there was variation in the severity of the disorder within the trial, and, in one of the blocks, the higher nitrogen caused a depression in yield in the absence of copper of 3·3 cwt. This yield depression was associated with more pronounced symptoms of blackening.

As in 1961, the average 1000-grain weight was much lower than that for good quality grain and differences between treatments were negligible.

TABLE V

Yield of grain—cwt/acre at 85% dry matter

	Cu_0	Cu_1	Cu_2	Mean
N_1	24·1	25·5	25·6	25·1
N_2	23·5	28·4	27·8	26·6
N_3	24·8	29·5	29·8	28·0
Mean	24·1	27·8	27·7	
S.E. for body of table 0·7 cwt				
S. E. for means 0·4 cwt				

General Discussion

Melanism

Frequently, melanism has been related to fungal infection, the pigment being produced by the plant tissue as a reaction to the infection.[1,7,8] It has been noted in association with infection by both stem rust (*Puccinia graminis tritici*)[9,10] and *Alternaria tenuis*.[7] By inoculating wheat plants with the organism thought to be responsible for bacterial black chaff in wheat (*Phytomonas translucens* f. sp. *undulosum*) Hagborg[11] was able to demonstrate associated melanism 12–14 days later, thus confirming the earlier observations of Smith.[12]

Environmental factors, through their effect on plant growth, even in the absence of infection can be the cause of melanism in wheat. Broadfoot & Robertson[3] found that glume melanism in two varieties was accentuated by high light intensity, while Johnson & Hagborg[13] were able to demonstrate that ear and stem melanism could be caused by conditions of high temperature especially when combined with high humidity. Probably the condition referred to as 'brown neck' in which melanism occurs immediately below the ear is also caused by a physiological disorder[14]. In at least one instance melanism has been reported as being caused by late application of hormone weedkillers.[15] Since the association between melanism and copper has been demonstrated by the authors similar effects have been found in S. E. England, again associated with organic chalk soils.[16] After examination of the pigment formed in these cases it was reported to be melanin or a closely related compound.[17] Dutch references to the increased incidence of melanism on wheat growing on copper responsive soils have already been noted; in these cases melanism was attributed to infection by *Septoria nodorum*, but the present authors suggest that at least some of the pigmentation may have resulted directly from the low copper status.

Melanin-type pigments in plants are produced by the phenoloxidase groups, for example, melanin itself is formed by the oxidation of tyrosine and subsequent polymerisation of the products. Where plants are grown in copper-deficient soils a reduction in tissue phenoloxidase activity can occur;[18] this could be expected to lessen the probability of melanism. Several workers have reported a large increase in total amino acids in plants as a consequence of mineral deficiencies, and in a review of the subject by Hewitt[19] it is shown that copper deficiency also has this effect. Although there is no reference to this in wheat, there is sufficient evidence to suggest this to be a general phenomenon in plants, and it is not unreasonable to suppose that a copper-deficient crop may contain abnormally high amounts of amino acid melanin precursors. If at the same time the phenoloxidase activity of plants was not severely reduced, conditions would be favourable for the expression of melanism. Such may be the case in the chalk soil disorders reported here, for the

TABLE VI

Grain size—1000-grain weights, g

	Cu_0	Cu_1	Cu_2	Mean
N_1	32·0	31·5	31·0	31·5
N_2	31·0	32·0	32·0	31·7
N_3	32·0	33·0	31·5	32·2
Mean	31·8	32·2	31·5	

deficiency of copper is not as acute as in cases where vegetative as well as reproductive phases of growth are curtailed.

Following this reasoning, E. J. Hewitt and the authors have attempted to reproduce blackening symptoms and in particular melanism in the variety Atle grown in sand and water culture. The treatments included a range of inadequate copper levels with both high and low nitrogen levels; the extra nitrogen was designed to increase the soluble nitrogen content of the plant and so increase the supply of melanin precursors. Superimposed on these treatments by foliar spray and in solution, were small quantities of the melanin-type pigment precursors tyrosine (T), phenylalanine (PA) and dihydroxyphenylalanine (DOPA), introduced at certain growth stages. Although symptoms of copper deficiency and of nitrogen antagonism were observed on the lower copper treatments, no melanism appeared in the above-ground part of the plants even in the presence of high temperature and humidity. However in the lower copper treatments with PA and DOPA the plant roots went black, presumably due to melanin formation. A previous unsuccessful attempt to induce melanism in wheat by the addition of T and PA has been reported,[7] but in this case the copper level used was adequate for normal growth.

Factors associated with the occurrence of blackening in wheat

Soil type and plant analysis

The soils on which severe cases of this disorder occur are the high organic-matter phase of the Icknield soil series. The high humus content of these soils gives them a loose fluffy consistence, a characteristic absent when they have been under cultivation for many years. These soils are found on the Upper and Middle Chalk formations wherever drift influence is slight. Most areas of these soils are found on the tops of the Downs—the last areas to be reclaimed. It is difficult to discover the full extent of potentially copper-responsive land but this certainly exceeds 10,000 acres in Wiltshire and there are areas affected in the adjoining counties of Dorset, Hampshire and Berkshire and also further east in Sussex. The total copper content of the soil is invariably low, 3–7 ppm in topsoil, falling to 1·2–1·5 ppm in the unweathered chalk, and the EDTA—extractable copper content is also low (0·4–1·5 ppm).

In 1965 wheat plants (variety Opal), subsequently showing severe blackening symptoms, were analysed for copper and nitrate-N at four stages during the growing season, and the results can be compared with copper-treated plants from the same site (Table VII).

The copper content of both ear and stem fell markedly during the season and both treated and untreated plants contained very low levels of copper by the end of June. The difference in copper content between treated and untreated samples is apparent at each stage. The nitrate-N levels although rising through the season do not show significant differences between treatments until September when the levels in the ear and straw samples from copper-treated plots had dropped much further than in the samples from untreated plots.

Level of N applied to the soil

Although there are several references to the increased severity of copper deficiency following extra N [20,21,22] there are no previously recorded observations of the severity of melanism being influenced by N rate.

Past cropping

In the areas where blackening occurs kale is frequently grown to be grazed *in situ*, and observations have shown the severity of the disorder to be increased by a previous kale crop. Barley yields are also reduced after kale on these soils and in a trial in 1965 a 9 cwt reduction in yield was recorded where kale, ploughed in, was compared with barley as the previous year's crop. Mitchell *et al.*[23] in Scotland have compared copper deficiency in oats following turnips and potatoes. After turnips there was an increased severity of deficiency symptoms which he related to lower levels of available copper in the soil. In the case of blackening of wheat further work is required to elucidate this.

Climate

Blackening symptoms have been observed to be more severe in wet warm seasons than in dry seasons. This may be due to the increased nitrogen uptake in such years but the direct effect of environmental conditions cannot be discounted.[13]

Variety of wheat

All the varieties of wheat commonly grown on chalk soils during the years 1961–1965 were susceptible to blackening disorders, although the degrees of susceptibility have not been compared. The varieties include the winter wheats Hybrid 46, Capelle Desprez and Champlein, and the spring wheats Atle, Atson, Jufy II and Opal. Some variation in symptom expression has been noted mainly in the development of internodal melanism which is not found in Opal.

Blackening in wheat and copper deficiency

The typical symptoms of the two disorders are quite different and there is no evidence that blackening is found in

TABLE VII

Wheat plant analysis from blackening site 1965

Treatment	Analysis	May	June		July		September	
		Stem and Leaf	Ear	Stem and Leaf	Ear	Stem and Leaf	Ear	Straw
No copper	Cu, ppm	3·7	1·5	1·6	0·7	0·9	0·6	0·8
	NO_3-N ppm	42	150	225	169	262	138	369
Soil application of copper	Cu, ppm	5·4	2·1	2·4	1·7	1·9	1·2	1·2
	NO_3-N ppm	97	161	288	189	242	69	271

areas in which copper deficiency is prevalent, although the reverse in one instance has been recorded by the authors. Accounts of deficiency[24,25] note paling and wilting of the leaves in May during vegetative growth as the first visible symptom, followed by severe stunting and impaired reproductive development resulting in small partly emerged ears, and secondary tillering. Blackening is not evident until much later, usually late July or August. The appearance of melanism has no counterpart in copper deficiency, and the delayed ripening effect produced by the dark green plant coloration is a reversal of the early ripening mentioned above. Deficiency is more severe in dry seasons whereas blackening is worse in warm wet seasons. Whereas copper deficiency occurs on peats, highly organic mineral soils, and leached sandy soils, blackening is observed on chalk rendzina soils, and as far as the authors are aware this is the first record of copper responses in crops growing in soils of limestone or chalk origin.

Both conditions occur on organic soils with low available copper content, and extra mineral nitrogen accentuates the severity of symptoms and yield-depression in both cases. Similar remedial measures are effective for both disorders, and both foliar and soil copper applications are effective in increasing yield.

In the case previously described where blackening and copper deficiency were found on the same site, blackening occurred where a copper treatment had been applied to the soil, and deficiency symptoms occurred where there was no copper treatment. This phenomenon has not been recorded since, but it indicated a close relationship between the two disorders.

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References

1. Dienst in de jaren 1931, 1932, 1940 en 1944 Wageningen, *Versl. Werkzaamh. Plantenziektenkundige*, 1931
2. Ogilvie, L., private communication, 1963
3. Broadfoot, W. C., & Robertson, H. T., *Sci. Agric.*, 1933, **13,** 512
4. Mitchell, R. L., 'Chemistry of the soil', Ed. F. E. Bear (Reinhold Publishing Corporation: New York) 1955, p. 253
5. Kannenberg, H., *Wass. Boden.*, 1957, **9,** 360
6. Polukhina, I. N., & Masljanja, H. K., *Isv. timiryasev sel'-khoz Akad.*, 1961, **1,** 205
7. Johnson, T., & Hagborg, W. A. F., *Scient. Agric.*, 1942, **22,** 746
8. Dantuna, I. G., *Technisch Bericht*, 1954, **7,** Wageningen
9. Hart, H., & Allison, J. L., *Phytopathology*, 1943, **33,** 494
10. McFadden, E. S., *Phytopathology*, 1937, **27,** 801
11. Hagborg, W. A. F., *Can. J. Res.*, 1936, 14C
12. Smith, E. F., *Pl. Dis. Surv. Bull. U.S. Dept. Agric. Bur. Pl. Ind.*, 1917, **1,** 40
13. Johnson, T., & Hagborg, W. A. F., *Can. J. Res.*, 1944, 22C (1) p. 7
14. Bull Minist. Agric., Fish Fd, No. 129 'Cereal diseases, their recognition and control', 1961 p. 38
15. Evans, S. A., *N.A.A.S. Q. Rev. M.A.F.F.*, 1961, **53,** p. 16
16. Charlesworth, R. R., private communication
17. Nicholas, D. J. D., private communication, 1963
18. Nason A. Olderwuntel H. A., & Propst, L. M., *Biochem. Biophys.*, 1952, **38,** 1
19. Hewitt, E. J., 'The essential nutrient elements: requirements and interactions', Plant Physiology, Vol. III, 1963, p. 299 (Acad. Press: New York)
20. Mulder, E. G., 'Trace elements in relation to soil conditions', *Scr. varia pont. Acad. Sci.*, 1954, **14,** 215
21. Fleming, G. A., *Ir. J. Agric. Res.*, 1961, **1,** 1, 81
22. Brown, J. C., & Harmer, P. M., *Proc. Soil Sci. Soc. Amer.*, 1950, **15,** 284
23. Mitchell, R. L., Reith, J. W. S., & Johnson, I. M. (1956), 2nd Symposium: Ánalyse des plants et problems des engrais mineraux, *VIth Int. Congr. Soil Sci. Paris*
24. Wallace, T., 'The diagnosis of mineral deficiencies in plants', 3rd Edn. (H.M.S.O.: London) 1961
25. Pizer, N. H., Caldwell, T. H., Burgess, G. R., & Jones, J. L. O., *J. agric. Sci. Camb.*, 1966, p. 66, 303

APPENDIX

Typical profile description of Icknield Series (Organic Phase)

Site: Slight slope to N. E. drainage normal. Vegetation, grass–clover ley. Parent Material, Upper Chalk. Altitude 600 ft O.D.

Horizon 1: 0–8 in. Very flinty organic silty loam with very loose strongly developed crumb structure, abundant fibrous roots. Very dark brown, 10YR/2/2. Organic matter 12%. Narrow boundary.

Horizon 2: 8–16 in. Very stony, soft chalk rubble and flint, with some brown, 10YR/4/3, silty loam between stained chalk, common fibrous roots. Merging to

Horizon 3: 16 in + white chalk rubble 7·5 YR/8/1 with few roots. Profile drainage free—very permeable.

Laboratory Methods

Copper in plant material

Copper was estimated colorimetrically with zinc dibenzyldithiocarbamate following digestion with nitric, perchloric and sulphuric acids (Andrus, S., *Analyst, London*, 1954, **79,** 547).

Copper in soil

The copper extracted from the soil by 0·05 M diaminoethane-tetra-acetic acid (disodium salt) was estimated with zinc dibenzyldithiocarbamate in carbon tetrachloride at 440 μm. The extraction time was 1 hour, with a soil/extractant ratio of 1 : 5.

Nitrate in soil and plant material

The nitrate was determined in a 10% acetic acid extractant using the 2,4-xylenol method.

SURVEY OF *o*-PHENYLPHENOL RESIDUES FOUND IN MARKETABLE CITRUS FRUIT

By ANNA RAJZMAN and A. APFELBAUM

Residues of *o*-phenylphenol (OPP) were determined in peel, pulp and the whole fruit of Shamouti and Valencia oranges, grapefruits and lemons which were treated under industrial conditions with solutions of sodium-*o*-phenylphenate (SOPP). The OPP residues found in the peel of the different species of citrus fruit varied from 5·4 to 26·1 ppm OPP. In many cases OPP residues were not detectable in the pulp, and in other cases traces and 0·4 ppm of OPP were found.

The residues in the whole fruit were found to range from 1·8 to 8·3 ppm, with 80% of the samples analysed containing less than 5 ppm. These amounts were within the tolerance limits generally established for residues of OPP in citrus fruit (10 and 12 ppm of OPP).

The SOPP concentration of the disinfectant solutions ranged from 0·24 to 0·55% SOPP.$4H_2O$ and had little effect on the OPP content of the fruits. The fruits which were treated at a pH below 11·6 usually contained more OPP than those treated at pH values between 11·6 and 12·3.

Introduction

Aqueous solutions, based on sodium *o*-phenylphenate (SOPP), have been in use for several years as a disinfectant wash of citrus fruits.[1–3] Owing to its fungicidal action this treatment reduces the potential losses of fruit caused by rot which can develop during storage and transportation.

The solutions based on SOPP, which is an easily dissociable salt of a weak acid and a strong base, contain in equilibrium the ions of *o*-phenylphenate and undissociated *o*-phenylphenol (OPP). The OPP concentration of the disinfectant solution increases with the SOPP concentration, the hydrogen-ion concentration, and the temperature. Since OPP is lipophilic, during treatment a certain quantity of it is absorbed progressively by the peel of the fruit. The amounts of OPP actually absorbed by the fruit depend on numerous factors. The absorption capacity of citrus fruit for OPP is very high and quantities in the order of several thousand ppm have been found in the whole fruit.[4]

In industrial practice, nevertheless, the actual absorption of OPP is limited by the conditions under which the citrus fruit has to be treated. OPP is phytotoxic, and the treatment with SOPP solutions can cause severe injury to the fruit. This injury can be important and diminish the market value of the fruit. The two conflicting effects of treatment—fungicidal and phytotoxic—increase (the former less than the latter) with the concentration of OPP in the solution.[5] Thus it is possible, by suitable selection of optimum conditions in regard to SOPP concentration, pH, temperature of the solution and duration of treatment, to effect the desired disinfection of fruit and at the same time to avoid the appearance of scald.

The optimum conditions of treatment, which may vary with different industrial needs[2,3] but always tend to maintain the OPP solution concentration as low as possible, delimit the absorption of OPP by the fruit.

The amounts of OPP residues allowable for marketable citrus fruit are limited in certain countries by tolerance levels, usually corresponding to 10 ppm of OPP in the whole fruit.[6] Recently, a tolerance limit of 12 ppm was established by the member-countries of the European Economic Community.[7]

Data available on OPP content of marketable citrus fruit are very rare. In view of the lack of data on OPP content of Israeli citrus fruit and the importance attached by the citrus industry to the distribution of fruit with OPP residues within the fixed tolerance limits, it appeared necessary to make a number of determinations of OPP residues in citrus fruit destined for export which had been treated by SOPP solutions.

Experimental

Fruit

The fruits analysed were Shamouti and Valencia oranges, grapefruits and lemons which were harvested during the 1966/67 citrus season in various regions of Israel. One to three days after harvest the fruit underwent treatment with SOPP solutions. The fruit was treated in 22 packing houses located in different regions of the country. The SOPP treatment used by the packing houses in Israel consists of a 2–3 min. immersion of fruit in solutions containing 0·5% sodium *o*-phenylphenate tetrahydrate at a pH of 11·8 to 12·2. The temperature used for the treatment is usually 38° but it varies between 32 and 38° depending upon the fruit. After immersion, the fruit is rinsed with water.

The maintenance of optimum conditions during treatment of fruit, especially as far as pH is concerned, presents certain difficulties, and from time to time lower pH values are noted.

The sample lots to be analysed were taken from consignments destined for export and normally consisted of 20–30 fruits. The fruit was taken at random or by design from the packing house when the pH of the disinfectant solution was below 11·8. A number of samples were taken from commercially stored fruit in which injury attributed to SOPP treatment, had appeared during storage. The fruit taken from the packing houses was analysed either one to three days after treatment or after storage of the samples at 15°.

SOPP solutions

Samples of disinfectant solutions were taken from the packing houses for subsequent determination of their pH and SOPP content.

Determination of OPP residues in the fruit

The OPP residues were determined from an average sample consisting of five fruits taken at random from the previously mentioned lot of 20–30 fruits. The residues were determined in the peel and pulp of the fruit, and then calculated for the whole fruit.

Since the eventual migration of OPP from the peel to the pulp is progressive and relatively slow,[4] the determination of OPP in the pulp of the fruit analysed one to three days after treatment was omitted.

The OPP residues in the peel and pulp were determined by a colorimetric method[8] based on the characteristic rose

coloration given by very small quantities of OPP and SOPP with sulphuric acid in the presence of traces of formaldehyde and ferric ion.[8,9]

Determination of SOPP and pH in disinfectant solutions

SOPP in disinfectant solutions was determined colorimetrically[8] and expressed as g sodium *o*-phenylphenate tetrahydrate/100 ml of solution. The pH was determined with a pH meter, the solution being maintained at 38°.

Results and Discussion

Data on OPP residues found in fruit, as well as data concerning the corresponding disinfectant solutions, have been grouped according to the species of fruit in Tables I–IV.

SOPP solutions

The solutions with which the different species of citrus fruit were treated contained between 0·24 and 0·55 g SOPP.4 H_2O per 100 ml. Of 50 samples analysed, 23 contained between 0·24 and 0·40 g SOPP.4H_2O per 100 ml, i.e. less than the prescribed quantity.

The pH of the solutions varied between 10·6 and 12·6. In 19 of 50 analysed samples the pH was below 11·8 and in six cases even below 11·6. Therefore 40% of the fruit analysed was treated at a pH below the optimum values.

OPP residues in the fruit

The values of the OPP residues found in the peels of the fruit varied with Shamouti oranges between 5·9 and 23 ppm, with Valencia oranges between 8·8 and 21·3 ppm, with grapefruits between 7·0 and 26·1 ppm and with lemons between 5·4 and 15·6 ppm.

The quantities of OPP found in the pulp of the fruit analysed after storage were very low. In many cases no residues of OPP could be detected and in others, usually when the peel contained appreciable quantities of OPP, traces of OPP (Tables I–IV) and 0·4 ppm (Table I) were found.

The OPP residues found in the whole fruit varied with Shamouti oranges between 1·9 and 8·3 ppm, with Valencia oranges between 2·2 and 4·8 ppm, with grapefruits between 1·7 and 7·7 ppm and with lemons between 1·5 and 5·8 ppm. For all the species the residues found varied between 1·5 and 8·3 ppm, i.e. they were within the tolerance limits established. About 80% of the samples (Table V), namely 53 of 65 analysed, contained less than 5 ppm OPP. In the majority of cases the fruits containing more than 5 ppm were injured by the treatment (Tables I–IV and VI).

TABLE I

***o*-Phenylphenol residues in Shamouti oranges**

No.	Packing House	SOPP treatment: Date	SOPP solution: SOPP.4H_2O g/100 ml	SOPP solution: pH	Length of storage, days	Average fruit weight, g/fruit	OPP residues: Peel, ppm	OPP residues: Whole fruit, ppm
1	A	23.11.66	0·45	11·8	0	193	10·7	3·1
2	B	3.12.66	—	—	17	200	10·3	3·4[b]
3	B	14.12.66	0·53	11·9	0	198	16·0	4·7
4	C	20.12.66	0·53	11·6	1	166	6·6	2·0
5	D	30.12.66	0·31	11·7	1	202	7·6	2·4
6	E	1. 1.67	—	—	1	186	6·3	1·9
7	F	21. 1.67	—	—	90	181	11·7	3·4
8	F	23. 1.67	0·46	12·2	7	170	6·8	2·2
9	E	2. 2.67	0·55	12·2	0	186	6·9	2·1
10	E	3. 2.67	—	—	77	165	18·4	5·4
11	C	4. 2.67	—	—	47	216[a]	19·1	7·0[c]
12	G	7. 2.67	0·44	12·3	1	193	5·9	1·9
13	G	7. 2.67	0·44	12·3	76	181	13·6	4·2
14	H	7. 2.67	0·44	12·0	1	191	6·1	2·0
15	H	7. 2.67	0·44	12·0	76	156	9·9	3·1
16	I	13. 2.67	0·37	12·2	2	253	6·0	2·1
17	J	13. 2.67	0·53	12·1	2	230[a]	12·3	4·3
18	K	13. 2.67	0·44	12·1	2	240[a]	10·9	3·7
19	I	13. 2.67	0·44	11·9	67	262	9·4	3·3
20	J	13. 2.67	0·53	11·8	73	157	15·2	4·4[b]
21	L	17. 2.67	0·53	10·9	2	217[a]	23·0	8·3
22	L	18. 2.67	0·44	11·9	65	222	11·2	3·9
23	M	20. 2.67	0·35	12·2	29	224	7·2	2·6
24	—	20. 2.67	—	—	30	194[a]	22·2	7·2[b]
25	N	7. 3.67	0·31	11·6	12	198	13·6	3·4
26	O	29. 3.67	0·35	11·5	2	202[a]	18·3	5·4
27	O	29. 3.67	0·35	11·5	2	200	7·8	2·3
Minimum			0·31	10·9	0	156	5·9	1·9
Maximum			0·55	12·3	90	262	23·0	8·3

OPP in the pulp { a – injured fruit; b – traces; c – 0·4 ppm

TABLE II

o-Phenylphenol residues in Valencia oranges

No.	Packing House	SOPP treatment			Length of storage, days	Average fruit weight, g/fruit	OPP residues	
		Date	SOPP solution				Peel, ppm	Whole fruit, ppm
			$SOPP.4H_2O$ g/100 ml	pH				
1	P	19.4.67	0·39	11·6	80	195	16·7	4·3[b]
2	P	20.4.67	0·39	12·1	29	173	9·3	2·3
3	N	22.4.67	0·44	11·4	30	188[a]	21·3	4·8[b]
4	N	22.4.67	0·44	11·4	30	207	14·3	3·3
5	N	7.5.67	0·26	11·9	62	164	8·8	2·2
6	N	7.5.67	0·48	11·6	1	164	15·6	4·1
7	O	7.5.67	0·48	11·9	1	215	13·0	3·1
8	O	7.5.67	0·48	11·9	61	190	9·6	2·7
9	R	8.5.67	0·53	11·7	0	132	14·6	4·0
10	O	9.5.67	0·26	11·7	62	196	12·4	2·7
11	N	16.5.67	0·35	12·0	51	170	12·6	3·1
12	N	18.5.67	0·31	11·6	2	172	20·7	4·2
Minimum			0·26	11·4	0	132	8·8	2·2
Maximum			0·53	12·1	80	215	21·3	4·8

a – injured fruit

b – traces of OPP in the pulp

TABLE III

o-Phenylphenol residues in grapefruit

No.	Packing House	SOPP treatment			Length of storage, days	Average fruit weight, g/fruit	OPP residues	
		Date	SOPP solution				Peel, ppm	Whole fruit, ppm
			$SOPP.4H_2O$ g/100 ml	pH				
1	S	24.10.66	—	—	3	296	7·4	2·0
2	S	26.10.66	—	—	104	240	13·4	3·4
3	C	6.11.66	—	—	3	285	7·0	2·0
4	O	12.12.66	0·39	11·3	3	360	9·5	3·2
5	O	12.12.66	0·39	11·3	132	281	13·2	3·0
6	E	18. 1.67	0·39	12·6	2	340	7·4	1·7
7	D	22. 1.67	0·46	11·9	3	380	7·0	2·3
8	H	7. 2.67	0·46	11·8	2	352	10·3	2·8
9	H	7. 2.67	0·46	11·8	76	337	12·9	3·6
10	C	10. 2.67	0·24	11·4	2	324[a]	24·0	6·7[b]
11	C	10. 2.67	0·24	11·4	72	256[a]	22·8	6·3[b]
12	T	22. 2.67	0·39	10·6	129	376[a]	19·9	5·5[b]
13	U	22. 2.67	0·44	11·8	29	362	10·3	3·1
14	V	22. 2.67	0·44	11·7	30	404	13·3	3·3
15	E	18. 3.67	0·53	11·6	24	328[a]	22·6	5·5[b]
16	T	25. 3.67	—	—	43	426	11·7	3·7
17	T	25. 3.67	—	—	43	490[a]	26·1	7·7[b]
18	T	8. 4.67	—	—	30	469[a]	23·1	7·3[b]
Minimum			0·24	10·6	2	240	7·0	1·7
Maximum			0·53	12·6	132	490	26·1	7·7

a – injured fruits

b – traces of OPP in the pulp

TABLE IV

o-Phenylphenol residues in lemons

No.	Packing House	SOPP treatment: Date	SOPP solution: $SOPP.4H_2O$ g/100 ml	SOPP solution: pH	Length of storage, days	Average fruit weight, g/fruit	OPP residues: Peel, ppm	OPP residues: Whole fruit, ppm
1	C	20.10.66	0·37	12·0	8	87	5·4	1·5
2	C	20.10.66	0·37	12·0	8	78	11·0	3·1
3	C	20.10.66	0·37	12·0	8	73[a]	11·9	4·1[b]
4	W	2.12.66	—	—	0	125	9·6	2·7
5	O	12.12.66	—	—	14	126	7·2	2·1
6	N	12. 1.67	0·30	12·1	0	127	12·0	4·0
7	O	12. 1.67	00·26	11·9	2	145[a]	15·6	5·8
8	E	22. 4.67	—	—	30	230	10·5	3·4
Minimum			0·26	11·9	0	73	5·4	1·5
Maximum			0·37	12·1	30	230	15·6	5·8

a – injured fruit

b – traces of OPP in the pulp

TABLE V

Distribution of samples of whole fruits according to their o-phenylphenol content

Fruit Species treated	Number of samples	OPP residues, ppm: Min.	OPP residues, ppm: Max.	Number of samples with an OPP content of: 1·4–3·0 ppm	3·1–5·0 ppm	5·1–7·0 ppm	7·1–8·3 ppm
Shamouti Orange	27	1·9	8·3	10	12	3	2
Valencia Orange	12	2·2	4·8	4	8	—	—
Grapefruit	18	1·7	7·7	6	6	4	2
Lemon	8	1·5	5·8	3	4	1	—
Total	65	1·5	8·3	23	30	8	4

TABLE VI

Relation between the pH of disinfectant solutions, the o-phenylphenol content of fruit and the condition of fruits

Fruit species treated	Condition of fruit	Number of samples	OPP residues according to pH of disinfectant solutions: pH <11·0 No.	pH <11·0 ppm	pH 11·3—11·5 No.	pH 11·3—11·5 ppm	pH 11·6–11·7 No.	pH 11·6–11·7 ppm	pH 11·8–12·3 No.	pH 11·8–12·3 ppm	pH 12·6 No.	pH 12·6 ppm	pH unknown No.	pH unknown ppm
Shamouti Orange	A	20	—	—	1	2·3	3	2·0–3·4	13	1·9–4·7	—	—	3	1·9–5·4
	B	7	1	8·3	1	5·4	—	—	2	3·7–4·3	—	—	3	3·4–7·2
Valencia Orange	A	11	—	—	1	3·3	5	2·7–4·3	5	2·2–3·1	—	—	—	—
	B	1	—	—	1	4·8	—	—	—	—	—	—	—	—
Grapefruit	A	12	—	—	2	3·0–3·2	1	3·7	4	2·0–3·6	1	1·7	4	2·0–3·7
	B	6	1	5·5	2	6·3–6·7	1	5·5	—	—	—	—	2	7·3–7·7
Lemon	A	6	—	—	—	—	—	—	3	1·5–4·0	—	—	3	2·1–3·4
	B	2	—	—	—	—	—	—	2	4·1–5·8	—	—	—	—
Total	A	49	—	—	4	2·3–3·3	9	2·0–4·3	25	1·5–4·7	1	1·7	10	1·9–5·4
	B	16	—	5·5–8·3	4	4·8–6·7	1	5·5	4	3·7–5·8	—	—	5	3·4–7·7

A – without peel injury

B – with peel injury

No constant relation was found between the SOPP content of the solutions and the OPP quantities absorbed by the fruit. Among the samples treated at an optimum pH (11·8–12·2) with solutions containing for example 0·26 or 0·55% SOPP.$4H_2O$, fruits containing respectively 5·8 (Table IV, No. 7) and 2·1 ppm (Table I, No. 9) were found. A certain relationship was found between the pH of the solutions and the amount of OPP in the fruits, but this was not constant. The residues of OPP in the fruits treated at a pH below 11·6 (Table VI) were usually higher than in the fruits treated at an optimum pH, but between the first and last fruits, fruits containing respectively 2·3 ppm (Table I, No. 27) and 5·8 ppm (Table IV, No. 7) were found.

It has to be noted that, in view of the small number of samples analysed, the maximum OPP content of 8·3 ppm found in the whole fruit cannot be considered to be the maximum quantity which can occur in citrus fruit treated with SOPP solutions.

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References

1. Sharma, J. N., S. African P. No. 1255/35, 1935, U.S. P. No. 2,054,392, 1936
2. Souci, S. W., *Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1966, **57**, 43
3. Eckert, J. W., & Sommer, N. F., *Rev. Phytopath.*, 1967, **5**, 391
4. Rajzman, A., & Apfelbaum, A., Unpublished data
5. Long, J. K., & Roberts, E. A., *Aust. J. agric. Res.*, 1958, **9**, 609
6. F.A.O., Committee on Commodity Problems, F.A.O. Group on Citrus Fruit, Rome, 1963, CCP/Citrus/63/3, CCP/Citrus/63/11
7. E.E.C., Directives du Conseil 27 Juin 1967, 67/427/CEE, *Journal Officiel des Communautés Européennes*, 1967, 10e année, No. 148, p. 1
8. Rajzman, A., The National and University Institute of Agriculture, Rehovot, Israel, Preliminary Report No. 334, 1961
9. Rajzman, A., *Analyst. Lond.*, 1960, **85**, 116

AMINO ACID ANALYSES OF GERMINATING SEEDS OF SOME MEMBERS OF THE LEGUMINOSAE

By V. M. JONES* and D. BOULTER

The free and bound amino acid contents of soaked seeds and 7- and 14-day old seedlings of *Pisum sativum*, *Phaseolus vulgaris* and *Canavalia ensiformis* were determined. It was found that on germination the free amino acid content of the plants increased, while the total amount of amino acid decreased. Most of the essential amino acids were shown to be present in the plants.

Introduction

Legume seeds are known to be particularly rich in protein compared with the seeds of many other species: wild varieties contain 20–30% protein by weight, while cultivated varieties may contain 50% protein.[1] Extraction and analysis has shown that much of this protein is in the form of globulins with large molecular weights and that these globulins are distinguished from other proteins by their high proportions of glutamic acid, aspartic acid, amide nitrogen and high arginine content.[2–5] Since the work of Schulze and Umlauft in the last century it has been known that the protein content of the plant decreases on germination, and the present studies attempt to find some of the overall changes in the amino acid concentrations during germination of some members of the Leguminosae.

Experimental

Samples

Seeds of *Pisum sativum* cv. Onward and *Phaseolus vulgaris* cv. Canadian Wonder, were obtained from Forizo Co., Birkenhead, and seeds of *Canavalia ensiformis* (Jack bean) were obtained from Sigma London Chemical Co. Ltd.

Method

At least 20 seeds were grown in pots of sand with added nitrogen-free culture medium,[6] for periods of 7 and 14 days under natural conditions of light and heat, after which the plant material was freeze-dried, ground to a meal in a hammer mill and weighed (see Table I). Samples of meal were estimated for water content by heating them to 105° and weighing to constant weight, and the nitrogen content was also determined.[7,8] 2 g meal were extracted with 40 ml 0·2 M-KCl at pH 7·0 at room temperature for ½ h, 20 ml 0·02 N-NaOH at room temperature for 1 h and then 20 ml 70% (vol) ethanol at 70° for 1 h. The protein was precipitated by addition of an equal volume of citrate buffer at pH 1·5, leaving it to stand for ½ h at 2° and centrifugation at 18,000 × *g*.[9] The precipitate was washed at the centrifuge in 0·1 N-HCl. Samples of the precipitate and extract were hydrolysed in 6 N-HCl at 110° for 22 h. Samples of the extract, hydrolysed extract, and hydrolysed precipitate were analysed for amino acid content using a Technicon 'Auto-Analyser'.

TABLE I

Weight of freeze-dried plants

	Pisum sativum	*Canavalia ensiformis*	*Phaseolus vulgaris*
Seed	0·303	1·24	0·44
7 day-old plant	0·270	1·16	0·39
14 day-old plant	0·236	0·96	0·34

Results

A preliminary experiment using a standard amino acid mixture showed the 'AutoAnalyser' results to vary from one analysis to another by less than ±5%, except for methionine estimations, which showed a larger variation (±10%).

When different batches of seeds of *Pisum sativum* were extracted, hydrolysed and analysed, aspartic acid, serine, glutamic acid, alanine, valine, cystine, isoleucine, and leucine showed a variation of less than ±5% in replicate analyses; of the other amino acids, all except proline showed a variation of less than ±10%.

In the analyses the total amounts of the amino acids were obtained by adding together the figures obtained for the hydrolysed precipitate and the hydrolysed extract. In the 3 species studied the amount of free amino acid increased in the 14 days after germination, while the total amount of amino acid, including free and bound forms, decreased. Tables II–IV list the amounts of the main amino acids which were identified—other amino acids occurred in amounts too small to determine, and these were not identified.

The changes in individual amino acids were not always consistent through the species, but in all the species the total amounts of tyrosine, phenylalanine, lysine and arginine decreased over the period studied, while aspartic acid increased (this value may also include some amide). The total amounts of glutamic acid, glycine, serine, alanine and threonine remained virutally constant. In all the species the relative amounts of different amino acids were similar. In the ungerminated seed glutamic acid which will include any hydrolysed amide was the amino acid present in the greatest concentration. By the fourteenth day aspartic acid which may also include some amide was the predominant amino acid. Arginine, lysine and leucine were always present in large proportions of the total amino acid content. Cystine (perhaps also containing a component of cysteine oxidised by

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TABLE II

Amino acid content of *Pisum sativum*
(mg nitrogen/plant)

Amino acid	Seed		7 day-old plant		14 day-old plant	
	Free	Total	Free	Total	Free	Total
Cysteic	0·002	0·002	0	0	0	0
Aspartic	0·023	0·917	0·048	1·164	0·256	1·408
Threonine	0·027	0·438	0·156	0·260	0·334	0·230
Serine	0·038	0·541	0·106	0·495	0·217	0·378
Homoserine	0	0	0·078	0·078	0·235	0·235
Glutamic	0·083	1·531	0·206	1·215	0·115	0·630
Proline	0·006	0·293	—	0·072	—	—
Glycine	0·029	0·998	0·036	0·483	0·069	0·392
Alanine	0·016	0·747	0·102	0·486	0·157	0·465
Valine	0·005	0·481	0·059	0·312	0·148	0·352
Cystine	0·001	0·074	0	0·044	0	0·122
Methionine	0·001	0·062	0·011	0·021	0	0·065
Isoleucine	0·003	0·380	0·022	0·199	0·088	0·234
Leucine	0·033	0·671	0·027	0·425	0·069	0·315
Tyrosine	0·005	0·235	0·021	0·146	0·039	0·112
Phenylalanine	0·004	0·360	0·026	0·246	0·089	0·224
γ-Aminobutyric	tr	tr	tr	tr	0	0
Ethanolamine	tr	tr	0·051	0·051	0·225	0·225
Ornithine	0	0	tr	tr	tr	tr
Lysine	0·109	1·528	0·092	0·789	0·138	0·730
Histidine	0·012	0·585	0·125	0·519	0·274	0·466
Arginine	1·182	3·250	1·262	2·125	1·571	2·425
Ammonia	0·059	1·640	0·627	2·250	0·979	3·262
Total	1·549	14·723	3·055	11·380	5·003	12·270

tr = trace
— indicates that the amino acid was not estimated

TABLE III

Amino acid content of *Canavalia ensiformis*
(mg nitrogen/plant)

Amino acid	Seed		7 day-old plant		14 day-old plant	
	Free	Total	Free	Total	Free	Total
Cysteic	0·007	0·007	0·009	0·010	tr	tr
Urea	0	0	0·519	0·520	tr	tr
Aspartic	0·074	3·460	0·126	3·430	0·825	5·042
Threonine	0·075	1·628	0·639	1·630	2·580	1·216
Serine		2·493	0·318	2·860		1·889
Glutamic	0·103	3·175	0·349	3·850	0·061	2·083
Proline	—	1·325	0·062	0·670	—	0·223
Glycine	0·017	1·995	0·057	1·890	0·144	1·414
Alanine	0·018	1·894	0·154	2·170	0·374	1·451
Valine	0·006	1·515	0·144	1·200	0·417	1·403
Cystine	0	0·097	0	tr	0·055	0·096
Methionine	0·009	0·258	0·023	0·020	0·017	0·075
Isoleucine	0·003	1·274	0·071	0·860	0·220	0·928
Leucine	0·004	2·470	0·055	2·200	0·193	1·372
Tyrosine	tr	1·031	0·046	6·280	0·109	0·349
Phenylalanine	0·013	3·077	0·060	1·020	0·149	0·666
α-Aminobutyric	0	0	tr	tr	tr	tr
Ethanolamine	0	0	tr	tr	tr	tr
Ornithine	tr	tr	0·112	0·110	0·319	0·322
Lysine	0·016	3·077	0·051	2·910	0·222	1·901
Histidine	0·020	2·005	0·337	2·070	0·889	1·877
Canavanine	6·060	8·830	6·400	8·520	5·450	5·850
Arginine	0·091	4·615	0·301	3·710	0·350	2·647
Ammonia	0·920	10·780	0·521	6·130	1·750	17·161
Total	7·436	55·010	10·354	52·060	14·124	47·960

TABLE IV

Amino acid content of *Phaseolus vulgaris*
(mg nitrogen/plant)

Amino acid	Seed		7 day-old plant		14 day-old plant	
	Free	Total	Free	Total	Free	Total
Cysteic	0·004	0·004	0	0	0·002	0·002
Urea	0·279	0·280	0	0	0	0
Aspartic	0·205	1·211	0·094	1·218	0·159	2·350
Threonine		0·505	0·011	0·447	0·287	0·457
Serine	0·032	1·080	0·085	0·796	0·273	0·774
Glutamic	0·056	1·567	0·128	1·486	0·063	0·595
Proline	—	0·270	—	0·436	—	tr
Citrulline	0·012	0·012	0·066	0·066	0·028	0·028
Glycine	0·002	0·681	0·022	0·632	0·039	0·381
Alanine	0·001	0·765	0·078	0·640	0·198	0·525
Valine	0	0·496	0·033	0·448	0·391	0·555
Cystine	0	0	0	0·051	0	0·015
Methionine	0·001	0·005	0·006	0·027	0·036	0·086
Isoleucine	0·005	0·388	0·020	0·286	0·340	0·453
Leucine	0·040	0·875	0·031	0·756	0·344	0·627
γ-Aminobutyric	0·004	0·004	0	0	0	0
Tyrosine	0·002	0·216	0·009	0·215	0·080	0·155
Phenylalanine	0·004	0·471	0·023	0·432	0·136	0·227
α-Aminobutyric	0	0	0·005	0·005	0·015	0·015
Ethanolamine	0	0	0·004	0·004	0	0
Ornithine	tr	tr	tr	tr	0·008	0·008
Lysine	0·011	1·230	0·028	1·080	0·071	0·604
Histidine	0·076	0·790	0·101	0·706	0·802	0·861
Arginine	0·402	2·117	0·189	1·292	0·807	1·393
Ammonia	0·287	3·097	0·126	2·377	0·292	6·455
Total	1·420	16·060	1·060	13·400	4·370	16·570

the extraction and analytical procedures), methionine, cysteic acid, citrulline, γ-aminobutyric acid and ornithine, when present, were present in small quantities.

Homoserine was found only in *Pisum* and then only after germination. Canavanine was found only in *Canavalia* and was present in decreasing amounts the older the plant material was.

Discussion

Analyses of the seeds and seedlings showed that most of the nitrogen present was in the form of amino nitrogen. Assuming that on average proteins and amino acids contain 16% nitrogen, this would indicate that the seeds of *Canavalia ensiformis* contained approximately 35% protein or amino acid, *Phaseolus vulgaris* contained approximately 23%, and *Pisum sativum* contained 30%. These concentrations rose slightly as the seeds germinated because the freeze-dried plants lost weight with germination, while the nitrogen content remained virtually constant. When the weight of protein and peptides precipitated was considered, it was found that these remained steady or rose during the first 7 days of germination but had decreased by the fourteenth day in all the species.

Citrate buffer did not precipitate all the bound amino acids present, as on hydrolysis of the extract after protein precipitation, an increase was noted in the amino acids estimated on the 'AutoAnalyser'. This increase is included in the figures for the total amino acid content of the seeds and plants in this work. However, although citrate buffer is not the most efficient protein precipitant available, acetone and picric acid being more efficient, its choice depended upon the fact that it gives higher yields of free amino acids.[9] The analyses showed that the free amino acid content of the seeds and plants rose over the first 14 days of germination in all three species.

In all the seeds and seedlings, glutamic acid, aspartic acid, and leucine were present in large quantities in both the free and bound forms. The nitrogen-rich amino acids, arginine and lysine, were both present in high concentrations in the seed but decreased during germination. *Canavalia ensiformis* also contains the nitrogen-rich amino acid canavanine. This was not found in the protein precipitate, but the amount detected was found to increase slightly on hydrolysis of the extract supernatant; this suggests that canavanine is present not only in the free form, but also in some bound form such as a small peptide. The sulphur-containing amino acids, methionine and cystine, were present in the species studied but only in trace amounts in the free state. Their occurrence in the bound form shows no well defined pattern except that methionine increased between the seventh and fourteenth days of germination in all the species. Tryptophan was not detected in any of the analyses, but this may be due to its breaking down during the preparation and analyses of the extracts. The aromatic amino acids phenylalanine and tyrosine were present mainly in the bound form, and there was a decrease in these over the period studied. The essential amino acids isoleucine, valine and threonine were also present in the species; the former two showed a rise over the period studied while threonine decreased. *Pisum sativum* produced the amino acid homoserine on germination. It was not found in the seed, but by the fourteenth day of germination it represented one of the free amino acids in greatest concentration. It was only found as the free amino acid.

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References

1. Brohult, S., & Sandegren, C., 'The Proteins', 1954, Vol. 2A, p. 487 (New York: K. Acad. Press)
2. Tristram, G. R., & Smith, R. H., *Adv. Protein Chem.*, 1963, **18**, 227
3. Gerritsen, T., *Biochim. biophys. Acta*, 1956, **22**, 269
4. Grant, D. R., & Lawrence, J. M., *Archs Biochem. Biophys.*, 1964, **108**, 552
5. Werner, G., Jaffe, Hannig, K., *Archs Biochem. Biophys.*, 1965, **109**, 80
6. Folkes, B. F., Willis, A. J., & Yemm, E. W., *New Phytol.*, 1952, **51**, 319
7. Baker, P. R. W., *Tantala*, 1961, **8**, 58
8. Conway, E. J., 'Microdiffusion analysis and volumetric error', 1957, (London: Crosby, Lockwood)
9. Gerritsen, T., Rebberg, M. L., & Waisman, H. A., *Analyt. Biochem.*, 1965, **11**, 460

ERRATA

In the paper by Pianka, *J. Sci. Fd Agric.*, 1968, **19**

Page 476 Compound 12 *for* '5-Chloromethyl-2-isopropylthio-1,3,4-thiadiazole'
read '5-Chloromethylthio-2-isopropylthio-1,3,4-thiadiazole'

Page 504 Compound 16 *for* '2-Butoxymethylthio-4*H*-1,3,4-thiadiazolinethione'
read '2-Butoxymethylthio-4*H*-1,3,4-thiadiazoline-5-thione'

Page 510 Compound 17 *for* '2-(*p*-Nitrophenyl-4*H*-1,3,4-oxadiazoline-5-thione'
read '2-(*p*-Nitrophenyl)-4*H*-1,3,4-oxadiazoline-5-thione'

Page 512 Table X title *for* '2-(2-furyl-4*H*-1,3,4-oxadiazoline-5-thione'
read '2-(2-furyl)-4*H*-1,3,4-oxadiazoline-5-thione'

In the paper by Ranken & Shrimpton, *J. Sci. Fd Agric.*, 1968, **19**

Page 613 left hand column, line 3 of 5th paragraph:

for 'Table I shows that approximately 1% of the carcasses had Probe Readings $\geqslant 80$'
read 'Approximately 1% of the carcasses had Probe Readings $\geqslant 80$'

The table referred to was deleted in the revision of the paper

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

DECEMBER, 1968

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Basaltic soils of Bombay Deccan. R. V. Tamhane and R. L. Karale (*J. Indian Soc. Soil Sci.*, 1967, **15**, 269–279).—Chemical, physical and mineralogical characteristics of these soils are recorded and discussed in relation to their genesis and classification. A. G. POLLARD.

Influence of deforestation and agricultural utilisation on the morphology and properties of chernozem in areas of varying relief. J. Borowiec (*Annls Univ. Mariae Curie-Sklodowska, Agric.* E, 1966, **21**, 83–103).—Studies were carried out on two slopes with max. inclinations of ~22% in a district where chernozem (black fertile) soils prevailed. One slope was afforested while the other was deforested at least 130 years ago and has since been under cultivation; the slopes were only 4 km apart and thus experienced identical atm. and climatic conditions. The thickness of the top soil was measured at 25 m intervals and 28 samples of soil were examined for physical properties, mechanical composition, pH, and the contents of $CaCO_3$, humus (org. C), N, P and K. The results indicate that (1) in spite of considerable inclination, no distinct effect of erosion in the forest area was observed, (2) in cultivated areas there was great differentiation of the thickness of the top soil due to the influence of erosion and cultivation practices, (3) properties of the soil in both areas were distinctly different. (32 references.) T. M. BARZYKOWSKI.

Use of isotopic methods for determination of physical properties of soils in establishing their geotechnical cross-sections. M. Jakubowski and J. Sapula (*Nukleonika*, 1968, **13**, 412–429).—Isotope probes were used to determine *in situ* properties of soils such as bulk density and natural moisture, necessary data for the design and construction of water dams. Determination of bulk density is based on defining the intensity of γ-radiation dispersed in the examined medium, and determination of the moisture is based on defining the intensity of moderated and dispersed neutrons; this latter intensity is a function of H content per unit vol. of examined soil. Probes for use in cased boreholes of 1·5—2-in. dia. were home-made, but owing to their defective operation, the method is still in the experimental stage. T. M. BARZYKOWSKI.

Range of nuclear methods for measuring soil moisture and density. J. A. Czubek (*Nukleonika*, 1968, **13**, 517–533).—Three different definitions of the range of nuclear investigations are given, viz., the %, effective and standard error ranges. Calculations were made for the neutron method of moisture measurements, using different approximations which gave different values for the range of this method. However, practically all the neutrons detected came from the range equal to three migration lengths. To obtain this value for a sand/water mixture, all up-to-date experimental values were compared with the theoretical ones. At the same time the results given by different authors for the moderation length in sandstone/water and limestone/water were summarised. Considering experimental results of various authors for the γ–γ method for density measurement, the range of 50g/cm² for the case when practically all scattered γ quanta measured by the detector are taken into account, was obtained. (26 references.) (From English summary.) T. M. BARZYKOWSKI.

Effect of muriate of potassium on fixed ammonium in the soils of Madhya Pradesh. S. S. Chauhan and D. Singh (*J. Indian Soc. Soil Sci.*, 1967, **15**, 257–259).—Fixation of K by the black and alluvial soils lowered the amount of naturally fixed NH_4^+; the amount of K fixed and the associated release of fixed NH_4^+ increased with time and with the amount of K^+ added. The pH of the soils increased with increase in K^+ fixed whereas with NH_4^+ fixation the reverse relationship obtained. Max. fixation of added NH_4^+ occurred in 1 month. A. G. POLLARD.

Potassium fixation in some soils of Punjab, Haryana and Himachal. J. S. Grewal and J. S. Kanwar (*J. Indian Soc. Soil Sci.*, 1967, **15**, 237–244).—To 23 soils examined K was added in various proportions (K_2O, 0–2000 ppm) as aq. KCl (1:1 soil:solution). Water-sol. and exchangeable K were later removed by leaching with N-NH_4OAc (pH 7·0). Added K remaining in the soil after 24 h, and regarded as fixed, ranged from 22·3 to 260·6 ppm in accordance with the amounts added. Fixation was increased by alternate wetting and drying the soil. The capacity of the soils to fix K was diminished by presence of other exchangeable cations in the order Na > Ca > Mg > H > NH_4 and increased with rise in pH 4 to 10. Fixation was relatively rapid, nearly 90% occurring in 24 h and equilibrium being reached in 7 days. A. G. POLLARD.

Accumulation of nitrite in fresh soils after gamma irradiation. P. A. Cause and D. V. Crawford (*Nature, Lond.*, 1967, **216**, 1142–1143).—Reports and discusses the rapid increase of NO_2^- with corresponding decrease of NO_3^- in Broad and Ickniel soils, as revealed by extraction ~7 days after irradiation (5×10^4—$2{\cdot}5\times10^6$ rad). In Broad soil the concn. of nitrite-N increased rapidly from ~13 ppm at 8 h to ~30 ppm at 64 h after irradiation with 8×10^5 rad. The NO_2^- in the filtered extracts are quite stable up to 5 h at 20° and their concn. is much increased when soil of high water-content is irradiated. Evidence confirms that nitrite formation arises mainly from nitrate reduction. In some soils there is greater radiation damage to nitrification (by bacteria) compared to nitrate reduction so that the latter is dominant before being inhibited by increasing irradiation dose. The influence of micro-organisms is briefly noted. W. J. BAKER.

Inhibitory effects of sods on nitrification. S. S. Brar (*Diss. Abstr.* B, 1967, **27**, 2963).—Comparison was made of nitrification in continuously cultivated soils with that in adjacent soils which had been under grass for various periods. Incubation tests (soil+grass roots) in an atm. of O_2+He formed the basis of comparison. Air-drying the soil samples prior to incubation did not affect nitrification in Cecil soil (B) but did so in Bladen soil (A); in the latter NO_3^- production was greater after air-dry storage than when kept in a moist condition before incubation. Coastal Bermuda-grass depressed nitrification and mineralisation in the older sods whereas fescue stimulated the accumulation of NO_3^-. Much of the N in the org. matter of sods existed as amino-N. Living roots of grasses may cause O_2-deficiency in the rhizosphere and thus depress nitrification. Root excretions may provide H-donors to the denitrifying organisms. Under conditions of O_2-deficiency NO_3^- was superior to NO_2^- as a source of O_2. The no. of nitrifying organisms in soil A was low but could be increased by liming and incubation after inoculation. Nitrifying organisms from soil B needed a longer lag period to become established on soil A than did those developed in soil A. Nitrification was not stimulated by Mo. A. G. POLLARD.

Performance, transformation and movement of urea in acid soils. H. Sinha and K. Prasad (*J. Indian Soc. Soil Sci.*, 1967, **15**, 281–287).—Wheat and paddy were used in pot experiments with upland, intermediate and lowland soils. Growth, yields and N contents of the plants increased with the level of urea applied to the soils. The transformation of urea to NO_3^- was largely completed in 4 weeks. Rates of ammonification in the early stages were highest in lowland and min. in upland soils; the reverse relationship developed during later growth. The N transformation was retarded by the heavier application of urea. Leaching experiments showed that urea-N was present mainly in the upper 10 cm of soil columns, its retention in soil being largely dependent on the rate of conversion into NH_4^+. A. G. POLLARD.

Soil temperature control for field plot studies. N. M. Macleod (*Diss. Abstr.* B, 1967, **27**, 2952–2953).—The system of temp. control in field soils is based on heat exchangers placed at a depth of 3 in. and operated by sources of chilled water, electric immersion heaters, thermostatic controls, thermocouples and temp. recorders. With orchard-grass as test crop, responses to 50°, 70° and 90°F, lighting, fertility and cutting heights were observed. The effect of temp. on growth varied with the season, optima being 90°F in spring and 50°F in summer. Each temp. treatment produced an individual environment in soil, but had very little effect on the aerial environment. A. G. POLLARD.

Retention of zinc by soils as related to mineralogy and extraction methods. W. L. Hoover (*Diss. Abstr.* B, 1967, **27**, 3023).—In soils of 18 different types, the total Zn content was not indicative of the Zn status. Extraction of soil-Zn by 1% EDTA or by a solution of 0·1M–$CuSO_4$ and 0·1N-HCl, yielded results closely correlated with the Zn status of leaves of sorghum grown in the soils. In non-calcareous, but not in calcareous soils 0·1N–HCl effectively extracted Zn in proportions which were of diagnostic value. Water-sol. Zn was low even in soils treated with Zn at 500 ppm followed by incubation for 30 days. Fixation of Zn probably resulted from the formation of $Zn(OH)_2$ or $ZnCO_3$. Approx. a half of such fixed Zn was removed by 1% EDTA or the $CuSO_4$–HCl extractant. The non-available portion of Zn in soil occurred mainly in the clay fraction. Of five soils examined, four retained > 66% of their total Zn after destruction of their carbonates and org. matter, followed by 14 successive extractions with 0·1N–HCl. The amount of Zn held by soil org. matter is probably very small. The mineralogy of soil clays had less influence on the reactions of soil-Zn than did pH; the controlling factor was whether the soil was or was not calcareous. When soil carbonates were removed the equilibrium pH controlled the amount of extractable Zn present; with equilibrium pH > 5, $Zn(OH)_2$ and $ZnCO_3$ become increasingly influential.
A. G. POLLARD.

Movement of copper, molybdneum and selenium in soils as indicated by radioactive isotopes. G. B. Jones and G. B. Belling (*Aust. J. agric. Res.*, 1967, **18**, 733–740).—Radioactive tracer studies show that Cu is strongly retained near the surface of soils of moderate exchange capacity (*EC*) following fertiliser treatments and the equivalent of several years rainfall, but with soils of low *EC* some penetration of Cu occurs. A high proportion of Mo is usually readily leached through the soil except in the case of some laterite soils where up to 50% may be retained near the surface. Se, applied as Na_2SeO_3, was intermediate in behaviour between Cu and Mo, i.e. calcareous soils showed considerable retention for this element, but not necessarily at the surface. (11 references.)
J. L. WALPOLE.

Selectivity of erosion processes with respect to soil phosphorus in the alluvial tracts of Uttar Pradesh. R. N. Gupta and N. Singh (*J. Indian Soc. Soil Sci.*, 1967, **15**, 261–268).—Losses of different forms of soil P during the erosion of soils differing in degree and length of slope are examined. Losses were greatest from Ca-bound P and least from Al-bound P and Fe- and Al-occluded P. Humus lost in the run-off of the steeper slopes was relatively poorer in P, whereas in that from longer and less steep slopes the P content tended to increase. Except in the case of Al-bound and Fe- and Al-occluded P the relationship between loss of P and characteristics of slopes was almost linear.
A. G. POLLARD.

Plant nutrition studies: Soil nitrogen. Anon. (*A. Rep. Exp. Stn S. Afr. Sug. Ass.*, 1966–7, 69–72).—Loss of N by leaching may occur in sandy soils which can be serious for young cane plantings, and nitrate-N is more liable to loss than ammonium-N. Results obtained from the use of a nitrifying inhibitor ('N-Serve') were conflicting.
J. L. WALPOLE.

Mineral nutrition. Anon. (*A. Rep. Exp. Stn S. Afr. Sug. Ass.*, 1966–7, 77–79).—A nutrient survey of sugar-cane on Table Mountain sandstone soils showed that the only important deficiency among the major elements was of K and the only widespread minor element deficiency was of Zn. A rapid spectrometric procedure has been developed for the determination of Al in NH_4OAc soil extracts.
J. L. WALPOLE.

Effects of pH, calcium carbonate, texture and organic matter on the availability of manganese [in soils]. B. R. Tembhare and M. M. Rai (*J. Indian Soc. Soil Sci.*, 1967, **15**, 251–256).—The available (water-sol.+exchangeable) Mn contents of 54 soils examined varied from 1·2 to 10·3 ppm, the amounts increasing with decrease in clay contents of the soils but without any apparent significant relationships. The pH and the available Mn contents of the soils showed a significant negative relationship. With increasing proportions of $CaCO_3$ the available Mn diminished; the positive relationship between the org. matter content of the soil and the level of available Mn was not significant.
A. G. POLLARD.

Suitable chemical method for determination of available phosphorus for paddy growing soils of Burma. M. Thant and K. Win (*Un. Burma J. Life Sci.*, 1968, **1**, 4–11).—Use of one isotopic and five chemical extraction methods on 124 soils (pH 4·6–9·2) showed that results from Olsen's method (*Analyt. Abstr.* 1954, **1**, 2851) had a closer correlation than those from the other methods with results from the isotopic method ('A value'). Olsen's method is suitable for routine determinations. (16 references.)
P. S. ARUP.

Availability of nitrogen to plants in acid coal-mine spoils. S. M. Cornwell and E. L. Stone (*Nature, Lond.*, 1968, **217**, 768–769).—The total-N content of black pyritic shales (< 2 mm fraction, pH in water 2·8–3·9) averaged ~ 0·5% (by Kjeldahl digestion) and NH_4-N ranged from 5–12 ppm. Aq. percolates from heaps weathered for 11 months were high in Al^{3+}, NH_4^+ and SO_4^{2-}. Heaps of sandstones and grey calcareous shales were much lower in available N and sol. salts. Wastes containing little oxidisable S, or having high Ca+Mg/S, undergo negligible acid weathering of silicate lattices and hence release very low concn. of N. The N content of *Betula populifolia* foliage growing on the waste heaps was max. (2·5%) for the black, acid shales.
W. J. BAKER.

Salinity test for soil testing kits. M. S. Khera (*Fertil. News.* 1967, **12**, No. 11; 22–23).—A simple, cheap and convenient kit test for checking soil salinity is described. Test for chlorides: 5 g of soil are shaken vigorously for 3 min. with 10 ml of water and filtered; 3 drops of the filtrate are diluted to 4 ml and 3 drops of 5% aq. $AgNO_3$ are added. The white turbidity developing after 10–15 sec. shaking is matched against standards. Test for sulphates: 0·7 ml of the extract and 0·25 mg of $BaCl_2$ are diluted to 4 ml and shaken well; after 20 to 30 sec. the turbidity is matched against standards as above.
I. DICKINSON.

Potassium nitrate production by a molten salt technique. R. W. Pfeiffer, V. J. DiFranco and L. F. Albright (*J. agric. Fd Chem.*, 1967, **15**, 949–953).—The production of KNO_3 from KCl was studied in laboratory experiments in which HNO_3 was bubbled through molten mixtures (150–385°) of nitrates and chlorides of K, Na, and Li, continuously fed with KCl. Conversion of KCl to KNO_3 was 23–61%, the best results being obtained at low temp. and with max. concn. of HNO_3. The exit gases were Cl_2, NO_2, and NOCl. A flow diagram of a proposed process is presented. A yield of 90% is expected under optimum conditions. (12 references.)
P. S. ARUP.

Fire hazards of ammonium nitrate–sulphur systems. C. M. Mason, D. R. Forshey and F. J. P. Perzak (*J. agric. Fd Chem.*, 1967, **15**, 954–966).—The sensitising of NH_4NO_3 to shock by S (3%) was studied. The stimulus, crit. dia., and temp. required to detonate the mixture were much higher than those required to detonate commercial explosives. Additions of 20% of KCl or triple superphosphate to the mixture decreased the sensitivity but mixtures containing Cl^- were more sensitive than those without. The possibilities of transition to detonation under exposure to fire are considered.
P. S. ARUP.

Advice on using fertilisers 1861–1967. G. W. Cooke (*Jl R. agric. Soc.*, 1967, **128**, 107–124).—A review. (27 references.)
E. G. BRICKELL.

Liquid fertilisers. N. H. Pizer (*Jl R. agric. Soc.*, 1967, **128**, 26–37).—A review of fertilisers made from NH_3, NH_4NO_3, urea, $(NH_4)_2HPO_4$ and KCl is presented, covering properties, interactions with soils, and field experiments in the U.K. (17 references.)
E. G. BRICKELL.

Value and valuation of fertiliser residues. G. W. Cooke (*Jl R. agric. Soc.*, 1967, **128**, 7–25).—Large residues accumulated in soil from long periods of manuring with phosphate and/or potash fertilisers are useful to crops. Changes in residual values with time, given in current tables, are wrong for most individual fields yet no better compromise allows for phosphate and potash in a scheme of the kind now used. There may be a good case for recompensing farmers producing favourable changes in cropping potential through long term manuring. (35 references.) E. G. BRICKELL.

Agronomic evaluation of nutrients in fertilisers. G. L. Terman (*Fertil. News*, 1967, **12**, No. 9, 24–27).—Principles and their application to the evaluation of nutrients in various fertilisers are described. Three types of evaluation are discussed: (*a*) comparative effectiveness of a fertiliser per unit of specific plant nutrient, (*b*) product evaluation and (*c*) toxicity to seedling growth. (10 references.)
I. DICKINSON.

Behaviour of different magnesium fertilisers in soil. G. Hubert and G.-J. Ouellette (*Naturaliste can.*, 1967, **94**, 727–734).—The availability of Mg from five sources to potatoes or lucerne (on a gravel soil) ranked in the (descending) order: Epsom salt, Sul-po-mag, dolomite (I), brucite, and asbestos (II) tailings. I was fairly efficient for potatoes in soil at pH 5·0 but not for lucerne in soil at pH 5·7; the II was of little use. The critical foliar Mg level was 1000 ppm for lucerne and 2500 ppm for potatoes. (18 references.)
P. S. ARUP.

Relative efficiency of phosphatic fertilisers on paddy. M. Thant and A. Khin (*Un. Burma J. Life Sci.*, 1968, **1**, 1–3).—$(NH_4)_3PO_4$

was 33 and bone meal 19% more efficient than superphosphate for rice cultivation on soil rich in N. Farmyard manure and rock phosphate were less efficient than superphosphate, but much cheaper. P. S. ARUP.

Liming of rice soils. T. P. Abraham and O.P. Kathuria (*Fertil. News*, 1967, **12**, No. 11; 9–13).—The effects of lime on different soils and in different areas of India are discussed. Data available from the National Index of Field Experiments published by the Indian Council of Agricultural Research are used. It is concluded that rice crop responds to liming only in the highly acidic soils and when high doses of lime are applied. I. DICKINSON.

Fertiliser use studies. Anon. (*A. Rep. Exp. Stn S. Afr. Sug. Ass.*, 1966–7, 48–56).—Growth response curves and yield data for sugarcane are given for phosphate, N and limestone treatments. An experiment with four commercial varieties of cane at three levels of fertiliser application has shown no significant evidence for an interaction between fertiliser and variety. J. L. WALPOLE.

Availability of various fractions of urea–formaldehyde. G. C. Kaempffe and O. R. Lunt (*J. agric. Fd Chem.*, 1967, **15**, 967–971).—The fertilising effect of the fractions was tested by responses of N-deficient grass. The effect of the cold water-sol. fraction was nearly the same as that of NH_4NO_3, the response being slightly delayed in comparison. Mineralisation of the hot water-sol. fraction occurred at 15% per week during the first week, and dropped to 1·5% per week after 4–6 months. The hot water-insol. fraction was mineralised at the rate of 10% per year; its availability was increased somewhat by actively-growing grass owing to the stimulation of microbial activity. P. S. ARUP.

[Production of] fertilisers and agricultural stock feed [from excrement]. Hydraulic Developments Ltd. (Inventor: M. G. G. Roblin) (B.P. 1,083,611, 4.3 and 30.10.63).—Excrement (poultry), is dried by heating under controlled conditions until it is in the form of granular material with a moisture content of < 15 (< 10) wt.-%. It is then heated so that it is dried rapidly to form a crust enclosing wetter excrement, the whole being simultaneously stirred and cut, or otherwise mechanically worked with a comb having prongs extending downwards into the excrement supported on a heated base; this keeps the mass in an open friable condition and prevents it binding, or burning, and ensures the crust being broken and mixed with the wetter interior. The product, which contains $P_2O_5 \nless 10$ and $N \nless 4\%$ is used as a fertiliser or, after re-fortification, as agricultural stock feed. J. M. JACOBS.

Plant Physiology, Nutrition and Biochemistry

Rôle of carotenoids in photosynthesis of green plants. H. Lundegårdh (*Nature, Lond.*, 1967, **216**, 981–985).—Detailed analysis of conditions and activities in isolated chloroplasts (from spinach leaves) reveals that β-carotene (**I**) is responsible for the photic reduction of triphosphopyridine nucleotide (**II**) during the photosynthetic cycle of electron transfer and energy conversion, and that other proposed hypotheses are invalid. Confirmatory experiments were (1) spectrophotometric recording of ferredoxin (**III**) and **II** reductions, (2) action of oxidants, including effect of $K_3Fe(CN)_6$ on the oxidation of cytochrome *f* (**IV**) to chlorophyll by an electric flash (3) time course of the excitations as revealed by oscillography up to ~ 420 m sec (instantaneous oxidation of **IV** at 554 nm and reduction of **II** at 340 nm is followed by an after-effect lasting up to 150 m sec before the original 75% reduction of **IV** is restored), (4) *in vitro* reduction of **III-II** by **I** (5) effect of pre-illumination with different colours on O_2 production. All the data clearly show the irrelevance of certain objections against the view that **I** is an active promoter of system I. Strong photosynthetic production of O_2 in red light during shorter periods is ascribed to an independent activity of system II at the expense of stored reduced-**II** and by a moderate activity of system I in red light. W. J. BAKER.

Carbon dioxide compensation in members of the *Amaranthaceae* and some related families. E. B. Tregunna and J. Downton (*Can. J. Bot.*, 1967, **45**, 2385–2387).—Measurements carried out on 33 species and varieties of the *Amaranthaceae* and related families showed them all to fall into two categories: those with CO_2 compensation of about 5 ppm and those with compensation of about 50 ppm at 21% O_2 and proportional to the O_2 concn. J. L. WALPOLE.

Soil physical conditions of winter and the growth of ryegrass plants. II. Effects of soil atmosphere. M. W. Gradwell (*N.Z. Jl agric. Res.*, 1967, **10**, 425–434).—Pot trials are reported. Plants raised in soil flushed with air weighed more and had more leaves and tillers than those in soil flushed with N_2. Overall the growth of roots is not prevented, though it may be slowed, by levels of soil O_2 represented by a flux of 0·75 g cm^{-2}.$min.^{-1}$ to the Pt micro-electrode of Lemon and Erickson (*Soil Sci.*, 1955, **79**, 383–392). Reduction of top growth by this level of soil aeration appears much smaller for plants with established root systems than for seedlings. E. G. BRICKELL.

Distribution of plant roots in soil. R. Scott Russell and F. B. Ellis (*Nature, Lond.*, 1968, **217**, 582–583).—A quant. estimate of the distribution is obtained by injection of ^{86}Rb into the shoots or leaves and the concn. of ^{86}Rb in the ambient soil is measured ~ 24 h later. Reproducibility is ~3% and 1μCi of ^{86}Rb gives ~ 2300 counts/min. Results for barley-plant roots grown in rows 15 cm apart are discussed in terms of errors in sampling and analysis. The method is superior to injection of ^{32}P (*Can. J. Bot.*, 1965, **43**, 1359) because only low concn. (~ 12μCi) of tracer are needed and a larger soil sample (2·5–3 kg) can be used. W. J. BAKER.

Sodium and potassium uptake by barley roots. M. G. Pitman (*Nature, Lond.*, 1967, **216**, 1343–1344).—Conflicting results reported in the literature (*J. Gen. Physiol.*, 1965, **48**, 601; *Nature, Lond.*, 1966, **212**, 132; *Pl. Physiol.*, 1967, **42**, 319) are commented on, especially in respect of the presence of Ca, and some values recently obtained by Pitman are listed. These show that although K is selectively absorbed by roots grown over solutions containing $CaSO_4$, there are large changes in selectivity between early stages (rapid uptake of K and Na, especially of K) and later stages (very rapid uptake of K) until salt saturation. Reasons for this are given and the uptake of K and Na by whole plants after transfer from $CaSO_4$ solution to a salt solution is briefly discussed. Interpretation of the process depends on establishing a more precise relation between initial and later uptakes. W. J. BAKER.

Early requirement for plant nutrients by subterranean clover seedlings (*Trifolium subterraneum*). I. Krigel (*Aust. J. agric. Res.*, 1967, **18**, 879–886).—Growth studies of the seedlings showed that the seed reserves of N, P, K, Ca and Mg were quickly exhausted and additional supplies were required within approx. 7 days for Ca, 10 days for P, 14 for N and Mg and 21 for K. N-deficient plants recovered rapidly when transferred to a complete nutrient solution. but those with Ca deficiency did not recover. Plants grown in nutrient solution lacking P had longer roots than those affected by deficiencies in other essential elements. J. L. WALPOLE.

Culture on artificial medium. Mineral deficiencies in avocado pear. I. Growth and symptoms. J. N. Charpentier and P. Martin-Prével (*Fruits d'outre mer*, 1967, **22**, 213–233).—A detailed study, by means of sand cultures, is presented of the symptoms caused in the seedlings and grafted plants by deficiencies in each of 12 nutrient elements during the growth stage (8–10 months). During this stage the symptoms caused by deficiency in Fe were very slight; Mo deficiency was not detectable. (11 references.) P. S. ARUP.

Isotopic studies on the uptake of N by pasture plants. III. Uptake of small additions of ^{15}N-labelled fertiliser by Rhodes grass and Townsville lucerne. I. Vallis, K. P. Haydock, P. J. Ross and E. F. Henzell (*Aust. J. agric. Res.*, 1967, **18**, 865–877).—Using ^{15}N-labelled $(NH_4)_2SO_4$ and KNO_3, Rhodes grass (*Chloris gayana*) (**I**) was found to compete strongly with Townsville lucerne (*Stylosanthes humilis*) (**II**) for available soil N and took up much greater quantities of it when the two species were grown together in pots. Measurements were made of the uptake of soil N for **I** grown alongside **II** and of its variation with time. (14 references.) J. L. WALPOLE.

Influence of root temperature on absorption of foliar-applied labelled phosphorus and calcium. R. L. Phillips and M. J. Bukovac (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 555–560) —The absorption of ^{32}P-labelled PO_4^{3-} and $^{45}Ca^{2+}$ by leaves of bean and pea increased with temp. (7–24°) of the root medium. ^{32}P moved from the treated leaf and accumulated in the roots to a greater extent at the higher than at the lower temp. Movement of ^{45}Ca from the treated leaf was negligible. A. H. CORNFIELD.

Absorption, distribution, and metabolism of foliar applied urea by the peach. A. J. Bester, J. T. Meynhardt and D. K. Strydom (*S. Afr. J. agric. Sci.*, 1967, **10**, 761–765).—The achievement of these results was demonstrated by local applications of 2% and 5% aq. ^{14}C-labelled urea to the leaves of peach seedlings. The addition of Triton or Carbowax to the solutions had no effect on urea absorption, whilst Teepol or Tween reduced the absorption. P. S. ARUP.

Mineral nutrition and metabolic processes in young plants. I. Effects of manganese nutrition on the levels of α-keto acids in oats

(*Avena sativa*) **and wheat** (*Triticum sativum*). F. Scheffer, R. Kickuth and V. K. Saolapurkar (*J. Indian Soc. Soil Sci.*, 1967, **15**, 209–215).—The young water-cultured plants were placed in nutrient solutions, containing 0·1, 1·0 or 100 ppm of Mn, for 4 weeks, after which the keto-acids in the aerial parts were extracted and determined chromatographically. Plants having a normal Mn supply (1 ppm) contained the lowest proportions of α-keto-acids. Measurable amounts of α-ketoglutaric acid were found only when high (toxic) concn. of Mn were present in the nutrient. No consistent relationship was apparent between the level of Mn in the nutrient and the α-ketoisovaleric acid content of the plants.
A. G. POLLARD.

Synthesis of malate by carrot and beetroot tissues incubated with labelled bicarbonate and distribution of labelled carbon within the malate molecule. W. E. Splittstoesser (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 235–238).—When carrot and beetroot slices were incubated with ^{14}C-labelled HCO_3^- carrot discs incorporated 73% and beet discs 93% of the added ^{14}C into malate. The label was confined to the carboxyl groups of the malate mol. In beet tissue 85% of the ^{14}C was in C-4 of malate. The asymmetrical distribution of label between the carboxyl groups suggests that the malate formed in beetroot tissue from CO_2 fixation is not in equilibration with the respiratory pools. In carrot tissue 33% of the ^{14}C was in C-1 and 66% in C-4 of malate. The labelling pattern in malate isolated from carrot tissue was similar to that reported for Crassulacean leaves. This suggests that carboxydismutase, a photosynthetic enzyme, is present in non-green carrot tissue. A. H. CORNFIELD.

Carbohydrate accumulation in leaves of leaf roll infected potato plants. M. A. Harmey, M. P. Crowley and P. E. M. Clinch (*Scient. Proc. R. Dubl. Soc.*, A, 1968, **3**, 87–105).—Abnormal amounts of carbohydrates (*CH*) in leaves showing incipient leaf roll were translocated during an 18-h dark period more rapidly than were *CH* from sound leaves. Incipient leaf roll and *CH* accumulation were accompanied by abnormally high respiration, whilst photosynthesis remained normal. With increase of severity of symptoms, translocation and photosynthesis were inhibited. The primary cause of *CH* accumulation cannot be blockage by phloem necrosis, but may be due to decreased nutritional requirements caused by retarded growth. (33 references.) P. S. ARUP.

Diurnal variation of glycogen in plants. S. R. Erlander and J. P. McGuire (*Stärke*, 1967, **19**, 402–410).—Sweetcorn was hand-pollinated to establish a definite time of fertilisation and at two-hourly intervals on the 13th and 26th days kernels were collected for analysis, to determine diurnal variations in yields of glycogen (*G*) and starch (*S*). *G* from endosperm passed through a max. at the time when *S* was being produced at its most rapid rate, while the yield of *S* increased steadily throughout. These and other observations suggest that a precursor *G* is formed from sucrose; this forms a sol. amylopectin and a sol. amylose and this sol. *S* then forms insol. *S* granules, possibly by complexing with protein. (In English.) (36 references.) J. B. WOOF.

Biosynthesis of starch. X. Reaction kinetics of phosphorolysis. J. Holló, E. László and J. Juhász (*Stärke*, 1967, **19**, 285–290).—(10 references.) J. B. WOOF.

Soluble carbohydrates in seeds of tropical pasture species. L. K. Lehmann and R. J. McIlroy (*Nature, Lond.*, 1967, **216**, 1044–1045).—Results of paper chromatography in Et acetate–pyridine–water (10:4:3), with 4% *p*-anisidine hydrochloride in Bu^nOH–EtOH–water (4:1:1) as spray, show that raffinose and stachyose are present in the seeds (as 80% EtOH extract) of three tropical legumes (Townsville lucerne, *Glycine javanica*, *Phaseolus atropurpureus*) but not in those of the tropical grasses *Cenchrus ciliaris*, *Chloris gayana* and *Setaria sphacelata*. Fructan was present in the deproteinised, cold-water extracts of all the seeds after extraction with EtOH, so that de Cugnac's classification of Gramineae is inapplicable to seeds of tropical species. It is suggested that differentiation between grasses and legumes should be based on the presence or absence of the raffinose group. W. J. BAKER.

Determination and identification of non-structural carbohydrates removed from grass and legume tissue by various sulphuric acid concentrations, takadiastase, and water. R. D. Grotelueschen and D. Smith (*J. agric. Fd Chem.*, 1967, **15**, 1048–1951).—Aq. extracts of timothy grass contained fructosans and those from lucerne contained some dextrins in addition to sugars. For the hydrolysis of carbohydrates based on glucose, including starch, takadiastase gave better results than did 0·2N– or 0·8N-H_2SO_4. Fructosans, raffinose, and arabinose were hydrolysed by 0·005N-H_2SO_4, but not by takadiastase. The enzyme prep. obviously contained sucrase, maltase, oligo-1,6-glucosidase and (probably) melibiase. (17 references.) P. S. ARUP.

Composition of protein fraction of spring rye seed. P. S. Boronoyeva and E. D. Kazakov (*Pishch. Tekhnol.*, 1967, No. 6, [61], 9–11).— C. V.

Influence of fertilisation with KNO_3 on some nitrogen fractions in tomato plants. G. M. Ward and D. E. Hawkins (*Can. J. Bot.*, 1967, **45**, 2091–2102).—Tomato seedlings grown at a low level of N nutrition were fertilised with KNO_3 at two rates of application and the subsequent foliar N conversion was followed at intervals over a period of 48 h. The total N and nitrate N increased rapidly and markedly but the absorption of N applied late in the day followed a different pattern from that applied early in the morning. The quantities of 14 amino-acids separated chromatographically were estimated: some increased in concn. by more than twenty times and the total free amino-acid concn. increased by seven times. Of the 14 acids measured, aspartic acid, serine, glutamic acid and alanine accounted for 83–90% of the total. P. S. ARUP.

Mushroom ninhydrin-positive compounds. Amino-acids, related compounds, and other nitrogenous substances found in cultivated mushroom, *Agaricus campestris*. M. R. Altamura, F. M. Robbins, R. E. Andreotti, L. Long, jun. and T. Hasselstrom (*J. agric. Fd Chem.*, 1967, **15**, 1040–1043).—The isolation of the ninhydrin-positive compounds consisted essentially in the removal of compounds sol. in light petroleum from the anhyd. solids extracted from mushrooms with anhyd. EtOH and 90% EtOH, followed by removal of matter insol. in MeOH and in H_2O. Finally a fraction sol. in water-saturated Et_2O and insol. in anhyd. Et_2O was obtained that contained the ninhydrin-positive compounds. The following substances separated by high-resolution automatic amino-acid analysis were identified: α-aminoadipic acid, β-aminoisobutyric acid, canavanine, carnosine, creatinine, cystathionine, 2, 4-diamino butyric acid, homocystine, homoserine, hydroxylysine, kynurenine, and sarcosine. A no. of other compounds were tentatively identified. (28 references.) P. S. ARUP.

Biochemical changes in the lipids of high-oil ripening sunflower seeds. V. G. Shcherbakov and A. A. Malyshev (*Pishch. Tekhnol.*, 1967, No. 6, [61], 15–17). C. V.

Lipid biosynthesis in relation to chloroplast development in barley. L.-A. Åppelqvist, J. E. Boynton, P. K. Stumpf and D. von Wettstein (*J. Lipid Res.*, 1968, **9**, 425–437).—During greening of detached leaves from dark-grown barley seedlings, the linolenic acid (**I**) content of the lipids increased in the early stages of formation of the chloroplast lamellar system. Using ^{14}C-labelled acetate, incorporation at various stages is studied. At initial stages of greening 75% of the label is found in the steroids and other unsaponificable lipid; in advanced stages of chloroplast development, 75% of incorporated acetate is built into phospho-, sulpho- and galacto-lipid and only 25% is channelled into unsaponifiable-lipid. Experimental variation in physiological conditions resulted in ratio changes in the phospholipid and galactolipid found and variation in incubation also resulted in different degrees of labelling of the fatty acids. Labelling of **I** was highest in the monogalactosyl diglyceride fraction at all stages of greening. (37 references.) C. V.

Metabolism of lipids in outer and epidermal parenchyma of apples. I. Comparison with central parenchyma. P. Mazliak and A.-M. Justin (*Fruits d'outre mer*, 1967, **22**, 413–432).—With the use of g.l.c. and t.l.c. the principal fatty acids in the ripe peel were found to be palmitic, linoleic, oleic, stearic, and linolenic acids; the content of linoleic acid was 53% (of the total lipids) in the peel as against 20% in the inner tissue. Paraffins, waxes, tri-, di-, and monoglycerides, phospholipids, galactolipids, sterols, and triterpenes were found in the rind, polar lipids amounting to 15% of the total as against 75% in the inner tissue. In experiments *in vitro* with ripe tissues in a glucose medium (with inorg. salts) the ^{14}C of added Na ^{14}C-acetate was incorporated into C_{20}–C_{26} fatty acids (mostly saturated); in peel tissue the incorporation was ten times as rapid as in inner tissue. (30 references.) P. S. ARUP.

Enzymic formation of steryl glycosides by particle fractions from lettuce and spinach leaves. E. Eichenberger and E. C. Grob (*Chimia*, 1968, **22**, 46–48).—Tissue particles of the leaves of lettuce (*Lactuca sativa* L.) were incubated in a homogenised and buffered system with UDP-^{14} C-glucose at 20° for 3 h and the lipids were extracted and submitted to two-dimensional thin layer chromatography. The labelled glucose was incorporated into steryl glycosides and their esters. Optimal activity of the enzyme was at pH 8·5. The reaction, stimulated by ATP, depended on the presence of free sterols. Extracts of particle fractions from leaves of spinach

(*Spinacia oleracea* L.) converted α-spinasterol and also β-sitosterol and cholesterol into glycosides. M. SULZBACHER.

Relationship between enzyme activities and phenolic components in banana fruit tissues. G. H. de Swardt, E. C. Maxie and V. L. Singleton (*S. Afr. J. agric. Sci.*, 1967, **10**, 641–649).—Phenol polymerisation in the ripening tissue was indicated by the decrease in compounds extractable with 100% MeOH and increase in those extractable with aq. MeOH, the latter consisting chiefly of increases in the leucoanthocyanins. The decreases in phenols were accompanied by increases in the vanillin to leucoanthocyanin ratio in the 100% MeOH extracts, and decreases in this ratio in the aq. MeOH extracts. The leucoanthocyanins of low mol. wt. probably inhibit the pectin methylesterase in the preclimacteric fruit, whilst those of higher mol. wt. are inactive in this respect. The possible rôle of tannins is considered. (23 references.) P. S. ARUP.

Stability of wheat embryo glutamate decarboxylase under conditions of water stress. C. Nations (*Can. J. Bot.*, 1967, **45**, 1917–1925).—The carboxylase activities of germinating wheat grains before and after desiccation were measured and attempts made to relate the activity of the enzyme to various fractions obtained by differential centrifugation. The stability of glutamate decarboxylase to desiccation appears to arise from a capacity to dissociate into smaller protein components under water stress; this capacity may depend upon the presence of lipids. (17 references.) J. L. WALPOLE.

Changes of peroxidase and catalase activity in dying plant tissue. V. Šicho and J. Kaš. (*Sb. vys, Šk. chem.-technol. Praze, Potraviny*, 1967, **E14**, 5–10, 11–19).—

I. Potato tissues (fine slices, or grated) were held at 37° in air, O_2, N_2 or CO_2 for determination of peroxidase and catalase activity at intervals during 24 h. Changes in peroxidase activity were little affected by either type of tissue damage or atm. composition; in all cases activity fell by approximately 50% during 24 h. (25 references.)

II. Catalase activity in potato slices fell to approx. 50% of its initial value within 3 h at 37° and then remained substantially unchanged unless increased by contaminating micro-organisms. Changes in activity in slices were substantially unaffected by atm. composition. In grated potatoes changes were similar in atm. of air, O_2 or N_2, but in CO_2 activity fell more rapidly and to a lower final value. (In English.) (23 references.) E. C. APLING.

Extraction of peroxidase isozymes from bean leaves. K. K. Adatthody and D. Racusen (*Can. J. Bot.*, 1967, **45**, 2237–2242).—Both the pH and the ionic strength of the extraction medium played an important rôle in the recovery of peroxidase isozymes from bean leaves. A considerable proportion of the cationic peroxidase in the leaf tissue was strongly sorbed to naturally-occurring insol. polymers at low ionic strength and neutral pH. These polymers acted as ion-exchangers from which the enzymes could be progressively eluted with increasing concn. of NaCl. J. L. WALPOLE.

β-Glycerophosphatase and lateral root development. J. F. Sutcliffe and R. Sexton (*Nature, Lond.*, 1968, **217**, 1285).—The large increase in activity of β-glycerophosphatase around the developing lateral roots of *Pisum sativum* was investigated by enzyme assays of serial sections and by histochemical examination of enzyme localisation. Results show that β-glycerophosphatase and possibly other hydrolytic enzymes are induced by mechanical stimulation of cells surrounding the emerging lateral roots (2·5–4 cm from root apex), and that this activity assists the lateral root in penetrating the bordering cortex. W. J. BAKER.

Action of potato-peel extracts in modifying tuber dormancy. M. G. Walker (*Nature, Lond.*, 1968, **217**, 878–879).—Observed effects of tuber extracts, and also of gibberellic acid (1 ppm), on sprout-growth of whole tubers partly immersed for 36 days in the dil. solution, suggest that inhibitor–β complex definitely prolongs dormancy. The extracts were prepared by paper chromatography of EtOAc fractions to yield ten R_F 0·1–1·0 strips for application by a modification of Goodwin's technique (*Europ. Potato J.*, 1966, **9**, 53). Inhibition of sprout-growth was dominant under treatments R_F 0·7 (region of inhibitor–β complex) and R_F 0·2 (region of Varga and Ferenczy's neutral inhibitors). W. J. BAKER.

Biochemistry of some heterocyclic nitrogen derivatives. R. F. Lloyd (*Diss. Abstr.* B, 1967, **23**, 3013).—The possibility that the purine ring in some biologically active compounds could be replaced by the pteridine ring, is explored. Some H-substituted pteridine deriv. having benzylthio-, n-pentylthio-, benzylamino- or furfurylamino-groups as substituents were synthesised and compared with the corresponding purine deriv. which are effective analogues of kinetin. Unlike the purine deriv. the pteridine compounds did not stimulate seed germination. Some ω-substituted Me, Et, Pr and pentyl members of a series of 6-(1′, 4′-cyclohexadiene-1′-alkyl)-aminopurines were tested as analogues of kinetin. They stimulated the germination of lettuce seeds to extents closely similar to those caused by corresponding 6-(phenylalkyl)-aminopurine deriv. The 1, 4-cyclohexadienyl-group thus appears to be isosteric with the phenyl group. The prep. of **1**, 5, 9-tris-(6′-amino-9′-purinyl)-nonane is described and its effects on certain metabolic changes within the plant system are examined. The transfer of N from glutamine, essential for the biosynthesis of anthranilic acid, is investigated by the stepwise production of three isomeric *N*-(γ-*l*-glutamyl)-aminobenzoic acids, all of which could be hydrolysed enzymically. Evidence favoured the view that the *m*- but not the *o*- or *p*-deriv. served as a biological intermediate. A. G. POLLARD.

Halogen derivatives of 4-hydroxybenzoic acid as root-growth stimulants and importance of light in this response. R. L. Wain, H. F. Taylor, P. Intarakosit and T. G. D. Shannon (*Nature, Lond.*, 1968, **217**, 870–871).—Evidence advanced shows that 3, 5-dichloro, -dibromo-, and -di-iodo-4-hydroxybenzoic acids can modify the normal response of roots to environment, especially light, and that generally the order of activity is I > Br > Cl. Root-growth of cress seedlings in nutrient alone with exposure to light was stimulated by the di-iodo acid (5×10^{-7}—5×10^{-5}M), but not when the seedlings were grown in soil or sand. Light inhibits growth of untreated roots, the inhibition being removed by treatment with 10^{-5}M-di-iodo acid. The acid is metabolised to a glucose ester in the roots of rice and cress seedlings. W. J. BAKER.

Carbohydrate contents during germination of growth regulator treated groundnuts. D. N. Vyas, K. C. Patel and R. D. Patel (*Stärke*, 1967, **19**, 410–415).—Seedlings grown for different times in sand with addition of gibberellic acid, ascorbic acid, sucrose, maleic hydrazide or sulphanilamide were extracted first with 80% ethanol and then with $HClO_4$. The fractions were analysed for starch, amylose, total and reducing sugars. Starch and total sugar fell in the first day of germination as it was used as a source of energy and then rose to a max. at about 10 days, after which it declined again. Differences in the pattern were observed with the additives, especially sulphanilamide. (In English.) (19 references.) J. B. WOOF.

Fatty alcohol inhibition of tobacco axillary and terminal bud growth. G. L. Steffens, T. C. Tso and D. W. Spaulding (*J. agric. Fd Chem.*, 1967, **15**, 972–975).—Selective inhibition of the unwanted growths, without damage to mature tissues, was achieved with the C_9, C_{10}, and C_{11} fatty alcohols if applied as emulsions in 1·5% Tween-80. The selectivity was lost without the use of a suitable surfactant. The alcohols were (per mol. equiv.) more efficient than the corresponding fatty acid Me esters. Alcohols higher or lower than those mentioned were less efficient. (10 references.) P. S. ARUP.

Plant growth regulant. Biochemical behaviour of 2-chloroethyltrimethylammonium chloride [chlorocholine chloride] in wheat and rats. R. C. Blinn (*J. Agric. Fd Chem.*, 1967, **15**, 984–988).—The ^{14}C-labelled CCC that was absorbed by wheat leaves underwent no significant metabolic change, and very little was transported to the roots. CCC administered to rats was almost exclusively excreted in the urine. P. S. ARUP.

Determination of chlorocholine residues in wheat grain, straw, and green wheat foliage. R. P. Mooney and N. R. Pasarela (*J. agric. Fd Chem.*, 1967, **15**, 989–995).—The CCC is extracted with MeOH (or in the case of straw with methanolic HCl), purified by passing the extracts through two columns of Al_2O_3, and determined by measurement at 415 nm of the colour produced with dipicrylamine in CH_2Cl_2 solution. Recoveries were 76–90%. A field test in which CCC was applied at 4 lb/acre showed a biological half-life for CCC of 13 days. (17 references.) P. S. ARUP.

Anthocyanin pigments in peaches. L. O. van Blaricom and T. L. Senn (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 541–545).—The anthocyanin pigments in nine varieties, ranging from early to late season, of peach fruits are reported. The main pigment in all varieties was cyanidin 3-monoglucoside. A. H. CORNFIELD

Comparison of three methods for measuring the cation-exchange capacity of plant roots. P. L. Carpenter and V. N. Lambeth (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 550–554).—Methods involving the extraction of Ca^{2+} from Ca-saturated roots and the exchange of ^{40}Ca in saturated roots with ^{45}Ca gave more precise results for cation-exchange capacity of roots than did the method involving the titration of H-saturated roots with alkali. A. H. CORNFIELD.

Phenolic compounds in apples. Preliminary investigations of a special method for chlorogenic acid. J. J. Macheix (*Fruits d'outre mer*, 1968, **23**, 13–20).—The low temp. extraction of the phenols is carried out with 90% EtOH under N_2, giving an 80% solution in EtOH. Paper chromatography reveals a large no. of phenols, mainly chlorogenic acid. The determination of total phenols by the method of Swain and Hillis is unsatisfactory. (41 references.) P. S. ARUP.

Automated procedure for simultaneous determination of phosphorous and nitrogen in plant tissue. W. D. Basson, D. A. Stanton and R. G. Böhmer (*Analyst, Lond.*, 1968, **93**, 166–172).—The P is determined (as molybdovanadophosphate) in an aliquot of the acid-digested sample coming from an AutoAnalyzer+Kjeldahl Analyzer digestor unit. The flow diagram is shown; solutions of NaOH (to neutralise the acid solution), NH_4 metavanadate and heptamolybdate are introduced into the system for P, whilst $HgSO_4$, SeO_2, $HClO_4$ and H_2SO_4 are used for digesting the tissue (these four compounds have no effect on the P determination). Optimum reaction conditions ensuring satisfactory accuracy are reported for ~ 0·1–0·2% P. W. J. BAKER.

Effect of phosphorus-32 on rye plants grown under unfavourable conditions. P. G. Marais, J. Deist and C. F. G. Heyns (*S. Afr. J. agric. Sci.*, 1967, **10**, 843–845).—Evidence was obtained of harmful radiation effects on rye plants grown under unfavourable conditions in soil treated with KH_2PO_4 containing ^{32}P. P. S. ARUP.

Crops and Cropping

Ice in plants. D. B. Idle (*Science J.*, 1968, **4**, No. 1, 59–63).—Toleration of freezing and thawing is discussed with a view to the protection of vulnerable crops from frost and expanding the growing season. The genetic aspects of hardy strains are also considered. C.V.

Crop production under glass. G. F. Sheard (*Jl R. agric. Soc.*, 1967, **128**, 185–193).—A review of current statistics, glasshouses, environmental control, environment and plant growth, chemical control of plant growth, crop nutrition, composts and peat culture, pest and disease control, soil sterilisation, crop production methods, and new crops. (53 references.) E. G. BRICKELL.

Plant competition and crop yield. H. Farazdaghi and P. M. Harris (*Nature, Lond.*, 1968, **217**, 289–290).—The Shinozaki-Kira equation relating plant density and yield (*J. Inst. Polytech., Osaka City Univ.*, 1956, **D7**, 35) is derived from equations for population growth and for the law of constant final yield. Whilst the logistic equation of growth has proved valid for different crops, the law of constant final yield may not always be satisfied. A more adequate equation is therefore derived and, by various transpositions, is applied to meet different environmental growth factors and levels of plant treatment, e.g., to describe (1) the relationship between plant density and yield of separate species or varieties and (2) the way plant density affects the distribution of dry matter into plant parts. W. J. BAKER.

Crop behaviour. J. D. Ivins (*Jl R. agric. Soc.*, 1967, **128**, 159–169).—A review covering sugar-beet, potatoes, cereals and grassland. (56 references.) E. G. BRICKELL.

Break crops in cereal production. E. R. Bullen (*Jl R. agric. Soc.*, 1967, **128**, 77–85).—The potential importance of break crops under cereal-dominated arable farming, and their benefits, are reviewed. (11 references.) E. G. BRICKELL.

Crops and plant breeding. H. W. Howard (*Jl R. agric. Soc.*, 1967, **128**, 125–143).—A review of plant breeders' rights, index of names of plant varieties, statutory performance trials, cereals, wheat, barley and oats. (71 references.) E. G. BRICKELL.

Some aspects of drought resistance in small grains and means of enhancement or induction. M. H. Salim (*Diss. Abstr.* B, 1967, **27**, 2955).—Resistance to desiccation of wheat, barley, and oats was examined in relation to transpiration rate and age of seedlings. type of soil, irrigation regime and intensity of desiccation. Methods for measuring this resistance included the use of potted seedlings, uprooted whole or cut seedlings, or cut leaf sections with static or dynamic equilibrium techniques. Transpiration rates were lowest in barley which also made best use of soil water; oats showed the opposite tendencies in both respects. Different species and varieties reacted individually according to age and hardening by differences in the intensity of desiccation. Methods based on dynamic moisture equilibrium with cut leaves were as informative as were other, more tedious methods. Pre-soaking of seeds did not lead to uniform results in respect of uniform hardening resistance and not all varieties responded to the same extent or in the same direction. A. G. POLLARD.

Copper deficiency in crops in north-east Scotland. J. W. S. Reith (*J. agric. Sci., Camb.*, 1968, **70**, 39–45).—Spring sown oats and barley are more susceptible to Cu deficiency than are mixed herbage; potatoes and swedes do not respond at all to Cu. Applications of 10–20 lb $CuSO_4.5H_2O$ (**I**) per acre correct the disturbance and have a residual effect for at least 8 years. Foliar sprays of 1 lb per acre of **I** are less effective, although the Cu content of plant tissue is raised. Grain yields are appreciably higher where soil applications have been made. Soil Cu content, estimated with 0·05 M-EDTA, is significantly correlated with the increase in grain yield produced by application of Cu in both pot and field trials. In N. E. Scotland cereal yields were not affected providing the EDTA-extractable Cu exceeded 1·1 ppm. Response to soil applications was considerable where the level fell below 0·75 ppm. (17 references.) M. LONG.

Nutritional requirement of the wheat crop. I. Limitations of foliar diagnosis techniques and variations in plant composition due to fertiliser treatment. C. L. Mehrotra and L. K. Lehri (*J. Indian Soc. Soil Sci.*, 1967, **15**, 217–227).—The response of wheat plants to nutrient treatments was much greater in mature than in younger soils. Fertilisers increased the concn. of N, K and, particularly, of P in mature soils. In plants the higher concn. of N, P and K in early growth diminished progressively with advancing age towards the ripening stage. The rate of intake of nutrients was max. in plants at the tillering stage. High positive correlations were established between the nutrient contents of plants at tillering and the final crop yield, the relationship offering a basis for correcting nutrient treatments during crop growth. Use of foliar analysis for this purpose was not effective. A. G. POLLARD.

Late topdressing of winter wheat by means of aerial spraying with urea. G. H. Arnold and K. Dilz (*Neth. Nitrogen Tech. Bull.*, 1967, No. 5, 28 pp).—In 1963 and 1965 an aerial spray of 11 kg N per ha gave as great an increase (200–300 kg per ha) in grain yield as did 30 kg N as $Ca(NO_3)_2$ applied by conventional means. No increase in yield was obtained from later top dressing in 1964 but in this year $Ca(NO_3)_2$ by comparison, led to a decrease. Spraying at ear emergence gave the best results. E. G. BRICKELL.

Potassium and Japonica rice. Summary of twenty-five years research. Y. Noguchi and T. Sugawara (*Int. Potash Inst. Berne*, 1966).—The symptoms and physiological effects of K-deficiency are described in detail. K-manuring by top-dressings and foliar sprays gave considerably increased yields in degraded or badly-drained paddy fields. (73 references.) P. S. ARUP.

Maize fertiliser experiments in Western Tanzania. M. A. Scaife (*J. agric. Sci., Camb.*, 1968, **70**, 209–222).—Responses to N and P were very large in the S.W. half of the area where rainfall is fairly well distributed. Around Lake Victoria a response was obtained only to N. In drier areas response to N and P was small and no response was obtained to K in any area save one. Responses to N and P could be accounted for by the Mitscherlich-Baule equation and this equation could be used to define max. economic requirements for N and P necessary for various maize and fertiliser prices and potential yields. Empirical equations for estimating the parameter b from soil analyses of areas not adequately covered by these experiments are given. (22 references.) M. LONG.

Influence of time lag between pollen-shedding and silking on yield of maize. D. P. du Plessis and F. J. Dijkhuis (*S. Afr. J. agric. Sci.*, 1967, **10**, 667–674).—A negative correlation ($r = -0·975$) was found between the lag from 50% pollen shedding to 50% silking on the one hand, and the $\log_{10}$ of the yield per plant on the other. Predictions of yields can be made on this basis provided that moisture is the limiting factor. (13 references.) P. S. ARUP.

Variability in factors affecting yield of sweet-corn varieties. L. E. Watts (*N.Z. Jl agric. Res.*, 1967, **10**, 389–396).—Total yield of an open-pollinated variety of sweet-corn was as high as that of any F.1 hybrid, while its variability for most characters was low. E. G. BRICKELL.

Effect of superphosphate on the uptake of micro-nutrients by sorghum (*Jowar*) and maize plants. B. L. Basen and Ram Deo (*J. Indian Soc. Soil. Sci*, 1967, **15**, 245–249).—Various applications of superphosphate (P_2O_5, 0–67 kg/ha) were made to five different soils in pot culture tests and the effects on the uptake of Mn, Fe, Cu and B by maize and sorghum were examined. The Mn content of sorghum was lowered but no relationship was apparent between the P treatment and the uptake of Mn by maize. In both crops

the uptake of Fe was increased. In some of the soils the larger applications of P lowered the Cu and B contents of the plants. A. G. POLLARD.

Effect of tetrachloronitrobenzene (TCNB) on emergence and yield of potatoes. I. [Katahdin potatoes grown in] Maine. H. J. Murphy and M. J. Goven (*Am. Potato J.*, 1967, **44**, 272–276).—Seed potatoes which had been treated with 6% TCNB dust (2·72 g TCNB per bushel) in Dec. for control of sprouting showed no delay in emergence nor reduced yields when compared with sprouted or unsprouted seed. Sprouting control using dust, emulsion, or aerosol formulations of TCNB did not reduce yields, although the emulsion form delayed emergence. Pure TCNB did not affect emergence but reduced the yields. A. H. CORNFIELD.

Influence of oxygen concentration during storage on seed potato respiratory metabolism and field performance. M. Workman and J. Twomey (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 268–274).—The respiratory quotient (R.Q.) of bud tissue of potatoes stored in atm. containing 1–21% O_2 at 5–20° was > 1 only in 1% O_2 at 20°. Parenchyma tissue showed R.Q. values > 1 in higher O_2 concn. and at lower temp. Yields were the same irrespective of O_2 level during storage of seed potatoes. Only when storage was extended to 9 months did fewer sprouts appear after the low O_2 treatment. Seed potatoes stored under continuous ventilation produced more larger tubers than did those stored in still air. A. H. CORNFIELD.

Influence of pre-harvest factors in carbohydrates in carrots. W. A. Sistrunk, G. A. Bradley and D. Smittle (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 239–251).—Irrigation level usually had no effect on water-sol. pectin, total sugars, starch, or hemicellulose contents of canned carrots, although there were differences in these constituents due to variety. There were differences in total sugars, water-sol. pectin, Calgon-sol. pectin and starch due to planting date in the spring, and also between spring and autumn plantings. Firmness was not closely related to differences in pectic constituents. A. H. CORNFIELD.

Changes in the structure of a perennial sward frequently but leniently defoliated during the summer. L. A. Hunt and R. W. Brougham (*N.Z. Jl agric. Res.*, 1967, **10**, 397–404).—*Lolium perenne* L. was trimmed every seven days over a period of 49 days in late summer. Tiller no. per unit of ground area increased slightly during the first 7 days of growth respectively and then declined. Yields of sheath and dead matter increased progressively throughout the study. (26 references.) E. G. BRICKELL.

Interaction between nitrogen and water in the growth of grass swards. I. Methods and dry matter results. M. J. D'Aoust and R. S. Tayler (*J. agric. Sci., Camb.*, 1968, **70**, 11–17).—Irrigation of *Lolium multiflorum* had no effect on its response to N over the season as a whole when the rate of application of N was between 1 and 2 cwt/acre. At double this application there was a large increase in response to N with irrigation. The moisture status of the top few inches of the soil is an important factor in determining response to N. (20 references.) M. LONG.

Effect of time of cutting on the mineral content of S.23 ryegrass. J. G. Thomas (*J. Br. Grassld Soc.*, 1967, **22**, 282–288).—The % of Ca, P, K, Na, Mg, Cu, Co and Mn (dry basis) in S.23 ryegrass herbage grown on two sites, (an open moor of relatively poor fertility, pH 5·0, and a fertile site on magnesian limestone, pH 7·2) and cut at different periods through the growing season, were determined over 2 years. In many cases significant differences occurred between the levels of a particular element at different times during the year. Results are discussed in relation to the adequacy of minerals in the grass for animals. A. H. CORNFIELD.

Effect of nitrogen, phosphate and potash fertilisers on three grass species. K. M. Wolton, J. S. Brockman, D. W. T. Brough and P. G. Shaw (*J. agric. Sci., Camb.*, 1968, **70**, 195–202).—Annual applications of up to 300 lb N, 100 lb P_2O_5 and 200 lb K_2O/acre on cut swards of S.24 ryegrass, S.37 cocksfoot and S.215 meadow fescue were made for 3 years. Differences in response between species to N and K were inconsistent and negligible over three years. A high response to N was maintained only when P and K were also applied. No response to applications of P_2O_5 > 50 lb/acre occurred in any year; > 100 lb/acre K_2O was found necessary in 2 of 3 years. Visual symptoms of P and K deficiencies were evident when high N applications coupled with no P and K were made. (14 references.) M. LONG.

Influence of leguminous plants on the growth and nitrogen nutrition of associated grasses. J. M. M. de la Fuente (*Diss. Abstr.* B, 1967, **27**, 2953).—Legume-grass associations (soyabean–Sudan-grass; ladino clover–fescue; lucerne–fescue) were grown in soil cultures. Neither soyabean nor Sudan-grass showed any benefit from the association. Fescue had a detrimental effect on the clover but not on lucerne. Fescue was improved by association with the clover or with lucerne; benefit to the grass and amounts of N released by either legume increased in successive harvests. In sub-irrigation gravel cultures the grass and legume were grown in separate pots but with a common source of nutrient. Growth and N content of fescue increased when thus grown with either legume, this being attributed to the increased N supply although this was very small compared with the N requirement of the grass. In a further test the associated plants were grown in a specially controlled environment in an atm. containing ^{15}N. Under these conditions the grasses grew normally but the legume growth was poor for unknown reasons. The possible formation of a volatile toxic substance in the closed system is suggested. A. G. POLLARD.

Influence of times of sowing on the industrial value of the seed of *Dracocephalum moldavicum* L. (II). K. Szklanowska (*Annls Univ. Mariae Curie-Skłodowska, Agric.* E, 1966, **21**, 131–138).—*Dracocephalum moldavicum* L., a common honey-bearing plant, produces seeds containing up to 22% of fat of industrial usefulness. The effects of the time of sowing on the yield of seeds and on their contents were studied in three consecutive years (1959–61) on test plots, sowing the seeds on 15th and 30th of April and May and on 15th of June each year. The results indicate that (1) sowing date has no significant influence on the fat content or chemical composition of seeds and (2) max. yield was obtained from three early sowings. The yield from later sowing was lower and the seeds showed lower germination power and energy, but there was no marked difference in seeds wt. (18 references.) T. M. BARZYKOWSKI.

Jute responds well to higher levels of nitrogen. S. N. Pandey and N. N. Goswami (*Fertil. News*, 1967, **12**, No. 11; 19–21).—Trials are described in which the effects of different levels of N on different varieties of jute are studied. Response to higher levels of N is more pronounced with high yielding varieties of jute. It is concluded, that for the JRC–212 variety under normal soil fertility levels, a dose of N up to 90 kg N/ha may be given with advantage until depression in yield is noticed. Under high fertility conditions, or with low yielding varieties a N dose of 60 kg/ha may be the safe and economic limit. I. DICKINSON.

Fruit growing. E. W. Hobbis (*Jl R. agric. Soc.*, 1967, **128**, 194–205).—A review covering modernisation of orchards, soft fruits, national fruit trials, pollination, fruit nurseries, rootstocks, herbicides, pest and disease control, plant nutrition, mechanisation, harvesting, and marketing. (51 references.) E. G. BRICKELL.

Prevention of apple blossom freezing by two fatty acids. M. T. Hilborn (*Phytopathology*, 1967, **57**, 341).—Low-temp. injury (−6° for 2 h) to McIntosh apple blossom was prevented by treatment with n-decenyl-succinic acid (**I**) or stearic acid (**II**) (10^{-3}M). All **I**-treated blossoms survived and many set fruit at −3°. Blossoms treated with **II** did not set fruit. With Golden Delicious blossoms fatty acid treatment, to be effective, must be made at least 4 h prior to exposure to low temp. Applications made 8–48 h earlier did not prevent injury. A. G. POLLARD.

Effect of treating apple trees with Alar (*N*-dimethylamino succinamic acid) on optimum harvest dates and keeping quality of apples. G. D. Blanpied, R. M. Smock, and D. A. Kollas (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 467–474).—Treatment of apple trees with Alar increased fruit firmness and delayed the best harvest date for three varieties in Ireland, but not for McIntosh in New York. The treatment delayed the onset of respiratory climacteric and ethylene peak and decreased post-peak levels. Scald was frequently decreased, whilst core browning in regular storage, but not in controlled-atm. storage, was increased. Red colour was increased, but susceptibility of McIntosh to fungus infection was not affected by the treatment. A. H. CORNFIELD.

Is *Prunus Brompton* suitable as root stock for apricots? H. Plock (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert*, 1967, **17**, 492–494).—Although the graftings appeared during the first few years to have been successful, the 6- to 8-year old trees developed swellings at the junction between the root-stock and scion, which resulted in the death of the tree. Numerous cases of wind-breakage indicated unsatisfactory union between the stock and the graft. P. S. ARUP.

Growing American bunch grapes. Anon. (*Fmrs' Bull., U.S. Dep. Agric.*, 1968, No. 2123, 21 pp.).—Climate, soil, varieties, vineyards, propagation, planting, training and pruning, soil management, chemical weed control, winter protection, harvesting and fruit maturity, are described. E. G. BRICKELL.

Results of a two-year experiment with strawberries. M. Bauckmann (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18,** 56–68).—Trials with 20 varieties were carried out with respect to yields, suitability for processing for preserves, and resistance to *Botrytis cinerea* and to herbicides. P. S. ARUP.

Compatibility and tissue assimilation in walnut grafting. K. J. Maurer (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17,** 481–491).—Symptoms of incompatibility are discernible only on examination of longitudinal sections of the cuts made for the introduction of the graft, and not by immediately visible outer symptoms; persistent effects are manifested by reduced yields during the latter half of the life of the tree. Many failures can, however, be traced to faulty grafting. (14 references.) P. S. ARUP.

Vegetable production. T. Laflin (*Jl R. agric. Soc.*, 1967, **128,** 170–184).—A review covering plant breeding, cultural techniques, seed beds and seeding, spacing, nutrition, irrigation, pests and diseases, weed control, harvesting, and storage and management. (102 references.) E. G. BRICKELL.

Effect of manurial treatments on leaf area index in spinach. B. Singh Panwar, J. P. Agarwal and G. B. Singh (*Fertil. News*, 1967, **12,** No. 11; 38–40).—Data on the leaf area index (*LAI*) and the various components as influenced by three levels of N and two levels of P are summarised. Trends in *LAI* values indicate that the balanced nutrition of plants is responsible for higher photosynthetic activity; higher values of *LAI* are associated with the application of N in combination with P. I. DICKINSON.

Irrigation studies with onions. E. Strydom (*S. Afr. J. agric. Sci.*, 1967, **10,** 767–779).—In experiments over 3 years with moisture regimes at 45–90% depletion of available water, the best yields were obtained at the 60% level (~ 1·2 bar stress). At the 90% level yields were decreased, but were still of good quality. The drier regimes tended to promote earliness. (17 references.) P. S. ARUP.

Inheritance of factors related to earliness in pepper, *Capsicum annuum*. N. S. Mansour (*Diss. Abstr*, B, 1967, **27,** 2953).—In a study of various hybrids and reciprocal crosses a high level of dominance is shown for the shorter duration to first anthesis, fewer nodes to the first furcation and the greater no. of red-ripe fruit per plant before the first killing frost. One major gene controlled the genetic variation. Similar though not identical relations were shown by the product of one of the former parents in a different cross. Correlation coeff. were established between the various parameters measured. A. G. POLLARD.

Cultural and environmental parameters for mechanically harvested cucumbers. F. D. Morrison (*Diss. Abstr.*, B, 1967, **27,** 2954).—Production of cucumbers for a once-over harvest is examined. Highest yield in terms of money value per acre was with Spartan Dawn planted at 9 in. intervals in rows 9 in. apart. N applied at the rate of 60 lb/acre was adequate for this type of harvesting. Moisture requirements were critical and supplementary irrigation was necessary. Best yields were obtained when the cucumbers reached 2–2·5 in. in dia. The critical min. temp. was 50°F. Growth was retarded by exposure of the plants at any age to 40–50°F for 4 h; plants from larger seeds showed greater tolerance to cold. Plants maintained at 50°F for 36 h showed delay in leaf development and time to anthesis, compared with those kept at 60°F for 36 h. Exposure to 45°F for 12 h altered the location of fruit set by drying-out basal pistillate buds and flowers. Elongation of fruit was reduced by 50% after 24 h at 45°F. A. G. POLLARD.

Plant physiology: [Observations made in the] root laboratory. Anon. (*A. Rep. Exp. Stn S. Afr. Sug. Ass.*, 1966–7, 31–38).—The growth rate of sugar-cane crops is largely controlled by the vigour and growth rate of the root system, which in turn is governed by the resistance offered by the soil and the amount of moisture, warmth and nutriment it holds. J. L. WALPOLE.

Sucrose studies. Anon. (*A. Rep. Exp. Stn S. Afr. Sug. Ass.*, 1966–7, 57–58).—Measurements were made of the sucrose content of sugar-cane of four varieties harvested at different ages and also of a single variety (N: Co. 376) under varied nitrogen fertiliser applications. J. L. WALPOLE.

Pest Control

Insecticidal constituents of *Chrysanthemum cinerariaefolium*. III. Their composition in different pyrethrum clones. S. W. Head (*Pyrethrum Post*, 1967, **9,** No. 2, 3–7).—Analysis of mature pyrethrum flowers by g.l.c. and the AOAC method showed that the proportions of the six insecticidal constituents may vary widely between different clones although these proportions are characteristic of a particular clone. No regular relationship could be found between the ratios of Pyrethrin I and Pyrethrin II fractions nor between the cinerin/jasmolin/pyrethrin proportions. J. L. WALPOLE.

Organic insectofungicides. Kinetics of the reaction of esters of thio- and dithio-acids of phosphorus with tertiary aliphatic phosphines. N. N. Mel'nikov, A. F. Vasil'ev, B. A. Khaskin, N. N. Yuturina and T. M. Ivanova (*Zh. obshch. Khim.*, 1968, **38,** 1745–1751).—The kinetics of alkylation of PEt_3 with Me_3 thio- and dithio-phosphate in n-heptane and MeCN were studied. Rate constants and activation energies were calculated. It is shown that (1) alkylation of t-phosphines is more rapid than that of t-amines, (2) in non-polar solvents dithiophosphates have higher alkylating capacity than analogous esters of thiophosphoric acid and (3) alkylation in MeCN is more rapid than in n-heptane. (16 references.) R.J.M.

Synthesis and herbicidal activity of some amides of *O*-arylmethyl-, dichloromethyl- and trichloromethyl-phosphonic acids. A. F. Grapov, N. V. Lebedeva and N. N. Mel'nikov (*Zh. obshch. Khim.*, 1968, **38,** 1751–1754).—The effect of the structure of the acyl residue and substitution in the Me group on biological activity was studied. Amides of *O*-2-chlorophenyl-, *O*-4-chlorophenyldi- and chlorophenyl-methylphosphonic acids are prepared from an appropriate chlorophenol, $RPOCl_2$ (R is $CHCl_2$ or CCl_3) and NEt_3. E.g., Me $POCl_2$ is heated with 2-chlorophenol for 5 h at 150° and then treated in $CHCl_3$ with NH_2Et giving *O*-2-chlorophenyl *N*-ethylamido-methylphosphonate. Amides of *O*-2-chlorophenylmethylphosphonic acid were less active as herbicides than the 4-compounds. Of the *O*-4-chlorophenyl *N*-isopropylamidomethyl-, chloromethyl- (**I**), dichloromethyl- and trichloromethyl-phosphonates **I** is most active. On further substitution of H by Cl activity is lost perhaps due to steric factors. R.J.M.

Synthesis of thiazoline compounds and their mercurated derivatives and their use as fungicides. B. C. Dash and G. N. Mahapatra (*J. Indian chem. Soc.*, 1967, 44, 939–942).—Ten symmetrical diarylthioureas were condensed with ethyl acetoacetate by refluxing in benzene in the presence of I_2 to give compounds (**I**), formulated as 2-arylimino-3-aryl-4-methyl-5-carbethoxy-4-thiazolines. **I** were mercurated by dissolving in 1:1 HOAc/EtOH and treating with $Hg(OAc)_2$ in EtOH acidified with HOAc, when the Hg deriv. was pptd. The Hg deriv. had one -HgOAc group attached *p* to the 3-aryl group. **I** were prepared with the following aryl groups (m.p.): Ph (111–2°), *p*-Me-Ph (82–3°), *o*-Cl-Ph (90°), *p*-Cl-Ph (96–7°), *p*-Br-Ph (86–7°), *p*-COOH-Ph (188–190°), *m*-NO_2-Ph (165°), *p*-NO_2-Ph (200°), α-naphthyl (240°) and β-naphthyl (230°). Fungicidal tests against *Piricularia oryzae* showed complete inhibition at 100 ppm in the case of **I** and at 50 ppm with the mercurated deriv. J. I. M. JONES.

Fungicidal activity of *N*-trichloromethylthio-derivatives of cyclic carbamates. E. Czerwińska, H. Stefaniak, A. Ziółkowska, R. Kowalik, J. Plenkiewicz and Z. Eckstein (*Bull. Acad. pol. Sci., Sér. Sci. Chim.*, 1967, **15,** 473–477).—Benzoxazolin-2-one (**10**) and 5-aryl-1, 2, 4-oxadiazol-3-one (**9**) deriv. of *N*-trichloromethylthio cyclic carbamates were synthesised. As the trichloromethylthio group in these compounds is similar to that present in cyclic imides such as captan (**I**), the new compounds were tested for similar properties. Tests carried out on *Fusarium culmorum*, *Alternaria tenuis* and *Rhizoctonia solani* proved that all the compounds showed some fungicidal activity; four of them showed activity equal to that of **I** and completely inhibited fungal growth at concn. as low as 0·005%. T. M. BARZYKOWSKI.

Introduction, culture, liberation, and recovery of parasites of *Sirex noctilio* in Tasmania, 1962–1967. K. L. Taylor (*Tech. Pap. Div. Ent. C.S.I.R.O. Aust.*, 1967, No. 8; 19 pp).—Biological control work is reported, with particular reference to the establishment of the parasites *Rhyssa persuasoria* and *Ibodia leucospoides* previously introduced. Details of nine other imported parasite species are also given, of which *Megarhyssa nortoni nortoni* and *I. ensiger* appear promising. E. G. BRICKELL.

Lawn insects. Anon. (*Home Gdn Bull., U.S. Dep. Agric.*, 1968, No. 53, 24 pp.).—Pests that infest soil and roots, feed on leaves and stems, suck plant juices, inhabit but do not damage lawns, are described together with methods for their control by insecticides. E. G. BRICKELL.

The golden nematode of potatoes and tomatoes. Anon. (*Leafl., U.S. Dep. Agric. Res. Serv.*, 1967, PA-816, 4 pp.).—Control methods are briefly described. E. G. BRICKELL.

The tomato fruit worm [control]. J. Wilcox and A. F. Howland (*Leafl., U.S. Dep. Agric.*, 1967, No. 367, 6 pp.).—Control by insecticides and by culture methods is described. E. G. BRICKELL.

Aphids on leafy vegetables. W. J. Reid, jun. and F. P. Cuthbert, jun. (*Fmrs' Bull., U.S. Dep. Agric.*, 1967, No. 2148, 16 pp.).—Various kinds of aphids are described together with methods for their control by natural means, cultural practices, and insecticides. E. G. BRICKELL.

Nematodes. C. D. Green (*Jl R. agric. Soc.*, 1967, **128**, 206–216).—A review describes the potato cyst nematode, nematodes feeding on sugar-beet and damaging cereals, the pea cyst nematode, and viruses transmitted by nematodes. E. G. BRICKELL.

Pests of cane. Anon. (*A. Rep. Exp. Stn S. Afr. Sug. Ass.*, 1966–7, 90–100).—Field studies were made of the movement and populations of *Numicia* in various sites and the results of insecticide trials using malathion, fenthion and endosulfan are discussed. Techniques for the study and separation of soil arthropods and nematodes are described. J. L. WALPOLE.

Effects of phloem temperature and moisture content on development of the Southern pine beetle. G. C. Gaumer and R. I. Gara (*Contr. Boyce Thompson Inst. Pl. Res.*, 1967, **23**, 373–377).—Phloem moisture content and temp. regimes necessary to produce vigorous broods of *Dendroctonus frontalis* Zimm. were determined, the development being measured in terms of average pupal wt., time to adult emergence, and ratio of increase. In cut logs, temp. of 20 to 22° and R.H. 50 to 60% were most satisfactory. (12 references.) E. G. BRICKELL.

Effect of pruning on silver-leaf disease (*Stereum purpureum* (Pers. Fr.)) and yield of peach and nectarine trees. M. H. Dye (*N.Z. Jl agric. Res.*, 1967, **10**, 435–444).—A field trial established that 26% of peach trees and all nectarine trees receiving standard winter pruning were infected with silver-leaf disease four years after pruning. No infection occurred in trees pruned shortly after harvesting, irrespective of the degree of pruning and whether wound dressing was used or not. E. G. BRICKELL.

Factors and practices related to the occurrence of blotchy ripening in tomato. J. W. Berry, jun. (*Diss. Abstr.* B, 1967, **27**, 2950–2951).—The influence of soil moisture content and other factors affecting transpiration in tomato plants on the occurrence of blotchy ripening is examined. Effects of treating the plants with HgPh acetate (alleged to close stomates) under field or glasshouse conditions or with glycerol, $CuSO_4$, octahexadecanol, water mist, reduction of air movement, or with i.r. heating lamps in the glasshouse on fruit quality (% dry matter, sol. solids, uniformity of colour) are reported. Blotchy ripening and blossom end rot were associated with conditions, respectively, of low and high water stress in the plants. Seed density beneath the blotchy tissue was lower than that beneath normal tissue, diminution in auxin content being a possible contributing factor. The incidence of blotchy ripening was affected by practices causing poor pollination, coupled with adverse weather conditions. A. G. POLLARD.

Bacterial wilt of teak seedlings. S. C. Doo (*Un. Burma J. Life Sci.*, 1968, **1**, 43–45).—The cause of the disease (not widespread) is traced to bacterial accumulations in the xylem consisting of short, motile gram-negative rods that obstruct the flow of the sap. The disease occurs in seedlings in acid soils with low N, P and K contents. Precautionary measures are recommended. P. S. ARUP.

Tomato bushy stunt virus from *Prunus avium* L. I. Field studies and virus characterisation. W. R. Allen and T. R. Davidson (*Can. J. Bot.*, 1967, **45**, 2375–2383).—Tomato bushy stunt virus (TBSV-P) has been isolated for the first time from sweet cherry trees and recovered from the leaves, flowers, seeds and fruit flesh. Characteristic symptoms associated with the virus were pitted fruit flesh, veinal necrosis, leaf twisting and severe shoot stunt. Attempts to discover the means of transmission of the virus proved negative. TBSV-P was distinguishable from the type strain in *Datura* and tomato because although both viruses had the same particle size and morphology they differed slightly in sedimentation rates and in antigenic composition. (14 references.) J. L. WALPOLE.

Factors affecting virulence and pigment production of *Xanthomonas phaseoli* var. *fuscans*. P. K. Basu and V. R. Wallen (*Can. J. Bot.*, 1967, **45**, 2367–2374).—Isolates of *Xanthomonas phaseoli* var. *fuscans* obtained from infected bean seeds stored at 10° for 2–4 months were more virulent than isolates obtained from similar seeds kept for 7–24 months. No correlation was found between the virulence of the organism and either its rate of growth or its ability to produce a brown diffusible pigment. Growth rate and pigment production of all isolates in nutrient broth were similar and reached a maximum after 36 and 48 h respectively, but in a medium containing inorg. salts and yeast extract, the growth rate was relatively slow and pigment development depended on additions to the medium. Tyrosine enhanced while glucose retarded pigment production but neither affected the growth of the pathogen. The formation and colour of the pigment, were unaffected by pH changes between 5·5 and 9·0 and its melanoid nature was demonstrated. (16 references.) J. L. WALPOLE.

Isolation, incidence and virulence of *Ascochyta* spp. of peas from the soil. V. R. Wallen, S. I. Wong and J. Jeun (*Can. J. Bot.*, 1967, **45**, 2243–2247).—*Ascochyta pinodella* (**I**) cause of footrot of peas and *A. pinodes* (**II**), cause of blight, were isolated from field soil. **I** was present in most soils, even in some where peas had not been grown for up to five years but soil cultures of it were only weakly virulent. **II** was isolated less frequently but soil cultures were strongly virulent and inoculated plants showed disease symptoms in five days. (12 references.) J. L. WALPOLE.

Prolificacy, height and spreading ability of some weed species growing among crops cultivated on loess soil. F. Pawłowski (*Annls Univ. Mariae Curie-Sklodowska, Agric.* E, 1966, **21**, 175–189).—50 specimens of each of 26 types of common weeds were collected in almost ripe stage and examined for the no. of seeds and side shoots, and height. They were taken from fields on which 8 crops were cultivated in loess soil (wheat, rye, barley, oats, rape, clover, potatoes and sugar-beet). Almost all weed species produced more seeds in root crops than in other plants. *Capsella bursa-pastoris* was the most prolific and produced 2424 seeds/plant in wheat, 1987 in rye, 1262 in barley, 1012 in oats, 3800 in rape, 2000 in red clover and 8000 in root crops (potatoes and beet). Other major seed producers were *Tripleurospermum inodorum*, *Cirsium arvense*, *Sinapsis arvensis*, *Anthemis arvensis* and *Chenopodium album* which was the most prolific in root crops with 13,000 seeds/plant. Those weed species which are able to produce side shoots did so more abundantly in root crops than in other plants. Almost all spring weeds grew higher in root crops than in cereals, rape or clover, but perennial and wintering species showed a reverse habit of growth. T. M. BARZYKOWSKI.

Effect of aphid numbers and stage of plant growth in determining tolerance to barley yellow dwarf virus in cereals. H. C. Smith (*N.Z. Jl agric. Res.*, 1967, **10**, 445–466).—Inoculation with low aphid numbers at later stages would appear necessary to breed cereals with high resistance to barley yellow dwarf virus for New Zealand conditions. E. G. BRICKELL.

Metabolism of 3′, 4′-dichloropropionanilide: 3, 4-dichloroaniline–lignin complex in rice plants. R. Y. Yih, D. H. McRae and H. F. Wilson (*Science, N.Y.*, 1968, **161**, 376–377).—*Oryza sativa* L. var. Bluebonnet 50 metabolises this herbicide (**I**) to 3, 4-dichloroaniline (**II**); this in turn conjugates with carbohydrates but sol. aniline–carbohydrate complexes account for only a small fraction of the hydrolysed **I**. The major portion of **II** is found complexed with polymeric cell constituents, chiefly lignin. The aniline is lignin-bound as **II** and not as **I**. C.V.

Influence of simazine on growth and nitrogen metabolism of plants. J. A. Tweedy (*Diss. Abstr.* B, 1967, **27**, 2956).—The increased growth and N content of several species of plants following the application of simazine (*S*) was investigated using maize grown in culture solution as test plant, the culture nutrient being low in NO_3^- and maintained at low temp. Neither the dry wt. nor the N content was increased by *S* treatment if the NO_3^- level or the temp. were optimal for growth or if NH_4^+ was substituted for NO_3^- in the nutrient. *S* did not affect the respiration rate of excised roots of barley, cucumber or maize seedlings germinated at high or low temp. Higher NO_3^--reductase activity occurred in *S*-treated maize plants grown at low temp. with low levels of NO_3^-; leaves of such plants when exposed to atm. containing $^{14}CO_2$ during photosynthesis, contained larger proportions of ^{14}C-labelled aspartic and glutamic acids. A. G. POLLARD.

Oxidation of methyl- and dimethylcarbamate insecticide chemicals by microsomal enzymes and anticholinesterase activity of the metabolites. E. S. Oonnithan and J. E. Casida (*J. agric. Fd Chem.*, 1968, **16**, 28–44).—Treatment of 33 of the insecticides (**I**) with a rat liver microsome-$NADPH_2$ system gave metabolites from each **I** by one or more of the following types of reaction: *N*-demethylation; conversion of *N*-methyl to *N*-formamide or to *N*-hydroxymethyl groups; aromatic ring hydroxylation or formation of a dihydrodihydroxy deriv.; *O*-dealkylation; alkylhydroxylation of an aralkyl

substituent; sulphoxidation. The anticholinesterase activities of the metabolites were sometimes greater and sometimes less than those of the original **I**. Hydrolysis of the carbamate ester was not a major type of reaction, but some of the metabolites were hydrolysed (spontaneously or enzymically) when formed. (36 references.) P. S. ARUP.

Excretion of DDT by migratory birds. J. M. Harvey (*Can. J. Zool.*, 1967, **45,** 629–633).—Starlings fed with 4·75 mg radioactive DDT per day for 5 days absorbed < 25% of the insecticide. Concn. remained high in the body and liver for 1 week but decreased more quickly in the brain. After 10 days < 10% of the ingested DDT remained. E. G. BRICKELL.

Metabolism of TOK herbicide in the dairy cow. W. H. Gutenmann and D. J. Lisk (*J. Dairy Sci.*, 1967, **50,** 1516–1518).—No residues of the herbicide 2,4-dichloro-4′-nitrodiphenyl ether (TOK) were found in the milk, urine, or faeces of a cow fed 5 ppm of the herbicide in its feed. *In vitro* tests showed that TOK rapidly disappeared in rumen fluid with the production of a metabolite having a retention time identical with that of 2,4-dichloro-4′-aminodiphenyl ether. This metabolite was not detected in the milk, urine, or faeces. M. O'LEARY.

Possible new approach to chemical control of plant-feeding insects. C. E. Dyte (*Nature, Lond.*, 1967, **216,** 298).—Discusses briefly, by reference to the literature on plant-host relationships, the possibility of overcoming or interfering with the detoxication mechanism in resistant insects by application of a synergist to enhance the action of any naturally occurring protective substances in the plant. This synergistic approach has interesting possibilities either against fungi or in plants containing potentially toxic compounds chiefly in parts not eaten by man, e.g. solanin in potato-plant leaves. The synergistic properties of methylenedioxyphenyl compounds are highlighted. (21 references.) W. J. BAKER.

Temperature coefficient of insect susceptibility to insecticides. B. K. Rai (*Indian J. exp. Biol.*, 1967, **5,** 151–155).—The values of post-treatment temp. coeff. of trichlorphon (**I**), dichlorvos, malathion, Malaoxon, diazinon, Diazoxon, parathion, Paraoxon, aldrin, dieldrin, lindane, DDT, carbaryl and pyrethrins on *Locusta migratoria* at temp. of 15, 27, and 35° depended on their positive or negative rates of toxicant balance formation *in vivo* (balance of activation and detoxification *in vivo*) and rates of penetration. For topical application of DDT and injection of **I** the disc effect was greater at 27 and 35 than at 15°. With the other insecticides there was no significant difference. E. G. BRICKELL.

Use of Disulfoton against pineapple wilt pest, *Dysmicoccus brevipes* Ckl. A. Villardebo, R. Guérout, M. Barbier and Y. Gicquiaux (*Fruits d'outre mer*, 1968, **23,** 67–78).—Results better than those obtained with parathion were obtained with this systemic insecticide [*O, O*-diethyl-*S*-2-(ethylthio)ethyl phosphorodithioate]. The no. of applications per season (at 0·025 g per plant) was initially 8–12, but could eventually be reduced to 3–4. Residues found in treated plants were < 0·15 ppm. P. S. ARUP.

Recommendations for chemical control of pests of rubber plantations. Anon. (*Plrs' Bull. Rubb. Res. Inst. Malaya*, 1968, No. 95, 67–68).—A table gives the rates and methods of application of eleven standard pesticides for the control of termites, cockchafer grubs, mites, thrips, scale insects, mealy bugs, caterpillars, leaf-eating beetles, grass-hoppers and crickets. J. L. WALPOLE.

Effectiveness of standard and new compounds for control of bud mite *Aceria sheldoni* Ewing on Navel oranges. A. Schwartz and F. J. Riekert (*S. Afr. J. agric. Sci.*, 1967, **10,** 609–616).—Standard applications of parathion, Rogor (dimethoate) or Thiodan (hexachlorohexahydromethanobenzodioxanthiepin-3-oxide) gave temporary control of the mite; further applications during summer were often necessary. Kelthane [1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol] was quite effective for ~ 2 months. The best results were obtained with chlorobenzilate (ethyl 4, 4-dichlorodiphenylglycollate) and with Kelthane which was also effective against citrus red mite; both of these are less harmful than other insecticides to beneficial species. The fungicide Morestan (6-methyl-2,3-quinoxaline dithiol cyclic carbonate) gave promising results as a safe multipurpose insecticide. P. S. ARUP.

Control of powdery mildew with chemical antisporulants. R. J. Lukens and J. G. Horsfall (*Phytopathology*, 1967, **57,** 342).—Antisporulants, 2, 4-dinitro-6-caprylphenyl-1-(4-pentenoate) (**I**), tetraiodoethylene (**II**) and hexachloro-2-propanol (**III**) were applied as sprays (200 ppm) to leaves of various affected plants. **I** controlled *Erysiphe polygoni* on inoculated bean, natural infection on knotweed and *E. cichoracearum* on dandelion and plantain; **II** was effective against bean and dandelion mildews, whereas **III** had very little antisporulant action. **I** and **II** reduced both sporulation and the mycelial growth of the pathogens. A. G. POLLARD.

Three-year effects of systemic fungicides applied for the control of *Cytospora* in Italian plum trees. A. W. Helton and W. J. Kochan (*Can. J. Bot.*, 1967, **45,** 2017–2020).—Italian plum trees (*Prunus domestica* L.) infected with *Cytospora cincta* were treated with 8-quinolinol benzoate, Phytoactin L-456 and CTS (cycloheximide thiosemicarbazone) and observed over a three-year period. All three materials gave significant reductions in canker-expansion rate but CTS gave the best results, causing a near halt to the canker expansion and providing increases in yield. The effects of the treatments showed little diminution at the end of the three years. J. L. WALPOLE.

Hot-water and chemical treatments to control scald on Stayman apples. R. E. Hardenburg (*Proc. Am. Soc. hort. Sci.*, 1967, **90,** 484–490).—Scald on Stayman apples was controlled by dipping the fruit for 30–60 sec. in water at 54°, 1–2 days after harvest. A 10-sec. dip in either 2,000 ppm diphenylamine or in 2,700 ppm ethoxyquin was equally effective. A. H. CORNFIELD.

Phenoxy herbicides. D. L. Klingman and W. C. Shaw (*Fmrs' Bull., U.S. Dep. Agric.*, 1967, No. 2183; 24 pp).—Methods are described for the most effective use of 2:4-D, 2:4:5-T, MCPA, Silvex, and 2:4-DB. Tabular data of the susceptibility of common weeds to these products are appended. E. G. BRICKELL.

Comparison of the effects of arsenic and amitrole (3-amino-1, 2, 4-triazole) in the control of *Eriocereus martinii* (Lab) Rice. G. Diatloff (*J. Aust. Inst. agric. Sci.*, 1967, **33,** 109).—Amitrole alone was unsatisfactory but combined with NH_4CNS it gave a kill of 50%. However, although low in mammalian toxicity and easy to apply, it was unreliable compared with a 10% As_2O_5 and 10% chlorate combination. E. G. BRICKELL.

Yield increases of wheat following the control of skeleton weed with picloram. V. Molnar, T. W. Donaldson and W. T. Parsons (*J. Aust. Inst. agric. Sci.*, 1967, **33,** 345–346).—Application of 0, 3, 4, 6 and 12 oz/acre of picloram (4-amino-3, 5, 6-trichloropicolinic acid) (**I**) to wheat at the 3- to 4-leaf stage to control skeleton weed (*Chondrilla juncea* L.) seriously reduces the yield but spraying skeleton weed with low rates of **I** in the year prior to sowing can result in a considerable increase in the yield of wheat. J. L. WALPOLE.

Phytotoxicity and mechanism of action of herbicide and herbicide–adjuvant combinations on quackgrass (*Agropyron repens*, L., Beauv.) A. R. Putnam (*Diss. Abstr.* B, 1967, **29,** 2954–2955).—Various adjuvants were evaluated for increasing the herbicidal action of simazine (*S*), diuron (*D*) amitrole-T, (*A*) and paraquat (*P*). Use of the adjuvants with *S* or *D* for application to cucumber beds under glass increased the herbicidal action but did not give effective control of quackgrass. The increased activity of *A* and *P* found in field trials was due, in part, to the increased wetting of the plants. Certain herbicide combinations, e.g. *P* at 0·5 lb/acre with *S* or *D* at 3–4 lb./acre, exhibited synergistic effects on quackgrass which provided an acceptable long-term control. The apparent synergism did not result from increased absorption of either herbicide; *P* destroyed the aerial parts of the grass, leaving the plants more susceptible to simazine absorbed through the roots. *P* moved both upward and downward in the grass leaves, more being translocated in light than in darkness. *S* moved only acropetally; *P* did not increase this movement appreciably. Application of *A* followed after 7 days by that of *P* resulted in phytotoxicity exceeding that produced by the two herbicides applied simultaneously. Foliar destruction by *P* or by cutting, following the application of *A*, diminished regrowth. Pretreatment with *A* increased the basipetal movement of *P* in the plants but simultaneous application lowered the absorption and translocation of both *P* and *A*. A. G. POLLARD.

Influence of doses and times of application of Afalon on weed control on potato plantations. F. Pawłowski (*Annls Univ. Mariae Curie-Sklodowska, Agric.* E, 1966, **21,** 191–206).—The action of Afalon (West-German urea-based herbicide) as a weed killer on potato plantations, and its influence on the yield and starch contents of tubers were investigated. Afalon, in doses of 2, 3 and 4 kg/ha, was applied at two different times—at potato tubers planting time and sprouting time. Afalon killed almost all annual and checked the growth of perennial weeds. The most effective doses were 3 and 4 kg/ha, which reduced the no. of weeds by 60% and their air-dry mass by 93%. The yield of tubers from the plot sprayed at planting time was increased by ~70 quintals/ha, i.e. by 23%; all doses applied at sprouting time were about half as effective. The

starch contents in tubers decreased slightly, but considering the increase in the bulk of tubers, the total amount of starch was still higher. The application of 2–3 kg/ha of Afalon, immediately before planting the tubers is recommended. If Afalon has to be applied after sprouting of tubers, however, the dose must not excede 2 kg/ha. (11 references.) T. M. BARZYKOWSKI.

Comparison of weed-killing action of some herbicides in orchards. J. Lipecki (*Annls Univ. Mariae Curie-Sklodowska, Agric.* E., 1966, **21**, 207–218).—The herbicidal action of simazine and atrazine was compared in two orchards. Prolonged application of these herbicides, by killing some annual weeds, creates favourable conditions for stronger development of weeds resistant to herbicides and of perennial weeds, especially *Convolvulus arvensis*. Development of other resistant species, e.g., *Cirsium arvense*, *Equisetum arvense*, *Sonchus arvensis* and *Taraxacum officinale*, depends partly on the amount of pptn. Good reduction of weeds in orchards was achieved by application of complementary herbicides, e.g., simazine + Weedazol T-L, or simazine + Gramoxone, combined with manual hoeing. (19 references.) T. M. BARZYKOWSKI.

Chemical control of weeds in pineapple plantations. State of the problem in the light of recent trials. C. Py (*Fruits d'outre mer*, 1968, **23**, 3–12).—None of the herbicides under test gave complete satisfaction when applied during the preparation of the soil. Diuron is recommended for use during the planting. After a preliminary application to the soil, the shoots are planted with as little disturbance of the soil as possible; a second application is made between the rows. During the growth of the crop ametryne may be used as a spray, applied only to the weeds between the rows. A scheme of instructions is presented. P. S. ARUP.

Herbicides. Anon. (*A. Rep. Exp. Stn S. Afr. Sug. Ass.*, 1966–7, 60–68).—Some results of herbicide screening trials on sugar-cane are presented and discussed, but give no reason for departing from the standard recommendation of a pre-emergent spray with 2, 4-D. J. L. WALPOLE.

General non-selective weed control with monosodium acid methane arsonate (MSMA) based mixtures in rubber plantations. Anon. (*Plrs' Bull. Rubb. Res. Inst. Malaya*, 1968, No. 95, 52–55).—Useful alternatives to Na arsenite in rubber plantations are provided by mixtures of MSMA and 2, 4-D amine with and without dalapon or $NaClO_3$. The rates of use and spraying frequency of these mixtures are given together with a table of susceptible weeds. J. L. WALPOLE.

Pesticide transformations: production of chloroazobenzenes from chloroanilines. R. Bartha, H. A. B. Linke and D. Pramer (*Science, N.Y.*, 1968, **161**, 582–583).—Aniline (**I**) and 11 different chloroanilines (**II**) were added to soil. No azo compound was formed from **I** but all monochloro- and some dichloro-**I** were transformed to their corresponding dichloro- and tetrachloro-azobenzenes. Other dichloro-**I** and trichloro-**I** were stable in soil. Peroxidase catalysed the formation of azo compounds by some **II**. The results are tabulated. C.V.

Silicates as carriers and diluents for insecticides. I. Raw materials. D. Alvarez-Estrada and J. Espinosa de los Monteros (*Revta Agroquim. Tecnol. Aliment.*, 1967, **7**, 464–475).—Chemical composition and physico-chemical characteristics (sp. surface, ion-exchange capacity, sp. wt., bulk density, pH of 10% suspension, i.r. and *X*-ray spectra, thermogravimetric and differential thermal analysis curves) are reported for typical samples of atapulgite, bentonite, kaolin, halloysite, talc and sericite. Formulation techniques for the use of these materials as carriers of solid or liquid insecticides are briefly discussed, with evaluations of the homogeneity, fluidity and absorption capacity of formulations with endrin and malathion. (16 references.) E. C. APLING.

'Booby-trapping' as alternative to sterile males for insect control. M. J. Whitten and K. R. Norris (*Nature, Lond.*, 1967, **216**, 1136).—Discusses possible applications of a booby-trapping system, e.g., exposing dieldrin-treated female flies (*Lucilia cuprina*) of resistant strain to males from a susceptible strain so that each female kills ≤ 100 males by contact during attempted mating. With such a system both sexes are effective in destroying or sterilising their partners, multiple matings are advantageous, only very low concn. of insecticide are required, and the range of possible insecticides is increased. Disadvantages are the probable entry of genes for insecticide resistance into the gene pool of field populations and the emergence of pesticide resistance into other regions under selection pressure, but use of a suitable stable chemosterilant as a non-lethal topical dose should resolve these problems. Insect characteristics most suited for booby-trapping systems are indicated, and the Australian sheep blowfly is shown to possess all these characteristics. W. J. BAKER.

Fate of atrazine in maize, cotton and soyabeans. D. R. Roberts (*Diss. Abstr.* B, 1967, **27**, 3002).—The resistance of maize (**I**), cotton (**II**) and soyabean (**III**) to the intake (via roots or by threads sewn through stems), translocation and degradation of atrazine (*A*) diminished in the order named. Seedlings were placed in nutrient solutions containing ^{14}C-labelled *A* for 3 h and subsequently examined at intervals up to 96 h. The distribution of *A*, hydroxy-*A* and some unidentified ^{14}C-labelled metabolites in the seedlings was examined. In another experiment the absorption of *A* via roots or stems of **III** exceeded that by **II** or **I**, expressed as rate or amount per unit fresh wt. Degradation of *A* was more rapid in **I** than in **II** or **III**; roots played no part in the degradation. In **I** the % of unknown metabolites of *A* remained at approx. the same level through the 96 h experimental period, as also did that of hydroxy-*A* over a 10 h period following the intake of *A*. Root-absorbed ^{14}C-*A* was much more mobile in **III** than in **II** or **I**; it moved towards leaf margins and interveinal areas of **III** where toxic effects first appeared. In **II** ^{14}C accumulated in the lysigenous glands. In **I** ^{14}C appeared evenly distributed. Movement of ^{14}C after applications to shoots was largely acropetal but small amounts moved basipetally in all species. In **III** movement of ^{14}C after treatment with hydroxy-*A* was much slower than after *A* but in **I** the rate of movement was similar for both. A. G. POLLARD.

Absorption and translocation of dieldrin by forage crops and its extraction from plant tissues. W. B. Wheeler (*Diss. Abstr.* B, 1967, **27**, 3018).—The possible intake of dieldrin (*D*) by roots of forage plants and its subsequent translocation within the plant systems are examined by growing the plants in sand or soil containing isotopically-labelled *D*. Maize, wheat, orchard-grass and lucerne absorbed *D* which was distributed throughout the plants. Quant. extraction of the radioactive material from the plants by a blending technique was unsuccessful. By first blending the fresh plant material in n-hexane–isopropyl alcohol and then extracting (Soxhlet) with $CHCl_3$–CH_3OH all radioactive matter was removed and shown to be dieldrin. In all cases Soxhlet extraction removed 10–50% of the total insecticide found. Absorption and translocation of *D* by all four plant species was much greater in sand- than in soil-cultures. More *D* was present in the second than in the first cutting of the forage. In sand-cultured wheat the young plants contained relatively high concn. of *D* expressed as ppm but only moderate total amounts expressed as μg. With advancing growth the relative concn. of *D* diminished but the total intake increased. Intake of *D* tended to increase with rise of temp. No metabolic products of *D* were found in the plants. A. G. POLLARD.

Thin-layer chromatography system for detecting 2, 6-dichloro-4-nitroaniline (Botran) and related compounds. C. L. Keswani and D. J. Weber (*Phytopathology*, 1967, **57**, 462).—The method serves to separate substituted nitroanilines related to Botran using SiO_2-gel G on glass plates and, as solvent, a 3:1 mixture of hexane and acetone. Diazo-colour is developed by spraying successively with 5% $NaNO_2$ in N-HCl, 5% phenol in water and 7% Na_2CO_3 in water. Fourteen substituted and non-substituted nitroanilines may be separated by this method: *o*-, *m*-, and *p*-nitroaniline may also be separated. The method is utilised in an investigation of the resistance of strains of *Rhizopus arrhizus* to Botran. A. G. POLLARD.

Determination of dimethoate insecticides (*O*, *O*-dimethyldithiophosphorylacetic acid *N*-methylamide) in technical products and in liquid formulations. B. Bazzi, L. Abbruzzese, R. Fabbrini, G. Galluzzi and M. Radice (*Chimica Ind., Milano*, 1968, **50**, 902–904).—Two methods for the determination of the dimethoate (Rogor) and its main impurities are suggested. T.l.c. on SiO_2 is used to determine dimethoate and the impurities methyl *O*,*O*-dimethyldithiophosphorylacetate and methyl *O*,*O*,*S*-trimethyldithiophosphate. The same compounds may also be determined by g.l.c. using a column of 5% QF1 on chromosorb W at 170° with He as carrier gas. Impurity concn. of 0·5–5·0% are determined. (11 references.) C. A. FINCH.

Gas chromatographic determination of captan residues. W. W. Kilgore, W. Winterlin and R. White (*J. agric. Fd Chem.*, 1967, **15**, 1035–1037).—Residues are extracted from fruits with C_6H_6, and from cottonseed products with MeCN. Residues from cottonseed products require a column chromatographic cleanup on Florisil. Determinations are carried out by electron capture g.l.c. As little as 0·01 ppm could be detected in 100 g of the macerated material. Recoveries were 92%. P. S. ARUP.

Bioassay of captan by zebrafish larvae. Z. H. Abedi and W. P. McKinley (*Nature, Lond.*, 1967, **216**, 1321–1322).—Method is based

on selective abnormal sensitivity of the larvae (4-days old) of *Brachydanio rerio* to ppm concn. of captan (I). Within 90 min. ~98% of the larvae die, mainly from severe head-injury, when exposed to concn. of 1 ppm I in acetone. Rapid assay of μg amounts of I is thus obtained by preparing a standard dosage-mortality curve. There is no response with other pesticides.

W. J. BAKER.

Dinitrocarbonates, carboxylates, and thioesters and pesticidal or herbicidal compositions [containing them]. Murphy Chemical Co. Ltd. (Inventors: M. Pianka and J. D. Edwards) (B.P.1,080,282, 22.6., 12.7., and 20.11.63).—Compounds claimed have herbicidal, pesticidal, and especially acaricidal properties and the formula 4, 6, 5, 2, 1-$(NO_2)_2$ $C_6HMeR.O.CX.YR'$ where X is O or S; Y is O, S, or a bond; R is branched alkyl of 3–12 C; and R′ is saturated or unsaturated aliphatic hydrocarbon residue(which may contain halogen, aryl, alkoxy, alicyclyl, heterocyclyl) or is a (substituted) aryl, heterocyclyl or cycloalkyl group. In an example, a mixture of 4, 6, 5, 2, 1-$(NO_2)_2$ $C_6HMeBu^t.OH$ (prep. described), acetone, and K_2CO_3 is boiled during 80 min., then a solution of $ClCO_2Me$ is added. After a further 5·25 h at the boil the filtered solution is worked up to give 4, 6-dinitro-5-methyl-2-t-butylphenyl Me carbonate, m.p. 102·5° (EtOH) in 94·5% yield. F. R. BASFORD.

Isohydrazone derivatives. Whiffen and Sons Ltd. (Inventors: M. D. Hinchliffe and J. Miller) (B.P. 1,085,794, 20.8.63).—Possessing pesticidal, herbicidal and pharmacological properties and useful as intermediates for the corresponding hydrazines, the title compounds have the formula $R^3R^2.A:(X)(R^1)$, where R^1 is H, X, alkyl or aryl, R^2 and R^3 are (same or different) H, alkyl, aryl or together form a carbocyclic ring, A is a C:N_2 ring, R^2 and R^3 being joined to the C atom and X and R^1 to separate N atoms; X is $C(R^4)(R^5)\cdot(CH_2)_n\cdot C(R^6)(R^7)OH$, where R^4—R^7 are (same or different) H or alkyl and n=0–3. They are prepared by reacting an isohydrazone with the appropriate cyclic ether. Thus, *N*-(β-hydroxyethyl)-methyl ethyl ketone isohydrazone b.p. 91–94°/0·5 mm, is prepared from ethylene oxide and methyl ethyl ketone isohydrazone. S. D. HUGGINS.

Isonitrile [derivatives]. Farbenfabriken Bayer A.-G. (Inventors: U. Fetzer, I. Ugi, G. Unterstenhofer and I. Hammann) (B.P. 1,086,417, 13.5.65. Ger., 21.5.64).—The title compounds have the formula $(CN)_mA^1N(X):NA^2(CN)_n$, wherein A^1 and A^2 are, independently, optionally substituted aromatic groups, optionally substituted arylene-carbonyls or arylene-sulphonyls; X is a free electron pair, O or N connected to A^2 to form a ring and m and n are 0, 1 or 2 and $(m+n)=1$ or 2. They are prepared by reacting a formamide of formula $(OHCNH)_mA^1N(\cdot X):NA^2(NHCHO)_n$ with a water-eliminating acyl halide in the presence of a base at −20 to +60°. Thus, 4-formylamino-2, 3′-dimethylazobenzene is mixed with CH_2Cl_2, NEt_3 and reacted with $COCl_2$ at 20°. After neutralisation with NH_3, 4-isocyano-2,3′-dimethylazobenzene, m.p. 108–110°, is recovered. The products have insecticidal, acaricidal and fungicidal properties, and low mammalian toxicity.

S. D. HUGGINS.

Phenazines and biocidal compositions containing them. Shell Internationale Research Mij. N.V. (Inventors: J. T. Hackmann, J. T. W. Montagne and B. Cross) (B.P. 1,086,522, 3.9.64).—Compounds with fungicidal, acaricidal, and herbicidal activity comprise phenazines (and mono- and di-oxides thereof) substituted in one or both benzene nuclei by up to 4 halogen, alkyl, aryl, aralkyl, or alkaryl of >25C (optionally substituted by halogen or OH), OH, CNS, N_3, NO_2, NRR^1 (R and R^1 are H or alkyl of > 25C), or alkoxy of >25C, or a benzo fused ring which may contain halogen and/or alkyl. A representative compound is 2-ethylphenazine, m.p. 59–60°, prepared in 12% yield by heating a mixture of 4-ethylcatechol and *o*-$(NH_2)_2C_6H_4$ at 240° during 3 days; then heating a solution of the product in toluene with HgO during 4 h at 50°; filtering the cooled mixture; concentrating and purifying the filtrate by treatment with acidic Al_2O_3, and recrystallising recovered material from light petroleum. The activity of many products is recorded. F. R. BASFORD.

Phosphorus-containing esters. Farbenfabriken Bayer (Inventor: W. Lorenz) (B.P. 1,080,434, 19.4.66. Ger., 18.5.65).—Possessing insecticidal and acaricidal properties, the esters have the formula $C_6H_4\cdot A\cdot CH(Me)SP(X)(R)OR^1$ wherein R is alkyl or alkoxy with 1–4C, or Ph, R^1 is alkyl with 1–4C, X is S or O and A is the azimide ring (CON_3) condensed with the benzene nucleus. A salt, of formula $(R)R^1O\cdot P(X)SM$, wherein M is NH_4 or monovalent metal equiv. is reacted with the halide $C_6H_4(CON_3)\cdot CH(Me)\cdot Z$ where Z is halogen, in an inert org. solvent at 50–70°.

S. D. HUGGINS.

Phosphorus-containing esters. Farbenfabriken Bayer A.-G. (Inventors: K-J. Schmidt and I. Hammann) (B.P. 1,081,249, 20.4.66 Ger., 26.5. and 14.7.65).—P-containing esters of 2-hydroxyquinoxaline (which have insecticidal and acaricidal properties) of the formula $Q\cdot OP(X)(OR)R^1$, where Q is the quinoxaline radical; R and R^1 are alkyl radicals containing 1–4 C atoms or R^1 is an alkoxy or phenyl radical; X=O or S; are prepared by reacting 2-hydroxyquinoxaline (I) with an ester halide, $(RO)(R^1)P(:X)\cdot Z$ (Z=halogen), at 20–120° in a solvent (e.g. C_6H_6) and the presence of an acid acceptor. Thus, I is reacted with *O*, *O*-diethylphosphoric acid ester chloride in acetonitrile, in presence of K_2CO_3 to give *O*,*O*-diethylphosphoric acid-*O*-[quinoxalyl-2]ester.

J. A. SUGDEN.

Phosphorus-containing esters. Farbenfabriken Bayer A.-G. (Inventors: C. Metzger and I. Hammann) (B.P. 1,081,277, 25.4.66, Ger., 24.7.65).—P-containing esters of the formula $RCO\cdot O\cdot CH_2\cdot S\cdot CH_2SP(:S)(OR^1)(R^2)$ possess insecticidal and acaricidal properties. They are prepared when a thioether $RCO\cdot O\cdot CH_2\cdot S\cdot CH_2\cdot Z$ is reacted with $HS\cdot P(S)(OR^1)(R^2)$ either in the form of a monovalent metal or an ammonium salt in the presence of an acid-binding agent at 40–100°. R=a straight chain or branched alkyl radical of 1–6 C, or a cycloalkyl; R^1 is a straight chain or branched alkyl radical and R^2 an alkoxy radical and Z a halogen atom. Thus: α-acetyloxy-α′-*O*,*O*-dimethylthionothiolphosphoryldimethyl thioether. b.p. 116–120/2 mm Hg, is prepared by adding α-acetyloxy-α′-chlorodimethyl thioether dropwise at 60° to a solution of Na *O*,*O*-dimethylthionothiolphosphate in acetonitrile.

E. ENOS JONES.

Organophosphorus insecticides. Shell Internationale Research Mij., N.V. (Inventors: G. O. Osborne, S. B. Webb, and J. Wood) (B.P. 1,086,048, 1.10.65).—Compounds with insecticidal properties (active against, e.g., *Musca domestica*, *Aedes aegypti*, *Phaedon cochlearia*, *Plutella maculipennis*, *Acyrthosiphon pisum*, *Tetranychus telarius*) have the formula $R^{III}R^{IV}P(X)\cdot O\cdot C_6H_{3-n}R_nR^VR^{VI}$ wherein R is halogen or alkoxy; n is 0–3; R^{III} and R^{IV} are alkenyloxy or optionally substituted alkoxy, NH_2, or substituted NH_2; X is O or S; and R^V and R^{VI} together represent a $CR^IR^{II}\cdot O\cdot CX_2$ chain in the 1, 2-position (R^I and R^{II} are H or alkyl or together are alkylidene). In an example, a mixture of 5-hydroxyphthalide, $(OMe)_2PSCl$, K_2CO_3, and $COMeBu^t$ is boiled during 5 h, then cooled, and filtered. The filtrate is worked up to give 5-(dimethoxyphosphinothioyloxy)phthalide (63%), m.p. 59·5–61·5 (hexane). A further 21 compounds are described. F. R. BASFORD.

Substituted acetamides and pesticidal compositions thereof. Allied Chemical Corp. (B.P. 1,086,295, 23.6.65. U.S., 25.6.64).—The title compounds, viz., $(p\text{-}ClC_6H_4)_2CCl\cdot CONRR^1$ (R and R^1 are H, org. radicals, or NRR^1 is heterocyclyl) are active against, e.g., *Prodenia eridania* (Southern armyworm) larvae, and *Tetranychus telarius* (2-spotted spider mite). In an example, a solution of $COCl\cdot CCl(C_6H_4Cl\text{-}p)_2$ in ether is added during 1 h to a solution of $p\text{-}NH_2C_6H_4Cl$ in ether at −10° to 0°, then after heating to room temp. the ppt is filtered off. The filtrate is evaporated and the residue is diluted with light petroleum, with separation of chloro-di-(*p*-chlorophenyl)acet-*N*-*p*-chloroanilide m.p. 113°.

F. R. BASFORD.

Phosphoric and thiophosphoric acid ester derivatives. Sandoz Ltd. (Inventors: H. Helfenberger and K. Lutz) (B.P. 1,085,340, 16.8.66. Switz., 3.9.65).—Possessing insecticidal, acaricidal and nematocidal properties, the claimed P compounds are quinoxalines, substituted in the 2-position by R^3(H, Me, OH or C_1-C_4 alkyl carboxylate), in the 3-position by $OP(Y)(R^1)R^2$ [Y is S or O, R^1 is alkoxy (C_1-C_4) and R_2 is also alkoxy or -*N*(alkyl C_1-C_4)$_2$] and the benzene ring is substituted by $(R^4)_n$ [R^4 is Me, halogen, NO_2 or H and n=1, 2, 3 or 4]. The corresponding quinoxaline, substituted by OZ in the 3-position [Z is a cation] is reacted with the P compound $R^1(R^2)P(Y)X$, where X is a halogen atom, in the presence of $MeBu^tCO$ at 30–80°. Compounds claimed include *O*,*O*-diethyl-*O*-quinoxalinyl-2-thionophosphate. S. D. HUGGINS.

Nematocidal compositions. Farbenfabriken Bayer A.G. (Inventors: B. Homeyer, K. Swincicki, S. Petersen and K. H. Mayer) (B.P. 1,081,259, 25,7.66. Ger., 2.8.65).—Nematodes are destroyed or controlled by adding to the soil ~5–80 ppm of 4-chloropyridine-oxide (I), or a mixture thereof with liquid or solid diluents. Surface active compounds may also be added. A number of phytopathogenic nematodes, including leaf nematodes, and free-living root nematodes are specifically mentioned. Besides possessing a very good compatibility with plants, I possesses appreciable growth-promoting properties. H. L. WHITEHEAD.

Dithiolphosphoric acid esters. Farbenfabriken Bayer A.-G. (Inventors: K. Mannes, G. Schrader and B. Homeyer) (B.P.

1,081,270, 20.6.66. Ger., 8.7.65).—The esters, $(RS)_2PO\cdot OEt$, possessing nematocidal activity, are produced when an *O*-ethyl phosphoric acid ester dihalide of the formula: $EtOP(O)Z_2$ (Z is a halogen atom and R is Pr or Pr^i) is reacted with PrSH or Pr^{i}SH at −20° to the b.p., in the presence of an acid-binding agent, or with a corresponding alkali metal- or ammonium-mercaptide.

E. ENOS JONES.

[Plant fungicide] basic copper sulphate compositions. Takeda Chemical Industries Ltd. (B.P. 1,080,738, 19.8.64. Jap., 20.8.63).—$Cu_4(OH)_6SO_4.H_2O$ with *X*-ray diffraction bands at 6·95 (very strong) 3·47, 2·70, 2·62, 2·42, 2·33, 2·26, 2·02, 1·99 (medium) and 1·54Å (weak) is prepared by reacting aq. $CuSO_4$ with an alkali or NH_4OH in presence of H_3PO_4, or an alkali or NH_4 salt thereof. A typical composition is Cu 51-57, SO_4 7–21, PO_4 0·15–9, OH 22–26 and H_2O 2–4 wt.%. The compositions are more active than natural brochantite or langite against e.g. *Colletotrichum lagenarium*, *Phytophthora infestans* and other blights and mildews attacking vegetables.

J. A. SUGDEN.

Heterocyclic compounds as fungicides. Imperial Chemical Industries Ltd. (Inventor: L. A. Summers) (B.P. 1,080,864, 26.2.63).—The alkyl deriv. (**I**) of 3-alkyl-4-arylazo-2-isoxazolin-5-ones are obtained e.g. by alkylating 3-alkyl(1-4C)-4-arylazo (optionally substituted aryl)-2-isoxazoline-5-ones with diazoalkane or an alkyl halide or sulphate in inert org. solvent. The fungicidal compositions contain 10–80% by wt. of **I**. They are specially effective in the control of powdery mildew on cucumbers, apples and oats, rice blast, chocolate spot on beans and late blight on tomatoes. They are effective in protecting seeds from certain soil- or seed-borne diseases such as foot rot of wheat and peas.

S. D. HUGGINS.

Fungicidal compositions. Sankyo Co. Ltd. and Dainippon Ink and Chemicals Inc. (B.P. 1,080,989, 2.11.64; Jap. 1.11.63 and 25.9.64).—Pentachlorobenzyl alcohol, or aliphatic monocarboxylic esters of it, together with an agriculturally acceptable carrier, are used. The treatment of rice blast, caused by *Piricularia oryzae* is specifically mentioned.

S. D. HUGGINS.

Fungicidal compositions. Farbenfabriken Bayer A.-G. (Inventors: E. Degener, H. Scheinpflug and H.-G. Schmelzer) (B.P. 1,085,474, 22.7.66. Ger., 30.7.65).—The pentachlorobenzoylamino compounds of formula $C_6Cl_5\cdot CONHR$, wherein R is H, NH_2, OH, 1-OH-2,2,2-trichloroethyl, hydroxymethyl, alkoxymethyl or a radical of formula $HO(CH_2CH_2O)_n\cdot CH_2$-, where $n=1$, 2 or 3, are applied in a solid or liquid carrier, optionally containing a surface-active agent. In an example of prep., *N*-(2, 2, 2-trichloro-1-hydroxyethyl)pentachlorobenzamide is obtained almost quant. by heating pentachlorobenzamide with excess anhyd. chloral at 80–110°. These compounds have excellent effect when used for combating *Piricularia oryzae* in rice. Several other species are mentioned.

S. D. HUGGINS.

Fungicidal carboxylic acid hydrazides. Schering A.-G. (B.P. 1,085,697, 30.8.66. Ger., 14.9.65).—The claimed fungicides have the formula $4,3,1\text{-}(X)(Y)C_6H_3\cdot NH\cdot NH\cdot CO\cdot R$, wherein R is vinyl, Bu^n or n-amyl and X and Y are H, Cl or NO_2. The hydrazine of formula (X) (Y) $C_6H_3\cdot NH\cdot NH_2$ is reacted with a carboxylic acid or its deriv. of formula RCOZ, wherein Z is OH, *O*-alkyl, OCOR or a halogen atom. E.g., valeryl 3,4-dichlorophenylhydrazide, m.p. 121–122° (light petroleum/benzene) is prepared by reacting valeryl chloride with 3,4-dichlorophenylhydrazine in acetonitrile, in presence of NEt_3. Experimental results against *Fusarium nivale* on rye, *Helminthosporium gramineum* disease in barley and effectiveness against *Ustilago avenae*, are shown.

S. D. HUGGINS.

Bis-quaternary salts of 4,4′-bipyridyls. Imperial Chemical Industries Ltd. (Inventors: J. E. Colchester and J. H. Entwisle) (B.P. 1,073,081, 9.4.64 and 8.9.64).—Used as herbicides, the claimed *N*, *N*′-disubstituted 4,4′-bipyridylium salts are obtained by treating an *N*,*N*′-disubstituted tetrahydrobipyridyl with an org. oxidising agent, e.g. quinone, which has a redox potential more positive than −1·48V with respect to the standard Hg_2Cl_2 electrode. Thus, a solution of 1,4-benzoquinone in Et_2O is added to *N*,*N*′-dimethyl tetrahydro-4,4′-bipyridyl in Et_2O and in absence of air. The bright blue solid is separated from Et_2O and dissolved in MeOH; 10% HCl in EtOH is added, giving a deep red solid that is dissolved in water to show a 40% yield of *N*,*N*′-dimethyl-4,4′-bipyridylium ion on colorimetric testing. The red solution yields a deep red 1:1 additon compound of quinol and *N*,*N*′-dimethyl-4,4′-bipyridylium dichloride (**I**). If the red aq. solution is extracted with Et_2O, and the extract evaporated to dryness, the residue consists of quinol, while evaporation of the aq. layer gives **I**.

S. D. HUGGINS.

Glycol amide derivatives for controlling weed growth. Stauffer Chemical Co. (Inventors: D. R. Baker, S. Yang Chung Soong and B. H. Lake) (B.P. 1,081,471, 23.2.65).—The new herbicidal amide deriv. are compounds $R^1SO_2O\cdot CH_2C(O)N(R^2)R^3$ where R^1 is alkyl, alkenyl, halo-alkyl or -alkenyl, Ph that may be substituted by halogen, alkyl, or NO_2, or is a thiophene, phenylalkyl, or naphthyl radical; R^2 and R^3 are (like or different) H, alkyl, alkenyl, cycloalkyl or -alkenyl, phenyl, phenylalkyl, furfuryl, alkoxyalkyl, or cyclic alkylether groups, e.g. *O*-benzenesulphonyl-*N*-Pr^i-glycolamide (m.p. 64–66°) which is prepared by reacting *N*-Pr^i-glycolamide with benzenesulphonyl chloride in presence of NEt_3. Over 200 examples are given of R^1, R^2 and R^3 reactants, the activity index (max. 21) being indicated in each case.

H. L. WHITEHEAD.

Triazine derivatives. Badische Anilin- und Soda-Fabrik A.-G. (Inventors: G. Steinbrunn and A. Fischer) (B.P. 1,084,941, 8.1.65. Ger., 11.1.64).—Herbicidal action is possessed by the reaction products of an anhydride of a dibasic carboxylic acid (e.g. maleic anhydride) and a triazine, substituted in the 1-position by R^1 (Cl, OMe or SMe), in the 3-position by NR^2R^3 (R^2 is hydroxy-alkyl, -alkenyl or -alkynyl, all of 2-4C and R^3 is H, alkyl, allyl or alkynyl of 1–4C) and in the 5-position by NR^4R^5 (R^4 is alkyl or alkynyl of 1-4C, chloroalkyl of 2–3C or ethyl substituted by OMe, OEt, SMe or SEt; SMe or alkyl having 2-3C and having SH as substituent and R^5 is H, allyl or alkyl of 1-3C). A reaction product of maleic hydrazide with e.g. 2-isopropylamino-4-β-hydroxypropylamino-6-chloro-*s*-triazine is among the compounds claimed.

S. D. HUGGINS.

Selective weed control of barley or wheat. Farbenfabriken Bayer A.-G. (Inventors: H. Hack, L. Eue and W. Schäfer) (B.P. 1,085,430, 7.9.66. Ger., 18.9.65).—Each hectare is treated with 1–5 kg 1-(2-benzothiazolyl)-1, 3-dimethyl urea, alone or in admixture with a solid or liquid diluent or carrier, before or after emergence of the wheat or barley plants. The herbicide destroys monocotyledonous and dicotyledonous weeds, and is toxic to cultivated plants (oats, millet, rice, rye and maize) other than those mentioned above.

S. D. HUGGINS.

Substituted pyridazones. Badische Anilin- und Soda-Fabrik A.-G. (B.P. 1,085,883, 15.1.65. Ger., 16.1.64).—Used as weed controllers, the pyridazones (**I**) are substituted in the 1-position by R^1 (Ph or cyclohexyl), in the 4-position by NH_2 and in the 5-position by OMe, the ketonic group being at the 6-position e.g. 1-phenyl-4-amino-5-methoxy-pyridazone-(6). **I** are prepared e.g. by hydrogenation of the corresponding 4-nitro-5-alkoxypyridazones or by alkylation of the corresponding 4-amino-5-hydroxypyridazones. Unlike previously known substituted chloropyridazones, **I** have very strong action on grasses such as wild oats and slender foxtail.

S. D. HUGGINS.

N-Substituted chloroacetamides. Monsanto Co. (B.P. 1,086,507, 16.11.64. U.S., 15.11.63).—Used in herbicidal compositions, the title α-chloroacetamides (**I**) have the formula $ClCH_2CONR^1(CH_2OR)$, wherein R is alkyl, alkenyl or alkynyl of 1-12C and R^1 is H, alkyl, alkenyl or alkynyl of 1-4C. The compounds can be prepared e.g. by heating the α-chloroacetamides of formula $ClCH_2\cdot CON(CH_2Cl)R^2$, where R^2 is R^1 (other than H), with the alcohol ROH. Where R^1 is H, **I** are obtained by heating *N*-hydroxymethyl α-chloroacetamide with ROH in presence of e.g. H_2SO_4. Specific compounds claimed include *N*-methyl-*N*(n-butoxymethyl)-α-chloroacetamide, which when used at 0·25 lb/acre inhibits several types of grasses but does not affect cotton, sugar-beet etc.

S. D. HUGGINS.

Animal Husbandry

Production of odourless fish meal. M. Carranza M. and E. Jimenez C. (*Infciones Grasas aceit.*, 1966, **4**, 274–278).—A procedure for treating the fish with EtOH to remove water and part of the oil, followed by hexane to remove the rest of the oil, leaving an odourless fish meal, and obtaining the oil and various byproducts from the extracts is described.

L. A. O'NEILL.

Rapeseed meal. XI. Effect of heating on the spectra of extracts of rapeseed extraction meals. J. Pokorný and A. Rutkowski (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E14**, 73–84).—Absorption spectra of the ethanolic, aq., salt and alkaline extracts of rapeseed extraction meals given a variety of heat treatments (30–90 min at 105–135°) are reproduced and discussed in relation to the evaluation of the previous thermal history of meal samples. A relatively sensitive criterion was found to be the ratio of extinctions of the ethanolic extract at 335 and 275 nm. (In English.) (26 references.)

E. C. APLING.

Effect of the addition of arachis oil on the conservation of heavily-wilted herbage sealed in polyethylene film. N. Jackson and W. O. Brown (*J. Br. Grassld Soc.*, 1967, **22**, 214–220).—The addition of 4·9% arachis oil (on dry matter basis) to wilted herbage (46% dry matter) before ensiling in polyethylene film bags increased the metabolisable energy content but reduced the digestibility of the mineral matter of the silage. There was little change in the composition of the fatty acids of arachis oil during ensiling.
A. H. CORNFIELD.

Influence of nitrogen top dressing on the yield and quality of fodder comfrey (*Symphytum peregrinum*). S. Tabin, S. Berbeć and H. Wrębiakowski (*Annls Univ. Mariae Curie-Sklodowska, Agric.* E, 1966, **21**, 139–153).—The response of fodder comfrey to N top dressing was examined in field experiments, carried out for four consecutive years (1959–62). Two ecological types of *Symphytum peregrinum* were used, planted on plots, situated on two levels. Before planting and also each year in spring, the plots were fertilised with customary doses of P_2O_5, K_2O and N and, independently, already growing plants also received a N top dressing in two separate doses of 30 and 60 kg/ha. The fodder comfrey thrived better on low-lying fields and responded readily to N top dressing. The average yield of green mass obtained was 280–300 quintals/ha and the highest yield, 600 q/ha, was harvested during the second year of cultivation. The crude protein content was 15·6–16·4% and that of crude fibre 24·3–25·8%. (23 references.)
T. M. BARZYKOWSKI.

Evaluation of lucerne–brome forage stored as wilted silage, low-moisture silage and hay. R. E. Roffler, R. P. Niedermeier, and B. R. Baumgardt (*J. Dairy Sci.*, 1967, **50**, 1805–1813).—Lucerne–brome forage preserved as hay (*H*) was found to contain less protein, ether extract and ash than that preserved as low-moisture silage (*LMS*) or wilted silage (*WS*). Carotene content was greatest in *WS* and least in *H*. Trials with cows showed that those fed *LMS* or *H* gained more body weight than those fed *WS*. Production of 4% *FCM* was greatest with cows fed *LMS* and lowest with those fed *H*. (38 references.) M. O'LEARY.

Prediction factors for maize silage production. W. F. Craig (*Diss. Abstr.* B, 1967, **27**, 2988).—Possible relationships between the breeding of maize and the quality of silage made from it are examined. From eight inbred strains, all possible single-crosses and a sample of the double-crosses were compared over a 4-year period. Results obtained were more reliable for prediction purposes than were those from a single season's observations. The latter were considerably influenced by environmental conditions, notably rainfall. Factors affecting the value of data for predicting silage quality from strains of different breeding lines are recorded. Predictions based on non-parental single-cross performance afford sound estimates of double-cross performance. No successful segregation of breeding lines yielding high and low stalk-sugar concn. were found. A. G. POLLARD.

Dry molasses product using waste paper as a base for a possible feed for cattle. E. M. Kesler, P. T. Chandler and A. E. Branding (*J. Dairy Sci.*, 1967, **50**, 1994–1996).—The development of a dry molasses–paper product is described. Feeding trials with yearling heifers showed that the animals gained 0·8 kg daily on a ration which included 1·1 kg of paper and 1·7 kg of molasses.
M. O'LEARY.

Xanthophyll and carotene loss during pilot and industrial scale alfalfa [lucerne] processing. A. L. Livingston, R. E. Knowles, J. W. Nelson, and G. O. Kohler (*J. agric. Fd Chem.*, 1968, **16**, 84–87).—Losses of xanthophyll were 28–73% and of carotene 0–33% during drying. An inverse correlation was found between the moisture content of the meal and the loss of xanthophyll during drying. Lutein was more stable than neoxanthine or violaxanthine; the total xanthophyll analysis of the meal reflects chiefly its lutein content, and results indicate that a lucerne dehydrator could, by maintaining a meal moisture level of 7–9%, produce a product containing up to 77% more lutein, with corresponding improved poultry pigmenting ability. (13 references.) P. S. ARUP.

Combination of nitrite and hexamine (hexamethylenetetramine) as an additive in the ensiling of herbage. A. Hellberg (*J. Br. Grassld Soc.*, 1967, **22**, 289–297).—When a mixture of $NaNO_2$ at 0·15% and hexamine at 0·25% of fresh herbage wt. was added the quality of the silage was superior to that made with commercial additives used in Sweden and the losses were considerably less. Results in 15 experiments with small steel cylinders and in 13 with plastic sacks were consistently good over 8 years. A. H. CORNFIELD.

Dynamics of the development of some micro-organism groups during ensiling of the rumen content. A. Szember (*Annls Univ. Mariae Curie-Sklodowska, Agric.* E, 1966, **21**, 219–226).—Laboratory experiments are described for the ensilage of the rumen content of abbatoir cattle, alone or mixed with molasses (**I**), steamed artichokes (**II**) or potato tubers (**III**) which help to propagate the fermentation in a desired direction, i.e. development of lactic bacteria. Addition of 5–15% of **I** led to production of an aromatic and stable silage. Addition of 5–10% of **II** or **III** stimulated development of lactic bacteria, but not in adequate quantity to produce a stable silage. The silage produced may be useful in pig fodder.
T. M. BARZYKOWSKI.

A factor limiting the ruminant's voluntary consumption of silage. J. H. Ternouth (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 263–264).—The reduction in voluntary food consumption when silage rather than hay is fed to ruminants is probably due in part to an increase in osmolality in the rumen caused by consuming pre-fermented food.
J. L. WALPOLE.

Use of non-protein nitrogen in animal feeding. D. G. Armstrong (*Chemy Ind.*, 1968, 894–898).—The sources and values of non-protein nitrogen (NPN) as food for simple-stomached and ruminant animals during the next few decades are discussed. Sources of NPN may even replace vegetable protein concentrates now used in some rations for dairy cows. In pastoral tropical and sub-tropical regions NPN supplements should keep livestock healthy during the dry season. NPN could be used together with straw as food for animals having low nutritional demands and in low-protein raw or processed plant material indigestible to humans. Main source of NPN will still be urea and possibly biuret, with NH_4 acetate for milk production and $(NH_4)_2SO_4$ in S-deficient regions. Essential amino-acids will be important for pigs and poultry. (34 references.)
W. J. BAKER.

Nitrogen utilisation by the ruminant—appreciation of its nutritive value. H. R. Conrad and J. W. Hibbs (*J. Dairy Sci.*, 1968, **51**, 276–285).—The uses and nutritive values of urea and other non-protein nitrogen sources are reviewed. (85 references.) M. O'LEARY.

Nitrogen metabolism in the ruminant. D. R. Waldo (*J. Dairy Sci.*, 1968, **51**, 265–275).—The pathways of the major N fractions through the ruminant body and their interactions with other feed components in the rumen are reviewed. (115 references.)
M. O'LEARY.

Digestion of chopped and ground roughages by sheep. I. Movement of digesta through the stomach. II. Digestion of nitrogen and some carbohydrate fractions in the stomach and intestines. J. P. Hogan and R. H. Weston. (*Aust. J. agric. Res.*, 1967, **18**, 789–801; 803–819).—I. Digestion studies were conducted with two types of hay (lucerne and wheat) prepared either by chopping or by grinding and pelleting and fed to sheep at 3-hourly intervals. The rates of flow of water and the rumen vol. were measured by means of a water-sol. marker (^{51}Cr-EDTA). Significant differences were found between each type of hay both in respect of consumption and the rate of flow from the rumen and abomasum. Sheep spent less time ruminating and eating when the roughage was ground and more metabolisable energy was therefore available for growth production. (26 references.)

II. Grinding permitted a substantial increase in consumption of each type of hay but grinding *per se* had little effect on the relative importance of the stomach and intestines as sites of digestion of any of the feed components. Grinding, however, affected the digestibility of the hays in different ways and altered the relative intakes of org. matter as well as the proportion of N in the total org. matter digested. For lucerne hay, 16–20% of the org. matter digested in the rumen was sol. carbohydrates against 40–50% for wheaten hay. (38 references.) J. L. WALPOLE.

Factors limiting the intake of feed by sheep. II. Studies with wheaten hay. R. H. Weston (*Aust. J. agric. Res.*, 1967, **18**, 983–1002).—The sheep's intake of a wheaten diet having an org. matter digestibility of about 57% and containing 4·4% of crude protein and a mineral supplement is reported. Voluntary feed consumption (*VFC*) of chopped hay increased when protein or urea was infused per abomasum, or when the protein content of the diet was increased by addition of wheat gluten. When the hay was ground, or ground and pelleted, the *VFC* increased and digestibility declined. Addition of protein to ground and pelleted hay increased the intake to 189% of the chopped feed figure. It is concluded that the primary factor limiting wheaten hay intake is N deficiency; next in importance is the resistance of the diet to removal from the rumen. (20 references.) J. L. WALPOLE

Rate of passage of food particles through digestive tract of sheep. W. J. Stielau (*S. Afr. J. agric. Sci.*, 1967, **10**, 753–760).—When sheep

had been fed with fine, medium, or coarse lucerne hay at two levels, the mean time required for the appearance of stained particles was ~16 h and the mean times for the passage of 5% and 95% of the marked particles were ~28 and 130 h, respectively. The average time for particle retention in the digestive tract was ~72 h. The only factor to affect the rate of passage was the level of feed intake, the rate being higher at the higher level of intake. The results indicate that neither the feeding level nor the fineness of the feed has much effect on the rate of passage. (11 references.)
P. S. ARUP.

Effect of non-protein nitrogen supplementation on the performance of laying hens on low-protein rations. E. T. Moran, jun., J. D. Summers, and W. F. Pepper (*Poult. Sci.*, 1967, **46**, 1134–1144).—Addition of 5% 'protein' as $(NH_4)_2$ citrate (**I**) or urea to 10% protein diets fed to laying hens or chicks did not improve the performance of the birds; **I** was toxic. Addition of 2–6% **I** to a semi-purified diet containing sufficient artificial amino-acids just to meet the min. requirements of laying hens did not improve the performance of the birds. Calculation of essential and non-essential amino-acid requirements for egg formation and maintenance indicates that a dietary inadequacy of non-essential N is improbable when normal feedstuffs are employed. A. H. CORNFIELD.

Report of the Committee of the World's Poultry Science Association on nutrient requirements for poultry. Anon. (*Poult. Sci.*, 1967, **46**, 1053–1055).—Tables of amino-acid, vitamin, and mineral requirements for chicks (0–4 weeks), layers, and breeders used in Australia, France, Japan, the United Kingdom, and the United States are presented. A. H. CORNFIELD.

Unextracted soyabeans for chicks. I. Comparison of infra-red cooked, autoclaved and extruded soyabeans. C. L. White, D. E. Greene, P. W. Waldroup, and E. L. Stephenson (*Poult. Sci.*, 1967, **46**, 1180–1185).—Infra-red cooking, extrusion and autoclaving significantly improved the feeding value of raw soyabeans for chicks. The material produced by extruding and pelleting supported wt. gains and feed efficiencies equal to those of the control, commercially processed soyabean meal diet. A. H. CORNFIELD.

Sequestering phosphatic solution as a phosphorus source for ruminants. R. R. Johnson and K. E. McClure (*J. Dairy Sci.*, 1967, **50**, 1502–1504).—Feeding trials with steers and a balance digestion trial with sheep indicated that a sequestering phosphatic solution (ammonium polyphosphate) is as good a source of phosphorus for ruminants as is dicalcium phosphate. M. O'LEARY.

Effect of calcium sulphate and different protein levels on [egg] albumen quality and other production characteristics. E. W. Pawson (*S. Afr. J. agric. Sci.*, 1967, **10**, 735–739).—Additions of 3·7% $CaSO_4$ to diets for laying hens, with 13 or 15% protein levels had no effect on the quality of the egg albumen. The $CaSO_4$, used as a supplement, caused diarrhoea, but when used as a source of Ca instead of limestone it had no harmful effects. (In Afrikaans.)
P. S. ARUP.

Acetyl-*p*-aminophenol and vitamin C in heat-stressed birds. D. V. Subaschandran and S. L. Balloun (*Poult. Sci.*, 1967, **46**, 1073–1076).—Addition of 110–220 ppm acetyl-*p*-aminophenol (**I**) to the diet significantly improved wt. gains of heat-stressed (32–38°) cockerels in only one of two experiments. **I** and 66 ppm vitamin C did not affect blood pressure, but tended to reduce body temp. in heat-stressed birds. A. H. CORNFIELD.

Effect of acetylsalicylic acid and some chemical analogues on chick growth and biochemical parameters. J. M. Thomas, H. S. Nakaue, and B. L. Reid (*Poult. Sci.*, 1967, **46**, 1216–1219).—Addition of 0·05–0·30% acetylsalicylic acid (**I**) to the feed had no effect on wt. gains or feed efficiency of chicks from 4 to 6 weeks of age. 0·24–0·32% methylenedisalicylic acid and 0·34% *p*-aminosalicylic acid had no effect, whilst 0·335% acetyl-*p*-aminophenol improved wt. gains. All dietary levels of **I** decreased plasma-uric acid and increased liver-glycogen and plasma-glucose.
A. H. CORNFIELD.

Effect of erythromycin thiocyanate on the performance of laying hens. S. C. Nivas, M. L. Sunde, and H. R. Bird (*Poult. Sci.*, 1967, **46**, 1103–1108).—Egg production and feed efficiency with respect to egg production were increased by addition of 20–100 ppm erythromycin thiocyanate to the diet of hens, particularly in the winter months. In two of three experiments hatchability was increased by the higher levels of antibiotic supplementation. The treatments had no effect on egg wt. or interior quality, blood spots, fertility of eggs, or mortality of chicks. A. H. CORNFIELD.

Ryegrass varieties in relation to dairy cattle performance. III. Comparison of milk yield and composition from a tetraploid and two diploid ryegrass varieties. G. F. Wilson and R. M. Dolby (*N.Z. Jl agric. Res.*, 1967, **10**, 415–424).—During winter groups of Friesian cows on Western Wolths (*WW*) and Paroa ryegrass gave a higher yield of milk than those on Ruanui ryegrass (*RR*), but the butterfat content for the *WW* group was significantly lower. In the spring, lactating monozygous twin cattle, milk yields and % butterfat contents were similar for the three groups, but the solids-not-fat content of the *RR* group was significantly depressed. (16 references.)
E. G. BRICKELL.

Wafered and baled lucerne hay harvested at different stages of maturity for lactating cows. D. E. Waldern and N. O. Baird (*J. Dairy Sci.*, 1967, **50**, 1430–1436).—Digestion trials with heifers and lactation trials with cows indicated that there is some advantage in wafering lucerne hay of $\frac{1}{10}$-bloom and greater maturity. Increased dry matter intake, body weight gain, and 4% *FCM* production are obtained compared to feeding the same hay in a long form as baled hay. (30 references.) M. O'LEARY.

Coastal Bermuda-grass as pellets and silage compared to oats + ryegrass + crimson clover, Sudan-grass, and corn silages with high and low grain levels for lactating cows. C. M. Clifton, W. J. Miller and N. W. Cameron (*J. Dairy Sci.*, 1967, **50**, 1798–1804).—Cows fed Coastal Bermuda-grass (*CB*) pellets consumed more forage dry matter than those fed silages made from (*a*) *CB*; (*b*) Tift Sudan-grass; (*c*) oats, ryegrass, and crimson clover (*ORC*) and (*d*) maize and produced more milk and *FCM* than those given the *ORC* silage. Type of forage fed had no significant effect on the *SNF* content of milk. Forage dry matter intake per kg of milk was much higher for cows fed pellets than for those fed the silages. Increasing the grain level of the rations resulted in an increase of 0·46 kg milk and 0·48 kg *FCM* and in a decrease of 0·28 kg forage dry matter intake for each additional kg of concentrate dry matter. (21 references.)
M. O'LEARY.

Frequency of feeding lucerne as a protein supplement. G. M. Ward and M. Z. A. Nomani (*J. Dairy Sci.*, 1967, **50**, 1509).—Feeding trials with dairy steers showed that once-daily feeding of lucerne is sufficient even when the hay is the major source of protein in the ration. M. O'LEARY.

Feed processing. II. Effect of feeding expanded grain and finely-ground hay on milk composition, yield, and rumen metabolism. V. F. Colenbrander, E. E. Bartley, J. L. Morrill, C. W. Deyoe and H. B. Pfost (*J. Dairy Sci.*, 1967, **50**, 1966–1972).—Holstein cows fed expanded sorghum grain (*SG*) and finely ground lucerne hay, produced more milk but less fat-corrected milk than cows fed a control ration of cracked *SG* and coarse chopped hay. % of milk protein, lactose, ash, and solids-not-fat were similar for both groups. Conversion of feed protein to milk protein was more efficient in the experimental group. Molar % of *VFA* for the control and experimental groups, respectively, were: acetic, 68·1, 53·7; propionic, 17·4, 31·7; butyric, 12·3, 10·6; isovaleric, 1·2, 0·4; and valeric, 1·1, 3·6. Rumen ammonia-nitrogen was considerably lower in the experimental group. There was no difference in blood glucose levels, but body weight gains were significantly higher in the experimental animals. (33 references.) M. O'LEARY.

Effects of multiple feeding upon performance of Guernsey heifers fed urea-treated corn silage. D. M. Fletcher, A. G. Lane, J. R. Campbell and F. A. Mortz (*J. Dairy Sci.*, 1968, **51**, 202–204).—Four times daily feeding of urea-treated corn [maize] silage to Guernsey heifers resulted in a nonsignificant (at $P < 0{\cdot}05$) 18·4% greater wt. gain than twice daily feeding. No significant effect (at $P < 0{\cdot}05$) on growth was observed between twice or four times daily feeding of both grain and urea-treated maize silage. Four times daily feeding of urea-treated silage caused a significant ($P < 0{\cdot}05$) 13·7% decrease in estimated kg of *TDN* required per kg of wt. gain compared to twice daily feeding. Heifers receiving grain 4 times daily and silage twice daily required 14·4% less *TDN* per kg of gain than animals receiving grain and silage twice daily. Blood urea nitrogen and rumen *VFA* were not significantly affected by the various treatments but their trends favoured more frequent feeding of silage. (17 references.) M. O'LEARY.

Effect of dietary cadmium and ethylenediaminetetra-acetate on dry matter digestibility and organ weights in zinc deficient and normal ruminants. J. M. Hiers, jun., W. J. Miller and D. M. Blackmon (*J. Dairy Sci.*, 1968, **51**, 205–209).—Feeding of 350 ppm of Cd (as $CdCl_2$) or 300 ppm of EDTA (as Na_2 salt) to Zn-deficient calves and goats had no effect on digestibility of a purified diet. Testicles of Zn-deficient goats were reduced in size and in general tibiae were slightly larger in Zn-deficient animals. Livers of deficient goats were enlarged but not those of deficient calves. Lungs of Zn-deficient animals fed Cd were larger relative to body size than those of

animals given other treatments. Sizes of kidney, heart, or spleen were not significantly affected by either EDTA, Cd or the Zn deficiency. The predominant influence of EDTA and Cd on health, performance and metabolism of young ruminants is not through an effect on dry matter digestibility and probably not through gross effects on sizes of the organs studied. (17 references.) M. O'LEARY.

Influence of a high level of dietary cadmium on cadmium content in milk, excretion, and cow performance. W. J. Miller, B. Lampp, G. W. Powell, C. A. Salotti, and D. M. Blackmon (*J. Dairy Sci.*, 1967, **50**, 1404–1408).—Administration of 30g/day of Cd for a two-week period to three Holstein cows resulted in a significant drop in milk production and a significant rise in milk fat %. Cows given Cd lost considerable weight but there were no other clinical symptoms of toxicity. Faecal excretion of Cd averaged 82% of that administered (during the second week of the experiment). Cd level in the urine was < 0·5 ppm and that in milk was < 0·1 ppm. (14 references.) M. O'LEARY.

Influence of grazing intensity on caesium-137 levels on milk. F. J. Burmann (*J. Dairy Sci.*, 1967, **50**, 1891–1896).—Trials with Holstein cows indicated that ^{137}Cs and ^{90}Sr concn. in milk were increased as a result of increasing the intensity of grazing. M. O'LEARY.

Effect of high temperature and dietary fat on performance of lactating cows. E. G. Moody, P. J. Van Soest, R. E. McDowell, and G. L. Ford (*J. Dairy Sci.*, 1967, **50**, 1909–1916).—The results of trials in which Holstein cows were fed high levels of fat under environmental temp. of 15–24° or 32·2° at 60% R.H. are presented. Elevated temp. caused a marked depression in milk yield, milk fat %, *SNF*, milk protein, body weight, feed intake, rumen *VFA*, acetic: propionic acid ratio, and gross efficiency. Water intake and rectal temp. increased. Significant ration effects were observed only with *FCM* production which was significantly increased by feeding of fat. (34 references.) M. O'LEARY.

Changes in secretion of the main fatty acids in milk and the concentration of free fatty acids in blood of cows at the start of lactation. C. Decaen and M. Journet (*Annls Biol. anim. Biochim. Biophys.*, 1967, **7**, 131–143).—Changes in the fatty acid (*FA*) composition of milk fat, the *FFA* content of the blood plasma, and in milk yield, milk fat content, energy balance and live wt. were studied during the first 6 weeks of lactation in nine normally-fed cows, of which two had calves prematurely. In all cases the *FFA* content of the plasma increased greatly at calving and subsequently fell gradually after normal calving, but continued to increase after premature calving. Changes in the proportion of long-chain *FA* ($C_{18:0}$ and $C_{18:1}$) and fat content of the milk roughly paralleled changes in plasma *FFA*. It is suggested that the latter may be important precursors of milk fat at the beginning of lactation. (31 references.) E. C. APLING.

Influence of frequency of feeding and of urea supplementation on sheep under conditions of drought. D. H. van Niekerk, W. D. Basson and A. M. Mulder (*S. Afr. J. agric. Sci.*, 1967, **10**, 687–695).—Contrary to the findings of *C.S.I.R.O. Aust.*, 1958, *Leafl. Ser.* 23, daily supplementation with ~0·5 lb of maize gave far better results as regards body wt. and survival rate than when the same total amount was fed twice or once per week. Possible reasons for the disagreement are considered. Supplementation with urea was ineffective. (In Afrikaans.) P. S. ARUP.

Digestion of two diets of differing protein content but with similar capacities to sustain wool growth. J. P. Hogan and R. H. Weston (*Aust. J. agric. Res.*, 1967, **18**, 973–981).—Two diets, one of high protein content and one low, were fed to sheep at 500g/day and measurements made of their digestion processes in the stomach and intestines. The similar levels of wool growth obtained from these dissimilar diets are consistent with the hypothesis that the growth is limited by the amount of amino-acid-N absorbed from the alimentary tract. The site and extent of digestion of the org. matter, cellulose, N and sol. carbohydrate fractions of the feeds are discussed. (14 references.) J. L. WALPOLE.

Wool fibre shedding in some Merino sheep. M. L. Ryder (*Aust. J. agric. Res.*, 1967, **18**, 683–687).—Four sheep were investigated by monthly skin sampling for 3 years in Australia and four sheep by quarterly sampling for 3 years in Scotland. Those in Australia had shedding in 30–50% of the samples while those in Scotland had shedding in only 15–30%, but in both the incidence of brush ends was < 1%. (10 references.) E. G. BRICKELL.

Interrelationship between fleece and fibre characteristics in the merino. IV. Wool production in the merino. J. J. Venter (*S. Afr. J. agric. Sci.*, 1967, **10**, 529–542).—The total wt. of raw wool produced by two-tooth merino ewes was positively correlated with the wt. of clean wool; total wool production was positively correlated with body wt., fibre thickness, and skin area occupied by fibres. Raw wool was negatively correlated with clean yield and the no. of crimps per in. Clean wool production was negatively correlated with the % of wool wax while raw wool production was positively correlated with this factor. Results are discussed in connection with the selection of animals to increase wool production. P. S. ARUP.

Feeding meat-type pullets and breeders. Methods of lowering the live weight of meat-type pullets at point of lay and the portion and energy needs of meat-type breeders. J. D. Summers, W. F. Pepper, S. J. Slinger and J. D. McConachie (*Poult. Sci.*, 1967, **46**, 1158–1164).—When compared with a control diet, feeding 80% wheat bran or using an abnormally high calorie/protein ratio in the feed resulted in a significant lowering of live wt. at point of lay, but did not delay sexual maturity. Egg production was similar irrespective of the growing diet. During the breeding period diets containing 14–16% protein gave optimum performance. Energy requirements of meat-strain birds was > 2·54 kcal per g during the breeding periods. A. H. CORNFIELD.

Amino-acid requirements of laying hens with special reference to methionine. A. H. Spandorf (*Diss. Abstr.* B, 1967, **27**, 2941).—Data obtained with individual hens by N-balance techniques was utilised in determining factors suitable for the prediction of amino-acid requirements. Five diets containing methionine (*M*) concn. 0·57–0·98 mg/kcal metabolisable calories were used; the hen/day/% production averaged 50–71% for the diets with the lowest to highest *M* levels. On the basis of egg clutch length, differences between the five treatment groups were greater (1·68–3·21). Periods between laying clutches were not greatly affected by the diets; with the three lowest dietary *M* levels few clutches exceeded four eggs. Egg-wt. and quality did not differ significantly between the five diet groups. Most of the hens lost body-protein during the test. The relationship, % protein in carcass = 0·0361 × [% egg production in 28 days] + 17·94 was established. Metabolisable energy determinations made simultaneously with the N balance and during the 32nd week of the investigation showed no change between periods or between diets differing only in *M* concn.; N balances were improved by adding *M*. N retention was significantly correlated with egg production but not with changes in body-wt. Hen feathers contained 21–23% of the total protein of the carcass. Experimental data are utilised in a discussion of amino-acid requirements; in hot weather neither egg production nor N balances were sensitive criteria. N retentions of diets varying in amino-acid adequacy are more easily shown when environmental temp. is lowered, excreta is collected in aq. 4% boric acid and when samples for N are analysed without heating during prep. A. G. POLLARD.

Results of feeding trials with young chickens. P. Smetana and O. Thwin (*Un. Burma J. Life Sci.*, 1968, **1**, 12–19).—The compositions are given of four rations based on locally produced ingredients (mainly maize, rice, rice bran and groundnut, sesame and prawn meals) that gave very satisfactory results as regards vitality and growth in tests with 1024 day-old chicks. P. S. ARUP.

Protein requirement of Coturnix quail to five weeks of age. C. W. Weber and B. L. Reid (*Poult. Sci.*, 1967, **46**, 1190–1194).—Coturnix quail fed a soyabean meal diet supplemented with methionine required 24% protein in the diet for max. wt. gains to 5 weeks of age. The most efficient protein retention, feed conversion and body wt. gains were obtained with a calorie/protein ration of 36–38. A. H. CORNFIELD.

Determination of the available lysine requirement of chickens and the relationship between serum-protein level and body composition. O. P. Thomas (*Diss. Abstr.* B, 1967, **27**, 2941–2942).—A maize-maize gluten (15·8% protein) diet supplemented with arginine and tryptophane and calculated to contain available lysine 0·27% and various proportions of added lysine (0·0–0·340%) as hydrochloride just failed to maintain egg production, egg size, feed consumption or body wt. at levels obtained with a control diet. The differences between the two rations in respect of the four parameters measured, diminished progressively as the dietary level of lysine was increased to 0·534%; total serum-protein and albumin levels increased slightly with increase in dietary lysine; haemoglobin levels were unaffected. An equation for predicting the lysine requirement is established. A. G. POLLARD.

Radiometric measurement of the rate of active transport of *l*-lysine across the intestinal wall of the fowl. J. R. Fearon and F. H. Bird (*Poult. Sci.*, 1967, **46**, 1037–1041).—The method, which utilises the *in vitro* everted sac technique, is described. A. H. CORNFIELD.

Effect of photoperiodism, rearing period and feed restriction on the performance of five Leghorn strains. F. G. Proudfoot and R. S. Gowe (*Poult. Sci.*, 1967, **46**, 1056–1072).—There were interactions among strain, light treatment and feed treatment with respect to egg production and monetary returns in experiments with five Leghorn strains using four light treatments and restricted *versus* full feeding. A. H. CORNFIELD.

Influence of ambient temperature on reproductive traits of male and female chickens. C. E. Clark and K. Sarakoon (*Poult. Sci.*, 1967, **46**, 1093–1098).—The effects of various constant and fluctuating temp. on fertility and semen characteristics of four strains of White Leghorns were studied. A. H. CORNFIELD.

Influence of thermal incubation stresses on chicken embryos. W. Morgan and W. L. Tucker (*Poult. Sci.*, 1967, **46**, 1172–1176).—A 3-h exposure of White Leghorn chicken embryos at 7, 8, 9, and 10 days of age did not significantly alter the sex-ratio when temp. of 6°, 21°, and 41° were compared with the 35° control temp. The 41° treatment caused high mortality, particularly in 9–10-day old embryos. A. H. CORNFIELD.

Essential fatty acid deficiency of the growing domestic cock. H. M. Edwards, jun. (*Poult. Sci.*, 1967, **46**, 1128–1133).—When male chicks received a fat-free diet their wattles did not grow and they had pale combs. By 15 weeks of age they resembled hens and had small testes and spleen and large pancreas, and could not be made to ejaculate semen at maturity. Addition of methyl oleate to the fat-free diet resulted in normal development of the chicks, but only for the first few weeks, whilst methyl linoleate and maize oil stimulated development until maturity. A. H. CORNFIELD.

Absorption of riboflavin in chickens. N. A. Cordona and I. R. Payne (*Poult. Sci.*, 1967, **46**, 1176–1179).—*In vitro* studies using the everted sac method showed that injection of 0·025g of β-oestradiol-3,17-dipropionate in mature and immature males and females did not affect the rate of absorption of total riboflavin (**I**) in the small intestine. Flavin mononucleotide accounted for a greater proportion of the total **I** in females than in males, as measured by the quantity of **I** in the serosal fluids. A. H. CORNFIELD.

Effect of cottonseed oil on egg production and quality. A. R. Kemmerer, B. W. Heywang and B. W. Lowe (*Poult. Sci.*, 1967, **46**, 1165–1167).—Addition of 2–5% cottonseed oil (containing 0·6% cyclopropenoid fatty acids) to the diet of laying hens reduced egg production and the quality of fresh and stored eggs. Another oil containing 0·45% cyclopropenoid fatty acids added at the above levels did not affect egg production but lowered egg quality after 3 months of storage. A. H. CORNFIELD.

Effect of eliminating machine stripping of dairy cows on milk production, residual milk and mastitis. K. R. Goff and G. H. Schmidt (*J. Dairy Sci.*, 1967, **50**, 1787–1791).—Elimination of machine stripping (*S*) had no significant effect on daily milk production or on milk fat %. % residual milk was significantly higher ($P < 0·05$) where *S* was not carried out. Elimination of *S* did not affect leucocyte counts or California Mastitis Test scores. (11 references.) M. O'LEARY.

Drug movement between bovine milk and plasma as affected by milk pH. G. E. Miller, N. C. Banerjee and C. M. Stowe (*J. Dairy Sci.*, 1967, **50**, 1395–1403).—Various weak org. acids and bases were infused into lactating cows and the front right quarter of each animal was infused with sterile bicarbonate buffer solution to establish a pH of 8·0. Blood, milk from normal quarters and milk from the bicarbonate-treated quarters were sampled simultaneously at hourly intervals. Experimentally determined milk/plasma ultrafiltrate ratios agreed well with theoretical ratios for nonionic diffusion both in the normal and in bicarbonate-treated quarters. (18 references.) M. O'LEARY.

Bloat in cattle. XIII. Efficacy of molasses-salt blocks containing poloxalene in control of lucerne bloat. D. A. Stiles, E. E. Bartley, A. B. Erhart, R. M. Meyer and F. W. Boren (*J. Dairy Sci.*, 1967, **50**, 1437–1443).—Trials with Holstein and Brown Swiss cows, Angus × Holstein steers and heifers, and Brown Swiss heifers indicated that molasses–salt blocks containing 66 g/kg poloxalene (**I**) are effective in controlling bloat. Though **I** intake varied from day to day, min. daily intakes were sufficient and also a carry-over effect provided protection against a usually low daily intake. M. O'LEARY.

Anabolic steroids for veal production. D. L. MacFadden and D. Belden (*J. Dairy Sci.*, 1967, **50**, 1848–1851).—The influence of anabolic agents on the growth of veal calves was investigated. Treatment with 17β-hydroxy-17α-methylandrostano(3,2-*c*)pyrazole (Win 14,833 or Stanozolol) or with stanzol, 17 β-(3-cyclohexylpropionoxy)androstano(2,3–5)isoxazole (Win 18792) resulted in significant increases in the rate of body weight gain and in feed conversion. Carcass data indicated no significant changes in the proportion of major cuts or in the proportion of lean, bone and fat in the round. (20 references.) M. O'LEARY.

Sensitivity of bovine staphylococci, streptococci and corynebacteria to cloxacillin and various other antibiotics. A. Jones, T. M. Higgs, F. K. Neave and A. Smith (*J. Dairy Res.*, 1967, **34**, 249–255).—Growth of various strains of *Staphylococcus aureus* was shown to be inhibited by the following range of concn. (μg/ml) of antibiotics: cloxacillin (**I**), 0·07 to 0·6; penicillin G (**II**), 0·018 to > 250; streptomycin, (**III**) 1·25 to > 250; novobiocin (**IV**), 0·15 to 25; chlortetracycline (**V**), 0·6 to 10. Concn. (μg/ml) of **I** required to inhibit growth of *S. agalactiae* were 0·15 to 1·25; *S. dysgalactiae*, 0·07 to 0·3; and *S. uberis*, 0·15 to 0·6. The corynebacteria were shown to be generally sensitive to ampicillin, **II**, phenethicillin but less so to **I**, **V**, neomycin and oleandomycin and still less to chloramphenicol, **III**, and **IV**. M. O'LEARY.

Effect of some pre-slaughter treatments on the Salmonella population in the bovine rumen and faeces. F. H. Graw, L. E. Brownlie and E. A. Roberts (*J. appl. Bact.*, 1968, **31**, 157–163).—Salmonella spp. were detected commonly in railway waggons, yards and abattoir holding pens. The % of animals with Salmonellae in the rumen was greater the longer the period between leaving farm and slaughter. Feeding once during this period increased the incidence and numbers of this organism in the rumen and the incidence in the faeces. (10 references.) C.V.

Abnormalities in electrocardiograms of young sheep and lambs grazing natural pastures low in selenium. K. O. Godwin (*Nature, Lond.*, 1968, **217**, 1275–1276).—Changes in the electrocardiograms of animals suffering from muscular dystrophy either on S. Australian farms (Se < 0·01 ppm in pasture) or produced experimentally (*Idem, ibid.*, 1966, **209**, 1030) have the same aetiology. In both instances the electrocardiogram patterns are associated with low intake of Se by the lambs, and show lesions typical of white muscle disease. W. J. BAKER.

Control and therapy of foot-rot in sheep in Friesland. G. D. van der Werff (*Versl. landbouwk. Onderz. Ned.*, 1968, No. 704, 100 pp.).—Factors governing the incidence of the disease are examined. The preferred treatment was a footbath in 2% formalin for 1 h followed by treatment with a 10% solution of chloromycetin in industrial EtOH and a plastic dressing. Recovery is greatly favoured by removal of the sheep to a well-drained clean pasture. (100 references.) (From 7-page English summary.) P. S. ARUP.

Susceptibility of domestic swine to influenza B virus. Gy. Takátsy, J. Romváry and E. Farkas (*Acta microbiol. hung.*, 1967, **14**, 309–315). C.V.

Effect of environmental temperature on healthy chicks and chicks inoculated with infectious bronchitis virus. R. P. Prince, J. J. Whitaker, R. E. Luginbuhl and L. D. Matterson (*Poult. Sci.*, 1967, **46**, 1098–1103).—Healthy chicks and those infected with infectious bronchitis virus responded similarly with respect to wt. gains and feed efficiency due to different environmental temp. (12·6–23·8°). A. H. CORNFIELD.

Effect of the insect chemosterilant Apholate on the reproductive performance of Leghorn hens. R. B. Herrick, M. Sherman and T. R. Batra (*Poult. Sci.*, 1967, **46**, 1045–1050).—Apholate [2,2,4,4,6,6-hexakis(1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphosphorine] at 100–500 ppm in the diet of laying hens depressed egg production and fertility in proportion to the level added. Body wt. decreased with 300–500 ppm Apholate in the feed and marked leukopenia occurred with all levels of the drug. Reproductive performance of the birds returned to normal following termination of administration of the drug. 50 ppm Apholate in the diet had no adverse effects. A. H. CORNFIELD.

Chemotherapeutic efficacy of sulfadimethoxine against fowl cholera and infectious coryza. M. Mitrovic (*Poult. Sci.*, 1967, **46**, 1153–1158).—Addition of 0·05% sulfadimethoxine [*N'*-(2,6-dimethoxy-4-pyrimidinyl)sulphanilimide] to the drinking water at time of infection or 24–48 h later was very effective in arresting *Pasteurella multocida* and *Hemophilus gallinarum* infections in chickens. The drug at 0·025% in the drinking water was also highly effective against *P. multocida* infection in turkeys. A. H. CORNFIELD.

Efficacy of *p*-ureidobenzenearsonic acid against blackhead (histomoniasis) in chickens. D. L. Peardon and H. J. Eoff (*Poult. Sci.*, 1967, **46**, 1108–1112).—Addition of 0·025–0·050% *p*-ureido-

benzenearsonic acid to the diet was very effective in eliminating the ill-effects due to exposure of birds to *Histomonas meleagridis*. With the higher level of the drug wt. gains were as high as in non-infected controls and caecal and liver lesions were prevented entirely.
A. H. CORNFIELD.

Effect of buquinolate on broiler chicks in floor pen trials. D. W. Rosenberg, W. D. Woodward and A. E. Kline (*Poult. Sci.*, 1967, **46**, 1113–1116).—The use of buquinolate (75–100g per ton of feed) and 0·0125% amprolium plus 0·0004% ethopabate in the diets of broiler chicks in floor pen trials had no effect on wt. gains, feed efficiency, or livability in four trials. None of the birds developed coccidiosis. Results showed that the use of these drugs to prevent coccidiosis did not affect the performance of healthy birds.
A. H. CORNFIELD.

Resistance of turkeys to haematologic effects of *S*-(1,2-dichlorovinyl) *l*-cysteine. P. E. Waibel, J. H. Sautter, V. Perman and M. O. Schultze (*Poult. Sci.*, 1967, **46**, 1144–1148).—No evidence of the development of blood dyscrasia in turkeys was obtained following daily injection of *S*-(1,2-dichlorovinyl)*l*-cysteine (I) at 0·02g per kg of body wt. for 30 days. The kidneys of birds receiving 0·035–0·050g I per kg of body wt. showed severe degenerative changes of the tubular epithelium. A. H. CORNFIELD.

Atlantic salmon disease fungus. L. G. Willoughby (*Nature, Lond.*, 1968, **217**, 872–873).—Further researches on the *Saprolegnia* isolates from diseased fish in Windermere are reported. Isolates produced sporangia directly on dry cholesterol agar (a possible medium for recognition of fish saprolegnias from mixed natural fungi populations). A tentative mycological description is given of oogonia formed from only a few of the isolates but all in cultures containing aerial fungal contaminants. The responsible species seems more closely related to the *S. delica–S. diclina* complex than to the *S. ferax–S. monoica* one. W. J. BAKER.

Resistance of rainbow trout to ulcerative dermal necrosis. J. T. Carbery and K. L. Strickland (*Nature, Lond.*, 1968, **217**, 1158)—Rainbow trout do not show clinical signs of ulcerative dermal necrosis nor are they symptomless carriers of the disease. The evidence for this was obtained by the siphoning technique in tanks at 6–13°, wherein trout exposed to salmon affected by the disease remained healthy, uninfected salmon exposed to water in which the trout from the first experiment had been placed remained healthy, but uninfected salmon exposed to water which had contained salmon affected by the disease soon contracted the disease and died.
W. J. BAKER.

***In vitro* susceptibilities of the lobster pathogen *Gaffkya homari* to various disinfectants and antibiotics.** J. E. Stewart and J. W. Cornick (*J. Fish. Res. Bd Can.*, 1967, **24**, 2623–2626).—Four strains of *G. homari* and one of *G. tetragena* were studied, the latter as a control. Susceptibilities of the two species to disinfectants, although not precisely equal, were in the same general range. The majority of the strains were sensitive to most of the antibiotics but not to the sulpha compounds. In contrast to previous results (Aaronson, *J. gen. Microbiol.*, 1956, **15**, 478), sensitivity of *G. tetragena* differed slightly from *G. homari*, chiefly with regard to streptomycin and oxacillin. The disinfectants which are effective at low levels, even in presence of an appreciable amount of org. matter, should be useful in disinfecting lobster storage units. E. G. BRICKELL.

Inhibition of multiplication of foot and mouth disease virus in adult mice pretreated with Freund's complete adjuvant. D. S. Gorhe (*Nature, Lond.*, 1967, **216**, 1242–1244).—The experiments reported previously establishing inhibition have been extended and the result confirmed by determining the *FMDV* titres in pancreas and the % animals showing viraemia in control and adjuvant-pretreated mice inoculated with a lethal and a non-lethal attenuated strain of *FMDV*, type 'O'. Comparative concn. of antibodies and of interferon (I) were also determined. Results indicate that substances having similar actions with respect to Freund's complete adjuvant can induce an effect that inhibits virus multiplication, and that these substances are more likely to be of the nature of I than antibodies. It is probable that, in pretreated mice, I is produced under the stimulus of the injected virus before or in the initial stages of virus multiplication, whereas in the control mice it follows the multiplication. The increased immunological response and early production of I most probably stem from stimulation of the reticulo-endothelial system. W. J. BAKER.

New aminoquinoline with schistosomicidal activity. N. W. Bristow, B. Lessel, H. C. Richards and G. A. H. Williams (*Nature, Lond.*, 1967, **216**, 282–283).—Some 5-aminoquinolines have schistosomicidal activity, but that shown by 6-chloro-5-β-diethylamino-ethylamino-8-methylquinoline (I) is outstanding. Tested against *S. mansoni* (maintained in *A. glabratus*) injected into mice, I was active at 30 mg per kg and killed 90% of the worms at one-eighth of the sub-chronic LD_{50} (300 mg per kg) against ≯ 70% by lucanthone at its LD_{50} (200 mg per kg). Acute administration of I produced little toxicity in mice, rats, guinea-pigs, rabbits and monkeys, but induced damage in cats and dogs. Chronic administration induced histopathological or other damage in all these species except monkeys, which tolerated 120 mg per kg daily for 5 days without serious lesions. W. J. BAKER.

Some aspects of ammonia toxicity in animal cells. W. J. Visek (*J. Dairy Sci.*, 1968, **51**, 286–295).—NH_3 toxicity in animals is reviewed. Special reference is made to pH and the diffusion of NH_3 across tissue barriers. (102 references.) M. O'LEARY.

Sinapine and related esters in seed meal of *Crambe abyssinica*. F. L. Austin and I. A. Wolff (*J. agric. Fd Chem.*, 1968, **16**, 132–135).—The isolation of sinapine (I) thiocyanate involved extraction of the defatted meal with aq. $COMe_2$, removal of the polypeptide crambin pptd. from the conc. extract, removal of epiprogoitin (the major thioglycoside) by adsorption on Al_2O_3, and pptn. of the I-thiocyanate by addition of aq. KCNS from the conc. aq. eluate. The method of Tzagoloff with improvements, was used for the assay of I. The occurrence in the meal of 1-sinapoyl β-D-glucose was demonstrated by t.l.c. The meal contained ~0·5% of I, being ~50% of the content in rapeseed meals. I had no harmful effects when fed to rats. (21 references.) P. S. ARUP.

Determination of trace amounts of copper, zinc and magnesium in animal feeds by atomic-absorption spectrophotometry. A. G. Roach, P. Sanderson and D. R. Williams (*Analyst, Lond.*, 1968, **93**, 42–49).—An automatised procedure, including Technicon sampler and a recorder, permits 60 samples to be analysed per h, with a max. coeff. of variation of ~4% for each element. Use of a dry-ashed sample makes it possible to use only one solution for Cu and Zn (ppm), with one dilution stage for Mg (0–5%). Method is claimed to be superior to the 2,2′-diquinolyl colorimetric determination of trace amounts of Cu and to the dithizone titrimetric method for Zn. Many results are reported. (20 references.) W. J. BAKER.

Residues in pasture following application of a granular heptachlor preparation. J. T. Hughes and P. G. Fenemore (*N.Z. Jl agric. Res.*, 1967, **10**, 261–271).—Residues of heptachlor and heptachlor-epoxide were found in fresh grass 9 to 12 months later even after several mowings. (13 references.) E. G. BRICKELL.

Rapid method for gas chromatographic determination of volatile fatty acids in rumen fluid. B. G. Cottyn and C. V. Boucque (*J. agric. Fd Chem.*, 1968, **16**, 105–107).—The fluid containing the acids (2–5 C) is prepared for g.l.c. by filtration, addition of HPO_3 (to precipitate proteins) and formic acid (to eliminate 'ghost' patterns in g.l.c.), and centrifugation. The g.l.c. is carried out with double flame ionisation detection and an integrator for evaluation of the peaks in comparison with standards. (15 references.)
P. S. ARUP.

[A] **Metabolic studies with *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (Dursban) insecticide in a lactating cow.** W. H. Gutenmann, L. E. St. John, jun. and D. J. Lisk. [B] **Determination of hydrolytic metabolites of organophosphorus insecticides in cow urine with an improved thermionic detector.** L. E. St. John, jun. and D. J. Lisk (*J. agric. Fd Chem.*, 1968, **21**, 45–47, 48–49).—[A] Dursban (I) fed at 5 ppm daily in the feed during four days failed to appear in the milk or the urine, but 1·7% of the total amount fed appeared in the faeces. Diethyl thiophosphate and diethyl phosphate found in the urine (see under B) would account for a further 62·7%. I was stable when incubated with rumen fluid or beef liver. I in forage at the 5 ppm level could be considered as safe. (10 references.)

[B] A g.l.c. method is described for the determination of phosphate, thiophosphate and dithiophosphate (Me_2 and Et_2 esters), in which use is made of the thermionic detector of Giuffrida *et al.*, modified to improve its stability during extended use. The method is sensitive to ~0·2 ppm of the metabolites in urine.
P. S. ARUP.

Retention of DDT and DDE by the bovine. G. F. Fries and E. A. Kane (*J. Dairy Sci.*, 1967, **50**, 1512–1514).—Calves were fed 2 g/day of DDT, DDE or a mixture of the two for 75 days. After slaughter the av. concn. of DDT, DDE and DDD in perirenal fat were 3·3, 1500, and 0 ppm for the calf receiving DDE; 240, 26, and 30 ppm for the calf receiving DDT and 195, 1500 and 24 ppm for the calf receiving the mixture. Concn. in the other tissues showed similar relationships. (10 references.) M. O'LEARY.

Effect of thyroprotein and a low-energy ration on removal of DDT from lactating dairy cows. D. D. Miller (*J. Dairy Sci.*, 1967, **50**, 1444–1447).—Experiments in which Holstein cows were contaminated with 600 mg/day of crystalline DDT for 30 days indicated that prepartum contaminated cows produce a condition of insecticide contamination more difficult to alleviate than do postpartum contaminated animals. There was no difference between the rate of decline of DDT content in milk of cows receiving normal rations and that of cows receiving thyroprotein. Cows on a low-energy ration showed a significant increase in the rate of decline compared to those on normal rations. (13 references.) M. O'LEARY.

Effect of even or uneven applications of DDT prills to pasture on DDT residues in stock. D. L. Harrison and V. Shanks (*N.Z. Jl Agric. Res.*, 1967, **10**, 253–260).—The residues found in sheep fat or in milk fat from stock on areas treated unevenly were similar to those in stock grazed on uniformly top dressed areas. This suggests the pattern of grazing was random rather than selective on the unevenly treated areas. E. G. BRICKELL.

Relation between DDT in diets of laying birds and viability of their eggs. F. J. S. Jones and D. D. B. Summers (*Nature, Lond.*, 1968, **217**, 1162–1163).—Studies on ingestion of DDT (200 ppm in food) by adult Japanese quail after start of laying showed that eggs can carry amounts of DDT or its metabolites sufficient to cause high mortality (60%) among chicks hatched from them, but without seriously affecting their hatchability. As found for dieldrin, the situation in which newly hatched chicks rely for some days on stored yolk for nutrition is also a hazard with birds contaminated by DDT. W. J. BAKER.

Oral toxicity of dieldrin to crowned guinea fowl, *Numida meleagris* (L.) I. H. Wiese and N. C. J. Basson (*S. Afr. J. agric. Sci.*, 1967, **10**, 697–705).—Guinea fowl proved to be more susceptible than chickens to dieldrin, and either more or less susceptible than various wild birds. Both the survival time and the dose ingested were inversely proportional to the dietary level. It appears highly improbable that poisoning of the guinea fowl would result from crop protection against the harvester termite with dieldrin. (15 references.) P. S. ARUP.

Production of products of nutritional value. Astra Nutrition AB (B.P. 1,080,054, 28.8.64. Sweden, 30.8.63).—Products of nutritional value for fish and animals are prepared by mixing fish or animal foodstuff of $\not< 2$ wt.-% of fat with $\not< 0{\cdot}01$ wt.-% (based on lipid content) of $CH_2OH{\cdot}CHOH{\cdot}CH_2OR$ (wherein R is aliphatic hydrocarbon of 14–24 C) or a non-toxic ester thereof, e.g., natural or synthetic glycerol ether (such as a mixture containing 15–50% of selachyl alcohol). The additives stimulate blood formation, confer increased resistance to infection, etc. F. R. BASFORD.

Benzimidazole, benzoxazole and benzthiazole derivatives. Merck & Co. Inc. (B.P. 1,074,640, 11.11.64. U.S., 19.11. and 23.12.63).—The deriv., which have a (substituted) amino group attached at the 5 or 6 position, and benzazoles having a halogen atom attached in one of these positions, are used for treatment and prevention of helminthiasis in animals. The benzimidazoles can be prepared e.g. by reacting a nitroaniline with a heterocyclic carboxylic acid (deriv.) (**I**), reducing the NO_2 group, and cyclising the anilide, or by reacting an *o*-phenylenediamine with **I** in polyphosphoric acid at 175–275°. The benzoxazoles and benzthiazoles are similarly prepared from **I** and an *o*-aminophenol or *o*-aminothiophenol respectively. Thus, 2-nitro-4-fluoroaniline is reacted in toluene with furoyl chloride; the anilide intermediate is reduced over Pd/C, refluxed with aq. HCl and neutralised with NH_4OH to give 2-(2′-furyl)-5 (6)-fluorobenzimidazole, m.p. 220–221° (aq. EtOH). E. ENOS JONES.

Benzimidazole, benzthiazole, and benzoxazole derivatives. Merck & Co., Inc. (B.P. 1,085,634, 11.11.64. U.S., 19.11.and 23.12.63).—The title compounds are effective against helminths, especially swine ascarids, and are substituted in the 2-position by pyrrolyl, pyridyl, furyl, thienyl, thiazolyl, isothiazolyl, or thiadiazoyl and in the 5- or 6-position by imidazolyl, thiazolyl, isothiazolyl, thiadiazolyl, thienyl, furyl, or pyrrolyl and (in the case of the benzimidazoles) optionally in the 1-position by alkyl of 1–5 C, aralkyl, or acyl. In an example, a mixture of 2-nitrothiazole-4-carbon-*N*-(*p*-imidazol-4-yl)-anilide, MeOH, conc. aq. HCl, and 5% Pd-on-C, is hydrogenated at room temp./40 p.s.i.; the filtered liquor is concentrated/<1 atm., then a solution of the residue in water, EtOH and conc. aq. HCl is boiled during 4 h, and volatile matter is removed/<1 atm. The residue is dissolved in alcohol and the solution is diluted with ether, with pptn. of 2-(thiazol-4-yl)-5(or 6)-(imidazol-4-yl)benzimidazole. F. R. BASFORD.

[A and D-F] 5-nitroimidazole compounds, [B] imidazole derivatives, [C] 1-alkyl-5-nitroimidazole-2-carboxylic acids and their preparation [and use]. Merck & Co., Inc. (B.P. 1,077,691–6, [A, B, D–F] 4.8, [C] 11.11.64. U.S., [A, B, D-F] 7.8., [C] 27.11.63).—Compounds claimed are active against histomoniasis and trichomoniasis and comprise 1-R-2-R^I-5-nitroimidazoles wherein R is [A-C, E, F] alkyl of 1-5 C or [A, B, F] $[CH_2]_nOX$ (*n* is 2-4; X is C_{1-6}-alkanoyl or [A, B] H) and R^I is [A] $CONR^{II}R^{III}$ (R^{II} and R^{III} are H, alkyl of 1–5 C, or R^{III} is OH-alkyl, optionally substituted Ph, heterocyclyl, NH_2, NHPh, glucosamino, or CH_2CONH_2, or $NR^{II}R^{III}$ is morpholino, piperazino, piperidino, or pyrrolidino), [B] CN, [C] CO_2H (or salt), [E] COZ (Z is OM, Cl, or Br; M is H, alkyl of 1-5 C, or metal), or [F] SO_2R^{IV} (R^{IV} is hydrocarbon radical, preferably alkyl of 1-5C, aryl, aralkyl, or cycloalkyl); or [D] R and R^I together form a $[CH_2]_nO{\cdot}CO{\cdot}$ chain (*n*=2-4). Compounds of [A] are prepared by interaction of $NHR^{II}R^{III}$ with compounds (**I**) of [E] (Z=Cl, Br, or alkoxy) or those (**II**) of [D]. E.g., crude 5-nitro-1-methylimidazole-2-carbonyl chloride (**III**) is reacted with NH_3 to give 5-nitro-1-methylimidazole-2-carbonamide, m.p. 222–224. Compounds of [B] are prepared by reacting compounds **IV** (of [F]) with a cyanide, and on hydrolysis with a base are converted into the carbonamides of [A]. They may also be reacted with alkanols in presence of a strong base to form 2-iminoethers which on treatment with mineral acid afford the esters of [E]. Where R is $[CH_2]_nOX'$ (X′ is alkanoyl), hydrolysis of compounds of [B] gives the free alcohols which with a strong base are converted into cyclic iminoethers—and these with acid yield **II**. E.g., 5-nitro-2-methylsulphonyl-1-methylimidazole (**V**) and KCN are reacted to give 5-nitro-2-cyano-1-methylimidazole. [C] Compounds claimed are prepared by oxidation of corresponding 2-hydroxymethyl analogues at $\not< 60°$ with an acidic oxidising agent in H_2SO_4 in presence of $< 25\%$ of water. E.g., 5-nitro-1-methyl-2-(hydroxymethyl)imidazole (**VI**) is stirred into conc. H_2SO_4, then 70% aq. HNO_3 is added. After 60 h at 75–80° the mixture yields 5-nitro-1-methylimidazole-2-carboxylic acid m.p. 84–85°, the K salt (**VII**) of which is converted into derivatives **I** of [E]. E.g., **VII** (prepared in [E] by oxidation of **VI** in acetone with $KMnO_4$) is reacted with $(COCl)_2$ to give **III**. [D] Lactones claimed, and mentioned in [B], are prepared in [D] by sulphonating a 5-nitro-2-(2 phenylvinyl)-1-(ω-hydroxyalkyl)imidazole, then oxidising the resulting 5-nitro-2-(2-phenylvinyl)- 1-(ω-sulphonyloxyalkyl)imidazole. [F] Compounds **IV** claimed are prepared by oxidation of the corresponding 2-SR^{IV}-analogues. E.g., 5-nitro-2-methylthio-1-methylimidazole is oxidised in AcOH with 30% H_2O_2 to give 5-nitro-2-methylsulphonyl-1-methylimidazole m.p. 91–92·5°. F. R. BASFORD.

2-Substituted benzimidazoles. Merck and Co., Inc. (B.P. 1,080,786, 15.10.64. U.S., 15.11.63).—The title compounds of formula BZ-R, wherein BZ is the benzimidazole (**I**) nucleus and R is aryl, heteroaromatic, aralkyl or alkyl radical, are obtained by oxidising an amidine of formula $Ph{\cdot}NH{\cdot}CR{:}NH$ (where Ph is optionally substituted with one or more substituents resistant to oxidation) with $Pb(OAc)_4$, an alkali metal ferricyanide, or S. Thus, 2-(4′-thiazolyl)benzimidazole is prepared by reacting aq. *N*-phenyl(thiazole-4-amidine)-hydrochloride with $K_3Fe(CN)_6$ in presence of $NaHCO_3$ and acidifying with conc. HCl. The 2-aryl and 2-heterocyclic **I** compounds possess anthelmintic properties; others are useful antimetabolites. S. D. HUGGINS.

Anthelmintic compositions. Harshaw Chemical Co. and Dr. Mayfield Laboratories. (B.P. 1,081,260, 25.7.66. U.S., 23.7.65).—A method to prevent, or control, the infestation of domestic animals and poultry with large roundworms is claimed. The composition is obtained by mixing Co arsenate, Co phenyl arsonate and Co substituted-phenyl arsonate with their water or feedstuffs; the substituents on the phenyl group are halogen, amino, methyl, phenyl, chlorophenyl, nitro, hydroxy or ureido groups. In general the dosage is 0·25–1 lb. Co-salt per ton dry solid feedstuff or an equivalent concn. if administered in oil, other feed or water. H. L. WHITEHEAD.

1,3-Ethanopiperazine and its derivatives. Merck & Co., Inc. (B.P., 1,082,061, 1.2.65. U.S., 3.2.64).—1,3-Ethanopiperazine (3,6-diazabicyclo[1,2,2]octane) (**I**) and deriv. thereof substituted in the 6-position by alkyl of 1-5 C, alkanoyl of 2-6 C or Bz are claimed. They are anthelmintic agents, especially useful in combating roundworms in animals. A solution of 2-(2-hydroxyethyl)pyrazine in MeOH is hydrogenated in presence of PtO at room temp./40 p.s.i. during 20 h, to give 2-(2-hydroxyethyl)piperazine (**II**) (dihydrochloride, m.p. ~ 210°). The **II** is cooled in solid CO_2, then $SOCl_2$ is added at $< 40°$. The mixture is boiled during 5·5 h, cooled to room temp., and filtered. The filter cake yields 2-(2-chloroethyl) piperazine dihydrochloride (decomp. at 348–350°). A suspension

of this in water is treated with NaOH and the solution is extracted with $CHCl_3$. Distillation of the extract yields **I**, b.p. < 100°/3 mm (dihydrochloride, m.p. 348° with decomp.). It is converted with BzCl in presence of aq. NaOH into 4-benzoyl-1, 3-ethanopiperazine, m.p. 95–97°. F. R. BASFORD.

Bacitracin metal methane sulphinates. Commercial Solvents Corp. (B.P. 1,072,337, 19.11.65. U.S., 22.1.65).—Useful as an animal feed additive, with reduced nephrotoxicity, the title compounds are obtained from the HCHO-sulphoxylates of metals, preferably Na and aq. bacitracin. Thus, four different concentrations (0·75%, 1·5%, 3·0% and 6·0% by wt.) of Na-HCHO-sulphoxylate (**I**) in saline are prepared and 0·169 g regular bacitracin added to 10 ml of each. Controls of 0·169 g bacitracin and/or 6·0% by wt. **I** are also prepared and all mixtures are allowed to stand at room temp. for approx. 2 h. Each of the mixtures is then injected into mice at a dose of 500 units/mouse. With the bacitracin control all mice show typically bleached kidneys, with the solution containing 0·75% **I** spotted kidneys are shown, but with the 1·5% and 3·0% damage is reduced. Solutions containing the higher amount (6%) of **I** were toxic. S. D. HUGGINS.

Thiopenicillins. Bristol-Myers Co. (B.P., 1,078,078, 4.9.64. U.S., 4.9.63).—Used as antibacterial agents, supplements for animal feeds and for treating mastitis in cattle, the N-substituted 6-ureidothiopenicillanic acids (and salts) are prepared by converting the 3-carboxylic acid group of the starting penicillin to a reactive acylating deriv. and then reacting this with a source of sulphydryl groups, e.g. H_2S. A specific compound claimed is 6-(benzoylureido)thiopenicillanic acid (salts). S. D. HUGGINS.

Erythromycin salt, compositions incorporating it, and its preparation. Roussel-Uclaf (B.P., 1,084,830, 30.6.66. Fr., 2.7. and 30.9.65).—The salt claimed is the glutamate (**I**). A solution of basic anhyd. erythromycin (**II**) in aq. COMeEt is treated with glutamic acid, then after 10-18 h, the pptd. **I** solvated with 5·5% of water, is collected (m.p. 148–150°). It is effective against gram-positive organisms, especially those resistant to **II**, and is useful in human and veterinary medicine. F. R. BASFORD.

17α-Substituted 17β-hydroxyoestrenes. Abbot Laboratories. (B.P. 1,073,387, 10.12.64. U.S., 30.12.63).—Compounds claimed include 3-methoxy-17α-(2-hydroxyethyl)- (**I**), 3-methoxy-17α-(2-acetoxyethyl)-, and 3-benzyloxy-17α-(2-hydroxyethyl)-**17β-hydroxyoestra-1, 3, 5 (10)-triene,** also **17α-(2-hydroxyethyl)**-17β-hydroxy-3-methoxy-oestra-2,5(10)-diene, and -17β-hydroxy-3-oxo-oestr-4-ene and -3,17β-dihydroxy-oestra-1,3,5(10)-triene. They have oestrogenic and growth-promoting activity in warm-blooded animals. In an example, a mixture of ether, benzene, acid-washed electrolytic Zn sponge and I_2 is boiled until colourless, then $CH_2Br \cdot CO_2Et$ is added, followed by a solution of oestrone Me ether in benzene. The mixture is boiled during 5 h, with addition of 5 lots of Zn at 45-min. intervals and 2 lots of ester at 90-min. intervals, then after cooling in ice a 1:5-mixture of AcOH–MeOH is slowly charged. The mixture is filtered; the filtrate is worked up to give Et (17β-hydroxy-3-methoxy-oestra-1,3,5(10)-trien-17α-yl)-acetate, m.p. 101-103° (aq. MeOH). A solution of this in benzene is added during 15 min. to a boiling mixture of $LiAlH_4$ and tetrahydrofuran, then after a further 2 h, EtOAc is added, followed by water and conc. aq. HCl. Solvent is evaporated off, and the product is recryst. from aq. MeOH, to give **I**, m.p. 140–143° and 156–159° (dimorphic). F. R. BASFORD.

A choline derivative and therapeutic compositions containing it. H. E. J.-M. Meunier (B.P., 1,077,039. 31.5.66. Fr., 21.10.65).—The compound claimed is choline dipropylacetate and it is used in animals (other than man) to reduce cholesterol level of the blood level or to cure liver damage. F. R. BASFORD.

Chromans. CIBA Ltd. (B.P. 1,086,101, 10.1.66. Switz., 19.1.65).—Used pharmaceutically or in veterinary preparations, the claimed optionally substituted 3-guanidinochromans have the formula $R_nC_6H_{4-n}(C_3H_4O)NH \cdot C(NH)NH_2$ wherein R is lower alkyl, alkoxy, CF_3, or a halogen atom, n=0–1, and are prepared by reacting a chroman with a NH_2 group in the 3-position, with a compound of formula XN:C(Y)Z (wherein Z is optionally substituted amino, Y is an etherified mercapto and X is H or a substituent or X and Y with the CN bond forms a triple bond) followed by N-substitution or conversion to the acid addition salt. Thus, 3-aminochroman hydrochloride is reacted in EtOH with cyanamide to give 3-guanidinochroman hydrochloride. An animal feedstuff containing 3-guanidinochroman is among the products claimed. S. D. HUGGINS.

Compositions for treating coccidiosis. Sumitomo Chemical Co., Ltd. (B.P. 1,085,738, 21.1.66. Japan, 23.1.65).—The active agent of the compositions is 4-(*p*-aminobenzene-sulphonamide)-6-methoxy-2-methyl-3(2*H*)-pyridazinone (of B.P. 1,063,884). Preferably it is administered during 3-4 days as a 0·1–0·2% additive to poultry feed. F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Hordein and gliadin in ripe and unripe grain. XVII. Work of E. Waldschmidt-Leitz *et al.* on seed proteins. H. Kling (*Brauwissenschaft*, 1968, **21**, 182–184).—Earlier electrophoretic observations that all the prolamine components present in ripe barley and wheat are also present in the unripe grain are confirmed. However, their proportions differ considerably. During 5 weeks storage after ripening, considerable variations in the proportion of hordein components occurred which (in barley) mainly took the form of increases in the α, β components. Higher than normal storage temp. produced variations in composition; these were not of a uniform pattern. I. DICKINSON.

Silicon, the neglected element, and its occurrence in cereal products. L. Tunger and M. Rothe (*Ernährungsforschung*, 1967, **22**, 471–483).—A review with 102 references. P. S. ARUP.

Hydrophilic properties and soluble substances [found] in intermediate fractions of wheat milling. I. F. Kr'yuk (*Pishch. Tekhnol.*, 1967, No. 4, [59], 29–31). C.V.

Viscosity, molecular weight and starch amylose content [observations] in heat drying of maize. L. V. Masenko, N. V. Romensky and V. A. Yakovenko (*Pishch. Tekhnol.*, 1967, No. 5, [60], 119–121).— C.V.

Starch as a raw material in dietary bakery and confectionery. A. Rotsch (*Stärke*, 1967, **19**, 349–354).—Ungelatinised starch is the main factor which controls crumb formation in bread and flour based confectionery. To demonstrate this bread has been baked from artificial doughs containing starch and gel-forming carbohydrates for dough binding. In biscuit production, starch from wheat, maize, rice and probably other sources, can replace wheat flour in amounts determined by the baking conditions with a resultant increase in bread vol., crumb texture and tenderness; however, the rate of staling is also increased. The significance of this in the production of dietary bread (for use in cases of sprue, coeliac disease, etc.) is discussed. (14 references.) J. B. WOOF.

Effect of temperature on adsorption of water and water-soluble material on ungelatinised potato starch. A. H. A. de Willigen and P. W. de Groot (*Stärke*, 1967, **19**, 368–372).—As the temp. falls, adsorption of water and amino-acids in solution increases considerably. Therefore starch should be purified at as high a temp. as possible, and never close to the freezing point. J. B. WOOF.

Variation in the physical and chemical properties of starch. IV. Artificial ageing of potato and cereal starch granules. F. Schierbaum (*Stärke*, 1967, **19**, 309–316).—Artificial ageing by moisture treatment has been carried out in model systems and changes in the chemical and colloidal properties followed. The moisture content was found to be the decisive factor. The splitting of amylophosphoric acid esters of potato starch appeared to be acid catalysed and dependent on cation content. (27 references.) J. B. WOOF.

Instant dextrin. R. A. van Linge (*Stärke*, 1967, **19**, 300–302).—Dextrins, especially from potato starch, have a tendency to form lumps during the dispersing and dissolving processes. Wetting agents cannot be used since they adversely affect the stability of the finished product. Instant products have distinct grain dimensions; these may be varied within certain limits under the influence of spray drying. Older methods, in which acid catalyst was added to the starch slurry and after maturation, the product was spray dried and roasted in a modified flash drier, are therefore reconsidered. Remoistening and agglomeration could then be achieved by using e.g. the Cherry-Burell process. J. B. WOOF.

Acid and fermentation hydrolysis of maize starch. V. A. Yakovenko, N. V. Romensky and L. V. Masenko (*Pishch. Tekhnol.*, 1967, No. 6, [61], 20–22).— C.V.

Phosphatase action on phosphate in potato starch. I. Purification and properties of phosphatase from *Aspergillus awamori* var. *kawachii*. Y. Ohta, Y. Sumie and S. Ueda (*Stärke*, 1967, **19**, 327–330).—Three different types of phosphatase have been isolated from an enzymic prep. provided by a filtrate of *Aspergillus awamori* var. *kawachii* culture on wheat-bran. One of the purified enzymes

was more active towards glucose-6-phosphate (I) than towards β-glycerophosphate (II) (the usual substrate for phosphatase); the other two enzymes were more active towards II than towards I. (In English.) (16 references.) J. B. WOOF.

Phosphatase action on phosphate in potato starch. II. Mechanism of complete degradation of phosphodextrin by amylase and phosphatase. Y. Ohta and S. Ueda (*Stärke*, 1967, **19**, 363–368).—Phosphodextrin with a degree of polymerisation (*DP*) of 9–10 glucose units was prepared from potato starch using bacterial α-amylase (I) according to the procedure of Posternak, Whelan and Fukui. This dextrin was found to be completely degraded by the combined I and phosphatase (II) activity of prep. from the black koji mould, *Aspergillus awamori* var. *kawachii*. I was inactive against the phosphodextrin with a *DP* of 3–4 units. The actions of bacterial α-I, potato β-I and malt-I were in agreement with those observed by Posternak, Whelan and Fukui. The smaller the dextrin unit the easier it was for the II to liberate phosphate. Both types of enzyme were necessary for total degradation. (In English.) (10 references.) J. B. WOOF.

Autolytic degradation of cereal starches by their own amylases. G. Wahl (*Stärke*, 1967, **19**, 322–327).—The activity of the amylase bound to the starch (*S*) granule, which causes saccharification, varies with the origin of the *S*. There is a direct relationship between the amylase activity of the raw material and the degradation of the *S* extracted from it. In wheat, different qualities of *S* show different tendencies towards autolysis. The breakdown can be decisively affected by steeping conditions; this was found to be at a min. in maize-*S* when the pH was < 3·2 and the SO_2 content was > 0·2%. Drying temp. which damage the *S* granule increase the tendency to autolytic degradation. (12 references.) J. B. WOOF.

Enzymic liquefaction of starch. W. Nierle (*Stärke*, 1967, **19**, 389–393).—The enzymic saccharification and liquefaction of starch has been studied under different conditions. The course of amylolytic hydrolysis is determined by substrate concn., pH and temp. changes. On a pilot scale it was shown that with high concn. of starch mechanical stirring was impracticable. However this proved unnecessary and results of experiments using a multistage impulse agitator are quoted. J. B. WOOF.

Enzymic methods for liquefying starch. H. Barfoed (*Stärke*, 1967, **19**, 291–295).—In the production of dextrose and glucose syrups by enzymic methods, efficient gelatinisation of the starch is essential if the hydrolysate is to be easily filtered. Heat gelatinisation methods are limited by the heat stability of the enzymes used. The methods employed are those of Rohm and Haas, the steam injection process, those operated by the Continental Engineering Co. and by the Karl Krøyer Co. (*Ibid.*, 1966, **18**, 311). J. B. WOOF.

Modern methods of purification of starch hydrolysates. J. Karkalas (*Stärke*, 1967, **19**, 338–345).—Starch hydrolysates (syrups) from the converter contain sol. and insol. impurities which must be removed. At pH 1·7 the protein and fatty substances are present in a colloidal state but if the pH is raised to 4·5, flocculation occurs and the ppt. can be removed by a rotary drum filter or continuous centrifugal separators. Conditions of operation are discussed with reference to pilot plant data. Minor components remaining in solution can have a major effect on colour development, turbidity, foaming and inversion of sucrose during sweet manufacture. Pilot scale experiments have shown the effects of Mg^{2+}, Ca^{2+} and phosphate in causing considerable inversion. Activated charcoal and ion-exchange resins can be used to remove protein and colouring compounds and the undesirable ions respectively. (In English.) (11 references.) J. B. WOOF.

Enzymic determination of starch in preparations of apple pectin. W. Bock, H. Ruttloff and R. Friese (*Ernährungsforschung*, 1967, **12**, 457–470).—A qual. test for starch or dextrin is carried out by the colour reactions of a 1% solution of the sample with 0·02 N-I_2, in comparison with standards; the min. detectable amounts are 70 μg of starch or 300 μg of dextrin per ml. The starch is first hydrolysed with glucose-amylase at 55°, the resulting glucose being determined by the glucose–oxidase–peroxidase method of Taufel *et al.* and Ruttloff *et al.* Errors possibly due to interference by the hydrolytic products of pectin are negligible. The min. detectable amount of starch is 0·19%; the accuracy is within ±2·4%. (17 references.) P. S. ARUP.

Wheat dough: Influence of intensity of mixing on colloidal and biochemical processes. A. I. Skorikova and I. M. Roiter (*Pishch. Tekhnol.*, 1967, No. 6, [61], 50–58).—(81 references.) C.V.

Rheological testing of wheat glutens and doughs. P. W. Heaps, T. Webb, P. W. Russell Eggitt and J. B. M. Coppock (*Chemy Ind.*, 1968, 1095–1096).—Variation of relaxation time with level of work input during dough mixing was determined in the Brabender Extensograph (for doughs) and the Instron Tensile Tester (for glutens). Results confirm that physical properties of dough are a reflexion of gluten properties. The compressive stress-relaxation test is proposed for routine purposes, especially by the plant breeder, as it is simple and requires only a 2·5 g sample. W. J. BAKER.

Comparison of physical properties of wheat dough and gluten after mixing in rotary and stationary bowl dough mixer. A. I. Skorikova and I. M. Roiter (*Pishch. Tekhnol.*, 1967, No. 4, [59], 47–51).— C.V.

Influence of defatted dried-milk additives on the properties of the dough and quality of bread. A. M. Kal'yuzhnaya, S. G. Sergeyeva and M. F. Muzyka (*Pishch. Tekhnol.*, 1967, No. 4, [59], 52–53).— C.V.

Baking properties in wheat flour blends. K. Möttönen (*Valt. tek. Tutkimusl. Julk.*, 1967, No. 117, 114 pp.).—Eight Finnish commercial wheats and Manitoba wheat were milled locally, blended in the ratios 2 : 1 and 1 : 2 and used in large scale baking trials for producing pan bread and round type loaves, using different bromate additions and dough times. The baking properties were evaluated statistically by analysis of variance, calculation of the factorial effects and by regression analysis. Analysis of variance showed no significant differences in baking behaviour of the two types of bread. The results show that if practical behaviour is to be predicted on the basis of conventional analyses, the type of bread and wt. and dimensions of the loaf must be taken into account. The rating of a wheat in blends is so complicated by baking conditions that total correlations varied between the extremes 0·999 and 0·223. (In German.) (55 references.) J. B. WOOF.

Determination of standards for vitamin B_1 content of loaf bread from flour of different extractions. II. Vitamin B_1 content of rye bread as influenced by dough production and baking. III. Comparison between rye and wheat breads. B. Thomas and L. Tunger (*Ernährungsforschung*, 1967, **12**, 625–639).—Further to Part I (*Idem, ibid.*, 231–247) no differences in B_1 content were caused by variations in the dough-making process, even when 1% of yeast was added. Although losses of B_1 during the baking of wholemeal rye bread were 25% as against 19% for light-coloured rye bread, the B_1 content of the wholemeal bread is, nevertheless, greater than that of bread of lower extraction-%, owing to its greater initial content of B_1; the difference is due to the longer baking times required by the latter types of bread. Max. B_1 contents (mg/100 g) occurred in wholemeal wheaten bread (0·229) and in wholemeal rye bread (0·172). (20 references.) P. S. ARUP.

Sugars and confectionery

Decomposition of glucose in sulphuric acid under high temperatures. V. G. Kul'nevich and B. N. Ershov (*Pishch. Tekhnol.*, 1967, No. 4, [59], 43–46).— C.V.

Relationship between refractive index and specific gravity of aqueous sucrose solutions. H. B. Basker (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1370–1371).—Differences between tabulated values of sp. gr. (Domke; International Scale) and those predicted by calculation from the regression of refractive index on concn. by vol., or *vice versa*, are systematic though small, being equivalent to about 0·1% of sucrose. A. A. ELDRIDGE.

Preparation of lactulose. L. Gatzsche and H. Haenel (*Ernährungsforschung*, 1967, **12**, 641–647).—The prep. of this ingredient for infants' foods (β-D-galactopyranosyl-α-D-fructose) (I) by the method of Montgomery was modified by the use of KOH instead of $Ca(OH)_2$. The KOH was added to the solution of lactose to give a concn. of 0·025 N-KOH, and further equiv. amounts were added after 6, 12, and 18 h to give a conversion of 30% of the lactose into I. The solution was passed over a cation- and an anion-exchanger, concentrated *in vacuo* to 25–30% and then spray-dried. A prep. of lactose containing 60% of I was obtained by concentration of the solution and pptn. with MeOH. A modification of the Montgomery method is described for the prep. of pure I, and a paper chromatographic method is described for the determination of I. P. S. ARUP.

Fermentation and Alcoholic Beverages

Bakers' yeast metabolism. I. Pyridine nucleotide reduction and oxygen utilisation during alcohol oxidation. II. The rôle of adenine

nucleotides and inorganic phosphates in the control of respiration during alcohol oxidation. III. Oxidation of acetaldehyde. P. K. Maitra and R. W. Estabrook (*Arch. Biochem. Biophys.*, 1967, **121**, 117–128; 129–139; 140–146).—I. Fluorometric measurement of pyridine (**I**) reduction reflected qualitatively the state of reduced diphospho-**I** nucleotide (*N*) produced by the action of ethanol in the cell. This has been assessed by comparative spectrophotometric and direct chemical analysis of **I**-*N* and shows a fourfold-fluorescence for reduced **I**-*N*, presumably associated with a binding to some cellular constituent. Differences in steady-state pattern of **I**-*N* reduction with saturated and unsaturated alcohols arise from oxidation to aldehyde (**II**). Measurement of CO_2 evolution during this indicates a delay in CO_2 production and **II**-formation with cinnamyl alcohol shows a stoicheiometric formation of **II** with O_2 utilised. Determination of **II** concn. during ethanol oxidation in short-time experiments suggests that 70% O_2 utilised arises from oxidation to CH_3CHO, the remainder being formed into acid. In long-term experiments, ethanol is completely oxidised. (23 references.)

II. Respiratory inhibition (*RI*) following within 40 sec. after addition of ethanol to a starved bakers' yeast cell suspension is studied. Glucose (**III**), 2-deoxy-**III** or **III** in the presence of iodoacetic acid show that *RI* is caused by the unavailability of adenosine-5′-diphosphate; using a PO_4-trap such as 2-deoxy-**III** or **III** it is shown that *RI* arsises from the lack of inorg.-PO_4. A true Crabtree effect could not be demonstrated. (20 references.)

III. The kinetics of respiration and **I**-*N* reduction during CH_3CHO oxidation are studied and it is shown that the reducing equivalents generated in the oxidation of CH_3CHO to acetic acid are capable of activating oxidative phosphorylation. Such acid yields H ions that permeate out of the cell in stoicheiometric exchange with extracellular K which permeates into the cell. (12 references.) C.V.

Biological action of 3-hydroxypyridine derivatives. E. N. Odintsova (*Dokl. Akad. Nauk SSSR*, 1967, **176**, 717–718).—The inhibiting properties of seven newly synthesised deriv. of 3-hydroxypyridine, similar in structure to vitamin B_6 (pyridoxine), were tested against cells of selected yeast organisms (*Saccharomyces cerevisiae*, *S. carlsbergensis*). They mostly inhibited cell growth, but were not toxic. The dose was 2000 μg/ml with 5 μg of inositol (**I**) and 0·25 μg pantothenic acid (**II**) per ml of medium. 2-ethyl-3-hydroxymethylpyridine hydrochloride had the same physiological activity as pyridoxine. A table shows the inhibiting action (24–60 h) of 4-dimethylaminomethyl-2,6-dimethyl-3-hydroxypyridine hydrochloride (**III**) on the multiplication of cells in two yeast cultures, one of which does not synthesise **I** and the other not **II**. **III** was the most active substance prepared. The other seven deriv. are not named. P. W. B. HARRISON.

The varieties of yeast in two different wine regions and their use in the wine industry. O. Šafar (*Bull. scient. Cons. Acads RSF Yougosl.*, 1967, **12**, 263).—The types of yeast found in two wine regions, Pljesivica and Peljesac, were investigated, 72 types of grape, 72 grape juices, 30 wines and 77 wine sediments were examined, 1682 different yeasts were isolated and their value in producing quality wines was studied. (In German.) W. E. ALLSEBROOK.

Effect of different steeping and germination times on the characteristics of spring barleys. II. A. Fritz, E. Ulonska and W. Lenz (*Brauwissenschaft*, 1967, **20**, 407–412).—Experiments were made to ascertain how far results of extract differences of malts agree with the result of the Brabender hardness testing procedure, described in Part I. Widely differentiated malts from different barley varieties were used and different variations of steeping and germination times were employed. A very good correlation was found between the Brabender hardness values and the extract differences ($r = +0·93$); this suggests that the Brabender measurements of malts can be used to assess their level of modification. I. DICKINSON.

Trial brews with hops of various origins and different chemical compositions. P. Hautke and D. Petříček (*Mschr. Brau.*, 1968, **21**, 91–96).—A series of brewing trials was carried out using the Saaz hop, Clone No. 72, widely grown in Czechoslovakia, as the standard. The beers, containing 21–33 mg of isohumulone/kg, were evaluated by a tasting panel using a points system but the ratings showed some divergence from the values given by spectrophotometric determinations of the isohumulones. The discrepancies may be due to differences in the compositions of the α-acids (cohumulone fraction) or in part to the levels of tannins and essential oils. J. L. WALPOLE.

Continuous fermentation of a continuously prepared decoction wort. J. Mostek, J. Dyr and M. Kahler (*Brauwissenschaft*, 1967, **20**, 397–407).—Worts, green beers and finished beers prepared continuously had a higher content of nitrogenous substances and of anthocyanogens, a higher colour, approx. the same tannin content, and a lower content of bitter substances than beer produced conventionally. They possessed a fine flavour and aroma and had excellent foam stability. (90 references.) I. DICKINSON.

Gas chromatographic determination of diacetyl and pentane-2,3-dione in beer. B. Drews, G. Bärwald and H.-J. Niefind (*Mschr. Brau.*, 1968, **21**, 96–100).—A gas chromatographic method using electron capture detection is described, Separation is best carried out on a 4·2-m column of 5% Igepal (nonylphenoxypolyethylenoxyethanol) absorbed on Chromosorb W-NAW (80–100 mesh) at 70°. Glass or stainless steel (but not Cu) should be used for the column. The diacetyl and pentanedione contents of a number of typical beers were determined and their variations during fermentations followed. The results are compared with those from a colorimetric diacetyl determination. J. L. WALPOLE.

Influence of enzymatic extracts from *Trichothecium roseum* strains on the content of extract and protein fractions in beer during its storage. C. Szajer (*Annls Univ. Mariae Curie-Sklodowska, Agric.* E, 1966, **21**, 253–261).—Protection of beer against turbidity during its storage was studied. Application of conc. enzymic extracts from cultures of the fungus *Trichothecium roseum* caused quant. changes in the protein fraction of beer and exerted a favourable influence on the reaction which led to the completion of the fermentation process. Result of analyses are presented in two tables. (29 references.) T. M. BARZYKOWSKI.

Catecholase activity of musts. Yu. D. Tagunkov (*Pishch. Tekhnol.*, 1967, No. 4, [59], 37–40).— C.V.

Action of glucose oxidase in grape vines and must. A. A. Merzhanian and Yu. D. Tagunkov (*Pishch. Tekhnol.*, 1967, No. 6, [61], 89–92).—(11 references.) C.V.

Gas chromatographic investigation of ciders. C. Reinhard (*Dt. LebensmittRdsch.*, 1968, **64**, 251–254).—Pentane/ether extracts of a number of ciders made from fresh fruit and syrups and having markedly different bouquets have been compared using gas chromatography. In syrup ciders the 1-butanol and 1-hexanol contents were between 0 and 0·4 mg/l whilst 98% of the fresh fruit ciders contained 3–9 mg/l of butanol and 3–6 mg/l of hexanol. It is suggested that these two compounds are the essential carriers of the special cider aroma; their quant. analysis is described. (17 references.) J. B. WOOF.

Removal of sulphurous acid from wine distillates. H. Haushofer and W. Meier (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 437–448).—The removal was achieved satisfactorily and without adverse organoleptic effects by boiling the distillates under reflux for 10–20 min., with or without the passage of a current of air through the liquid. Satisfactory results were also obtained by treatment with an anion-exchanger containing α-hydroxyethylsulphonic acid (**I**) (Lewatit MP 500); the efficacy of the treatment is inversely proportional to the EtOH %; treatment of the wine before distillation is, therefore, recommended. The organoleptic threshold value for **I** in brandies was determined as 85–175 mg/l (as SO_2), according to the aroma intensity. (11 references.) P. S. ARUP.

Accuracy of pyknometric alcohol determination in spirits containing extract. B. Matzik (*Dt. LebensmittRdsch.*, 1968, **64**, 247–250).—Laboratory determinations by three operators have been compared statistically. For all the determinations, a standard deviation of ±0·060 was obtained which for 90% certainty limits gives a dispersion range of ±0·15% by vol. For duplicate determinations the range for the same degree of certainty was ±0·11%. Results by the three operators were examined separately and showed up certain personal factors in the errors. J. B. WOOF.

Fruits, Vegetables, etc.

Combined effects of temperature and atmospheric composition on fruit ripening, with the use of a variety of pear [*Passe Crassane*]. S. Güçlü (*Fruits d'outre mer*, 1967, **22**, 433–442, 503–516; 1968, **23**, 79–106).—The importance of the determination of both the O_2 absorbed and the CO_2 evolved in respiration studies is pointed out. Laboratory apparatus for the determination of gaseous compositions, for respiration studies on small no. of fruits, and for the determination of total CO_2 in fruits is described. The effects of storage temp. on respiration and the onset of the climacteric are investigated. Effects of changes in the CO_2 and O_2 content of the storage atm. are largely dependent on the storage temp. A

preliminary storage period at 0° in an atm. of low O_2 and high CO_2 content retards the softening of the fruit in air at 15°; the longer the period at 0° the more rapid will be the softening; after storage in N_2 the softening is greatly retarded. (118 references.)
P. S. ARUP.

Flavonoids of apples and pears. Y. G. Skorikova and E. I. L'yashenko (*Pishch. Tekhnol.*, 1967, No. 6, [61], 40–45).—(14 references.) C.V.

Biochemical changes of apples in the production of a concentrate. V. B. Usheva and E. V. Scherbakova (*Pishch. Tekhnol.*, 1967, No. 5, [60], 108–111). C.V.

Volatiles in controlled-atmosphere apple storage–evaluation by gas chromatography and mass spectrometry. P. Angelini and I. J. Pflug (*Fd Technol. Champaign*, 1967, **21**, 99–102).—The C_2H_4 content of storage atm. could be readily controlled by g.l,c. Equilibria in C_2H_4 concn. differed for various apple varieties, and were independent of the method of CO_2-removal used (water, aq. NaOH, or active C). Concentrates obtained by the low temp. high vacuum process from active C that had been exposed to storage atm. were analysed by g.l.c. and mass spectrometry; a large no. of saturated and unsaturated hydrocarbons, and smaller no. of alcohols, esters, and aldehydes were identified. (16 references.)
P. S. ARUP.

Quality control of canned grapefruit segments. H. B. Basker (*Israel J. Technol.*, 1967, **5**, 221–222).—Means and variations in net wt., headspace, drained wt., cut-out ° Brix, and tare wt. for 68 consecutive cans of grapefruit are reported and discussed in respect of difficulties in determining compliance of a batch with standard specifications. From 5 to 15 cans in a batch (one day's production) are tested destructively for significance of correlation of parameters, but for an accurate estimation of true mean Brix a larger no. of cans are tested non-destructively by using the correlation with net wt. If the mean Brix is unsatisfactory, sub-division of the batch on the basis of net wt. is necessary, each unit having a different mean Brix. Validity of the correlations found is discussed. True average drained-wt. cannot be accurately estimated by non-destructive means. W. J. BAKER.

Some physical constants of tomato products. V. A. Maslikov and A. K. Medvedev (*Pishch. Tekhnol.*, 1967, No. 4, [59], 78–79).—
C.V.

Storage temperatures and chemiluminescent activity of potatoes. B. I. Popov, V. V. Tsvetkova and A. A. Ponomarenko (*Pishch. Tekhnol.*, 1967, No. 4, [59], 27–28).— C.V.

Nitrate content of edible vegetables and vegetable products. W. A. Jackson, J. S. Steel and V. R. Boswell (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 349–352).—The NO_3^- contents of 33 common vegetables in fresh, frozen and canned condition are presented. Results were not unduly different from those reported for vegetables in 1907. A. H. CORNFIELD.

Rapid method for colour evaluation of vegetable food products. J. J. Monselise (*Israel J. Technol.*, 1967, **5**, 222–223).—The juice or powdered sample is shaken for a few sec. with approx. twice its wt. of 95% EtOH and the mixture filtered. The colour of the virtually liquid-free and uniformly thick pulp is then compared either with standards prepared similarly or with permanent standards. Minor modifications apply to different foodstuffs, e.g., green peas are shaken with water instead of EtOH, and the method is limited to evaluation of water-insol. pigments and not to anthocyanins, etc.
W. J. BAKER.

Non-alcoholic beverages

Factors influencing hydroxymethylfurfuraldehyde content of fruit juices. E. Dworschák and E. Erdélyi (*Ernährungsforschung*, 1967, **12**, 417–426).—From the results of model experiments with sucrose solutions an equation has been developed for the calculation of the concn. of hydroxymethylfurfuraldehyde formed, in terms of the initial concn. of sucrose, the pH of the solution, and the temp. and time of heating. The results agree with the calculated data within 20% and can be used in practice provided that the conditions of heat treatment are known with sufficient accuracy. (18 references.)
P. S. ARUP.

Detection of adulterations in citrus juices. X. Identification of sugars in orange juices and commercial sucroses by gas–liquid chromatography. J. Alberola, A. Casas and E. Primo (*Revta Agroquim. Tecnol. Aliment.*, 1967, **7**, 476–482).—G.l.c. studies of the sugar composition of genuine juices from four varieties (Comuna, Sanguina, Pineapple and Valencia) are reported, and the procedures used (cf. Brobst & Lott, *Cereal Chem.*, 1966, **43**, 35) are described in detail. All the juices contained α- and β-glucose (**I, II**), fructose, galactose (not previously reported as components of orange juice), sucrose (**III**) and several unidentified sugars. Parallel examination of the five commercial samples of **III** showed the major sugar impurities to be **I, II** and fructose. For juice of a particular variety the peak area ratio of major monosaccharides to **III** is a sensitive indicator of adulteration with **III**, but inter-varietal differences are large. (26 references.) E. C. APLING.

Improvement in quality of orange juice by aromatisation. B. L. Flaumenbaum, D. Al'saadi and I. M. Soboleva (*Pishch. Tekhnol.*, 1967, No. 4, [59], 80–82).— C.V.

Correlations between orange-juice quality parameters as obtained by different conditions of extraction. M. T. Danziger and H. C. Mannheim (*Israel J. Technol.*, 1967, **5**, 216–220).—The results relate to Shamouti (*S*) and Valencia (*V*) juices, the parameters determined being acidity, total sol. solids, ascorbic acid, ash, clarification, colour, suspend pulp, and recoverable oil. Quality of *S*-juice correlated with acidity, total sol. solids, clarification, and Hunter values Rd and *a*, but only minor changes, caused mainly by fruit variability, were found in *V*-juice. Changing extraction pressure in an FMC in-line juice extractor considerably influenced the quality parameters of *S*-juice; decreasing pressure increased the overall quality (up to 10%), colour, flavour, total sol. solids and acidity, besides decreasing the contents of vitamin C, ash and *d*-limonene. Higher quality was, however, obtained with a loss in yield of 3–4·5%. An equation for expressing overall-quality by one value is developed. W. J. BAKER.

Preservation of Moscatel and Bobal grape juices by the D.S.D.A. [dearomatisation-sulphiting-desulphiting-aromatisation] method. F. Gasgue, B. Lafuente and E. Primo (*Revta Agroquím. Tecnol. Aliment.*, 1967, **7**, 493–498).—Comparative assessments are reported for grape juices of the varieties Moscatel (white) and Bobal (red) preserved (*i*) by using the D.S.D.A. procedure involving aroma recovery from the must before addition of SO_2, separate storage, and re-addition of the aroma concentrate to the desulphited must before use, and (*ii*) by sterilisation and storage under N_2. For storage periods of up to 10 months differences were not significant, but after 14 months acceptability of the juices preserved by D.S.D.A. was the same as that of frozen controls stored at −18° while the sterile juices had suffered a considerable loss of quality.
E. C. APLING.

Tea, coffee, cocoa

Chemistry and biochemistry of black tea manufacture: Fact and speculation. R. L. Wickremasinghe (*Tea Q.*, 1967, **38**, 205–209).—
C.V.

An antioxidant for tea. A.S. L. Tirmanna, R. L. Wickremasinghe and K. P. W. C. Perera (*Tea Q.*, 1967, **38**, 36–40).—Tea flush is rich in tocopherols (**I**), γ- being the most active in this respect and its presence may protect tea from oxidation during manufacture, thus preserving flavour. A comparative table of I-concn. is given, 0·387 mg/g dry wt. being recorded for tea leaf. (10 references.)
C.V.

Coffee substitute from *Gundelia tournefortii* L. M. A. Kagitci (*Fette Seifen AnstrMittel*, 1968, **70**, 73–74).—The use of the ripe fruit of *Gundelia tournefortii* L, a plant indigenous to Turkey, is described as a satisfactory coffee substitute. The ripe seeds contain 37·8% of fatty matter (which is reduced to 14·70–15·95% after roasting) together with 5·2–6·6% water, 20·12% protein, 10·5% carbohydrate and 20·3% extract. Some analytical data are provided for 16 other coffee substitutes. G. R. WHALLEY.

New constituents of roasted coffee. J. Stoffelsma and J. Pypker (*Recl Trav. chim. Pays-Bas*, 1968, **87**, 241–242).—30 new constituents occurring among volatiles extracted from steam-distillates of coffee are tabulated. Separation was carried out by g.l.c. and identification, in comparison with authentic compounds, by g.l.c. retention times and by i.r. and mass spectrometry. A further list is given of 6 compounds that were only tentatively identified.
P. S. ARUP.

New constituents of roasted cocoa. B. van der Wal, G. Sipma, D. K. Kettenes and A. T. J. Semper (*Recl Trav. chim. Pays-Bas*, 1968, **87**, 238–240).—81 new constituents are tabulated. Separations were accomplished by g.l.c. and identifications, in comparison with authentic compounds, by g.l.c. retention times and by i.r. and mass spectrometry. P. S. ARUP.

Cocoa aroma. II, III. M. Stoll (and P. Dietrich, E. Sundt, M. Winter II), and I. Flament, B. Willhalm (III) (*Helv. chim. Acta*,

1967, **50**, 2065–2067; 2233–2243).—II. A new component of the aroma fraction of cocoa has been isolated by fractional distillation and gas chromatography and identified by i.r., u.v. and mass spectra. It is the practically inodorous 4-methyl-5-(β-hydroxyethyl)thiazole, which is accompanied by trace amounts of its dehydration product, 4-methyl-5-vinylthiazole; this latter has a strong nut-like odour.

III. The volatile aroma substances of a cocoa concentrate were isolated by codistillation with propylene glycol; the head fraction was diluted with water, extracted with pentane, and the extract fractionated by gas chromatography. Retention data, mass spectrometry, combined in many cases with i.r. spectrometry and paper chromatography, were employed for identification. A list of 62 constituents, 42 of which are recorded for the first time, is given. Hydrocarbons, alcohols and keto alcohols, aldehydes, ketones, acids, esters and lactones, phenols, and pyrazines were present, but several minor constituents could not be identified. (15 references.) (In French.) M. SULZBACHER.

Milk, Dairy Products, Eggs

Preference and acceptance of solids-enriched milk in homes. J. J. Janzen and J. M. Rogers (*J. Dairy Sci.*, 1967, **50**, 1882–1885).—Consumer studies revealed a highly significant preference at the $P < 0{\cdot}01$ level for whole milk enriched with 1% solids-not-fat as compared with conventional market milk. M. O'LEARY.

Important sources of variation in milk flavour. D. D. Kratzer, C. F. Foreman and A. E. Freeman (*J. Dairy Sci.*, 1967, **50**, 1384–1389).—The stage of lactation was found to have significant effects on flavour score and on feed and salty flavour incidence. The first 45 days of lactation was found to be the most susceptible stage for development of undesirable flavours. The season of sampling affected flavour characteristics significantly and this was attributed to variations in management practices between winter and summer. (12 references.) M. O'LEARY.

Flavour of recombined milk. A. Tamsma, F. E. Kurtz, E. Berlin and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 1878–1881).—Recombined milk made from anhyd. butter oil and nonfat milk powder had a flavour score relatively close to that of fresh pasteurised milk. Storage of the milk components at −18° in an N_2 atm. for 6 months produced no detectable flavour change in the recombined milk compared with fresh pasteurised milk. Higher storage temp. caused changes in the butteroil which resulted in a stale flavour appearing in the recombined milk. (17 references.) M. O'LEARY.

Environmental influences on monthly variation in milk constituents. P. W. Spike and A. E. Freeman (*J. Dairy Sci.*, 1967, **50**, 1897–1904).—The effects of age of cow, stage of lactation, month of year, and the two factor interactions of age with stage and month with stage, on milk and its constituents were estimated using least-square techniques. Traits considered were the test day production of milk, milk fat, solids-not-fat, total solids, and the % composition of the three constituents. The reduction due to fitting the set of constants for each main effect and each of the interactions was large and gave a highly significant ($P < 0{\cdot}001$) *F*-ratio for all traits. Stage of lactation accounted for the largest % of the total variance in all yield traits with age, and age by stage interaction ranked next in importance. Interactions of month of year and stage of lactation accounted for a very small proportion of the variance in all traits. (19 references.) M. O'LEARY.

Presence of inhibitors and activators of xanthine oxidase in milk. Q. Hwang, K. S. Ramachandran and R. McL. Whitney (*J. Dairy Sci.*, 1967, **50**, 1723–1737).—The presence in milk of activators and inhibitors of xanthine oxidase (*XO*) was demonstrated. Heating milk to 91·4° for 4·2 sec resulted in inactivation of the enzyme but not in the destruction of activators or inhibitors. All the major milk protein fractions were shown to be activators or inhibitors, depending on their concentration in the reaction mixture. A nonionic dialysable inhibitor was also found in the natural-protein-free milk system. An explanation of the action of these substances on *XO* is advanced. A procedure is described for determining the *XO* content of the various fractions of cows' milk obtained during fractionation procedures. (25 references.) M. O'LEARY.

Physical and chemical properties of bovine milk and colostrum whey M-1 glycoproteins. A. Bezkorovainy (*J. Dairy Sci.*, 1967, **50**, 1368–1375).—The M-1 acidic glycoprotein fractions isolated from colostrum and whey were shown to contain several *N*-terminal amino-acids and, in some instances, several bands on gel electropherograms. The fractions were homogeneous by ultracentrifugal and boundary electrophoretic criteria. Av. mol. wt. were 10,000 with high β-values and negative sp. rotations. The fractions were shown to contain high % of glutamic acid, proline, threonine, and isoleucine and very small amounts of basic amino-acids. (12 references.) M. O'LEARY.

Relation of heat-induced changes in protein–salt constituents to astringency in milk systems. R. V. Josephson, E. L. Thomas, C. V. Morr and S. T. Coulter (*J. Dairy Sci.*, 1967, **50**, 1376–1383).—Astringency (*A*) was produced by heating various skim-milk whey and ultrafiltrate systems. *A* development in whey and ultrafiltrate systems was accompanied by aggregation and formation of large protein–salt and salt particles, respectively. *A* development in skim-milk was accompanied by association of Ca phosphate and whey proteins with the caseinate system without noticeably altering the size, shape, or electron density of the caseinate–phosphate micelles. (20 references.) M. O'LEARY.

Effect of hydrogen peroxide treatment on heat induced interaction of κ-casein and β-lactoglobulin. N. L. Fish and R. Mickelsen (*J. Dairy Sci.*, 1967, **50**, 1360–1362).—Modification of β-lactoglobulin (I) by H_2O_2 treatment resulted in partial retardation of the heat induced interaction of I with κ-casein. (10 references.) M. O'LEARY.

Gel filtration of acid casein and skim-milk on Sephadex. M. Yaguchi and N. P. Tarassuk (*J. Dairy Sci.*, 1967, **50**, 1985–1988).—The results of a study of the fractionation of whole casein on Sephadex are presented. (10 references.) M. O'LEARY.

Separation of β-lactoglobulin from other milk serum proteins by trichloracetic acid. K. K. Fox, V. H. Holsinger, L. P. Posati, and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 1363–1367).—A description is given of the isolation of β-lactoglobulin (I) from milk. The casein is first pptd. with HCl and then the remaining proteins, other than I, are pptd. with CCl_3CO_2H and filtered. The filtrate is concentrated by negative pressure dialysis, dialysed free of low mol. wt. materials, and lyophilised. (14 references.) M. O'LEARY.

Sources of variation affecting relationships of milk protein determinations by the Orange G dye and Kjeldahl methods. F. N. Dickinson, S. N. Gaunt and D. J. Hankinson (*J. Dairy Sci.*, 1967, **50**, 1841–1843).—A procedure is used to compare the Kjeldahl and Orange G methods of determining milk protein. The procedure may also be used to compare other methods of protein determination with the Kjeldahl method. (11 references.) M. O'LEARY.

Dilatometric examination of milk fat. S. S. Gul'ayev-Zaitsev and K. V. Obyedkov (*Pishch. Tekhnol.*, 1967, No. 4, [59], 164–166).— C.V.

Turbidimetric method for milk fat determination. P. Walstra (*J. Dairy Sci.*, 1967, **50**, 1839–1840).—The accuracy of a turbidimetric method for milk fat determination, originally described by Haugaard, was investigated. The accuracy of the method (coeff. of variation = 2·5%) is not sufficient to justify its application in practice. M. O'LEARY.

Rapid method for isolation of unesterified sterols and its application to detection of milk fat adulteration with vegetable oils. I. Katz and M. Keeny (*J. Dairy Sci.*, 1967, **50**, 1764–1768).—A rapid method for the isolation of unesterified sterols from milk fat is described. The method permits the detection of adulteration of milk fat with 1% of corn, cottonseed, soyabean, or peanut oil and with 2% of coconut or safflower oil. The method is slightly less sensitive in detecting adulteration of ice-cream fat. M. O'LEARY.

Simple ultrasensitive test for detecting penicillin in milk. J. M. A. Palmer and F. V. Kosikowski (*J. Dairy Sci.*, 1967, **50**, 1390–1394).—A procedure for screening large numbers of milk samples for the presence of penicillin (*P*) is based on the growth of *B. subtilis* spores on nutrient–spore–dye paper discs in a small quantity of test milk, and evaporation in air. Evaporation of the milk increases the concn. of any *P* present. Colour changes in the disc, dependent on spore growth, indicate the presence or absence of *P*. The method was shown to be capable of detecting 0·002 IU/ml *P* in 4 to 6 h. (11 references.) M. O'LEARY.

Photometric determination of carbonyl compounds. G. A. Muck, N. R. Sundararajan, J. Tobias and R. McL. Whitney (*J. Dairy Sci.*, 1967, **50**, 1983–1985).—A description is given of a modification of the method of Henich *et al.* (*J. Am. Oil Chem. Soc.*, 1954, **31**, 88) for the analysis of carbonyl compounds, e.g., in milk. M. O'LEARY.

Determination of non-fat dry milk in meat products with a specific enzymatic assay. L. Hankin (*J. Ass. off. analyt. Chem.*, 1967, **50**,

1342–1348).—The filtrate from an extract of the sample in dil. HCl, which has been treated with phosphotungstic acid, is treated at pH 7 with the chromogen (*o*-tolidine) and then with galactose oxidase and peroxidase. After incubation at 37° and addition of glycine buffer the extinction is measured at 425 nm and compared with standards. Recoveries were 90 to 120%. The procedure is specific for measurement of lactose (converted to galactose by HCl hydrolysis), even in presence of other sugars.
A. A. ELDRIDGE.

Occurrence of antibiotic-producing strains among lactic bacteria. A. Szember and E. Szulga (*Annls Univ. Mariae Curie-Skłodowska, Agric.* E, 1966, **21**, 227–233).—370 Strains of Streptococci and 103 strains of milk-fermenting bacilli were isolated from various media, e.g., fresh and sour milk, curd cheese and sauerkraut, and examined. Amongst the isolated lactic bacteria, a number of strains can always be found which are capable of producing antibiotics, checking the growth of *Bacillus subtilis* or *Escherichia coli*. 11·6% of Streptococci and 4·8% of Lactobacilli are capable of producing antibiotics. The greatest number of such strains was isolated from sauerkraut and from effluents (feed silage sewage) from a starch plant. (33 references.) T. M. BARZYKOWSKI.

Influence of carbon and nitrogen sources in the culture medium on the antibiotic activity of milk Streptococci. A. Szember and B. Raczkiewicz (*Annls Univ. Mariae Curie-Skłodowska, Agric.* E, 1966, **21**, 235–243).—Investigated strains of *Streptococcus lactis* were inoculated on a modified nutrient medium to which lactose, glucose or arabinose were added as the source of C and broth, casein hydrolysate, peptone and asparagine as N sources. Antibiotics produced in these circumstances acted to different degrees as growth inhibitors of gram-positive *Bacillus subtilis* or gram-negative *Escherichia coli*. Under the conditions used, lactose was the best source of C and peptone the best source of N.
T. M. BARZYKOWSKI.

Influence of vitamins contained in the culture medium on the intensity of the production of antibiotic substances by some strains of milk bacteria. A. Szember and M. Małka (*Annls Univ. Mariae Curie-Skłodowska, Agric.* E, 1966, **21**, 245–251).—Addition of a vitamin B complex and also separately of thiamine, riboflavine, niacinamide and folic acid to the culture medium increased the antibiotic activity of the examined strains of milk bacteria (*Streptococcus lactis*) against *Bacillus subtilis* and *Pseudomonas fluorescens*. Niacinamide is the most potent stimulant for production of antibiotics. However, the presence of vitamins in the culture medium, especially of thiamine, protects *Escherichia coli* against the action of antibiotics to some extent. The results are of use in finding suitable culture media on which strains of milk bacteria will continue to be capable of producing antibiotics.
T. M. BARZYKOWSKI.

Thermophysical indices of creams of different fat content. Y. P. Andrianov and G. V. Tverdokhleb (*Pishch. Tekhnol.*, 1967, No. 6, [61], 72–77).— C.V.

Influence of antioxidants on increased stability of melted butter under different conditions. A. N. Val'eyeva and M. I. Gor'yaev (*Pishch. Tekhnol.*, 1967, No. 6, [61], 23–25).— C.V.

Flavour chemistry of Swiss cheese. J. E. Langler (*Diss. Abstr.*, B, 1967, **27**, 2625).—Neutral volatile flavour compounds were isolated from Swiss cheese fat by low-temp. low-pressure distillation, and the compounds separated by temp. programmed gas chromatography. Direct analysis of cheese fat and whole cheese from four domestic and two imported good flavoured cheeses by gas entrainment and on-column trapping provided a further means of isolation of volatile flavour compounds, and rapid scan mass spectrometry and relative retention time data were used to identify compounds. These are listed. It appeared that satisfactory reproduction of Swiss cheese flavour could be achieved only if the mixture contained free fatty acids, volatile constituents, and free amino-acids and was adjusted to the pH of natural cheese.
F. C. SUTTON.

Proteolytic and microbial changes during ripening of Cheddar cheese using bacterial enzyme as milk coagulating agent. Ajaib Singh, Ajit Singh, R. K. Kuila, S. M. Dutta, I. J. Babbar, R. A. Srinivasan and A. T. Dudani (*J. Dairy Sci.*, 1967, **50**, 1886–1890).—Different stages of ripening of Cheddar cheeses, made from milk coagulated with enzymes from selected strains of *Bacillus sublilis*, *B. megatherium* and *B. cereus*, were chemically and bacteriologically studied. (23 references.) M. O'LEARY.

The rôle of ethanol and certain ethyl esters in the fruity flavour defect of Cheddar cheese. D. D. Bills (*Diss. Abstr.*, B, 1967, **27**, 2582).—Isolation and identification of the components responsible for the fruity flavour defect were attempted. The rôle of certain cheese starter cultures in the development of this defect was studied. Volatiles were isolated by distillation from fat expressed from a typically fruity cheese after centrifuging. These were then separated by gas–liquid chromatography. Ethyl-butyrate, -hexanoate and -octanoate were found to be the only compounds with detectable fruity odours. Single-strain cultures of *Streptococcus lactis*, *S. diacetilactis*, and *S. cremoris* as well as three mixed-strain commercial cultures were evaluated for ethanol and acetaldehyde production in non-fat milk medium. Certain cultures are thought to be directly responsible for this defect. F. C. SUTTON.

Unusual flavour defect in cheese similar to the odour of feline urine (*Felis libyea domestica*). W. A. McGugan and D. B. Emmons (*J. Dairy Sci.*, 1967, **50**, 1495–1496).—A flavour defect of Cheddar cheese, characterised by an odour of feline urine, was found to be associated with the presence in the cheese fat of a C_2 mono-unsaturated ketone (**I**). **I** did not possess this odour which appeared to be due to an unidentified component slightly less volatile than **I**.
M. O'LEARY.

Causes of Ribes flavour in cheese. H. T. Badings (*J. Dairy Sci.*, 1967, **50**, 1347–1351).—Ribes flavour in cheese was caused by the formation of 2-mercapto-2-methylpentan-4-one. The mechanism of its formation in cheese was not elucidated but it is thought that the cheese coating, the microflora of the cheese rind, the surface of the cheese tray, and storage conditions are important factors.
M. O'LEARY.

Increase in soluble nitrogen and bitter flavour development in cottage cheese. W. K. Stone and D. M. Naff (*J. Dairy Sci.*, 1967, **50**, 1497–1500).—Neither the soluble N compounds produced during curd formation by culture bacteria and rennet nor those from the dressings used were of the type that caused bitter flavour (*BF*) in cottage cheese. Samples of cheese which had developed a *BF* had significantly higher soluble N values than normal cheese, indicating that the soluble N had increased in the defective cheese during storage and handling. Development of *BF* was also associated with an increase in the no. of psychrophilic bacteria in the cheese. (12 references.) M. O'LEARY.

Solubilisation of cottage cheese curd by wash water and its causes. F. F. Schmidt, H. T. Roth and R. T. Marshall (*J. Dairy Sci.*, 1967, **50**, 1769–1771).—The approx. quantities of hydroxides in distilled water necessary to produce gelatination in cottage cheese curd were shown to be 500 ppm NaOH and 300 ppm $Ca(OH)_2$. Hydroxyl concn. of 0·0125 N caused gelatination. The presence of chlorides reduced the amount of hydroxide necessary to produce gelatination. 1% solutions of Mg, Fe(III) and Al sulphates failed to produce gelatination as did 1000 ppm NaClO and NaCl solutions.
M. O'LEARY.

Moisture analysis of freeze-dehydrated cottage cheese. J. L. Fowler and C. H. Coleman (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1279–1283).—An attempt to substitute the use of a mechanical convection air oven, used at 100–102° for 16–18 h, for that of a vac. oven (*Official Methods of Analysis, A.O.A.C.*, 10th Ed., 1965, 15.119) gave unsatisfactory results. A. A. ELDRIDGE.

Ion-exchange chromatography of egg yolk. W. E. Seideman (*Diss. Abstr.*, B, 1967, **27**, 2741).—A method for separating the proteins and lipoproteins of egg yolk using a carboxymethylcellulose chromatographic column was developed and used to study changes due to freezing, spray drying, and variations in yolk composition. A combination gradient and stepwise elution increasing both pH and ionic strength gave a chromatogram from native yolk with four major and several minor peaks. The quantity of one of these major peaks varied between two strains of chickens. Freezing and spray drying yolk causes the complete disappearance of one of the major peaks, reduction in the size of several others, and the appearance of several new peaks in the chromatogram. These peaks are probably combinations or complexes of proteins and lipoproteins. F. C. SUTTON.

Ether extract of shell membrane and albumen as affected by spray and dip oiling. D. Fromm and S. U. Gammon (*Poult. Sci.*, 1967, **46**, 1222–1224).—Treatment of shell eggs by oil spraying or dipping had no significant effect on the % ether extract of the albumen 2–20 days after treatment. The outer shell membrane retained all the oil which penetrated the shell. A. H. CORNFIELD.

Measuring egg shell strength by beta-ray backscatter technique. P. E. James and H. J. Retzer (*Poult. Sci.*, 1967, **46**, 1200–1203).—A technique for measuring the backscatter of β-rays from shell eggs is described. Backscatter counts were highly correlated with impact resistance of the shell. A. H. CORNFIELD.

Edible Oils and Fats

Hydrogenation of sunflower oil on a stationary nickel–chrome catalyst. I. 'Jet' method. II. Drop method. D. V. Sokol'sky, V. I. Komarov, K. A. Zhubanov [and Y. P. Kiyushnikov, II only] (*Pishch. Tekhnol.*, 1967, No. 6, [61], 64–66; 66–68). C.V.

Hydrogenation of soyabean oil using 'jet' method and stationary nickel–chrome catalyst. D. V. Sokol'sky, V. I. Komarov and N. K. Nadirov (*Pishch. Tekhnol.*, 1967, No. 5, [60], 129–130).— C.V.

Tocopherols of β-cytosterol of walnut oil. V. I. Dorodneva and A. L. Shinkarenko (*Pishch. Tekhnol.*, 1967, No. 5, [60], 101–104).— (16 references.) C.V.

Fatty acid composition of palm kernel, illipe and shea nut oils by urea fractionation and programmed temperature gas chromatography. J. L. Iverson and P. G. Harrill (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1335–1338).—By means of the procedure applied to the methyl esters, saturated fatty acids containing C_6 to C_{28} were identified. Mono-unsaturated acids containing C_{14} to C_{24} and dienes of even chain length from C_{14} to C_{24} were also detected. (12 references.) A. A. ELDRIDGE.

Trace elements in edible fats. XIV. Application of demetallisation to refined olive oils. A. Vioque, T. Albi, M. A. Albi and M. Nosti (*Grasas aceit.*, 1968, **19**, 81–88).—The demetallisation of olive oils by cation-exchange resins (H^+-form) as an additional step in the refining process offers only minor advantages, but since the demetallisation decolorises the oil the operation may be used in place of the bleaching process, thereby giving oils which retain their tocopherols, and are of greater stability. Macro-reticular anion-exchange resins remove free fatty acids but these are weakly held and large ion-exchange beds are required. No improvement in stability is obtained compared to oils neutralised normally with soda. L. A. O'NEILL.

Determination of the hydroxyl value of the unsaponifiable matter of olive and sansa oils by near infra-red spectrophotometry. J. Gracián and J. Martel (*Grasas aceit.*, 1968, **19**, 99–109).—The oil is saponified and the unsaponifiable matter extracted with hexane and washed. The hexane is removed by evaporation, a few drops of acetone added and the residue heated under vac. at > 40° to constant wt. The i.r. spectrum of the sample is determined in CCl_4 solution. The unsaponifiable matter from olive oil gives a max. at 2773 nm and that from sansa oil at 2771 nm. The hydroxyl content is calculated with reference to β-sitosterol, n-hexadecanol and homo-olestranol as standards, which give max. at 2773, 2763 and 2762 nm respectively. Results compare well with those obtained by acetylation. L. A. O'NEILL.

Organoleptic properties of food vegetable oils. A. M. Goldovsky and Z. D. Patrakova (*Pishch. Tekhnol.*, 1967, No. 6, [61], 137–141). C.V.

Volatile oxidation products responsible for the alteration of flavour [of fats]. M. Loury (*Alteration oxydative des Corps Gras*, [Marseilles, Inst. Corps Gras], 1967, 11–23).—The various types of compounds produced on autoxidation of fats, and the mechanism of their formation are discussed. L. A. O'NEILL.

Non-volatile oxidation products responsible for reversion [of fats]. M. Naudet (*Alteration oxydative des Corps Gras*, [Marseilles, Inst. Corps Gras], 1967, 25–36).—The formation of oxidised fatty acids on autoxidation of fats, and their responsibility for reversion of colour and flavour are discussed. L. A. O'NEILL.

Rancidity of lard bakery products. II. R. Vaśquez Ladrón and J. M. R. de la Borbolla y Alcalá (*Grasas aceit.*, 1968, **19**, 93–99).—The stability of lard bakery products is mainly related to that of the lard from which they are prepared, although the flour has some influence. It can be considerably improved by using lard containing antioxidants, and the best results are obtained with a mixture of butylated-hydroxyanisole and -hydroxytoluene (0·01% of each). Cutting out harmful irradiation (< 450 nm) in storerooms is also helpful. L. A. O'NEILL.

Effect of batter ingredients on changes in fatty acid composition of fats used for frying. M. Bennion (*Fd Technol., Champaign*, 1967, **21**, 94–98).—When a fritter-type batter was fried in maize oil or a hydrogenated soyabean oil shortening (with antioxidants) for 8·5 h, the I_2 val. were progressively decreased with time of use in both the frying fat and the fat absorbed by the fritters. The presence of egg in the batter caused little change in the maize oil but a slight increase in the I_2 val. of the hydrogenated oil. With maize oil the lowest I_2 val. occurred in the presence of both egg and baking powder, but with the hydrogenated fat the max. I_2 val. were found. The presence of baking powder accelerated the decrease of the linoleate content of the oil. (14 references.) P. S. ARUP.

Positional distribution of fatty acids in triglycerides of animal depot fats. H. Brockerhoff, R. J. Hoyle and N. Wolmark (*Stud. Fish. Res. Bd Can.*, 1966, Pt. 2, 1967, 87–92; *Biochim. biophys. Acta*, 1966, **116**, 67–72).—The positioning is non-random and that between position 2 and positions 1 and 3 would appear to be governed by chain length and degree of unsaturation. The shorter the chain, and the more unsaturated the acid, the greater is the tendency to occupy position 2. This rule would also appear to apply to the apparent exceptions, the fats of pig and marine mammals; in these however, the influence of chain length overrides that of unsaturation. In the three birds investigated, unsaturation would seem to be the only directing factor. Both symmetrical and asymmetrical distributions were found. All mammalian fats were asymmetrical as were those of one amphibian, one fish and one arthropod, whereas the fats of one fish, one arthropod and three birds were symmetrical. All symmetrical fats have an excess of palmitic acid in the 1 and 2 positions with oleic acid in position 3. (11 references.) C.V.

Apparatus for extraction of fat [in foods]. J. H. N. Levy and A. Lifshitz (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1340–1342).—By means of the glass apparatus (illustrated) consisting of three parts attached by ground-glass joints, the liquid or solid sample is kept immersed in the solvent throughout the extraction period. A. A. ELDRIDGE.

Improved clean-up method for the detection of chick œdema factor in fats and fatty acids by electron capture gas chromatography. P. Neal (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1338–1340).—The clean-up procedure employing H_2SO_4 is used instead of the saponification step since it is more rapid (50% reduction in sample clean-up time). A. A. ELDRIDGE.

Meat and Poultry

Autolytic processes in meat during treatment. L. N. Gordzievsky (*Pishch. Tekhnol.*, 1967, No. 4, [59], 32–36).— C.V.

Effect of micro-organisms on emulsifying capacity and extract release volume of fresh porcine tissues. R. J. Borton, N. B. Webb and L. J. Bratzler (*Fd Technol. Champaign*, 1968, **22**, 94–96).—Two cuts of muscle tissue were taken with precautions to avoid infection. One of the cuts was sprayed with a suspension of bacteria from meat and both were kept in bottles at 4–6° for 17 days. The effect of the presence of a relatively large no. of bacteria was to decrease the extract release vol. and the emulsifying capacity of the tissue when blended with M-NaCl solution and soyabean oil. (11 references.) P. S. ARUP.

Fish

Ultrastructure of the white striated myotomal muscle of the cod, *Gadus morhua*. C. M. Bishop and P. H. Odense (*J. Fish. Res. Bd Can.*, 1967, **24**, 2549–2553).—Peripheral fibrils are ribbon-like and rectangular in cross-section with the long axis normal to the sarcolemma; inner fibrils are mainly polygonal in cross-section. Most of the mitochondria and nuclei are peripheral to the fibrils and next to the sarcolemma. A distinct N-band is apparent with indications of branching and re-orientation of the actin filaments. E. G. BRICKELL.

Phospholipase A activity in rainbow trout muscle. R. E. E. Jonas and E. Bilinski (*J. Fish. Res. Bd Can.*, 1967, **24**, 2555–2562).—A sensitive method for the assay of phospholipase A is described. ^{14}C-labelled lecithin is converted to lysolecithin (**I**) by the enzyme, unreacted lecithin removed by silicic acid column chromatography, and the **I** recovered by thin-layer chromatography. The results are discussed in relation to the enzymic activity previously demonstrated in fish muscle. (26 references.) E. G. BRICKELL.

Radioactive ^{54}Mn and ^{65}Zn in euryhaline fish. P. F. Gustafson, S. S. Brar, D. M. Nelson and S. E. Muniak (*Can. J. Zool.*, 1967, **45**, 729–735).—Euryhaline fish (alewife, char, eel, salmon, shad, smelt, and sturgeon) retain ^{54}Mn and ^{65}Zn to a significantly greater degree than stenohaline species, even when they are from the same area and eat a similar diet. Accumulation of ^{65}Zn appears to occur only in a marine (salt-water) environment. E. G. BRICKELL.

Protection of dried sea-fish from infestation by *Dermestes frischii* Kug. A. A. Green (*Pyrethrum Post*, 1967, **9**, No. 2, 24–33).—Dried sea-fish is commonly heavily infested with *Dermestes*

frischii Kug. and other insects, but infestation can be avoided if the fish is well salted and thoroughly dried. Insecticidal treatment at curing time gives relatively little protection during storage but can be highly effective for fish which has been stored for some time. Immersion of the fish in an aq. emulsion containing 0·02% pyrethrins and 0·08% piperonyl butoxide controls an existing infestation and gives protection for six weeks. Malathion at 0·0625% also gives protection but may leave an undesirably high residue in certain fish. J. L. WALPOLE.

Toxicity of 1,2-dichloroethane-extracted fish in protein concentrate. J. C. Munro and A. B. Morrison (*Can. J. Biochem.*, 1967, **45**, 1779–1781).—Evidence was obtained of the toxicity to rats of cod fillets extracted with 1,2-dichloroethane (I), and of the non-toxicity of fillets extracted with Pr^iOH. One product of reaction of I with solids components, *S*,*S*′-ethylenebiscysteine, has been shown to be non-toxic (cf. *ibid.*, 1967, **45**, 1049), and the toxicity of I-extracted fillets was not explainable on the basis of the content of another reaction product, chlorocholine chloride. Although further extraction of the I-extracted fillets with MeOH removed most toxic material, the fillets thus treated still remained toxic, even when supplemented with cystine and histidine. P. S. ARUP.

Fish protein concentrate. VI. Quintero fish protein concentrate—protein quality and use in foods. E. Yanez, I. Barja, F. Monckeberg, A. Maccioni and G. Donoso (*Fd Technol. Champaign*, 1967, **21**, 60–66).—The manufacture of the concentrate in Chile and its composition are described. The results of experiments on its use as an additive to bread, pasta, and roasted whole wheat meal are described and discussed. The product should provide a useful and safe source of protein and lysine, and thus serve as a substitute for imported milk. (16 references.) P. S. ARUP.

Spices, Flavours, etc.

Comparison of methodology used in determining flavour effect of 5′-ribonucleides on processed foods. E. F. Stier, F. M. Sawyer and P. E. Fergenson (*Fd Technol. Champaign*, 1967, **21**, 83–86).—Organoleptic assessments by the paired and multiple comparison techniques were made of the flavour enhancement effects of additions at three levels of Na_2 5′-inosinate (I) and Na_2 5′-guanylate (II) to a dry beef bouillon, a condensed chicken noodle soup, and a dry beef gravy mix. The results indicated that a mixture (1 : 1) of I and II was more efficient than I alone. The results of the two methods were essentially the same. The relative merits of the methods are considered. P. S. ARUP.

Meat flavour. II. Procedures for the separation of water-soluble beef aroma precursors. L. L. Zaika, A. E. Wasserman, C. A. Monk, jun. and J. Salay (*J. Fd Sci.*, 1968, **33**, 53–58).—A method is described in which water sol. low mol. wt. beef aroma precursors were separated by column chromatography on Bio-Gel P-2, Amberlite XAD-2 and DEAE-Sephadex. Separations based on gel filtration, adsorption and anion exchange gave a number of fractions that developed roast beef aroma on pyrolysis, but differed in composition. Sugar phosphates or free sugars are involved in aroma development. Tyrosine, phenylalanine, taurine and glutamic acid may be removed without affecting the aroma. Amino-acids in trace amounts were present in the aroma-producing fractions, and creatine, creatinine and the purine deriv. inosinic acid, inosine and hypoxanthine may also be removed without affecting aroma development. (24 references.) I. DICKINSON.

Preservatives

Stabilisation and decomposition of sorbic acid. E. I. Petropavlovsky and A. V. Ustinova (*Pishch. Tekhnol.*, 1967, No. 6, [61], 36–39).— C.V.

Utilisation of sorbic acid for storage of granular curds. N. L. Druzhinina and A. Y. Sulin (*Pishch. Tekhnol.*, 1967, No. 6, [61], 78–79).— C.V.

Spectrophotometric determination of citric acid. N. A. Zhabolovskaya, L. M. Ageyev, G. A. Malysheva and V. B. Kaplan (*Pishch. Tekhnol.*, 1967, No. 4, [59], 170–172).— C.V.

Spectrophotometric determination of BHA and BHT in vegetable oils. D. P. Johnson (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1298–1304).—To determine butylated hydroxyanisole (BHA) cottonseed oil is treated with $NaNO_2$ and HCl, the product is extracted into light petroleum/acetone (9 : 1), thence into aq. NaOH and, after acidification, again into light petroleum/acetone which is then passed through a Florisil column. After extraction into aq. NaOH the extinction is measured at 480 nm. Butylated hydroxytoluene (BHT) is extracted into acetonitrile, then into pentane, the concentrate from which is diluted with iso-octane and passed through a column of Al_2O_3 before measurement of the extinction at 283 nm. Recoveries ranged from 97·14 to 101·40% and 91·43 to 106·00%, respectively. A. A. ELDRIDGE.

Antioxidants and prevention of oxidation of fats. C. Paquot (*Alteration oxydative des Corps Gras*, [Marseilles, Inst. Corps Gras], 1967, 37–56).—The various types of antioxidants and their mechanism of action are discussed. (55 references.) L. A. O'NEILL.

Preservation of fish by antibiotics. H. L. A. Tarr (*Stud. Fish. Res. Bd Can.*, 1966, Pt. 2, 1967, 101–109; *Proc. Indo-Pacific Fish. Council*, 1965, **11**, 265–273).—Earlier work is summarised and reviewed. The current position is assessed. C.V.

Pesticides in Food

Spectrophotometric determination of Temik residues in citrus. W. R. Meagher, R. Hendrickson and B. G. Shively (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1242–1246).—For the elimination of interfering substances in the determination of Temik [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime], the use of a Florisil column replaces the coagulation procedure of Johnson and Stansbury (*ibid.*, 1966, **49**, 399). At 0·002 to 0·2 ppm the average recovery of Temik and its metabolites was 98·5 ± 2·2%. As little as 0·0006 ppm of Temik could be determined on a 500-g sample. A. A. ELDRIDGE.

Rapid specific method for gas chromatographic determination of organophosphate pesticides in cold pressed citrus oils. R. K. Stevens (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1236–1242).—Two glass tubes, 6 ft × 4 mm, were packed with 10% SE-30 and 10% SE-52 on 80–100 mesh Chromosorb W, respectively. For injection the sample was diluted with hexane. The flame photometric detector of Brody and Chaney (*J. Gas Chromat.*, 1968, **4**, 42) was used with N_2 as carrier gas and H_2+O_2 as burner gas, the flow of the latter mixture being precisely controlled. The column temperature was 190°. The Florida and Californian oils contained 1·5 to 20 ppm of thiophosphate pesticide. A. A. ELDRIDGE.

Determination of Difolotan residues in fruits by electron-capture gas chromatography. W. W. Kilgore and E. R. White (*J. agric. Fd Chem.*, 1967, **15**, 1118–1120).—This new fungicide *N*-[(1,1,2,2-tetrachloroethyl)sulphenyl]-*cis*-cyclohex-4-ene-1,2-dicarboximide, can be directly determined by g.l.c. in conc. C_6H_6 extracts from the fruits. At levels of 0·5–2·0 ppm recoveries were 84–100%. By applying a cleanup process with charcoal and Attaclay to the C_6H_6 extract, the min. detectable amount could be reduced to 0·01 ppm with an average recovery of 83%. The presence of Botran (2,6-dichloro-4-nitroaniline) or of captan caused no interference. P. S. ARUP.

Problems incurred in the analysis for trace chemical residues in foods, with particular reference to some chlorinated hydrocarbon insecticides in milk. M. Kroger (*Diss. Abstr.* B, 1967, **27**, 2938).—Analysis of traces of undesirable materials present in foods in amounts in the ppb range are discussed and the utilisation of modern techniques, e.g., of gas–liquid chromatography, is noted. A study of the contents of dieldrin and heptachlor-epoxide in milk is outlined. A modified method of fat extraction for use in these determinations is compared with that recommended by the A.O.A.C. The error for determinations of both substances in milk-fat at the 0·10 ppm level or above was < 20% in 13 of 14 duplicate determinations. A. G. POLLARD.

Effect of milk processing methods on endosulfan, endosulfan sulphate and chlordane residues in milk. T. A. McCaskey and B. J. Liska (*J. Dairy Sci.*, 1967, **50**, 1991–1993).—Experiments in which milk containing endosulfan (I), endosulfan sulphate (II), or chlordane (III) was manufactured into condensed, whole dried, or evaporated milk showed that the greatest loss of insecticide occurred during manufacture of drum-dried milk. The % decreases as a result of drum-drying were 49·7, 70·4, and 44·9 for I, II and III respectively; III was the most stable in all the milk processing methods. (16 references.) M. O'LEARY.

Selective residue determination of sulphur-, halogen- and phosphorus-containing pesticides [in foods] by helium-plasma emission spectrometry. C. A. Bache and D. J. Lisk (*J. Ass. off. analyt. Chem.* 1967, **50**, 1246–1250).—In the He-plasma procedure (*Idem, Analyt. Chem.*, 1967, **39**, 786) He at 5–10 mm Hg was used. The gas-chromatographic column, 6 ft × ⅛ in., was packed with 10%

DC-200 on 80–100 mesh Gas Chrom Q and operated isothermally in the range 130° to 210°. Recoveries of 62 to 115% are reported. A. A. ELDRIDGE.

Examination of foodstuffs containing [pesticide] residues. R. Engst, H. Ackermann and B. Nickel (*Ernährungsforschung*, 1967, **12**, 403–415).—Results of determinations of pesticide residues in ~400 samples of plant products and milk point to the necessity for organised control. Proposed tolerance limits are tabulated for 40 pesticides in 20 types of foodstuffs. (30 references.) P. S. ARUP.

Determination of residual acrylonitrile, carbon disulphide, carbon tetrachloride and ethylene dichloride in cereals after fumigation. S. G. Heuser and K. A. Scudamore (*Chemy Ind.*, 1968, 1154–1157).—In the recovery analyses reported, the vapour of a known wt. of each compound is kept hermetically in contact with the cereal for up to 70 h, the loosely-held and free vapour is then removed in a stream of air and absorbed in acetone–water (5 : 1) (but in ethanolic KOH for CS_2) for determination by g.l.c. The firmly-held fumigant is then determined by extraction of the whole cereal with acetone–water at 20° followed by g.l.c. A long column of 10% Carbowax 1540 on Teflon 6 is used at 60° with N_2 as carrier-gas and a flame detector (but an electron-capture detector is used for CS_2 and the extract is considerably diluted). Sensitivities for acrylonitrile, CS_2, CCl_4 and $C_2H_2Cl_2$ are (in ng) 0·5, 0·02, 2·5 and 1, respectively; a 100-fold increase in sensitivity is obtained for $C_2H_2Cl_2$ with column temp. 120° and an electron-capture detector. Results listed show the method to be reliable and that the residual fumigant migrates almost completely into the solvent, generally within a few h (maize needs 24–48 h extraction). No vol. correction for water in the cereal is necessary, and none of the fumigants reacts to any extent with cereal constituents. W. J. BAKER.

Food Processing, Refrigeration

Diffusion rates in desalting of pickles. I. J. Pflug, P. J. Fellers and D. Gurevitz (*Fd Technol. Champaign*, 1967, **21**, 90–94).—Diffusion rates for NaCl from salt stock pickles of various sizes were evaluated in a running water experiment and an equilibrium test. The diffusion coeff. were fairly close in both tests, but not close enough for the use of the same coeff. in designing the two types of systems. P. S. ARUP.

Effect of post-harvest γ-irradiation on orange fruit. F. P. Guerrero, E. C. Maxie, C. F. Johnson, I. L. Eaks, and N. F. Sommer (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 515–528).—Waxed fruit withstood irradiation damage better than unwaxed fruit when irradiated with 50–600 krad of γ-radiation. Rate and severity of rind breakdown increased with higher storage temp. of irradiated fruit. Irradiated fruit had a lower incidence of decay, a higher respiratory rate and more rapid rind breakdown than did unirradiated fruit. A dose of 200 krad increased pH and decreased sol. solids % and crushing resistance of the pulp. A. H. CORNFIELD.

Use of steam injection as a means of rapid heating for sterilisation of liquid foods. M. C. Jones and G. S. Larner (*Chem. Engr, Lond.*, 1968, CE 4–CE 9).—A bath process for heating 5 lb batches of viscous liquids with apparent η up to 5 P from 65 to 130° in 30 sec by direct steam injection, and also a process for continuous heating, are described. Heat transfer coeff. for steam injection were determined. The maintenance of temp. for sterilisation by stable steam injection in flowing systems is discussed. Certain materials go 'off-flavour' with the use of high temp., short time sterilisation as opposed to slower, more conventional methods. (17 references.) J. LAMBORN.

Regeneration of thermally inactivated enzymes in pH-adjusted chlorophyll-containing vegetables processed by H.T.S.T. methods. R. Resende (*Diss. Abstr.* B, 1967, **27**, 2740–2741).—Fresh spinach (*S*) and green beans (*GB*) were puréed, the pH adjusted to about 8·5, and stored at minus 20°F. For processing, the purée was de-aerated and packed in capillary tubes (outside dia. 1·5 to 2 mm). After filling the headspace with N_2, the tubes were sealed, and the purée processed in a glycerol bath in a modified Stern and Proctor apparatus. Thermal resistance characteristics of peroxidase (**I**) and chlorophyllase (**II**) were determined in spinach purée by processing over two different ranges of temp., 170 to 198°F., and 184 to 205°F., respectively. *S*-**I** and -**II** thermal destruction time curves showed *z* values of 29 and 22 respectively. *GB*-**I** gave a *z* value of 88. It was proved that on the basis of the guaiacol–hydrogen peroxide assay, a process of F_0 equal to 5, usually given to low-acid vegetable products for sterilisation, was not sufficient to inactivate *GB*-**I** to the point at which regeneration did not take place during storage. Processes of F_0 equal to 31 and 245 were required at 270°, and 290°F., respectively, to prevent regeneration. *S* gave no reason for concern about regeneration of either **I** or catalase at temp. as high as 290°F. A procedure was developed for integrating lethality in the whole capillary tube for proper correction of heating times. F. C. SUTTON.

Contact sterilisation of fruit compote. V. I. Perepeka (*Pishch. Tekhnol.*, 1967, No. 6, [61], 80–82).— C.V.

Influence of various methods of peeling on new varieties of tomato. A. Reig Felíu and A. Albert Bernal (*An. Inst. nac. Invest. agron.*, 1967, **16**, 201–217).—A comparative evaluation of five proposed methods of peeling applied to nine varieties of tomatoes for canning is reported. After storage of processed cans for one year at ambient temp., the contents were examined for dissolved solids, acidity, pH, vitamin C, total reducing sugars, colour, % entires, appearance and texture. Best results were obtained following conventional immersion blanching for 40 sec. or tunnel-freezing at −30° for 10 min. The worst results were found with blast-freezing at −20°/30 min. followed by immersion in water at 40°. The pear-shaped varieties Roma and San Marzano were better suited than round varieties to peeling. E. C. APLING.

Corrosion test of chrome-tin alloy in milk media. A. V. Izmailov, N. P. Chernyshova and V. G. Pron'yuk (*Pishch. Tekhnol.*, 1967, No. 6, [61], 26–27).— C.V.

'Smoked-fish-in-oil' canned foods: Interaction of nitrogenous substances with phenols during storage. I. I. Lapshin and T. G. Rodina (*Pishch. Tekhnol.*, 1967, No. 6, [61], 30–35).— C.V.

Comparative aspects of the dehydration and freeze-drying of vegetables. I. Green beans. B. Lafuente, F. Piñaga, J. Chamarro and J. Carbonell (*Revta Agroquím. Tecnol. Aliment.*, 1967, **7**, 483–492).—The effect of various factors on quality deterioration (colour, vitamin C content, organoleptic acceptability) of freeze-dried (*FD*) and conventionally dried 'Blue Lake' green beans was studied during storage for 3 months. Initial quality of *FD* beans was close to that of quick frozen beans; the conventionally dehydrated product was acceptable, but of markedly lower quality. Effect of increased storage temp. (0–37°) was greater for *FD* than for conventionally dried beans, but both products remained acceptable after 3 months at 37° provided moisture content was less than 2%. Replacement of air by N_2 gave no advantage, but light exercised a marked deteriorative effect, particularly on the colour of the *FD* product. E. C. APLING.

Application of a new salt-free cooling fluid in the brewery. W. Müller (*Brauwissenschaft*, 1967, **20**, 389–397).—$CaCl_2$ solution used as cooling fluid is compared with a new salt-free fluid called Antifrogen N (**I**). **I** is based on ethylene glycol with added inhibitors for protection against corrosion. It was found to have a slightly better heat transfer performance (*HTP*) and induces much less corrosion than $CaCl_2$ solution. *HTP* was studied in a plate cooler used to cool wort; corrosion behaviour was determined by the ASTM procedure. Trials were carried out by replacing the $CaCl_2$ in the whole of the cooling system by **I**. The changeover of a cooling system to glycol in an American brewery is described in *Brewers' Dig.*, 1965, **40**, No. 853, 55. I. DICKINSON.

Diacetyl test as quality control tool in processing frozen concentrated orange juice. D. I. Murdock (*Fd Technol. Champaign*, 1968, **22**, 90–94).—An account is given of the use of the test (*Idem, ibid.*, 1967, 643–646) in process control. Diacetyl values in the plant products vary within narrow limits during normal working; sudden increases indicate lack of cleanliness, admixture of stagnant residues or the use of low quality fruit. (11 references.) P. S. ARUP.

Influence of precooling on refrigerated storage of green vegetables. A. Albert Bernal, A. Reig Felíu and C. Perez-Nievas (*An. Inst. nac. Invest. agron.*, 1967, **16**, 219–246).—Cauliflowers, endive, lettuce and cabbage were precooled by (1) air-cooling at 0–1°, 75% R.H. (2) vacuum-cooling at 5 mm Hg (3) water-cooling at 0·8° and (4) crushed ice, and stored together with untreated samples for 9 weeks at 2–3°, 90% R.H. Changes were followed by determination of wt. loss, alcohol insol. solids, and total reducing sugars and by organoleptic evaluation and observation of physiological and microbiological condition. Method (4) was satisfactory for cauliflowers and lettuce, method (1) or (2) for cabbage and methods (2) and (3) for endive. E. C. APLING.

Effect of liquid nitrogen freezing on the taste, tenderness, and keeping qualities of dressed turkey. L. D. Pickett and B. F. Miller (*Poult. Sci.*, 1967, **46**, 1148–1153).—Taste, tenderness, and keeping quality of turkey carcasses frozen in liquid N_2 immediately

after evisceration were very similar to those treated by a commercial freezing process. A. H. CORNFIELD.

Chilling and freezing salmon and tuna in refrigerated sea water. S. W. Roach, H. L. A. Tarr, N. Tomlinson and J. S. M. Harrison (*Bull. Fish. Res. Bd Can.*, 1967, No. 160, 40 pp.).—Engineering aspects are considered for systems employing mechanical refrigeration, ice and salt mixtures, brine-spray freezing, and partial freezing. Changes in fish such as wt., flesh salt content, free drip, loss of sol. components, development of rancidity and proteolysis, discoloration and bacterial spoilage are discussed in relation to the different procedures. (43 references.) E. G. BRICKELL.

Packaging

Testing requirements for plastics. Migration behaviour of cadmium pigments from food packaging materials. H. Woggon, U. Köhler and W.-J. Uhde (*Dt. LebensmittRdsch.*, 1968, **64**, 243–247).—Test strips of polyethylene, polystyrene and polyamide plastics containing a number of Cd-based pigments were placed in contact with water or 5% AcOH for 6 h at 80° or extracted under reflux with ether and EtOH. The Cd extracted was then determined polarographically. There was little tendency for Cd to be lost from the polyethylene and polystyrene samples but acids (and hence probably acid foods), were able to extract almost all the metal from polyamide plastics even when present in small amounts (0·1%). (11 references.) J. B. WOOF.

Criteria for selection of packaging materials for roasted macadamia kernels. C. G. Cavaletto and H. Y. Yamamoto (*Fd Technol. Champaign*, 1968, **22**, 97–99).—The kernels can be expected to keep well for at least 6–7 months, provided the flexible packaging material has a water vapour transmission rate < 0·02 g/100 in.²/24 h at R.H. 90% and 38°. Kernels stored in glass or tin containers showed no flavour change after 12 months at room temp.
P. S. ARUP.

Plastic materials in the dairy industry—a critical study. IV. Effects of chemical composition and thermal conductivity of plastics. G. Wildbrett (*Fette Seifen AnstrMittel*, 1968, **70**, 107–118)—The effect of direct contact between plastic containers and dairy products is reviewed, and it is shown that diffusion can occur with low mol. wt. plastics which can affect the organoleptic properties of the foodstuff. In specific cases, microbiological changes can occur, and some dairy products are affected by the mechanical properties of plastic compositions. Special techniques are considered which allow milk to be cooled rapidly in plastic containers. (143 references.) G. R. WHALLEY.

Experimental equipment for cooling and packaging foam-spray-dried milk in the absence of oxygen. F. P. Hanrahan, R. L. Selman and B. H. Webb (*J. Dairy Sci.*, 1967, **50**, 1873–1877).—A description is given of pilot scale equipment for cooling and packaging dried whole milk in an inert atm. Tests showed that the equipment enabled an O_2 level of < 0·01% in the head space gas of the packaged products to be attained. (18 references.)
M. O'LEARY.

Carbon dioxide preservation of fresh poultry and related studies with corrugated and wirebound wooden containers. C. J. Wabeck (*Diss. Abstr.*, B, 1967, **27**, 2741).—CO_2 was used to assess its value as a preservative and super-coolant. Permeability of corrugated materials to CO_2 was determined gravimetrically (by increase in weight of soda lime). CO_2 atm. had a stimulatory effect on the microbial flora of fresh poultry over a period of two days but when CO_2 was used for the entire storage period at 1°, final microbial population was lower than for controls. Coliforms remained constant, but psychrophilic, lipolytic and proteolytic organisms were inhibited by CO_2. Wirebound containers had a tendency to absorb large quantities of water. Lacerations resulted from sharp blows on the edges of the rigid corrugated containers.
F. C. SUTTON.

Evolution of space feeding concepts during the Mercury and Gemini space programmes. R. A. Nanz, E. L. Michel and P. A. Lachance (*Fd Technol. Champaign*, 1967, **21**, 52–54, 56, 58).—A review is presented comprising descriptions of arrangements for food storage, of developments in packaging, of the designs of food and water containers and dispensers for rehydratable foods, and of problems of orientation. (12 references.) P. S. ARUP.

Printing ink compositions. Union Carbide Corp. (Assee. of G. M. Adams) (B.P. 1,086,525, 2.10.64. U.S., 4.10.63).—A rapid drying ink for use on polymeric films (particularly on regenerated cellulose sausage casings) consists of a solution (in a volatile org. solvent) of (*i*) a reactive mixture of a polymer containing OH groups, e.g. an alkylene oxide–polyol adduct and a prepolymer containing NCO groups, (*ii*) a polymeric resin hardener, e.g. a partially hydrolysed vinyl chloride-vinyl acetate resin and (*iii*) a pigment and/or dye. The inks possess a long pot life.
H. L. WHITEHEAD.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Effect of time factor on nitrogen sparing effect of dietary carbohydrate. M. N. Rao and J. M. McLaughlan (*Can. J. Biochem.*, 1967, **45**, 1653–1658).—Rats receiving protein (*P*) and carbohydrate (*C*) in the same meal retained more N during the first day than did rats receiving the *C* separately 8 h after the *P*, but no such difference between the groups was observed during the subsequent 9 days. The former group developed increased plasma-amino-acid levels which decreased to the level of rat-carcase *P* when *C* was given 8 h later. Rats fasted for 16 h had low levels of liver-glycogen; *P* given alone gave a partial increase in the glycogen, whilst *C* fed 8 h after the *P* restored the glycogen to normal levels and spared the *P* fed at the next meal. Some comments on aspects of human nutrition are included in this paper. (16 references.)
P. S. ARUP.

Effect of heat treatment on quality and utilisation by rat of protein in wheat germ meal. E. M. Olsen (*Can. J. Biochem.*, 1967, **45**, 1673–1679).—Losses of arginine and lysine and decreases in nutrient value occurred when the meal was toasted for 45 min. or autoclaved at 121° for 45 or 90 min. The treatments, especially autoclaving, decreased the growth rate when the products were fed to rats. The raw meal was non-toxic to rats; its nutrient value could not be improved by supplementation with any amino-acid, except methionine (I). I-supplemented raw meal was superior to I-supplemented soyabean meal. Heat treatment during processing should be kept to a min. (14 references.) P. S. ARUP.

Plant seeds as protein sources for food or feed. Evaluation based on amino-acid composition of 379 species. C. H. VanEtten, W. F. Kwolek, J. E. Peters and A. S. Barclay (*J. agric. Fd Chem.*, 1967, **15**, 1077–1089).—Further to previous reports on 214 plant species, amino-acid compositions are tabulated for 165 additional species. The combined results are surveyed with regard to the provisonal patterns recommended by the Food and Agricultural Organisation of the U.N.O. as part of the search for additional food sources. (14 references.) P. S. ARUP.

Use of ascorbic acid in fruit processing techniques, with particular regard to its rôle in prevention of enzymic browning and the occurrence of non-enzymic browning. T. Lovric, J. Debicki, F. Ipsa and R. Vucic (*Kemija Ind.*, 1967, **16**, 115–126).—Natural non-conc. and conc. apple juice, frozen apple cuttings in syrup, and stewed pears are studied. Results show that the treatment of fruit with ascorbic acid in the preliminary phase of the processing has a positive influence on individual organoleptic properties, but factors promoting the process of non-enzymic darkening, must be reduced to a minimum. (20 references.) T. M. BARZYKOWSKI.

Vitamin E determination in sunflower oil. A. G. Malsheva (*Pishch. Tekhnol.*, 1967, No. 4, [59], 160–163). C.V.

Unclassified

Effect of processing method on oxidative off-flavours of soyabean milk. W. F. Wilkens, L. R. Mattick and D. B. Hand (*Fd Technol. Champaign*, 1967, **21**, 86–89).—An acceptable bland milk was produced by grinding the hulled beans (without previous soaking) with water at 80–100° and holding at the grinding temp. for 10 min. in order to destroy the lipoxidase. Yields of milk solids were increased by lowering the temp. from 100° to 60°, but at < 80° it was necessary to use nordihydroguaiaretic acid (an antioxidant) to destroy all enzymic activity. P. S. ARUP.

Biological effects of oxidised fatty acids. J. Raulin (*Alteration oxydative des Corps Gras*, [Marseilles, Inst. Corps Gras], 1967, 57–70).—A review. (118 references.) L. A. O'NEILL.

Technological study of yeast production with n-alkanes as sources of carbon and energy. B. Erdtsieck (*Proefschr. Verkrijg. Graad Doct. tech. Hoogesch. Eindhoven*, 1967, 153 pp.).—Experiments are described in which strains of *Candida tropicalis* and *C. lipolytica* were grown in shaking flasks or vats (with stirring and aeration) in aq. inorg. media, using as source of C C_{11}-C_{19} alkanes dissolved in

a liquid paraffin of sp. gr. 0·883 (pristane). On a mineral salt–n-alkane medium fortified with yeast extract, adaptation was accomplished within 24 h after transfer from a carbohydrate medium. Apparatus and methods used for the study of the mechanism of the utilisation of the alkanes are described. (193 references.) P. S. ARUP.

Bio-stimulants of bakers' yeast from wastes from vitamin, citric acid, brewing, meat and milk plants (*Pishch. Tekhnol.*, 1967, No. 6, [61], 59–63).— C.V.

Formation of aflatoxin derivatives. E. V. Crisan and A. T. Grefig (*Contr. Boyce Thompson Inst. Pl. Res.*, 1967, **24,** 3–8).—Procedures are given for preparing the oximes and 2,4-dinitrophenylhydrazones of aflatoxins B_1 and B_2. Aflatoxins G_1 and G_2 are unreactive as they lack a carbonyl group, so that formation of the aflatoxin B deriv. can be used to remove traces of the B toxins from G toxin prep. E. G. BRICKELL.

Formation of aflatoxin in Cheddar cheese by *Aspergillus flavus* and *Aspergillus parasiticus*. J. L. Lie and E. H. Marth (*J. Dairy Sci.*, 1967, **50,** 1708–1710).—Formation of aflatoxins B_1 and G_1 by *Aspergillus flavus* and *A. parasiticus* when grown on three-month-old Cheddar cheese at room temp. for one week, and upwards, was demonstrated. The aflatoxin was not formed more than 1·3 cm from the cheese surface. (13 references.) M. O'LEARY.

Separation of aflatoxin on selectively deactivated silicic acid. E. V. Crisan and E. Mazzucca (*Contr. Boyce Thompson Inst. Pl. Res.*, 1967, **23,** 361–365).—Activated Silic AR, Mallinckrodt type CC-4, permitted recovery of 87% of the aflatoxin B, in a crude extract. Methods of purifying the remaining aflatoxin G by selectively deactivated adsorbents are described. E. G. BRICKELL.

Thermal resistance of spores of *Clostridium tyrobutyricum* and *Clostridium butyricum*. P. O. Cerf, J.-L. Bergere and J. Hermier (*J. Dairy Res.*, 1967, **34,** 221–229).—Survivor curves of *Clostridium tyrobutyricum* spores (three strains) heated at 90° in 0·033 M phosphate buffer (pH 7·0) or in skim-milk were logarithmic. D at 121° was 0·003–0·012 min. and *z* was 8·4–10° with the phosphate buffer, and with milk D at 121° was 0·006–0·008 and *z* was 9·5–9·9°. Survivor curves of *C. butyricum* spores (two strains) heated at 85° in 0·005 M phosphate buffer (pH 7·0) were logarithmic but 'tails' were observed after heating in skim-milk. D value at 85° was lower than that obtained with *C. tyrobutyricum* spores. (17 references.) (In French.) M. O'LEARY.

Increasing water solubility of 3,4-benzpyrene by addition of 1,3,7-trimethylxanthin (caffein). III. Effect of addition of organic compounds containing nitrogen to caffein solutions. J. Eisenbrand and K. Emrich (*Dt. LebensmittRdsch.*, 1968, **64,** 217–218).—Solutions of 5·82% caffein containing in addition phenyldimethylpyrazolone (10%), thiosinamine (5%), urethane (10%), urea (20%) and thiourea (5%) were found to dissolve 592, 540, 500, 330 and 345 mg/l of 3,4-benzpyrene respectively. None of the additives quenched the fluorescence of the hydrocarbon. J. B. WOOF.

Gravimetric procedure or the quantitation and identification of Polysorbate 80 [polyoxyethylene sorbitan mono-oleate emulsifier] in pickle products. P. Barcklow (*J. Ass. off. analyt. Chem.*, 1967, **50,** 1265–1268).—The sample is heated first with HCl, then with KOH; a $CHCl_3$ extract of the acidified liquid is evaporated, and the residue dissolved in water. After removal of fatty acids with light petroleum, the glycol, recovered from a $CHCl_3$ extract, is pptd. by boiling with HCl, aq. $BaCl_2$ and silicotungstic acid. The Polysorbate 80 (**I**) is determined gravimetrically by reference to a standard curve, and is identified by its i.r. spectrum. Recoveries of **I** (0·01–0·02%) from pickles were 80–98·5%. A. A. ELDRIDGE.

Survey of organic impurities in food grade hydrochloric acid. J. G. Cummings and K. T. Zee (*J. Ass. off. analyt. Chem.*, 1967, **50,** 1262–1265).—Samples of HCl obtained from various food manufacturing processes contained 3·9 to 10·2 ppm of organic chloride extractable with light petroleum. Examination by g.l.c. showed the substances to be aliphatic chlorohydrocarbons; chlorinated pesticides were not found. A. A. ELDRIDGE.

Carbonyl compounds and gas chromatography. I. G. Mokhnachev, V. S. Kovtunov and S. V. Kamenshchikova (*Pishch. Tekhnol.*, 1967, No. 5, [60], 112–115).— C.V.

Micro determination of copper in some foods. L. E. Gotsuly'ak and I. I. Traskov (*Pishch. Tekhnol.*, 1967, No. 6, [61], 142).— C.V.

3.—SANITATION, WATER, etc.

Rapid identification of coliform organisms from extra-intestinal infections. E. Whitefield (*J. med. Lab. Technol.*, 1967, **24,** 171–178).—Tests were evolved which enable these organisms to be assigned to their genera either on the day of isolation or on that following. (20 references.) C.V.

Isolation of *Vibrio parahaemolyticus* from the Northwest Pacific. J. Baross and J. Liston (*Nature, Lond.*, 1968, **217,** 1263–1264).—Enrichment of water, sediment, and homogenised oyster prep. with *V. parahaemolyticus* (**I**) (which causes gastroenteritis) yielded two bacteriophages specific for **I** and both from Pacific oyster homogenates. The related biotypes *V. alginolyticus* and *V. anguillanum* were not lysed by these phages. Biochemical characteristics of Japanese and Puget Sound isolates of **I** are listed; except for their ability to ferment sucrose the Puget Sound isolates are physiologically identical with Japanese strains. The Pacific oyster is thus a potential carrier of **I**. A satisfactory, semi-selective medium for isolation of **I** is based on the invariant ability of **I** to utilise starch with use of penicillin to inhibit contamination by *Bacillus* types. Differentiation from the other two biotypes is based on salt tolerances as described by Sakazaki. W. J. BAKER.

Selective feeding of tubificids on bacteria. R. A. Coler, H. B. Gunner and B. M. Zuckerman (*Nature, Lond.*, 1967, **216,** 1143–1144).—Selective feeding rather than continuous indiscriminate sapropelic ingestion is postulated as the nutrition mechanism of tubificid worms in benthic ooze of sewage outfalls. Evidence for this is based on behavioural studies and on analyses of homogenates of mixed worm populations for presence of diazinon (**I**) incorporated by bacteria in their feed. **I** is present in worms showing tolerance to *E. coli, S. natans* and *A. aerogenes*, but is absent in worms showing avoidance of *Arthrobacter* sp., *Chromobacterium* sp. and *M. flavus*. Avoidance is tentatively ascribed to presence of one or more readily diffusible water-sol. metabolites. Results are discussed briefly in terms of the relevance of food preferences to population distributions. W. J. BAKER.

Continuous monitoring of high concentrations of atmospheric fluoride. D. C. MacLean, L. H. Weinstein and R. H. Mandl (*Contr. Boyce Thompson Inst. Pl. Res.*, 1967, **24,** 9–10).—An SO_2 analyser/recorder was adapted to gaseous HF analysis and proved satisfactory for air analyses of plant fumigation chambers containing concn. of 0·5 to 10 ppm. E. G. BRICKELL.

Pyrethrum as an insect repellent. III. Persistence of pyrethrin films, when applied in three types of formulation, as insect repellents to human skin. N. K. Sylvester and A. J. S. Weaving (*Pyrethrum Post*, 1967, **9,** No. 2, 18–24).—Alcohol-, mineral oil- and water-based formulations of synergised pyrethrum were applied to the skin and measurements taken at hourly intervals. The oil-based prep. showed the highest apparent loss of pyrethrins although it gave the fastest knockdown of female *Aedes aegypti* and was the most efficient in repellency. The pyrethrin losses were almost certainly due to absorption into the deeper tissues of the skin. J. L. WALPOLE.

Amidino-phosphoric, -phosphonic, -thiophosphoric and -thiophosphonic acid esters. Farbenfabriken Bayer A.-G. (Inventors: H. Malz and G. Hermann) (B.P. 1,080,450, 22.2.66. Ger., 22.2.65).—The P-containing esters have the general formula $p\text{-}Z\cdot C_6H_4\cdot YP(X)R^2\cdot N{:}C(NH_2)R^1$, wherein R^1 is H or alkyl of 1–4 C, R^2 is alkyl or alkoxy of 1–4 C, X and Y are O or S and Z is halogen atom. The ester halide $p\text{-}Z\cdot C_6H_4\cdot YP(X)R^2Z$ is reacted with the amidine $HN{:}CR^1NH_2$ in a solvent, in the presence of an acid acceptor at −50 to +100°. These claimed compounds possess outstanding rodenticidal properties. They can be used in the usual formulations, emulsifiable concentrates, etc. S. D. HUGGINS.

4.—APPARATUS AND UNCLASSIFIED

Farm implements and machinery. C. Culpin (*Jl R. agric. Soc.*, 1967, **128,** 144–158).—A review is presented of tractors, mechanisation of crop production, fodder conservation, grain harvesting and storage, root harvesters, horticultural engineering, livestock engineering, and mechanisation management. (57 references.) E. G. BRICKELL.

Respiration apparatus for determining the energy expenditure of livestock in cold environments. A. J. F. Webster and A. M. Hicks (*Can. J. Anim. Sci.*, 1968, **48,** 89–92).—An open-circuit respiration

apparatus is described, in which air is drawn over a restricted area enclosing the face of the animal, at a rate ensuring that all expired air passes into the ventilating stream. Illustrations are given and operational details are described. A. G. POLLARD.

Rind tester, a new device to measure pressure required to break the oil cells of citrus rind. R. A. Christ (*S. Afr. J. agric. Sci.*, 1967, **10**, 741–745).—The instrument comprises a small dial gauge operated by a spring connected to a steel ball (0·125 in. dia.) which is pressed against the rind through an intervening piece of tissue paper. The rupture is immediately indicated by a stain on the paper. Susceptibility of the rind to cell-rupture increases the danger of fungal penetration. P. S. ARUP.

New words in biology. N. W. Pirie (*J. med. Lab. Technol.*, 1967, **24**, 253–275).—Based on earlier lists (Pirie and Talboys, *J. Inst. Biol.*, 1964, **11**, 92; 1965, **12**, 146; **13**, 150), the new words, together with definition and source, are reproduced as one full list. C.V.

Photosynthetic bacterium growing under carbon monoxide. P. Hirsch (*Nature, Lond.*, 1968, **217**, 555–556).—Reports the anaerobic growth of purple bacteria (*Rhodopseudomonas* sp.) under incandescent light at ~ 22° in enrichment cultures (NaOAc) for CO-oxidising bacteria under an atm. of CO 70, O_2 20, CO_2 1 and N_2 9%. Growth was accelerated only after other CO-bacteria had removed O_2 from the mixture. The enrichment and isolation procedure described was successful with garden soil (pH 6·8) and sewage mud (pH 7·2). The mode of formation of these photo-organotrophically produced bacteria is briefly discussed. W. J. BAKER.

Bacterial lipids. J. Asselineau (*Hermann, Paris*, 1966, 372 pp.).—This is a revised edition of the original French book *Les lipides bacteriens* published in 1962. Isolation, fractionation, analysis, methods of degradation and synthesis are dealt with. Simple specific constituents, hydroxy, carbonyl, amino and acidic compounds, are examined and their biosynthesis is discussed. The lipid groups are reviewed in considerable detail together with their biological properties. (1464 references.) C.V.

Comparison of the cardiovascular effects of regular and filter-tip cigarette smokes. S. Kaymakçalan, R. K. Türker, B. K. Kiran and S. Kayan (*Arzneimittel-Forsch*, 1968, **18**, 817–819).—A simple method is described for use in investigating the influence of a paper filter on the degree of absorbance of nicotine and thus on the blood pressure and heart-rate changes in anaesthetised dogs. Both Turkish and American cigarettes were tested by the method, and no significant difference is observed in either blood pressure or heart-rate with filtered or unfiltered inhalation of cigarette smoke. Nicotine can therefore be absorbed from both types of cigarette smoke to produce similar effects. (In English.) G. R. WHALLEY.

Carbohydrates of tobacco smoke. I. G. Mokhnachev and S. V. Kamenschikova (*Pishch. Tekhnol.*, 1967, No. 4, [59], 41–42).— C.V.

Validation study of a method for determining pesticide residues in foods and animal feeds. C. E. Wells (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1205–1215).—The A.O.A.C. method having limited application (*ibid.*, 1966, **49**, 222) was used in collaborative studies of the determination of residues of 65 pesticides in numerous foods and feeds. Recoveries of 32 pesticides were from 80 to 110%; others were less satisfactorily recovered, or not recovered. Carrots, parsnips, onions and lettuce interfere when an electron capture detector is used. A. A. ELDRIDGE.

Temperature-programmed gas chromatography of twenty phosphorus-containing insecticides on four different columns and its application to the analysis of milk and corn [maize] silage. M. C. Bowman and M. Beroza (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1228–1236).—The Melpar flame-photometric detector, which can sense compounds containing P or S, is particularly suitable for analysing multi-component mixtures of pesticides containing P. Retention times of 20 such compounds were determined on four different column packings; with flame photometric detection the limit of sensitivity was 0·01 ppm. Recoveries from silage were > 90% (often > 95%) and those from milk were > 80% (often > 90%). (32 references.) A. A. ELDRIDGE.

Identification of azinphos-methyl in human tissues and in figs. F. Sa. and M. E. da Silvá (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1258–1260).—A benzene extract of stomach tissue or figs (suspected of causing poisoning in cattle) is concentrated and purified by t.l.c. on silica gel G plates with n-hexane/acetone (2 : 1) as developing solvent. The spot is located on one plate by spraying with 0·5% $PdCl_2$ in 5% HCl. An unsprayed spot is extracted into methanol and identified by t.l.c., u.v. and i.r. spectrophotometry. A. A. ELDRIDGE.

Flame spectrophotometric determination of phosphorus. R. K. Skogerboe, A. S. Gravatt and G. H. Morrison (*Analyt. Chem.*, 1967, **39**, 1602–1605).—Optimum conditions for excitation of the atomic spectrum of P are reported and discussed in respect of different flames and detection limits attainable. Use of the band at 246·4 nm, an air–H_2 flame, and 90% EtOH as solvent ensures a sensitivity of ~ 5 μg P per ml for most biological materials. Interfering elements (mainly Fe, Ca, Ba, K, etc.) must be removed initially by cation-exchange, and the H_3PO_4 standards must also be equilibrated with the resin. Results for cherry leaves, liver, and bone (P 0·16–9·8%) agree with those obtained by colorimetry, the coeff. of variation for the photometric measurements being 2–3%. (4 analyses of each sample). W. J. BAKER.

Determination of strontium in bone, milk, and vegetable ashes using the Unicam SP 90 atomic absorption spectrophotometer. Anon. (*P.G. Rep. U.K. atom. Energy Auth.*, 1967, 775 (CA), 6 pp.).—An acid solution of the ash is used to coprecipitate Sr and Ca as oxalates; the ppt. is ignited and the oxide/carbonate residue is dissolved in HCl. Excess acid is vaporised and the residue is dissolved in a standard vol. of water such that the Ca content is 0·5%. The Sr content of the solution is determined by comparison with suitable solutions using atomic absorption spectrophotometry. The limit of Sr detection is 50 ppm relative to Ca; the coeff. of variation is 2·5% at the 300 ppm level of Sr relative to Ca. J. W. TAYLOR.

Spectrographic determination of lead in agricultural [plant tissue, soils] and related [other biological] materials. K. S. MacLean, D. L. Byers and M. H. Brown (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1366–1369).—After removal of org. matter by ashing, the sample (20 mg) is mixed with Li_2CO_3 and graphite (2 : 1, 40 mg) containing 0·015% Bi as internal standard, and arced in a d.c. arc between graphite electrodes. The lines Pb 2833 Å and Bi 2898 Å are examined densitometrically and compared with standards. The results are as accurate as those obtained by chemical methods. (13 references.) A. A. ELDRIDGE.

Determination of arsenic in animal tissues, using a dry ashing procedure. L. R. Stone (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1361–1362).—The sample is ashed at 550–600°, and As is determined by the A.O.A.C. method (*Official Methods of Analysis*, 10th ed., 1965). A. A. ELDRIDGE.

Bird repellent. Bugges Insecticides Ltd. (Inventor: F. W. Lepine) (B.P. 1,073,210, 2.6.66. Pat. of Addn. to B.P. 974,641).—The original patent claims the use of a mixture of a bis(thiocarbamoyl)disulphide and a film-forming polyacrylate for protecting fruit trees and bushes against attack by birds. The present addition claims the use of similar compositions for protection of buildings and other artifacts. S. D. HUGGINS.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

DECEMBER, 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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Kinetics of the reduction of silicon tetrachloride and trichlorosilane by hydrogen
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Application of thermal analysis in investigation on calcium silicate bricks
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Observations on calcium sulphate dihydrate formed in media rich in phosphoric acid. I. Precipitation of calcium sulphate dihydrate
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