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RAPID METHOD FOR THE DETERMINATION OF STEAM-VOLATILE FATTY ACIDS IN RUMEN LIQUOR

By F. G. YOUSSEF and D. M. ALLEN

A method is described for the rapid separation of steam-volatile fatty acids in rumen liquor by gas–liquid chromatography and automatic titration of the eluted acids. The method consists of a pressure programme for nitrogen gas which allows the separation of acids up to n-valeric in 22 minutes.

Introduction

In a previous paper Youssef & Allen[1] described improvements to the method of Storry & Millard[2] for the determination of steam-volatile fatty acids (VFA) in rumen liquor by gas–liquid chromatography, and automatic titration of the separated free acids. Lough, Reid, Murray & Black[3] compared two gas–liquid chromatography procedures for the separation of VFA, one of which operated in conjunction with an automatic titration device[2] and the other with a flame ionisation detector.[4] They found that flame ionisation detection was more accurate and yielded results more rapidly than did automatic titration. However, the difference in accuracy between the two procedures may have been largely caused by the malfunction of columns such as the one described by Youssef & Allen.[1]

Though the method of Youssef & Allen[1] has shown very similar precision to flame ionisation detection, it is very time consuming. Attempts to increase the rate of elution of VFA by raising the temperature of the column to 135°[3] were vitiated when the reproducibility of results was found to be poor under those conditions. Lanigan & Jackson[5] used a temperature and nitrogen pressure programme successfully on a behanic and orthophosphoric acids column but the separation of acids up to caproic still took 70 minutes.

This paper describes a nitrogen pressure programme to increase the rate of elution of VFA.

Experimental

Apparatus

The apparatus of Storry & Millard[2] as modified by Youssef & Allen[1] was used.

Nitrogen pressure programme

The extracted sample of VFA was injected on to the column and nitrogen gas passed through at 13–14 lb/in.^{2}. Acetic acid was titrated manually. The pressure of nitrogen gas was reduced to 9–10 lb/in.^{2} just before the elution of propionic acid but, as soon as the emergence of iso-butyric acid commenced, it was increased to 18 lb/in.^{2} for the separation of acids up to, and including, n-valeric.

Results and Discussion

Table I shows the precision obtained in replicated analyses of a known standard solution and a sample of rumen liquor. The retention times of various acids relative to n-butyric acid and the times required for the elution of each acid are presented in Table II. It can be seen that by using the nitrogen pressure programme the total time required for an analysis was reduced to less than half of that taken when a constant nitrogen pressure of 9 lb/in.^{2} was used. The resolution of the acids was not adversely affected, and it may have been improved for the higher acids. Caproic acid emerged after approximately 33 minutes if the pressure of nitrogen gas was increased to 22 lb/in.^{2} at the elution of iso-valeric acid.

TABLE I

Analyses of standard solution of volatile fatty acids and rumen liquor sample (6 replicates)

Acid	Composition of standard solution %	Found (mean % ± S.E.)	Rumen liquor (mean % ± S.E.)
Acetic	62·5	62·2 ± 0·17	59·6 ± 0·18
Propionic	18·2	18·3 ± 0·10	19·5 ± 0·11
Isobutyric	2·2	2·3 ± 0·07	1·6 ± 0·07
n-Butyric	10·4	10·5 ± 0·09	13·3 ± 0·10
Isovaleric	2·2	2·2 ± 0·08	4·2 ± 0·09
n-Valeric	4·5	4·5 ± 0·09	1·8 ± 0·09

TABLE II

Retention times relative to n-butyric acid and the times required for the elution of each acid

Acid	Retention	Elution times (minutes)	
		Variable pressure programme	Constant pressure 9 lb/in.^{2}
Acetic	0·36	5	9
Propionic	0·57	8	15
Isobutyric	0·71	10	18
n-Butyric	1·00	14	26
Isovaleric	1·21	17	33
n-Valeric	1·57	22	48

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EFFECT OF SOLID HYDROCARBONS AS ADDITIVES IN BREADMAKING

By G. A. H. ELTON and N. FISHER

A study of pure saturated hydrocarbons as experimental breadmaking additives has shown a critical dependence of loaf volume on chain length of the additive, addition of two methylene groups converting a highly significant volume reduction to a highly significant volume increase. The results are discussed in the light of current hypotheses of the mechanism of action of lipids in breadmaking, none of which is regarded as capable of explaining all the observed phenomena. A 'reservoir' of crystalline additive was shown not to be essential to the improving effect, and factors other than melting point of the additive were found to be involved, including a 'flour factor'.

Introduction

Modern breadmaking processes such as the Chorleywood Bread Process (CBP)[1] in which bulk fermentation is replaced by a brief period of intense mechanical work depend critically on the inclusion of fat in the recipe for the attainment of those loaf characteristics regarded as desirable in British and American practice. In bulk fermentation processes, however, use of fat is less critical, though its improving effects on loaf volume and crumb properties justify its widespread utilisation. The volume of fat-containing loaves produced by mechanical development may be 20% higher than that of fat-free controls, and the increased volume is also accompanied by finer crumb texture. The effects have been shown by work from a number of laboratories[1–4] to vary with the slip-points of the fats used, and a requirement for the presence of solid fat during dough-mixing or at the end of final proof has been inferred.[5] Addition of flakes of hardened fat has been shown to impart the required properties to an otherwise ineffective fat.[3,4]

The mechanism by which fats exert their improving effects in conventional breadmaking as well as mechanical development processes has been attributed to lubrication of starch granules or 'gluten filaments',[6,7] modification of water transfer,[7,8] and sealing of pores in the protein structure,[9] and a hypothesis involving structural support of the rising crumb structure by fat has recently been advanced.[10]

None of the hypotheses so far advanced appears entirely convincing, and the purpose of the present communication is to give evidence, confirming that the improving effect need not be related to the functional groups present in the fats, and demonstrating that it can be produced by pure saturated long-chain hydrocarbons. Evidence is also presented that there is a critical influence of hydrocarbon chain length in the 18–24 C range on loaf volume, a chain extension of only two methylene groups (involving a melting point increase of 7°C) converting a highly significant reduction in loaf specific volume (compared with a control) to a highly significant increase in specific volume. It has also been shown that factors other than the melting point of the additives are involved in the mechanism(s) by which their effects are exerted.

Experimental

Materials

'Pure' hydrocarbons n-octadecane, n-eicosane, n-docosane and (later) n-tetracosane were purchased from Koch-Light Laboratories Ltd., Colnbrook, Bucks. Infra-red spectra of these products agreed with the corresponding Sadtler Standard Spectra; after the first baking test had been carried out it was found that a slight impurity giving rise to a peak at 1885 cm^{-1} could nevertheless be removed by ether recrystallisation. This was carried out and the baking test was repeated, with similar results to those obtained previously. The recrystallised products each gave a single spot on thin layer chromatography in light petroleum : ether : acetic acid 70 : 30 : 3[11] and also in carbon tetrachloride.[12] Melting points (hot stage, uncorrected) and elementary analyses of the purified materials are shown in Table I.

TABLE I

Melting points and elemental analyses* of saturated straight-chain hydrocarbons

n-Hydrocarbon	M.p. (°C)		Found		Required by formula	
	Observed	Published[13]	C	H	C	H
Octadecane	28–28·5	28	85·03	14·84	84·95	15·05
Eicosane	36·5–37	36·8	84·83	15·02	85·04	14·96
Docosane	43·5–44	44·4	84·80	14·92	85·09	14·91
Tetracosane	51–51·5	51	84·99	14·94	85·11	14·89

* Dr. G. Weiler, Dr. F. B. Strauss, Banbury Road, Oxford

1 : 1 mixtures of octadecane and eicosane, and of eicosane and docosane were prepared, and 6 g of the mixture were used in the same way as the individual hydrocarbons.

In order to provide a basis for comparison of the effects of the hydrocarbons with those produced by fats of the type normally used in bread, a commercial shortening with slip point of 43° was included in the tests.

Previous experience had shown the need for use of a dispersing agent if solid fats were to be adequately mixed into a dough. Preliminary tests showed that this was also true for the hydrocarbons. Liquid paraffin B.P. ('Nujol') was found to cause highly significant loaf volume reduction and was therefore unsuitable. A commercial sample of groundnut oil (GNO) gave loaves with identical specific volumes to 'no-fat' controls, and was therefore used in all these tests.

Doughmaking procedure

Doughs were prepared according to the following recipe:

	g	% of flour
Flour ('Baker's grade')	840	—
Yeast	18	2·14
Salt	15	1·78
Fat (or hydrocarbon)	6	0·71
GNO (if hydrocarbon used)	6	0·71
Ascorbic acid	0·063	0·0075
Water	495–513 ml (according to 'CBP water absorption')[14]	

Doughs were mixed to a work input of 5 Wh/lb in a water-jacketed Morton mixer, the final dough temperature being 30·3° (standard deviation 0·45°). Hydrocarbons were added in an equal weight of groundnut oil, after the mixture had been warmed until the hydrocarbons had just melted. In the final experiments involving tetracosane (Flour E in Table II) the hydrocarbon–groundnut oil mixtures were heated prior to addition to the dough as follows: octadecane/GNO and eicosane/GNO to 41°; docosane/GNO, tetracosane/GNO and shortening to 54°.

Doughs were divided into 454 g pieces, machine moulded, rested for 10 minutes while covered with moisture-proof film, remoulded as before and proved to a height of 3·9 in. in the baking tins in a cabinet held at 40·5°. When differences in mean proof times of doughs treated with different additives were present, these were not significant. Loaves were baked at 223° for 25 minutes in a 'Despatch' reel oven. 3 mixings each yielding 2 replicate loaves were prepared for each experimental variant. Loaf volumes were determined by seed displacement.

Results

Results are shown in Table II and the appearance of the (cut) loaves in Fig. 1.

Discussion

In practice fats would not be replaced by hydrocarbons even if this were legally permissible (which it is not), because of the undesirable nutritional and pharmacological effects this might involve. The use of hydrocarbons in this work was solely dictated by the need to demonstrate whether the presence of ester or other functional groups in a lipid additive was essential to its effect on loaf volume. Solid hydrocarbons have previously been used[9, 15] for this purpose, but the commercial materials used were mixtures, and the effect of chain length could not be studied. (The concentrations employed were also very much higher than those used in the present work.) It was felt to be important to use materials with sharp melting points since the presence of solid over a wide range of temperatures might complicate the interpretation of the results. The use of these compounds would also be a test of the necessity for a 'reservoir' of solid fat during baking.[2, 9]

It may be seen from Table II that the effect of saturated hydrocarbons on loaf volume is a direct function of the chain length. This determines the melting point of the hydrocarbon, but melting point may not be the critical factor since the hydrocarbon mixtures gave loaf volumes less than, or not significantly different from, those given by the components with lower m.p., although here the concentration of hydrocarbon of higher m.p. may have fallen below a critical limit lying between 3 and 6 g (the greater volume depression given by Ei + Do + GNO than by Ei + GNO is not paralleled by a corresponding effect for Oc + Ei + GNO mixtures); further reasons are that the liquid GNO gave higher loaf volume than GNO–hydrocarbon mixtures where the hydrocarbon had a chain length <20, and in two cases the docosane/GNO mixture also gave a lower volume than GNO alone. (The 'solids content' of the GNO used was a negligible proportion

TABLE II

Effect of hydrocarbon additives on the specific volume of bread

(specific volumes are expressed as ml/g)

	Flour A		Flour B				Flour C		Flour D		Flour E			
Proof temp °C	40·5		40·5		32		40·5		40·5		40·5			
Additives	Sp. Vol.	△GNO†	Sp. Vol.	△GNO	Sp. Vol.	△GNO	Sp. Vol.	△GNO	Sp. Vol.	△GNO	Sp. Vol.	△GNO	Sp. Vol.	△GNO
None	3·31	−0·05NS	3·82	−0·03NS	3·78	−0·12NS	3·73	−0·15**	3·12	−0·03NS	3·37	0·03NS	3·50	0·07NS
Octadecane (Oc) + GNO	2·96	−0·40***	3·13	−0·72***	3·05	−0·84***	2·95	−0·93***	3·03	−0·12***	3·07	−0·27*	3·05	−0·38***
Eicosane (Ei) + GNO	3·02	−0·34***	3·50	−0·35**	3·43	−0·46***	3·43	−0·45***	3·00	−0·15***	3·07	−0·27*	3·12	−0·31**
Docosane (Do) + GNO	3·76	0·40***	3·73	−0·13NS	3·84	−0·05NS	3·76	−0·12**	3·07	−0·08*	3·46	0·12NS	3·60	0·17*
Oc + Ei + GNO	2·93	−0·43***	3·20	−0·65***	—	—	3·17	−0·71***	2·97	−0·15***	—	—	—	—
Ei + Do + GNO	3·01	−0·35***	3·09	−0·76***	—	—	3·11	−0·77***	2·91	−0·24***	—	—	—	—
Tetracosane + GNO	—	—	—	—	—	—	—	—	—	—	3·75	0·41**	3·91	0·48***
GNO	3·36	—	3·85	—	3·89	—	3·88	—	3·15	—	3·34	—	3·43	—
Shortening	4·06	0·70***	4·14	0·29**	4·09	0·20*	4·08	0·20***	3·98	0·83***	4·08	0·74***	4·19	0·76***
Shortening + GNO	—	—	—	—	4·14	0·25**	—	—	—	—	—	—	—	—

† △GNO is the difference between the mean of the specific volumes of loaves produced with the given additive and the corresponding mean using groundnut oil

* Significant at $P = 0·05$

** " " $P = 0·01$

*** " " $P = 0·001$

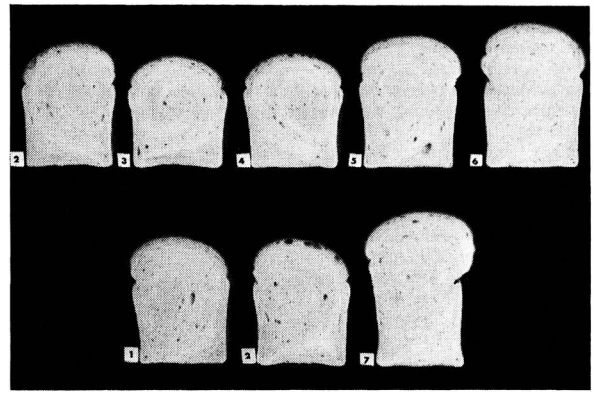

FIG. 1. *The appearance of cut surfaces of loaves prepared with* (1) *no additives*, (2) *GNO*, (3) *octadecane*, (4) *eicosane*, (5) *docosane*, (6) *tetracosane*, (7) *shortening*

The upper series shows the effect of increasing carbon chain length of the added n-hydrocarbon. The lower series shows the effects of GNO (added as a dispersing agent for the hydrocarbons) and normal commercial shortening.

of the oil[16,17] although it may be noted that the presence of 7% C_{20}–C_{24} saturated fatty acids has been reported.[17])

The shortening used had an extremely low solids content at the proof temperature used (40·5°) and a slip point of 43°. (Another sample of this bakery fat had a solids content of 1% at 43°.) It nevertheless gave much higher loaf volumes than either docosane or tetracosane with their higher melting points.

The results show that the possession by a hydrocarbon of a melting or slip point above the proof temperature is not by itself sufficient to ensure that loaf volume improvement will occur, since it will be seen that when the proof temperature was lowered from 40·5° to 32° for flour B, neither eicosane nor docosane gave loaf volumes greater than the control, and the former gave a significantly lower volume. Further, the effect of the shortening was not significantly affected by reducing the proof temperature.

Since the same hydrocarbon had different effects on different flours, one factor involved is a function of the flour properties, as has also been established in the case of improvement by fat.[5] Thus interaction between the hydrocarbons and other dough components must occur. Interaction between hydrocarbons and proteins as an explanation of the effect of hydrocarbons has been suggested previously[18] and other interactions of this type have been reported.[19,20] The binding of heptane to protein has been held to be incapable of influencing the binding of free fatty acid.[21] Work on the effect of varying chain length on the influence of fatty acids on starch gelatinisation[22] suggests that interactions with starch may also be worthy of investigation.

Ponte *et al.*[23] have demonstrated that lower aliphatic hydrocarbons reduce loaf volume, and the present work suggests that this effect may persist up to eicosane. Thereafter, volume increases with chain length, though a possible falling off in the improving effect may occur with compounds of much higher m.p.[15] (cf. also effect of excess hard fat in ref. 3). These observations, coupled with the reversal of the effect of the lower hydrocarbons in the presence of fat, indicate that the hydrocarbons are not simply acting as inert compounds but are interacting with other dough components. These interactions must, by virtue of the absence of reactive functional groups, be physical in character (e.g. hydrophobic bonding), and the improving effects must likewise involve physical rather than chemical mechanisms. Several physical hypotheses have already been advanced.

The 'lubrication' mechanism of improvement[6,7] applied to fats (lubrication of starch granules and gluten protein fibres allowing increased slip) has often been questioned in the past, and the volume-reducing effects of the octadecane and eicosane coupled with the variable effects of docosane which gave significant volume reduction with some flours and significantly increased volume with others would appear to invalidate any such hypothesis. No plausible explanation for the opposing directions of the effects of closely similar hydrocarbons on a single flour appears possible on the basis of retardation of 'labile water' transfer between gluten and starch,[7,8] another of the suggested mechanisms for the action of fats in baking. The 'pore-sealing' hypothesis first proposed by Baker & Mize[9] and later extended by Cotton and his co-workers,[2] according to which the melting of a reservoir of solid fat fills pores in the gluten created by the expansion of the dough during baking is widely accepted, but it is difficult to understand why an adequate supply of a molten additive fails to have a similar effect to a mixture of solid and the derived melt, or a dispersion of solid in a carrier. It also fails to overcome the objection that much of the expansion of the dough takes place well after all the fat has completely melted.[24] In the case of the hydrocarbons in this study, the sharp melting points of the higher pair of hydrocarbons, which, moreover, were very close to the proof temperature, would ensure that these were completely melted within a very short time after entry into the oven and could not on the basis of this hypothesis cope with any further expansion. Clearly such a hypothesis is incapable of explaining the reduced volume

obtained with the lower-melting solid hydrocarbons studied. The doughs containing these hydrocarbons proved to the same height in the same time as those containing the higher hydrocarbons, so that this reduced volume was due to a failure to rise adequately in the oven.

This objection also applies to the 'structural support' hypothesis,[10,15] although where very high concentrations of additive with very high m.p. are employed this may be a factor. The liquid form of these additives must play some as yet obscure part in the performance of doughs during baking. This must be the subject of future work. The improving effect of sharply melting hydrocarbons shows that there is no necessity for a 'fat reservoir',[2] and an explanation of the mechanism of fat improvement must be sought in the phenomena occurring during the early stages of baking.

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MINERAL COMPOSITION OF APPLES

VI*.—Changes in the composition of seeds and stems of Cox's Orange Pippin apples during development and near picking time

By M. A. PERRING and B. G. WILKINSON

Mineral analyses have been made of the seeds of developing Cox's Orange Pippin apples in three seasons. Some results are also given for stems and flesh. There are unexplained variations in the Na content of seeds in that the Na concentration appears to be at a minimum in the three or four weeks near harvest.

Changes in mineral composition of the fruit as a whole, though similar in direction, were different in magnitude from those previously reported for fruit from the same source. This further emphasises the marked effect of season on the chemical composition of apples.

Introduction

Analyses of the fleshy tissue of apples picked over a period of several weeks have shown that variations in the concentrations of most of the major mineral elements and of Mn are negligible near picking time. Exceptions are Ca, the concentration of which falls, and Na and B, the concentrations of which rise rapidly.[1,2]

The following analyses of apple seeds were made in order to ascertain whether changes in mineral concentrations occur at the time when the colour of the seeds is changing. Flesh and stems were also analysed in the first experiment so that a better picture of the major nutrient changes could be obtained. Experiments with seeds taken from fruit near picking time, and with seeds and stems taken from fruit earlier in their development were also made in subsequent years to confirm some results and to complete the picture of the uptake of the major nutrients.

Experimental

Sampling

The fruit analysed to determine changes near picking time was taken from a row of 23 trees in an orchard (D3) which has already been described.[1,3] In 1964 a random sample of 25 apples was taken from several hundred apples picked at weekly intervals for storage and respiration experiments, and in 1965 duplicate samples of 25 apples were taken in the same way. Samples were picked earlier in 1966 and at more frequent intervals. Most samples were of 46 apples (2 per tree) but a few were of 23 apples. All these samples were picked at about 9 a.m. and, in 1965 and 1966, they had been sorted, seeds had been removed, and subsamples had been weighed and frozen by 11 a.m. The sampling of the 1964 apples took about an hour longer as they were washed in distilled water and allowed to dry naturally before they were cut. Stems were found to dry out very rapidly when detached from the apple and it was necessary to weigh them immediately they were detached and again immediately before freezing or subsampling so that a correction could be made to the analytical results.

Seeds and stems of the 1964 samples were removed and weighed and then frozen at $-20°$ together with the weighed flesh. The flesh was grated at $-20°$ before subsamples were weighed for analysis. The samples of seeds and stems were homogenised, by addition of liquid nitrogen and repeated crushing in a press. The resulting small particles were well mixed before subsamples were weighed.

The seeds were not crushed in 1965. They were removed from one of the two samples, weighed and counted, and then random subsamples of about 2 g were weighed for analysis. The seeds of the second sample were divided into three groups, brown, brown/white, and white, and all the seeds of a group, or 2 g subsamples taken at random from a group, were weighed for analysis.

In 1966 seeds were removed from all the samples and divided into three colour groups as in 1965. Since most samples were of 46 apples, each group was larger and it was possible in most instances to take from each group random subsamples of about 1 g for analysis. In one sample brown and brown/white seeds were combined to provide sufficient material for analysis. One group of brown/white seeds weighed less than 0·5 g and was not analysed.

Five samples of immature apples were picked at intervals from a row of six trees in another orchard (D) during 1965. Seeds and stems were removed and weighed. Each stem was cut into about 8 transverse sections, and the sections of the whole sample were well mixed before subsamples were weighed for analysis. All of the seeds from the earliest sample were ashed, but later, random samples of about 1 g were ashed and some were digested for N determinations.

Ashing

Subsamples of flesh were ashed with nitric acid, and subsamples of seeds and stems were ashed at 900° in platinum crucibles; the ashes were dissolved in nitric acid exactly as described in Parts I and V of this series.[4,5] To prevent loss of samples due to explosions, whole seeds were punctured before being ashed. The ashings of seed and stem samples were duplicated when sufficient material was available.

Analysis

Ash constituents were determined by methods previously described.[5,6] K and Na were determined with an E.E.L.

* Part V. *J. Sci. Fd Agric.*, 1967, **18**, 265

flame photometer, as was Ca after precipitation as oxalate. P and Mg were determined colorimetrically by the molybdenum blue and Titan Yellow methods. Subsamples were digested and analysed for N by the method of Williams.[7] Dry matter was determined by drying subsamples in an oven at 50° for 44 hours.

Respiration

The physiological state of the fruit as picked was assessed by plotting the climacteric curves of respiration. These were obtained by a modification of a simple method previously described.[8] From these curves the dates of commencement of the climacteric rises were estimated.

Results

Fig. 1 summarises the data obtained from the 1964 samples from orchard D3 and indicates the composition of seeds, flesh, and stems. As regards the flesh, the most obvious changes with time were the rises in Na concentration and dry matter and the fall in Ca concentration. In the stems there was a rise in Na, N and P concentrations and a fall in K concentration. The diagrams for the seeds show initial rises in N and dry matter, and an unusual pattern of Na in that an initial fall in concentration is followed by a rise. The K concentration remains level. The beginning of the climacteric rise in respiration was found to coincide with week 5.

Fig. 2 shows the changes in Na and K concentrations in seeds of fruit picked at weekly intervals from the same orchard (D 3) in 1965 and 1966. In these samples the seeds were separated into three categories according to colour. Concentrations of N in the mixed seeds of 1965 are given in Table I.

In 1965 there was an initial fall in Na concentration followed by a later rise; there was a continuous rise in N concentration and also a rise in K. The beginning of the climacteric rise in respiration was estimated to be about 21 September, which is at 35 days on the time scale shown.

In 1966 the apples were picked somewhat earlier. The climacteric rise in respiration was estimated to start at about 19 September, which would be 46 days on the time scale shown and is beyond the limits of the information given on the diagram. The Na pattern was somewhat confused, particularly in the separated seeds, but the concentration again drops to a minimum value at about day 20. The rise in K concentration is repeated.

Table II contains the data for average number and size of seeds from D 3, 1966, classified according to colour.

Table III shows the analytical data for seeds and stems from fruit from another orchard (D) picked very early (June and July) in 1965. The growth results show that the seeds and stems reached full size when the fruit was only about one quarter grown. At this stage the K and Na concentrations in the seeds were similar to those in the seeds of mature fruit. Ca, N, Mg and P concentrations were low in the seeds, but N was rising continuously. In the stems there were large initial falls in N and Mg whilst Ca concentration rose later.

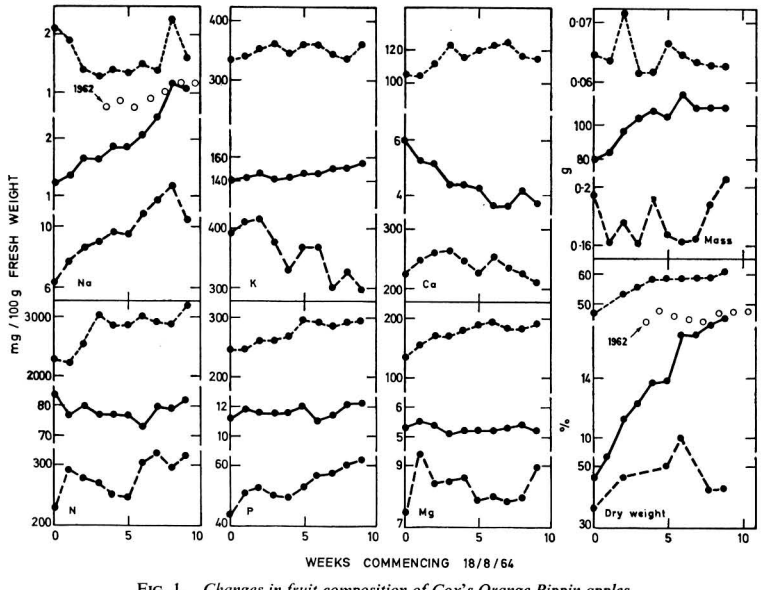

FIG. 1. *Changes in fruit composition of Cox's Orange Pippin apples near picking time*, 1964

Mineral concentrations as mg per 100 g fresh weight. Masses in g are the means per fruit without seeds and stem, per seed and per stem
Dry weight as % fresh weight

Seeds
Flesh
Stems

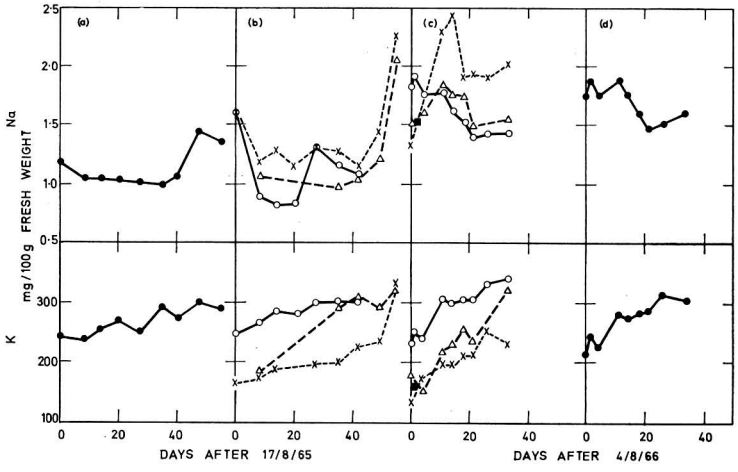

FIG. 2. *Effect of time of picking on Na and K in apple seeds*

(a) Mixed seeds
(b) Separated seeds
(c) Separated seeds
(d) Mixed seeds (calculated)
○ White, △ Brown/white, × Brown, ■ Mixed brown & brown/white

TABLE I

Orchard D 3, 1965. Concentrations of N in seeds (mg/100 g fresh weight)

Pick	1	2	3	4	5	6	7	8	9
N	1630	1885	—	2120	2130	2350	2380	2500	2550

First picking on 17 August. Sample for picking 3 lost in digestion
Figures for pickings 6, 7 & 8 are means of two digestions

Discussion

Flesh

A rise in Na concentration and a fall in Ca concentration during development (Fig. 1) have been reported previously.[1] In the nine weeks during which the mass of the flesh increased from 77 to 114 g the Na concentration rose from 1·3 to 2·8 and the Ca concentration fell from 6·0 to 3·9 mg per 100 g. The total amount of Na in the flesh therefore rose from 0·96 to 3·25 mg, and the total amount of Ca showed little change

TABLE II

Mean number of seeds per apple, and mean mass per seed (g) in apples from Orchard D 3, 1966

Date Picked	White		White/Brown		Brown		Mean	
	No.	Mass	No.	Mass	No.	Mass	No.	Mass
4.8*	3·38	0·075	0·50	0·061	0·42	0·060	4·30	0·072
5.8	3·56	0·075	0·31	0·060	0·15	0·047	4·02	0·074
8.8	3·02	0·078	0·57	0·056	0·50	0·050	4·09	0·072
15.8	3·04	0·070	0·50	0·054	1·06	0·045	4·60	0·063
18.8	2·31	0·083	0·35	0·059	1·04	0·045	3·69	0·070
22.8	2·64	0·084	0·28	0·058	1·13	0·047	4·05	0·072
25.8*	2·35	0·086	0·35	0·066	0·74	0·048	3·44	0·076
30.8	2·52	0·084	0·17	0·046	1·28	0·045	3·97	0·070
6.9	1·76	0·088	1·11	0·082	1·59	0·046	4·46	0·071

46 apples per sample except for dates marked * when 23 apples were picked

TABLE III

Orchard D, 1965. Composition of seeds and stems during development (mg/100 g fresh weight). 25 apples per sample

Date Picked	Mean mass per apple (g)			Seeds						Stems					
	Flesh	Seeds	Stems	K	Na	Ca	Mg	P	N	K	Na	Ca	Mg	P	N
9.6	2·2	0·002	0·060	333	1·6	58	41	100	—	385	4·0	371	27	61	584
23.6	8·3	0·171	0·080	566	1·6	36	58	144	492	417	2·8	352	9	55	321
7.7	16·3	0·287	0·113	246	0·8	17	32	78	660	388	3·7	379	10	62	349
21.7	27·2	0·346	0·137	210	1·1	43	56	99	840	357	4·1	457	12	66	269
18.8	56·1	0·326	0·132	250	1·1	98	81	224	1830	376	6·2	460	13	82	310
9.9	76·0	0·323	—	244	1·1	—	—	—	—	—	—	—	—	—	—

(4·6 to 4·3 mg). The rise in total Na was therefore greater than that observed in 1962 in fruit from the same trees. In that year the total amount of Na in the flesh rose from about 2·5 to 3·5 mg in the last six weeks of the experiment. In 1964 the rise over the same period was from 1·9 to 3·2 mg.

Another difference between the two seasons was that in 1962 the dry matter was at its maximum (18 to 19%) two or three weeks before the commencement of the climacteric rise. In 1964 the dry matter rose from 14 to 18% after the start of the climacteric. This suggests that the development of the fruit was quite different in the two seasons.

Stems

In the stems the Na concentration rose later in the season (Fig. 1 and Table III) and this could perhaps be expected because the concentration in the flesh was rising. However, this argument is not necessarily valid because the K concentration fell in the stems at the time when it was rising in the flesh. Admittedly this rise was small compared with that of Na. Both N and P concentrations rose in the stems in the later stages although there was no obvious change in the concentrations of these two elements in the flesh.

Seeds

It can be seen from Table II that the mean mass of the seeds (last column) was virtually constant with time. However, the number of brown seeds was increasing with a consequent reduction in the number of white seeds. Moreover the brown seeds were always smaller than the brown/whites which were in turn smaller than the whites. The mean mass remained constant because the white seeds of the later picks were larger although fewer. Similar figures were obtained for seed samples in 1965.

These changes in size (presumably due to loss of water as the seeds age and change colour) would result in higher concentrations of minerals in brown seeds if the total amount per seed remained the same. On the whole, Na concentrations were higher in the brown seeds than in the white ones (Fig. 2) but K concentrations in the brown seeds were lower than in the white despite loss of water. Thus K seems to continue to move into the white seeds after the brown have ceased to accumulate it.

The Na pattern in 1965 (Fig. 2) for both mixed and separated seeds followed the trend of the mixed seeds of 1964 (Fig. 1). The concentration of Na in the mixed seeds fell to a minimum value just before the climacteric rise in respiration and then rose to a high level in the later weeks. The fall was found again in 1966. Since the mean mass of the mixed seeds was constant with time in all of these years the changes in concentration reflect the change in the amount of Na per seed and it can be seen that Na was actually leaving the seeds during the first few weeks of each experiment. At this stage no explanation can be offered for this movement out of, and later, into the seeds. The amount of Na involved is negligible compared with the total amount of Na in the fruit.

The explanation of the changes in the concentration patterns of the separated seeds is complicated by the ripening and probable loss of water by the brown seeds, and by the conversion of white to brown/white and then brown seeds whilst nutrients are also moving into and out of the seeds. The concentration of Na in white seeds is usually lower than in brown seeds although this was not so in the samples picked earlier in 1966 (Fig. 2). The rapid increase in concentration of Na in the brown and brown/white seeds after 40 days in 1965 was probably due to drying, colour change and sodium movement into the seeds. It is apparent the Na is able to move out of the white seeds more readily than out of the brown ones in the same way that K is able to move more easily into the white seeds. The amounts of K and Na *per seed* are less in the brown seeds than in the white seeds and it seems possible that seeds containing lower amounts of these elements might turn brown first.

The day on which the Na concentration in the mixed seeds reaches a minimum occurs at no fixed time before the beginning of the climacteric rise; and the cessation of increase in concentration of N (Fig. 1) which occurred just before the climacteric rise in 1964 (week 5) did not occur in 1965 when N concentration continued to rise throughout the experiment (Table I).

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MINERAL COMPOSITION OF APPLES

VII*.—The relationship between fruit composition and some storage disorders

By M. A. PERRING

There is evidence that the incidence of each of three storage disorders of Cox's Orange Pippin apples (senescent breakdown, low-temperature breakdown and bitter pit) is affected by the mineral composition of the fruit. These effects have been studied by comparing initial average compositions of bulk samples with the storage behaviour of bulk replicates and by experiments with individual apples in which apples were analysed after their storage record was known.

It is suggested that if the Ca level in an apple is less than 3 mg/100 g fresh weight it will be liable to senescent breakdown at an early stage of storage. A P concentration of less than 8 mg/100 g may have the same result even if the Ca level is high.

Low-temperature breakdown is less likely in apples with high levels of K, P and Mg than in apples with low levels of these elements.

The results confirm that low average Ca concentration is associated with bitter pit. It is suggested that if the average Ca concentration exceeds 5 mg/100 g fresh weight the sample will probably be free from this disorder. The relationship between Ca and bitter pit in apples stored individually was not clearly defined.

Introduction

Cox's Orange Pippin apples from two commercial orchards were stored in air at 0° and 2·8° in 1959 and samples were removed at frequent intervals for analyses of total and inorganic P. The fruit from one orchard was known to be susceptible to low-temperature breakdown at 0° whereas the fruit from the other was more resistant. However, after two months' storage at 2·8° a few individual apples in samples from the second orchard had not only bitter pit, but also physiological breakdown which was identified as senescent breakdown. The first apples to break down were analysed separately and were 15 to 17% lower in concentrations of total P than the remainder of the sample.

Analyses made in the following years of sound apples and apples with breakdown indicated that low Ca concentrations might also be associated with early senescent breakdown. The results of these experiments, together with additional evidence from other experiments, are given below.

Experimental and Results

All the experiments were with Cox's Orange Pippin apples from commercial and experimental orchards. Samples from store were cut transversely and examined for internal disorders as described previously,[1,2] Seeds and stems were removed before samples were frozen and grated for subsampling and analysis.[3–5] In some experiments apples were weighed before and after storage and it was thus possible to calculate the initial concentrations of minerals.

Commercially grown apples, 1959/60

Apples from each of the two orchards (R) and (H) were randomised and divided into samples of 25 which were stored in air at 0° and 2·8°. Samples were periodically taken for examination and analysis. The chemical composition of these apples at the time when they were put into store is shown in Table I. Apples from orchard (H) were larger than those of orchard (R) and with the exceptions of Ca (lower), N (lower) and alcohol-soluble matter (about the same), had higher concentrations of the constituents measured.

Some of the storage results of this experiment indicated in Table II illustrate the differences in susceptibility to physiological disorders in fruit from the two orchards, (R) being less resistant to low-temperature breakdown and (H) being less resistant to bitter pit and senescent breakdown.

Individual apples, 1961–62

When apples from a manurial trial were examined in 1962 after storage in air at 2·8° a few individuals with senescent breakdown were removed from samples which had received the same orchard treatment. These apples were analysed individually and the results are shown in Table III together with analytical data for an initial bulk sample from the four trees receiving the same treatment, i.e. P, K and Sward[2]. The apples were not weighed initially and the results for the individual apples are uncorrected for weight losses in store; results will therefore be somewhat high (possibly 5 to 10%) in comparison with the initial figures. Even so, it can be seen that none of the P concentrations in the damaged apples were particularly low, but that Ca concentrations of all these individuals were lower than the initial mean figure.

Commercially grown apples 1960–61

Four samples of commercially grown fruit which were severely affected by senescent breakdown were selected when the East Malling Research Station survey samples were examined in January 1961. These samples had been stored in air at 2·2°. Twenty sound apples and twenty apples with senescent breakdown were taken from each sample and

* Part VI. Preceding paper.

TABLE I

The chemical compositions of bulk samples of Cox's Orange Pippin apples from two orchards when stored at 24 September, 1959. Mass as g, mineral elements as mg/100 g fresh weight and other results as % fresh weight

Orchard	Mass of 25 apples	K	P	Mg	Ca	N	Na	Inorganic P	Dry matter	Alcohol-insoluble matter	Alcohol-soluble matter
R	2788	116	9·9	5·4	5·7	57	2·1	5·0	18·4	4·4	14·0
H	3587	157	14·2	6·4	4·3	43	3·0	7·7	21·0	7·2	13·8

TABLE II

Storage characteristics of fruit from orchards (R) and (H), 1959–60. The Breakdown Index indicates severity in addition to incidence.[2]
(No breakdown = 0; maximum breakdown = 300)

Time	0°						2·8°					
	R			H			R			H		
	% Bitter pit	Breakdown		% Bitter pit	Breakdown		% Bitter pit	Breakdown		% Bitter pit	Breakdown	
		%	Index		%	Index		%	Index		%	Index
Mid-October	0	0	0	0	0	0	0	0	0	0	0	0
Mid-November	0	0	0	11	0	0	0	0	0	8	8	8
Mid-December	0	4	4	8	0	0	0	0	0	7	4	4
Mid-January	0	64	84	5	0	0	0	0	0	8	8	8
Mid-February	0	92	230	7	20	20	0	0	0	9	16	16

TABLE III

The mineral composition of a bulk sample and individual apples of treatment PKS, 1960/61. Mass as g, mineral elements as mg/100 g fresh weight initially and on removal from store respectively

Sample	Mass of one apple	K	P	Ca
Mean for treatment PKS (100 apples)	105	154	14·5	4·6
Tree C1	110	156	14·0	3·7
Tree E5	61	181	14·0	3·4
Tree F3	101	198	18·3	2·3
Tree F3	133	186	16·7	3·3

frozen and grated in bulk for analysis. Results of these analyses are shown in Table IV. As in the previous experiment the fruit was not weighed before and after storage so all the analytical results are somewhat high. Subsequent experience has shown that there is no reason to suppose that water losses from the damaged apples were very much in excess of those from the sound apples and so it is reasonable to compare directly the results for damaged and sound fruit.

The most noteworthy feature of Table IV is that if corrections for weight losses in store had been made, 7 out of the 8 P results would have been below the lowest concentrations which had previously been recorded for Cox's Orange Pippin apples.[4] The P level in sample A was exceptionally low, the damaged fruit having 10% less than the sound fruit. In this sample also, the K concentration was about 20% lower in the damaged apples but Ca concentrations in these apples were 20% higher than in the sound fruit. The mean level of Ca in this sample was high. In the other three samples the damaged fruit had *higher* P concentrations than the sound fruit in the same sample. In these samples there was about 20% less Ca in the damaged apples than in the sound apples from the same sample. Bitter pit was recorded in both sound and damaged apples of sample D.

Apples stored individually, 1961–62

Fruit from two treatments—cultivation (O) and grass (S)—of a manurial trial[2] were stored in air at 2·8° in 1961. Two samples of 25 apples were taken from each of 4 trees in each treatment and every apple was weighed separately before and after storage. The apples were removed from store, cut and examined for injuries on two occasions, but even at the second examination (177 days in store) the majority of the apples were sound. A few apples had bitter pit, two were cracked and one apple had senescent breakdown. These apples were analysed and after correction for losses the results were compared with those of bulk samples taken initially from each tree. The results may be summarised briefly as follows: with the exception of one very small apple (51 g initial mass), which had a high Ca concentration (8·8 mg/100 g) and was from a cultivated plot, all of the apples with bitter pit were from 3 trees of the grass treatment. The bulk samples from these trees were lower in Ca (4·7–5·0 mg/100 g) than were those from the other tree and those from trees of the cultivated plots (5·3–5·8 mg/100 g). Twelve of the pitted apples had lower Ca concentrations (2·2–4·3 mg/100 g) than the bulk samples whilst the concentrations in the other three were similar to those of the bulk samples from the grassed plots (4·8–5·1 mg/100 g). The apple with breakdown was low in Ca (2·5 mg/100 g) and the two cracked apples were high in Ca (6·1 and 6·6 mg/100 g).

TABLE IV

The chemical composition of sound apples and apples with severe senescent breakdown, 1960–61. Mass as g, mineral elements as mg/100 g fresh weight on removal from store

Sample	Condition	Mass of 20 apples	K	P	Mg	Ca
A	Sound	2100	118	8·3	7·7	5·0
	Senescent	1900	97	7·5	6·7	6·0
B	Sound	2500	124	9·2	6·7	4·3
	Senescent	2500	138	12·7	9·2	3·3
C	Sound	2000	143	8·6	—	4·7
	Senescent	1900	128	9·6	6·9	3·9
D	Sound	2200	163	8·9	5·6	4·0
	Senescent	2100	154	9·7	5·7	3·1

Apples stored individually, 1962–63

The total crop from each of three trees of an orchard (P 10) at the East Malling Research Station[1] known to produce apples with a low average Ca concentration[6] was picked in 1962 and every apple was weighed separately before being put into random groups of 25 and stored in air at 2·8°. A few apples, when picked, were found to be mis-shapen and severely pitted and all had watery patches internally as shown in Fig. 1. These were immediately frozen for analysis. Samples of 25 apples from each tree were removed from store on four occasions (there was insufficient fruit from tree 14.8 for more than 3 examinations) and reweighed before examination for disorders. Individuals or bulk samples of fruit with the same disorder were frozen for analysis. Two apples with severe breakdown at an early date were removed earlier than the rest of the group to avoid losses by rotting.

Analyses were made for K, P, Mg and Ca and the results of these, together with the calculated mean results for each tree, are given in Tables V, VI and VII. Although the mean fruit size and K, P and Mg concentrations were about the same in fruit from all three trees the mean Ca concentration in apples from each tree was different, and if these results are compared with the storage results for each tree given in Table VIII it can be seen that most senescent breakdown occurred in samples with the lowest mean Ca and that practically no breakdown occurred in the samples with the highest mean Ca. For the purposes of Table VIII apples removed from store early are included with the results for other apples in their groups. Mean Ca concentrations appear to be unrelated to the amount of bitter pit per sample.

Tables VI and VII show that Ca concentrations in the deformed apples were lower than those in the means for the trees, but that concentrations of K and Mg were very much higher than the means, and P concentrations were also high.

Analyses of apples with breakdown from trees 14.3 and 14.16 were made, and Tables V and VII show that all of these apples were lower in Ca concentration (2·3–3·1 mg/100 g) than the means. The suggestion of lower P concentrations in these apples from tree 14.3 is not apparent in similar apples from tree 14.16. It must be noted that one apple (tree 14.16, 162 days) with low concentrations of all elements remained clear from disorders.

The relationship between Ca concentration and bitter pit is less clearly defined. Most of the apples from tree 14.3 (Table V) with bitter pit had lower Ca concentrations than the corresponding clear apples and the Ca results for these sound apples were higher than the mean, the highest results occurring at the later examinations. This trend is shown in the earlier examinations for trees 14.8 and 14.16, but at 162 and 189 days apples from both of these trees had similar concentrations of Ca whether they were clear or pitted.

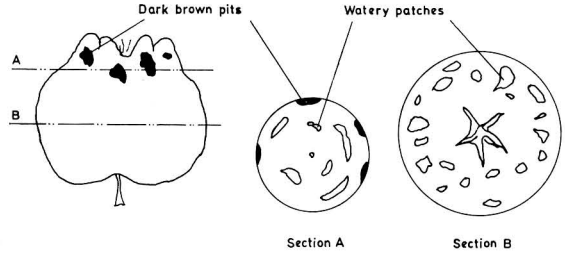

FIG. 1. *Pitting and watery patches in mis-shapen apples from orchard P 10*

TABLE V

The mineral composition of apples from the tree 14.3 of orchard P 10, 1962–63

Days at 2·8°	Condition when analysed	Number analysed	Initial mass per apple (g)	Minerals (mg/100 g initial fresh weight)			
				K	P	Mg	Ca
95	Sound	16	114	146	14·4	5·9	3·7
95	Bitter pit	1	162	140	11·9	5·2	3·2
		1	158	141	16·3	6·8	3·9
		1	138	161	13·6	6·2	3·1
		1	132	143	13·8	5·8	3·4
		1	121	147	15·8	5·8	3·7
		1	104	136	13·5	5·0	3·4
		1	103	154	12·4	4·8	3·1
		1	98	154	14·0	5·3	3·3
		1	88	140	13·9	4·9	3·1
95	Breakdown (from 127 day sample)	1	100	145	12·5	4·7	2·7
127	Bitter pit	1	134	155	14·5	5·3	3·0
		1	117	143	13·1	5·9	3·8
		1	112	154	13·4	5·8	3·2
		1	96	157	14·3	6·0	3·1
		1	86	143	13·8	5·1	3·4
		1	78	140	15·0	5·7	4·2
127	Breakdown	1	170	160	13·8	5·7	2·6
		1	140	150	13·9	5·6	2·8
		1	135	154	13·6	6·0	2·3
		1	93	164	14·5	5·5	3·1
162	Sound	10	115	146	14·8	5·9	3·9
	Bitter pit	6	118	167	14·8	5·8	3·2
	Breakdown	8	134	141	13·4	5·5	2·9
189	Sound	1	130	138	17·0	6·1	4·2
		1	128	161	13·8	6·8	5·2
		1	111	146	13·2	5·6	4·0
		1	106	168	16·3	6·9	3·9
	Means for 95 day sample	(25)	117	146	14·2	5·8	3·6
	Means for 162 day sample	(24)	122	149	14·3	5·7	3·4

TABLE VI

The mineral composition of apples from tree 14.8 of orchard P 10, 1962–63

Days at 2·8°	Condition when analysed	Number analysed	Initial mass per apple (g)	Minerals (mg/100 g initial fresh weight)			
				K	P	Mg	Ca
0	Deformed, pitted, watery patches	3	128	192	14·5	7·4	2·7
95	Sound	21	103	133	13·9	5·9	4·6
	Bitter pit	4	112	166	13·3	5·9	3·3
127	Bitter pit	10	139	147	12·5	5·9	3·2
162	Sound	16	117	134	13·5	5·9	3·7
	Bitter pit	9	121	141	13·0	5·8	3·7
189	Sound	7	85	137	14·3	5·7	4·1
	Means for 95 day sample	(25)	104	139	13·9	5·9	4·4
	Means for 162 day sample	(25)	118	137	13·3	5·9	3·7

TABLE VII

The mineral composition of apples from tree 14.16 of orchard P 10, 1962–63

Days at 2·8°	Condition when analysed	Number analysed	Initial mass per apple (g)	Minerals (mg/100 g initial fresh weight)			
				K	P	Mg	Ca
0	Deformed, pitted watery patches	5	149	191	16·5	7·2	3·2
74	Breakdown (from 162 day sample)	1	161	171	16·3	6·4	2·6
127	Bitter pit	13	134	140	13·6	5·6	3·1
127	Breakdown	1	196	135	14·2	5·6	2·8
127	+ bitter pit	1	184	154	15·4	6·3	2·6
127	+ bitter pit	1	134	134	11·7	4·9	2·8
162	Sound	1	120	119	12·7	4·8	2·8
162	Sound	1	105	115	13·7	5·2	3·7
162	Sound	1	101	151	16·1	6·5	3·8
162	Sound	1	94	167	17·7	6·9	5·4
162	Bitter pit (including some breakdown)	19	120	145	14·6	5·9	3·7
189	Sound	1	166	121	12·4	5·0	3·6
189	Sound	1	120	98	12·2	4·6	3·1
189	Sound	1	111	135	13·2	5·4	3·6
189	Sound	1	105	133	13·2	6·0	3·6
189	Sound	1	94	144	15·4	6·1	4·2
189	Sound	1	90	115	12·2	5·4	3·8
189	Sound	1	68	161	15·3	6·2	4·8
189	Sound	1	50	118	16·0	5·1	3·9
	Means for 162 day sample	(25)	119	145	14·7	5·9	3·7

TABLE VIII

Senescent breakdown indices (maximum = 300: none = 0) and % bitter pit in bulk samples of apples from 3 trees of orchard P 10, 1962–63

Days at 2·8°	Tree 14.3 (Ca = 3·5)		Tree 14.8 (Ca = 4·1)		Tree 14.16 (Ca = 3·7)	
	Breakdown index	% Bitter pit	Breakdown index	% Bitter pit	Breakdown index	% Bitter pit
95	0	36	0	16	—	—
127	48	20	0	24	12	56
164	64	40	4	36	28	80
189	112	20	0	54	52	48

Trials with calcium sprays, 1962–63

In 1962, experiments to determine the value of Ca sprays in reducing bitter pit were made jointly by the N.A.A.S., the East Malling Research Station and the Ditton Laboratory. In one of these, the Ca concentration in the fruit was increased considerably and the amount of bitter pit which developed during storage was consequently reduced.[7] Fruit from untreated plots developed senescent breakdown in store (2·8°) and some of these individuals, in addition to individuals with bitter pit, were analysed for K, P, Mg and Ca after the fruit had been examined in December. Once again the apples with breakdown were low in Ca (2·1–2·6 mg/100 g) and the apples with bitter pit were lower in Ca (2·7–3·3 mg/100 g) than the remainder of the apples (3·7). The apples which had been sprayed were more susceptible to core flush.

Discussion

Two forms of physiological breakdown are being considered here. It is not always possible to distinguish between them when apples have been held at the temperatures of these experiments. The low-temperature breakdown of the R and H experiment was of the classical type starting with either coreflush, or with browning around the vascular bundles. This browning then spread towards the outer parts of the fruit. Senescent breakdown in these experiments was nearly always first seen near the surface of the fruit and towards the calyx end. The browning then spread inwards towards the centre of the fruit. The difference in the development of the two disorders can be seen in Table II.

The pattern of bitter pit development in the (H) samples at 2·8° was similar to that of senescent breakdown. The level

rose suddenly to about 30% in November and remained fairly constant for several months. This level was reached at the same time by (H) samples stored at 0°. It was concluded that susceptibility to bitter pit and senescent breakdown might be determined by the composition of the individual apple.

It has been shown that concentrations of K, P and Mg are inversely related to amount and severity of low-temperature breakdown in bulk samples of apples,[2] and Table I shows that apples from orchard (R) (badly affected by low-temperature breakdown) were particularly low in these elements. There is, however, little evidence that concentrations of these elements in an individual apple determine the resistance of that particular apple, within a sample from the same tree, to this disorder (Wilkinson, B. G., & Perring, M. A., unpublished results). In contrast to this the Ca concentration in an individual apple appears to have a marked effect on its resistance to the onset of senescent breakdown. The results of analyses of individual apples (Tables III, IV, V, VII, individuals from the spraying trial, and the one apple in four hundred which had breakdown in the abortive experiment of 1961) indicate that a lower concentration of Ca in an apple, when picked, than 3 mg/100 g fresh weight is likely to lead to breakdown in that apple at an early date in storage. It also seems that an exceptionally low concentration of P (of the order of 7 mg/100 g) can have the same effect even when the Ca concentration is fairly high (Table IV, sample A).

Since the mean concentration of an element in a bulk sample of apples is dependent upon its concentration in the individual apples, it follows that a low level of Ca or P in a bulk sample will predispose that sample to senescent breakdown early in its storage life. There is sufficient evidence to calculate this level for Ca assuming a two-fold variation in concentration within a sample.[8] The mean concentrations of Ca in samples with apples containing as little as 2·0, 2·5 and 3·0 mg/100 g would be 3·0, 3·8 and 4·5 mg/100 g respectively. A small amount of Ca can therefore make a considerable difference to the amount of senescent breakdown in a sample. Samples with more Ca than 4·5 mg/100 g should remain free of the disorder until late in the storage season when the fruit would probably be unsaleable for other reasons. Table I shows that apples of orchard (H) had less Ca than this, whereas those of orchard (R) had a much higher level. It is unfortunate that the individual (H) apples with senescent breakdown were not analysed for Ca. Decrease in the amount of senescent breakdown in bulk samples with increasing amounts of Ca is shown in Table VIII. The mean fruit size and concentration of other mineral constituents in these samples differed only slightly (Tables V, VI and VII). A reduction of senescent breakdown from 16% to none in fruit samples examined in March was achieved in the spraying trial by raising the mean Ca level from 3·7 to 5·4 mg/100 g. Once again the differences in the concentrations of other constituents measured in these samples (K, P, Mg, N, Na, dry matter, alcohol-soluble and -insoluble matter and starch) were negligible. The reduction of breakdown by spraying with Ca has been recorded by other workers.[9–12]

The mean level of P for sample A in Table IV, on the basis of fresh weight at time of picking, is probably just above 7 mg/100 g. This appears to have resulted in a very high incidence of senescent breakdown at an early date. In the previous year a similar level of K in sample R and a somewhat higher level of P (9·9) had no such effect. It would seem that a level of P below or above about 8 or 9 would determine whether a bulk sample of apples would break down early in storage or remain sound for a longer period.

Results of an experimental trial with fertilisers indicated that orchard treatments with high levels of K reduced not only the amount of low-temperature breakdown in the fruit but also that of senescent breakdown.[13] The effect of applying K was to raise its level in the fruit by about 25%. The level of Ca was not affected by K treatment but there was about 10% more P in apples from the treated plots. P concentrations in apples from this orchard were not, however, in the 'dangerously low' level quoted above.

It is concluded that if the levels of K, P and Mg are raised in bulk samples of fruit the amount of low-temperature breakdown will be reduced, but that an adequate level of Ca is necessary if senescent breakdown is to be reduced. It is not claimed that these mineral elements are the only factors involved. Indeed, if this were so the apple from tree 14.16 which had lower levels of all these elements than the mean for apples from the tree (Table VII, 162 days), should have surely broken down at a very early date instead of remaining sound.

It is generally agreed in recent reviews of work in many countries[14–17] that low levels of Ca result in bitter pit in apples and that this is further increased by raising the levels of K and Mg. On the whole, results from these experiments are in agreement with this. The (H) samples had low Ca and very high K and Mg concentrations; sample D of 1960 had a higher K level than the other samples (Table IV); the (S) samples of 1961 had lower Ca and higher K and Mg concentrations than the (O) samples; and spraying with Ca in 1962 reduced the amount of bitter pit from 21% to 1% at the March examination. In the three trees of the 1962 trial however, the fruit with the intermediate level of mean Ca had more bitter pit than that with higher and lower levels (Tables VIII, V, VI, VII) and, as stated before, size and concentrations of other elements in the apples from the three trees differed very little.

When individual apples are considered there seems to be no clearly defined relationship between Ca concentration and bitter pit as there is with senescent breakdown. Several apples of tree 14.3 (Table V) had higher levels of Ca than the mean yet they were pitted. The pitted apple from an (O) treatment in 1961 had a very high Ca concentration. Moreover, differences between Ca concentrations in pitted and sound apples were very small in most instances. It must be noted that the degree of bitter pit was not assessed. An apple with one pit was recorded as equal with another with many. The large variations of mineral composition within the individual fruit[18,19] might easily have resulted in a localised mineral imbalance with no measurable change in the composition of the whole apple. These imbalances have been recorded in pitted tissue.[20]

When K and Mg concentrations were extremely high in relation to that of Ca, pitting occurred on the tree (deformed apples, Tables VI and VII) and it is possible that the watery patches were due to this mineral imbalance.

The (R) results (Table I), the results of 1961, and the spray trial results all indicate that if the concentration of Ca in a bulk sample is above 5 mg/100 g it is probable that the sample will be unaffected by bitter pit.

Some reservations must be made about the figures quoted. The results refer to the variety Cox's Orange Pippin only. The analyses were made on the whole apple with the seeds and stem removed. Ca results depend to some extent upon the method of analysis. The determinations quoted here

were made with an E.E.L. flame photometer[5] and recent work has shown that had they been made with a Unicam SP.900 flame spectrophotometer they would have been about 5% lower.

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The work described in this paper formed part of a programme of research on the relation between the chemical composition and storage disorders of apples directed by Dr. B. G. Wilkinson. Most of the fruit was supplied by the East Malling Research Station. Mr. R. D. Jones assisted with the analyses.

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METHOD FOR QUANTITATIVE DETERMINATION OF *p*-HYDROXYBENZYL ISOTHIOCYANATE IN DIGESTS OF SEED MEAL OF *SINAPIS ALBA* L.

By E. JOSEFSSON

A method has been developed for the quantitative determination of *p*-hydroxybenzyl isothiocyanate in enzymic digests of seed meal of white mustard (*Sinapis alba* L.). The isothiocyanate was treated with sodium hydroxide and the released thiocyanate was determined by use of ferric nitrate reagent. A sample in which the ferric thiocyanate complex was destroyed by addition of mercuric chloride was used as a control for the colorimetric determinations. The yields of isothiocyanate obtained after hydrolysis of *p*-hydroxybenzylglucosinolate at different pH have been studied. A relative standard deviation of 0·9% was found when the method was applied to micro- quantitities. The simplicity and rapidity of the method and its applicability to small samples make it well suited for use in connexion with plant breeding.

Introduction

Seed meal of white mustard (*Sinapis alba* L.) contains *p*-hydroxybenzylglucosinolate, which upon enzymatic hydrolysis yields glucose, hydrogen sulphate and *p*-hydroxybenzyl isothiocyanate.[1,2] The toxic properties of the latter substance limit the use of white mustard seed meal as animal feed. In efforts to select plant material with a lower *p*-hydroxybenzylglucosinolate content, a method for quantitative determination of the isothiocyanate was sought. In order to be applicable to plant breeding, the method should be suitable for rapid serial analysis of small quantities of meal.

For estimating *p*-hydroxybenzyl isothiocyanate Mühlenfeld[3] suggested adding ammonia and silver nitrate. The silver nitrate was thought to react with the sulphur of the thiourea derivative of the isothiocyanate and the surplus silver could be titrated with ammonium thiocyanate. The same principle was used by Beer *et al.*[4] It has been shown, however, that when *p*-hydroxybenzyl isothiocyanate is treated with alkali it is rapidly and quantitatively split to *p*-hydroxybenzyl-alcohol and thiocyanate ion.[5–7]

Scharrer *et al.*[8] and Ettlinger & Thompson[9] determined the thiocyanate ion released from *p*-hydroxybenzyl isothiocyanate by treatment with alkali. The methods described, however, do not seem to be well adapted for analysing a large number of samples of plant breeding material.

Bauer & Holle[10] and Terry & Corran[11] made estimations of *p*-hydroxybenzylglucosinolate content by determination of released sulphate. Barothy[7] extracted the glucosinolate with methanol, evaporated the solvent, incubated with myrosinase and titrated the hydrogen ion released. In this laboratory a rapid serial method based on determination of sulphate was tested but it lacked precision when applied to quantities of meal lower than 30 mg.

Since colorimetric determinations are very suitable for serial analysis, and estimation of thiocyanate ions with ferric nitrate reagent is a very sensitive method, this principle was used in the present study.

Bowler[12] investigated the optimum conditions for determination of thiocyanate in blood serum with ferric nitrate reagent. He found that a large excess of reagent should be used. His method was adapted by the Central Veterinary Laboratory, Weybridge,[13] for use in analysis of fresh kale. In such material as well as in seed meal of white mustard, the ferric nitrate reagent reacts with phenolic compounds in the sample, giving rise to a yellowish coloration interfering with the colour of the ferric thiocyanate complex. This problem was overcome by preparing duplicate aliquots of each sample and adding to one set a little mercuric chloride which destroys the ferric thiocyanate and provides a control.[13] In the present studies it was found that when the absorption spectrum in the interval 380–550 nm was measured against the control it was the same as that of a sample of pure thiocyanate.

Experimental and Results

Materials

Seed material was obtained from the Oil Crops Division at the Swedish Seed Association, Svalöf.

Reagents were of analytical reagent grade.

Myrosinase was prepared using the method outlined by Wrede.[14,15]

p-Hydroxybenzylglucosinolate was obtained as the tetramethylammonium salt from Calbiochem, Los Angeles.

Conditions for optimum release of *p*-hydroxybenzyl isothiocyanate and thiocyanate ions

In studies of analysis of isothiocyanates of *Brassica* seed meals, it was found that the yield of isothiocyanates was highly dependent on the pH at which enzymatic hydrolysis of the glucosinolates was performed.[16] At pH 7 there were considerable losses, which could, however, be avoided by heating the seed and treating the meal with hot buffer.[15,17] Fig. 1 shows that when incubated at pH > 5 there are losses in yield of thiocyanate from white mustard. These losses could be avoided either by applying the treatments used for *Brassica* seed meals or by the use of hot buffer treatment alone (Table I). Since a pH of 4·5 gives an optimum yield without heat treatment, this pH was used in further studies.

After studying the incubation times needed for the complete enzymic release of isothiocyanate from *p*-hydroxybenzylglucosinolate and the release of thiocyanate ions after addition of different quantities of sodium hydroxide, the following analytical method was found to be suitable.

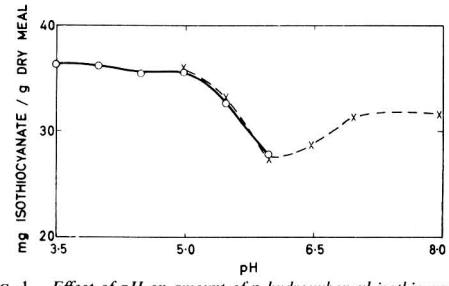

FIG. 1. *Effect of pH on amount of* p-*hydroxybenzyl-isothiocyanate obtained from digests of Seco white mustard meal*

(o) acetate buffer, (x) phosphate buffer

The samples were incubated with myrosinase at room temperature for 90 min and treated with sodium hydroxide for 15 min

TABLE I

Effect of heat treatments of a single batch of seed and seed meal of white mustard, cv. Seco, on the amount of *p*-hydroxybenzyl isothiocyanate obtained

The samples were incubated with myrosinase at room temperature for 90 min and treated with sodium hydroxide for 15 min

Exp. No.	Seed treatment	Meal treatment	Incubated in buffer	mg *p*-hydroxybenzyl isothiocyanate per g dry meal
1	None	None	Phosphate, pH 6·0, 0·05 N	$M_2 = 27·5$
2	Kept at 90°C for 15 min in a closed vessel	None	Phosphate, pH 6·0, 0·05 N	$M_2 = 30·5$
3	None	Treated with hot buffer, pH 6·0	Phosphate, pH 6·0, 0·05 N	$M_2 = 36·0$
4	Kept at 90°C for 15 min in a closed vessel	Treated with hot buffer, pH 6·0	Phosphate, pH 6·0, 0·05 N	$M_2 = 35·0$
5	None	None	Phosphate, pH 7·0, 0·05 N	$M_4 = 31·3$
6	Kept at 90°C for 15 min in a closed vessel	Treated with hot buffer, pH 7·0	Phosphate, pH 7·0, 0·05 N	$M_2 = 35·0$
7	None	None	Phosphate, pH 8·0, 0·1 N	$M_2 = 31·5$
8	Kept at 90°C for 15 min in a closed vessel	Treated with hot buffer, pH 8·0	Phosphate, pH 8·0, 0·1 N	$M_2 = 35·1$

Method

150 mg of defatted seed meal is placed in a 250 ml Erlenmeyer flask and 100 ml 0·1 M acetate buffer, pH 4·5, is added together with 1 ml 0·5% aqueous myrosinase solution. The flask is stoppered with a greased glass stopper and shaken for 15 min at room temperature. Then 12 ml 1 N sodium hydroxide are added and the sample is shaken for a further 5 min. The sample is filtered and 15 ml of the filtrate are treated with 15 ml 10% trichloroacetic acid. The precipitate is filtered off. From each filtrate are taken two 5 ml aliquots, and 5 ml 0·4 M ferric nitrate in 1 N nitric acid is added to each in artificial light. To one are added 2 drops of 5% mercuric chloride solution. This sample is used as a control and the colorimetric measurement is made at 460 nm in 1 cm cuvettes. In the present experiments the measurements were made in a Zeiss PMQ II spectrophotometer.

The method has been successfully adapted to analysis of microquantities. In this case about 15 mg of meal are incubated in centrifuge tubes with 10 ml acetate buffer and 0·1 ml myrosinase solution. During incubation, the tubes are shaken.

Precision and accuracy of the method

Studies of the precision of the method were made using the micro-modification. From Table II the standard deviation of ±0·3 is less than 1% of the average value.

TABLE II

Determination of *p*-hydroxybenzyl isothiocyanate in digests of a single seed meal of Seco white mustard according to different methods

Method	mg *p*-hydroxybenzyl isothiocyanate per g dry meal Means ± S.D.
Gravimetric determination of enzymatically released sulphate	$m_4 = 31·7 \pm 0·7$
Ettlinger & Thompson[9]	$m_8 = 35·8 \pm 0·7$
Present, micro- modification	$m_{10} = 36·8 \pm 0·3$

The accuracy of the method has been tested by comparing it with gravimetric determination of enzymically released sulphate, with the method of Ettlinger & Thompson,[9] and by observing the recovery upon addition of known amounts of potassium thiocyanate or *p*-hydroxybenzylglucosinolate. Table II shows that the present method gives a significantly ($P < 0·001$) higher isothiocyanate value than the gravimetric sulphate determination. The gravimetric method is, however, rather complicated and its standard deviation relatively large. The method of Ettlinger & Thompson[9] gave slightly lower values than the present one. The difference in values between the two methods (2·6%) was significant at the 1% level. As the method of Ettlinger & Thompson includes several extractions and washings, it seems probable that as a result of minor losses, low rather than high values would be obtained.

Addition of potassium thiocyanate to the meal gave a recovery of 95·7% (mean value of three determinations). Recovery tests were also made with *p*-hydroxybenzylglucosinolate added to the meal as the tetramethylammonium salt. The mean value of five determinations was 87·9% recovery if the glucosinolate added was regarded as 100% pure. It is, however, probable that its purity was lower. Thiocyanate analysis of the glucosinolate by itself gave a mean value (five determinations) of 89·9% of the theoretical. Calculated from 89·9% purity of the glucosinolate, the recovery with meal was 97·8%.

Discussion

The reason for the lower values for *p*-hydroxybenzylisothiocyanate when unheated meal is analysed at pH >5·0 has not been clearly established. The minimum of estimated isothiocyanate at pH 6·0 may indicate the existence of an isothiocyanate-destroying enzyme with a pH optimum at that pH and sensitive to a low pH. André & Carbouères[18] report that an enzyme which destroys isothiocyanates exists in seeds of black mustard, rape and turnip rape but not in white mustard seed. At pH >5 isothiocyanates react with proteins,[19,20] but this reaction would be supposed to proceed more quickly at pH 7·0 than at pH 6·0. It has been found, however, that at pH 7 considerable amounts of thiocyanate will be formed from *p*-hydroxybenzyl isothiocyanate.[6] Since thiocyanate ion is inert to protein this reaction will reduce the consumption of isothiocyanate by protein at higher pH.

As the analyses are carried out in test tubes and the operations are simple, the present method is very well adapted for rapid serial analysis. By still further reducing the volumes used in the analysis, it would be possible to analyse samples of 2–3 mg of white mustard meal.

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INSECTICIDES ON SINGLE SEEDS TREATED WITH LIQUID DRESSINGS

By K. A. JEFFS, K. A. LORD and R. J. TUPPEN

A method for determining insecticide on seeds treated with liquid formulations by extraction and gas chromatography of the extract was devised and used to estimate the amount of the insecticide on single seeds.

Eight solvents were tested for their ability to extract aldrin from wheat seeds treated with a liquid formulation. Acetone–hexane was chosen for further investigation because in 24 hours at room temperature it extracted at least 95% of the aldrin from treated seeds. This solvent almost quantitatively extracted chlorfenvinphos and carbophenothion at room temperature and γ-BHC at 45° in 24 hours, without extracting substances which interfere with gas chromatography of the insecticides.

The amount of insecticide found on single seeds varied widely, in seeds dressed both in the laboratory with commercial preparations (range 4- to 7-fold) and in 2 commercial plants (range 40- to 200-fold).

Introduction

Seedlings may be protected from soil insects such as wireworms and wheat bulb fly by treating the seeds with insecticides. Protection is sometimes unsatisfactory and determination of insecticide on seeds treated with dry powders showed that methods of application needed improving. Little is known about the efficiency of methods of applying liquid seed dressings. The simple and rapid method used for seeds treated with dry powders cannot be used for seeds treated with liquids, because short immersion in hexane does not quantitatively remove insecticide from these seeds. To check the efficiency of a method of treating seeds it is necessary to determine not only the average amount of insecticide on seeds but also the variability of the dose received by individual seeds, therefore the method should be applicable to single seeds. This excludes the accepted way of making exhaustive extracts by crushing or grinding followed by prolonged Soxhlet extraction, which is cumbersome and laborious. A better solvent than hexane and a better method were needed.

This paper describes the search for such a solvent and method. The method was used for preliminary investigations on the amount of insecticide on single seeds taken from batches treated by different methods and the results obtained are included.

Experimental

Gas chromatography

Insecticides extracted from seeds were assayed by gas chromatography using a 2 ft × ¼ in. column of 5% SE 30 silicone gum supported on Chromosorb W, treated with hexamethyl disilazine. The carrier gas was nitrogen. An electron capture detector was used to measure aldrin and γ-BHC by comparison of the heights of peaks from the unknown samples with those of standards. The column temperature was 180°. Carbophenothion and chlorfenvinphos were assayed with a Varian-Aerograph phosphorus detector by comparison of heights of peaks from unknown samples with those of standards. The column temperature was 200°.

With both types of detector care was taken to ensure that they were used with amounts of insecticide giving a response directly proportional to the amount of insecticide used.

Extraction procedures

Single seeds and batches of 10 seeds were extracted by immersion in 10 ml of solvent in glass-stoppered 10 ml graduated flasks. Larger batches of seeds (25–50) were extracted in 1 ml solvent per seed in 100 ml glass-stoppered conical flasks; aliquots were taken at intervals up to 48 hours. Unless other treatment is specified, the containers were shaken by hand during the extraction and immediately before aliquots were removed.

To test the efficiency of extraction of insecticide by a solvent from whole seeds they were rinsed with acetone and then crushed in a pestle and mortar and transferred to a Soxhlet extractor and extracted continuously for at least 8 hours with Analar acetone. The extraction temperature in the Soxhlet was 55–56°; the solvent was changed five times per hour. Negligible amounts of insecticide were detected if the broken seed was extracted in a Soxhlet for a second period of eight hours.

Materials

Four samples of seed were obtained from two commercial seed-treating plants. In one plant (A) Cappelle wheat seeds were treated by being sprayed with γ-BHC or aldrin formulated in dimethyl formamide. In the other plant (B) treatment was by trickling a solution of aldrin in alkyl benzenes on to Cappelle wheat seeds, and a solution of γ-BHC in alkyl benzenes on to Maris Widgeon wheat seeds. The time lapse between seed treatment and extraction was 1–6 months.

Two batches of Cappelle recleaned seed were treated in the laboratory with formulations of 60% carbophenothion in alkyl benzenes or 35% w/v chlorfenvinphos in dimethyl formamide. The seeds were treated by placing 50 g in a 9 cm diameter crystallising dish arranged with its axis at approx. 50° from the vertical and rotating this at about 30 rev/min about its axis. 10 μl of formulated insecticide was injected at 1 minute intervals into the moving seeds from an Agla microsyringe having a fine needle held at right angles to the line of flow of the seed. The process was repeated until the required amount of insecticide had been added, after which rotation was continued for a further 10 minutes. The volume of carbophenothion preparation used was 100 μl, and that of chlorfenvinphos was 200 μl. The time lapse between seed treatment and extraction was 2 days to 4 months.

Results

Choice of solvent

For the tests to select a solvent the sample of seeds dressed with aldrin in plant A was used. Preliminary tests showed that hexane extracted most of the aldrin from seeds in 30 minutes but little more in 24 hours. The remaining 10–20% could be extracted from crushed seeds in a Soxhlet using either acetone or dichloromethane.

Two criteria were used to select solvents to be tested for extracting insecticides from seeds. Firstly, that the solvents should not interfere with electron capture detection of chlorinated insecticides; secondly that the solvents (or mixtures) should be more hydrophylic or water-soluble than hexane and thus more likely to diffuse into the seeds. Because the amounts of aldrin on the seeds differed, the solvents were tested as follows: batches of 10 seeds were extracted for 30 minutes (swirled every 5 minutes) at room temperature with 10 ml hexane, which was then decanted and replaced with 10 ml of the solvent being tested. The concentrations of aldrin extracted by the solvent after 30 minutes and 24 hours were determined and expressed as percentages of the aldrin extracted by hexane. In this way it was possible to compare extractions done on batches of seeds carrying widely differing amounts of aldrin. All the solvents tested extracted more aldrin after 24 hours than after 30 minutes. The two most effective solvents were a mixture of 2 : 1 acetone–hexane, and isobutyl methyl ketone, both of which extracted about 20% more aldrin in 24 hours than hexane alone. Of the other solvents, ethanol, acetone, n-propanol, n-hexanol and cyclohexanol extracted about 10% more aldrin, and ethylcellosolve about 4% more aldrin (Table I).

The completeness of extraction by isobutyl methyl ketone and acetone–hexane mixtures (2 : 1 and 1 : 1) was checked by extracting 2·5 g of seed with 50 ml of solvent and finally crushing the seeds and extracting them with acetone in a Soxhlet for 8 hours. Both acetone–hexane mixtures and the isobutyl methyl ketone extracted most of the aldrin from the seeds, but left 2–3% which was extracted from the crushed seeds in a Soxhlet (Table II).

In order to extend the method to other insecticides and methods of application, the number of possible solvents investigated was limited arbitrarily. The 1 : 1 acetone–hexane mixture was chosen in preference to isobutyl methyl ketone because organophosphorus insecticides are less stable in this solvent than in hexane, but there is no reason to suppose that this mixture is more or less suitable than other untried solvents such as benzene or toluene, or solvent mixtures such as ethanol–hexane.

TABLE I

Comparison of solvents for the extraction of aldrin from samples of 10 seeds treated in Plant A

μg per seed extracted by hexane after 30 min	Solvent	Percentage increase in aldrin extracted by the second solvent after 30 minutes	24 hours
10·9	Ethanol	5·0	13·2
40·3	Acetone	5·5	9·1
21·3	Acetone–hexane 2 : 1	12·2	18·6
10·3	Isobutyl methyl ketone	7·3	20·6
47·0	n-propanol	3·4	6·1
26·3	n-hexanol	3·8	9·9
44·7	Ethyl cellosolve	—	4·1
21·0	Cyclohexanol	0·8	7·8

TABLE II

The completeness of extraction of aldrin from samples of 50 seeds treated in Plant A, using selected solvents

Solvent	μg/seed extracted by cold solvent in 24 h	μg per seed remaining	Residue in seed as a percentage of the total
2 : 1 acetone–hexane	30·0	0·7	2·3
,, ,, ,,	36·5	1·2	3·2
1 : 1 acetone–hexane	38·2	0·8	2·2
,, ,, ,,	41·6	0·7	1·8
Isobutyl methyl ketone	38·2	0·8	2·1
,, ,, ,,	46·0	1·5	3·4

Timed extraction of γ-BHC and aldrin from seeds

To test the rate of extraction, batches of 10 treated seeds were extracted with 1 : 1 acetone–hexane, and at intervals aliquots of the solvent were removed and assayed for insecticide. Much of both aldrin and γ-BHC was removed from the surface of the seed in the first 3 hours, but after this the amount of insecticide extracted increased only slowly. With both methods of application the amount of aldrin extracted increased significantly for 24 hours but extraction of γ-BHC seemed to continue for 48 hours (Fig. 1). Each point in Fig. 1 represents a mean of the amounts extracted from 3 batches of 10 seeds. Replicates of the same treatment differed considerably as a result of uneven distribution of insecticide on seeds. Both aldrin and γ-BHC applied by Plant A were more rapidly removed from seeds than when applied by Plant B.

Another test confirmed that immersion in 1 : 1 acetone–hexane for 24 hours did not extract all γ-BHC from seeds treated by Plant B, and extraction of the crushed seed with acetone in a Soxhlet removed a further 10–15%. The proportion of γ-BHC extracted from batches of 50 seeds by 1 : 1 acetone–hexane in 24 hours was not changed when the solvent was continuously shaken or was decanted from the seeds after 3 hours and replaced by an equal volume of solvent.

Warm solvent extracted γ-BHC faster than did cold solvent (Fig. 1). Continuous shaking did not increase the speed of extraction. Isobutyl methyl ketone, kept at 45° for several hours, extracted substances from the seeds which interfered

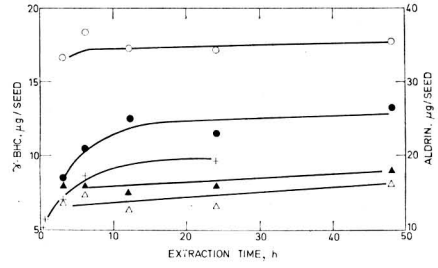

FIG. 1. *The rate of extraction of aldrin and γ-BHC from samples of 10 seeds treated in Plants A and B, using 1 : 1 acetone–hexane*

Plant A Aldrin ○ γ-BHC △ Plant B Aldrin ● Room temperature γ-BHC ▲ Room temperature γ-BHC + 45°

with the gas chromatographic assay of aldrin and γ-BHC but 1 : 1 acetone–hexane did not. 45° was chosen as the highest temperature at which the evaporation of the solvent was negligible if the vessel was closed with a loosely fitting stopper. Higher temperatures were not tested because the water condensers needed would make the method unsuitable for analysing many samples.

Extension of the method to organophosphorus insecticides

Batches of 50 seeds treated in the laboratory with commercial formulations of either carbophenothion or chlorfenvinphos were extracted with 50 ml 1 : 1 acetone–hexane mixtures, at room temperature. These insecticides were extracted from seeds faster than with aldrin or γ-BHC, and the extract was about 90% complete after 3 hours and was almost quantitative after 24 hours.

When seeds, extracted whole for 24 hours, were crushed and extracted in a Soxhlet with acetone, about 0·2% carbophenothion was extracted and 0·3% more chlorfenvinphos.

Examination of the amount of insecticide on individual seeds

Single seeds were extracted in 10 ml 1 : 1 acetone–hexane mixture for 24 hours at 45° for γ-BHC, and at room temperature for aldrin, carbophenothion and chlorfenvinphos, and the insecticide was then assayed. About 50 seeds treated with aldrin and γ-BHC by Plants A and B and about 25 seeds treated in the laboratory with carbophenothion and chlorfenphinphos were examined. The efficiency of the extraction was checked by crushing the seeds and extracting them in the Soxhlet; in all cases less than 10% was extracted. In all samples the amount of insecticide on individual seeds varied widely (Table III, Fig. 2). The distribution of insecticide on individual seeds (Fig. 2) was calculated as a percentage of the mean amount found on a single seed for each treatment to facilitate comparison between different loadings. The amounts of insecticide found on single seeds varied least on the seeds treated in the laboratory. With carbophenothion there was a 7-fold range, and with chlorfenvinphos a 4-fold range (see Table III). Commercially treated seeds from Plant A showed a 100-fold range for aldrin and a 200-fold range for γ-BHC. The amount on seeds treated in Plant B varied over a 40-fold range for both aldrin and γ-BHC.

TABLE III

Ranges and mean values of the amounts of insecticides found on individual seeds treated by different methods

Treatment	Insecticide	μg of insecticide per seed			
		Expected	Determined		
			Mean	Min.	Max.
A	Aldrin	40	36	3	315
A	γ-BHC	20	10	1	200
B	Aldrin	40	27	2	97
B	γ-BHC	20	8	2	84
Laboratory treatment	carbophenothion	60	83	25	165
Laboratory treatment	chlorfenvinphos	74	57	30	115

Discussion

Much of the insecticide (80–90%) on seeds treated with liquid dressings is readily removed by immersion in solvents for 30 minutes and the problem was to find a way of quantitatively extracting the remainder. Preliminary tests indicated that a 1 : 1 acetone–hexane mixture was useful although the results were not absolute because the variable amount of aldrin on individual seeds made it necessary to compare the efficiency of the different solvents indirectly (Table I).

Because later tests confirmed that this cheap and convenient solvent extracts insecticides quantitatively (Table II, Fig. 1) without extracting substances which interfere with the electron capture detector, other solvents were not investigated extensively, although some may be as good. Although 80–90% of aldrin and γ-BHC is removed during the first 3 hours in the solvent at room temperature, the remainder is removed more slowly, and the extraction nears completion only after 24 hours for aldrin and 48 hours for γ-BHC. Extraction cannot be speeded by continuous shaking of the seeds and solvent, or by increasing the volume of solvent. Warming speeds extraction, indicating that a small part of the insecticide penetrates deeply into the seed and suggesting that complete extraction in a short time by any solvent is unlikely.

The following method was chosen for general use. Treated seeds were immersed in 1 : 1 acetone–hexane mixture at 45° for 24 hours in a loosely stoppered tube or flask (although aldrin is adequately extracted at room temperature). For batches of 10–50 seeds 1 ml solvent per seed and for single seeds 10 ml per seed was used. The container was shaken at convenient intervals and immediately before a sample of extract was taken for assay by gas chromatography. The method was satisfactory for the assay of chlorfenvinphos and carbophenothion, which were extracted more rapidly than aldrin and at room temperature.

The method of analysis was applied to seeds treated in two commercial plants and in the laboratory as a preliminary investigation of the distribution of insecticides in seed samples. The mean amount of insecticide found using small numbers of seeds (10–50) varied greatly in all samples, although the proportions of insecticide extracted by the acetone–hexane mixture always exceeded 90%. These variations indicate that the amount of insecticide on individual seeds differed greatly, which was confirmed by the assay of insecticide on single seeds. This shows the need to examine several large samples of seed to ascertain reliably the mean amount of insecticide in a batch of treated seed and the importance of examining the distribution of insecticide on individual seeds.

Although only small numbers (25 or 50) of seeds from each treatment were examined, the pattern of distribution of insecticide appeared to differ between seeds dressed by the different methods. Seeds treated in the laboratory carried a moderately uniform load of insecticide and nine-tenths of the seeds carried a dose not more than twice than, or less than half of, the mean dose. About half the seeds had more, and half less, than the average dose. The amount of insecticide on single seeds was much more variable in the commercially treated batches, and in all four samples more than half the seeds had less than the average dose. Of the seeds treated in Plant B, about half had less than half the mean dose and of those treated in Plant A almost half had less than one quarter of the mean dose (Fig. 2).

These results indicate that liquid formulations are not always uniformly applied to seeds, so that distribution of

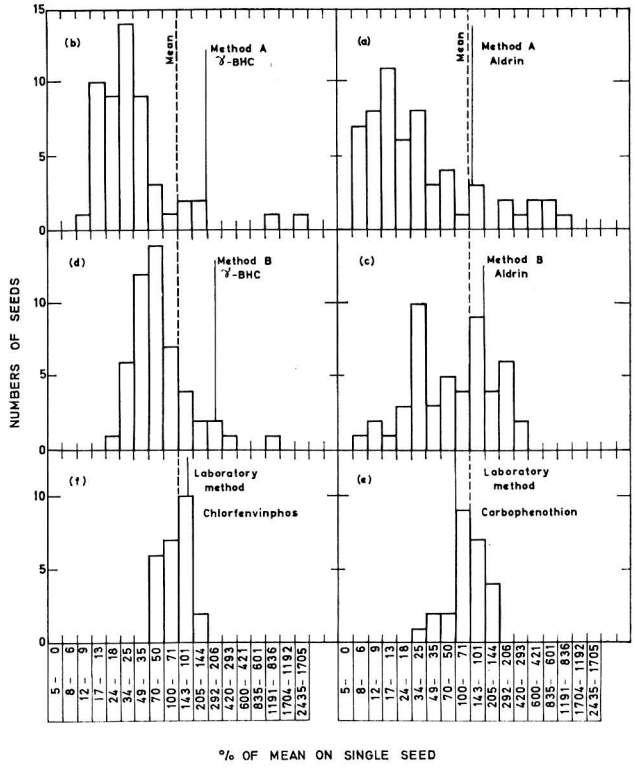

FIG. 2. *The distribution of insecticide on single seeds treated in Plants A and B and in the laboratory, expressed as a percentage of the mean dose*

(a) commercial Plant A — Aldrin
(b) ,, ,, A — γ-BHC
(c) ,, ,, B — Aldrin
(d) ,, ,, B — γ-BHC
(e) Laboratory treated — Carbophenothion
(f) ,, ,, — Chlorfenvinphos

Mean dose found | Mean dose expected

insecticide on seeds, as well as the average dose, must be assessed. A distribution of insecticide such as found on samples treated in Plant A may waste both chemicals and seeds. At best, if control is good and the seeds are not harmed by this treatment, much less than the mean dose applied would suffice since about half the seeds carry less than a quarter of the mean dose. At worst, if control is poor and the insecticide kills heavily loaded seeds, then very many seeds (half carry less than a quarter of the mean dose) have no protection and most of the insecticide is useless and kills seed (half the insecticide is on the one-fifth of the seeds with a dose greater than the mean). None of the four samples examined showed a reasonably good distribution of insecticide, and in three of the four samples the average dose was less than the theoretical mean, assuming that all the applied dose of insecticide adhered and was evenly distributed (Table III, Fig. 2).

Although only four samples, which may not be truly representative of commercial practice, were examined the results indicate that further investigations of methods of treating seeds with liquid formulations are needed.

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CHANGES IN LEAF PROTEIN LIPIDS *IN VITRO*

By F. H. SHAH

The lipids were responsible for the development of rancidity in leaf protein concentrates from wheat, kale, maize and red clover on prolonged exposure to air. Phospholipids were shown to oxidise more rapidly than neutral fats. The enzyme systems causing changes in the leaf protein concentrate were inactivated by heating the protein in nitrogen at 100°, but non-enzymic oxidation of the lipids continued.

Addition of ascorbic acid failed to prevent oxidation, but dried 'amla' (*Emblica officinalis*) fruit reduced oxidation considerably. Dried 'amla' leaf powder was more effective and 'jantar' (*Sesbania aegyptica*) leaf powder was less effective.

Introduction

The lipids present in leaf protein concentrates deteriorate extensively on prolonged exposure to air or oxygen; this results in the development of unpleasant flavours which render the protein unpalatable. Fresh preparations (dry matter content, 30–40%) become mouldy after 3 days at room temperature. However, the concentrate keeps well when stored at −10°,[1] and is completely stable for 15 months when kept at −20° under oxygen-free nitrogen in a gas-tight container.[2] The freeze-dried product is fairly stable at room temperature for 2–3 months, but the lipids should be removed if it is stored in this way for a longer time.

Lea & Parr[1] found an active enzyme system in leaf protein prepared by the method of Morrison & Pirie[2] which rapidly oxidised the lipids. Lipoxidases have also been found in wheat (*Triticum vulgare*), groundnut (*Arachis hypogaea*), urd bean (*Phaseolus radiatus*) and lucerne (*Medicago sativa*) by Siddiqui & Tappel.[3] A fairly high concentration of antioxidants was required to control development of rancidity in leaf protein concentrates, but heating at 90° for 30 min in oxygen-free nitrogen reduced the rate of oxygen uptake to one-third.

Santhakumari & Pillay[4] observed that addition of 'amla' (*Emblica officinalis*) powder increased the shelf life of coconut oil. Ahmed *et al.*[5] found that 'amla' products were better than synthetic antioxidants in retarding the onset of rancidity in 'ghee' (butter oil). Damodran & Nair[6] and Bramchari & Gupta[7] reported the presence of tannins in 'amla' which had a protective action on ascorbic acid even when heavy metals were present. Antibacterial activity has been reported in 'amla' powder by Khorana *et al.*[8]

The present work was undertaken to determine whether it is possible to prevent the oxidation of leaf protein lipids either by addition of antioxidants or by enzyme inactivation.

Experimental

Preparation of leaf protein concentrates

The proteins used in these experiments were extracted from the leaves of wheat (*Triticum vulgare*), kale (*Brassica oleracea*), maize (*Zea mays*) and red clover (*Trifolium pratense*) by the method of Morrison & Pirie.[2]

Some of each concentrate was washed well in water (pH of the suspensions were 6·25, 6·4, 6·9 and 6·7, respectively). Other samples were suspended in 20 times their weight of water and the pH adjusted to 4·0 by addition of dilute hydrochloric acid. In both the cases, the protein was filtered off and pressed to remove excess water.

Freeze-drying

All the protein preparations were freeze-dried by the quick freezing process of Morrison & Pirie.[9]

Extraction of lipids

Samples (50 g) of freeze-dried protein concentrates were soaked for 1 hour in a volume of water sufficient to bring the moisture content to about 80% and were then extracted three times with five volumes of a 2 : 1 chloroform–ethanol mixture following the method of Folch *et al.*[10] The extracts were combined, shaken with 0·2 volumes of water, and allowed to separate; the upper (methanol–water) and lower (chloroform) layers contained the water-soluble substances and lipids, respectively.

Samples (1·0 g) of extracted lipid were dissolved in 10·0 ml chloroform, and 100·0 ml acetone were added slowly with stirring. The mixture was left for one hour and then centrifuged at 2500 g for 15 minutes to remove the acetone-insoluble matter. After standing at +4° overnight, the supernatant was centrifuged again to remove further precipitate. The combined precipitates were re-dissolved in 10·0 ml chloroform and re-precipitated with cold acetone.

The material precipitated by acetone will be referred to as phospholipid and that soluble in acetone as neutral lipid.

Inactivation of plant enzymes

Samples of wheat protein concentrate (dry matter 38·6%) were heated in the presence of oxygen-free nitrogen for 10, 20 or 30 min.

Maize protein concentrate was steamed for 10 min immediately after precipitation.

Measurement of oxygen uptake

Oxygen uptake was measured at 37° using Warburg manometers. The lipid (50–70 mg) was spread in a thin layer around the main compartment of a Warburg flask, the centre well of which contained a filter paper fan moistened with 0·15 ml 5 N-KOH solution to absorb the carbon dioxide.

Control experiments without leaf protein concentrates were run concurrently.

Natural antioxidants

Dried 'amla' (*Emblica officinalis*) fruit was ground in a porcelain mortar after removal of stones. The powder which passed through a 40 mesh sieve was retained.

Leaves of 'amla' and 'jantar' (*Sesbania aegyptica*) trees were ground and sieved after being dried at 80° for 3 h. Powder passing through at 40 mesh sieve was retained for use.

Synthetic antioxidants

Citric acid (A.R.), α-tocopherol and l-ascorbic acids (A.R.) were mixed with the protein concentrates.

Analyses

Dry matter: Leaf protein concentrates were dried at 100° for 40 h. Lipid fractions were concentrated at 40° under reduced pressure, and dried to constant weight over sulphuric acid *in vacuo*.

Nitrogen: A micro-Kjeldahl procedure was used; catalyst $K_2SO_4 : CuSO_4 : SeO_2$, (9 : 1 : 0·02).

Phosphorus: Total phosphorus was determined by the method of Holden & Pirie.[11]

Chlorophyll: The chlorophyll content of the protein concentrates was determined by the method of Arnon,[12] using an E.E.I. colorimeter, filter 607.

Results and Discussion

Table I shows the composition of the total lipid fraction extracted from wheat and kale protein concentrates; Table II, the composition of kale and wheat phospholipids. The lipids present in all four leaf proteins oxidise rapidly when kept at 37°; this is shown by the rate of oxygen uptake (Figs 1–4). One of the factors probably responsible is the presence of unsaturated fatty acids in the crude protein; Lima *et al.*[13] reported that unsaturated fatty acids formed 53·4–78·7% of the total fatty acids present in leaf protein lipids. Oxygen uptake studies on lipids extracted from kale protein (Fig. 1) show that phospholipids are probably responsible as they are oxidised more rapidly than the neutral fractions. Chargaff, Ziff & Rittenberg[14] have reported on the high rate of oxidation of phospholipids isolated from various tissues, and Lea[15] has observed that phosphatide ethanolamine, separated from egg yolk, absorbed one mole of oxygen per mole of phospholipid per hour.

TABLE I

The composition of kale and wheat protein lipids

	mg/g of protein	
	Wheat	Kale
Total extractable lipid	252	232
Lipid-N	3·20	3·22
Lipid-P	0·22	0·37
Lipid chlorophyll	1·22	1·46

TABLE II

The composition of kale and wheat phospholipids

	Wheat	Kale
As % of total lipid	18·2	25·0
P (as % of total lipid)	4·13	3·68
N (,, ,, ,, ,, ,,)	0·43	0·48
Chlorophyll (,,)	0·09	0·12

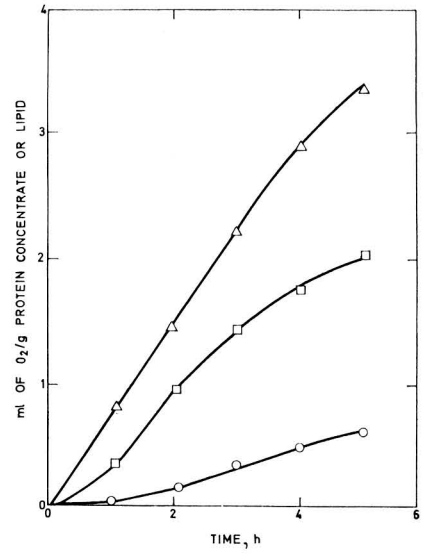

FIG. 1. *Comparative study of oxygen uptake by kale protein concentrate and its lipids*

□ Whole kale protein concentrate
○ Neutral lipids } Isolated from kale protein.
△ Phospholipids }

Deterioration of leaf protein concentrates is also due to the presence of oxidative enzyme systems in the freshly prepared product. These are more active at pH 4·5 than at pH 6·9 (Fig. 2). Their presence is demonstrated by the diminution in oxygen uptake following inactivation of the enzyme systems by heating wheat protein concentrate in oxygen-free nitrogen at 100° for 10 min (Fig. 3). Since heating for longer periods has relatively small effect on oxygen uptake the oxidation still taking place after 10 min would appear to be non-enzymic.

Steaming of maize protein concentrate for 10 min in air also resulted in a decrease in the absorption of oxygen (Fig. 2), but the decrease was less than that caused by heating the wheat protein in nitrogen. It would appear that heating in the presence of air helps in the formation of peroxides which accelerate the rate of non-enzymic oxidation. A similar observation was made by Tappel[16] in a study of the effect of adding linoleate peroxide to a system containing haematin,

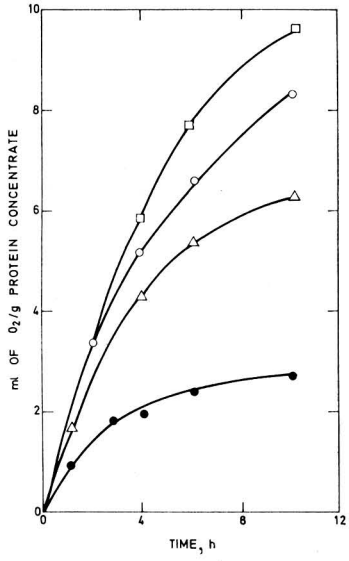

FIG. 2. *Comparison of oxygen uptake by maize protein concentrate following various treatments*

□ Protein concentrate acid-washed (pH 4·5)
○ Protein concentrate (pH 6·9)
△ Protein concentrate steamed for 10 minutes.
● Protein concentrate containing 2·5% 'amla' powder.

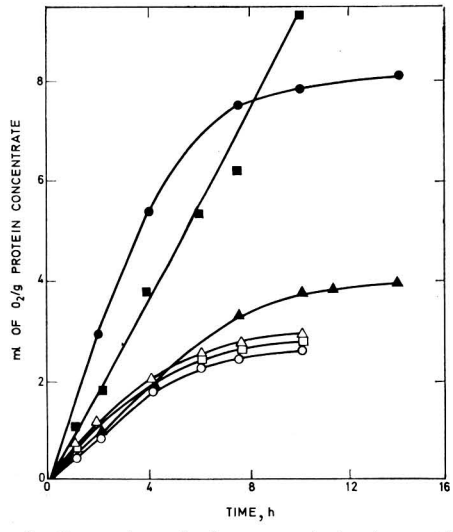

FIG. 3. *Comparative study of oxygen uptake by wheat protein in the presence of antioxidants or after steaming*

■ Protein concentrate (wet).
● Protein concentrate (wet) + {(0·1% ascorbic acid / (0·1% citric acid.
▼ Protein concentrate (wet) + 2·5% 'amla' fruit powder.
△ Protein concentrate (wet) steamed for 30 min in N_2.
□ Protein concentrate (wet) steamed for 10 min in N_2.
○ Protein concentrate (wet) steamed for 20 min in N_2.
+ (% on dry weight basis)

cytochrome *c* or haemoglobin. A recent study [17] has shown that heat sterilisation of milk causes destruction of essential fatty acids, in part by oxidation.

Though washing the protein concentrate with dilute acid removes much soluble carbohydrate and non-protein N material, the rate of oxidation increases (Fig. 2): this could be due to contamination with heavy-metal ions or removal of antioxidants during washing.

Stabilisation of the protein by the addition of antioxidants, synergists and metal deactivators seemed to offer a possible solution, but Lea & Parr[1] reported failure to achieve satisfactory results with most of those they tested. Moreover, the use of synthetic antioxidants in human food is restricted by law.

Addition of ascorbic acid plus citric acid to the protein concentrate increased the rate of oxidation instead of retarding it (Fig. 3) and ascorbic acid in combination with citric acid and tocopherol had little effect on the final amount of oxygen absorbed (Fig. 4). This corresponds to the findings of Marcuse[18] who found that small amounts of ascorbic acid accelerated rancidity in salted herrings. The autoxidation of chlorophyll is also accelerated by ascorbic acid.[19–21]

Figs 2 & 3 show that when 'amla' fruit powder (2·5% concentration) was added to maize and wheat protein the rate of oxygen uptake was diminished to approximately one-third and one-half, respectively. 'Alma' leaf powder

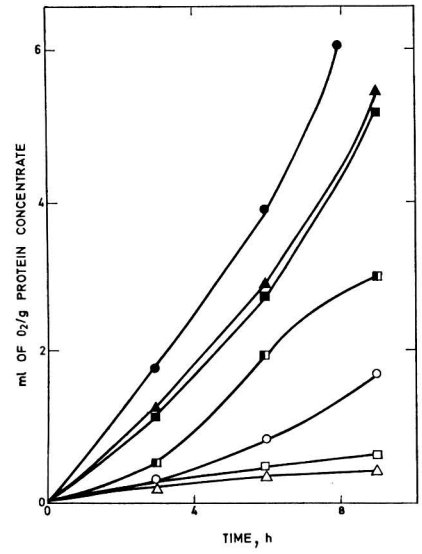

FIG. 4. *Comparative study of oxygen uptake by red clover protein conc. (wet) in presence of antioxidants**

● Protein conc. without antioxidants
▲ Protein conc. + {0·1% citric acid / 0·1% α-tocopherol
■ ,, ,, + {0·1% citric acid / 0·1% ascorbic acid / 0·1% α-tocopherol.
◧ ,, ,, + 5% 'jantar' leaves powder.
○ ,, ,, + 1% 'amla' leaves powder.
□ ,, ,, + 2% 'amla' leaves powder.
△ ,, ,, + 5% 'amla' leaves powder.
* (% on dry weight basis)

(5% concentration) diminished the oxidation of clover protein (Fig. 4) to about one-twelfth of that of the untreated protein; 'jantar' leaf powder at the same concentration was not so effective. This suggests that 'amla' is inactivating the system slowly as well as inhibiting it. These results support those of Pratt & Watts[22] who found antioxidant activity in several vegetable extracts. The effectiveness of the 'amla' powders is probably due to the combined presence of gallic acid, its condensation products and high vitamin C content, which between them possess antioxidant, antibacterial and chelating properties. It may be noted that the 'amla' leaf powder is a more effective antioxidant than the fruit powder; this may be due to the presence of flavenoids which are known to occur more abundantly in the inedible parts of a number of plants.[23–25]

'Amla' fruit powder is a very rich source of vitamin C; as well as retarding the onset of rancidity in leaf proteins it could also enhance its value as a food.

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FRACTIONATION OF PLANT MATERIAL

III.—Two schemes for chemical fractionation of fresh leaves, having special applicability for isolation of the bulk protein*

By A. C. JENNINGS†, A. PUSZTAI, R. L. M. SYNGE‡ and W. B. WATT

Nearly all the protein of broad-bean leaves other than cell-wall protein was readily extracted into phenol–acetic acid-water mixtures, and further purified by free-solution electrophoresis therein, using the continuous apparatus of Hannig. Under these conditions RNA migrated cationically in association with the protein. Adding salt did not greatly improve the separation, but it reversed the relative cationic migration rates of phaeophytin *a* and protein. Liquid–liquid partition between phenol and aqueous buffer phases gave good separation of the protein from RNA.

Scheme I involved successive extraction of the leaves with 5% (w/v) aqueous trichloroacetic acid, ethanol and phenol–acetic acid–water; the bulk of the protein went into this last solvent mixture and did not require further electrophoretic treatment. In Scheme II, components of low molecular weight were washed out from the plasmolysed leaves with water. Extraction was then carried out with phenol–acetic acid–water, and this extract was further fractionated by electrophoresis. The non-migrating fraction should be valuable starting material for studies of a variety of leaf constituents. The extraction residues are promising starting material for studies of the leaf polysaccharides and other cell-wall components.

The protein preparations contained little phosphorus or carbohydrate but were brown and had high C/N ratios and higher ultra-violet absorption than corresponded to the aromatic amino acids present. This was decreased, but not abolished, by keeping the work under nitrogen. Model experiments showed good electrophoretic separation of protein from polyphenols and tannic acid under the same conditions. The extraneous aromatic material is therefore presumed to be bound covalently to the protein. Coupling of oxidatively polymerising quinones to side-chains of amino acid residues, especially lysine, seems probable. The occurrence of such reactions in leaves has implications for the nutritive value of their proteins. Such products may also contribute directly to the 'humic acid' fraction of soil.

Introduction

In the development of the procedures discussed in this paper, the almost complete extraction of protein (as well as other herbage constituents) by phenol–acetic acid–water mixtures[1] and the fact that proteins dissolved in such mixtures have rather similar cationic mobilities regardless of their content of basic groups[3,4] have been used. Thus, on electrophoresis, nearly all the protein of the extract can be collected as a single zone largely freed from non-protein materials. The initial electrophoresis can conveniently be done in the continuous electrophoresis apparatus of Hannig.[5] Although the mobility of the protein is low, the low conductivity of the solvent mixture permits the application of high voltage gradients without excessive heating. Preliminary experiments along these lines with materials from leaves have been mentioned at a symposium.[6] Staron[7] has used phenol–ethanol mixtures for extracting protein from a wide range of biological materials. Menke & Jordan[8] subjected the bulk protein of chloroplasts to phenol–water partition at 65°, but most of it failed to go into solution. Two methods for the extraction of bulk protein have been developed, and the various steps in this development and the conclusions reached will be described first, while a detailed description of the experimental methods will be given in Appendix I.

Experimental

Extraction procedures

Scheme I (Fig. 1)

As a first step in working up leaf material, trituration in a medium of 5% (w/v) aqueous trichloroacetic acid is convenient because enzymes are immediately inactivated and good extraction of water-soluble substances of low molecular weight is obtained; the extraction of the free amino acids is particularly effective.[9] The presence of trichloroacetic acid in the residual mass does not seriously hinder, and may even assist, the subsequent solution of the protein in phenol–acetic acid–water.[1] However, the presence of trichloroacetic acid in such extracts interfered with the electrophoretic migration of at least the protein and the phaeophytin *a*;[10] very broad diffuse zones resulted, with a sharp trailing edge which scarcely migrated towards the cathode.

Extraction of the residue I.R_1 with ethanol decreased its trichloroacetic acid content to such a level that the electrophoresis was unimpaired; at the same time most of the lipids, pigments, etc. were extracted. Unfortunately a small proportion of the protein (< 10%)[11] was extracted at the same time (cf. Racusen & Foote,[12] Munro & Downie[13]).

The subsequent treatment of I.R_2 with phenol–acetic acid–water gave a good yield of protein, mixed with nucleic acid but with little other contamination. The final residue, after being washed with ethanol to remove phenol, was fluffy and fibrous in character.

Scheme II (Fig. 2)

A proportion of the small molecules was removed by repeated gentle rinsing in water and squeezing, after plasmoly-

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sing the leaf by freezing and thawing. The residue II.R_1 is then triturated in, and extracted with, phenol–acetic acid–water and then with ethanol, and leaves a fibrous residue as in Scheme I.

Separation of protein from RNA

On electrophoresis in phenol–acetic acid–water, RNA is carried along by the protein in a single cationic zone. It was hoped that they might be separated electrophoretically by the addition of strong univalent electrolyte to the phenol–acetic acid–water.[4,6] Preliminary experiments in such solutions, on Flodin–Porath columns packed with cellulose powder, confirmed quantitatively the results of Brattsten *et al.*,[4] that cytochrome *c* is readily separable by this means from 'soluble' RNA. However, with RNAs of higher molecular weight (ribosomal and viral), substantial overlapping of the protein and RNA zones occurred. Furthermore, cationic mobility of the protein is depressed to a greater extent than that of phaeophytin *a*, so that their relative positions are reversed by the addition of salts. This effect is presumably due to the polyelectrolyte nature of the protein (Table I). Under these conditions the electrophoresis is too slow, particularly as the increased conductivity limits the voltage which can be applied. Accordingly, this approach was abandoned, and the protein-rich fractions were subjected to phenol–water partition at controlled pH.[14] Protein was then recovered from the phenol-rich phases either by precipitation with ethanol or by successive dialysis against aqueous acetic acid and water,[15] followed by freeze-drying. The fractionation procedures used are outlined in Fig. 3.

TABLE I

Cationic migration velocities in free solution of phaeophytin *a* and horse-heart cytochrome[4,10] at 5–7°

Solvent mixture	cm^2V^{-1} sec^{-1}	
	Phaeophytin *a*	Cytochrome *c*
Phenol–acetic acid–water (1 : 1 : 1, w/v/v)	$1{\cdot}9 \times 10^{-5}$	$3{\cdot}5 \times 10^{-5}$
Phenol–acetic acid–water (2 : 1 : 1, w/v/v) containing 2% NaBr (w/v)	$1{\cdot}2 \times 10^{-5}$	$0{\cdot}8 \times 10^{-5}$

Aromatic contamination of the protein isolated: need for exclusion of oxygen

The first protein preparations made by these procedures were brown, and had a high ultra-violet absorption around 280 nm. The contamination was the greater the slower the preparation had been. At first, the contamination was attributed to secondary-valence association of the protein with polyphenolic material of the leaves, but the components could not be separated. However, in model experiments in

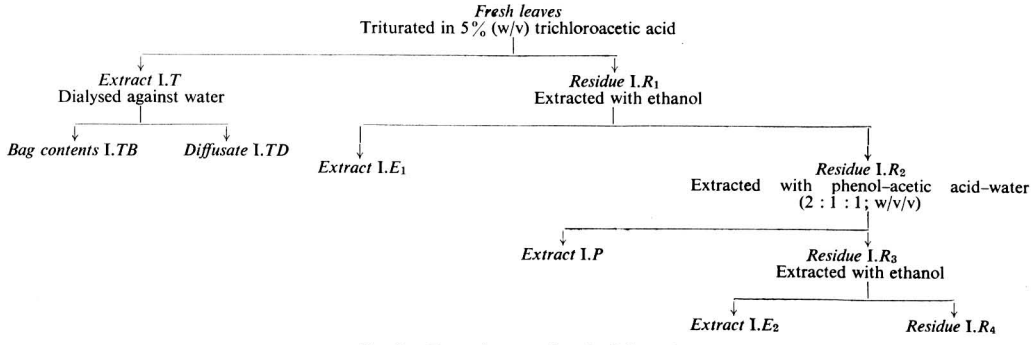

FIG. 1. *Extraction procedure for Scheme I*

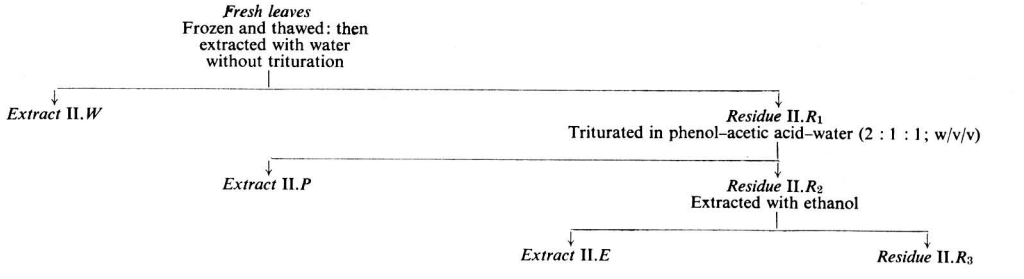

FIG. 2. *Extraction procedure for Scheme II*

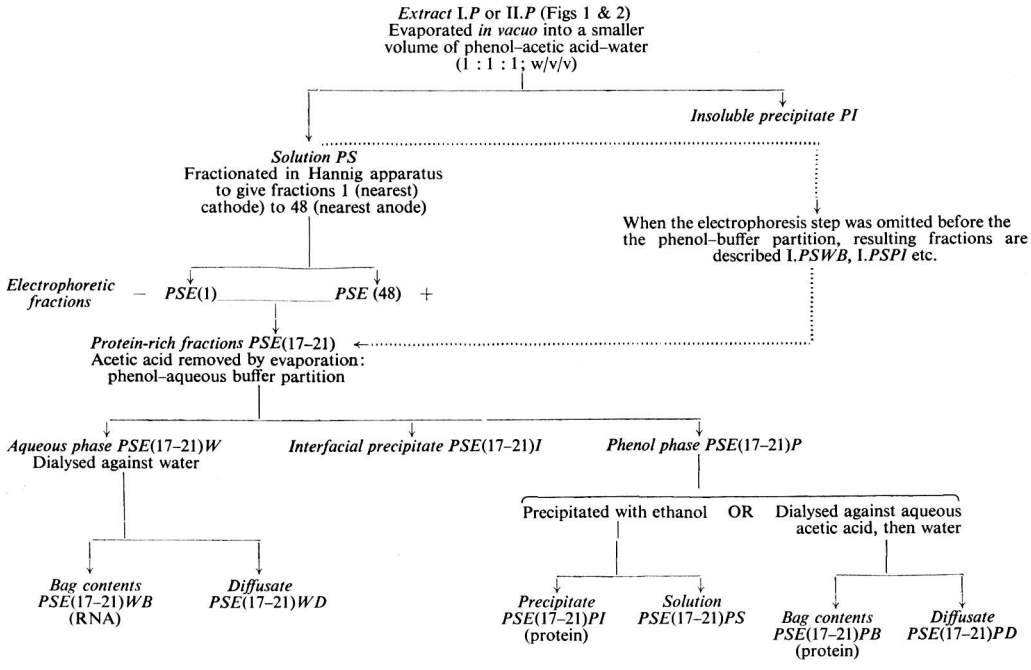

FIG. 3. *Procedure for electrophoresis and further working up of phenol–acetic acid–water extracts of leaves*

which cytochrome *c* was subjected to diagonal crossing filterpaper electrophoresis[17,18] with polyphenols such as quercetin and 'tannic acid' (Chinese gallotannin) in phenol–acetic acid–water (1 : 1 : 1 ; w/v/v), the polyphenols did not migrate and the cytochrome *c* zone migrated cationically[3,4] across the stationary polyphenol zone, the zones remaining unaffected in form or velocity.* Therefore, since the leaf protein fractions had already been subjected to electrophoresis in the same solvent mixture, it seemed unlikely that the aromatic contamination was held to the protein by secondary valences alone. Professors Nicolaus and Piattelli, Naples, suggested that during the fractionation quinones are formed from the polyphenols by chemical or enzymic oxidation, and these coupled to the proteins by primary-valence linkage, chiefly to the free amino groups. Such reactions have been reviewed[22–24] and have also been the object of recent experimental study.[22,25–31] Brown coloration of proteins worked up from leaves has often been noted, and prevented by addition of various reducing agents. Cohen *et al.*[32] found that it could be avoided by keeping the work under nitrogen. In the present work preparations that were nearly colourless and had ultra-violet absorption closer to that attributable to their content of aromatic amino acids were obtained in this way.

* These observations suggest a possible electrophoretic approach to the breaking up of secondary-valence protein–polyphenol complexes. In a different approach, it has been found (unpublished results of A. Pusztai) that, on partitioning between phenol and aqueous buffers, tannic acid enters the aqueous phase much as do other polyanions.[14] Caffeine, nicotine, urea and poly-(vinyl-pyrrolidone) have also been used for dissociation of such complexes.[19–22]

Results and Discussion

Choice of fractionation procedures in relation to aim of preparative or analytical work

Scheme II is considerably simpler than Scheme I, and avoids extraction by both trichloroacetic acid and ethanol. The freezing-and-thawing followed by extraction with water was intended to effect removal of substances of low molecular weight which might interfere with electrophoretic fractionation by raising the conductivity, but this preliminary extraction may not be necessary.

Tables II and III show the distribution of dry matter, ash, N and P obtained by the two procedures when applied to broad-bean leaves. Details of the distribution of amino acid residues are given and discussed by Clarke *et al.*[11] Figs 4–6 show the distribution of dry matter, N and P in the electrophoretic runs following on the two extraction procedures. It seems that very little except protein and RNA is present in extracts I.*PS* (Figs 4 and 5). This, in suitable cases, allows the electrophoretic fractionation to be dispensed with, as is seen by comparing the yield and purity of protein preparations made with and without it (Tables III and IV) (B.I. *PSE* (17–21) (*PI* or *PB*) and B.I. (*PSPI* or *PSPB*)). The yield and purity of protein obtained by Scheme II (where solution II.*PS* has to be subjected to electrophoresis) is also good;

in this scheme there is no preliminary loss of protein into the ethanol extract (I.E_1).

As for components other than bulk protein, the following points should be made about Scheme I. There is a good yield in the trichloroacetic acid extract (I.*T*) of the water-soluble materials of low molecular weight (including much of the inorganic components), that in Scheme II are found in the phenol–acetic acid–water extract (II.*P*) and in the residue (II.R_3). The trichloroacetic acid moreover extracts a substantial proportion of the carbohydrate material that in Scheme II is left in the residue (II.R_3). At least some of this can be recovered from the trichloroacetic acid by dialysis,[1] and this largely polysaccharide fraction (I.*TB*) yields on hydrolysis an amino acid mixture rich in hydroxyproline.[2,11] The treatment with trichloroacetic acid also renders the RNA more readily soluble in phenol–acetic acid–water,[1] perhaps by partly hydrolysing it, so that it is removed from the final residue (I.R_4) to a much greater extent than from II.R_3 in Scheme II. Finally, the ethanol extract (I.E_1) contains, besides trichloroacetic acid and some protein, those leaf components usually isolated by extraction with organic solvents.

Besides yielding the bulk protein more or less in a single fraction (II.*PSE* (17–21)) and phaeophytin *a* separate from

TABLE II

Distribution of dry matter, N, P and ash of broad bean leaves on extraction by Schemes I and II

	Distribution, % of amount in original leaf								Distribution, % of dry matter of fraction					
	Dry matter		N		P		Ash		N		P		Ash	
Starting material	A 100	B 100	A 100	B 100	A 100	B 100	A 100	B 100	A 8·1	B 8·9	A 0·86	B 0·75	A 10·5	B 10·6
Scheme I (*Fig.* 1)														
I.*T* TCA extract	—	—	34·3	39·8	61·5	66·0	85·5	88·5	—	—	—	—	—	—
I.*TB* TCA extract (dialysed)	2·92	4·7	0·54	0·60	0·43	0·78	—	—	1·58	1·16	0·13	0·13	—	—
I.E_1 First ethanol extract	—	—	7·35	5·3	8·4	11·3	Nil	0·6	—	—	—	—	—	—
I.*P* PAW (2 : 1 : 1) extract	—	—	56·4	53·5	26·7	19·5	10·9*	7·7*	—	—	—	—	—	—
I.E_2 Second ethanol extract	0·08	0·22	0·01	0·04	—	—	—	—	1·1	1·6	—	—	—	—
I.R_4 Final residue	12·7	11·4	1·90	1·42	3·5	3·2	3·6	3·2	1·27	1·1	0·25	0·21	3·0	3·0
Scheme II (*Fig.* 2)														
II.*W* Aqueous extract	—	23·0	—	26·0	—	37·1	—	39·6	—	10·0	—	1·21	—	18·3
II.*P* PAW (2 : 1 : 1) extract	—	56·0	—	69·7	—	44·0	—	52·3*	—	11·0	—	0·59	—	9·9*
II.*E* Ethanol extract	—	0·07	—	0·02	—	—	—	—	—	2·5	—	—	—	—
II.R_3 Final residue	—	20·8	—	4·3	—	18·9	—	8·1	—	1·85	—	0·68	—	4·1

* Determined by difference

TABLE III

Distribution of dry matter, N and P when extracts *P* were worked up

(Figures relating to final bulk-protein fractions are shown in bold type)

	Distribution, % of amount in original leaf									Distribution, % of dry matter of fraction					
	Dry matter			N			P			N			P		
Starting material Extraction Scheme	A I	B I	B II	A I	B I	B II	A I	B I	B II	A I	B I	B II	A I	B I	B II
Fractions (Fig. 3)															
PI	10·5	3·2	1·5	4·9	1·2	0·8	7·6	2·9	3·4	3·8	3·2	4·8	0·62	0·68	1·73
PS	41·3*	36·1*	54·5	51·5	52·3	68·9	19·1	16·6	40·6	10·1*	12·8*	11·2	0·40*	0·34*	0·56
PSE(17–† or 18‡–21)	23·6	30·0	32·4	37·8	42·5	48·7	10·1	13·9	1·11	13·0	12·5	13·4	0·37	0·35	0·02
PSE(17–† or 18‡–21)*WB*	0·68	0·46	0·1	1·07	0·71	0·06	5·38	2·9	0·03	12·7	13·7	5·3	6·8	4·7	0·2
PSE(17–† or 18‡–21)*WD*	—	—	—	0·64	—	—	Nil	—	—	—	—	—	—	—	—
PSE(17–† or 18‡–21)*I*	0·34	4·48	0·1	0·38	0·62	0·1	0·53	0·03	Nil	9·0	1·2	8·9	1·34	0·005	Nil
PSE(17–† or 18‡–21)*PI*	**21·8**§	**22·0**	**18·5**	**31·1**	**35·1**	**31·0**	**0·75**	**4·26**	**0·45**	**11·6**§	**13·6**	**14·3**	**0·03**	**0·13**	**0·02**
PSE(17–† or 18‡–21)*PS*	1·3§	—	—	0·53	1·01	0·5	Nil	0·35	0·28	2·9§	—	—	Nil	—	—
PSE(17–† or 18‡–21)*PB*	—	**24·8**	**18·5**	—	**39·2**	**31·6**	—	—	**0·77**	—	**13·5**	**14·7**	—	—	**0·0**
PSWB	—	2·9	—	—	3·1	—	—	13·1	—	—	9·5	—	—	3·4	—
PSI	—	5·5	—	—	5·4	—	—	0·4	—	—	8·7	—	—	0·05	—
PSPI	—	**22·9**	—	—	**35·3**	—	—	**0·66**	—	—	**13·7**	—	—	**0·02**	—
PSPS	—	—	—	—	0·95	—	—	0·68	—	—	—	—	—	—	—
PSPB	—	**21·1**	—	—	**38·2**	—	—	—	—	—	**15·5**	—	—	—	—

* Residual trichloroacetic acid as well as leaf material present in these fractions
† Starting material B
‡ Starting material A
§ Probable contamination by borate and metabisulphite

phaeophytin *b*,[10] Scheme II gives in the non-migrating fraction II.*PSE*(27–31) (Fig. 6) a great variety of neutral and weakly acidic compounds already freed from proteins, salts and other troublesome contaminants. These compounds include lipid[16] and glycosidic materials, which occur here in a form much more suitable for detailed study than when they are obtained in fractions I.*T* and I.E_1. Some chemically bound amino acid residues are present in this fraction, and are discussed by Clarke *et al.*[11]

The final residues, either I.R_4 or II.R_3, should prove suitable starting material for studies of polysaccharides. These residues may also turn out to be valuable for assessments of nutritive value of herbages in the same way as the 'detergent-fibre' preparations of Van Soest.[33]

Properties of the isolated bulk protein

Table IV gives the analytical results. The preparations mostly have a N content in the lower part of the range covered by proteins. They are low in P (which sets a limit to contamination by nucleic acids), carbohydrate, hexosamine and lipid (fatty acid) content.[16] The Cl figures set a limit for contamination by trichloroacetic acid. The ash content will be mostly due to contamination by the salts used in the phenol–water partition, and is least in those preparations worked up by dialysis. The S content, which is variable, is not all explicable as methionine and cystine;[11] some of it may be due to the bisulphite introduced at the phenol–water partition stage (cysteine-*S*-sulphonate formation, etc.). Clarke *et al.*[11] give detailed amino acid analyses.

Table IV also shows a wide range of ultra-violet absorption values for the preparations, those made without exclusion of

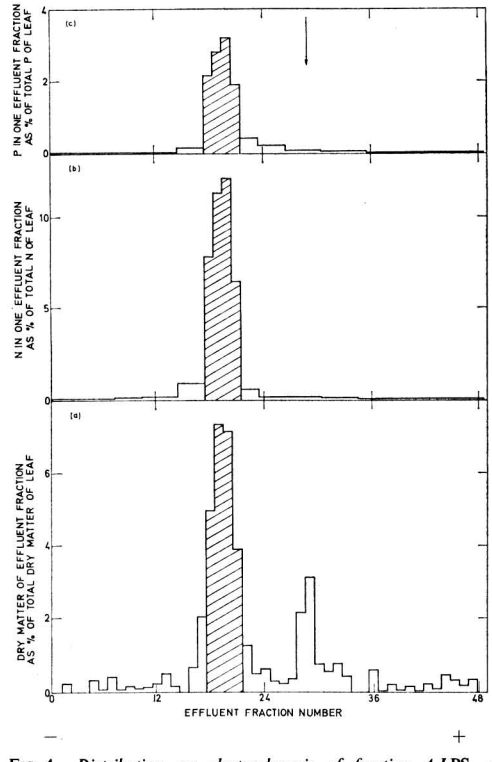

FIG. 4. *Distribution, on electrophoresis of fraction A.I.*PS, *of (a) dry matter, (b) nitrogen, (c) phosphorus*

The hatched area shows fractions 18–21, from which protein was worked up. The arrow indicates that sample was introduced opposite outlet No. 29

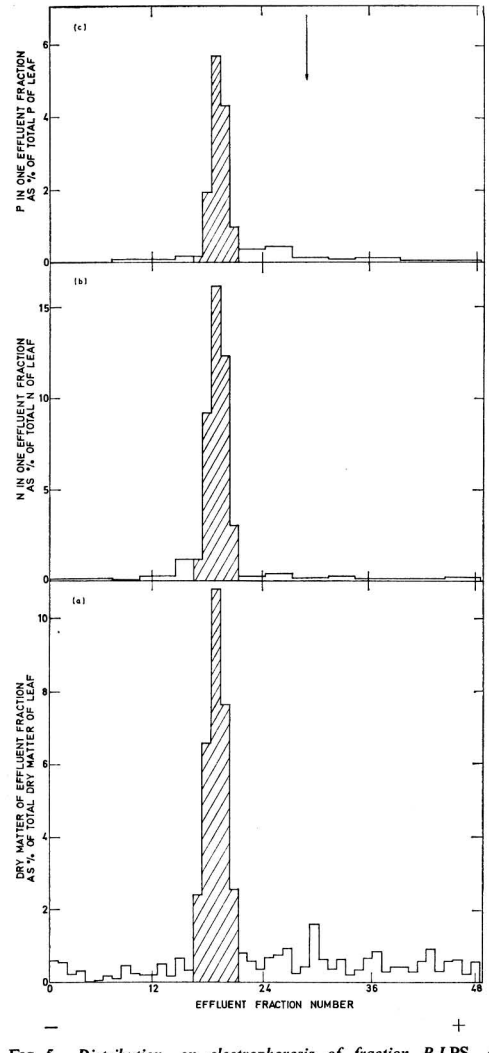

FIG. 5. *Distribution, on electrophoresis of fraction B.I.*PS, *of (a) dry matter, (b) nitrogen, (c) phosphorus*

The hatched area shows fractions 17–21, from which protein was worked up. The arrow indicates that sample was introduced opposite outlet No. 29

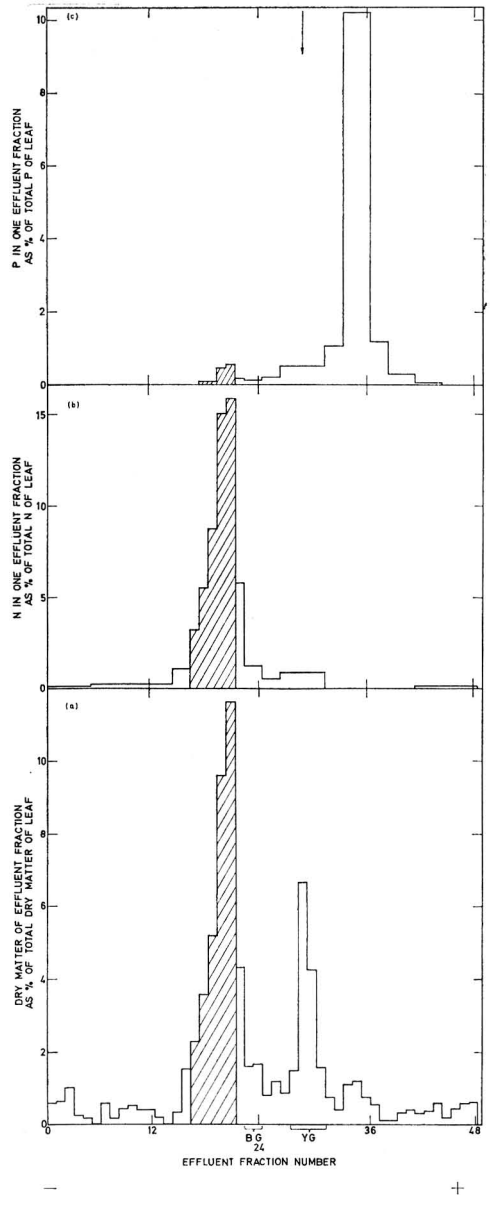

FIG. 6. *Distribution, on electrophoresis of fraction B.II.*PS, *of (a) dry matter (b) nitrogen, (c) phosphorus*

The hatched area shows fractions 17–21, from which protein was worked up. BG, blue-green zone (phaeophytin *a*); YG, yellow-green zone (phaeophytin *b* and carotenoids). The arrow indicates that sample was introduced opposite outlet No. 29

oxygen giving the highest values. Fig. 7 shows the absorption spectra of the two extreme bean-leaf protein preparations, compared with that calculated from the content of tyrosine and tryptophan.[11] The difference spectra are typically aromatic. C/N ratios in Table IV are correlated with ultra-violet absorption and tend to be higher than that (3·23) calculated from the amino acid analysis.[11] Furthermore, various fractions, obtained from the highly absorbing preparations by ether extraction at different stages of the fatty acid determination, had ultra-violet absorption greatly in excess of that found in corresponding fractions from casein or from less absorbing bean-leaf preparations (see Table V). These differences are attributed to varying amounts of covalently linked non-amino acid aromatic contaminants, perhaps arising by reaction of the protein with quinones present in the leaf initially, even when air has been excluded during the preparation. This suggests that the addition of reducing agents such as sulphur dioxide or ascorbic acid at the beginning of the extraction might be useful.

Implications of chemical coupling of aromatic plant constituents with proteins

The fractionations described have so far only been used with a limited range of materials. However, leaves are of extreme chemical complexity, and similar procedures may effectively handle other kinds of biological material, including

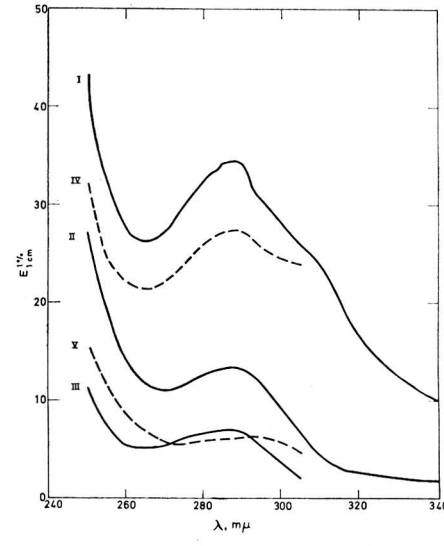

FIG. 7. *Ultra-violet absorption curves for protein preparations in* 0·3M-*KOH, compared with that calculated on basis of tryptophan and tyrosine content*[11] *of A.I.*PSE(18–21)PI

I, preparation C.II.*PSE*(18–21)*PI*; II, preparation A.I.*PSE*(18–21)*PI*; III, calculated according to Goodwin & Morton[46]; IV, difference of I and III; V, difference of II and III

TABLE IV

Analytical results on final bulk-protein fractions
Air was excluded during the preparation of the first 6 fractions in this Table

Starting material / Extraction Scheme / Fraction	Analysis, % of dry matter of fraction: C	H	O	N	S	Cl	P	Ash	Carbohydrates (as mannose)	Hexosamine	$E^{1\%}_{1cm}$ at 290 nm in 0·3 M-KOH	$\frac{E^{1\%}_{1cm}\ (290\ nm)}{N\%}$	$\frac{C\%}{N\%}$ (wt. ratio)
A. I. *PSE*(18–21)*PI*	42·07	6·04	26·53	11·8	2·37	0·58	0·03	16·68	0·51	0·08	13·5	1·10	3·45
B. I. *PSE*(17–21)*PI*	42·94	6·25	28·75	13·6	1·40	0·58	0·13	3·47	0·86	—	18·8	1·39	3·16
B. I. *PSE*(17–21)*PB*	50·68	7·67	—	13·5	1·26	0·39	—	1·63	—	—	22·1	1·64	3·75
B. I. *PSPB*	50·95	6·53	—	15·5	1·44	0·51	—	1·28	—	—	20·6	1·33	3·28
B. II. *PSE*(17–21)*PI*	47·34	6·14	—	14·3	2·35	0·09	0·02	5·15	0·51	—	22·7	1·68	3·31
B. II. *PSE*(17–21)*PB*	49·81	6·79	—	14·7	1·39	0·38	0·03	0·81	0·74	—	26·3	1·80	3·39
B. II. *PSE*(17–21)*PB**	50·91	7·35	—	14·1	—	—	0·05	1·23	0·95	—	26·7	1·90	3·61
C. II. *PSE*(18–21)*PI*	45·36	6·65	—	11·9	—	—	0·02	5·91	0·52	—	34·3	2·88	3·82
(Tea) D. I. *PSE*(15–23)*PB*†	49·67	6·70	23·14	12·4	1·43	1·17	0·09	0·34	3·8	—	46·0	3·7	4·00

* B.II.*PS* oxygenated at room temperature before electrophoresis
† Fraction contained 23% of N of the tea

TABLE V

Distribution of ultra-violet absorbing material in extracts made after alkali treatment of protein

Protein preparation	$E^{1\%}_{1cm}$ at 290 nm in 0·3M-KOH	The maximum value of E_{1cm} in the range 270–290 nm calculated for that amount of each fraction in 1 ml as was derived from 10 mg of protein preparation: First ether phase	Second ether phase	Second aqueous phase	Ether extract after acid hydrolysis of first aqueous phase
Casein†	6·9	0·08	0·3	0·3	0·1
A.I.*PSE*(18–21)*PI*	13·5	0·08	0·24	0·03	0·24
B.I.*PSE*(17–21)*PI*	18·8	0·04	1·7	0·2	0·1
B.II.*PSE*(17–21)*PB**	26·7	0·41	13·6	3·3	0·6
C.II.*PSE*(18–21)*PI*	34·3	1·7	7·3	13·3	4·0

* B.II.*PS* oxygenated at room temperature before electrophoresis
† Hammarsten (British Drug Houses Ltd.)

subcellular components isolated by less drastic methods (cf. Takayama *et al.*[34], Braunitzer & Bauer[47]).

Although these procedures, especially the use of meta-bisulphite during partition between phenol and aqueous buffer, may be presumed to lead to chemical alteration of the proteins isolated, there seems no reason why such methods could not be adapted as a stage in the isolation of intact individual proteins. The phenol–acetic acid–water mixtures do not readily split peptide bonds;[9] Pusztai has recovered bovine plasma albumin,[15] chymotrypsinogen[15] and aldolase (unpublished) unchanged from such mixtures.

Enzymic or non-enzymic oxidation and coupling to protein of aromatic plant components probably occurs outside the laboratory whenever wilting or drying of plant material occurs. Preparation of 'protein' from Indian black tea (D.I.*PSE*(15–23) *PB*) is an example, perhaps extreme, of an end-product of such reactions. Since lysine, histidine, and cystine residues are thought to be particularly involved, such reactions could have far-reaching effects on the digestibility and nutritive quality of the protein of a variety of vegetable products.* Since these preparations are largely free from carbohydrate, it should not be difficult to determine the extent of substitution of their functional groups.

Coupling and polymerisation reactions of quinones are probably important for incorporating proteins, amino acids, etc., into the more persistent fractions of the organic matter of the soil.[38] For this reaction, ε-NH_2 groups of lysine residues are the functional groups which bulk largest in proteins; should these serve as the point of attachment of protein to a more resistant moiety formed by oxidative polymerisation of quinones and polyphenols, lysine residues might be expected to have a special stability to microbial degradation and weathering. Carles & Decau[39] found the lysine content of humic acid from a range of soils to be

* Secondary-valence tanning of proteins has been studied by Leroy and colleagues[35–37] as a means of influencing the course of their digestion by ruminants

greater the warmer the climate in which the soil had been formed (cf.[48]). In plot experiments with different fertilisations and cultivations, Stevenson[40] found a higher proportion of lysine to total amino acid residues, when the carbon content of the soil was lower. Degens *et al.*[41] found a high proportion of lysine in the top layers of ocean sediments. Such results could also be explained by persistence of bacterial cell-wall material rich in lysine, although Stevenson[40] found no increase of α, ε-diaminopimelic acid to parallel that of lysine. Analytical differentiation of the two possibilities should not be difficult.

The thought prompted by the extreme blackness of withered broad-bean leaves is that coupling of plant protein and amino acids to polymerising quinonoid material could take place extensively in withering leaves before they became incorporated into the soil. Micro-organisms may thus play a smaller part in the formation of humic acid than Kononova[38] supposed.

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APPENDIX I

The detailed experimental work involved in the procedures outlined in the paper is described below.

Materials

Phenol and acetic acid were A.R. quality, redistilled immediately before use. Other solvents and reagents were A.R. quality. Nitrogen gas (British Oxygen Co., 'white spot') was $>99{\cdot}9\%$ N_2 (v/v) (O_2 $<0{\cdot}001$ % v/v).

Analytical procedures

Dry matter of fresh leaves was determined after drying them at 105° for 16 h; all other materials were dried to constant weight in a vacuum desiccator over sulphuric acid and soda-lime.

Ash was determined by incineration at 800° to constant wt. in a platinum crucible, except for the determinations in Table IV, which were by Drs. F. & E. Pascher, Bonn.

N and P were determined as described by Jennings & Watt.[1]

C, H, O, S and Cl were determined by Drs. F. & E. Pascher, Bonn.

Carbohydrate was determined as described by Dubois *et al.*[42]

Hexosamine was determined as described by Pusztai.[43]

RNA determinations were on fractions already freed from lipids and material of low molecular weight, starting with the KOH extraction and thereafter according to Fig. 3 of Smillie & Krotkov.[44] It was found that, in the eluates from the Dowex-1 columns, P and pentose values often exceeded those calculated for RNA from the ultra-violet absorption; this last was therefore regarded as the best indication of RNA content (cf. Holdgate & Goodwin[45]).

Apparatus for continuous electrophoresis in phenol–acetic acid–water mixtures

For this purpose the continuous electrophoresis apparatus of Hannig[5] (Elphor model VaP1; Dr. Bender & Dr. Hobein GmbH, Lindwurmstr. 71, Munich) had to be modified in some respects. The plastic parts in contact with the solvent mixture or its vapour had to be of unplasticised PVC, polyethylene or silicone rubber. Phenol is appreciably sorbed by these materials, and only slowly released, so it is advisable to have duplicate sets of plastic parts for use with aqueous electrolytes and phenolic mixtures respectively. Cellophane membranes were used between the separation and electrode chambers. The electrode chambers were perfused with the same solvent mixture as that used in the separation chamber, each at approx. 2 ml/min from separate Mariotte bottles. The effluent from each electrode chamber was allowed to drain to waste. The conductivity of the mixtures used[4] is such that, with phenol–acetic acid–water (1 : 1 : 1 , w/v/v), the current is only approx. 20 mA when the operating voltage (2,500 V) is applied to the apparatus. The current stabiliser provided does not function for currents less than about 50 mA, so repeated manual adjustment of the applied voltage was necessary. A safety device to switch off the apparatus in the event of a failure of the vacuum used for periodic transfer of effluent fractions to collection vessels was added. Some of these modifications have now been adopted by the manufacturers.

Fractionation procedures

Material was evaporated *in vacuo* below 40°. Mixtures containing phenol were evaporated with repeated additions of water to maintain two liquid phases until near the end. If acetic acid was present, the liquids were evaporated with additions of water until two liquid phases resulted. Additions of water were usually continued until the residue no longer smelt of phenol. Where air was to be excluded, a rotary evaporator with central feed tube, through which water was introduced as required from a storage vessel through which nitrogen was bubbled, was used. To release the vacuum, nitrogen was admitted through the feed tube.

Fractions were dialysed on a mechanical rocker at +1° in Cellophane sacs. Trichloroacetic acid extracts were dialysed against approx. 2 l portions of water for several days, the water being changed twice daily. Other aqueous extracts were treated in the same way, except that 2 ml chloroform were added to each change of water as an antiseptic. Phenol solutions containing protein were dialysed first against aqueous acetic acid, then against water, as described by Pusztai.[15]

Fractions in solution that had been prepared with exclusion of air were stored in stoppered vessels in the dark at +1°; the vessels were thoroughly flushed with nitrogen after removal of each sample. Solvent mixtures for the extractions were likewise flushed with nitrogen before transfer to the glovebox.

Extraction—Scheme I (*Fig.* 1)

A glovebox set up in a cold laboratory (+1°) contained nitrogen maintained from a cylinder at positive pressure of a few cm of water. Broad-bean leaflets (25 g fresh wt.) were cut into large segments in the cold laboratory, and transferred immediately to the glovebox for treatment with 25 ml 10% (w/v) aqueous trichloroacetic acid in a MSE top-drive homogeniser having stainless-steel blades running in a vortex beaker. After being blended for 30 min the resulting slurry was transferred, using a little 5% (w/v) aqueous trichloroacetic acid, to four 40 ml polypropylene centrifuge tubes and centrifuged for 10 min at top speed in a MSE 'Angle' bench centrifuge also located in the glovebox. The pale yellow supernatant was removed and the precipitates were stirred and washed each with 5 successive 35 ml portions of 5% (w/v) trichloroacetic acid. The combined supernatants and washings were Fraction I.*T*. The residues (I.R_1) were each washed in the same manner with 5 successive 35 ml portions of ethanol, and the highly pigmented ethanol washings were combined to give Fraction I.E_1. The very pale green residues I.R_2 were each stirred with 35 ml phenol–acetic acid–water (2 : 1 : 1 ; w/v/v) which caused them to swell and take on a gelatinous consistency and translucent appearance. The tubes were kept overnight, then centrifuged; the supernatant was removed and each residue was washed in the same manner as above with four successive 35 ml portions of the phenol–acetic acid–water mixture. The combined supernatants and washings were very pale yellow-green, and constituted Fraction I.*P*. Extracts I.*T*, I.E_1 and I.*P* were stoppered before removal from the glovebox and stored at +1° in the dark. The residues I.R_3 were removed from the glovebox to the laboratory at room temperature and transferred with ethanol from the centrifuge tubes to a tared sintered-glass Gooch crucible (porosity 3), and further washed with ethanol, the combined filtrate being approx. 500 ml (Fraction I.E_2). The final residue I.R_4 was allowed to dry in air before being stored in a desiccator. It was colourless, fibrous and fluffy.

Extraction—Scheme II (*Fig.* 2)

Broad-bean leaflets (25 g fresh wt.) cut into large segments were placed in a muslin bag in a wide-mouthed Dewar flask containing solid CO_2 in the nitrogen glovebox set up as above in the cold laboratory. After some hours of freezing, the bag was removed from the Dewar flask and placed for 10 min in 120 ml water. The thawed leaf material was freed from excess water by gentle squeezing of the bag and was stirred gently in a fresh 120 ml portion of water. This was repeated a further four times; the six nearly colourless aqueous extracts were combined to give fraction II.*W*, to which 2 ml chloroform were added. The resulting flaccid leaf material II.R_1 was blended for 30 min in the homogeniser with 45 ml phenol–acetic acid (2 : 1, w/v). The dark green gelatinous slurry was transferred as above to centrifuge tubes, using phenol–acetic acid–water (2 : 1 : 1, w/v/v) for the transfer, and kept overnight. It was then centrifuged and washed 5 times with phenol–acetic acid–water (2 : 1 : 1; w/v/v) as for

the corresponding stage in Scheme I, and gave the dark green extract II.*P*. The same final procedure as in Scheme I resulted in the colourless washings II.*E* and final residue II.R_3. The latter was somewhat softer and bulkier than I.R_4.

Electrophoresis of phenol–acetic acid–water extracts and working-up for protein (*Fig.* 3)

Extracts (I.*P* or II.*P*) were evaporated as described above in a tared flask, until the phenol-rich phase, at a moment of disappearance of aqueous phase, weighed approx. 35 g. This was assumed to contain 70% of phenol by weight; still under nitrogen, acetic acid and water were added to make the ratio of phenol–acetic acid–water to 1 : 1 : 1, w/v/v); after being shaken well and kept overnight at room temperature, the mixture was transferred using further small portions of the same solvent mixture to the graduated sample bottle of the Hannig apparatus, which was well flushed with nitrogen. This gave extracted material at a convenient volume for electrophoresis and in the solvent mixture in which the model electrophoreses with known substances had been made. Precipitate forming in the rotary evaporator, the evaporation flask, the sample bottle of the Hannig apparatus and (sometimes) in the separation chamber of the Hannig apparatus was bulked as fraction *PI*. This was very variable in quantity (Table III) and was largely carbohydrate in nature, although significant quantities of protein[11] and RNA were also present. The electrophoresis was conducted in phenol–acetic acid–water (1 : 1 : 1; w/v/v) with the outlet tubes numbered, from cathode side to anode side, 1 to 48, and with the sample pumped in at 1 ml/hr into the inlet opposite outlet no. 29. The solvent mixture was pumped through the apparatus at such a rate that the sample stayed in the separation chamber for 105 min; 2,500 V were applied to the apparatus. The sample bottle and the solvent mixture supplying the separation chamber were flushed with nitrogen throughout the run. The cooling air was maintained between 2° and 6°. The effluent fractions were not protected from air as they emerged from the apparatus and in later fractionations. They were transferred daily to tared stoppered bottles after a check, from the distribution of pigment or of (protein + RNA) (dioxan-precipitation test),[1] that there had been no fluctuation of the electrophoresis since the previous collection. For longer-term storage, the contents of each bottle (*PSE*(1) to *PSE*(48)) were flushed with nitrogen and stored at 0–2° in the dark. The clear supernatant remaining in the sample bottle at the end of the run was regarded, for analytical purposes and further study, as fraction *PS*.

After distribution of dry matter, pigment, N and P had been established by analysis of suitable samples of the effluent fractions, larger portions of the fractions containing the main protein–RNA zone (*PSE*(17–21) or *PSE*(18–21)) were pooled for separation of protein from RNA. An amount of pooled material containing approx. 150 mg dry matter was evaporated with additions of water until acetic acid had been removed, and the volume of phenol-rich phase at the moment of disappearance of aqueous phase was approx. 20 ml. An equal volume of sodium borate buffer (0·2 M, pH 9·2) was then added, and the fractionation was conducted by the 'standard partitioning procedure' of Pusztai.[14] Since, on cooling, a bulky interfacial precipitate formed, the third extraction was carried out substituting 1% (w/v) aqueous sodium metabisulphite for the borate buffer, which greatly decreased the precipitate. The resulting pooled aqueous phases were dialysed against water and then freeze-dried (*PSE*(17– or 18–21)*WB*), the diffusate usually being discarded. The insoluble material (*PSE*(17– or 18–21)*I*) was washed with ethanol to remove phenol before being dried for analysis. The protein in the phenol phases was worked up either by successive dialysis against aqueous acetic acid and water or by precipitation with ethanol. In the former case, diffusates were discarded and bag contents were freeze-dried (*PSE* (17– or 18–21)*PB*). In the latter case, the phenol phase was evaporated to 5–10 ml and added dropwise to a centrifuge tube containing 5 vol. ethanol. After being kept overnight at 0–2°, the precipitate was centrifuged and then washed thrice with 40 ml portions of ethanol with thorough stirring before being dried (*PSE* (17– or 18–21)*PI*). The combined supernatant and washings, after evaporation to dryness, were *PSE* (17– or 18–21)*PS*.

Effect of salt on electrophoretic migration rates

Before electrophoretic separation of the protein and RNA was abandoned, experiments were carried out in the Hannig apparatus on the effect of the addition of salt on the migration of phaeophytin *a* and cytochrome *c* in phenol–acetic acid–water mixtures. Table I shows that the relationship of the cationic migration velocities is reversed on the addition of sodium bromide. A similar relative depression of the migration velocity of the bulk of the leaf protein was found, so that, on electrophoresis of extracts II.*PS* in phenol–acetic acid–water (2 : 1 : 1; w/v/v) containing 2% (w/v) NaBr, the main protein–RNA zone lay between the faster-cationic zone of phaeophytin *a* and the non-migrating zone containing phaeophytin *b* and the carotenoid pigments.

Growth, harvesting and origin of leaf material

Broad beans (*Vicia faba* L. cv. Windsor Improved) were grown in pots in a glasshouse in John Innes compost and harvested 4–6 weeks after seedling emergence, when there were about four well-developed leaflet pairs on each plant. Leaflets were detached in the cold laboratory immediately before treatment. Dates of harvesting of the various lots were as follows: A, 8 Nov. 65; B, 28 Feb. 66; C, 19 Feb. 65. C was whole subaerial portions of plants and contained 6·2% N in the dry matter. D was an Indian black tea (Fine Darjeeling Flowery Orange Pekoe) obtained commercially. It contained 4·0% N in the dry matter.

Leaflets A were worked up according to Scheme I. Leaflets B were used for a detailed comparison of Scheme I with Scheme II. Further subsidiary comparisons were made of the effect of passing oxygen for 39 days through extract B.II.*PS* at room temperature (in the dark) and of omitting the electrophoresis step in isolating protein from B.I.*PS*. Material C was worked up by Scheme II and the black tea D by Scheme I, except that this was done at room temperature and without exclusion of air at any stage. C had been stored three days at −20° and thawed for 90 min before being worked up.

Course and results of fractionations

The results of the fractionations are set out quantitatively in Tables II and III and in Figs 4–6. Due account has been taken at all stages of samples removed for analysis etc. Extracts I.*PS* were nearly colourless, and during electrophoresis gave only a trace of greenish-yellow colour as a

non-migrating zone, largely due to phaeophytin *b*, as judged by the absorption spectrum. Extracts II.*PS* were dark green, and on electrophoresis gave a narrow blue-green zone of phaeophytin *a* emerging centred between outlets no. 23 and 24 (see Fig. 6) and a non-migrating yellow-green zone which, besides phaeophytin *b* and carotenoid pigments, contained much lipid and other material. These were the only coloured zones visible on electrophoresis of extracts from which air had been excluded. When this had not been done, the protein-rich zones were coloured brown; the non-migrating zone also acquired a brown colour, and there were intermediate brown zones. The preparation from tea showed maxima of brown colour associated with the non-migrating zone and with the 'protein', with some brown colour at all intermediate positions.

Figs 4 and 5 show that, when Scheme I was followed, extracts A.I.*PS* and B.I.*PS* contained little material that did not migrate on electrophoresis in the main protein–RNA zone, especially as a proportion of the non-migrating zone of dry matter is attributable to trichloroacetic acid. When Scheme II was followed (Fig. 6), extract B.II.*PS* gave a much more complicated picture, in which the additional zones are attributable to substances extracted by the trichloroacetic acid and ethanol when Scheme I is followed.

Most of the P in fractions A.I.*PSE*(18–21)*WB*), B.I.*PSE* (17–21)*WB* and B.I.*PSWB* was held by and later eluted from the anion-exchange resin in the RNA determination,[44] and the ultra-violet absorption of these eluates agreed qualitatively and quantitatively with this P being in nucleotide form. This, as well as the partition behaviour and the non-diffusible nature of these fractions, established that they were rich in RNA.

Properties of isolated bulk-protein fractions

Preparations from bean leaves made with exclusion of air were white or very pale yellow. Those made in air were brown; that from tea was very brown. The analytical results are set out in Table IV. It is unfortunate that preparation A.I.*PSE*(18–21)*PI*, which had the lowest ultra-violet absorption and was studied in greatest detail,[11] had the highest ash content. Fig. 7 shows the ultra-violet absorption spectra in alkali of the bean leaf preparations that had the lowest and highest absorption together with the spectrum calculated as being due to the presence of tyrosine and tryptophan residues.[11]

A further test for extraneous ultra-violet absorbing material was applied to fractions resulting during the saponification and isolation of fatty acids from the bulk-protein fractions.[16] Material in each fraction was read in the spectrophotometer against a reference sample from a blank saponification in which the same reagents and solvents had been used at all stages. Casein served as a control protein low in extraneous aromatic contamination. The acidified second aqueous phases were subjected to spectrophotometry after being made up to standard volume with ether-saturated water. The first and second ether phases were evaporated to dryness and the residues ('unsaponifiable and saponifiable lipid fractions respectively) were dissolved in methanol for spectrophotometry. Furthermore, to liberate aromatic material in the first aqueous phases from possible glycosidic combination and to make it more readily extractable into ether, the first aqueous phases were made N in respect of H_2SO_4 and refluxed for 1 h. After being cooled, each was extracted with 50 ml ether, and the ether was washed with two 10 ml portions of water. It was then evaporated to dryness, and the residue was dissolved in methanol for spectrophotometry as above. The results are set out in Table V. A.I.*PSE*(18–21)*PI* gave little more ultra-violet absorption in any of the derived fractions than did casein. The other, more heavily absorbing, bean-leaf protein preparations all showed increases. Thus this approach, while not reliable in the present instance for detection of aromatic contamination of proteins, did yield positive evidence that such contamination could occur.

FRACTIONATION OF PLANT MATERIAL

IV*.—Distribution of amino acid residues in fractions obtained from leaves of broad bean (*Vicia faba* L.)

By EILEEN M. W. CLARKE, GABRIELLE M. ELLINGER and R. L. M. SYNGE†

The quantitative distribution of amino-acid residues amongst some fractions derived from broad-bean leaves was determined. Among the free amino acids, asparagine predominated and 3,4-dihydroxyphenylalanine (DOPA) was abundant. DOPA was absent from the protein fractions. Aggregated N recovery and amino acid composition resembled those found for leafy materials by previous workers. Material extracted by ethanol from the TCA-precipitated fraction resembled the bulk protein in molecular size and amino acid composition. A fraction not migrating during electrophoresis in phenol–acetic acid–water gave, on hydrolysis, amino acids presumed to be bound in neutral or weakly acid compounds, mostly of low molecular weight.

Introduction

Amino acid analyses of the following materials described in the preceding paper[1] are given: the main fractions resulting from application of Scheme I (Fig. 1 of preceding paper)[1]; some fractions arising during a comparison of Schemes I and II. It was intended that ethanol-extracted material obtained by Scheme I (B.I.E_1) should be compared with electrophoretically non-migrating material obtained by Scheme II (B.II.*PSE*(27–31)) (Figs 1, 2, 3 and 6 and Tables II and III of preceding paper).[1] The two fractions contained similar proportions (about 5%) of the total N of the leaf, and closer characterisation of their amino acid-yielding components, particularly as to molecular weight, was required. For this purpose, the materials were further fractionated by molecular-sieve chromatography in phenol–acetic acid–water.

Experimental

Preparation of broad-bean leaf fractions

The preparation of the trichloroacetic acid (TCA)-soluble fraction (A.I.*T*), the TCA-soluble non-diffusible fraction (A.I.*TB*), the ethanol-soluble fraction (A.I.E_1), the fraction soluble in phenol–acetic acid–water (PAW) (2 : 1 : 1, w/v/v) (A.I.*P*), the final residue (A.I.R_4), and the final protein fraction (A.I.*PSE*(18–21)*PI*), has been described in the preceding paper.[1]

Two fractions from other preparations described there, the ethanol-soluble fraction B.I.E_1 and the portion of PAW-soluble material that emerged on continuous electrophoresis in the neutral region, B.II.*PSE*(27–31), were subjected to further fractionation in PAW (1 : 1 : 1, w/v/v) by the method described by Bagdasarian, Matheson, Synge & Youngson,[2] using columns made from 1 g Sephadex G–75 (Batch To 5784). Portions of B.I.E_1 (0·511 mg N) and of B.II.*PSE*(27–31) (25·1 mg dry matter and 0·636 mg N) (see Jennings *et al.*,[1] Table II and Fig. 6) were evaporated to dryness *in vacuo* under nitrogen, dissolved in 0·5 ml PAW (1 : 1 : 1, w/v/v) and transferred quantitatively to the columns. Distribution of dry matter in the effluent is shown in Fig. 1. Both columns showed a green zone (phaeophytins) emerging with its centre at approx. 6·5g effluent, followed closely by a yellow zone

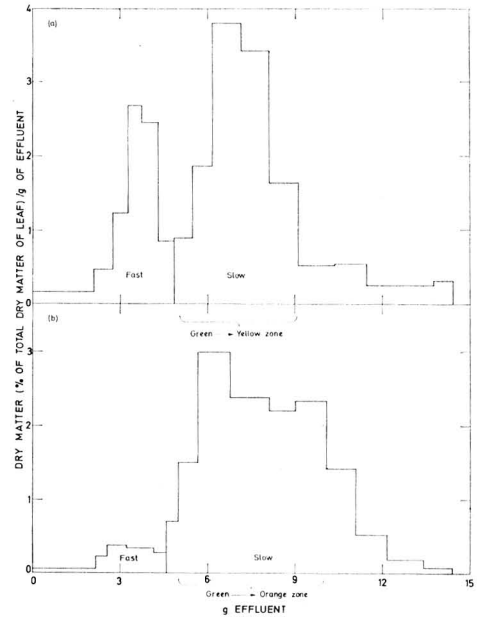

FIG. 1. *Chromatography on Sephadex G-75 in phenol–acetic acid–water* (1 : 1 : 1, *w/v/v*) *of fractions from broad-bean leaves*

(*a*), ethanol extract in Procedure I (B.I.E_1) (trichloroacetic acid is present in this fraction); (*b*), electrophoretically non-migrating fraction of PAW extract in Procedure II (B.II.*PSE*(27–31)). The vertical lines between 4 and 5 g effluent show the cuts between sub-fractions pooled as 'fast' and 'slow'

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(carotenoids). The effluent fractions were pooled on the basis of being before ('fast') or after ('slow') a point between 4·5 g and 5·0 g effluent, as shown in Fig. 1. This should separate substances having mol. wt. greater and less than 1000, respectively.[2,3]

Methods of analysis

Hydrolysis

The general procedure, using constant-boiling hydrochloric acid (5·4–5·6 N) for these analyses, has been described by Clarke & Ellinger.[4] Fractions suspected of a high lipid content were initially dispersed in glacial acetic acid[5] and the final hydrolysing medium was a mixture of 80 ml glacial acetic acid and 120 ml concentrated hydrochloric acid. After it had been boiled under reflux for 24 h, cooled and filtered through sintered glass (Grade G3), the hydrolysate was reduced in volume to about 5 ml on a rotary evaporator operating at 40°. Water was added and evaporated 5 to 10 times, until the acetic acid had been removed. The residue dispersed readily in a mixture of equal volumes of diethyl ether and constant-boiling hydrochloric acid before extraction with three further successive portions of diethyl ether. The aqueous phase was then evaporated to dryness, and the residue was made up to volume in citrate buffer, pH 2·2.[4] The procedure adopted with individual fractions is shown in Table I. All fractions described in Table II were hydrolysed in this manner.

Amino acid analyses

A Beckman Spinco Amino Acid Analyser was used as described by Clarke & Ellinger.[4] Conditions 'for the analysis of physiological material' were adopted for the acidic and neutral amino acids of hydroxyproline-containing fractions and for the basic amino acids of the TCA-soluble fraction, A.I.*T*.

The cystine and methionine contents of the final protein fraction A.I.*PSE*(18–21) *PI* were estimated as cysteic acid and methionine sulphone according to the procedure of Moore.[6] No specific precautions against losses during hydrolysis were taken for the other analyses given for the sulphur amino acids; hence they must be considered minimum values. Methionine values represent the sum of methionine and methionine sulphoxides that were found. The values obtained for ammonia were omitted. When the samples hydrolysed were very small their contribution of amide N was small compared with the control and the use of concentrated

TABLE I

Amino acid composition of some bean leaf fractions expressed as: I, moles/100 moles estimated; II, g amino acid N/100 g total leaf N

Fraction	TCA-soluble, not dialysed		TCA-soluble, non-diffusible		Ethanol-soluble		PAW-soluble		Residue		Final protein from phenol/borate partition		Amino acid N in whole leaf
	A.I.*T*		A.I.*TB*		A.I.E_1		A.I.*P*		A.I.R_4		A.I.*PSE*(18–21)*PI*		
N of fraction as percentage of total N of leaf	34·3		0·54		7·35		56·4		1·90		31·1		100
Hydrolysis	5·6 N-HCl		5·6 N-HCl		Conc HCl glacial acetic acid (3 : 2, v/v)		5·6 N-HCl		5·6 N-HCl		5·4 N-HCl		
	I	II	I	II	I	II	I	II	I	II	I	II	II
Lysine	1·1	0·309	7·0	0·028	5·8	0·456	6·5	5·13	8·9	0·165	6·3	3·02	6·06
Histidine	3·0	1·372	3·3	0·020	1·4	0·162	2·1	2·48	2·6	0·072	2·2	1·59	4·09
Arginine	0·8	0·480	2·3	0·019	4·0	0·632	4·9	7·67	4·4	0·162	4·7	4·42	8·94
Aspartic acid	67·3	10·084	7·4	0·015	8·0	0·316	9·6	3·78	8·0	0·074	9·4	2·24	14·25
Threonine	0·6	0·103	7·0	0·014	4·9	0·191	5·7	2·26	5·4	0·049	5·4	1·28	2·60
Serine	1·9	0·274	9·8	0·019	5·3	0·206	5·6	2·20	6·0	0·055	5·2	1·21	2·74
Glutamic acid	6·8	1·029	7·0	0·014	10·1	0·397	10·5	4·12	9·2	0·086	10·1	2·40	5·63
Proline	0·4	0·069	5·6	0·011	5·9	0·228	5·3	2·09	5·0	0·046	5·3	1·24	2·43
Glycine	1·6	0·240	10·6	0·021	9·2	0·360	9·8	3·89	15·5	0·142	9·4	2·21	4·63
Alanine	1·3	0·206	5·9	0·012	9·0	0·353	9·0	3·55	7·3	0·068	8·9	2·12	4·18
Valine	1·0	0·137	5·5	0·011	6·5	0·257	6·7	2·65	5·2	0·048	7·2	1·71	3·09
Methionine	trace		0·5	0·001	1·4	0·051	1·7	0·68	0·9	0·008	1·6	0·40	0·74
Isoleucine	0·5	0·069	3·0	0·006	5·3	0·206	5·2	2·03	4·5	0·042	5·2	1·21	2·35
Leucine	0·4	0·069	4·4	0·009	10·8	0·426	9·3	3·67	7·0	0·065	9·0	2·12	4·23
Tyrosine	0·3	0·034	3·6	0·007	3·3	0·132	3·4	1·35	3·2	0·030	3·4	0·81	1·55
Phenylalanine	0·5	0·069	1·6	0·003	5·8	0·228	4·7	1·86	3·0	0·027	4·6	1·09	2·18
Cystine	0·6	0·103	0·8	0·002	0·2	0·007	trace		trace		1·1	0·25	> 0·36
Hydroxyproline	trace		14·5	0·029	nil		nil		3·9	0·036	nil		0·06
Dihydroxyphenylalanine	5·1	1·749	nil		nil		nil		nil		nil		> 1·75
Ethanolamine	trace		n.d.		2·9	0·118	n.d.		n.d.		n.d.		0·12
Tryptophan	n.d.		n.d.		n.d.		n.d.		n.d.		1·2	0·56	> 0·56
Amino-acid N recovered as % of total N of fraction	47·8		44·7		64·3		87·6		61·8		96·0*		71·5

*Amide N (kindly determined by Mr. W. B. Watt[9]) was 4·8% of the total N of this fraction

hydrochloric acid in some hydrolyses further increased the control values that were obtained.

Tryptophan N was determined on fraction A.I.*PSE*(18–21)-*PI* and was 1·8% of the N of this fraction by the method of Brieskorn & Scheide[7] and 2·1% by procedure N of Spies & Chambers.[8]

Results

The amino acid composition of fractions obtained from preparation A.I. is presented in Table I. Columns I give the relative composition in terms of moles per 100 moles estimated. On this basis the compositions of the principal protein fractions, A.I.E_1, A.I.*P*, and the derived A.I.*PSE*(18–21) *PI* were very similar, with the exception of the relatively high phenylalanine content of the ethanol-soluble fraction. Fractions A.I.*TB* and A.I.R_4 were distinguished from the other fractions by the presence of hydroxyproline, and A.I.*TB* had high threonine and serine contents and low isoleucine, leucine and phenylalanine contents. The residues contained an exceptionally high level of glycine, and also, for a plant material, of lysine. Fraction A.I.*TB* contributed less than 2% to the nitrogen of A.I.*T*. Hence the amino acid composition of A.I.*T* was largely that of the free amino acids (and possibly of small peptides). Aspartic acid represented 67% of all amino acid residues, histidine, glutamic acid and 3,4-dihydroxyphenylalanine (DOPA) a further 15%.

Amino acid N in the proportion in which it occurs in each fraction in the leaf is given in columns II. The final column is the sum of these values for A.I.*T*, A.I.E_1, A.I.*P* and A.I.R_4, which is the amino acid composition of the whole leaf in g amino acid nitrogen per 100 g total nitrogen.

The analytical results for the two high-lipid, low-N extracts that were further fractionated on Sephadex G-75 are presented in a similar manner in Table II. The very small amounts of N that were available for most of these analyses may have impaired their precision.

By comparison with the bulk protein soluble in PAW, the high-molecular-weight fraction from B.II.*PSE*(27–31) had a low basic amino acid content, very high serine and high glycine contents and contained no tyrosine. The isoleucine content of the corresponding low-molecular-weight fraction was exceptionally high and exceeded the leucine content. The high- and low-molecular-weight portions of ethanol-soluble material (B.II.E_1) did not differ much from each other, but the serine content of the latter was much higher. Compared with the PAW-soluble protein, both had low histidine contents and high phenylalanine contents.

TABLE II

Amino acid composition of fractions resulting from molecular-sieve fractionation expressed as: I, moles/100 moles estimated; II, g amino acid N/100 g total leaf N

Fraction	PAW-soluble, electrophoretically neutral B.II.*PSE*(27–31)						Ethanol-soluble B.I.E_1			
	without sub-fractionation		sub-fractionated				sub-fractionated			
mg N	0·637		0·636				0·511			
N of fraction as % of total leaf N	4·47		4·47				5·3			
			'fast' fraction		'slow' fraction		'fast' fraction		'slow' fraction	
mg dry matter	25·1		1·4		23·7		4·54		13·55	
dry matter as % of total leaf dry matter	15·4		0·9		14·5		4·5		—*	
	I	II	I	II	I	II	I	II	I	II
Lysine	2·4	0·012	3·8	0·010	6·2	0·032	5·2	0·272	4·7	0·042
Histidine	0·7	0·006	0·9	0·004			0·6	0·044	0·9	0·012
Arginine	1·3	0·013	1·7	0·009	2·8	0·028	4·0	0·423	3·4	0·061
Aspartic acid	8·1	0·021	9·1	0·012	9·2	0·023	8·8	0·231	8·7	0·039
Threonine	4·8	0·013	6·8	0·009	3·8	0·010	5·2	0·138	5·6	0·025
Serine	8·7	0·022	13·2	0·018	10·1	0·025	5·7	0·150	9·9	0·045
Glutamic acid	16·2	0·041	5·6	0·008	18·2	0·046	10·0	0·264	9·9	0·045
Proline	4·0	0·010	5·6	0·008	4·2	0·010	6·1	0·162	4·0	0·019
Glycine	16·0	0·041	13·3	0·018	15·1	0·038	10·0	0·266	12·4	0·056
Alanine	8·2	0·021	9·5	0·013	6·7	0·017	9·9	0·261	9·2	0·041
Valine	5·8	0·015	8·2	0·011	4·3	0·010	7·2	0·191	6·3	0·028
Methionine	0·7	0·001	0·6	0·001	0·7	0·001	0·8	0·023	0·5	0·002
Isoleucine	9·6	0·024	6·2	0·008	8·3**	0·021	5·6	0·147	5·1	0·023
Leucine	7·8	0·020	11·1	0·015	6·0	0·015	11·8	0·311	11·0	0·050
Tyrosine	1·4	0·004	nil	nil	1·4	0·004	2·6	0·068	2·0	0·009
Phenylalanine	4·2	0·010	4·4	0·006	2·9	0·007	6·2	0·165	6·3	0·028
g amino acid N recovered/ 100 g leaf N	0·274		0·150		0·287		3·116		0·525	
N recovery as % of total N of fraction	6·1		9·8				68·7			

* not calculated, because TCA present

** This value is calculated, using the isoleucine colour factor, from a double peak composed of isoleucine and an unknown ninhydrin-positive material that emerges just ahead of and adjacent to isoleucine. The contribution of colour from the two components is similar

Discussion

Technical aspects of the analyses

The precision of amino acid analysis is largely determined by the preceding hydrolysis. Not all amino acids are equally affected. It is accepted that cystine is subject to large losses and, to determine it precisely, a preliminary oxidation to cysteic acid[6] is essential. The same applies, to a lesser degree, to methionine. During the normal preparation of hydrolysates methionine is partly converted to sulphoxides. When the areas of the three small peaks representing methionine and its sulphoxides on the elution diagram are summed, errors, apparent in poor reproducibility, are introduced. After preliminary oxidation the value obtained for methionine as the sulphone has invariably been greater, indicating that after normal hydrolysis small proportions of the methionine are not recovered as such nor as the sulphoxides or sulphone. Losses during hydrolysis of serine and threonine are well recognised. Rees'[9] corrections of 10·5 and 5·3% respectively have not been applied here. The stability of both trans-4-hydroxyproline and DOPA during hydrolysis must also be considered. Losses of bound hydroxyproline have been mentioned.[10,11] When the hydrolysis of residues A.I.R_4 was extended to 48 hours, the amount of hydroxyproline found was 16% less than that found after 24 hours' hydrolysis. It could be assumed that a similar loss of hydroxyproline occurred during the first 24 hours of hydrolysis.

The behaviour of DOPA during hydrolysis is exceedingly variable. Heating of DOPA alone in 6 N-HCl resulted in 92% recovery. Only 66% of DOPA found in the unhydrolysed TCA-soluble material could be recovered after hydrolysis. These results resemble those of Sborov *et al.*[12] However, Rolland *et al.*[13] reported that free DOPA in the presence of ribonuclease was totally destroyed in 6 N hydrochloric acid. A 50% recovery could be achieved by hydrolysis under highly specific reducing conditions. Yet their peptide-linked DOPA, formed by the controlled oxidation of tyrosine residues of ribonuclease, was 73% recoverable from hydrolysates prepared without specific precautions with 6 N-HCl. Consequently, the values presented for DOPA content of the TCA-soluble fraction are minimum values. On the other hand, the absence of DOPA from the hydrolysates of the protein fractions may be presumed not to be the result of total destruction during hydrolysis. After hydrolysis of the TCA-soluble fraction an unidentified peak emerged on the 50 cm column at the position of ornithine and α,γ-diaminobutyric acid. This was suspected of being the principal breakdown product of DOPA. Apart from losses during hydrolysis, Rolland *et .al.*[13] observed that DOPA was unstable on ion-exchange resins unless oxidising impurities had been carefully removed from them. No such precautions were taken in these experiments. This may account for the low, though reproducible, colour factor of 13·5 obtained in calibration chromatograms.

Distribution of amino acids in main fractions (Table I)

One third of the nitrogen of the leaf was extracted by TCA, less than half of this being recoverable as amino acid nitrogen. Yet this fraction contained 70% of the total of aspartic acid and asparagine in the leaf. This bears out the reports of high asparagine contents of legumes (Czapek,[14] McKee.[15]) By qualitative two-dimensional filter-paper chromatography the predominance of asparagine and DOPA among the free amino acids of the leaf was confirmed. Neither Petronici[16] nor Boulter & Barber[17] observed such a predominance of aspartic acid and its amide amongst the free amino acids of *Vicia faba* shoots. Glutamic acid and glutamine were present in largest amounts in their analyses. There is, however, agreement over the relatively large amount of free histidine. On a molar basis it is the only amino acid that occurs free in proportions similar to that bound in the bulk-protein fraction. Torquati[18] and Guggenheim[19] reported the presence of free DOPA in fresh pods of *Vicia faba.* Chodat & Evard[20] studied its distribution within the plant and found the highest concentration to be in the leaves. Neither Petronici[16] nor Boulter & Barber[17] mention its presence but according to the published chromatogram[17] it seems likely that the peaks attributed to argininosuccinic acid are in fact those of DOPA. Under the chromatographic conditions, DOPA emerged on the 150 cm column in the close, but fully separated, sequence: leucine, norleucine, DOPA. On the 50 cm column it emerged immediately ahead of β-alanine which precedes the combined tyrosine-plus-phenylalanine peak. Cusworth & Westall[21] describe the chromatographic behaviour of argininosuccinic acid and its accompanying anhydrides under similar conditions; if present it could have coincided with glucosamine on the long column and DOPA on the 50 cm column. While it has been shown by Andrews & Pridham[22] that DOPA occurs as a glucoside in the testa and in both dormant and germinating cotyledons of *Vicia faba*, these authors found no evidence that it exists in this form in either roots or shoots of young plants, or in any part of the mature plant other than the pods. No γ-aminobutyric acid was found in the fraction A.I.*T* although it was present in a similar fraction obtained from another preparation. In the latter, the concentration of γ-aminobutyric acid was the same both before and after hydrolysis. Hence its absence from A.I.*T* is not attributable to the hydrolysis procedure.

The amino acid composition of the TCA-soluble non-diffusible fraction and of the extracted residues was discussed in Part II[4] of this series.

The bulk protein extracted by PAW and the final protein prepared from it did not differ markedly in their amino acid compositions nor did the ethanol-soluble protein. Without exception, their amino acid values fell within the ranges for the large number of leaf-protein extracts described by Chibnall *et al.*[23] and Gerloff *et al.*[24] The absence of DOPA is in agreement with the findings of Sborov *et al.*[12] on a bean protein.

The proportion of total N recovered as amino acids was very different for the different fractions. For the aggregated fractions, representing the whole fresh leaf, (Table I, last column) amino acid N accounted for 71·5% of total N, a recovery that agrees well with the 66% reported by Wilson & Tilley[25] for analyses of whole oven-dried lucerne herbages. 87% of the PAW-soluble N was recovered in the form of amino acids. 96% of the N of the final protein fraction was accounted for as amino acid N and the rest as amide N (cf. Chibnall *et al.*[23])

The other fractions fall into two groups. About 60% N is recovered as amino acids from the ethanol-soluble fraction and from the residues, but less than 50% from the whole TCA-soluble fraction and from its non-diffusible fraction. High amide content and the presence of other nitrogenous compounds are some of the factors contributing

to the low N recovery. Others may include destruction of amino acids during hydrolysis in the presence of large proportions of carbohydrate, as in the TCA-soluble non-diffusible fraction, and possibly also in the residues.

Amino acid residues in the fractions studied by molecular-sieve chromatography (Fig. 1 and Table II)

The ethanol-extract fractions A.I.E_1 and B.I.E_1 yielded a good proportion of their N as amino acids after acid hydrolysis, and the proportions in which these occurred did not differ much from those in the bulk protein, although histidine was lower and phenylalanine higher. Most of the phospholipid N and phaeophytin N must also have been present in these fractions. When B.I.E_1 was subjected to molecular-sieve chromatography, most of the amino acid residues emerged in the 'fast' fraction, suggesting protein character with molecular weight of the order of several thousand. A proportion, however, emerged in the 'slow' fraction along with TCA, lipid and phaeophytins. This was presumably low-molecular-weight material and did not exceed in amount the low-molecular-weight fractions ($b + c$) studied by Bagdasarian *et al.*,[2] which likewise did not differ markedly in amino acid composition from the bulk protein of the leaf. It is reasonable to suppose that the TCA renders part of the proteins soluble in ethanol (cf. Munro & Downie,[26] Racusen & Foote[27]).

The fraction not migrating on electrophoresis in PAW (B.II.*PSE*(27–31)) yielded only a small proportion of its N as amino acids after acid hydrolysis. Their mutual proportions differed significantly from those in the bulk protein. Besides N of phaeophytin *b* and phospholipids, some additional nitrogenous compounds, not yielding amino acids, must have been present. On molecular-sieve chromatography, most of the amino acid residues migrated in the 'slow' fraction, suggesting low molecular weight. Since typical proteins, peptides and amino acids would have been removed by cationic migration during the electrophoresis step, it is reasonable to regard B.II.*PSE*(27–31) as comprising neutral or weakly acidic compounds, comparable with the low-molecular-weight fractions from ryegrass studied by Synge & Wood[28] (*PM*, *PVPM*, *PWPM* etc). These authors considered these fractions to be a complicated mixture of acyl amino acids. However, coupling products of amino acids with quinones, discussed in Part III,[1] would behave similarly, and further work is required to establish the chemical nature of such fractions. There was a significantly greater recovery of amino acids from the chromatographic fractions than from the material taken for chromatography. These fractions are all rich in polyphenolic and glycosidic material, which may have been destructive to the small amounts of amino acids during acid hydrolysis; these amino acid figures should therefore be treated as minimum ones.

The excess of amino acids recovered after chromatography may have arisen from the Sephadex used (W. M. Laird, M. Smith, & R. L. M. Synge. To be published).

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ORGANOCHLORINE INSECTICIDE RESIDUES IN EARTHWORMS FROM ARABLE SOILS

By G. A. WHEATLEY and J. A. HARDMAN

Six species of earthworms from an arable soil were analysed for residues of aldrin, dieldrin, DDT and γ-BHC. The ratios between the concentrations of γ-BHC, aldrin + dieldrin and *p,p'*-DDT + *p,p'*-DDE in the earthworms and in the soil (concentration factors) were similar but the residue concentrations were consistently higher in the smaller more shallow-living species *Allolobophora caliginosa*, *A. chlorotica* and *A. rosea* than in the larger deeper-living species *Lumbricus terrestris*, *A. longa* and *Octolasion cyaneum*.

In arable field plots, dieldrin residues in *A. longa* and *A. chlorotica* increased with increasing concentrations in the soil but the concentration factors decreased. The concentrations of residues in earthworms (W) appear to be related to those in the soil (S) by an equation of the form $W = aS^b$ where a and b are constants, the latter being about 0·79 for a wide range of residue data embracing the uptake of aldrin–dieldrin, DDT components, and γ-BHC by earthworms.

Introduction

The relatively low concentrations of organochlorine insecticide residues present in many arable soils in central England[1,2] are unlikely to have directly adverse effects on earthworm populations.[3–8] As earthworms are eaten by a wide range of vertebrates, however, and may thus introduce residues into certain food chains,[9,10] information on their residue content in different environments is of value in assessing their potential as residue vectors.

Several quantitative field investigations of DDT uptake by earthworms have been reported[8,9,11] but these related to situations where relatively high concentrations of insecticide occurred, or would be expected to have occurred, at or near the soil surface or in superficial herbage and debris. In arable fields, such conditions will normally occur infrequently, and then for only a few months after insecticides have been applied, because subsequent ploughing and other cultivations will dilute and re-distribute the residue throughout the cultivated layer of the soil.

From two fields treated with aldrin (in fertiliser), Raw[12] found concentrations of dieldrin in earthworms to be several times greater than those in the soil itself, and Davis & Harrison[2] reported that concentrations of dieldrin in four samples of earthworms of mixed species from arable sites were two to ten-fold greater than those in corresponding soil samples. However, different species of earthworms can apparently accumulate both dieldrin and DDT residues to different extents.[12,13]

The results of more detailed investigations into the uptake of organochlorine insecticide residues by earthworms from two arable sites are described here. One site represented a relatively high residue environment and the other site contained dieldrin at a range of concentrations in the soil.

The earthworm nomenclature adopted was that of Gerard.[14]

Experimental

Sources of earthworms

For developing and assessing the efficiency of the analytical technique, adult *Allolobophora longa* Ude were collected from an untreated sports turf at Wellesbourne by spreading Mowrah meal over the turf and flooding it with water to bring the worms to the surface.

Earthworms were obtained from two arable sites where residues of organochlorine insecticides in the soil had previously been assessed.

One was a field near Great Rollright, Oxfordshire, the soil of which was a shallow stony light loam of calcareous origin. Organochlorine insecticides, particularly aldrin, had been used excessively on this site for eight years and the soil contained high residues of aldrin, dieldrin and DDT components.[15]

The other was a field at Wellesbourne with a light friable sandy-loam soil. An experiment in progress in this field on the effects of dieldrin on beetle predators of the cabbage root fly[16] comprised six 30 × 30 yd plots separated by untreated strips 10 yd wide. Four plots had been treated with dieldrin at either 0·5, 0·75, 1·0 or 1·25 lb a.i./ac on 8 April, 1963, the insecticide being applied to the soil surface as a coarse spray and then mixed into the upper 8–10 cm soil by two rotary cultivations. The other two plots had received no insecticide but were otherwise cultivated similarly. The earthworm samples were collected from an area 15 × 2 yd under swedes growing in part of each plot.

Sampling

Soil

The soil samples taken at both sites each comprised 25 cores, 2·59 cm dia. and approximately 22 cm deep. Each sample was mixed by sieving five times and subsampled to

leave about 1 kg which was stored at $-8°$ to $-10°$ for four weeks prior to analysis.

The field at Great Rollright was divided arbitrarily into four similar-sized strips, each several acres in extent, and a soil sample was obtained over the area of each strip on 20 August, 1963.[15] In the Wellesbourne experiment, duplicate samples were taken from the central 15 × 15 yd area of each plot on 10 October, 1963.

Earthworms

Samples were obtained by hand-digging the soil to a depth of 15–20 cm. In the laboratory, they were quickly sorted into species, immature forms being discarded, and rinsed in tapwater, excess water being removed with filter paper before they were counted and weighed. Residue concentrations were calculated on the basis of these fresh weights. The samples were stored at $-8°$ to $-10°$ for not more than two weeks pending analysis.

Samples of *Lumbricus terrestris* L. (totalling 29 worms of mean weight $3{\cdot}22 \pm 0{\cdot}200$ g) and *Allolobophora longa* Ude (95 worms of mean weight $1{\cdot}05 \pm 0{\cdot}028$ g) were obtained from the four strips of the field at Rollright on 2 October, 1963. In addition, a 10 g sample of *Octolasion cyaneum* (Sav.) (5 worms) were obtained from one strip. On 29 October, 1963, samples of *A. caliginosa* (Sav.) (170 worms of mean weight $1{\cdot}05 \pm 0{\cdot}028$ g), *A. chlorotica* (Sav.) (324 worms of mean weight $0{\cdot}17 \pm 0{\cdot}004$ g) and *A. rosea* (Sav.) (92 worms of mean weight $0{\cdot}16 \pm 0{\cdot}010$ g) were collected from the four strips.

From the Wellesbourne experiment, duplicate 25 g samples of *A. longa* and *A. chlorotica* were collected from each dieldrin-treated plot and one of the control plots on 25–28 November, 1963. The samples of *A. longa* contained 9–16 worms and those of *A. chlorotica* 123–153 worms per 25 g, the samples of the latter being particularly uniform in size/weight composition.

Soil moisture

To avoid losses of the more volatile residue components, the soil samples were not air-dried but were analysed in their naturally moist condition. The residue concentrations were calculated on the basis of the dry weights determined from a 50 g portion of each sample dried at 105° to constant weight.

Preparation of extracts

Soil

Extracts were prepared by the same methods used previously for assessing organochlorine insecticide residues in soil by paper partition chromatography.[1] A 50 g portion of each sample was refluxed with 30 ml acetone in a Bolton extractor. The resulting aqueous acetone extract was partly cleaned-up on a cellulose powder column from which an appropriate fraction was collected and allowed to partition with hexane. Further clean-up was effected by passing the hexane extract through a small bed of decolorising charcoal (B.D.H., Poole, Dorset) from which the residues were eluted with hexane, yielding a dilute extract. This was then concentrated to 5 ml and stored at $-8°$ to $-10°$ until required. Suitable duplicate aliquots of this extract were subsequently concentrated under reduced pressure to about 50 μl for application to paper chromatograms. A third aliquot was used for gas chromatography after being subjected to an additional cleanup on a partly de-activated Florisil/3% charcoal column. The paper and gas chromatography systems used for the soil extracts were the same as those described for the earthworm extracts.

Earthworms

The analytical method was devised on the basis of the extraction of a 25 g sample. Except for samples of *A. longa*, those from Great Rollright were generally less than 25 g and the amounts of reagents used to prepare the crude extracts were therefore reduced proportionally.

The frozen earthworm samples were chopped up and placed in a 100 ml vortex beaker (M.S.E. Homogeniser) with 15 g powdered anhydrous Na_2SO_4 and 20 ml purified redistilled acetone. The beaker was kept chilled while the contents were macerated at high speed for three min. 50 + 1 ml redistilled hexane fraction (b.p. 68–70°) was then added (the additional 1 ml compensating for subsequent vapour losses) and the contents of the beaker were stirred slowly on the macerator for two min. After equilibration for 20 min, the contents were again stirred slowly and 25 ml of the supernatant liquid were decanted and transferred to a 100 ml separatory funnel. The extract was washed three times with 50 ml 1% w/w Na_2SO_4 solution and the resulting crude hexane extract was dried by percolation through 2–3 g powdered anhydrous Na_2SO_4. A 2 ml portion of the extract was cleaned-up using a 10 mm diam. three-layer column comprising 0·3 g Celite 545 (Johns-Manville Co. Ltd.), 1·0 g partly deactivated Florisil containing 3% w/w decolorising charcoal (B.D.H.) and 2 g anhydrous granular Na_2SO_4. The Florisil-charcoal mixture was partly deactivated by exposure in a desiccator for at least 24 hours at 52–54% R.H. over a saturated solution of $Mg(NO_3)_2$. The three-layer adsorption column (hold-up volume 2·5 ml) was pre-washed with 10 ml hexane before addition of 2 ml crude hexane extract ($\equiv$1 g worm tissue). This was rinsed into the upper layer with 2 × 0·25 ml hexane without collection of eluent. When drainage ceased, the organochlorine insecticide residues were eluted into a 10 ml calibrated B14 test tube with 10 ml 2 : 1 v/v hexane : benzene mixture. Cleaned-up extracts were stored at $-8°$ to $-10°$ until used first for gas chromatographic analysis (2 × 5 μl) and finally for paper chromatography (2 × 5 ml concentrated to about 50 μl).

Gas–liquid chromatography

The apparatus used was a Shandon FB-4 Ionisation Chromatograph modified to improve oven temperature distribution and injection port performance. The parallel-geometry tritiated foil electron-capture detector was operated at a polarising potential of 5 volts. Both column and detector temperatures were 157° and the injection port was maintained at about 220°. The column was 40 cm × 2 mm i.d. stainless steel, packed with 2½% w/w E301 silicone gum + 1½% w/w Apiezon L grease + 0·4% w/w Epikote 1001 resin on 180–200 mesh Celite. The nitrogen flow was either 60 or 90 ml/min and 5 μl aliquots were injected. An A.E.I. 1 mV 2 sec f.s.d. potentiometric recorder was used throughout.

The column operated with an efficiency equivalent to 400–430 theoretical plates measured at the aldrin peak. Retention times relative to aldrin (137 sec = 100) were 53, 245 and 639 for γ-BHC, dieldrin and *p,p'*-DDT, respectively. The stationary phase mixture had been found to give separations intermediate between those obtained with the usual silicone or Apiezon L columns and hence it is a useful compromise when only one single-column gas chromatograph is available and there is adequate prior or confirmatory information about the identity of the residue components.

Quantitative measurements on the gas chromatograms were based on a linear double-reciprocal relationship found between peak height (h) and the weight of solute injected (w), namely $1/h = a + b/w$ where a and b are the intercept and slope constants, respectively. Under given operating conditions, this relationship was found to hold over the full range of the recorder response so that the non-linear quantitative response commonly associated with peaks exceeding 60–70% scale deflection on the recorder[17] could be taken into account and the useful working range extended correspondingly. To facilitate the use of this transformation, J. A. Nelder (N.V.R.S. Statistics Section) devised a computer programme to accept peak height data in the random order of injections, to fit standard curves, to indicate their precision and to calculate the concentrations of solutes present in the extracts. The gas chromatograph results presented here have all been calculated in this way.

Paper chromatography

The method was similar to the ascending aqueous system described by Evans[18] using pre-washed Whatman No. 3 chromatography paper impregnated above the origin line with 10% v/v light paraffin B.P. in diethyl ether. Appropriate volumes of standard solutions and aliquots of extracts were concentrated under reduced pressure (250 mm Hg, 57°) to about 30–50 μl in 5 ml conical-bottomed B14 test tubes. 2–3 μl of the ether–paraffin solution was applied to each origin spot to minimise volatilisation losses, and standards and extract concentrates were spotted in random order by multiple transfer of 2–3 μl volumes, ensuring that the origin spots did not exceed 5–6 mm dia.

The ascending mobile phase was a 70 : 30 v/v acetone : water saturated with paraffin and chilled to about 5°–8° for the first 30 min of the 3½–4 hour development period. The chilling improved sensitivity and resolution by keeping the spots small and well defined. When the solvent front had ascended about 20 cm above the origin line, the paper was dried in warm air (~40°) and sprayed on both sides with Mitchell's silver nitrate–2-phenoxyethanol reagent.[19] After being dried again the paper was rotated for 30 min per side at 55 rev/min around the element of a Hanovia Chromatolite (filter removed) at right angles to the axis of development. This precaution ensured uniform exposure to the u.v. light at any given R_f position. The reflectance density of the spots[20,21] was measured on both sides of the paper with an Evans Electroselenium Ltd reflectometer. The amounts of the residue components in the sample extracts were found by interpolation from standard curves relating mean spot reflectance density to four known levels of reference compounds present on each paper. The limit of detection was about 0·1 μg per spot, corresponding to about 0·2 ppm in worm tissue or 0·005 ppm in soil for extract aliquots used, but the system was most sensitive quantitatively to amounts of 0·5–8 μg per spot. The R_f values for analytical grade specimens of the principal residue components were usually about 0·25, 0·29, 0·35, 0·39, 0·46 and 0·63 for aldrin, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, dieldrin and γ-BHC respectively, the *o,p'*- and *p,p'*-DDT spots being incompletely resolved.

Efficiency of the analytical techniques

The efficiencies of the analytical methods were assessed by adding known amounts of analytical grade compounds to uncontaminated soil or earthworm tissue 1–2 h before extraction and then determining the amounts recovered. The results were corrected proportionally in accordance with the levels of recovery obtained.

The addition of analytical grade specimens of the residue components to soil in amounts equivalent to concentrations in the range 0·04–0·5 ppm had been found to average 87±4% recovery when assessed by paper chromatography.[1] For the gas chromatographic analysis of the soil extracts, the levels of recovery found were 86·0, 93·5, 100 and 77·4% for aldrin, dieldrin, *p,p'*-DDT and γ-BHC respectively. Other work (unpublished) had indicated that the recoveries of *o,p'*-DDT and *p,p'*-DDE from soil were similar to those for *p,p'*-DDT and dieldrin, respectively, namely 100 and 93·5%, when assessed by gas chromatography.

Recoveries were determined for amounts of aldrin, dieldrin *p,p'*-DDT and γ-BHC equivalent to 1 and 3 ppm added to earthworm tissue (*A. longa*). Paper chromatography indicated 104, 106, 99·9 and 116% recovery, respectively; this suggests that differential losses occurred on the origin spots as a result of traces of co-extractives reducing the volatility of the compounds in the earthworm extracts relative to those of standard compounds. The recoveries assessed by gas chromatography (96·8, 101, 95·0 and 102%, respectively) did not differ significantly from 100%, and no corrections were made to results obtained by this method. The importance of an efficient clean-up of extracts was demonstrated during the preliminary investigations into the analytical method for residues in earthworms. Abnormally high 'recoveries' (up to 150%) were experienced when relatively concentrated inadequately cleaned-up extracts were gas chromatographed. This appeared to be caused by co-extracted material, not itself strongly electron-capturing, enhancing the detector response to the residue components (cf. Lovelock[22]).

Calculations and statistical evaluation

As in most field residue data,[23] the variances of the analytical results and derived values were proportional to the mean residue levels. Hence, the analytical data and derived variates were transformed to logarithms before being statistically evaluated. The calculated geometric means given are correct to two significant figures. Standard errors were retransformed and are expressed in a multiplicative form in the tables, rather than as additive percentages which are less appropriate when relatively large standard errors are involved. Critical comparisons of the means should be based on their logarithms, using least significant differences calculated in logarithmic units.

Analyses of variance of the residues in both the soil and earthworm samples showed, as was expected,[23] that the random errors were accounted for mainly by the sampling error components. Random analytical errors contributed negligibly to the variance of the results. The between-sample variance component was used to calculate the standard errors which therefore reflect the sampling rather than the analytical variation.

The ratios of the residue concentrations in the earthworms to those in the corresponding soil samples were derived from the basic analytical data, and the geometric mean values are shown in Tables II and IV as concentration factors. The amounts of residue components in different species of earthworms are of use when their relative importance as residue vectors is assessed, and these have been computed as μg per worm from the measurements of fresh weights and residue

concentrations (Tables III and IV). The above derived variates were calculated from the basic sample data and hence the values given for concentration factors and μg per worm do not necessarily agree *exactly* with those calculable from the summarised analytical results in Tables I and IV.

The soil samples were taken to a depth of 22 cm, although at Wellesbourne the dieldrin was unlikely to have been incorporated deeper than about 9 cm.[24] The concentrations of dieldrin found in these soil samples were therefore corrected by the factor 22/9 to obtain the concentration in the upper 9 cm of soil.

Results

Great Rollright

The residue concentrations in Table I are means of duplicate determinations by both chromatographic methods for the samples from the four strips of the field. Certain residues could be assessed only by gas chromatography. Interfering co-extractives prevented aldrin being determined in extracts of *L. terrestris* by paper chromatography and the resolution of *o,p'*-DDT from *p,p'*-DDT in the earthworm extracts was insufficient for quantitative measurements of the *o,p'*- isomer to be made by this method. Although detectable on paper chromatograms, γ-BHC residues in both soil and earthworm extracts were too small to be measured in this way.

The variation in the residue concentrations in the samples from different parts of the field is reflected in the relatively large standard errors, and very critical comparisons between means are probably not justified. For the earthworm samples, differences between the mean residues (Table I) of less than about 70% for dieldrin, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE and about 45% for γ-BHC are not statistically significant ($P = 0{\cdot}05$) but, nonetheless, certain smaller differences are consistent with general trends and appear to be valid.

The results in Table I show that the residue components did not occur at the same level in all the earthworm species. The largest concentrations were found in *A. chlorotica*, except

TABLE I

Concentrations (ppm) of organochlorine insecticide residues in six species of earthworms from Great Rollright in relation to the residue content of the soil

Except where indicated, the values are averages for duplicate assessments by both paper chromatography and gas–liquid chromatography on extracts of four samples

	aldrin	dieldrin	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDE	γ-BHC
Soil[15]	0·72	0·64	0·63	0·14	0·17	0·004
S.E. of mean (d.f.)	$\overset{\times}{\div}$1·087 (6)	$\overset{\times}{\div}$1·152 (6)	$\overset{\times}{\div}$1·152 (6)	$\overset{\times}{\div}$1·132 (6)	$\overset{\times}{\div}$1·121 (6)	$\overset{\times}{\div}$1·130 (3)
Earthworm species						
L. terrestris	*0·053	1·6	0·54	0·068	0·49	*0·0064
A. longa	0·28	2·2	0·77	0·19	0·38	*0·0060
A. caliginosa	0·52	3·8	1·5	0·35	0·65	*0·011
A. chlorotica	0·98	4·6	2·9	0·72	1·0	*0·013
A. rosea	0·64	3·9	1·6	0·30	0·70	*0·017
†*O. cyaneum*	0·84	2·4	0·67	0·19	0·38	*0·0076
S.E. of means	$\overset{\times}{\div}$1·292	$\overset{\times}{\div}$1·185	$\overset{\times}{\div}$1·192	$\overset{\times}{\div}$1·194	$\overset{\times}{\div}$1·195	$\overset{\times}{\div}$1·130
(d.f.)	*$\overset{\times}{\div}$1·438 (12)	(15)	(15)	(15)	(15)	(15)

* Analysis by g.l.c. only
† One sample; S.E.'s do not apply

TABLE II

Mean concentration factors (ppm in worms/ppm in soil) calculated for the six species of earthworms from Great Rollright

Residue component	aldrin (A)	dieldrin (D)	*p,p'*-DDT (T)	*o,p'*-DDT	*p,p'*-DDE (E)	γ-BHC	A+D	T+E
Species								
L. terrestris	0·073	2·5	0·85	0·50	2·9	1·6	0·97	1·3
A. longa	0·40	3·4	1·2	1·4	2·3	1·5	1·8	1·5
A. caliginosa	0·71	5·9	2·4	2·6	3·9	2·7	3·1	2·7
A. chlorotica	1·3	7·2	4·6	5·3	6·3	3·3	4·0	5·0
A. rosea	0·89	6·1	2·5	2·2	4·2	4·2	3·3	2·9
**O. cyaneum*	1·5	6·4	1·6	2·0	2·5	1·9	3·5	1·8

S.E. (110 d.f.) = $\overset{\times}{\div}$1·282

* 1 sample only; S.E. does not apply

TABLE III

Mean amounts of organochlorine insecticide residues in the six species of earthworm from Great Rollright (μg per worm)

Residue component	aldrin	dieldrin	*p,p'*-DDT	*o,p'*-DDT	p,p'-DDE	γ-BHC	Total
Species							
L. terrestris	0·17	5·2	1·7	0·22	1·6	0·020	8·9
A. longa	0·30	2·3	0·82	0·20	0·40	0·006	4·0
A. caliginosa	0·18	1·4	0·53	0·12	0·23	0·004	2·5
A. chlorotica	0·16	0·77	0·48	0·12	0·18	0·002	1·7
A. rosea	0·097	0·59	0·24	0·046	0·11	0·003	1·1
**O. cyaneum*	1·7	4·8	1·3	0·38	0·76	0·015	9·0
S.E. (110 d.f.) = $\overset{\times}{\div}$1·263							

* 1 sample only; S.E. does not apply

TABLE IV

Mean dieldrin concentrations in the soil (ppm/9 cm depth) and in the two species of earthworms (ppm) from the Wellesbourne experiment
Except where otherwise indicated, the values are averages for duplicate assessments by both paper and gas–liquid chromatography on duplicate samples. The concentration factors and amounts of dieldrin per worm are averages calculated from the results for the individual samples

Nominal dose of dieldrin applied to the soil lb/ac	Dieldrin concentrations			Concentration factors (ppm worm/ppm soil)		μg dieldrin per worm	
	Soil	*A. longa*	*A. chlorotica*	*A. longa*	*A. chlorotica*	*A. longa*	*A. chlorotica*
0	*0·0030	*0·033	*0·028	11	9·3	0·077	0·0053
0·50	0·50	0·70	1·8	1·4	3·6	1·2	0·31
0·75	0·85	1·0	2·0	1·2	2·4	2·9	0·38
1·00	1·1	1·3	2·9	1·2	2·6	2·7	0·51
1·25	1·2	1·3	2·1	1·0	1·8	3·3	0·36
S.E. of means (d.f.)	$\overset{\times}{\div}$1·116 (3)	$\overset{\times}{\div}$1·134 (3)	$\overset{\times}{\div}$1·134 (3)	$\overset{\times}{\div}$1·108 (11)	$\overset{\times}{\div}$1·108 (11)	$\overset{\times}{\div}$1·109 (11)	$\overset{\times}{\div}$1·109 (11)
	* $\overset{\times}{\div}$1·169 (1)	* $\overset{\times}{\div}$1·227 (2)	* $\overset{\times}{\div}$1·227 (2)				

* Analysis by g.l.c. only

possibly for γ-BHC in *A. rosea*, although this difference was not statistically significant (P = 0·05). In general lower concentrations of residues of dieldrin, *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and γ-BHC occurred in the larger species (*L. terrestris*, *A. longa* and *O. cyaneum*) than in the smaller species (*A. caliginosa*, *A. chlorotica* and *A. rosea*), the tendency being apparently less marked for *p,p'*-DDE, and possibly also for dieldrin, than for the other components.

As a consequence of the large natural variation in residues in both soil and earthworm samples, the standard errors of the derived concentration factors shown in Table II are correspondingly large. Only differences greater than two-fold between concentration factors for the individual residue components (Table II: columns 1–6) were statistically significant (P = 0·05) but certain smaller differences may be valid since they conform with general trends in the data. The concentration factors for the individual components were in the order dieldrin > *p,p'*-DDE ≫ *p,p'*-DDT = *o,p'*-DDT = γ-BHC ≫ aldrin for all species except possibly *L. terrestris*. However, by grouping the analytical results for those components which are obviously interdependent in the system, namely aldrin + dieldrin and *p,p'*-DDT + *p,p'*-DDE, and calculating the concentration factors for these groups (Table II: columns 7 and 8), it is apparent that only minor differences occurred in the uptake and overall behaviour in the residues from the three types of insecticide present (aldrin–dieldrin, DDT and γ-BHC). Consistent differences in the extents to which the different earthworm species stored the residues are also made more obvious by grouping the components, the concentration factors being generally greatest for the three smallest species, particularly *A. chlorotica*. The relatively large concentration factors for aldrin + dieldrin in the single small sample of *O. cyaneum* appears to be an exception, but this needs to be confirmed.

The amounts of the individual residue components were mainly dependent on the mean weights of the species, although the results for aldrin and *o,p'*DDT residues in *O. cyaneum* were anomalous in this respect. Thus the residue contents were generally *L. terrestris* = *O. cyaneum* > *A. longa* > *A. caliginosa* > *A. chlorotica* > *A. rosea*. The total residue contents ranged from about 1 μg in *A. rosea* to about 9 μg per worm for *O. cyaneum* and *L. terrestris*, the greatest amount of any one component being 5·2 μg dieldrin in the latter species.

Wellesbourne

The background levels of dieldrin in the soil from the untreated plot were detectable, but not measurable, by paper chromatography, and the small concentrations of dieldrin in the corresponding earthworm samples could be detected and measured only by gas chromatography.

As in the samples from Great Rollright, dieldrin concentrations were greater in *A. chlorotica* than in *A. longa* from all the treated plots (Table IV). The concentrations of dieldrin in both species tended to increase with increasing concentration in the associated soil but they were relatively less at the higher than at the lower concentrations. This is reflected in the trends of the concentration factors (Table IV) which declined systematically with increasing dieldrin concentrations in the soil.

The residue contents for average specimens of *A. chlorotica* (Table IV) were generally one-third to one-tenth those for corresponding samples of the larger species and, as would be expected, the greatest amount of dieldrin per worm (3·3 μg) occurred in the samples of *A. longa* from the soil containing 1·2 ppm dieldrin. The reason for the unusually low residue in *A. chlorotica* from this plot is not known, the average weights of the worms in these samples being comparable with those from other plots so that samples themselves did not appear to be obviously atypical.

Discussion

Small amounts of soil remaining in the alimentary tracts of the earthworms may have led to slight underestimates of the differences between the residue concentrations in the tissues and in the soil. This is unlikely to have been an important source of error, except possibly for the aldrin, which is transient in other animals because it is not stored as such but is rapidly converted into dieldrin.[25] The residual gut-soil may therefore have contributed appreciably to the aldrin found in the earthworms from Great Rollright. Davis & Harrison[2] also recorded only low concentrations of aldrin in earthworms from arable soils, and Raw[12] mentioned dieldrin, but not aldrin, as a residue in earthworms from an aldrin-treated soil. Such evidence indicates, therefore, that aldrin is rapidly epoxidised to dieldrin by earthworms, as by other animals.

As a corollary, dieldrin derived from aldrin should enhance the dieldrin content of animals exposed to mixed residues of the two pesticides. In such circumstances, the concentration factor for dieldrin would appear to be greater than that for animals exposed solely to dieldrin residues at comparable concentrations. This is supported by the fact that the dieldrin concentration factors for *A. longa* and *A. chlorotica* from Great Rollright were two to three times larger than those for the same species at Wellesbourne. However, when the combined aldrin + dieldrin residue was the basis for comparison at Great Rollright, the difference in the concentration factors at the two sites was less marked and not statistically significant.

The calculation of absolute concentration factors depends on a true assessment being obtained of the actual or effective residue concentration in the environment, in this case soil. Although no analytical information was obtained on the depth distribution of the residue components at Great Rollright, it is a reasonable assumption that applications of aldrin or dieldrin to the field in the early summer of 1963 would have left higher concentrations of residues in the upper than in the lower part of the sampled depth. The average aldrin–dieldrin concentrations in these soil samples may thus have been underestimates of those actually present in the upper layer of the soil and, as a result, the concentration factors may have been overestimated. At Wellesbourne, the dieldrin should have been evenly distributed to a depth of about 9 cm,[24] and the concentration factors calculated for the earthworms from this experiment are therefore likely to be more reliable as absolute values than are those for the Great Rollright samples.

As a further complication to the difficulties of assessing concentration factors, the Wellesbourne results show that these decreased with increasing concentrations of dieldrin in the soil. A graphical examination of these results suggested that the logarithm of the dieldrin concentrations in the earthworms (log W) was linearly related to the logarithm of the concentration in the soil (log S) and hence that $W = aS^b$ where log a and b are the intercept and slope of the line drawn on log–log co-ordinates. The concentrations of aldrin + dieldrin, *p,p'*-DDT + *p,p'*-DDE and of γ-BHC in the earthworms from Great Rollright were also found to approximate closely to the trend established for the Wellesbourne data. The results of Stringer & Pickard[11] for DDT and those of Davis & Harrison[2] for γ-BHC appeared to permit valid comparisons to be made between residue concentrations in earthworms and in associated soil, and these results also agreed closely with, and extended, the relationship. A line was fitted by the method of least squares to 90 points taken from the Great Rollright and Wellesbourne results combined with those of Stringer & Pickard[11] and Davis & Harrison[2] discussed above. For clarity, only the geometric mean values for the sets of data used are shown in Fig. 1 but the 95% confidence limits calculated for individual observations (not the fitted line) indicate the limits within which 95% of further samples would be expected to lie. The species

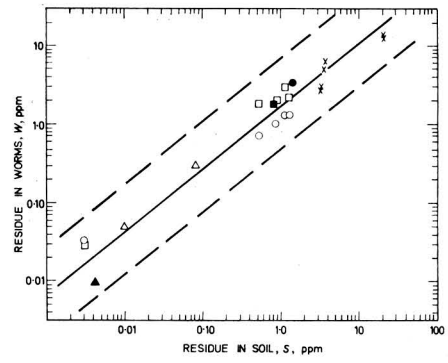

FIG. 1. *General relationship between the concentration of organochlorine insecticides in earthworms and in associated soil, calculated from 90 paired values.*

Geometric mean values for sets of data:
○ = Wellesbourne expt.; dieldrin; *A. longa*
⊡ = Wellesbourne expt.; dieldrin; *A. chlorotica*
● = Great Rollright; aldrin + dieldrin
■ = Great Rollright; *p,p'*-DDE
▲ = Great Rollright; γ-BHC
× = Stringer and Pickard[11]; DDT
△ = Davis and Harrison[2]; γ-BHC
– – – = 95% confidence limits for individual samples
Equation for the relationship: log $W = 0{\cdot}268 + (0{\cdot}798 \pm 0{\cdot}072) \log S$

differences observed in this work indicate also that results for the shallow-living species would tend to fall above, and those for the deeper-living species below, the fitted line.

In view of the composite nature of the data, the trend is remarkably consistent over four orders of magnitude of residue concentrations in the soil. The slope of the fitted line ($b = 0{\cdot}794 \pm 0{\cdot}032$) is appreciably less than unity, residues tending to be relatively greater in the earthworms when the concentrations in the soil are low than when higher concentrations are present, substantiating the results of the Wellesbourne experiment. It is apparent from Fig. 1 that concentration factors would be expected to change from about 5 to 10-fold when residue concentrations in the soil are 0·001–0·01 ppm to less than unity when concentrations exceed about 10 ppm in the soil; this is also evident from Stringer & Pickard's results for the uptake of DDT by earthworms in orchards. When high residue concentrations are encountered by earthworms, it is possible that their metabolic degradation and excretory mechanisms may be stimulated into relatively greater activity than when only low concentrations are present. The mechanism of uptake and storage may thus be to some extent self-regulatory.

The consistent differences in uptake of residues between the earthworm species can be accounted for by known differences in their habits in relation to the position of the residues in the field. The largest concentrations of residues were found in the small species, *A. caliginosa*, *A. chlorotica* and *A. rosea*, which live and feed mainly in the upper few inches of soil[14] where residues are likely to be most concentrated in both arable and orchard sites. In contrast, *L. terrestris* lives in well defined burrows frequently 4–6 feet deep and probably feeds almost entirely on surface debris.[14] Residues in this species are thus likely to be greatest when high concentrations occur over the soil surface or in plant litter, conditions typical of grassed orchards of the type in which residues in *L. terrestris* have been found to be similar to those in associated 'other species'.[11] Under arable conditions, this species may have relatively little contact with residues incorporated shallowly into the soil and hence it should not acquire residues to the same extent as the more shallow-living species. Furthermore, the latter are not only in more continuous contact with residues but they also probably ingest soil more freely when feeding. Residues were intermediate in *A. longa* and *O. cyaneum*, the former living mainly in the upper 12 inches of soil and the latter having temporary but well defined burrows about 6 inches below the surface.

The foregoing results and discussion emphasise the importance of defining carefully the composition and distribution of residues in an environment and of taking into account the habits of species when investigating problems of residue uptake and storage equilibria. A history of pesticide usage and of cultivations can only be a guide to residue distribution, and it is probable that the earthworms themselves may be better indicators of the effective residue concentrations in their environment than would simple soil samples taken only to one specified depth.

Acknowledgments

The authors are indebted to the late Dr. F. Raw for instruction in the identification of the earthworm species and also for confirming the identity of the species analysed. Mr. T. H. Coaker kindly made available the facilities provided by the Wellesbourne experiment. The invaluable technical assistance of Mrs. C. M. Ackley and Mrs. C. E. Padbury is gratefully acknowledged and we wish to thank Mr. J. A. Nelder, Mr. G. Berry and Mr. J. T. Wood for advice on statistical matters.

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IDENTIFICATION OF FISH SPECIES BY THIN-SLAB POLYACRYLAMIDE GEL ELECTROPHORESIS OF THE MUSCLE MYOGENS

By W. P. COWIE

Electrophoretic band patterns for twenty commercially important British fish species have been obtained by electrophoresis of their muscle myogens on thin slabs of polyacrylamide gel. Since each band pattern is characteristic of one species and is practically unaltered as a result of ice storage or frozen storage of the fish, it is possible to identify any fresh and frozen fillet belonging to these twenty species by comparing its band pattern with the standards obtained. The method can also be used to identify two species of fresh fish in admixture.

Cooked fish cannot be identified by this method.

Introduction

Frozen fish fillets are being used increasingly in the preparation of fish products and it is often necessary to identify the species of fish being used in order to conform with labelling laws. Identification on the basis of visual appearance alone might be impossible especially if the fillets have been skinned. A reliable, easy and rapid method is therefore needed. Several papers have described such methods, all of which involved electrophoresis of the water-soluble proteins (myogens) extracted from the raw fish flesh. The different media used for the electrophoresis have included starch gel,[1,2] polyacrylamide gel,[3,4] cellulose acetate strips[5] and agar gel.[6] In each case the protein band patterns given by extracts from fish of known species, identified by the external characteristics of the whole fish, were established under known electrophoresis conditions. The pattern given by extracts from the unknown fillet under the same conditions was then compared with the standards, and identification was then possible because each species gave a unique protein band pattern.

In none of the above publications was a systematic study undertaken to find out if either ice storage or frozen storage of fish resulted in changes in the electrophoretic protein band patterns. If storage of the fish were to result in gross changes in the band patterns, then any electrophoresis technique would have limited commercial application for species identification. It has already been shown[7] that starved and unstarved cod gave very similar electrophoretic band patterns, indicating that seasonal variation in the composition of the fish muscle will not invalidate this method of species identification. In this work the electrophoretic band patterns given by twenty commercially important British fish species have been established, and the effect on these patterns of ice storage and frozen storage of the fish has been investigated for some of the species. The new method of thin-slab polyacrylamide gel electrophoresis developed by Akroyd[8] has been employed throughout this work.

Experimental

Apparatus and method of electrophoresis

The construction of the glass cell and the buffer compartments used for the electrophoresis has been fully described by Akroyd.[8] The preparation, running, staining, washing and photography of the gels closely followed the instructions detailed in that report.

10% polyacrylamide gels prepared in Tris–citrate buffer (76 mM 2-amino-2-(hydroxy methyl)-propane-1 : 3 diol titrated to pH 8·6 with citric acid) were used throughout this work. Borate buffer (26 mM boric acid titrated to pH 8·9 with sodium hydroxide) was used in the electrode compartments. This gave a discontinuous system of buffers for the electrophoresis which lasted for approximately two hours for each run at a constant current of 50 mA, and voltage of 500 V. Under these conditions the fish muscle proteins migrated downwards through the gel towards the anode.

Preparation of muscle extracts

Fish muscle was extracted with distilled water (1 : 1, w/v) in a vortex beaker using a top-drive M.S.E. homogeniser. The homogenate was centrifuged at 7,000 g for 30 minutes at 0°, and the clear aqueous supernatant was decanted off. 1 ml of the extract was treated with a few drops of a saturated sucrose solution containing a trace of Amido Black B to act as a marker for the electrophoresis run. 10 μl of the coloured dense extract were pipetted into position between the sample spacers on the top of the gel. Up to 16 samples could be accommodated with ease in each run.

Fish species examined

Fresh gutted whole fish (1–5 days on ice) of the following species were obtained from Aberdeen Fish Market: cod (*Gadus morhua* L.), haddock (*Gadus aeglefinus* L.), lemon sole (*Pleuronectes microcephalus* Day), plaice (*Pleuronectes platessa* L.), coal fish (*Gadus virens* L.), hake (*Merluccius merluccius* L.), witch (*Glyptocephalus cynoglossus* L.), tusk (*Brosme brosme* Müller), dory (*Zeus faber* L.), whiting (*Gadus merlangus* L.) ling (*Molva molva* L.), megrim (*Lepidorhombus whiff-iagonis* Walbaum), dab (*Limanda limanda* L.), pollock (*Gadus pollachius* L.), squid (*Loligo forbesi*), skate (*Raja batis* L.), redfish (*Sebastes marinus* L.), gurnard (*Trigla cuculus* L.), dogfish (*Acanthias vulgaris*), and monk fish (*Squatina squatina* L.).

Effect of ice storage of fish on electrophoretic band pattern

Four cod, haddock, lemon sole and plaice were obtained in a very fresh condition (1 day on ice) from Aberdeen Fish Market. They were kept stored in ice in a chill room. At weekly intervals further batches of similar fish were purchased and stored in ice. The electrophoretic band patterns of four lots of four fish of each species stored in ice for 0, 1, 2 and 3 weeks, respectively, were then determined in four separate electrophoresis runs.

Effect of frozen storage of fish on electrophoretic band pattern

Fresh haddock fillets were blast-frozen and wrapped closely in aluminium foil, and different samples were stored at $-7°$, $-14°$ and $-29°$ for different periods. The electrophoretic band patterns given by these samples and by fresh unfrozen haddock were determined simultaneously.

A similar experiment was carried out using lemon sole and plaice.

Results

Identification of individual species

Fig. 1 shows the electrophoresis patterns given by fresh unfrozen samples of fourteen different species of commercially important British sea fishes. Each species gave a unique protein band pattern which could be reproduced from run to run.

Fig. 2 shows duplicate band patterns given by six other different species. The duplicate separations are identical but separations given by each species are unique to that species.

Identification of two species in admixture

If equal quantities of two species of fresh fish are present in a product, then it is possible to identify those species. Fig. 3 shows that the electrophoretic protein band pattern of a mixture of species is equivalent to that obtained by superimposing the band patterns given by the individual species. It should be noted that, since the faster-moving proteins in some of the 15 μl samples have migrated off the gel, certain bands are missing from the patterns given by those samples.

Fresh and ice-stored fish

The patterns given by fresh cod, haddock, plaice and lemon sole were also given by samples of these species which had been stored in ice for 1, 2 and 3 weeks (Fig. 4 illustrates the result for haddock). Thus it is possible to identify fish species by this technique even though the fish have been held in ice for a period representing maximum shelf-life. There are minor variations in the intensities of certain bands from different fish of the same species, but the basic pattern of bands characteristic of these species can always be distinguished irrespective of how long they have been stored in ice.

Fresh and frozen fish

Figs 5 and 6 show that haddock, lemon sole or plaice frozen-stored for different periods at different temperatures give band patterns practically identical to those given by their fresh unfrozen counterparts. There may be some variation in the intensity of some of the bands in the frozen samples but the patterns of bands do not change appreciably.

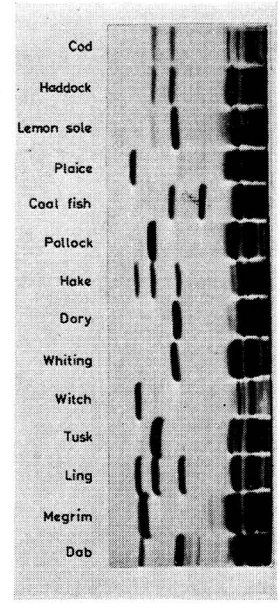

FIG. 1. *Electrophoresis patterns for 14 samples of fresh unfrozen fish*

In Figs 1–6, direction of migration, from origin to +, is from right to left of photographs

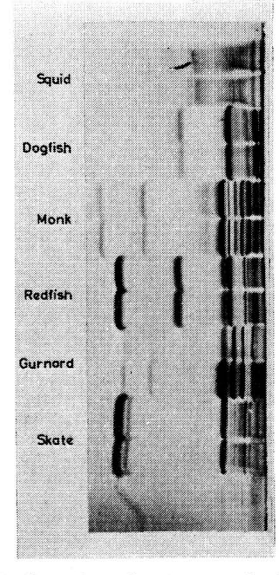

FIG. 2. *Duplicate electrophoresis patterns for 6 fish species*

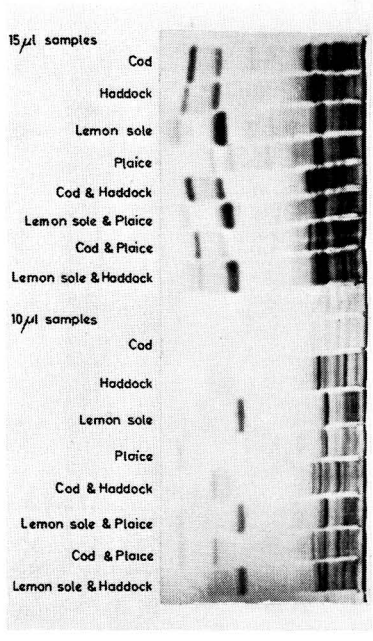

FIG. 3. *Electrophoresis patterns of 4 fish species individually and in admixture*

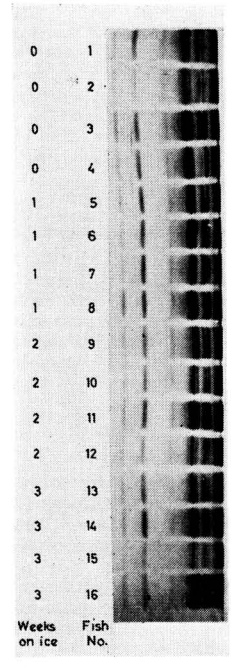

FIG. 4. *Electrophoresis patterns for haddock stored in ice for 0, 1, 2 and 3 weeks*

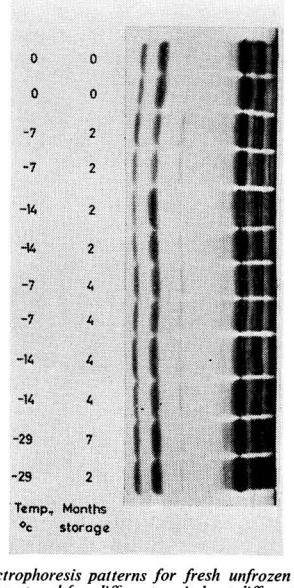

FIG. 5. *Electrophoresis patterns for fresh unfrozen haddock and haddock frozen stored for different periods at different temperatures*

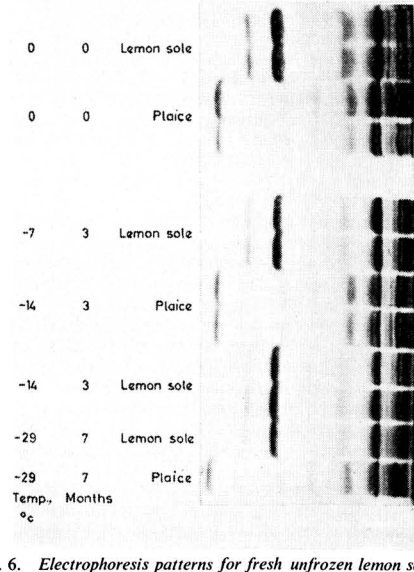

FIG. 6. *Electrophoresis patterns for fresh unfrozen lemon sole and plaice and for these species frozen stored for different periods at different temperatures*

Discussion

This work shows that extracts from different species of commercially important British fish separated under identical electrophoresis conditions side by side on the same gel, give different patterns, each pattern being characteristic of that species. Thus it is possible to distinguish cod and other varieties such as coal fish, tusk, ling or pollock which may be substituted for cod in a fish product. Likewise, megrim and witch can be distinguished from lemon sole, the myogen electrophoretic band pattern for which has not previously been reported.

Identification of fish species is thus very easily accomplished using the polyacrylamide thin slab technique since it is possible to separate simultaneously on one gel extracts from the unknown sample and from known species. Direct visual comparison of the electrophoresis patterns thus obtained affords a reliable means of identification. Another method is to add a pure protein such as bovine serum albumin to the extract of the unknown sample and to measure the migration of each of the bands of the unknown sample relative to this added protein. These relative migrations can be established for all the different species likely to be encountered, and by comparison of the values for the standard sample with those of the unknown, identification is possible. The same procedure can be used to identify two species in admixture.

The fact that ice-stored and frozen-stored fish give the same patterns as fresh unstored fish means that this technique has wide commercial application as a reliable method for fish species identification.

Since cooking denatures proteins, cooked fish cannot be identified by this technique.

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GEL FILTRATION ON SEPHADEX® LH-20.—A GENERAL CLEAN-UP METHOD FOR PESTICIDES EXTRACTED FROM GRAIN

By D. F. HORLER

Gel filtration on Sephadex LH-20 in ethanol was shown to be a practical method for the single stage clean-up of insecticide residues from grains. The advantage over previous column clean-up procedures is that one set of conditions is applicable to most insecticides. The process is thought to be particularly applicable where routine screening procedures are operated. The clean-up is adequate for thin-layer chromatography.

Introduction

In analysis for the pesticide residues that may contaminate grain when it is grown and stored, several column materials have been used for the 'clean-up' stage, notably Florisil, Celite, alumina, activated charcoal and silica.[1,2] Generally these materials have the disadvantage that the conditions vary from one pesticide to another since their effectiveness depends upon the chemical properties of the insecticide.

It was thought that gel filtration might offer possibilities as a general method of clean-up because hydrophylic gels enable the separation of solutes in aqueous solution on a basis of molecular size.[3–7] Most pesticides have molecular weights in the range 200–600. Gel filtration on Sephadex G has been widely used in biochemical work over the past few years.

Recently the introduction of the alkylated Sephadex LH-20

has enabled this technique to be extended to organic solutions. Because of the limited range of molecular sizes it was expected that most pesticides would elute from Sephadex LH-20 in the same region. Grains contain a high percentage of polymeric material and it was thought that gel filtration might be a feasible method for the clean-up of grain extracts.

Experimental

Column

Sephadex LH-20 was left to swell overnight in absolute ethanol. The gel was then poured into a glass column, the outlet of which was plugged with cotton wool. When most of the gel had been added the upper glass joint was put in position and the rest of the gel was added through the inlet tube until the gel filled the column. About 40 g of gel filled a tube 15 × 3·7 cm. This bed volume was used throughout. The flow rate was maintained at 2 ml/min by use of a screw clamp. A head of 30 cm ethanol was used.

Extracts

No attempt was made to use an optimum extraction procedure because the problems of extraction were not being considered. A standard procedure was used for all samples. 25 g of the ground or crushed material was macerated in an MSE macerator for 3 minutes with 100 ml n-hexane. The suspension was filtered and washed with a further 2 × 50 ml n-hexane and the filtrate and washing were evaporated in a stream of air in a 250 ml flask. The residue was dissolved in 2–5 ml ethanol and transferred quantitatively to the column. It was then passed through the column in ethanol. Generally 10 ml fractions were collected. Insecticide (50–200 μg) was added to the residue before dissolution in ethanol. The weight of material in each fraction was determined by evaporating aliquots at 100°.

The insecticides were determined by g.l.c. using an electron capture detector.

Materials

Wheat and barley were harvested in England in 1966, maize in South Africa in 1966, sorghum in Nigeria in 1961, and white rice and paddy in British Guiana in 1964.

Sephadex LH-20 was obtained from Pharmacia of Sweden.

Results

Fig. 1 shows the distribution of the weight in each fraction for grain extracts.

Fig. 2 shows a typical distribution of three insecticides in the presence of barley. The separations are good. Table I shows the percentage clean-up, i.e. the percentage of plant material eluted before that particular insecticide starts to elute from the column, and the elution volume for a range of insecticides.

Other plant materials examined, pimento, copra, cocoa beans, apples, carrots, haricot and broad beans did not give satisfactory clean-up.

Apart from α-allethrin (56%) and diazinon (78%) the clean-up for all the others examined was above 90%. All the insecticides were recovered quantitatively from the column. There was no indication of irreversible adsorption on to the gel.

The positions of the eluted insecticides were constant from one column to another as was the weight distribution of the grain extracts. Only one determination was done for the

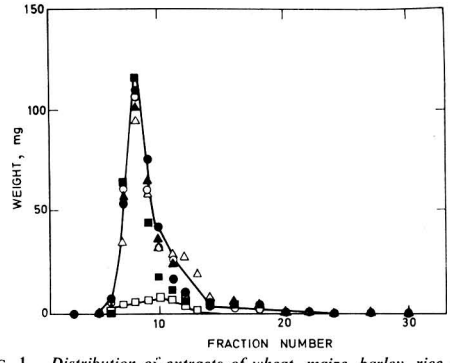

FIG. 1. *Distribution of extracts of wheat, maize, barley, rice and sorghum on a 15 cm × 3·7 cm diameter column of Sephadex LH-20 in ethanol*

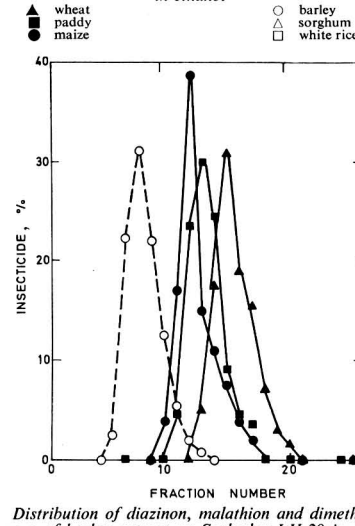

FIG. 2. *Distribution of diazinon, malathion and dimethoate in the presence of barley extract on Sephadex LH-20 in ethanol*

● diazinon
■ malathion
▲ dimethoate
○ barley

TABLE I

Percentage clean-up of insecticides from barley extract on 15 × 3·7 cm column Sephadex LH-20

Insecticide	% Clean-up	Peak elution vol. ml.
Malathion	91	130
Diazinon	78	120
Dicapthon	>99	165
Fenchlorphos	96	142
Fenitrothion	>99	165
DDT	99	142
Heptachlor	91	130
Lindane	>99	165
Dieldrin	96	155
Endrin	91	150
Carbaryl	>99	157
α-Allethrin	56	105
Dimethoate	99	150

other vegetable extracts. For different columns of similar sizes, there was no difficulty in obtaining agreement for the peak elution volumes to within less than 10 ml.

Increasing the sample volume above 5 ml caused peak broadening; below this, sample volume had very little effect. Adding lindane separately in 5 ml dimethyl formamide, benzene and acetone caused the insecticides to elute in a band from fraction 5–15.

Very little contamination of the columns occurred with grain extracts. Any that did occur affected the top layer, which could periodically be removed and replaced with fresh gel.

Discussion

These results show that Sephadex LH-20 can be used to clean-up insecticide residues in grain extracts. The clean-up is efficient enough for the residue to be concentrated for thin-layer chromatography.

Although this technique has been considered essentially as a method of separation, the positions of the peaks give some indication of the nature of the insecticide. If the ratio elution volume/total column volume (V_e/V_t) is plotted against $\log_{10}$ molecular volume, most of the insecticides examined fall on a straight line (Fig. 3). This is similar to the results obtained with proteins on the Sephadex G series.[3]

In order to consider the theoretical side more fully it would be necessary to know more of the properties of the gel. In particular the value for the void volume needs to be determined. In the G series a blue dextran of high molecular weight is used to measure this quantity. This substance is not soluble in ethanol so that it has not been possible to use it. The manufacturers give the excluded weight for Sephadex LH-20 as about 4,000. From the distribution of the plant extracts it would seem that the void volume for this size column is 60–70 ml. The total volume is 170 ml. Extrapolation of the straight line on Fig. 3 to a V_e/V_t of 0·38, i.e. $V_e = 65$, gives an exclusion limit of about mol. wt. 2,000 for the gel in ethanol. Various materials are being investigated as possible markers to determine void volumes for this material.

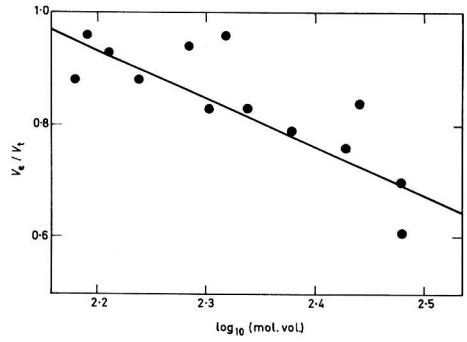

FIG. 3. *Plot of ratio of elution volume/total column volume (V_e/V_t) against log (molecular volume) for each insecticide*

The separation of the peaks of different insecticides suggests the use of Sephadex LH-20 or a similar gel for automatic analysis of mixtures of pesticide. This possibility is being examined.

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ERRATA

In the paper by Sawyer *et al.*, *J. Sci. Fd Agric.*, 1967, **18,** 283,

On page 284 rt hand column lines 12, 13 and 16, the quantities of reagents shown as 5 ml additions should read as 2 ml additions

i.e. '2 ml zirconium reagent'
'2 ml solochrome cyanine R reagent'
'2 ml dye reagent'

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(Revised 1965)

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Experimental.—The methods and materials used should be clearly stated in sufficient detail to permit the work to be repeated if desired. Only new techniques need be described in detail, but known methods should have adequate references.

Results.—These should be presented concisely, using tables or illustrations for clarity. Adequate indication of the level of experimental error and the statistical significance of results should be given.

The number of illustrations and graphical chemical formulae used must be kept to a minimum and only in exceptional cases will tables *and* graphs derived from them be accepted for publication.

Discussion.—In general the discussion and interpretation of results should follow their presentation, in a separate section.

Conclusions.

Acknowledgments (*see* VI).

Address where the work was carried out.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

APRIL, 1968

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Green humic acid and its possible origin as a fungal metabolite. K. Kumada and H. M. Hurst (*Nature, Lond.*, 1967, **214,** 631–633).—P-type humic acid (**I**) (cf. *J. Soc. Soil and Manure, Japan*, 1965, **36,** 373) has now been isolated from $A_{1,2}$ and B_{1-3} soil horizons in Britain and presence of the green pigment, identical with that found up to 10% in Japanese soil, is confirmed. This green **I**, probably a perylene quinone, is obtained simply as the second fraction in the elution (with 0·1 N-NaOH) of a column of Sephadex G-25 through which **I** extracted from soil are passed. The pure pigment has λ_{max} at 430, 450, 570 and 615 nm in aq. NaOH; its properties are described and it is suggested that it is not chemically an intrinsic part of the brown **I** complex. Since it has also been isolated from fungal sclerotia, this green component is probably a fungal metabolite. Its importance in the study of soil **I** is discussed. (10 references.) W. J. BAKER.

Selectivity characteristics of montmorillonite clay. A. Chatterjee and S. K. Mukerjee (*J. Indian chem. Soc.*, 1966, **43,** 673–678).—Exchange reactions are carried out between H-resin and varying concn. of montmorillonite clays saturated with Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+} and Ba^{2+} in aq. media, to show that the selectivity coeff. decrease with increased clay suspension concn., the decrease being most pronounced with K^+-saturated clays. It is considered that the selectivity coeff. depends on the affinities of the ions towards the exchanger and the decrease in value with clay concn. is due to the overlapping of the electrical double layer resulting in the immobilisation of ions. Experiments carried out in 50% aq. EtOH further reduced the selectivity coeff., particularly in the case of Na^+ and K^+, probably due to the low dielectric const. of aq. EtOH. S. D. HUGGINS.

Diffusion of potassium from mica-like clay minerals. J. H. Chute and J. P. Quirk (*Nature, Lond.*, 1967, **213,** 1156–1157).—The rate of release of K from the fraction of Fithian illite less than 2 μ and an illite from Tumut, N.S.W.; has been measured. Samples were shaken in 0·3 and 0·03 N-NaCl at 20°, 40° and 60° and the solution analysed for K with a flame photometer. The mean diffusion coeff. calculated from the results were $7{\cdot}7 \pm 2{\cdot}8 \times 10^{-20}$ and $1{\cdot}1 \pm 0{\cdot}5 \times 10^{-20}$ cm²/sec for Fithian illite in 0·3 and 0·03 N-NaCl respectively at 60°; those for Tumut illite were the same for 0·3 N-NaCl and a factor of two higher for 0·03 N-NaCl. These values are comparable with those for H_2 in Ni or Fe hydroxides but 10 times lower than for K release from biotite. S. A. BROOKS.

Sources of error in advisory soil tests. II. Temporal field variance. N. S. Mountier and C. During (*N.Z. Jl agric. Res.*, 1966, **9,** 964–971).—In the Ca and Truog P quick test the long-term laboratory by soil variance was by far the most important component. In pH and in the K soil quick test, however, field temporal variance was appreciable on improved and grazed pastures but not on mown pastures. (11 references.) E. G. BRICKELL.

Sources of error in advisory soil tests. III. Spatial variance. IV. Discussion of total variance. N. S. Mountier and C. During (*N.Z. Jl agric. Res.*, 1967, **10,** 134–138, 139–142).—III. On Marton loam, variance was higher under grazing for pH and K, but not for P and Ca soil tests. On Judgeford silt loam the spatial variance of the K test was higher on samples taken when the pasture was being grazed by sheep than on samples taken two or more weeks after grazing.

IV. Different sources of variation in soil tests estimated in the previous paper are discussed. Increasing the no. of core samples taken gives only a slight improvement in precision. E. G. BRICKELL.

Effect of freezing on the free sugars in soil. K. C. Ivarson and U. C. Gupta (*Can. J. Soil Sci.*, 1967, **47,** 74–75).—Freezing soils for three weeks at −14° caused a 2-5-fold increase in glucose and a 2–3-fold increase in mannose and low R_F compounds. Pentoses increased considerably in a peat. Identification was achieved by using aniline phthalate spray on paper chromatograms. A. H. CORNFIELD.

Effect of herbicides on soil algae. L. S. Balezina (*Mikrobiologiya*, 1967, **36,** 163–167).—Na trichloroacetate (**I**) has little effect under field conditions in autumn but in spring before cultivation the number of algae is reduced three times. Under laboratory conditions of high constant moisture level **I** stimulated the algae, the effect increasing at higher **I** doses. Dicotex-80 and kresonite stimulated algal development especially the latter at dose levels of 2 kg/ha. Simazine (2 kg/ha) was highly toxic, especially for blue-green algae. (16 references.) (From English summary.) R. J. M.

Atmospheric nitrogen fixation by hydrocarbon-oxidising bacteria. V. F. Coty (*Biotechnol. Bioengng*, 1967, **9,** 25–32).—*Pseudomonas*, *Mycobacterium* and *Azotobacter* utilising the hydrocarbons methane, n-butane, n-tetradecane, toluene and a naphthenic acid (cyclohexane carboxylic acid) have been grown in the presence of $^{15}N_2$. A gain of 5–10 μg/ml was taken as acceptable evidence for N fixation. Results indicate a wide range of hydrocarbon-oxidising bacteria able to fix N at levels of 20 to 130 μg/ml. This explains high N in soils exposed to gas leaks, and suggests the possibility of applying petroleum residues to soil to increase N fixation. J. B. WOOF.

Occurrence of microbial forms of unusual morphology in European and Asian soils. S. W. Orenski, V. Bystricky and K. Maramorosch (*Contr. Boyce Thompson Inst. Pl. Res.*, 1966, 2069, 3 pp).—Specimens from cultures derived from all the European soils and from most of the Asian soils collected, revealed the presence of 'polysperoids' in varying numbers. E. G. BRICKELL.

Soil fumigation for control of *Verticillium* wilt of eggplant. C. D. McKeen and W. B. Mountain (*Can. J. Pl. Sci.*, 1967, **47,** 1–10).—The results of treating a sandy loam soil, highly infected with *Verticillium dahliae*, with six soil fumigants showed that *Verticillium* wilt of eggplant is effectively delayed and reduced in incidence. Vorlex (methyl isothiocyanate 20% : 1,3-dichloropropene and related compounds 80%), and EP201 (chloropicrin 17% : Me isothiocyanate 15% : 1,3-dichloropropene and related compounds 68%) were generally the most effective fumigants and in addition markedly reduced the numbers of weeds in the plots. Yield increases due to the treatments varied from year to year and ranged from 5 to >100 times the yields from control plots. Some comments are made on the economics of field fumigation. (15 references.) J. L. WALPOLE.

Determination of nitrate in soil solutions by ultra-violet spectrophotometry. P. A. Cawse (*Analyst, Lond.*, 1967, **92,** 311–315).—The sample (1 ml) is mixed with Al_2O_3-cream (4 ml), centrifuged, and aq. 2% sulphamic acid (1 ml) is added to 1 ml of the supernatant, followed by 5% $HClO_4$ to make 10 ml; the extinction is then measured at 210 nm *vs.* a blank. In absence of org. matter the Al_2O_3-cream treatment is omitted. The working range is 0·5–10 ppm in absence of org. matter and 5–50 ppm in its presence; the usual nitrification inhibitors do not interfere in perfusate samples. Results agree well with those for NO_3-reduction and NH_3-distillation methods. About 40 samples were analysed in 2·25 h with a standard deviation of $\pm 0{\cdot}048$ μg. (16 references.) W. J. BAKER.

Use of iron (II) sulphate for reduction of nitrate to ammonia in the microdiffusion method for determining nitrate in soil extracts. P. R. Premi and A. H. Cornfield (*Analyst, Lond.*, 1967, **92,** 196–197).—It is established that $FeSO_4$ (**I**) can replace $Ti_2(SO_4)_3$ (**II**) in the Bremner-Shaw method. In comparison with the variable nature of commercial **II**, **I** is of const. quality, is stable for <2

months in a stoppered bottle at 2–4°, and gives a low blank value for NH_3. For the determination of a max. of 200 μg of nitrate-N in soil extract (5 ml), **II** should be replaced by M-$FeSO_4$ (1 ml) (in 0·5 M-H_2SO_4) plus saturated aq. Ag_2SO_4 (0·1 ml).
W. J. BAKER.

Radiochemical determination of plutonium in soil and other environmental samples. M. C. de Bortoli (*Analyt. Chem.*, 1967, **39,** 375–377).—The bulk of the Fe in an HCl extract of soil (containing added ^{236}Pu tracer) is removed by complexation with 0·1 M-EDTA and slow passage of the solution (9 M-in HCl) through Dowex 50–8 resin (Na^+ form). The resin is eluted with 3 M-HCl and Pu in the eluate is decontaminated by successive hydroxide pptn. at pH 13 (to remove Al) and pH 9 (to remove Mn, Mg and residual Fe), followed by ion-exchange on Dowex 1 × 8 (Cl^- form), from which Pu alone is eluted with 6 M-HCl–0·2 M-HF and determined by electrodeposition on a Ta disc. The plated disc is submitted to α-spectrometry. Average recovery is ~68%; <10% Pu is unextracted by HCl. W. J. BAKER.

[Comparison of] extraction of soil organic matter by alkali and chelating resin. M. Levesque and M. Schnitzer (*Can. J. Soil Sci.*, 1967, **47,** 76–78).—A comparison of 0·5 N-NaOH and Dowex A-1 resin (Na^+-form) as extractants for soil org. matter, did not confirm a previous report that the Na-resin was able to extract more high-mol. wt. compounds than did 0·5 N-NaOH. Na-resin may be the most suitable extractant for metal-org. matter complexes, but 0·5 N-NaOH is preferable if purified org. matter is required.
A. H. CORNFIELD.

Comparison of two methods for determination of organic carbon in soil. S. F. du Plessis and W. P. Burger (*S. Afr. J. agric. Sci.*, 1966, **9,** 721–723).—The Walkley and Black method (cf. Piper, *Soil and Plant Analysis*, Adelaide, 1942) was used in the examination of 24 soils; the results showed a close correlation ($r = 0·99$) with those obtained by Heck (*Soil Sci.*, 1929, **28,** 225). P. S. ARUP.

Evaluation of methods for the determination of total phosphorus in soils. C. G. Sherrell and W. M. H. Saunders (*N.Z. Jl agric. Res.*, 1966, **9,** 972–979).—Two colorimetric procedures were found to be satisfactory; the molybdivanadophosphoric acid yellow method, following HNO_3/HF decomposition, and a molybdenum blue method using ascorbic acid as reductant, following Na_2CO_3 fusion. E. G. BRICKELL.

Rapid method for determination of nitrate in plant and soil extracts. R. H. Lowe and J. L. Hamilton (*J. agric. Fd Chem.*, 1967, **15,** 359–361).—The method depends on the reduction of NO_3^- to NO_2^- by soyabean root-nodule bacteria using 0·1 M-K succinate as exogenous electron-donor. An automatic analyser is described through which the plant extract and the bacterial suspension pass, under pressure of N_2. After passage through a time-delay coil, the mixture passes through a continuous-flow dialyser, a coil where it is mixed with a colour-producing reagent for NO_2^-, and finally through a colour-recording assemblage. The method is sensitive to 0·01 μg of NO_3^- and shows very satisfactory recoveries.
P. S. ARUP.

Anaerobic incubation of soil and production of ammonium. J. B. D. Robinson (*Nature, Lond.*, 1967, **214,** 534).—Correlation of anaerobic (ΔNH_4-N) and aerobic [$\Delta(NH_4+NO_3+NO_2)$-N] topsoil values with soil analyses and data from maize trials in the field shows that the aerobic index (Bremner, *Methods of Soil Analysis*, 1965, p. 1241) is more satisfactory than the anaerobic index (Waring and Bremner, *Nature, Lond.*, 1964, **201,** 951) for determining potentially available mineral-N in soil. Possible reduction of any NO_3-N during anaerobic incubation tests was studied in terms of ΔNH_4-N found and soil type. Values of ΔNH_4-N are higher for strongly org. soils, e.g., a humic ferrisol, especially when the values are determined in the steam distillate of soil plus extract instead of in the filtered extract. Anomalous correlations are ascribed to this source of variation. For certain soils, more reliable results in the anaerobic soil-incubation method are obtained by distillation of the NH_4-N from a filtered extract. W. J. BAKER.

Rapid procedure for the estimation of nitrogen availability in soils. S. A. Waring (*J. Aust. Inst. agric. Sci.*, 1967, **33,** 39).—Mineralisable N in soils is determined by measuring the production of NH_3 during incubation under waterlogged conditions. Results correlate closely with mineralisation during incubation under aerobic conditions. E. G. BRICKELL.

Determination of assimilable potassium, magnesium, and phosphoric acid content of soils. F. Grill and H. Schlosser (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17,** 1–4).—The simultaneous extraction of these nutrients with aq. H_2CO_3 is proposed. As the proposed ratio of aq. H_2CO_3 to soil is 10 : 1, correction factors, based on 54 comparative analyses, are given for the conversion of results to a basis of a ratio of extracting solvent to soil of 40 : 1. P. S. ARUP.

Transformation of cyanamide in sterile soil. O. T. Rotini, C. Galopinni and P. G. Belli (*Chimica Ind. Milano*, 1967, **49,** 374–379).—Transformations of cyanamide (**I**) in two soils, with different autoclaving treatment, are studied. Residual **I**, urea, pH of soil and sterility are determined, showing that transformation of **I** is unaffected by autoclaving. A comparison of the velocity const. of **I** breakdown shows that a catalytic reaction is involved. (19 references.) (From English summary.) C. A. FINCH.

Soil and root copper. Evaluation of copper fertilisation by analysis of soil and citrus roots. J. G. A. Fiskell and C. D. Leonard (*J. agric. Fd Chem.*, 1967, **15,** 350–353).—Rectilinear regression relationships were found between root- and soil-Cu added to the soil as CuO or $CuSO_4$. Foliar Cu-deficiency symptoms occurred when the root-Cu was <3 ppm of the fresh wt., and the root-Cu extracted with N-HCl was <2 ppm. P. S. ARUP.

Silicate–phosphate inter-relationships in soils. S. F. du Plessis and R. du T. Burger (*S. Afr. J. agric. Sci.*, 1966, **9,** 525–534).—Yields of wheat grown in pot experiments on two out of seven sandy soils were increased by the addition of Na silicate alone. Combined applications of silicate and phosphate increased the total available P to an extent that indicated a decrease in P-fixation. Indications were also obtained that isomorphous substitution of phosphate for silicate might occur in some soils. (15 references.)
P. S. ARUP.

Effect of amelioration and fertilisation on ammonification and nitrification in salt soils. V. Rankov (*Mikrobiologiya*, 1967, **36,** 144–149).—Simultaneous application of $CaSO_4$, org. and mineral fertilisers increased the number of ammonifying and nitrifying bacteria and accelerated the corresponding soil processes. $CaSO_4$ alone (especially at high doses ~25 ton/ha) tends to inhibit the processes immediately after application but after a while development of bacteria occurs, probably due to improvement of soil properties. Application of all substances together increases the number of *Pseudomonas, Bacillus megaterium, B. cereus* and to a lesser extent *B. mycoides*. (14 references.) (From English summary.) R.J.M.

Red phosphorus as fertiliser. VI. White clover yield and phosphorus uptake from red phosphorus and monocalcium phosphate over three years. J. P. Widdowson (*N.Z. Jl Sci.*, 1966, **9,** 882–888).—Applied as a top-dressing on a P-deficient silty loam in pots, red P (**I**) was inferior to $Ca(H_2PO_4)_2$ (**II**), the production of dry matter at 10, 40 and 160 lb per acre being only 78, 63 and 51%, respectively, of that produced by **II**. Yields of clover from **I**-treated soil tended to increase with time relative to those from **II** treated soil, but only 16% of **I** was recovered from clover tops during 3 years in comparison with 50% of **II**. Recovery of both sources was max. at the 10-lb level. There would be little gain in using the slow-acting **I** for pasture top-dressing. W. J. BAKER.

Urea–monocalcium phosphate, a component of mixed fertilisers. A. W. Frazier, J. R. Lehr and J. P. Smith (*J. agric. Fd Chem.*, 1967, **15,** 345–347).—The adduct $Ca(H_2PO_4)_2 \cdot 4[CO(NH_2)_2]$, formed by the addition of urea to a saturated solution of $Ca(H_2PO_4)_2$ is stable and non-hygroscopic at R.H. >60%. Mixtures in which the adduct has formed could probably be dried with satisfactory results. The crystallographic properties and *X*-ray pattern of the adduct are described. (10 references.) P. S. ARUP.

Serpentine and dunite as magnesium fertiliser. E. T. Chittenden, D. J. Stanton, J. Watson and K. J. Dodson (*N.Z. Jl agric. Sci.*, 1967, **10,** 160–171).—Compared to dolomite and talc-magnesite, dunite was the most efficient fertiliser; serpentine was equal to dolomite in certain cases but superior in pot trials with tobacco and clover. E. G. BRICKELL.

Effect of cattle dung patches on pasture growth, botanical composition, and pasture utilisation. W. C. Weeda (*N.Z. Jl agric. Res.*, 1967, **10,** 150–159).—Effect on pasture growth was generally small. White clover (*Trifolium repens* L.) was more prevalent on dung patches and often remained so for one to one-and-a-half years. Grazing was most uneven in spring, herbage around dung patches being left 2–3 in. higher than unaffected pasture. Chain-harrowing after each grazing depressed pasture growth but improved pasture utilisation in spring by grazing steers. (14 references.)
E. G. BRICKELL.

Phosphate manuring of soils in depth. E.-M. Batisse (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53,** 1079–1085).—It was previously

shown (cf. *ibid.*, 1966, **52**, 246, 464) that the P contained in Ca hypophosphite (**I**) and Na_2HPO_3 (**II**) is not fixed by soil unless **I** or **II** become oxidised to the corresponding phosphates. In percolation experiments with columns of a 25% clay soil, considerable amounts of P were fixed as P_2O_5 during a 180-day experiment (36% of the total when introduced as **I**, and 88% of the total when introduced as **II**), and presumably deposited at various depths.
P. S. ARUP.

Coprophagous beetles in pasture ecosystems. P. Gillard (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 30–34).—Dung beetles of the subfamilies *Coprinae* and *Aphodiinae* should be considered an integral part of a pastoral community in that they assist in the decomposition of faeces and speed up the circulation of plant nutrients. They are present in profusion in South Africa but do not occur in such abundance in Australia and are not so aggressive in colonising freshly deposited faeces. The implications are considered; at present it appears that a large source of plant nutrients remains immobilised in undecomposed faeces. (24 references.)
E. G. BRICKELL.

Iron and aluminium compounds in commercial superphosphates. A. W. Frazier and J. R. Lehr (*J. agric. Fd Chem.*, 1967, **15**, 348–349).—The salt $Ca(Al,Fe)H(PO_4)_2 \cdot 4H_2O$ has been found to occur in amounts up to 25% in some wet-process superphosphates. The presence of significant amounts of this and similar salts interferes with the ammoniation of the superphosphate, but does not prevent its direct use as a fertiliser. The crystallographic properties, *X*-ray diffraction pattern, and i.r. spectrum of the new salt are presented; the salt is aq. citrate sol., but water-insol. P. S. ARUP.

Agricultural value of anhydrous ammonia on grassland. Experments 1963–1965. P. F. J. Van Burg, G. D. Van Brakel and J. H Schepers (*Neth. Nitrogen Tech. Bull.*, 1967, 2, 31 pp).—Anhydrous NH_3 has no commercial future in intensive grassland farming with the equipment at present available. E. G. BRICKELL.

Influence of fineness on effectiveness of phosphorous fertilisers with particular reference to liquid phosphoric acid. P. F. J. Van Burg (*Neth. Nitrogen Tech. Bull.*, 1966, 1, 9 pp).—Highly water-sol. P fertilisers should be rather coarsely granulated for acid soils (*AS*) but rather fine for calcareous soils (*CS*). A marked interaction was found between soil type and P source. Liquid H_3PO_4 proved to be the most effective on *CS* and less effective on *AS*.
E. G. BRICKELL.

Comparison of potassium content of fertilisers by atomic absorption and sodium tetraphenylboron. M. L. McCrackaw, H. J. Webb, H. E. Hammar and C. B. Loadholt (*J. Ass. off. analyt. Chem.*, 1967, **50**, 5–7).—The correlation coeff. for triplicate determinations of K in fertilisers by atomic absorption spectrophotometry at 4038 Å and by the Na tetraphenylboron (**I**) method was 0·99 (101 samples). The coeff. for 1190 samples analysed once by each method was 0·96. **I**-method is the more precise. A. A. ELDRIDGE.

Effect of sample size and environmental conditions on evaporation of water from soil. H. R. Gardner and R. J. Hanks (*Cons. Res. Rep. U.S. Dep. Agric. Res. Serv.*, 1966, 9, 14 pp).—Data are presented concerning water evaporation from soil in the laboratory in which different depth and dia. of sample containers were used with various environmental conditions, including a hexadecanol surface treatment. A min. dia. of 10 cm and a min. height of 24 cm is recommended and all samples should be wetted and dried at least once before the evaporation data are taken.
E. G. BRICKELL.

Influence of hydrothermal activity on element content of soils and plants. N. Wells and J. S. Whitton (*N.Z. Jl Sci.*, 1966, **9**, 982–991).—The trace element content of topsoils (0–3 in.) in ppm from pumice, org. and alluvial soils are indicated. Li 26, 0·88, 48, Rb 146, 5·2, 143, Cs < 5, < 5, < 5, Sr 320, 70, 160, Ba 1200, < 10, 1200, B < 10, 11, < 10, Ge < 10, < 10, < 10, As 5, 120, 4·7, Ti 1500, < 10, 2500, V 18, < 10, < 10, Cr 2·5 , < 1, 8, Mn 820, 100, 800, Cu 16, 4, 8, Zn 34, 1·5, 29, Zr 200, < 10, 160 and Mo 2·5, < 1, < 1. Analyses of sweet vernal grass, white-clover leaves and manuka leaves grown on such soils indicate abnormally high concn. of Cs, Li, Rb, B and Mo, the max. increases above normal being in white-clover leaves (Cs 1000, Li 300). These soils associated with hot springs are now well-drained and used for dairy farming all the year round. W. J. BAKER.

Evaluation of agricultural hydrology by monolith lysimeters, 1956–62. L. L. Harrold and F. R. Dreibelbis (*Tech. Bull. U.S. Dep. Agric.*, 1967, 1367).—Lysimetric studies of the retention of water by soil, percolation, evaporation and consumption by crops under various conditions are described. Conditions limiting the application of lysimeter data to watershed hydrology are considered, together with those affecting the losses of Ca, Mg, K, S and N from the soil. Typical analytical data for lysimeter percolates are tabulated. (187 references.) A. A. ELDRIDGE.

Infiltration rate as related to rainfall energy. F. Bisal (*Can. J. Soil Sci.*, 1967, **47**, 33–37).—The rate of decrease in infiltration, with increasing amount of rainfall, was much greater in a clay than in a fine sandy loam when the soil was saturated before the rainfall treatment. A loam had a low infiltration rate when saturated before treatment. The amount of energy of rainfall required to reduce the infiltration rate of the clay and loam to a min. was similar whether the soil was dry or saturated before treatment.
A. H. CORNFIELD.

Effect of ripening stage irrigation on the growth and maturity of spring paddy cane. R. C. Lin (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 42, 79–86).—Ripening of spring paddy cane (chosen for its shortened period of growth) is promoted by lowering the soil moisture level during the maturing stage. The treatment gave a 6·3% to 8·4% increase in sugar content when applied at the early maturing stage, but only 4·6% to 6·5% when applied at the mid-stage of maturing. J. L. WALPOLE.

Methods of improving sandy soil. D. S. Cheng, S. S. Wan and Y. J. Hsia (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 42, 71–78).—Applications of filter cake at 250, 500 or 1000 tons/ha or clay at 250 or 500 tons/ha of sandy soil gave significant increases in yield of sugarcane and ratoon. The heaviest applications gave the greatest yields and the response to filter cake was particularly good owing to its extra nutrient content. (15 references.) (From English summary.) J. L. WALPOLE.

Effects of pulverised fuel ash on moisture characteristics of soils. P. J. Salter and J. B. Williams (*Nature, Lond.*, 1967, **213**, 1157–1158).—Pulverised fuel ash, at rates ≡ 0, 50, 100 and 200 tons/acre and containing 65% of particles of size 0·2–0·02 mm, was incorporated into the top 12 in. of coarse sandy Newport loam and a crop of red beet grown. After harvest the upper and lower limits of available water were determined. The largest addition of ash significantly increased the available water content and there were small non-significant increases with lower rates of application. The effect of ash on four other coarse textured soils was assessed in laboratory experiments. The available water content of all soils increased progressively with increasing quantities of ash, the largest increases occurring with the coarsest textured soils.
S. A. BROOKS.

Bringing back the waste lands. N. Weymouth (*Rubb. J.*, 1967, **149**, No. 5, 130–135).—A blend of oil and rubber latex (Unisol 91) when sprayed on to shifting sands and soils affords a practicable means of stabilising soil against wind erosion and of reclaiming land lost by over-grazing. Examples of the use of Unisol 91 are given and the prerequisites for its successful employment are listed.
J. L. WALPOLE.

[Concentrated] fertilisers [containing ammonium phosphate]. Fisons Fertilizers, Ltd. (Inventors: G. G. Brown, K. F. J. Thatcher and V. C. Vinyard) (B.P. 1,048,134, 15.5.63).—Monocalcium phosphate 2–2·5, NH_4NO_3 6–30, NH_3 2–6 (3·5–5) and H_2SO_4 < 3 (1·5–2·3) moles, together with an amount of water ⩾15 (⩾12) wt.-% of the total wt. of these materials are caused to react together to form a melt of pH 2·5–5·5, at 130° to the b.p. of the melt. The melt is cooled to form flakes which are broken to granules and blended with, e.g., granular KCl to give a non-caking fertiliser.
J. M. JACOBS.

Concentrated complex fertilisers. Deputy Minister in the Ministerul Industriei Petrolului Şi Chimiei (Inventor: E. Jonescu) (B.P. 1,048,269, 17.9.63).—A conc. fertiliser containing > 80% of total P_2O_5 in water-sol. form is prepared by reacting phosphatic rock with HNO_3, selectively extracting H_3PO_4 and HNO_3 with a partially water-miscible org. solvent (e.g., BuOH, t-BuOH, COMeEt, cyclohexanone), and reacting the extract with aq. NH_3. Any $Ca(NO_3)_2$ remains in the aq. portion. F. R. BASFORD.

Ammonium sulphate nitrate [fertiliser]. Fisons Fertilizers, Ltd. (Inventors: K. S. Barclay, P. Doran and E. J. Smith) (B.P. 1,049,782, 22.5.62).—A molten mixture of NH_4NO_3 and NH_4HSO_4 in the mol. ratio 0·9–4 (1–2·2) : 1 and optionally, $(NH_4)_2SO_4$ (⩾16 wt.-% of the mixture), is flaked or granulated, e.g., by running the molten mixture on to the cooled surface of flaking rolls or by spraying it on to granules containing fertiliser or inert materials and/or recycle fines maintained at 60–90°, in a rotating drum, and simultaneously ammoniating the mixture to form ammonium sulphate nitrate. J. M. JACOBS.

Slow-acting fertilisers. Badische Anilin-u. Soda-Fabrik A.-G. (Inventors: G. Daumiller, H. Pohlemann, B. Lehmann, K. Opp and O. Detmer) (B.P. 1,053,336, 12.3.64. Ger., 14.3.63).—A fertiliser (consisting of granules of a multi-purpose fertiliser) is rendered slow-acting by applying thereto 2–20 wt.-% of a coating composition consisting of a polyene with free vinyl groups, e.g., a butadiene polymer (45–85), drying oil containing <2 conjugated double bond systems or a group readily convertible thereto (e.g., oiticica, castor, or wood oil) (10–50 wt.-%), and optionally a lipophilic aminoplast precondensate (5–45 wt.-%), then curing.
F. R. BASFORD.

Granular fertilisers. Scottish Agricultural Industries Ltd. (Inventors: D. G. Cowan and R. S. Stevenson) (B.P. 1,055,816, 23.11.64).—There is claimed a granular fertiliser composition containing 15–75 wt.-% of a cocoa waste product consisting of particles passing an 8 mesh BS sieve. F. R. BASFORD.

Slow-acting fertilisers. Badische Anilin-u. Soda-Fabrik A.-G. (Inventors: O. Detmer, J. Jung, H. Mueller and H. Seibt) (B.P. 1,056,457, 20.11.63. Ger., 24.11.62).—These are obtained by coating fertilisers with 2–20 wt.-% of a drying oil consisting of a polyene polymer, e.g., a metal-isomerised polybutadiene.
F. R. BASFORD.

Humic and nitrohumic salts as soil improvers. S. Tomioka (B.P. 1,058,763, 19.7.63).—A soil nutrient is produced by reacting $MgCO_3$, MgO, $Mg(OH)_2$, serpentine (optionally calcined), and/or dolomite with lignite, peat, turf etc. or with nitrohumic acid (obtained by nitration of lignite, etc.) at $>120°$ for <2 h in presence of 30–100% of water. F. R. BASFORD.

Fertilisers. Imperial Chemical Industries Ltd. (Inventors: N. Watchorn and F. M. Tayler) (B.P. 1,060,182, 28.1.65).—There is claimed a blended fertiliser in which one component consists of rounded discrete particles of NH_4NO_3 containing a compatible desiccant salt, e.g., $Mg(NO_3)_2$ (0·1–5% of MgO on NH_4NO_3), $FeSO_4$, Fe^{III} alum, or $FeSO_4(NH_4)_2SO_4$, to confer free-flowing properties. F. R. BASFORD.

Mixed fertilisers containing ammonium nitrate and a chloride. Verenigde Kunstmestfabrieken Mekog-Albatros N.V. (Inventor: G. Perbal) (B.P. 1,076,284, 25.3.65).—A mixed fertiliser composition containing NH_4NO_3 and a chloride, and, optionally, one or more other components, the composition being liable to a self-sustaining exothermic decomposition, is produced by mixing at least two different fertiliser blends. These blends contain the same components, but in themselves, have a composition such that they are not liable to a self-sustaining exothermal decomposition, i.e., a NH_4NO_3 content outside the range of (preferably) 15–65 wt.-%.
E. ENOS JONES.

Liquid fertilisers. Fisons Fertilizers Ltd. (Inventors: K. S. Barclay and P. Doran) (B.P. 1,076,901, 1.8.64).—The fertilisers comprise 3–10% by wt. of K^+, 2–20% by wt. phosphate (expressed as PO_4^{3-}), 10–30% by wt. urea, 0–2% by wt. NH_4^+ and 40–85% by wt. water, and are produced by reacting wet process H_3PO_4 with KOH or K_2CO_3 in presence of water, filtering the mixture and adding urea to the filtrate. The fertilisers do not form a ppt. on dilution. E. ENOS JONES.

Plant Physiology, Nutrition and Biochemistry

Rates of photosynthesis and respiration in leaves of the cranberry with emphasis on rates at low temperatures. F. R. Forsyth and I. V. Hall (*Can. J. Plant Sci.*, 1967, **47**, 19–23).—Rates of apparent photosynthesis of cranberry leaves increased over the range 3·5–25° when measured both manometrically and by means of an O_2 analyser. At all temp. (3·5, 5, 10 and 15°) the O_2 analyser method showed that the rate of photosynthesis exceeded the rate of respiration which is an important factor in the winter survival of cranberry plants, especially the meristematic tissues.
J. L. WALPOLE.

Effect of daylength on the growth of wheat. D. J. C. Friend, V. A. Helson and J. E. Fisher (*Can. J. Bot.*, 1967, **45**, 117–131).—Increasing the daylength over the range 8–24 h increased the leaf area per plant, the total number of leaves and tillers and the total plant dry weight of Marquis wheat. During the early stages of growth the leaf area ratio decreased with increasing daylength mostly because of increased leaf thickness; in the later stages earlier flowering at long daylengths reduced the leaf area ratio because of the rapid stem-growth associated with inflorescence formation. This latter was a photoperiodic effect independent of the level of total daily radiation. (20 references.) J. L. WALPOLE.

Retention of water by plant cell walls and implications for drought resistance. T. S. Teoh, L. A. G. Aylmore and J. P. Quirk (*Aust. J. biol. Sci.*, 1967, **20**, 41–50).—Water-retaining properties of root cell wall material from two monocotyledons (*Ehrharta calycina* and *Triticum vulgare*) and two dicotyledons (*Salicornia australis* and *Vicia faba*) have been investigated. Young root cell walls were obtained by standard methods and formed into a 30-mg core under 500 lb/sq. in. Sorption–desorption isotherms were constructed from which it appeared that drought resistant species endure prolonged water stress because of the greater tenacity with which cell wall water is held when compared with mesophytes. (32 references.) J. B. WOOF.

Moisture content of living trees. C. M. Stewart (*Nature, Lond.*, 1967, **214**, 138–140).—Based mainly on published data an attempt is made to explain systematic variations in water-content within the sapwood of angiosperm (*A*) and gymnosperm (*G*) species, especially during different seasons and during transition of sapwood into heartwood. The variation seems to be controlled firstly by changes of pressure arising from transpiration demand within the dead water-conducting cells and secondly by the water demand of living parenchyma cells, especially those of the rays. Distribution of water across stems of *A* and *G* from cambium to pith is discussed and shown in graphs. (16 references.) W. J. BAKER.

Effect of water stress on translocation in relation to photosynthesis and growth. I. Effect during grain development in wheat. I. F. Wardlaw (*Aust. J. biol. Sci.*, 1967, **20**, 25–39).—Wheat plants (*Triticum aestivum* cv. Gabo) were grown in perlite with a 16 h photoperiod. Tillers were removed 5 weeks after planting, at anthesis and also before treatment. After anthesis plants were transferred to an artificially lit cabinet for 15 days before water stress was introduced by terminating watering and feeding. The rate of drying out was not controlled. Dry wt. increases were followed for a 6 day period after treatment, and photosynthetic measurements were made on a single leaf in a perspex chamber by following CO_2 changes with an i.r. gas analyser. Relative stomatal aperture was obtained by measuring leaf resistance to air flow with a porometer. Turgidity and rate of movement of ^{14}C labelled assimilates were determined. From onset of wilting there was a progressive decrease in photosynthesis rates even where light was limiting. The difference between fully turgid and wilted leaves was not reduced by CO_2. Grain growth was unaffected for several days though assimilates were distributed from lower parts of the plant to the grain. Velocity of translocation was little affected by stress. In this case water stress appeared to act directly on the leaf rather than through changes in growth or sugar movement. (49 references.) J. B. WOOF.

Boron deficiency and ribonuclease activity in plants. Y. P. Abrol (*Indian J. Biochem.*, 1966, **3**, 263–264).—Ribonuclease (**I**) activity has been measured in sunflower (*Helianthus annuus* cv. Mammoth Russian) plants grown in B-deficient nutrient solutions. A progressive increase in **I** activity occurred with increase in time for which the plants were grown in the solutions. This was reversed when plants were transferred to normal nutrient media after four days. S. A. BROOKS.

Boron deficiency in white clover (*Trifolium repens* L.) seedlings grown in an organic soil. C. G. Sherrell (*N.Z. Jl agric. Res.*, 1966, **9**, 1025–1031).—In a pot trial using Rukuhia peat, the addition of borax at 20 lb per acre increased the dry matter five-fold at the first harvest. This response diminished in subsequent harvests and was absent after three. N content was affected similarly, from which it is concluded the effect of B is indirect and operates through N nutrition. (13 references.) E. G. BRICKELL.

Growth changes in subterranean clover during recovery from phosphorus and sulphur stresses. D. Bouma (*Aust. J. biol. Sci.*, 1967, **20**, 51–66).—Clover seeds were germinated and grown on basal nutrient medium for 14–19 days after which some plants were transferred to solutions lacking P or S and others to a complete nutrient medium. Whilst under these conditions for 7 days, growth was followed by means of leaf area and plant dry wt. After removal of the stress, preferential distribution of assimilates to leaves formed subsequently was observed; this was more pronounced as the stress applied was increased. Reduction of growth rates in the early stages of recovery were related to lower net assimilation rates as well as to lower leaf area ratios. Rates of photosynthesis per unit leaf area were much reduced during and after stress; this was shown by direct measurement of CO_2 exchange. Recovery from S stress was slower than from P stress. (21 references.) J. B. WOOF.

Effect of chemical inhibition of nitrification on phosphorus absorption by wheat. K. F. Nielsen, F. G. Warder and W. C. Hinman (*Can. J. Soil Sci.*, 1967, **47**, 65–71).—When nitrification in pot tests was inhibited by application of 2-chloro-6-(trichloromethyl-pyridine), the uptake of N by wheat was reduced but the uptake of P was increased in comparison with pots where nitrification proceeded normally. A. H. CORNFIELD.

Nickel, iron and manganese in oat-plant metabolism. P. C. Williams (*Nature, Lond.*, 1967, **214**, 628).—Different ratios of Fe and Mn were applied to oat-plants in sand culture in presence of 0–20 ppm Ni. Results showed that ~300 ppm Mn greatly intensified toxicity due to Ni (~10 ppm) and that both Ni and Mn increased the rate of intake of Fe by the plant although they inhibited Fe metabolism inside it. In both pot- and field-plants the concn. of Ni, Fe and Mn were much higher in affected plants than in healthy plants; the most affected plants in the field were devoid of chlorophyll. (Cf. Crooke *et al.*, *Ann. appl. Biol.*, 1954, **41**, 311; 1955, **43**, 454; Hewitt, *A. Rev. Pl. Physiol.*, 1951, **2**, 25.) W. J. BAKER.

Physiological assessment of the nutrient status of plants. IV. Effect of interaction between nutrient elements on leaf area responses. D. Bouma and E. J. Dowling (*Aust. J. agric. Res.*, 1967, **18**, 223–233).—Experiments with subterranean clover in water cultures, supplied with varying amounts of P, S, and N, showed that when elements were deficient to differing degrees, leaf area responses depended on the relative intensities of the deficiencies. Transfer of plants deficient in only one element confirmed that there is no simple relation between N status of the plant and the induced leaf area responses. Results also confirmed the feasibility of at least a qual. assessment of the P or the S status of plants. E. G. BRICKELL.

Leaf absorption of nitrogen by vine plants. S. Silva and P. Fontana (*Chimica Ind., Milano*, 1967, **49**, 162–166).—Leaf absorption and transportation of the different forms, e.g. $(NH_4)_2SO_4$, NH_4NO_3 and urea under which N was applied to the leaves vary considerably and it appears that such behaviour can be connected with the different sectors within the plant where the various forms of N are utilised. It is shown that the plant hydrolyses urea before transporting and utilising the NH_3 but as opposed to the N derived from $(NH_4)_2SO_4$, the N is easily transported to the upper section of the plant. (15 references.) M. DUDLEY.

Protein synthesis in developing wheat endosperm. J. K. Raison (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 38).—Protein granules are composed mainly of slow-moving cationic compounds. There appear to be two independent systems for the synthesis of storage proteins and for the synthesis of sol. (cytoplasmic) proteins of endosperm. E. G. BRICKELL.

Metabolism of glycosides, organic acids, and amino-acids in *Vitis vinifera* L. G. Ribéreau-Gayon (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 631–635).—A summary is presented of a study carried out by the introduction of radio-active labelled nutrients into the plant and their distribution. Consideration is given to the mechanism of the synthesis of malic and tartaric acids. P. S. ARUP.

Comparative investigation of lipids present in seeds and tubers. G. Lotti and V. Averna (*Riv. ital. Sostanze grasse*, 1967, **44**, 297–305).—Chemical characteristics, including fatty acid composition (by gas chromatography), and in some cases spectroscopic characteristics, of the fat from the seeds, tubers and tuberised roots of 16 vegetable families (29 species) have been determined. In general, the total N and ethereal extract were higher for the seed, but the unsaponifiable content of the fat was lower. The fatty acid composition of the fat from the seed was appreciably different from that of the tubers or tuberised roots. (19 references.) L. A. O'NEILL.

Comparison of lipids present in seeds and bulbs. G. Lotti and V. Averna (*Riv. ital. Sostanze grasse*, 1967, **44**, 336–340).—A comparison has been made of the chemical and spectroscopic characteristics, including fatty acid composition of the fat from the seeds and bulbs of 17 species of wild or cultivated plants. The fatty acid composition of the seed fat bore no general relation to that of the bulb, but the number of different acids found was less for the former. L. A. O'NEILL.

Fatty acid composition of lipids of *Pinus radiata* and *Pinus nigra*. R. P. Hansen (*N.Z. Jl Sci.*, 1966, **9**, 801–805).—GLC of lipid extracts showed that unsaturated fatty acids constituted 85% and 94%, respectively, of the 'combined' fatty acids from sawdust from these woods. Oleic and linoleic acids predominated, but 5,9-octadecadienoic, 5,9,12-octadecatrienoic and 5,11,14,eicosatrienoic acids (all reported by Finnish workers) were also tentatively identified. The chief saturated acid was palmitic, being ~10·5% and 2·5%, respectively, of the total fatty acids in *P. radiata* and *P. nigra*. (21 references.) W. J. BAKER.

Structure and function of lysozyme. D. C. Philips (*Proc. R. Instn Gt Br.*, 1965, **40**, 530–543).—A lecture. P. S. ARUP.

Study of phytase and fluoride effects in germinating corn seeds. C. W. Chang (*Cereal Chem.*, 1967, **44**, 129–142).—Properties of the phytase of the endosperm-scutellar tissue of germinating maize are reported. The enzyme had an optimum incubation temp. of 50°, optimum pH of 5·6, a Michaelis const. (K_m) of $0·8 \times 10^{-4}$, lacked a specific substrate specificity, and was activated by $CaCl_2$ (0·01 M) and significantly inhibited by NaF (10 to 0·05 mM). The highest total activity in the strained tissue homogenate was in the fraction separated at $1700 \times g$; the highest specific activity was in the supernatant at $20,000 \times g$. Enzyme activity increases rapidly during germination (× 40 in 96 h) accompanied by rapid decomposition of phytin and increase in inorg. phosphate. Fluoride prevents dephosphorylation of phytin and retards the rate of seedling growth. (17 references.) E. C. APLING.

Wheat leaf phosphatases. VIII. A preparation with phosphotransferase activity. D. W. A. Roberts (*Can. J. Biochem.*, 1967, **45**, 401–408).—The nucleoside phosphotransferase of wheat leaves has been separated from the acid β-glycerophosphatase (**I**) by chromatography on a weak base anion-exchange resin. It was contaminated with 3′-nucleotidase, acid pyrophosphatase (**II**) and a phosphotransferase that apparently catalyses the interconversion of adenosine 3′-phosphate and adenosine 5′-phosphate. The low level of acid **I** activity in these prep. shows the existence of an active acid **II** in wheat leaves distinct from classical acid phosphomonoesterase. (22 references.) S. A. BROOKS.

Phenol oxidase activity and flooding tolerance in higher plants. R. M. M. Crawford (*Nature, Lond.*, 1967, **214**, 427–428).—During tests on EtOH metabolism in relation to flooding tolerance (*Idem, J. Ecol.*, 1966, **54**, 403), the root extracts of plants susceptible to flood damage rapidly turned brown whilst those of flood-tolerant species remained almost colourless. Non-flood-tolerant plants also had a much higher catecholase activity at pH 8, whether grown under high or low water-table conditions. Results are considered briefly in respect of function of phenol oxidases, which are probably essential to plants relying on oxidative phosphorylation but inessential to those having a mainly anaerobic habitat for their roots (cf. *Pl. Physiol.*, 1956, **31**, 425). W. J. BAKER.

Enzymic degradation of pectic acid. IV. Action of carrot *exo*-polygalacturonase on pectic acids prepared by saponification of pectin with alkali and with pectinase. V. Mode of action of fungal saccharifying polygalacturonase. C. Hatanaka and J. Ozawa (*Ber. Ôhara Inst. landw. Biol.*, 1966, **13**, 161–174, 175–183).—IV. The *trans*-eliminating glycosidic cleavage of pectin proceeds rapidly in warm alkaline solutions but is practically stopped at 0°; ester hydrolysis, however, continues to proceed to completion at 0°. Reasons for the decrease in η observed in this case are discussed. Whilst ester hydrolysis of the pectic acids by the carrot enzyme was slow and incomplete, an esterase prepared from the peel of *Citrus unshiu* carried the process practically to completion. (14 references.)

V. Chromatographic evidence is presented that the alleged complete glycosidic hydrolysis of pectic acid by a fungal *exo*-enzyme prepared from the commercial prep. Sclase, was probably due to the presence of an *endo*-enzyme in the prep. P. S. ARUP.

Rôle of cobalt in storage of nitrogen by legumes. T. A. Danilova and E. N. Demkina (*Dokl. Akad. Nauk SSSR*, 1967, **172**, 487–490).—Favourable effect of Co on development of leguminous plants is known. To determine whether the host plant or only *Rhizobium* which develops in its roots needs Co, experiments were made on peas raised in sterile sand in a greenhouse. Plants were nourished with NPK mixture containing only $\frac{1}{8}$ of its normal content of sol. nitrogen. The treatment given was addition of 0·01 mg/kg of Co, with and without inoculation of the plant by the organism; a control test without inoculation or addition of Co was also included. The effect of Co (with and without inoculation) on height, raw and dry wt. of above-ground parts and roots; total and protein N and carbohydrate content of six plant organs are tabulated. Plants do not develop so well without inoculation and show clear signs of N deficiency. Uninoculated plants improve after application of Co. Co promotes size and development both of the host plant and of *Rhizobium* in its roots, intensifies N fixation by *Rhizobium*

bacteria and increases storage of nitrogenous matter and carbohydrate. P. W. B. HARRISON.

Development of quantitative methods for individual anthocyanins in cranberry and cranberry products. T. Fuleki (*Diss. Abstr.* B, 1967, **27,** 4439–4440).—The four pigments involved were the monogalactosides and mono-arabinosides of cyanidin and peonidin. For determination of total anthocyanin (*A*) content in cranberry cocktail a new method was developed, based on O.D. measurements made at pH 1·0 and 4·5. Data obtained (total *A*) could also be used to calculate a Degradation Index (ratio of degraded pigments to non-degraded). Experiments with the $AlCl_3$ shift indicated that it is feasible to apply it for the quant. determination of the *o*-dihydroxyl group-containing *A*. A new solvent consisting of the upper phase of 1-butanol–benzene–formic acid–water (100 : 19 : 10 : 25) was used for chromatographic separation of the pigments (applied as 4 cm streaks). Outlines of the developed methods are summarised. F. C. SUTTON.

Ultrastructure of the shoot apices and leaves of normal and physiologically dwarfed peach seedlings. I. Plastid development. F. Flemion, R. E. Dengler, N. G. Dengler and K. D. Stewart (*Contr. Boyce Thompson Inst. Pl. Res.*, 1967, **23,** 331–344).—The ultrastructure and development of plastids in green and chlorotic peach leaf tissues have been investigated and the apices of normal shoots and physiological dwarfs compared from germination to seedling stage. With dwarf seedlings there were more membrane-bound inclusions in the uppermost cell layers, a progressive accumulation of starch (**I**) basipetally into the pith rib meristem and more tubular lamellae in the leaf primordia. There was more **I** in chlorotic leaf tissue and a tendency for the adaxial mesophyll plastids to develop rudimentary grana. In entirely chlorotic leaves tubular lamellae and membrane-bound inclusions were present rather than other internal membranes and **I**. (24 references.) S. A. BROOKS.

Extraction procedure for quantitative determination of six elements in plant tissue. J. H. Baker and T. Greweling (*J. agric. Fd Chem.*, 1967, **15,** 340–344).—The extraction of Ca, Mg, K, Mn, Cu and Zn is carried out on the dried powdered tissue with 0·1 M-EDTA (NH_4 salt at pH ~9). The determinations by spectrographic and spectrophotometric methods, gave the same results as those obtained by the dry ashing method. P. S. ARUP.

Biochemical studies of dormancy and after-ripening of seeds. IV. Further studies on changes in contents of some amino-acids and organic acids. L. V. Barton and J. L. Bray (*Contr. Boyce Thompson Inst. Pl. Res.*, 1967, **23,** 311–318).—The amounts of most free amino-acids and amides produced upon transfer of tree peony seedlings to the greenhouse for one week, following periods of 1, 2, 4 and 9 weeks at 5°, were found to be increased as compared to controls. Similar results were obtained with apple seeds (I). Exogenous application of amino-acids to tree peonies, did not show any consistent effect as stimulators or inhibitors of after-ripening. No apparent differences in keto acid content of **I** were noted between dormant tissues held at 20° and those after-ripened at 5°. S. A. BROOKS.

Effects of temperature and moisture on viability of stored lettuce, onion, and tomato seeds. L. V. Barton (*Contr. Boyce Thompson Inst. Pl. Res.*, 1966, **23,** 285–290).—Viability during the first 18 years of a planned extended storage period is described. Moisture content was adjusted before storage to approx. 5–50% and temp. ranged from −18 to 30°. Higher moisture content was tolerated during storage at lower temp. Germination capacity of lettuce and tomato seeds was not impaired at −18° during the 18 years, but onion showed some signs of injury at this temp. compared with −2°. E. G. BRICKELL.

Effect of storage conditions on the viability of bean seeds. L. V. Barton (*Contr. Boyce Thompson Inst. Pl. Res.*, 1966, **23,** 281–284).—Sealing extended longevity when storage was in a humid room, even when the temp. was as low as 5°. Sealing was without effect at below freezing temp. of −18 and −2° up to 15 years. A temp. of 30° brought about rapid deterioration in both open and sealed storage. Impermeable coats developed during storage of var. 'Top Notch Golden Wax' principally in open storage at 10°. The no. of impermeable seeds increased with progressive storage from 8% after 6 months to 64% after 10 years. E. G. BRICKELL.

Physiology of stratification of cherry stones. Effects of imbibition on permeability to oxygen. D. Côme (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53,** 623–631).—A description is given of the apparatus previously employed for measurement of the permeability of seed coats to H_2 (Devaux effect) (cf. *ibid.*, 1961, **47,** 55). Cherry stones that had not been wetted were fairly permeable to H_2 (and therefore to O_2); the permeability was much reduced after wetting, and more than fully restored after subsequent drying. The main factor promoting germination is the increase in porosity of the stones, due to alternate wetting and drying, which facilitates the access of water to the embryo. (13 references.) P. S. ARUP.

Isolation and partial characterisation of the mitochondrial fraction from ripening banana pulp. N. F. Haard (*Diss. Abstr.*, B, 1967, **27,** 4440).—Mitochondria were isolated from preclimacteric, climacteric, and postclimacteric banana fruit by a new technique. It was found that there were differences in the stimulatory action of added Ca on O_2 consumption when succinate was substrate and the capacity of the mitochondria 'actively' to accumulate Ca^{2+} when succinate or ATP were substrate. Ca^{2+} (1 mM) stimulated succinoxidase activity 50% with the prep. from preclimacteric fruit, but had no influence on O_2 consumption rate of climacteric and postclimacteric mitochondrial fractions. Conversely, active accumulation of Ca^{2+} by climacteric mitochondria increased 4–5 fold over that of the preclimacteric prep. These observations suggest a relationship between membrane permeability and the post-harvest changes of fruit. The thesis here is that a change in Ca localisation (from the middle lamella to the intracellular environment) is directly related to the climacteric rise in fruit respiration. F. C. SUTTON.

Growth-accelerating substances in cotton fibres. J. W. Mitchell, G. D. York and J. E. Worley (*J. agric. Fd Chem.*, 1967, **15,** 329–333).—Substances capable of accelerating growth in the second internodes of young bean plants were extracted from raw cotton fibres or from a chromatographic filter paper with 2-propanol or EtOH mixed with aq. NH_3. Five growth accelerating factors were detected and these were partially purified by TLC. These factors were chromatographically and physiologically unlike indole-3-acetic acid and physiologically unlike six other endogenous compounds known to possess growth regulating properties. Chromatographically they were unlike gibberellic acid A_3 but they resembled it as regards stem elongation, which they induced. (19 references.) P. S. ARUP.

Development of sprouts in *Brassica oleracea* L. var. *gemmifera*, DC. induced by kinetin. M. Nieuwhof (*Naturwissenschaften*, 1967 **54,** 202–203).—In sprout buttons that had been dipped in talcum powder containing 1% of kinetin, root and apical development were inhibited whilst the axillary buds at the base were unusually thickened; the outgrowth of these buds occurred only when gibberellic acid had been applied together with the kinetin. (In English.) P. S. ARUP.

Growth of apricot fruit. II. Effects of temperature and gibberellic acid. D. I. Jackson and B. G. Coombe (*Aust. J. agric. Res.*, 1967, **18,** 95–106).—Application of heat during the first 10 nights after anthesis increased the initial growth rate of fruit and of cells in the mesocarp and produced more rapid cell division in this tissue. It did not affect final fruit size or the no. and dia. of cells in the mesocarp but higher temp. did hasten maturity of fruit. Gibberellic acid produced an increased drop of flower buds and fruit, raised the ratio of flower buds to leaf buds initiated that season, and resulted in elongated pedicels. There were no significant interactions between temp. and gibberellin in any parameter of apricot fruit growth. (26 references.) E. G. BRICKELL.

Gibberellins. N. Ya. Grigor'eva and V. F. Kucherov (*Usp. Khim.*, 1966, **35,** 2044–2071).—The structure and features of gibberellins (**I**) are discussed and progress made in gibberellin chemistry during the last 50 years reviewed. The biogenesis of **I**, their place in a series of other natural polycyclic compounds, and assessment of their action mechanism on higher plants, such as stimulating the biosynthesis of lignin, are considered. (176 references.) A.L.B.

Effects of gibberellic acid and aflatoxin in germinating seeds. H. C. Jones, H. S. Black and A. M. Altschul (*Nature, Lond.*, 1967, **214,** 171–172).—Comparative effects of gibberellic acid (**I**) (9×10^{-5} M) and aflatoxin (**II**) ($1{\cdot}5 \times 10^{-4}$ to $6{\cdot}5 \times 10^{-6}$ M) on lipase activity (*LA*) in distal halves of cottonseed during the first 5 days incubation are discussed. The whole pattern of development of *LA* was advanced ~15 h by **I**, which also reversed the inhibition caused by the max. concn. of **II** and produced a pattern similar to that of **I** alone or the median concn. ($6{\cdot}5 \times 10^{-5}$ M) of **II**. In combination with the min. concn. of **II**, **I** slightly accelerated *LA* in comparison with **II** alone. Only in low concn. does **II** stimulate *LA*. The effects of **I** and **II** on formation of chlorophyll parallel those for *LA*, but only **I** accelerates cell expansion. Most rapid

acceleration of all three processes was obtained with **I** plus lowest concn. of **II**; actinomycin D inhibited completely all three processes. An explanation in terms of RNA and DNA is attempted. (10 references.) W. J. BAKER.

New phenolic plant growth-regulating compounds. R. L. Wain and D. B. Harper (*Nature, Lond.*, 1967, **213**, 1155–1156).—In pea segment and pea curvature tests 2-chloro-, 2-bromo- and 2-iodo-6-nitrophenols have been shown to promote cell elongation; the corresponding 6-cyanophenols and 2-trifluoromethyl-6-nitrophenol were also active. It appears that for a phenol to show appreciable growth-regulating activity both positions *ortho* to the hydroxyl must be occupied by electron-attracting groups but the *para* position must be free. Some compounds active in pea and tomato tests were inactive in the wheat cylinder test; hydroxylation of the *para* position by wheat tissue is thought to cause this inactivity. S. A. BROOKS.

Derivatives of *d*-limonene. Detection and translocation of quaternary ammonium plant growth retardants in young grapefruit and bean seedlings. W. F. Newhall and A. P. Pieringer (*J. agric. Fd Chem.*, 1967, **15**, 488–491).—The translocation from the stem to the roots, and from the roots to the leaves, of two quaternary NH_4 deriv. of limonene (cf. *ibid.*, 1966, **14**, 23) was observed to occur in both plants. Evidence was obtained that the two deriv. were similarly metabolised in both, and that they were decomposed on the Al_2O_3 used for TLC to their common amine precursors. A special TLC procedure was based on the latter observation. P. S. ARUP.

Conversion of the plant growth retardant (2-chloroethyl)trimethylammonium chloride to choline in shoots of chrysanthemum and barley. E. F. Schneider (*Can. J. Biochem.*, 1967, **45**, 395–400).—Shoots of barley and chrysanthemum (*Chrysanthemum morifolium* 'Princess Anne') were treated with ^{14}C labelled (2-chloroethyl)trimethylammonium chloride (CCC) and plant extracts were examined by paper chromatography. Five radioactive products were obtained, one of which was identical chromatographically with choline (**I**). The four unknown products were all tertiary or quaternary amines, three of them being metabolites of **I**. Between 20 and 30% of the ^{14}C was found in the metabolites after 24 h. (11 references.) S. A. BROOKS.

Natural capacities of varieties of *Begonia rex* Putz to form shoots and leaves. C. Bigot (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1005–1010).—The capacities of leaf cultures of 14 varieties were compared under controlled cultural conditions. P. S. ARUP.

Crops and Cropping

Effect of soil organic matter content on the ^{137}Cs concentration in crops. E. J. Evans and A. J. Dekker (*Can. J. Soil Sci.*, 1967, **47**, 7–13).—The effect of varying org. matter content of a mineral soil on uptake of ^{137}Cs was obscured by variable exchangeable soil K content. In general there was high uptake with high org. matter content providing K was added. ^{137}Cs uptake in lettuce grown after oats, decreased with increasing soil org. matter content. A. H. CORNFIELD.

[A] **Interactions between treatment of winter wheat with chlorocholine chloride and nitrogen fertilisation.** [B] **Interaction between treatment of winter wheat with herbicide, amounts applied, and dates of application of nitrogenous fertiliser.** M. Raverdy (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 709–718, 718–723).—[A] Effects of these factors on the shortening of the internodes with increased yields to be obtained with increased N-fertilisation are statistically examined. (20 references.)

[B] Relationships between these factors are statistically examined with the use of the herbicide 2-methylthio-4-isopropylamino-6-(3-methoxy) propylamino-*S*-triazine (Gesaran 25). P. S. ARUP.

Wheat-crop physiology in relation to time of sowing, rate of sowing and fertiliser application. R. A. Fischer (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 40–41).—Time of sowing had little effect on soil moisture available to the crop at flowering though in later sown crops plant moisture stress occurred sooner after flowering. Increased fertiliser application and increased rate of sowing increased vegetative growth and potential yield at flowering but this was associated with small reductions in soil moisture at flowering and an earlier onset of plant moisture stress. E. G. BRICKELL.

Competition among wheat plants sown at a wide range of densities. D. W. Puckridge and C. M. Donald (*Aust. J. agric. Res.*, 1967, **18**, 193–211).—Four sequential stages in the growth of wheat (cv. Insignia 49) were noted; (1) a period of no interplant competition at any density, (2) a period in which crop growth rate showed a curvilinear relation to leaf area index and a linear relationship to light interception, (3) a period when wt. of ears showed a linear relationship to an expression involving wt. of green leaf in an earlier period and % survival of tillers, and (4) a period of grain filling and ripening in which some further relative changes in ear and grain weight occurred. (17 references.) E. G. BRICKELL.

Moisture conservation for wheat production in the Upper Snake River dryfarming area. T. W. Massee, F. H. Siddoway, H. C. McKay and S. J. Mech (*Cons. Res. Rep. U.S. Dep. Agric., Res. Serv.*, 1966, 10, 21 pp).— Studies to reduce run-off and erosion, conserve moisture, maintain soil fertility and org. matter, and increase soil moisture utilisation efficiency by crops, are described. Results are presented on stubble management, fall tillage after harvest, rotary subsoiling after planting, initial fallow tillage operations, and cropping systems. E. G. BRICKELL.

Influence of nitrogenous fertilisation on nutrition of crops as regards sulphur. G. Simon Sylvestre, J. Chabannes and N. Driard (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 691–697).—In an experiment with winter wheat, increasing applications of NH_4NO_3 increasingly stimulated the mineralisation of S and its uptake by the crop. In an experiment with flax, N-fertilisation had the opposite effects. Possible causes for these and previous conflicting results are discussed. (Cf. *ibid.*, 1967, **53**, 96, 99.) P. S. ARUP.

Variability of yield within commercial wheat crops. A. C. Taylor (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 51).—Sampling intensities of 225 to 300 quadrats per crop are needed to provide yield estimates with standard errors $\gtrsim \pm 1$ bushel per acre ($P = 0{\cdot}05$). Such intensities would be beyond practical limits in most circumstances. E. G. BRICKELL.

Growing grain sorghum. Anon (*Leafl. U.S. Dep. Agric.*, 1966, 478, 8 pp).—Varieties, seed and seed treatment, seedbed preparation, planting, cultivation, weed control, fertilising, irrigation, harvesting, storing, insects and diseases, and main uses are described. E. G. BRICKELL.

Rice and phosphates. A. Mariakulandai and A. Chamy (*Fertil. News*, 1967, **12**, (2), 20–25).—The effect of phosphates on rice yields in Madras State were studied in relation to limiting factors such as the effect of placement, response to indirect manuring, effect of combination with green manure, farmyard manure, nitrogen and potash, the effect of lime, chelates and micronutrients, and the effect of soil type and soil reaction. The general response of rice to phosphate is complicated, but the following basal dressing is recommended: 33·6 kg P_2O_5, 5,600 kg green leaf, 33·6 kg N and 11·2 kg $MnSO_4$ per hectare. (13 references.) I. DICKINSON.

Bacterial wilt and Stewarts leaf blight of corn. A. L. Robert (*Fmrs Bull., U.S. Dep. Agric.*, 1967, 2092, 12 pp).—Distribution, development, symptoms, spread and control of *Xanthomonas stewartii*, are discussed. E. G. BRICKELL.

Resistance in sweetpotato to the scurf and black rot pathogens. L. W. Nielsen and D. E. Yen (*N.Z. Jl agric. Res.*, 1966, **9**, 1032–1041).—Over 600 sweetpotatoes [*Ipomoea batatas* (L.) Lam.] were evaluated for resistance to black rot (*Ceratocystis fimbriata* Ell. & Halst.) and 506 to scurf (*Monilochaetes infuscans* Ell. & Halst.). No variety was immune from either pathogen. E. G. BRICKELL.

Production of turnips and rutabagas. Anon. (*Leafl., U.S. Dep. Agric.*, 1966, 142, 8 pp).—Climatic and soil requirements, varieties, fertilising, seeding, cultivation, diseases, pests, harvesting, marketing and storage, are briefly described. E. G. BRICKELL.

Variations in silica content of range grasses. A. Johnston, L. M. Bezeau and S. Smoliak (*Can. J. Pl. Sci.*, 1967, **47**, 65–71).—The silica (**I**) contents of range grasses from various sources were found to show significant differences between years and between species within years. No consistent relation could be found between **I** content and location or between **I** content and annual rainfall either current or long-term or over selected periods. Seasonal trends in percentage SiO_2 and in percentage sand + SiO_2, based on many determinations over several years, are shown. J. L. WALPOLE.

Effect of frequency of irrigation on soil moisture and turf quality. R. H. Turley (*Can. J. Pl. Sci.*, 1967, **47**, 89–97).—Plots of three lawn grasses were irrigated when the soil moisture budget (estimated by black Bellani plate atmometers) showed a deficit of 1·3, 1·9, 2·5 and 3·2 cm, all treatments receiving the same amount of water over the season. The 1·3 cm irrigation programme maintained the most satisfactory lawn growth based on yields and visual ratings, giving higher and more uniform soil moisture levels. Of the remainder, only the 3·2 cm irrigation led to reduced growth

and temporary browning of the turf during periods of severe drought. (10 references.) J. L. WALPOLE.

Effects of nitrogen fertilisation of bromegrass on solonetzic soils. R. R. Cairns, W. E. Bowser, R. A. Milne and P. C. Chang (*Can. J. Soil Sci.*, 1967, **47**, 1–6).—Over a five year period there was a 3-fold increase in bromegrass yields due to annual applications of 150 kg N as NH_4NO_3 per hectare, and a 4·5-fold increase due to 300 kg N per hectare. The treatments increased the % of N and K in the forage, but somewhat reduced that of Na, Al and Fe. The reduced Na content in the A horizon and accumulation of NO_3 in the subsoil due to the treatments, indicated increased permeability. A. H. CORNFIELD.

Effects of clipping management on forage yield and leaf streak reaction of five orchard grass varieties grown alone or with lucerne. I. T. Carlson (*Iowa St. J. Sci.*, 1967, **41**, 375–383).—Two early and three late varieties of orchard grass (*Dactylis glomerata* L.) were compared under different cutting regimes that simulated either rotational grazing or growing for hay. The grasses were either intercropped with lucerne or grown alone with two application rates of N. Cutting all varieties on the same day underestimated the annual yield of two of the late varieties which yielded significantly more when harvested on the basis of maturity. Average yields were increased by 57% at the high N rate compared with the low rate while in 14 cases out of 15 the grass-lucerne plots gave even higher yields than the high N plots. Higher yields were obtained under hay management than under simulated rotational grazing but the incidence of leaf streak was greater. Leaf streak was also less evident in mixed stands of orchard grass and lucerne. J. L. WALPOLE.

Promotion of winter growth in pastures through growth substances and photoperiod. G. W. Arnold, D. Bennett and C. N. Williams (*Aust. J. agric. Res.*, 1967, **18**, 245–257).—*Phalaris*-annual grasses–subterranean clover pastures were studied at Canberra in 1964. Responses to gibberellic acid (**I**) increased with decreased growth rate of normal pasture and were obtained subsequent to the first harvest. Response in growth to 4 g **I** could be increased to the level of that obtained with 20 g **I** by the addition of either 4 g naphthylacetic acid or 4 g kinetin. *Phalaris* showed greater response to **I** than did annual grasses or subterranean clover. Extending the photoperiod to 16 h gave a 40% increase in growth rate in winter associated with earlier floral initiation. (32 references.) E. G. BRICKELL.

Cobalt concentrations in some New Zealand fodder plants grown on cobalt-sufficient and cobalt-deficient soils. E. D. Andrews (*N.Z. Jl agric. Res.*, 1966, **9**, 829–838).—Marked differences in Co concn. occurred between fodder plants grown in association with each other on soils deemed to be Co-sufficient. In particular, lucerne and red and white clovers accumulated more Co than did grasses. On Co deficient soils, however, there was no significant difference in Co concn. between white clover and mixed grasses. (12 references.) E. G. BRICKELL.

Growing crimson clover. Anon. (*Leafl. U.S. Dep. Agric.*, 1967, 482).—Optimum cultural conditions are specified and the advantage of bacterial inoculation of the seed before planting is emphasised. A. A. ELDRIDGE.

Legume inoculation in New Zealand. A. Hastings, R. M. Greenwood and M. H. Proctor (*Inf. Ser. Dep. scient. ind. Res. N.Z.*, 1966, 58, 37 pp).—The principles of legume inoculation and types of inoculants, both pelleted and non-pelleted, are discussed. Pelleting has been used successfully with clover, lucerne, and lotus on many soils including the Northland gumlands, the pumice soils of central North Island, and various areas in Otago and Southland. E. G. BRICKELL.

Effect of creeping-rootedness on survival in lucerne. R. A. Bray (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 46–47).—Field trials in S.E. Queensland suggest that creeping-rootedness is a desirable characteristic and should prove useful in the breeding of persistent lucerne varieties. E. G. BRICKELL.

Appearance of sulphur-deficiency symptoms in winter rape in Champagne berrichonne. J. Dejou and J. Morizet (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 997–1005).—The symptoms, including chlorosis of the leaf-parenchyma, stunted growth, and reduced yields, could be relieved by applications of Na_2SO_4; they were particularly prevalent during a season of unusually heavy rainfall. Leaching of SO_4^{2-} from certain soils was an important factor causing the deficiency. P. S. ARUP.

[A] **Influence of time of pruning on bud-development and lengthening of vine shoots.** J. Bouard. [B] **Date of pruning.** M. Rives (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 635–638, 644–651).—[A] Comparative trials with the vine Ugni-Blanc in Charente showed that bud-development on vines pruned later than mid-Jan. was 20% less than on vines pruned during Nov.-Jan. In comparison with vines pruned in March, vines pruned early in May eventually produced longer shoots.

[B] Results obtained with the vine Merlot in Gironde over three years were not sufficiently concordant to form the basis of advice on the best time for pruning. Causes for differences in the results of various observers are discussed. P. S. ARUP.

Factors limiting crop production. VI. Grapes. W. B. Hewitt and D. J. Raski (*Span*, 1967, **10**, 56–59).—Grape production is reduced by adverse climatic conditions, unfavourable and mineral-deficient soils, nematode infestations, virus diseases transmitted by man, insects and nematodes, bacterial diseases and numerous fungus diseases. The symptoms, effects and distribution of many of these are discussed together with some of the control measures adopted. J. L. WALPOLE.

Fungal flora of shoots of fruit trees. P. Bondoux (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 723–730).—The fungal flora on the inside of the bracts of the shoots were examined by direct microscopy and by a cultural method. Data for the occurrence of 18 species on six different fruit trees are tabulated. Possible host-parasite (or saprophyte) relationships are discussed with reference to the different types of invading fungi. P. S. ARUP.

Observations on the pollination of apple trees (*Malus sylvestris* Mill.). II. Varieties Granny Smith, Sturmer, Jonathan, and Cox's Orange Pippin. T. Palmer-Jones and P. G. Clinch (*N.Z. Jl agric. Res.*, 1967, **10**, 143–149).—The only pollinating insects observed during the blossoming period in the Nelson district were honeybees and negligible numbers of *Bombus terrestris* Linn. A density of about 40 bees per 30,000 flowers per min. appears adequate. E. G. BRICKELL.

Nitrogen metabolism during storage in relation to the breakdown of apples. I. Changes in protein nitrogen level in relation to incidence. D. Martin, T. L. Lewis and J. Cerny (*Aust. J. agric. Res.*, 1967, **18**, 271–278).—High N level in the fruit, high R.H. in the storage atm., and advanced maturity at harvest, all increased susceptibility to breakdown; only the first of these factors influenced the protein N level. An inverse relationship between % water loss and breakdown incidence was demonstrated. (18 references.) E. G. BRICKELL.

Virus effect on union and growth of peach scions on *Prunus besseyi* and *P. tomentosa* understocks. G. N. Agrios and W. F. Buchholtz (*Iowa St. J. Sci.*, 1967, **41**, 385–391).—Equal numbers of necrotic ring spot virus (*NRSV*)-infected and non-infected peach, *Prunus besseyi* (**I**) and *P. tomentosa* (**II**) seedlings were budded with *NRSV*-free and *NRSV*-infected peach buds on three different dates. The results of this and a similar, smaller trial without peach seedlings showed that combination of *NRSV*-free stocks with *NRSV*-free buds gave higher percentages of bud take and growth of buds into trees than combinations involving infected scion and/or stock. There were better stands of trees on **I** than on **II** but more trees showed scion-stock incompatibility on **I**; both are relatively poor understocks on which to propagate dwarf peach trees. (12 references.) J. L. WALPOLE.

Phoney disease of peaches. Anon. (*Leafl. U.S. Dep. Agric.*, 1967, 515, 4 pp).—Control measures are described. E. G. BRICKELL.

Close planting of pineapples. I. The effect of different plant spacings upon fruit yield of two Cayenne varieties. K. H. Kwang and Y. M. Chiu (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 42, 125–146).—The closest planting (24 cm apart) gave the highest yield of pineapple fruit in the first crop but the lowest in the second crop while the best overall spacing of the plants was 30 cm apart with a density of 40,000 per ha. The percentage fruitage, and mean fruit weight, were higher for the variety Normal Smooth Cayenne than for Collar-of-slip but the size of fruit obtained from all the treatments was suitable for canning purposes. (11 references.) (From English summary.) J. L. WALPOLE.

Replacement of *Citrus aurantium* L. (bigaradier) and use of new stocks. L. Blondel (*Fruits*, 1967, **22**, 19–26).—Stocks resistant to the Tristeza disease and suitable for replacement, stocks worth further investigation, and unsuitable types of stock are enumerated. Three stocks which are resistant to Tristeza and to Gommose are particularly considered. (37 references.) P. S. ARUP.

Black rot of oranges caused by species of *Alternaria*. P. Joly (*Fruits*, 1967, **22**, 89–95).—The modes of attack of several species

and the consequent symptoms are described. Control by use of chemicals having proved inadequate, the cultivation of resistant varieties of trees, under optimum growth conditions, is recommended. (17 references.) P. S. ARUP.

Strawberry diseases. J. R. McGrew (*Fmrs' Bull., U.S. Dep. Agric.*, 1966, 2140, 27 pp).—Distribution, symptoms and damage, and methods of prevention for 31 diseases, are described. E. G. BRICKELL.

Strawberry culture. G. M. Darrow (*Fmrs' Bull., U.S. Dep. Agric.*, 1966, 1026, 36 pp).—Location, soil, planting and training, setting, tillage, mulching, frost prevention, fertilisers, irrigation and harvesting are discussed for the South Atlantic and Gulf Coast regions. Suitable varieties are indicated. E. G. BRICKELL.

Protection of melon against *Fusarium* by pre-infection with other strains of *Fusarium*. P. Mas (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1034–1045).—Infection, through the soil, of young melon plants with strains of *Fusarium oxysporum* that were non-pathogenic to melons but pathogenic to related botanical species, gave a certain measure of protection against the strain specifically pathogenic to melons. (11 references.) P. S. ARUP.

Effect of thinning and early harvesting on flowering and fruit set of 'flat white Boer' pumpkins. E. Strydom and J. Breytenbach (*S. Afr. J. agric. Sci.*, 1966, **9**, 651–660).—The removal of 25–40% of the pistillate flowers and young fruits decreased the natural (80%) natural abscission of these growths and resulted in the same (20%) yield as obtained without thinning. The harvesting of fully developed immature pumpkins resulted in increased fruit set and higher yields. The distribution of the sexual phases occurring on the main stems and side shoots was examined. P. S. ARUP.

The relationship between pan evaporation and irrigation requirements of sugarcane in Taiwan. I. Effect of different pan ratios for controlling irrigation on the yield of sugarcane. H. Chang, J. S. Wang and S. S. Lin (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 42, 51–69).—Field trials involving three types of soil and six different irrigation pan ratios (*PR*) (three in each of two years) showed no significant difference in the yields of cane and sugar between the various *PR* but suggests that a *PR* of 0·80 may be used as a standard for optimum irrigation. The poorest cane yield was given by the sandy soil with little difference between clay loam and sandy loam but the poor performance in sandy soil may be mainly due to the much heavier infestation of root-knot nematodes it contained. The trials stress the importance of water requirements for the cane crop. (11 references.) (From English summary.) J. L. WALPOLE.

Potassium requirements of sugarcane on liming an acid soil. Y. J. Hsia and C. M. To (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 43, 55–68).—Liming increases the pH and the sugarcane yield of acid soils but is effective for at the most 2 years, after which the soil becomes depleted of essential elements. Production of sugarcane can be restored and improved by the addition of potash provided the rate exceeds 150 kg/ha. (12 references.) (English summary.) J. L. WALPOLE.

The cultivation system of late spring planting and ratooning of sugarcane in gravelly, sandy soil. L. H. Lee and S. T. Liao (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 42, 111–124).—The late spring planting and ratooning system (*LSPR*) of planting sugarcane three months earlier than normal autumn planting (*AP*), harvesting at the first dry season and ratooning it afterwards has been tested on gravelly, sandy soil having its dry season in the winter and spring. The total yield of two harvests of *LSPR* was higher than one harvest of normal *AP* but the second ratoon yield of *LSPR* was low so that the total yield from three *LSPR* harvests was not superior to two *AP* with its succeeding ratoon. *LSPR* would be an acceptable system when the sugar price is high and the increased yield is imminent. Suitable cultural details of the *LSPR* system are given. (From English summary.) J. L. WALPOLE.

Studies on the cultivation of the newly released cane variety F.151. R. S. Chen and C. C. Tse (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 42, 97–109).—Planting trials on the sugarcane variety F.151 (1962–1965) showed that the highest plant cane yield was obtained from mid-July planting with late-August (*A*) planting second highest; mid-October (*O*) gave the lowest while higher total yields of plant and ratoon cane were achieved in *A* and *O* plantings. Max. yields were obtained from a spacing of 1·25 m between rows and 0·4 m between plants giving 20,000 double node cuttings per ha. (24 references.) (From English summary.) J. L. WALPOLE.

Study on the planting method of sugarcane at the tidal land of Yung Lin. I. Influence of row spaces on the yield of cane. W. T. Chen (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 42, 87–96).—Experiments with two varieties of sugarcane and three planting row spacings showed N : Co.310 to be the better yielding variety and for spring planting the cane rows should be about 1 m apart with about 40,000 seed cuttings per ha. J. L. WALPOLE.

Red-rot resistant mutant of sugarcane induced by gamma irradiation. J. T. Rao, K. V. Srinivasan and K. C. Alexander (*Proc. Indian Acad. Sci.*, B, 1966, **64**, 224–230).—Mutant clones from irradiated sugarcane, variety Co. 449, were morphologically indistinguishable from the original variety and possessed similar agronomic attributes also. They were resistant to strain D of the red-rot pathogen *Glomerella tucumanensis* and arose from the third bud of the cane arising from the irradiated bud. E. G. BRICKELL.

Studies on the ratoon stunting disease of sugarcane. III. Effect of thermal treatments on germination of sugarcane. H. P. Liu, S. M. Lee, W. S. Teng and P. T. Hsieh (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 42, 9–21).—The effects of hot water (*HWT*) and hot air (*HAT*) treatments for sugarcane ratoon stunting disease are reported and show that although there were varietal differences in heat tolerance the germination of all seed cane was reduced by increasing the treatment temp. particularly under *HWT*. Germination of seed cane from upper portions of the stalk was poor after *HWT* compared with medium and lower portions whereas *HAT* usually gave a higher germination rate for the higher portions. A new system of collecting spring top cuttings to be used for autumn planting has been developed and they can be safely given *HWT* at 50° for 2 h. (15 references.) (From English summary.) J. L. WALPOLE.

Seasonal evapotranspiration of irrigated cotton in a low-altitude environment. W. R. Stern (*Aust. J. agric. Res.*, 1967, **18**, 259–269).—Evapotranspiration (**I**) directly after irrigation (E_{tI}) was greater than the Penman estimate of potential evaporation (E) but less than the evaporation from a standard Australian tank (E_{tank}), the mean ratios being 1·2 for E_{tI}/E and 0·9 for E_{tI}/E_{tank}. Rate of **I** was a function of soil moisture, declining rapidly as the available moisture fell below 60%. It would appear that in this environment advection of energy persisted for most of the year, and that during periods of rapid height increases, particularly when ground cover was incomplete, crop surface roughness enhanced **I**. (32 references.) E. G. BRICKELL.

Preliminary physiological studies on the promotion of latex flow by plant growth regulators. S. G. Boatman (*J. Rubb. Res. Inst. Malaya*, 1966, **19**, 243–258).—Possible mechanisms whereby the plant growth regulators, 2,4-D and 2,4,5-T (**I**), promote the flow of latex in rubber trees are investigated. Changes in latex viscosity, or in latex vessel collapse, or bacterial contamination of the cut ends of the latex vessels are considered; these are unlikely to be involved in this effect. Repeated re-opening of the tapping cut showed that the flow is restricted fairly rapidly by some process close to the surface of the cut; this process is apparently delayed by **I** treatment. There is evidence to show a lowering of the rubber content after treatment with **I** and this cannot be entirely explained in terms of the dilution effect due to increased yields. This is not experienced in rested trees which have been treated. (12 references.) J. L. WALPOLE.

Biological coagulation of *Hevea* latex using waste carbohydrate substrates. C. K. John (*J. Rubb. Res. Inst. Malaya*, 1966, **19**, 286–289).—Near-completion (>99·5%) coagulation of latex was obtained in 16 hours by the addition of molasses (**I**) or waste pineapple juice (**II**) at the level of 0·2% sugar which effectively expedites natural coagulation of latex by microbial breakdown. The rubber treated with **II** is golden yellow; that from **I** molasses is paler, both rubbers having pleasant odours and fairly fast curing characteristics. Addition of latex serum as a seeding inoculum further speeded up coagulation but led to the production of malodorous rubber. This biological coagulation process is economically attractive and the resulting rubber is technologically satisfactory if processed with minimum delay. (12 references.) J. L. WALPOLE.

Particle aggregation following dilution of *Hevea* latex: A possible mechanism for the closure of latex vessels after tapping. S. W. Pakianathan, S. G. Boatman and D. H. Taysum (*J. Rubb. Res. Inst. Malaya*, 1966, **19**, 259–271).—*Hevea* latex samples diluted with water show aggregation and flocculation which lead to changes in sedimentation behaviour in the ultracentrifuge. Dilution causes progressive damage to a portion of the non-rubber bottom fraction

which tends to aggregate both with itself and with rubber particles. Flocculation seen in latex collected immediately after tapping is probably caused by osmotic shock (rapid decrease in osmolarity of the latex). The accumulation of flocculated particles at, or near, the cut ends of the latex vessels may be the mechanism for the formation of plugs which are thought to restrict the flow. Treatment of trees with 2,4,5-T prolongs the flow and results in a greater dilution and increased damage to the collected latex. (14 references.) J. L. WALPOLE.

Pest Control

Substituted phenyl *N*-methylcarbamates as temporary immobilising agents for birds. E. W. Schafer, R. I. Starr, D. J. Cunningham and T. J. Decino (*J. agric. Fd Chem.*, 1967, **15**, 287–289).—Temporary immobilisation and acute oral toxicity of 22 compounds were determined on red-winged blackbirds (*B*) and starlings (*S*). *B* were immobilised by 12, *S* by eight at doses 10 mg/kg. Three were exceptionally effective: *o*-(2-propynyloxy)phenyl-*N*-methylcarbamate on *B*, 6-chloro-3,4-xylyl-*N*-methylcarbamate on *S* while the 4-(methylthio)-3,5-xylyl deriv. was effective on both *S* and *B*. Structure correlation indicated that the presence of branched, low mol. wt. alkyl or ether groupings at the 2- or 3-positions or Me-groups at the 3- or 5-positions in the phenyl ring, or both together with a substituted S or N containing group at the 4-position of the phenyl ring, enhanced the temporary immobilising action. (16 references.) P. S. ARUP.

Protecting honeybees from pesticides. Anon. (*Leafl. U.S. Dep. Agric.*, 1967, 544, 6 pp).—Simple precautions for farmers and beekeepers are listed together with commonly used pesticides grouped according to their relative hazards to honeybees. E. G. BRICKELL.

Rapid method for [determination of] surface residues of organophosphorus pesticides by total phosphorus. E. J. Broderick, E. F. Taschenberg, L. J. Hicks, A. W. Avens and J. B. Bourke (*J. agric. Fd Chem.*, 1967, **15**, 454–456).—The matter extracted with light petroleum from the surface of fruits is digested with HNO_3 and H_2SO_4, followed by $HClO_4$. Diazinon or Guthion could be determined satisfactorily as inorg. PO_4 by this method, but Imidan required an extra preliminary treatment with HBr for complete ashing. (12 references.) P. S. ARUP.

Thin-layer chromatographic screening test for organophosphorus pesticide residues. D. C. Abbott, A. S. Burridge, J. Thomson and K. S. Webb (*Analyst, Lond.*, 1967, **92**, 170–175).—Traces of organo-P compounds are extracted with CH_2Cl_2; the extracts are cleaned-up on SiO_2-gel chromatoplates (250 μ) developed with hexane-acetone (5 : 1). After elution with hexane–acetone (3 : 1), the separated compounds are oxidised with $(NH_4)_2S_2O_8$ or HNO_3-$HClO_4$, for spectrophotometric determination of P at 820 mμ by Mo-blue procedures. Mobile solvents required for cleaning up some polar pesticides and metabolites on multi-band plates are listed. Method is most suitable for compounds having $R_F > 0{\cdot}03$ in the system. Recoveries of most pesticides in concn. of $\sim 0{\cdot}2$ ppm are usually >80%, with crop blank-values generally $< 0{\cdot}2$ ppm. W. J. BAKER.

Physical methods of pest control. W. M. Carleton and L. A. Liljedahl (*J. Wash. Acad. Sci.*, 1967, **57**, 61–69).—A review of methods based on changes in mechanical, thermal, sound or radiation environment. E. G. BRICKELL.

Assessment of efficiency of aerial application of sprays: use of kaolin-coated targets. M. R. Middleton (*Chemy Ind.*, 1968, 113–114).—The prep. of thin-layer plates (5 or 20 cm × 20 cm) coated with 0·02 in. of Spestone-grade kaolin is described. They can be used to collect both oil- and water-sprays without shatter of droplets >3000 μ; the kaolin is hard and absorbent and permits determination of droplet spectrum down to 20 μ (down to 5 μ when dyed with Disulphine Blue). An image of the deposits can be projected on to a screen or obtained photographically for printing on 'Dalcopy' paper. The dyed spray is easily removed from the kaolin for colorimetric determination. W. J. BAKER.

Use of aircraft and associated equipment [for crop spraying]. R. J. V. Joyce (*Chemy Ind.*, 1968, 117–121).—A brief account of the principles of target spraying is followed by a discussion of the following possible large-scale uses of it in Africa: (*i*) aerial spraying of chemicals for land reclamation and crop protection, including spraygear (the Micronair), (*ii*) surveys for pests and diseases, including establishment of a reliable sampling scheme and the technique of search and control in the air (as for the desert locust and army worm), (*iii*) exploitation of variable rainfall to establish an agricultural system based on aerial survey for well-watered sites, aerial seeding and application of fertilisers, and any necessary post-emergence weed control. Such a system has been proposed in Malaya for rice production, but ultra-low-vol. spraying techniques are essential. (17 references.) W. J. BAKER.

Evaluation of foliar sprays for control of weather fleck on flue-cured tobacco. E. K. Walker (*Can. J. Pl. Sci.*, 1967, **47**, 99–108).—Selected materials (32) comprising dithiocarbamates, quinones, secondary amines, various antioxidants and miscellaneous substances used with, and without, stickers and/or wetters were sprayed on susceptible tobacco plants in attempts to control weather fleck. Diphenylamine, phenothiazine, 1,4-naphthoquinone, dichlone and *N,N'*diphenyl-*p*-phenylenediamine gave good results, the first three being particularly effective. These three caused some leaf damage but not sufficient to depreciate the quality of the cured leaves. Dichlone sprays gave rise to excessive green fixation which reduces leaf value. (16 references.) J. L. WALPOLE.

Sampling and sizing of spray drops from water-in-oil [pesticide] emulsions. R. E. Ford and C. G. L. Furmidge (*Chemy Ind.*, 1968, 111–113).—Discusses conditions necessary for making accurate stain-size/drop-size measurements on invert emulsion sprays. Well-defined stains, with low spread-factor, are best obtained by impacting drops on glazed bromide paper and developing with 1% Nigrosine G 140 dye. The emulsion used for calibration should pass through a nozzle similar to that used in the field. For drops of dia. >1000 μ, single drops of known size are obtained from a micrometer-syringe so that they impact at terminal free-fall velocity, but for dia. <1000 μ a small spinning-cup atomiser (sketched) is preferred and the drops collected into spherical globules by use of 10% petroleum jelly in naphthenic acid. Viscosity is the main factor responsible for variations in spread factor. W. J. BAKER.

Studies on root-knot nematodes of sugarcane. I. Effect of soil temperature on the activity of *Meloidogyne* spp. C. H. Hu (*Rep. Taiwan Sug. Exp. Stn*, 1966, No. 42, 23–40).—Four species of root-knot nematodes were studied: *Meloidogyne incognita, M. incognita acrita, M. javanica* and *M. arenaria* of which *M. incognita* and *M. javanica* were the most important. The egg hatch which generally took 6–8 days at room temp. was about 99% and single egg masses contained an average of about 844 eggs. The larvae of *M.* spp. were able to survive in the soil for 2 months without food in the winter and no root knot formation was observed below a soil temp. of 16°. The life cycle of the root knot nematode took 56 days to complete in winter (19·2°), 28 days in autumn (22·1°). and only 25 days in summer (28·6°). The summer and autumn seasons were the most favourable to nematodes since 100% infection occurred 10 days after inoculation while in winter only 67·5% infection was observed 40 days after inoculation. (40 references.) (From English summary.) J. L. WALPOLE.

Study on the sugarcane yield-increasing effect of the soil fumigant DBCP compared with DD and EDB. T. K. Tsai and H. T. Chu (*Rep. Taiwan Sug. Exp. Stn.*, 1966, No. 42, 1–8).—Trials over the years 1961–1966 showed that DBCP (1,2-dibromo-3-chloropropane) applied as three commercial formulations, significantly increased the sugarcane yield to a greater extent than DD or EDB (ethylene dibromide) both for plant cane and ratoon cane. (From English summary.) J. L. WALPOLE.

Comparison of *Pythium* spp. causing sugarcane root rot. S. C. Hsu and Y. C. Liu (*Rep. Taiwan Sug. Exp. Stn*, 1966, No. 42, 41–46).—Sugarcane root rot is caused mainly by the pathogens *Pythium catenulatum, P. arrhenomanes* and *P. mamillatum*. The optimum growth temp. for *P. arrhenomanes* and *P. mamillatum* is 32° and for *P. catenulatum* is 34°; the optimum pH is 6·5 although growth can take place between pH 4·5 to 9·5. Corn meal agar and potato glucose agar were the best of six media tested for colony growth. *P. catenulatum* was the most harmful but a combined inoculation of all three caused the most severe damage. (From English summary.) J. L. WALPOLE.

Pathological causes of poor ratoon standing of sugarcane. H. T. Chu, S. C. Hsu and Y. T. Liu (*Rep. Taiwan Sug. Exp. Stn*, 1966, No. 43, 1–9).—Trials have shown that poor sugarcane ratoon stands are associated with the presence of root rot pathogens, particularly *Pythium arrhenomanes, P. catenulatum, P. mamillatum* and *Leucoporus* sp. Higher plant yields and better germination were obtained by steam-sterilising the soil and by the use of fungicides. (10 references.) (English summary.) J. L. WALPOLE.

Fungicidal activity of some fluoro-aryloxy fatty acids and their mercury salts. K. C. Srivastava (*Chem. Age India*, 1966, **17**, 1012–1013).—The 2-chloro-4-fluorophenoxy carboxylic acids $[ClFC_6H_2(OCHC_nH_{2n+1}COOH)_2$, $n = 1,2,3,4,6,8]$, the corresponding 2-bromo compounds, and the Hg salts show fungicidal activity against *Alternaria solani*. Only the Hg salts are effective at 35 and 350 ppm levels. K. GRAUPNER.

Industrial fungicides. N. J. Turner (*Encyl. Chem. Technol.*, 1966, **10**, 228–236; *Contr. Boyce Thompson Inst. Pl. Res.*, 1966, 2067, 9 pp).—A review. (23 references.) E. G. BRICKELL.

Dithiocarbamate fungicides. I. Preparation of *N*-cyanoalkyl and *N*-carboxyalkyl dithiocarbamates. G. Matolcsy and G. Josepovits (*Acta Chim. Hung.*, 1967, **51**, 319–325).—Sodium *N*-methyl-*N*-β-cyanoethyldithiocarbamate was made from β-methylaminopropionitrile and CS_2 in NaOH and its hydrolysis with NaOH gave Na *N*-methyl-*N*-β-carboxyethyldithiocarbamate. The Na salts were converted to the Zn salts. The S-substituted deriv. were obtained analogously to the similar deriv. of dimethyldithiocarbamic acid. A scheme of synthesis is presented. The contact and systemic fungicidal activities of the compounds will be reported elsewhere. (14 references.) (In English.) M. SULZBACHER.

Effect of exposure to radio-frequency electric fields on seed-borne micro-organisms. W. L. Seaman and V. R. Wallen (*Can. J. Pl. Sci.*, 1967, **47**, 39–49).—Exposure of various infected seeds to radio-frequency electric fields of 59–64 Mc/sec gave variable control of fungi and bacterial organisms. *Diaporthe phaseolorum* in soyabean seeds was controlled with little loss of germination whereas the fungi *Ascochyta pisi* and *A. pinodes* in field peas could not be eradicated without considerable reduction in germination. Bean seeds infected with *Xanthomonas phaseoli* var. *fuscans* and cabbage seed inoculated with *X. campestris* were killed by exposures which only reduced without eradicating the bacteria. The effect of varying the moisture content of treated pea seeds was also studied. (29 references.) J. L. WALPOLE.

Periodical cicadas. Anon. (*Leafl. U.S. Dep. Agric.*, 1966, 540, 8 pp).—Habits of the 17-year brood locusts (*Magicicada septendecim*, *M. cassini*, and *M. septendecula*, and the 13-year brood *M. tredecim*, *M. tredecassini*, and *M. tredecula*, are described. E. G. BRICKELL.

Ovariole number in brown locust (*Locustana pardalina*, Walker) in relation to environment. I. G. Venter (*S. Afr. J. agric. Sci.*, 1966, **9**, 629–638).—Significant correlations were found between variations in the mean ovariole no., 60·4–66·4, (observed as between different populations and successive generations), and the local and seasonal variations in nutritional conditions. The results confirm the previously observed lack of contact between neighbouring contemporaneous populations. P. S. ARUP.

Histology of fore gut and mesenteron of the larva of the sugarcane borer *Sesamia calamistis* Hmpsn. (Lepidoptera: Noctuidae). H. J. R. Dürr (*S. Afr. J. agric. Sci.*, 1966, **9**, 639–650).—A detailed histological description is presented of the fore gut and mesenteron of this major pest of maize in W. Cape, S. Africa. (23 references.) P. S. ARUP.

Studies on *Paratylenchus nanus*. II. Population changes around apple roots. J. M. Fisher (*Aust. J. agric. Res.*, 1967, **18**, 279–287).—In soil all stages of *P. nanus* showed a decline in numbers in winter and summer and an increase in spring (Sept.). At other times numbers remained stationary except for a decrease in larval numbers in Oct. and an increase in females between Nov. and Jan. Males always exceeded females in number and larvae always exceeded adults. Survival of the different stages under an adverse environment suggested that high temp. in summer and low temp., with high moisture levels in winter, were partly responsible for decreases in number at these times. E. G. BRICKELL.

Effect of attack by *Costelytra zealandica* (White) larvae on ryegrass varieties. J. M. Kelsey (*N.Z. Jl agric. Res.*, 1967, **10**, 172–175).—Tests with four varieties of ryegrass showed no significant differences in numbers of *C. zealandica* larvae attacking, or in dry matter wt., but there was a very strong correlation between increase in larval numbers and decrease in plant dry matter. E. G. BRICKELL.

Relations of insect and host plant. I. Effects of water stress in host plants on infestation by *Aphis fabae* Scop., *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.). II. Effects of water stress in host plants on the fecundity of *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.). C. H. Wearing (I and II) and H. F. van Emden (I) (*Nature, Lond.*, 1967, **213**, 1051–1052; 1052–1053).—I. Bean plants infected with *Aphis fabae* Scop., marigolds infected with *A. fabae* and *Myzus persicae* (Sulz.) and brussels sprouts infected with *M. persicae* and *Brevicoryne brassicae* (L.) were grown under controlled degrees of water stress for four weeks. No effect of water stress was observed with *A. fabae* whereas *B. brassicae* decreased with increasing water stress; *M. persicae* showed a decreased reproduction at a water deficit of 90% but a highly significant increase in reproduction at a water deficit of 50%. A further experiment has shown that *M. persicae* feeding on a pressureless diet obtains greater benefit from an increase in sucrose concn. than the other two aphids so that it may be less dependent on a food supply under pressure. (10 references.)

II. The fecundity of *Myzus persicae* and *Brevicoryne brassicae* has been studied on young, mature and old leaves of brussels sprouts growing in three water regimes. On young and mature leaves the fecundity of both species increased with water shortage. On old leaves the fecundity of *B. brassicae* diminished with increasing water shortage while that of *M. persicae* was greater on plants in the medium regime but less in the dry regime. *M. persicae* appears to be less dependent on turgor pressure for feeding than *B. brassicae* though both appear to benefit from sap enrichment during host plant water shortage. S. A. BROOKS.

Fate of 3-hydroxy-*N*-methyl-*cis* crotonamide dimethylphosphate in cotton plants. D. A. Lindquist and D. L. Bull (*J. agric. Fd Chem.*, 1967, **15**, 267-272).—In the plants the initial oxidation of the insecticide Azodrin (**I**) to the *N*-methylol deriv. is less important than in insects or animals. The primary sites of hydrolytic degradation here are at the vinyl-phosphate bond and at one Me-phosphate bond. The half-life of **I** in cotton leaves was 7 days. **I** rapidly evaporated from the surface of the leaves. P. S. ARUP.

Effect on honey bees of diazinon and phenthoate applied as sprays to white clover (*Trifolium repens* L.). P. G. Clinch, T. Palmer-Jones and I. W. Forster (*N.Z. Jl agric. Res.*, 1966, **9**, 980–984).—Diazinon at 1-lb and phenthoate (94–96% pure) at 1·25-lb active ingredient per acre, proved highly toxic to honey bees, and are thus unsuitable for application to flowering crops. (10 references.) E. G. BRICKELL.

DNA, RNA, and protein synthesis in HeLa S cells exposed to DDT and dieldrin. R. A. Chung, I-Lo Huang and R. W. Brown (*J. agric. Fd Chem.*, 1967, **15**, 497–500).—In cultures of HeLa S cells total cell-counts decreased as the concn. of DDT or dieldrin were increased from 0–0·5 ppm, but the decrease became less marked as the concn. were increased up to 50 ppm, though the total protein content remained constant. Incorporation of ^{14}C-leucine increased over the lower range of added DDT and dieldrin, but decreased over the range up to 50 ppm. Differences in the effects at various levels of DDT and dieldrin were observed as regards the synthesis of DNA and RNA. (12 references.) P. S. ARUP.

Retention of vaporised lindane by plants and animals. D. M. Whitacre and G. W. Ware (*J. agric. Fd Chem.*, 1967, **15**, 492–496).—Almost all of the tissues of test animals and plants stored γ-BHC during exposure to the vapour over 46 days. Visible effects were observed in fish only, all of which died during exposure. (11 references.) P. S. ARUP.

Selectivity of Sumithion compared with methyl parathion. [A] Influence of structure on anticholinesterase activity. [B] Metabolism in the white mouse. [C] Metabolism in susceptible and resistant houseflies. H. M. Hollingsworth, R. L. Metcalf and T. R. Fukuto (*J. agric. Fd Chem.*, 1967, **15**, 235–241; 242–249; 250–255).—[A] Whilst the toxicities of Sumithion (**I**) and Me-parathion (**II**), and the closely related analogues to susceptible houseflies and the German cockroach showed slight variations, their toxicities to white mice differed considerably. The reasons for these variations are considered in relation to structural differences as between the insecticides and as between insect and mammalian cholinesterases. (29 references.)

[B] The metabolism of ^{32}P-labelled **I** and **II** in the mouse was studied with the object of explaining the comparatively low mammalian toxicity of **I**. The reason is probably to be sought in the ability of the digestive system to cleave the P-O-alkyl bond in the **I**, whereas very little demethylation was observed in the analogous phosphonothionates. (32 references.)

[C] Penetration, activation, and degradation mechanisms functioned almost identically as regards both compounds, penetration rates being slower in the resistant than in the susceptible flies. Resistance was mainly attributed to enhanced phosphatase activity in the resistant flies, resulting in increased degradation of the activation products Sumioxon and Me-Paraoxon. Further possible factors are considered. (16 references.) P. S. ARUP.

Antidotes in anticholinesterase poisoning. T. Nishimura, C. Tamura and Y. Uchida (*Nature, Lond.*, 1967, **214**, 706–708).—The irreversible inhibition of acetylcholinesterase (**I**) by some alkyl phosphates (including insecticides) is prevented by reactivation of **I** with nucleophilic substances, e.g., pyridine-2-aldoxime methiodide (**II**) or trimethylene bis (4-hydroxyimino methyl pyridinium) dibromide (**III**), which displace the phosphoryl group from **I**. Fifteen oxime compounds, all 1-(quaternary NH_4)-3-(4-hydroxyiminomethylpyridinium) propane dibromides, were examined for their effectiveness in reactivating **I** inhibited by Et_4 pyrophosphate, their acute toxicity, and their ability to save the lives of mice poisoned by parathion, Me-parathion (**IV**) or Dimetilan. Results are listed and discussed in respect of correlations between reactivating potency, toxicity and chemical structure. One or two compounds were as effective as **II** or **III** when injected intraperitoneally into mice poisoned by **IV** or 2,2-dichlorovinyl dimethyl phosphate. W. J. BAKER.

Photo-oxidation of two 4-dimethylaminoaryl methylcarbamate insecticides (Zectral and Matacil) on bean foliage and of alkylaminophenyl methylcarbamates on silica gel chromatoplates. A. M. Abdel-Wahab and J. E. Casida (*J. agric. Fd Chem.*, 1967, **15**, 479–487).—Column chromatography, TLC, and the detection of the carbamates are described. Whilst Zectran and Matacil do not readily decompose when exposed as spots on the plates to fluorescent light, toxic products are formed by extensive oxidation at the dimethylamino moiety when residues of the insecticides are exposed to sunlight on bean foliage. Chemical and physical properties (including TLC data) and biological activities are tabulated for Zectran and Matacil and for oxidation products of each with various *p*-substitutents. (20 references.) P. S. ARUP.

Studies on the stability of malathion in emulsifiable concentrates. Chung-sung Wu (*Rep. Taiwan Sug. Exp. Stn*, 1966, No. 43, 69–78).—Benzene and xylene are satisfactory solvents for malathion (**I**) emulsifiable concentrates (*EC*); polar cpds., such as isopropyl, n-butyl and isoamyl alcohols, lead to fairly rapid breakdown of the toxicant. Moisture content must be kept below 1% and emulsifying agents should contain few OH groups: thus polyoxyethylene (**II**)-mono-oleate, sulphonated castor oil and **II**-alkyl phosphate gave good results. The decomposition rates of **I**-*EC* kept at elevated temp. rise rapidly above 40° and the rate of decomposition is also accelerated by contamination with >0·05% of $AlCl_3$, $FeCl_3$ and $ZnCl_2$; org. acid anhydrides (e.g. Ac_2O at 4·8%) minimise decomposition at 50° over a period of one year. (English summary.) J. L. WALPOLE.

Heptachlor, heptachlor-epoxide and gamma chlordane residues in soil and rutabaga after soil and surface treatments with heptachlor. J. G. Saha and W. W. A. Stewart (*Can. J. Pl. Sci.*, 1967, **47**, 79–88).—Distribution of heptachlor (**I**) insecticide residues in soil and rutabagas (**II**) was studied by GLC which enabled **I**, **I**-epoxide and gamma chlordane to be determined separately. **I** in granule form was incorporated into the soil at 6·6 kg/ha and **I** emulsion was applied three times at 4·48 kg/ha to the soil surface. Only about 3% of the surface-applied insecticide was recovered after 3–4 months whereas 15% of the insecticide incorporated into the soil was recovered after 5 months. **II** grown in surface-treated soil contained 0·042 ppm of total residues while those from insecticide-incorporated soil had 0·060 ppm, 98% of the residue being found in the peel and only 2% in the pulp. The vertical and lateral distributions of **I** into the **II** were studied in detail. Boiling in water removed half of the pesticide residue from the peel and all of it from the pulp. (19 references.) J. L. WALPOLE.

Insecticide residues in wheat grown in soil treated with aldrin and endrin. J. G. Saha and H. McDonald (*J. agric. Fd Chem.*, 1967, **15**, 205–207).—Detectable amounts of endrin (**I**) (0·015–0·075 ppm) were found in the foliage of young wheat plants sown eight days after treatment of the soil with 2 or 8 lb **I** per acre; they were also found in the straw. Similar results were obtained with aldrin. No residues were found in the grain from 8 lb/acre plots. (16 references.) P. S. ARUP.

Residues of organochlorine insecticides and their metabolites in soils in the Atlantic provinces of Canada. J. R. Duffy and N. Wong (*J. agric. Fd Chem.*, 1967, **15**, 457–464).—The residues were obtained from 56 agricultural soils by extraction with hexane–2-propanol, and purification with Florisil. Analyses were carried out by GLC with two column types and electron capture detection, supplemented by TLC. About 45% of the soils contained 1–9 ppm of DDT and its metabolites (DDD and DDE), 32% contained 0·75 ppm of aldrin plus dieldrin, and 9% contained 0·06–0·86 ppm of heptachlor, heptachlor-epoxide, and γ-chlordane. (12 references.) P. S. ARUP.

Residue determination of GS 13005, a new insecticide. D. O. Eberle, R. G. Delley, G. C. Székely and K. H. Stammbach (*J. agric. Fd Chem.*, 1967, **15**, 213–216).—This insecticide is extracted with light petroleum from aq. homogenates of fruits, vegetables, and foliage. Descriptions are given of a cleanup process involving solvent-partition, the colorimetric determination as used for diazinon (cf. Zweig, *Pesticides, Plant Growth Regulators, and Food Additives*, Academic Press, 1964, Vol. 2, 109), and TLC and GLC methods. GS 13005 is the active ingredient of a new organo-P insecticide (Geigy S.A., Basel, Swiss P. 392,521 and 395,632) with a structure *O,O*-dimethyl-*S*-[2-methoxy-1,3,4-thiadiazol-5-(4*H*)-onyl-(4)-methyl]-dithiophosphate. P. S. ARUP.

Gas chromatographic analysis of chlordane by head gas. E. F. Corcoran, J. F. Corwin and D. B. Seba (*J. Am. Wat. Wks Ass.*, 1967, **59**, 752–756).—The application of the technique of the head gas analysis of volatile org. compounds to the analysis of a chlorinated pesticide (chlordane), using a H_2 flame ionisation detector, is described, and the potential value of this new method for the analysis of all non-polar pesticides is discussed. (16 references.) J. M. JACOBS.

Gas chromatographic analysis of 3-hydroxy-*N*-methyl-*cis*-crotonamide dimethylphosphate (Azodrin) and 3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide dimethylphosphate (Bidrin). M. C. Bowman and M. Beroza (*J. agric. Fd Chem.*, 1967, **15**, 465–468).—Residues of the insecticides were extracted from sweet maize with $CHCl_3$. Aliquots of the extracts were submitted directly to GLC with flame photometric detection and the use of a 526 nm filter to sense P. Reponses were rectilinear up to 250 ng. Recoveries were 94–101% in the range 0·05–5·0 ppm. (14 references.) P. S. ARUP.

Determination of residues of 4-amino-3,5-trichloropicolinic acid in cereal grains by gas chromatography. E. L. Bjerke, A. H. Kutschinski and J. C. Ramsey (*J. agric. Fd Chem.*, 1967, **15**, 469–473).—Residues of the herbicide (Tordon acid) are extracted from grain or straw with 0·1 N-KOH. The cleanup is accomplished by column chromatography on Al_2O_3 followed by regulated oxidation of impurities with acid $KMnO_4$. The acid is esterified with diazomethane for GLC with electron capture detection. Recoveries were 74% for straw and 86% for grain. Out of 24 samples of wheat and barley grown in Canada, U.S.A., and Australia, 75% contained 0·05 ppm of the acid, but residues amounting to 0·22–0·64 ppm were occasionally found. P. S. ARUP.

[A] **Quantitative extraction of root-absorbed dieldrin from aerial parts of forage crops.** [B] **Absorption and translocation of dieldrin by forage crops.** W. B. Wheeler, D. E. H. Frear, R. O. Mumma, R. H. Hamilton and R. C. Cotner (*J. agric. Fd Chem.*, 1967, **15**, 227–230; 231–234).—[A] Using ^{14}C- and ^{36}Cl-labelled dieldrin (**I**) it is shown that root-absorbed insecticide is not quant. extracted from the aerial tissues by the normal procedure of blending with successive portions of hexane and Pr^iOH. The remaining **I** can be extracted with $CHCl_3$–MeOH by Soxhlet. Testing of all extraction procedures involving labelled compounds is advised. (11 references.)

[B] The above procedures show that uptake of **I** from sand is greater than from soil. (12 references.) P. S. ARUP.

Insect population control by use of sex pheromones to inhibit orientation between the sexes. L. K. Gaston, H. H. Shorey and C. A. Saario (*Nature, Lond.*, 1967, **213**, 1155).—By permeating the atm. with synthetic *Trichoplusia ni* pheromone, *T. ni* males have been prevented from orienting to *T. ni* females. It is thought that economic control of an insect over large areas may be possible by this method. S. A. BROOKS.

Use of micro-organisms for control of insect pests. J. R. Norris (*Chemy Ind.*, 1967, 1941–1945).—Requirements of a successful insect pathogen are first enumerated and some laboratory and field experiences in the use of the two main types of control agent are then described. These are the bacteria, e.g., *Bacillus popilliae* and *B. thuringiensis*, and the viruses, e.g., polyhedroses and granuloses. Problems and difficulties in the successful mass production, storage, and application to specific pests are discussed in general. Some immediate and future developments are forecast. W. J. BAKER.

Advances in insect population control by the sterile-male technique. K. N. Mehrotra and G. R. Sethi (*J. scient. ind. Res.*, 1966, **25**, No. 12, 539–543).—History and basic principles of the sterile male technique for the eradication of insect pest species are described. An evaluation of the various methods of inducing sterility (γ-radiation, use of chemo-sterilants) is made and aspects of insect nutrition, with special reference to mass rearing of insects for

laboratory work are discussed. Effects of male-sterilisation on the screw-worm and fruit flies are specially noted. (78 references.)
J. LAMBORN.

The pickleworm. W. J. Reid jun. and F. P. Cuthbert jun. (*Leafl. U.S. Dep. Agric.*, 1966, 455, 8 pp).—Development, habits, and damage caused by *Diaphania nitidalis* is described together with methods of control by cultural practices and by insecticides.
E. G. BRICKELL.

Insects in farm-stored wheat. Anon. (*Leafl. U.S. Dep. Agric.*, 1966, 345, 8 pp).—Methods of control are described.
E. G. BRICKELL.

Pea aphid on lucerne. Anon. (*Leafl. U.S. Dep. Agric.*, 1967, 529, 5 pp).—Appearance, development, and control of *Acyrthosiphon pisum*, is described. E. G. BRICKELL.

Extraction of pyrethrin from pyrethrum. Yu. P. Volkov, M. P. Bogomolova and A. V. Starkov (*Zh. prikl. Khim.*, 1967, **40**, 417–420).—Experiments were undertaken with the object of obtaining a greater quantity of pyrethrum extracts from the dried flowers of the pyrethrum cultivated in the USSR (*Chrysanthemum coccineum* Willd., *C. carneum* Steud and *C. roseum* Ad) which contain e.g. 0·38–0·46% and 0·30–0·33% of pyrethrin. A fuller recovery of pyrethrin under optimum conditions at room temp. was obtained with a pyrethrum–solvent ratio of 1 : 5 (wt. : vol.), using light petroleum as solvent for a period of 6 days. Extracts obtained in these conditions were used for second, third and fourth extractions with fresh portions of pyrethrum. In this way, more concentrated extracts were obtained. A.L.B.

Ultra-low-volume [aerial] spraying [of insecticides]. C. D. Lindley (*Chemy Ind.*, 1968, 114–117).—This review covers droplet spectrum, spray coverage, application equipment and drift hazards for undiluted conc. formulations. Decreased cost is the main advantage (more acres sprayed per h, several hundred acres treated without reloading, elimination of water-tankers and mixing equipment), but careful attention must be given to application techniques, correct use of equipment, and spray-formulation. Disadvantages are increased contamination of adjacent crops by drift residues, possible greater toxicity to men and animals, risk of overdosing, and limited formulations in comparison with normal dil. spray. (20 references.) W. J. BAKER.

Safe and effective use of insecticides. E. Champion (*Baker's Dig.*, 1966, **40**, 70–75).—Sources of insect infestation and the use of methoxychlor–lindane, malathion, pyrethrum, diazinon, Divap, chlordane, Baygon, Kepone, DDVP, Cygon etc. for control are discussed. I. DICKINSON.

Influence of time and rate of application of barban on response of spring wheat varieties. P. F. Rylands and A. D. Mears (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 49–50).—Results demonstrate that the response of spring wheats to barban, 4-chloro-2-ynyl *N*-(3-chlorophenyl)carbamate, is conditioned by the growth stage at which the herbicide is applied; there is considerable difference in response between varieties. E. G. BRICKELL.

Effect of annual applications of diuron on seed yields of perennial grasses in Oregon. W. O. Lee (*Tech. Bull., U.S. Dep. Agric. Res. Serv.*, 1966, 1358, 23 pp).—Seven bluegrass, seven orchardgrass, and five tall fescue varieties showed considerable tolerance to five annual applications of diuron [3-(3,4-dichlorophenyl-1,1-dimethylurea] at rates up to 4 lb active ingredient per acre. Colonial bentgrass, Highland, also showed considerable tolerance but a second variety, Astoria, was only moderately resistant. Creeping bentgrass varieties were severely impaired as was ryegrass. Creeping red fescue and Chewings fescue varieties showed only fair tolerance to diuron. E. G. BRICKELL.

Relationship between partition data and adsorption of herbicides by soils. R. J. Hance (*Nature, Lond.*, 1967, **214**, 630–631).—Hydrophilic–hydrophobic balance of 29 non-ionic solutes (mainly *s*-triazines and phenylureas) was correlated with their adsorption by a humic loam and a chalky boulder-clay, respectively. Except for that of urea, the graphs of log x/m vs. log c were linear (x = solute adsorbed by wt. m of adsorbent when equilibrium solution concn. is c), so that comparisons of adsorption behaviour are valid at any concn. (10^{-4}–10^{-6} M). It is recommended that estimation of the hydrophilic–hydrophobic balance (by thin-layer partition chromatography) be included as a test in screening or evaluation programmes for pesticides. (Cf. Ward and Holly, *J. Colloid Sci.*, 1966, **22**, 221.) W. J. BAKER.

Interactions between soil and weedkillers. II. Influence of some electrolytes on adsorption–desorption of *s*-triazines. A. Malquon, P. Fusi and G. Staccioli (*Chimica Ind. Milano*, 1967, **49**, 279–284).—Adsorption and desorption of prometryne, simazine and atrazine are studied on kaolinite, illite and montmorillonite (H and Ca form), in presence of the numerous electrolytes occurring in chemical fertilisers. In saline media, 0·01–0·5 N concn., triazine (**I**) adsorption by H-mineral decreases, compared with aq. media; it increases at 3 N-concn. Ca-mineral adsorption of **I** increases in presence of primary phosphate in proportion to concn. of the latter; e.g., adsorption of atrazine on 1 g of mineral increases from 0 (water) to 168 γ (0·5 N-KH_2PO_4) and to 220 γ (1-N KH_2PO_4). Desorption of adsorbed **I** is favoured by electrolytes over a concn. in which adsorption is lower than it would be in the absence of salts. The results are tabulated. (24 references.) (From English summary.) C. H. FINCH.

Degradation of dimethyl tetrachloroterephthalate. H. Gershon and G. W. McClure jun. (*Contr. Boyce Thompson Inst. Pl. Res.*, 1966, **23**, 291–294).—GLC analysis of an extract of soil treated with a cumulative total of 94-lb of dimethyl tetrachloroterephthalate (**I**) per acre during 5 years showed the presence of **I**, monomethyl tetrachloroterephthalate and tetrachloroterephthalic acid as residues. E. G. BRICKELL.

Effect of introducing a sulphur bridge on herbicidal activity of diquat. L. A. Summers (*Nature, Lond.*, 1967, **214**, 381–382).—Evidence advanced shows that diquaternary salts (e.g., dimethiodide, diethyldibromide, etc.) of 2,2′-dipyridyl sulphide are reduced to radical cations at approx. the same potential as diquat and paraquat. However, in post-emergent herbicidal tests (7 lb/acre) on linseed, buckwheat, mustard, peas, sugar-beet and barley, they were virtually inactive. This difference in behaviour from that of the bipyridylium compounds is tentatively ascribed to differences in the stability and reactions (e.g., polarographic reduction, atm. oxidation) of the two series of radicals. W. J. BAKER.

Synthesis and herbicidal activity of small-ring compounds. T. R. Hopkins, R. P. Neighbors and L. V. Philips (*J. agric. Fd Chem.*, 1967, **15**, 501–507).—A large no. (130) of cyclo-aliphatic (chiefly cyclopropane) carboxamides was prepared, and structural requirements for activity were determined. The compound *N*-(3,4-dichlorophenyl)cyclopropanecarboxamide proved worthy of introduction as a selective post-emergence herbicide in maize. (18 references.) P. S. ARUP.

Benzthiadiazole derivatives. Shell Internationale Research Mij, N.V. (Inventors: P. Kirby, J. Roberts and J. H. Davies) (B.P. 1,059,800, 8.12.64).—Hydroxy-1,2,3-benzthiadiazoles (of use as in B.P. 1,059,799 for making pesticides) are prepared in good yield by diazotisation of the corresponding aminobenzthiazole and acid hydrolysis. E.g., 5-aminobenzthiazole in H_2SO_4 solution is diazotised and the mixture poured on to ice. The resulting solution is added rapidly to boiling aq. H_2SO_4, then 1·5 h later the cooled mixture is made alkaline with aq. NaOH, and the filtered solution adjusted to pH 4–5 with dil. H_2SO_4 and extracted with ether to give 5-hydroxy-1,2,3-benzathiadiazole (m.p. 160–161°) (after purification on SiO_2 gel from ether or $CHCl_3$).
F. R. BASFORD.

Benzthiadiazole esters of phosphorus acids as pesticides. Shell Internationale Research Mij N.V. (Inventors: J. T. Hackmann and P. Kirby) (B.P. 1,059,799, 8.12.64).—Compounds of high insecticidal activity have the formula $R^IR^{II}PX_2R$ wherein X is O or S; R^I and R^{II} are alkyl or alkoxy; and R is 1,2,3-benzthiadiazine residue which may contain up to 3 halogen, NO_2, or C_{1-4}-alkyl in the benzene ring. E.g., a solution of 5-hydroxy-1,2,3-benzthiadiazole in EtOH is boiled with aq. alcoholic KOH for 1 h then solvent is removed and the residue is boiled with benzene until anhyd., then benzene is distilled off, and $(OMe)_2PSCl$ is charged, followed by $COMeBu^t$. The mixture is boiled for several h, cooled, and the filtered solution is worked up to give 1,2,3-benzthiadiazin-5-yl *O,O*-Me_2 thionophosphate (65%), m.p. 60–61°. Its effect on *Musca domestica*, *Aedes aegypti* (larvae), *Phaedon cochleariae*, *Plutella maculipennis* (larvae), *Acyrthosiphon pisum* and *Tetranychus telarius* is described. F. R. BASFORD.

Thiophosphoric esters and pesticidal compositions containing them. Rhône-Poulenc S.A. (B.P. 1,059,707, 3.1.66. Fr., 5.1.65).—The title compounds have the formula $(OR)_2PS_2{\cdot}CH_2CO_2Bu^t$ wherein R is Me or Et. E.g., $(OEt)_2PS_2NH_4$ is added to a solution of $CH_2Cl{\cdot}CO_2Bu^t$ in acetone, then after 8 h at room temp. the mixture is worked up, to give O,O-*Et*$_2$ S-*carbo*-t-*butoxymethyl thiothionophosphate*, b.p. 125–127°/0·5 mm. Its activity against housefly, *Tribolium confusum*, cricket, red spider, *Plutella* caterpillar, *Aphis rumicis*, *Macrosiphum pisi* and *Tetranychus telarius* is tabulated. F. R. BASFORD.

Aromatic phosphoric, thiophosphoric, phosphonic or thiophosphonic acid esters. CIBA Ltd. (B.P. 1,057,609, 15.11.65. Switz., 20.11.64).—The claimed pesticides (insecticides, fungicides, nematocides) have the formula $R^{I}(R^{II}Y)\cdot X{:}PO\cdot C_6H_2Cl_2I$, where R^{I} is a lower alkoxy, alkylthio, alkyl, haloalkyl or optionally substituted aryl, R^{II} is Me or Et and X and Y independently are O or S. The alkali salt of 2,5-dichloro-4-iodophenol (**I**) is reacted with the appropriate phosphoric or phosphonic halide in presence of a solvent to give the claimed esters. Thus, $(EtO)_2P(S)Cl$ is added, dropwise, to a mixture of **I**, K_2CO_3 and Cu powder in MeEtCO and the reactants then heated to 70–80° for 8 h. After cooling and filtering, the filtrate is evaporated to give a residue that is dissolved in CH_2Cl_2 and washed with cold aq. NaOH. The org. solution yields the product $(EtO)_2\cdot P(S)O\cdot C_6H_2Cl_2I$, m.p. 47–48° (from MeOH). S. D. HUGGINS.

Nitrogen-containing dithiophosphoric acid ester derivatives and pesticidal compositions containing them. CIBA Ltd. (B.P. 1,057,130, 12.11.63. Switz., 22.11.62).—Compounds claimed have the formula $OR^{I}(OR^{II})PS_2\cdot[CH_2)\cdot NH\cdot COXR^{III}$ wherein R^{I} and R^{II} are alkyl of 1–4 C; X is O or S, R^{III} is C_{1-5}-alkyl (optionally interrupted by 1–2 O or 1 S, or SO_2), haloalkyl or alkenyl of >4 C, $(CH_2)_2CN$, CH_2Ph, Ph, C_6H_4Cl, C_{1-4}-alkylphenyl, or CH_2CO_2R (R is alkyl of 1–4 C). In an example, a solution of $CO_2Pr^{i}\cdot NH[CH_2]_2Cl$ in $COMeBu^{i}$ (**I**) is added at 65–70° to a solution of $(OEt)_2PS_2Na$ in **I**, then after 5 h at 75–80° the filtered solution is evaporated, and a solution of the residue in benzene is washed with water, then distilled to give *Et*$_2$ S-2-(*carboisopropoxyamino*)*ethylthiothionophosphate*, b.p. 150–153°/0·08 mm. Its effect on *Tetranychus urticae* is described. F. R. BASFORD.

Amido-thiol-phosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 1,057,090, 7.12.65. Ger., 15.1.65).—Esters of formula $NR^{II}R^{III}(OR^{I})PO\cdot SR$ are claimed and characterised by insecticidal, acaricidal, and anthelmintic properties (R is propenyl or propynyl; R^{I} is Me or Et, and R^{II} and R^{III} are H or Me). E.g., a solution of allyl bromide in MeCN is added dropwise at 70–80° to a solution of $NH_2(OEt)PO\cdot SNa$ in MeCN; after 1 h at the boil the filtered solution is evaporated and aq. solution of the residue is saturated with NaCl and extracted with CH_2Cl_2. This extract affords *S-allyl O-Et amidothiophosphate*, b.p. 128–130°/0·2 mm, m.p. 45–46°. F. R. BASFORD.

Organic phosphorus compounds of reduced toxicity and extended activity. CIBA Ltd. (B.P. 1,048,814, 21.6.63. Switz., 25.6.62 and 12.2.63).—Pesticides of formula $RR^{I}PZ\cdot O\cdot CR^{II}{:}CR^{III}Y$ are mixed with adsorbent C for prolonged effect and reduced toxicity to warm-blooded animals (R and R^{I} are C_{1-5}-alkyl or Ph which may contain Cl, and are joined to P directly or via O or S; Z is O or S; R^{II} is H or Me; Y is halogen; and R^{III} is halogen or C_{1-4}-alkyl or chloroalkyl; and the double bond may be saturated with 2 Cl). A preferred product is $(OMe)_2PO\cdot O\cdot CH{:}CCl_2$, and it is active against flies, spider mites, nematodes, snails, also helminths (e.g., in human and veterinary medicine), its toxicity being reduced as indicated. F. R. BASFORD.

Mixed phosphorus-containing acid anhydrides and pesticidal compositions containing them. Esso Research and Engng. Co. (B.P. 1,049,722, 10.2.64. U.S., 27.2.63).—A mixed anhydride of a dialkyldithiophosphoric acid (**I**), e.g., $(EtO)_2P(S)SH$ (**II**) or $(MeO)_2P(S)SH$ and a carboxylic acid is prepared by heating **I** (at 70° for 2 h) with an equimol. amount of an anhydride of an alkyl, aryl or aralkyl carboxylic acid C_{2-10} which may be substituted by nitro, amino or halogen and, in the case of an aryl carboxylic acid, may also have an hydroxy substituent. The products have outstanding pesticidal properties towards, e.g., cockroaches and houseflies. Thus, **II** is heated with benzoic anhydride or butyric anhydride to give products of b.p. 100°/1 mm and 85–87°/1 mm respectively. J. M. JACOBS.

Pesticidal compositions. CIBA Ltd. (B.P. 1,059,276, 19.6.64. Switz., 21.6.63).—Prolonged active life is claimed for the composition consisting e.g. of 120 pt. of a biocidal org. phosphoric acid ester, such as Me_2-dichlorovinyl phosphate (**I**), 100 pt. of finely dispersed silicic acid and 1·2 pt. of 8-hydroxy or 2,4-dihydroxyquinoline. After storing in sealed jars for 3 months at 35°, no loss of **I** occurs, while in absence of a quinoline compound, the loss is 8·3% in 2 months if the H_2SiO_3 is previously dried at 120°/50 mm Hg and is 17·7% if the H_2SiO_3 is used without preliminary drying. S. D. HUGGINS.

[A] **Isophthalonitriles** [B] **halogenated terephthalonitriles.** Diamond Alkali Co. Assee. of R. D. Battershell and H. Bluestone (B.P. 1,058,557–8, 31.5.64. U.S., 1.4.63).—Compounds claimed are fungicides, bactericides, and especially nematocides and have the formula [A] $1,3,2,4,5,6\text{-}(CN)_2C_6RR^{I}R^{II}R^{III}$, [B] $1,4,2,3,5\text{-}(CN)_2C_6HRR^{I}R^{II}$ wherein $X\text{–}X^{III}$ are H or halogen, at least 1 being halogen. Thus [A] a slurry of $2,4,5,6,1,3\text{-}C_6Cl_4(CH_2OH)_2$ in CCl_4 is gassed at the boil with Cl_2 for 18 h under u.v. light, then excess Cl_2, HCl and solvent are removed. The residue is tetrachloroisophthaloyl chloride, m.p. 99–102° (from heptane). This in dioxan is treated with 28% aq. NH_3 at a temp. of 40–50°. After 2 h the slurry is diluted with 1 vol. of water, to give a ppt. of tetrachloroisophthalamide, m.p. 398–400°. This is heated with $POCl_3$ at 75–110° for 3 h, then cooled, and poured on to ice; the ppt. is recrystallised from benzene, to give tetrachloroisophthalonitrile, m.p. 250–251°. F. R. BASFORD.

Water-dispersible pesticide concentrates. Union Carbide Corp. Assee of Union Carbide Australia Ltd. (B.P. 1,058,891, 19.7.63. Austral., 19.7.62).—The concentrates consist of 20–60 pt. by wt. of 1-naphthyl-*N*-methylcarbamate (**I**), 40–20 pt. of an *N,N*-dialkyl amide of formula $R\cdot CO\cdot NR^{I}R^{II}$ (R is alkyl or aryl, R^{I} and R^{II} are alkyl) of water solubility >0·1% by wt. at room temp., 40–20 pt. of an acid-stable, hydrophilic, nonionic surface-active compound/s and 0·1–0·5 pt. of an acid. Thus, 51 pt. of technical **I**, 22 pt. of lauryl polyoxyethylene ether (12 oxyethylene groups per mol.) 26·9 pt of *N,N*-dipropyl-2-ethylhexanoamide and 0·1 pt of AcOH are mixed to form a smooth paste, which is melted and poured into water. The dispersion when applied by spraying or dipping and allowed to dry is found to be resistant to washing or rain. S. D. HUGGINS.

Cyanothioformylarylamides and pesticidal preparations containing them. CIBA Ltd. (B.P. 1,058,622, 13.1.65. Switz., 13.1. and 12.8.64).—Compounds for use in combating pests (especially harmful insects, acarides, nematodes, molluscs, and phytopathogenic fungi and bacteria) and for controlling plant growth have the formula $NRR^{I}\cdot CS\cdot CN$ wherein R is mono- or binuclear aromatic residue which may contain halogen, alkyl, alkoxy, haloalkyl, alkylthio, NO_2, CN, SCN, CF_3, $NR^{II}R^{III}$, or $SO_2NR^{II}R^{III}$ (R^{II} and R^{III} are H or alkyl) and R^{I} is H, alkyl, alkenyl, aralkyl, acyl, aroyl, $CX\cdot NBB^{I}$, or $CX\cdot CXR^{IV}$ (X is O or S; B-B^{I} are H, alkyl, or aryl; and R^{IV} is alkyl, aryl, or aralkyl). E.g., a mixture of $3,4,1\text{-}C_6H_3Cl_2\cdot NCS$, KCN, EtOH and water is stirred at 30° for 4 h, then diluted with water and acidified with conc. HCl. Ppt. is recrystallised from benzene, to give *N-cyanothioformyl-3,4-dichloroaniline*, m.p. 164° (decomp.). Many more new compounds are described. F. R. BASFORD.

Manufacture of a pesticide [fungicide] by chlorination of a sulphur-containing gasoline. Pakistan Council of Scientific and Industrial Research (B.P. 1,059,449, 4.12.63. Pakistan, 26.4.63).—The pesticide is made from gasoline (obtained by gasification of sulphuraceous coal) by chlorinating it in presence of a Cl_2-saturated aq. solution of an alkali at a temp. >25° until the sp. gr. of the partly chlorinated product rises to 1·1–1·3. This product is separated from the aq. phase, and chlorination is continued in presence of fresh, Cl_2-saturated aq. alkali or of an anhyd. metal halide (or its Cl_2-saturated aq. solution) at a temp. >35° until the sp. gr. of the product rises to 1·4–1·5. The metal halide is e.g. NaCl, or a chloride of Al, Cr or Fe. E. ENOS JONES.

Oxiodinium and thia-iodinium compounds. Eli Lilly & Co. (Inventor: W. N. Cannon) (B.P. 1,056,418, 5.8.64).—Bis(dibenz)[b,e][1,4]-oxiodinium- or -thia-iodinium-salts (**I**) (sulphate, bisulphate, chloride), optionally substituted by 1–3 R groups on each aromatic ring (R is alkyl or haloalkyl of 1–3 C, halo, NO_2, amino or sulphamoyl), e.g., 3,7-dinitrodibenz[b,e][1,4]oxiodinium bisulphate, are prepared by cyclising corresponding iodosodiphenyl ether or thioether by treating it with conc. H_2SO_4 or conc. H_3PO_4. The iodoso compound is obtained by chlorination of the corresponding iodo compound to prepare the iododichloride, followed by alkaline hydrolysis. **I** have antibacterial, antifungal and antimildew activity (>30 micro-organisms tested); they can also be used as pesticides and herbicides. J. M. JACOBS.

Herbicides and thiobenzamide derivatives as active ingredients therefor. N. V. Philips' Gloeilampen-Fabrieken (B.P. 1,052,888, 9.7.63. Neth., 12.7.62).—*2,6-Dichlorothiobenz-N-hydroxymethylamide* (**I**) and derivatives thereof containing another Cl are prepared for use as herbicides. A mixture of $2,6,1\text{-}C_6H_3Cl_2\cdot CSNH_2$, 36% aq. CH_2O, dioxan, and 2 N-NaOH is stirred at room temp. for 2 h, then poured into aq. NaCl. Pptd. oil is extracted with ether, washed with 2 N-NaOH and with water, evaporated to half vol., and the concentrate washed again with 2 N-NaOH. The combined alkaline extract is washed with ether, then ether is evaporated, and the residue is cooled to 10° and treated with conc. aq. HCl, to give, after 16 h, a ppt. of **I**, m.p. 112–115°. F. R. BASFORD.

Quaternary ammonium derivatives of benzonitriles and herbicidal compositions containing them. May & Baker Ltd. (Inventors: B. J. Heywood, W. G. Leeds and R. F. Collins) (B.P. 1,056,235, 26.5.64).—The herbicidal agents have the formula 1,3,5,4-CN-$C_6H_2XX^I\cdot O^-N^+RR^IR^{II}R^{III}$ where X and X^I are Br or I and $R–R^{III}$ are Me or Et. In an example, a solution of NMe_4Cl in EtOH is added to a hot solution of 1,3,5,4-$CN\cdot C_6H_2I_2\cdot ONa$ in EtOH, then after 1 h at the boil the cooled solution is filtered; the filtrate yields the *NMe_4 salt* of *3,5-diiodo-4-hydroxybenzonitrile*, m.p. 195–198°. The salts are active against broad-leafed weeds (named). F. R. BASFORD.

Derivatives of 3,5-dinitro-2-hydroxybenzyl alcohol. Boehringer Ingelheim G.m.b.H. (B.P. 1,055,279, 12.12.63. Ger., 27.12.62 and 29.5.63) (83 claims).—Compounds claimed have pesticidal properties and the formula $3,5,2,1\text{-}(NO_2)_2C_6H_2(OY)\cdot CH_2X$ wherein X is halogen, ONO_2, OCOR, OCONHR, $OCONRR^I$, OCO_2R, OSO_2R, or OR and Y is H, cation, R, COR, CONHR, $CONRR^I$ SO_2R, $PZ(OR)\cdot ZR$, or CZ_2R, Z is O or S; R and R^I are hydrocarbon radicals which may contain halogen, NO_2, OH, NH_2, CN, or CO_2R^{II} (R^{II} is alkyl), aryl, substituted aryl, or $R^{III}\cdot R^{IV}$ (R^{III} is hetero atom; R^{IV} is optionally substituted aryl), or hetero atoms in an alkylene chain, but when X is OAc then Y is not Ac. E.g., a mixture of $3,5,2,1\text{-}(NO_2)_2C_6H_2(OH)CH_2OH$ and $SOCl_2$ is boiled for 6 h, then excess of $SOCl_2$ is removed. Recrystallisation of residue from benzene–light petroleum affords 3,5-dinitro-2-hydroxybenzyl chloride, m.p. 96–98° in 83·3% yield. Many more compounds are described. F. R. BASFORD.

9-Substituted fluorene-9-carboxylic acid esters. E. Merck A.-G. (B.P. 1,051,654, 16.9.63. Ger., 22.9.62).—The Me, Et and Bu^n esters of 2-chlorofluorene-9-ol-9-carboxylic acid, the Bu^n esters of 2,7-dichlorofluorene-9-ol-9-carboxylic acid and the Bu^n ester of 2,7,9-trichlorofluorene-9-carboxylic acid are claimed; the two latter products have m.p. of 109° and 114·5° respectively. They are made by direct esterification of the acids; the esters of fluorene-9-ol-9-carboxylic acids are also made by esterifying the 9-chlorofluorene-9-carboxylic acid and replacing the Cl at position 9 with OH (treatment with Ag_2O). The esters are plant growth inhibitors. S. D. HUGGINS.

Unsymmetrical 1,3-dicycloalkyl ureas. E. I. Du Pont de Nemours & Co. (B.P. 1,056,290, 4.5.64. U.S., 6.5.63).—Unsymmetrical dicycloalkyl ureas $R^I\cdot NH\cdot CO\cdot NH(CH_2)_tR^{II}$ (**I**) in which R^I and R^{II} are the same or different, and are cycloalkyl groups C_{5-8} or bicycloalkyl groups C_{7-8} which may be substituted by 1 or 2 halogen atoms, 1 or 2 Me or by 1 halogen atom and 1 Me, *t* is O or 1 when R^I and R^{II} are different and 1 when R^I and R^{II} are the same (*t* is 1 and R^{II} is cyclohexyl), are prepared by reacting a cycloalkyl- or bicycloalkyl-amine $R^{II}(CH_2)_tNH_2$, dissolved in, e.g., n-hexane, toluene or dioxan, with the corresponding R^INCO at 10–100 (25–45°). Various compositions containing **I** together with, e.g., emulsified oil or attapulgite clay, surface-active agents, etc., for retarding the growth of germinating and seedling weed grasses, are claimed. J. M. JACOBS.

Animal Husbandry

Storage losses in silage as affected by moisture content and structure. C. H. Gordon (*J. Dairy Sci.*, 1967, **50**, 397–403).—The effect of moisture content on storage losses of silage in various types of silo is reviewed. (51 references.) M. O'LEARY.

Ryegrass varieties in relation to dairy cattle performance. I. Influence of ryegrass varieties on milk yield and composition. II. Influence of ryegrass varieties on intake, digestibility, and on some characteristics of rumen fermentation. G. F. Wilson and F. H. McDowall (*N.Z. Jl agric. Res.*, 1966, **9**, 1042–1052, 1053–1063).—I. During the Spring period mean milk yields from different ryegrasses in decreasing order were:—Manawa : Ariki : Ruanui. In Autumn experiments cows fed on Ruanui produced higher milk and butterfat yields than those on Ariki ryegrass. (13 references.)

II. Ryegrass varieties differed in apparent digestibility, but there were no significant differences between total concn. of fatty acids or the proportions of the individual fatty acids in the rumen liquors. Milk and butterfat yields were closely related to the voluntary intake of individual animals. (12 references.) E. G. BRICKELL.

The intestinal enzymatic-splitting of saccharose fatty esters. H. Ruttloff, F. Linow and K. Täufel (*Fette Seifen AnstrMittel*, 1967, **69**, 231–235).—Saccharose esters of palmitic and hardened groundnut oil (**I**) fatty acids are decomposed by treatment with carbohydrate-, ester- and lipase-type enzymes; the rates of decomposition and the resultant products are determined; the latter by paper and TLC methods. The Me esters of poppyseed, sunflower, **I** and palmitic acid are similarly treated as controls. Results indicate that the fission of the ester bonds occurs in the intestinal tracts of pigs and rats, but at a much slower rate than the corresponding lipolysis of normal triglycerides. It is further shown, that after the ester breakdown, the saccharose mol. also undergoes hydrolysis. (28 references.) G. R. WHALLEY.

Separating the effects of digestibility and palatability on food intake in ruminant animals. J. F. D. Greenhalgh and G. W. Reid (*Nature, Lond.*, 1967, **214**, 744).—Digestibility was kept const. and palatability was varied by giving sheep part of their diet through a rumen fistula, i.e. straw (*S*) eaten and grass (*G*) through fistula and *vice versa*. After ⩽12 days of each treatment (*S,S/G,G/S,G*), food intake and digestibility were measured for periods of 9 days. Sheep varied considerably in response to the four different diets, and the combined effects of digestibility and palatability on intake were very great, e.g., those fed on dried *G* above ate >4 times as much as those fed on *S* alone. Relative intakes indicate that effects of digestibility and palatability are approx, the same, viz. changing the palatability without changing digestibility had an equally dramatic effect as the reverse change. (Cf. Blaxter, *et al.*, *Anim. Prod.*, 1961, **3**, 51.) W. J. BAKER.

The source of protein in calf diets. I. Comparison of dried skim milk and meat meal. II. Meat meal quality. J. Leibholz and F. P. Moss (II) (*Aust. J. agric. Res.*, 1967, **18**, 149–155, 157–168).—I. Tests on Friesian calves showed that meat meal resulted in significantly greater ($P < 0·05$) live weight gains but no change in the efficiency of feed conversion. (15 references.)

II. The source of meat meal resulted in a significant difference in live weight gain of calves; this was related to the Ca and cystine contents of the meat meals. For chickens the effect of meat meal source was dependent on the formulation of the diets. The free amino-acid, ammonia and urea concn. in the blood plasma of both species are presented. (26 references.) E. G. BRICKELL.

Utilisation of mixed fatty acids and calcium by healthy sucklings in the first quarter of their life in relation to the fatty acid content and the fatty composition of the milk mixtures. H. Stolley and W. Droese (*Fette Seifen. AnstrMittel.*, 1967, **69**, 291–292).—The daily intake of Ca and mixed fatty acids is recorded for 43 healthy sucklings from 4 to 90 days of their lives; each received usual cow's milk with different fat contents and varying fatty acid compositions. The results show that the Ca utilisation by newly-born, and young sucklings (even on feeding with mother's milk) is lower than in the later infant stage. A statistically proved relationship also exists between the fat level content and the fatty acid constitution of the feed as well as the Ca utilisation. G. R. WHALLEY.

Effect upon lipid metabolism of feeding alfalfa hay or concentrate *ad libitum* as the sole feed for milking cows. J. Opstvedt and M. Ronning (*J. Dairy Sci.*, 1967, **50**, 345–354).—Feeding trials with six Holstein cows showed that dry matter intake was similar when the animals were fed alfalfa hay only or concentrate only. With Jersey cows a higher dry matter intake was obtained with hay than with concentrate. The concentrate-only diet depressed percentage and yield of milk fat in both breeds. Milk secretion was higher with the concentrate diet but outputs of milk energy were similar on the two feeds. Solids-not-fat concn. and yield, and live weight gain, were higher on the concentrate diet. (35 references). M. O'LEARY.

Efficiency of wool growth. I. Comparison of differences between high- and low-producing sheep under restricted and under *ad libitum* feeding. M. Wodzicka-Tomaszewska (*N.Z. Jl agric. Res.*, 1966, **9**, 909–915).—Sheep which produced more wool at pasture also grew more wool in the pens both on controlled intake and when subsequently *ad libitum*. The difference in wool production between the two groups was, however, reduced under pen conditions. It is concluded that the higher wool-producers were more efficient converters of feed to wool. (13 references.) E. G. BRICKELL.

Diet of the grazing animal. II. Effect of physiological status in ewes and pasture availability on herbage intake. G. W. Arnold and M. L. Dudzinki (*Aust. J. agric. Res.*, 1967, **18**, 349–359).—Digestible org. matter intake of dry, pregnant and lactating ewes, was significantly related in asymptotic form to pasture conditions; about 40% of the variability is accounted for by the total dry matter per acre. Differences in grazing time and rate of eating, were found in breeds and between ewes of different physiological status, within breeds. (26 references.) E. G. BRICKELL.

Absorption of volatile fatty acids into rumen of fasted sheep and heat increment of feeding. A. Rakib and D. P. Sadhu (*Indian J.*

exp. Biol., 1967, **5**, 29–30).—The effects of Na salts of acetic (**I**), propionic (**II**) and butyric (**III**) acids administered to the rumen of fasted sheep on the absorption of volatile fatty acids and heat increment of feeding have been studied. The pH of rumen liquor remained alkaline suggesting the quick absorption of volatile fatty acid radicals leaving the Na behind. **III** disappeared most rapidly followed by **II** and then **I**. The heat increment of feeding due to the administration of **I** was greater than that for **II** which was greater than that for **III**. S. A. BROOKS.

Effect of time of shearing on onset of breeding activity in the ewe. J. L. Lees (*Nature, Lond.*, 1967, **214**, 743–744).—The observed correlation between mean ambient temp. for mid-July to early August and onset of oestrous activity of Clun Forest ewes is independent of whether the ewes are shorn or unshorn. It is thus a natural temp. effect, probably associated with a period of instability in the ewe as it emerges from anoestrum. W. J. BAKER.

Nutritional evaluation of meat meals for poultry. V. Effect of addition of antioxidants during and after processing on growth-promoting value of high and low quality meat meals. B. S. Sathe and G. L. McClymont (*Aust. J. agric., Res.* 1967, **18**, 183–191).—Butylated hydroxytoluene and ethoxyquin were studied. Only the addition of 0·02% of the latter to both high and low quality meat meals significantly increased growth rates of chicks (by about 7%) but only with fresh samples. Other treatments were without effect. It would appear that oxidation in the course of processing or autoxidation during storage is unlikely to be the major determinant of the relative growth-promoting ability of meat meals. (27 references.) E. G. BRICKELL.

Effect of dose and period of administration of oxytetracycline (OTC) to hens upon level of OTC in eggs, meat, and giblets. Hygienic evaluation of these products. E. M. Rutczynska-Skonieczna and M. Nikonorow (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1966, **57**, 377–381).—Eggs from OTC-dosed hens were free from OTC 5–8 days after the dosage period, depending on the amounts administered. As regards the meat and giblets, the min. permissible periods between the end of dosage and slaughtering varied from 1–6 days, but no OTC was found in these products after cooking for 1 h. OTC in liver, however, was only partly destroyed by frying. P. S. ARUP.

Trans-lipids: Hen egg yolk lipids. H. P. Kaufmann and A. Mankel (*Fette Seifen AnstrMittel.*, 1967, **69**, 107–110).—The introduction of *trans*-fatty acids in the form of edible glycerides into the diet of hens resulted in a 10% increase in the *trans*-fatty acid composition of the egg yolk lipids; when such a diet is replaced with one of lower *trans*-acid content the observed increase disappears within 14 days. It is further shown that the triglycerides contain in each case more *trans*-unsaturated fatty acids than the corresponding phosphatide fractions; also the triglycerides contain more oleic acid than the phosphatide fatty acids, whereas stearic acid as well as polyunsaturated components are concentrated chiefly in the phosphatide fraction. G. R. WHALLEY.

Farm poultry management. Anon (*Fmrs Bull., U.S.Dep. Agric.*, 1966, 2197, 32 pp).—Profitable poultry management, farm laying stock, housing, equipment, feed and water, brooding, rearing, management of layers, hatching-egg production, meat production, diseases, parasites, and pests, are described. E. G. BRICKELL.

Utilisation of xanthophylls from natural sources by the chick. P. N. Dua, E. J. Day, J. E. Hill and C. O. Grogan (*J. agric. Fd Chem.*, 1967, **15**, 324–328).—The xanthophylls (**I**) from commercial yellow maize (*M*) and from a new high-**I** yellow *M* were equally available for utilisation, and more so than those of lucerne meal or *M* gluten meal. Increasing dietary **I** levels increased the skin- and serum-**I** levels, but decreased their % uptake. Strains of *M* with differing proportions of lutein and zeaxanthin produced the same pigmenting effects. (12 references.) P. S. ARUP.

Determination of lipo-peroxides in meat meals and poultry feeds. G. M. Keeley, B. A. Peddie and L. Hartman (*N.Z. Jl Sci.*, 1966, **9**, 806–808).—Peroxide values are too high when samples are extracted and filtered in natural or artificial light. Meklenbacher's method ensures greater accuracy if these operations are conducted rapidly in the dark. A deaërated solvent and an inert atm. should be used. W. J. BAKER.

The fowl tick. Anon. (*Leafl. U.S. Dep. Agric.*, 1966, 382).—Control measures for *Argas persicus* by spraying, sanitation, and management practices, are described. E. G. BRICKELL.

New anti-coccidial drug. S. J. Ball, M. Davis, J. N. Hodgson, J. M. S. Lucas, E. W. Parnell, B. W. Sharp and D. Warburton (*Chemy Ind.*, 1968, 56–57).—Measurements of anticoccidial activity of 6,7-dialkoxy-4-hydroxyquinoline-3-carboxylic acid esters reveal that activity is unusually intense when in the alkoxy groups RO and R^1O, R is a straight C_8–C_{10} chain and R^1 simultaneously a smaller straight or branched alkyl group of 1–4 C; the esterifying alcohol is preferably MeOH or EtOH. Of some 150 compounds examined, the most active was Et 6-n-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate (M & B 15497), which at concn. of 0·0014 wt.-% in food completely inhibited oocyst production of 6 species of *Eimeria* infection in chickens, besides permitting normal growth, feed conversion and sexual maturity when added continuously (from day-old) to diet in concn. up to 0·32 wt.-%. W. J. BAKER.

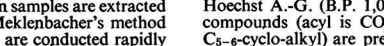
Cesticidal action of diphenyl sulphone 4,4′-di-isothiocyanate. J. C. Katiyar, A. B. Sen and B. K. Bhattacharya (*Nature, Lond.*, 1967, **214**, 708–709).—Preliminary results reveal that this sulphone compound exerts a strong cesticidal action against both immature and adult *Hymenolepis nana* in rats and mice; the min. effective dose is 100 mg per kg in rats and 10 mg in mice. Even in doses (daily for 5 days) of 250 mg per kg the compound was ineffective against ova or the cysticercoid stage. Its low toxicity and high therapeutic index should enhance its effectiveness as a drug against tapeworm infections. W. J. BAKER.

Detection of chick oedema factor and other chlorinated hydrocarbons in fats and oils by electron capture gas chromatography: use of thin layer chromatography for preliminary clean-up. A. Huang, D. Firestone and A. D. Campbell (*J. Ass. off. analyt. Chem.*, 1967, **50**, 16–21).—A fraction containing the oedema factor is isolated from the unsaponifiable matter (cf. *ibid.*, 217) by TLC, the solution in light petroleum being cleaned up by shaking with H_2SO_4 followed by $NaHCO_3$. After evaporation under N_2 the residue is dissolved in iso-octane and analysed by electron capture gas chromatography. The efficiency of this method of clean-up is comparable with that of Al_2O_3 column chromatography. This procedure also detects highly chlorinated hydrocarbons other than the chick oedema factor. (14 references.) A. A. ELDRIDGE.

Anticoccidial compositions. Merck & Co. Inc. (B.P. 1,046,947, 30.9.63. U.S., 1.11.62).—The active agent is a compound of formula 1,4,2-$CO_2R^1 \cdot C_6H_3(NO_2)XR$, added in 0·0005–0·0125% concn. to solid poultry feed (X is O or S; R is alkyl or alkenyl of $\geq$5 C; and R^1 is H, metal, or hydrocarbon radical of $\geq$8 C), e.g., 2-methoxy-4-nitrobenzoic acid. F. R. BASFORD.

Benzamidoalkylthiopseudo-ureas [and their use in veterinary compositions]. Baxter Laboratories Inc. (Inventor: B. M. Regan) (B.P. 1,048,000, 19.10.65).—Compounds of general formula $C_6H_4R^{III} \cdot CO \cdot NH \cdot [CH_2]_n \cdot S \cdot C(:NR)NR^IR^{II}$; also veterinary compositions containing them (for inducing local anaesthesia in animals) are claimed; R is H or alkyl of 1–4 C; R^I and R^{II} are H, allyl, alkyl of 1–4 C, or cyclohexyl, at least 1 of R–R^{II} being other than H; R^{III} is H, Cl, or Br; *n* is 2–3. A mixture of $BzNH[CH_2]_2Cl$, $CS(NHPr^i)_2$ and EtOH is boiled for 5 h, then diluted with EtOAc. On cooling, cryst. 2-(2′-*benzamidoethylthio*)-1,3-*diisopropylpseudourea hydrochloride* m.p. 181–183° is obtained. F. R. BASFORD.

2-Ethylthiophen derivatives. Pfizer Ltd. (Inventors: W. C. Austin, J. C. Danilewicz and L. H. Conover) (B.P. 1,049,047, 13.8.64).—Compounds claimed have the general formula



($R^I(CH_2)_2 \cdot C:N(CH_2)_nCHR$ (*n* is 2 or 3; R is H or Me; R^I is thien-2-yl), and are active against helminths, viz., *Ancylostomidae*, *Strongylidae*, and *Trichostrongylidae* in sheep and cattle. E.g., a mixture of Mg, ether, and 2-(2′-chloroethyl)-thiophen is boiled for 2 h, then a solution of $CN(CH_2)_4Cl$ in ether is added. After a further 30 min. xylene is charged and ether is distilled off. The residue is boiled for 1 h, cooled, and treated with aqueous 15% NH_4Cl solution; the org. layer contains 2-(2′-*thien-2″-ylethyl*-3,4,5,6-*tetrahydropyridine*, b.p. 68–69°/0·002 mm, (*H maleate*, m.p. 78–80°). F. R. BASFORD.

3,6-Diamino-2,5-diacylamino-1,4-benzoquinone. Farbwerke Hoechst A.-G. (B.P. 1,051,592, 29.7.63. Ger., 1.8.62).—The title compounds (acyl is CO·R where R is C_{2-5}-alkyl or alkenyl or C_{5-6}-cyclo-alkyl) are prepared from corresponding 3,6-dihalogenated analogues and are effective in the treatment and prophylaxis of coccidiosis. E.g., a solution of 3,6-dichloro-2,5-diacetamido-1,4-benzoquinone in BuOH is gassed at 120° during 1 h with NH_3, then the mixture is worked up, to give 3,6-*diamino*-2,5-*diacetamido*-1,4-*benzoquinone*. F. R. BASFORD.

Heterocyclic amides. May & Baker Ltd. (Inventor: E. W. Parnell) (B.P. 1,055,266, 11.9.62).—Pyridine-3-sulphonamide and

N-C_{1-4}-alkyl derivatives thereof are effective in the treatment of coccidiosis in poultry. The *N*-alkyl derivatives are new, e.g., *pyridine-3-sulphon-*N*-ethylamide*, m.p. 76–77° (EtOAc–light petroleum), made by adding pyridine-3-sulphonylchloride hydrochloride gradually to 33% (wt./vol.) NH_2Et in EtOH, then evaporating solvent after 30 min. at 10–20°. F. R. BASFORD.

Early diagnosis of pregnancy in farm animals. W. Jochle (B.P. 1,055,274, 4.10.63. Ger., 5.10.62).—Peroral or parenteral administration to a covered female animal of an androgen–oestrogen mixture in a ratio adjusted according to the species, in a dose adjusted as indicated, at a suitable time interval after service or artificial insemination, is followed by observation of oestrus symptoms. A suitable composition for use with cattle consists of testosterone oenanthate, oestradiol valerate, and sesame oil. F. R. BASFORD.

Phthalazinium compounds. CIBA Ltd. (B.P. 1,055,718, 6.4.64. Switz., 10.4 and 25.10.63 and 7.2.64).—A 2-(polyhalobenzyl)-phthalazinium compound, or a pseudo base thereof, is produced when a phthalazine is reacted with a compound R–X, where R is a polyhalobenzyl group and X a reactive esterified OH-group. The resulting quaternary salt (**I**) can be converted into the corresponding hydroxide or pseudo base by reaction with a base in presence of water, or the pseudo base converted into **I** by reaction with an acid. Thus 2-(2′,4′-*dichlorobenzyl*)-*phthalazinium chloride*, m.p. 208–210°, is obtained from phthalazine and 2,4-dichlorobenzyl chloride. The compounds are effective against tapeworms. E. ENOS JONES.

Carboxylic acid piperazides. Farbwerke Hoechst A.-G. (B.P. 1,056,421, 15.7.63. Ger., 21.12.62 and 5.2.63).—Carboxylic piperazides RCOX are claimed. X is piperazinyl substituted on one N by RCO and on the other by H, alkyl of 1–12 C (optionally substituted by alkoxy of 1–4 C, dialkylamino of 1–2 C in each alkyl, or OH) cycloalkyl of 5–8 C or phenyl alkyl. R is $R^{II}R^{III}R^{IV}C\cdot(CH_2)_n$ (**I**) or $R^{V}R^{VI}C{:}CH\cdot CR^{VII}{-}CH$, R^{II} is H or Ph, R^{III} and R^{IV} are Ph or $PhCH_2$ or 2-alkylbenzyl or R^{II} and R^{III} are benzylidene, R^{V} and R^{VI} are Ph (optionally containing alkyl or alkoxy), R^{VII} is Me or 2-phenylvinyl, R^{I}–R^{VII} may contain halogen and all alkyls have 1–2 C, *n* is 0 or 1. If R is **I**, $n = 1$ if R^{I} is alkyl of 1–4 C, R^{II} is H, and R^{III} and R^{IV} are halophenyl. Examples include *ββ-tris-*(4-*chlorophenyl*)*propionic acid-* and *bis-*(4-*chlorobenzyl*)*acetic acid-*N′-*methylpiperazides*, prepared by reacting carboxylic acids or reactive deriv. with a piperazine and, if necessary, hydrogenating or alkylating. The compounds have good anthelmintic properties, especially against lancet liver flukes *Dicrocoelium dendriticum*. J. M. JACOBS.

2,5-Dihalo-3-nitrobenzene derivatives. Sterling Drug Inc. (B.P. 1,057,498, 14.11.63. U.S., 17.12.62).—Used for the prevention and suppression of coccidiosis, the title compounds are substituted by -X in the 1-position (X is $CONH_2$, CONHR, $CONRR^{1}$, CONHNHCOR, CONHNRR^{1}, CONHOH or CN, R and R^{1} being alkyl radicals of 1–6 C, alkenyl (C_{2-6}), Ph, $PhCH_2$, pyridyl, thienyl, thiazolyl, etc.). Thus, a solution of 2,5-dichloro-3-nitrobenzamide and $SOCl_2$ in Ph.Me is slowly heated and then refluxed until HCl evolution ceases, to form 2,5-dichloro-3-nitrobenzoyl chloride, which is cooled and poured into excess of $MeNH_2$ in ice-water maintained at $<20°$ to give 2,5-dichloro-3-nitro-*N*-methylbenzamide, (**I**) m.p. 169·8–171° (from EtOH). A medicated feed composition contains of 0·00625–0·1% by wt. of **I**. S. D. HUGGINS.

Trimethoxychalcone hydrochloride derivative. W. H. Rorer Inc. (B.P. 1,059,715, 28.7.65. U.S., 6.8.64).—Conc. aq. NaOH is added to a solution of *o*-$CHO\cdot C_6H_4\cdot O(CH_2)_2NMe_2$ (prep. described) and 3,4,5,1-$C_6H_2(OMe)_3Ac$ in EtOH, then after 4–5 h at room temp. the mixture is poured on to ice and extracted with ether. The dried extract is treated with saturated ether–HCl, with pptn. of 2-(*o-dimethylaminoethoxy*)-3′,4′,5′-*trimethoxychalcone hydrochloride*, m.p. 168–170°. Its use as a hypotensive agent for animals other than man is claimed. F. R. BASFORD.

Choline chloride. Union Chimique-Chemische Bedrijven S.A. (Inventor: C. V. Eygen) (B.P. 1,060,256, 17.12.67).—A continuous economical process consists of introducing $\leq 99·5\%$ ethylene oxide (**I**) (containing $\geq 0·1\%$ of aldehyde) in 0·1–10 wt.-% excess and aq. NMe_3HCl at $\geq 120°/5$–20 atm. into a reaction zone with no gaseous phase (time of residence 30–120 min.), then passing the resulting solution at $\leq 60°/5$–20 atm. into a second gas-free zone (time of residence ≥ 40 min.), and elsewhere removing part of the water and most of unused **I** in a concentration zone, leaving aq. choline-HCl ready for use in animal and poultry foodstuffs. R. F. BASFORD.

5β,19-Cycloandrost-6-enes. Abbott Laboratories (B.P. 1,060,900, 27.1.66. U.S., 2.3.65).—The claimed androgenic and growth-regulating agents for warm-blooded animals are 3β-methoxy-5β,19-cycloandrost-6-enes with OH, oxo or Ac group in 17β-position, prepared by treating a 3β-methoxy-6-hydroxy-5β,19-cycloandrostan-17-one with $\leq$ mol. equiv. of $MeSO_2Cl$ (**I**) at -10 to 50°. Thus, a solution of 3β-methoxy-6-hydroxy-5β,19-cycloandrostan-17-one in C_5H_5N is cooled and reacted with **I**, with stirring, for 10 min. After standing at room temp. for 3 h the mixture is shaken with Et_2O and 10% aq. NaCl, the aq. phase being washed with 5% aq. $NaHCO_3$. The combined NaCl and $NaHCO_3$ extracts are diluted, continuously extracted with $CHCl_3$ for 4·5 days, and chromatographed (benzene–Al_2O_3) to give 3β-methoxy-5β,19-cycloandrost-6-en-17-one (**I**), m.p. 100–103° (Et_2O–pentane). The oxo group of **I** can be converted to OH or Ac in the usual ways. S. D. HUGGINS.

Anti-allergic animal tissue extracts. Canadian Patents & Development Ltd. (B.P. 1,076,514, 27.8.64. U.S., 27.8.63).—Anti-allergic material is isolated from animal tissue by subjecting it to acid hydrolysis (preferably aq. mineral acid); saturating the aq. phase (preferably after removal of fatty acid material by extraction with a non-polar solvent) with a salt, e.g., $(NH_4)_2SO_4$, with subsequent removal of ppt.; extracting the aq. phase with a low-mol. alkyl ester, e.g., EtOAc; drying and evaporating the solvent, then extracting the residue with a low mol. aliphatic halo-hydrocarbon, e.g., CCl_4; evaporating the solvent; neutralising the residual oil and extracting with a water-immiscible C_{2-10}-alkyl ether; then removing solvent from this extract to give an active oil product. Suitable animal tissue is gingiva, lung, or stomach. Therapeutic compositions for administration to animals are claimed. F. R. BASFORD.

Anti-allergic compositions containing vicinally dioxygenated alkanes. Canadian Patents and Development Ltd. (B.P. 1,076,515, 27.8.64. U.S., 27.8.63).—The title alkanes (**I**) are of formula $RC(R^{I})HCR^{II}\cdot R^{III}$ where R is Bu, C_5H_{11} or C_6H_{13}, R^{I} and R^{II} are OH, oxo and their hydrates (R^{I} and R^{II} must not both be OH) and R^{III} is H or Me. The therapeutic composition (for animals) comprises a sterile injectable aq. carrier or an orally administered solid or flavoured liquid carrier and **I**, e.g. 2-ketoheptanol-1, 2-keto-octanol-1 or 2-ketohexanol-1 or their tautomers, prepared by brominating the n-C_6-C_8-aldehydes and hydrolysing the product with dil. alkali, these products then being distilled *in vacuo* to give a fraction that is separated by chromatography. S. D. HUGGINS.

Animal foods. Cerebos Foods Ltd. (Inventors: J. Saunders and W. Murchison) (B.P. 1,076,676, 22.6.65) —Particularly suitable for dog foods, a mix of cereal and water (18–25% by wt.) is cooked at low temp. to cause gelatinisation of starch, the mixture being extruded during and/or after the cooking into masses, of $d \leq 1·1$g/cc, bonded by gelatinised starch. Thus, wheat flour, soya bean meal and fish meal (all of B.S. 22 mesh size) are mixed and water added to give 25% final moisture content. The ingredients are extruded at 60° and dried to a final moisture content of 12% and a *d* of 1·1–1·3 g/cc. S. D. HUGGINS.

Silage additives. Dr. Plate G.m.b.H. (B.P. 1.076,913, 19.4.66. Neth., 29.4.65).—A composition for adding to silage to preserve protein value and colour comprises a mixture of formate, nitrite, and 30–60 wt.-% of carob flour or other vegetable material containing various sugars. F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Comparative composition of waxy and non-waxy rice. A. J. Vidal and B. O. Juliano (*Cereal Chem.*, 1967, **44**, 86–91).—The content and composition of fat, protein and starch in the brown rice from three pairs of isogenic lines of rice, differing only in the waxy gene, are reported. Differences were slight, and non-consistent for the three pairs of lines. Gelatinisation temp. was unaffected by the waxy gene, but the amylopectin of waxy rice had a lower intrinsic η than the corresponding non-waxy amylopectin. (18 references.) E. C. APLING.

Effect of heating on brown rice composition and quality. T. Iwasaki and T. Tani (*Cereal Chem.*, 1967, **44**, 204–210).—Effects of temp. (50–80°), moisture content (14–18%) and heating time (up

to 4 h) on the properties of rice (**I**) were studied. Good indicators of quality changes were found to be measurements of enzymic activity (catalase, α- and β-amylase), reducing sugar content, and acidity of water extract. At 14·3% moisture viability was unaffected by heating for 1 h at 60°, but was destroyed by 1 h at 70°. Sensitivity to heating increased with the moisture content of **I**. Heating had a similar effect to ageing on the texture of cooked **I**, but had no effect on gelatinisation temp. or fat acidity. (16 references.) E. C. APLING.

Investigation of fat–protein complexes in cereals. I. Isolation of a proteo-lipid from wheat, rye and oats. M. Rohrlich and T. Niederauer (*Fette Seifen AnstrMittel.*, 1967, **69**, 63–67).—A review of published work on the nature and occurrence of fat–protein complexes, and their isolation from cereals by special solvent systems. Reproducible N- and P-values are obtained by using MeOH–$CHCl_3$ followed by treatment with water. An isolated fat–protein complex behaves like a single substance when examined chromatographically or by electrophoretic methods. (49 references.) G. R. WHALLEY.

Significance of thiol and disulphide groups in the selection of wheat varieties for baking quality. B. Belderok (*Getreide Mehl*, 1967, **17**, 20–23).—Relationships found between the SH- and S-S contents, determined by amperometric titration with $AgNO_3$ (Cf. Bloksma, *J. Sci. Fd Agric.*, 1963, **14**, 529) and baking quality for 66 samples of nine spring wheat varieties grown in 1963 and 1964 are reported and discussed. Suitable criteria for the preliminary selection of wheat varieties for good baking quality are considered to be: flour protein $\leqslant$11% (dry basis), S-S/SH- ratio 15–20. E. C. APLING.

Some properties of wheat proteins. G. A. H. Elton and J. A. D. Ewart (*Baker's Dig.*, 1967, **41**, (1) 36–44, 73).—Theories concerning starch gel electrophoresis, structure of glutenin, glutenin-gliadin relationship, the proteins from different cereals and dough rheology are reviewed in relation to some eight species. (37 references.) I. DICKINSON.

Amino-acid composition of wheat as related to quality. C. E. McDonald and K. A. Gilles (*Baker's Dig.*, 1967, **41**, (1) 45–49, 75).—Relationship between amino-acid (**I**) composition of wheat (*W*) and quality are reviewed. Formulae of **I** found in *W*, protein fractions of *W*-flour, change in composition of **I** during milling, **I** content of some mill products and free-**I** in flour and dough are tabulated. (25 references.) I. DICKINSON.

Weathering of mature wheat by rain and snow, and their influence on grain quality. C. A. Watson, F. H. McNeal, M. A. Berg and G. P. Hartman (*Cereal Sci. Today*, 1967, **12**, 86–87).—Adverse effects of weathering, varying according to locality, were concerned with test wt., protein content, and flour absorption. Favourable effects were observed with respect to flour yield, flour ash, farinograph stability, loaf vol. and loaf texture. P. S. ARUP.

Continuous quality control in grain processing. Y. Pomeranz and A. B. Ward (*Cereal Sci. Today*, 1967, **12**, 159–163, 167–168).—A review. (83 references.) P. S. ARUP.

Evaluation of the baking properties of grain and flour by plant laboratories. L. J. Auermann (*Brot Gebäck*, 1967, **21**, 5–7).—Routine methods used in the laboratories of grain receiving depots, mills and bakeries in the U.S.S.R. are reviewed. Emphasis is laid on speed, simplicity, reproducibility and avoidance of expensive equipment. The Brabender Quadrumat is used as the standard laboratory mill, and for measurements on dough and gluten, a compact, simple and relatively cheap automatic penetrometer (Type AR-4: 'Feinmess', Dresden) gives good results. (18 references.) E. C. APLING.

Extensive analyses of flours and millfeeds made from nine different wheat mixes. I. Amounts and analyses. II. Amino-acids, minerals, vitamins and gross energy. E. P. Farrell, G. D. Miller and (I) A. Ward, L. A. Lovett, (II) D. H. Waggle, M. A. Lambert, C. W. Deyoe) (*Cereal Chem.*, 1967, **44**, 39–47; 48–60, 229).—I. Results of 12 h milling tests under controlled conditions on the same pilot mill are reported for nine samples of wheat, of several classes and protein contents; these were grown in widely separated regions of the U.S.A. Results of proximate analysis of the whole wheat, and of proximate analysis, bulk *d* measurements and sieving tests for the flour, germ, shorts and red dog are reported for each sample. (10 references.)

II. The results of determinations of 17 amino-acids, nine vitamins and related compounds, 15 minerals and of total energy content are tabulated for the samples of whole wheat, and other fractions mentioned above. The protein values reported in Tables Tables I–VI are erroneous. These are correctly given in an *erratum* p. 229. (12 references.) E. C. APLING.

Incorporation of cysteine-^{35}S, cystine-^{35}S and *N*-ethylmaleimide-^{14}C into doughs made from wheat flour. C. M. Mauritzen (*Cereal Chem.*, 1967, **44**, 170–182).—Doughs were prepared under N_2 from four Australian wheat flours with addition of labelled compounds (singly or paired) and the specific activities of the gluten (**I**) and soluble proteins, and the sp. activities and amino-acid compositions of the fractions, separated by ion-exchange chromatography, were determined. The sp. activities of the fractions varied considerably and not simply in relation to their cysteine–cystine content, but the results obtained gave general indication of S-S/SH- interchange between **I** and diffusible compounds; they support the suggestion that -SH blocking and S-S/SH- interchange in **I** occur most rapidly during dough mixing. (20 references.) E. C. APLING.

Isolation and chemical comparison of different gamma-gliadins from hard red winter wheat flour. F. R. Huebner, J. A. Rothfus and J. S. Wall (*Cereal Chem.*, 1967, **44**, 221–229).—The separation of three distinct gamma-gliadins by a combination of ion-exchange and gel filtration chromatography (Cf. Huebner and Wall, J.S.F.A. abstr., 1966, ii–235) is reported. The separated gliadins showed slightly different electrophoretic mobilities and differed significantly in amino-acid composition. (19 references.) E. C. APLING.

Significance of wholemeal products on dental health. K. G. König (*Getreide Mehl*, 1967, **17**, 17–20).—Rats were fed diets based on bread prepared from wheat flours (70, 82 and 100% extraction) with or without the addition of Emmenthal cheese, raw or refined sugar. Development of dental caries was reduced by cheese, increased by sugar and generally increased with flour extraction rate. Additions of B vitamins were without significant effect. (25 references.) E. C. APLING.

Determination of amylase. G. Jongh (*Getreide Mehl*, 1967, **17**, 1–4).—A review of available methods for the specific determination of α-amylase activity in wheat and rye flours. (10 references.) E. C. APLING.

Derivation of comparative values for the vitamin contents of wheat and rye flours. B. Thomas and L. Tunger (*Getreide Mehl*, 1967, **17**, 4–8; 13–17).—Published figures for the thiamin (**I**), riboflavin (**II**), niacin (**III**), pantothenic acid, pyridoxine, folic acid and biotin contents of wheat flours and meals of various extractions and of the **I**, **II** and **III** contents of rye flours are collected and statistically analysed. Regression equations between content of each vitamin and milling extraction rate are calculated and average values for wheat and rye flours of differing extractions (from 60 to 100%) are tabulated. (90 references.) E. C. APLING.

Amino-acid composition of rye meal protein. M. Rohrlich (*Dt. LebensmittRdsch.*, 1967, **63**, 106–109).—Extractable and bound protein in rye flour was hydrolysed for 24 h with 5·7 N HCl and the liberated amino-acids determined quant. The content of the basic amino-acids lysine and arginine was higher in the bound than in the free protein. The proportion of these acids was even higher in the residue remaining after dissolving part of the bound protein in N-AcOH. Differences in glutamic acid and proline content of the two types of protein were also found. J. B. WOOF.

Lipids of wheat starch. I. Published work on cereal starch lipids. H. Schmitz and L. Acker (*Stärke*, 1967, **19**, 17–21).—A literature review. Data on starch lipids are incomplete, mainly because the fatty acid fraction forms inclusion compounds with the amylose fraction and this makes quant. recovery difficult. Phosphatides, although identified, have been little studied. (33 references.) J. B. WOOF.

Effect of genetic differences on the properties of maize starch. R. Sandstedt and B. D. Hites (*Getreide Mehl*, 1967, **18**, 8–12).—Studies of the properties of starches separated from maize homozygous for the recessive genes 'amylose extender' (*ae*), 'dull' (*du*), 'sugary-1' (su_1), 'sugary-2' (su_2) and 'waxy' (*wx*), individually, or in combination, and of mixtures of the separated starches, are reported. Water absorption, gelatinisation temp., amylograph max. and resistance to amylolytic attack varied greatly with genotype, and gene combinations produced further property variations. For example, homozygous *ae*-starch showed low water absorption (<4 g/g at 90°) and high resistance to enzymolysis, while *wx*-starch showed very high water absorption (>40 g/g) and low resistance to enzymolysis; the gene combination su_2wx showed higher water absorption, lower gelatinisation temp. and greater gel stability than normal waxy (*wx*) starch. The possibilities of producing starches with particular properties of industrial use are considered. (15 references.) E. C. APLING.

Heat–moisture treatment of starch. L. Sair (*Cereal Chem.*, 1967, **44**, 8–25).—The effects of moist heat (2–18 h at 95–110° and 100%

R.H.) on the properties of potato (*PS*) and maize starches (*MS*) are reported. The physical properties of *PS* were profoundly altered at 95–100° without changes in the physical appearance of the granules or appreciable chemical change, and above 100° chemical degradation was also appreciable. Changes in *MS* were similar but less in extent. Moist heat treatment generally increased gelatinisation temp., reduced the sorptive capacity for water, markedly altered viscosity behaviour in hot water or 1% NaOH, increased paste opacity, altered gel properties and reduced the rate of dispersion in aq. chloral hydrate, 3 or 5% NaOH and water at 120°. The physical changes are believed to be due to increased inter-mol. association resulting from rotation of starch mol. within the granule; some re-arrangement of the starch mol. is shown by changes in the *X*-ray diffraction pattern of *PS*. (18 references.)
E. C. APLING.

Fate of ADPG-alpha-glucan glucosyltransferase during amylolytic corrosion of starch granules, and its relation to starch granule structure. K. R. Chandorkar and N. P. Badenhuizen (*Cereal Chem.*, 1967, **44**, 27–38).—Measurements of the activity of adenosine diphosphate D-glucose: α-1,4-glucan α-4-glucosyltransferase (**I**) in the starch (**II**) of germinating maize, barley, smooth pea are reported. In all cases, activity of **I** in **II** fell during germination and activity in the juice of maize kernels also was lowered during germination. Activity in the juice of bean and tobacco leaves disappeared along with the disappearance of **II** following a period in darkness, and re-appeared on exposure of the plants to light. Electron micrographic studies of the corrosion of the granules of wrinkled pea and potato **II** during germination showed that loss of activity of **I** correlated with granule structure and the onset and extent of corrosion of the granules. It is concluded that the protein of **I** is an integral part of the structure of the **II** granule. (17 references.)
E. C. APLING.

Improvement of semolina quality through steam treatment of durum wheat. G. N. Irvine, J. W. Bradley and H. C. Black (*Cereal Chem.*, 1967, **44**, 230–231).—Traditionally it has been considered detrimental to heat durum wheat during processing, loss of colour being alleged. In this work, steam is applied both before and after washing, under properly selected conditions of pressure and time. The lipoxidase activity is reduced to zero; the yellow pigment content is essentially unchanged and loss of this during pasta processing is greatly reduced, giving a significant improvement in macaroni colour. The findings are tabulated.
C.V.

Macaroni brownness. R. R. Matsuo and G. N. Irvine (*Cereal Chem.*, 1967, **44**, 78–85).—Brownness arising from a varietal characteristic of durum (as distinct from that arising from a Maillard-type reaction, an enzymatic reaction, or from bran contamination) is shown to be due to a water-sol. protein. This component is extractable with water to give a reddish-brown solution, λ max. 400 mμ. Amino-acid analysis, ion-exchange chromatography, electrophoretic examination and trace-metal analysis showed it to be a basic protein, probably associated with Cu. (17 references.)
E. C. APLING.

Study of gluten properties as influenced by certain organic solvents. J. G. Ponte, jun., V. A. de Stefanis, S. T. Titcomb and R. H. Cotton (*Cereal Chem.*, 1967, **44**, 211–220).—Flour–water doughs made with the inclusion of methanol, $CHCl_3$, benzene (**I**), 1-hexanol, or different members of the alkane series (0·0074 g moles/100 g flour solids) yielded glutens (*G*) of variably reduced extensibility. Greatest decrease resulted with C_6 or C_8 alkane. Hexane (**II**) treatment also produced increased expansion during the baking of re-constituted commercial dry-*G* as well as improved grain structure, and in breadmaking gave slightly faster proof and substantially finer grain in the crumb. Both **II** and **I** reduced the solubility of *G*-protein, but when defatted flour was used as the source of *G*, solubility was increased by **II** and decreased by **I**.
E. C. APLING.

Influence of consistency on the dynamics of the development of the microflora in rye doughs and on the quality of the baked product. H. Opuszynska and M. Kowalczuk (*Brot Gebäck*, 1967, **21**, 7–13).—Influence of sour dough consistency (*DC*) on the multiplication of yeast and bacteria, gas production and dough expansion during fermentation, and on the organoleptic properties of the finished bread are reported. Reduction in *DC* increases the multiplication of both yeasts and bacteria, markedly increases gas production and dough expansion and improves the uniformity and elasticity of the bread crumb. The magnitude of the effects found varies with the ash content of the flour used; generally the use of lower sour *DC* in rye bread production is advocated on both microbiological and technical grounds. (27 references.)
E. C. APLING.

Use and function of intermediate proof in dough handling. O. Doose (*Brot Gebäck*, **21**, 29–32).—Times of intermediate proof used in current bakery practice are summarised (varying from 5 to 8 min. for wheaten bread to nil for rye bread) and the effects of variations in the time of intermediate proof on dough development, total proof time, and the texture and vol. of the baked goods are discussed. The major effect of intermediate proof is shown to be on crumb texture, and the crumb texture of bread from mechanically developed doughs is considered to be considerably improved by immediate handling of the dough, followed by two periods of intermediate proof.
E. C. APLING.

The influence of egg yolk lipoprotein–carbohydrate interactions on baking performance. J. R. Schultz and R. H. Forsythe (*Baker's Dig.*, 1967, **41**, (1), 56–62). Structure of the functional portions of egg yolk (**I**) is discussed and examples are given to illustrate the relationship between the bakery performance of **I** and changes in the proposed **I** model. Lipids of **I** probably do not function as free lipids until they are released late in the baking process as the yolk proteins are denatured. The most important rôle of the lipids in **I** is their rôle as a complex emulsifying agent rather than that normally associated with baking fats.
I. DICKINSON.

Bakery glazes and present-day packaging and delivery problems. F. J. Goebels (*Brot Gebäck*, 1967, **21**, 13–14).—Some proprietary glaze stabilisers used in formulation are discussed. These are primarily intended for wrapped goods or where cold storage is envisaged.
E. C. APLING.

Bread flavour and aroma—a review. J. R. Coffman (*Baker's Dig.*, 1967, **41** (1), 50–55).—Preparation of bread flavour/aroma concentrates and separation, isolation and identification of flavour components are discussed. A possible objective method for evaluation of bread aroma is considered. No definite conclusions were arrived at. (16 references.)
I. DICKINSON.

Fat absorption by doughnuts. F. G. Wheeler and J. G. Endres (*Baker's Dig.*, 1967, **41** (1), 63–65).—Controllable and uncontrollable factors affecting the adsorption and absorption of fat are discussed. Free fatty acid increase, due to gradual accumulation of extraneous material in the frying fat, and through the fatty acids collecting on the vents and dripping back into the fryer, is determined. The importance of frying temp. is stressed.
I. DICKINSON.

Flavoured fatty material. Procter and Gamble Co. (B.P. 1,061,364, 2.11.65. U.S., 2.11.64).—Used in the preparation of baked products (bread, rolls, buns), the process consists of fermenting an aq. nutrient containing sugar with *Saccharomyces* and/or *Torulopsis utilis* and contacting the resulting broth with a shortening in liquid form for 10 min.–4 h until an internal temp. of 130–275°F is obtained, followed by segregation of the flavoured shortening. Thus, *Saccharomyces cerevisiae* is added, with stirring, to aq. sucrose solution and fermented for 1·3 h at 90°F to give a broth at pH 4·5, which is then poured into an all-soyabean oil liquid shortening of I.val. ~100. After 55 min. heating at 350°F an internal temp. of 235°F is attained, when the decanted shortening will have a yeasty flavour. If the oven temp. is 350°F and internal temp. of 245° is attained after 1·5 h, the shortening will have a crusty flavour.
S. D. HUGGINS.

Stabilised cake batter systems. Procter & Gamble Co (B.P. 1,062,557, 5.11.64. U.S., 5.11.63).—A cake batter system comprising an emulsion of shortening, flour and sugar, containing 0·5–16 wt.-% of an α-phase crystal-tending emulsifier (containing ≮1 fatty acid radical of 12–22 C and ≮1 OH) and 0·1–8 wt.-% of a high-temp. batter stabiliser (specified) is further stabilised with 0·001–1 wt.-% of a non-toxic, water-sol. polyvalent metal ion salt (e.g., of Cu, Mg, Ca, Zn, Sr, Cd, Ba, Al, Ti, Sn, Mn, Fe, Co, or Ni).
F. R. BASFORD.

Doughmaking. H. Simon Ltd. (Inventors: G. H. Sugden and A. W. Pearson) (B.P. 1,076,922, 12.3.64).—In the manufacture of baker's dough from cereal grains by dry milling, sieving and classifying the product into two fractions, and mixing one of these with liquid to form a slurry which is subsequently divided to yield a product which in combination with usual additives (**I**) is mixed with flour from the other fraction, a product free from coloured impurities is obtained by classification at the sieving stage into three fractions, (*i*) non-fibrous endosperm fraction (amounting to ≮60 wt.-% of the whole grain and containing essentially all the flour produced by the dry milling); (*ii*) a bran free from endosperm

and (*iii*) residue (of >3% on grain) containing all the fibrous endosperm constituents. Fraction (*iii*) is agitated or impact milled in presence of water to yield a slurry which is classified to give three further fractions, (*a*) a fluid fraction which is recirculated; (*b*) a disposable fraction comprising all the wet, fibrous constituents; and (*c*) a semi-solid fraction comprising the endosperm constituents of the slurry (together with enough liquid to maintain semi-solid state). This last fraction is mixed together with **I** with enough flour from fraction (*i*) to maintain 44–49% of water in the dough produced by kneading. F. R. BASFORD.

Sugars and confectionery

Use of enzymes in the production of dextrose and starch syrup. H. Barfoed (*Stärke*, 1967, **19**, 2–8).—The advantages and disadvantages of enzymic, rather than acid, hydrolysis of starch are considered. Enzymes (*E*) only produce dextrose derivatives but the bitter by-products often formed by acids are absent. Since a range of *E* with different specificities is available, a greater range of products and more control is possible. The plant required for *E*-treatment is relatively simple and a high degree of acid resistance is not required. The cost of *E* is considerably greater than the acid; in some cases the increased yield may offset this, but reaction time is longer. Bacterial and fungal α-amylases are available with different characteristics and malt α- and β-amylases can be used. Amyloglucosidase is also described. J. B. WOOF.

Structural relationships, kinetics and molecular properties in the acid depolymerisation of amylose. H. L. Griffin, S. R. Erlander and F. R. Sentini (*Stärke*, 1967, **19**, 8–17).—A butanol fraction of dent corn starch was subjected to acid hydrolysis at pH 4·42–4·66 and 99° to give a series of amylose samples the intrinsic viscosity and weight average mol. wt. of which were determined. Staudinger eqn. for the amylose solutions in 4·2 M guanidine hydrochloride at 25° was $[\eta] = 1{\cdot}22 \times 10^{-3}\bar{M}_w^{0{\cdot}5}$. Assuming a random A-R-$B_2$ type of branching occurs in amylose, less than 0·01% branching is indicated by viscosity-mol. wt. studies and less than 0·07% by acid hydrolysis. The amylose separated by crystallisation of the butanol complex behaves as a linear A-B type of condensation polymer. The same radius of gyration is obtained in 0·1 N KOH, 0·5 N KOH, guanidine, DMSO and 0·33 M KCl but it is 33% lower at pH 12. (46 references.) J. B. WOOF.

Quebrachitol, a new component of maple sap and syrup. E. E. Stinson, C. J. Dooley, J. M. Purcell and J. S. Ard (*J. agric. Fd Chem.*, 1967, **15**, 394–397).—Quebrachitol (1-*O*-methyl-L-inositol) was found to be by far the most abundant monosaccharide in the sap and syrup (3·8—5·7% of solids); glucose + fructose were ≡ <0·1% of the solids. Analyses were carried out by GLC of the trimethylsilyl and acetyl deriv. of the sap constituents; other instrumental determinations were also carried out. (17 references.) P. S. ARUP.

Improved equipment for manufacture of preserves. B. S. Ramachandra, S. Ranganna, L. S. Subba Rao and S. S. Kalbag (*J. Fd Sci. Technol.*, 1966, **3**, 103–108).—The equipment has been developed for use in the hot, continuous syrup process. The syrup concn. is gradually raised in the preserve equipment itself and the fall in the syrup level is made up by continuous addition of conc. syrup. S. A. BROOKS.

Starch hydrolysing enzyme preparations. Grain Processing Corp. (B.P. 1,061,668, 29.9.65. U.S., 2.10.64).—A fungal enzyme prep. containing *trans*-glucosidase (**I**) and glucoamylase (and derived e.g., from *Aspergillus niger*) is purified (freed from **I**) by treating in aq. medium (at 5–45°) with an anion-exchange resin at pH >9 or a cation-exchange resin at pH <3. The resulting product, when used for the saccharification of starch, affords e.g., a hydrolysate of dextrose-equiv. 92·2 as compared with 84·5 for untreated enzyme material (after 36 h). F. R. BASFORD.

Sugar. Fabcon Inc. (B.P. 1,061,698, 29.12.65. U.S., 22.1.65).—An improved process for crystallising sugar from a sugar-containing solution (or a syrup or massecuite derived therefrom) in which boiling time can be reduced (e.g., by 35·3%) and productivity thereby increased, comprises addition of a water-sol. salt of a sulphosuccinate ester (the ester groups being water-insol.), e.g., the K salt of dioctyl sulphosuccinate (introduced as a 50% solution in water–EtOH (and/or propylene glycol) (1 : 1). F. R. BASFORD.

Fermentation and Alcoholic Beverages

Examination and assessment of bentonites for treatment of wines. I. Purity grading of commercial products. W. Kain (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 10–24).—Nine samples tested according to directions laid down by the Codex Oenologique Int. (Paris, 1964) and the Austrian Arzneibuch IX, failed to comply with the prescribed standards. Modified standards are suggested. (24 references.) P. S. ARUP.

Relationship between alcohol content of an unsweetened wine and the specific gravity of the original grape must. E. Gilbert (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 25–35).—A modified form of the Tabarié formula is presented. P. S. ARUP.

Treatment of wine distillery residues. M. Flanzy, J. Maugenet, P. Benard and J. Mourgues (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1088–1096).—A scheme is suggested for utilisation of the residues (a source of river pollution) as a medium for the production of food-yeast. The process comprises addition of $(NH_4)_2SO_4$ or $(NH_4)_2HPO_4$ to the clarified residues, aerobic fermentation with *Candida utilis* var. major at 25–30°, and separation of the yeast by centrifugation. A yield of 10 g of yeast (dry basis) per l was obtained with the possibility of further improvement. The recovery of the 5–10% of glycerol contained in the unfermented residues is also under consideration. P. S. ARUP.

Use of thiamine in experimental production of white liqueur wines. S. Lafon-Lafourcade, J. Blouin, P. Sudraud and E. Peynaud (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1046–1051).—The inactivation of SO_2 in wines from grapes affected by *Botrytis cinerea*, due to the presence of excessive amounts of pyruvic and ketoglutaric acids, was prevented by addition of commercial thiamine hydrochloride (0·5 g per l) before fermentation. Thiamine acclerates fermentation, and reduces the formation of the above acids and of volatile acids. P. S. ARUP.

Identification of ethyl acid tartrate and one isomer of ethyl acid malate in California Flor sherry. A. D. Webb, R. E. Kepner and L. Maggiora (*J. agric. Fd Chem.*, 1967, **15**, 334–339).—Ethyl acid tartrate and the ethyl acid malate having the free carboxyl group next to the carbinol group were isolated from the conc. wine by gradient elution with aq. formic acid from Dowex resin. Purification was effected by conversion of the acid esters into Et-Me esters, followed by gas chromatography. The identity of the purified esters was established by comparison of their i.r. and mass spectra with those of synthetic compounds of known structure. (19 references.) P. S. ARUP.

Determination of diethyl carbonate in wine. C. Reinhard (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 151–153).—After reviewing available methods, a modification of the method of Prillinger and Horwatitsch is described. 10 ml of wine is shaken with 2 ml CS_2 and centrifuged. The org. phase is dried with Na_2SO_4; 2 μl of extract is injected on to a 2 m polypropylene glycol column operated isothermally at 100° with a flame ionisation detector and N_2 as carrier. Solutions containing 0·01 and 0·005% Et_2CO_3 were used for calibration. Recovery at the extraction stage is 85·8% and the appropriate correction must be made. (12 references.) J. B. WOOF.

Gas chromatographic investigation of hop oils. II. Hop oil in wort. K. Silbereisen and E. Krüger (*Mschr. Brau.*, 1967, **20**, 389–396).—Hop oil was shaken with water or wort at increasing concn. until the turbidity started to increase. By this method the solubility of the oil was found to be about 0·4 mg/l in water and about 2 mg/l in wort. Hops were boiled with water with a boil-off rate of 10%/h and the distillate condensed. After extraction with ether–pentane, the volatile oils were separated by gas chromatography. No fractionation of the hop oil occurred, which accounted for the presence in the wort of residual amounts of both high and low boiling components. Further losses occur on both hot and cold break; the ppt. was steam distilled and the volatiles analysed. The composition of the oils removed on hot break resembled that in the wort but hydrocarbons only were detected on cold break. The hop oils from worts from three breweries using different boiling procedures were compared using liquid–liquid extraction, steam distillation and quant. gas chromatography. (29 references.) J. B. WOOF.

Evaluation of malt analysis with regard to beer colour. C. Kremkow and G. Krauss (*Mschr. Brau.*, 1967, **20**, 396–398).—Analytical results from 109 commercial brews have been evaluated, using a Siemens 2002 data processing system to determine the factors influencing the colour of beers produced from pale malts. There was no relation between wort colour at different stages and beer colour but in general the higher the sol. N the higher the colour. The product of malt colour and modification index gives

a less reliable indication. The 'boiling colour' (as defined by Kolbach and Zastrow) which is dependent on pH, formol N and sugar content gives a good indication of final beer colour.
J. B. WOOF.

Determination of oxygen by the Enzinger syringe method. Further development of apparatus and method. H. Kipphan and J. A. Herrmann (Enzinger-Union-Werke A.-G.) *Brauwissenschaft*, 1967, **20**, 291–293).—The cast metal fittings of the syringe are replaced by silicone rubber fittings which are more durable and tighter fitting and therefore more effective against gaps between the glass walls caused by temp. changes. The plunger and the cylinder are standardised and interchangeable, the needle is held firmly by a clasp. Two legs are added to the Holzblock colorimeter so that readings can be made without removing the needle and the plunger rod. The improved syringe has a needle 15 cm long, so that beer samples can be taken directly from a level below the middle of the beer bottle. I. DICKINSON.

Aromatic congener formation in maturing of alcoholic distillates. S. Baldwin, R. A. Black, A. A. Andreasen and S. L. Adams (*J. agric. Fd Chem.*, 1967, **15**, 381–385).—Products derived from interactions between EtOH–lignin (**I**) and vanillin, syringaldehyde, sinapaldehyde, scopoletin, were found by paper chromatography in concentrates of whisky that had been stored in oak (charred or uncharred) casks. **I**, the source of the above compounds, was obtained by filtration of the concentrates. These cogeners are similar to those found in matured brandies. (38 references.)
P. S. ARUP.

Treatment of malt. Enzymic Malt Co. Ltd. and Dixon Malt Co. Ltd. (Inventors: A. A. D. Comrie and B. Dixon) (B.P. 1,076,883, 8.9.64 and 1.4.65).—Anaerobic respiration is induced in green malt (and acidity thereby produced and enzyme activity increased) by keeping the malt (of $<75\%$ extract potential) in a sealed container (in which up to 100% of the free space is occupied by inert non-toxic gas) during <24 h at 15–35°. The treated malt (preferably made from barley or wheat) may be dried at 50–60° and can be used directly. F. R. BASFORD.

Fruits, Vegetables, etc.

Determination of pectin substances in presence of dextrins. M. A. Joslyn and Tung-Shan Chen (*J. agric. Fd Chem.*, 1967, **15**, 398–402).—Determination of pectins, without interference by dextrins, in maize syrups or canned fruit tissues, can be carried out by pptn. as Ca pectate (**I**), washing the ppt. with hot water and then dissolving the **I** in Versene for colorimetric determination by the carbazole method of McComb and McCreedy. (27 references.)
P. S. ARUP.

Influence of differences in carbon dioxide and oxygen concentrations in storage atmosphere on metabolism of non-volatile organic acids and nitrogen of apples (Golden Delicious). K. Klemm (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 50–55).—Decreases observed during storage at 0° in the content of shikimic acid were unaffected by the atm. composition. Decreases in malic acid occurring in air with increased concn. of CO_2 and decreased concn. of O_2 were somewhat less than those found after storage in normal air. The content of citric acid increased during normal storage but it decreased under the modified atm. conditions. Increases in the ratio of protein N to total N in air were somewhat greater than those occurring under the modified atm. conditions. The amino-acid content decreased considerably under all atm. conditions. (14 references.) P. S. ARUP.

Factors related to discoloration of canned Bon Chrétien pears. B. K. Nortjé (*S. Afr. J. agric. Sci.*, 1966, **9**, 681–689).—The pink discoloration was proportional to the leucocyanidin (**I**) content of the fruit which depended, not on the nature of the storage treatment, but on preharvesting conditions. Locality, habits of growth from different rootstocks, and exposure to sunlight are considered to be factors influencing the content of **I**. Cold storage in the presence of 5% CO_2 increased the acid content but did not alter the **I** concn. (11 references.) P. S. ARUP.

Analysis of recovered natural orange flavour enhancement materials by means of gas chromatography. R. W. Wolford and J. A. Attaway (*J. agric. Fd Chem.*, 1967, **15**, 369–377).—The GC techniques included temp. programming with flame-ionisation and electron-capture detection. Indications were obtained from the chromatograms as to the origin (e.g., peel or juice) and method of prep. of various orange products. About 70 constituents were identified, and the great majority confirmed. (28 references.)
P. S. ARUP.

Volatiles from grapes. *Vitis vinifera* **Linn., *cultivar* Grenache.** K. L. Stevens, J. L. Bomben and W. H. McFadden (*J. agric. Fd Chem.*, 1967, **15**, 378–380).—An isopentanol extract of the conc. aromatic essence of grape juice (Cf. Stevens, *ibid.*, 1966, **14**, 249; Bomben, *et al.*, *Fd Technol.*, 1966, **20**, 1219) was analysed by means of gas chromatography with temp.-programming and flame-ionisation detection together with mass spectrometry. About 60 constituents were identified, the most important being 1-hexanol, 3-methyl-1-butanol, *trans*-2-hexanal, hexanal, and 1-heptanol.
P. S. ARUP.

Quality control of dried bananas. P. Dupaigne (*Fruits*, 1967, **22**, 27–29).—Suitable manufacturing conditions are described. The control tests used include colour measurements in comparison with standards (prep. described) and penetrometric measurements of consistency. P. S. ARUP.

Tomato spotted wilt virus on *Arachis hypogaea*. P. L. Klesser (*S. Afr. J. agric. Sci.*, 1966, **9**, 731–736).—Symptoms developed after natural and artificial infection with the tomato virus are described for eight S. African varieties of groundnuts. The symptoms are compared with those due to the groundnut ring-spot virus. (12 references.) P. S. ARUP.

Some hormone-like properties of *Allium cepa* L. (Onion). A. Sharaf (*Qualitas Pl. Mater. veg.*, 1967, **14**, 267–275).—Onion juice fed to rats produced a testosterone-like effect; it increased the wt. of the seminal vesicles in both castrated and non-castrated animals, it possessed an insulin-like action reducing blood sugar level in experimental-hypoglycaemic animals and gave an increased growth rate in young rats. When tested *in vitro* on the uterus, there is an oxytocic effect. The peptide, vitamin and mineral contents of the onion are considered with a view to assessing their rôle in these effects. (16 references.) C.V.

Pink pigment in onion purées: [A] Reactions involved in formation. [B] Precursors. S. Shannon, M. Yamaguchi and F. D. Howard (*J. agric. Fd Chem.*, 1967, **15**, 417–422; 423–426).—[A] The formation of the pigment is traced to a colourless Et_2O-sol. precursor which combines with amino-acids to form an Et_2O-insol. colourless compound which, in its turn, reacts with natural carbonyls to form the pigment. The mechanism of the pigment formation is investigated and considered.

[B] Evidence is presented that the first Et_2O-sol. pigment precursor may possibly be *S*-1-propenylcysteine sulphoxide; this compound when acted on by alliinase gives rise to the second precursor that combines with amino-acids to form the compound that reacts with carbonyls to form the pigment. (16 references.)
P. S. ARUP.

Thermal diffusivity by finite differences and correlation with physical properties of heat treated potatoes. F. V. Matthews, jun. (*Diss. Abstr.*, B, 1967, **27**, 4365).—An investigation was made of the variations in thermal diffusivity (*TD*) of a potato section from the raw to the cooked state. Physical properties were also evaluated and correlated with *TD*. In the procedure used, a heated cylinder of silicone rubber or steel was placed in direct contact with a section of *Solanum tuberosum*. The temp. history at $X = O$ and $X = L$ of a given length of the potato section was used to calculate the *TD* by a finite difference method. Simultaneous recordings of temp. at $X = O$ and $X = L$ permitted measurement of the temp.–time area between the 160°F line and higher temp. up to 215°F. The degree–min. area was related to the heat exposure of the potato section and was a parameter of the study. Elastic modulus was determined for both raw and heat treated potato sections. Results are recorded. F. C. SUTTON.

Radio-active contamination of edible fungi. K. Rohleder (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 135–138).—Between July and October 1966 several varieties of edible fungi originating mainly from Luneburg heath but also from the Harz and the Elm regions, were studied. The content of ^{137}Cs was determined by γ-spectrometry; ^{90}Sr was determined by the method of Bryant. In general, contamination, after reaching a maximum in 1965, had only slightly declined. The ^{137}Cs content depended not only on the species and kind of soil but also upon the humus layer on which the mycelium grew. Fungi growing in woods or in symbiosis with trees contained less ^{137}Cs than others. Values for ^{90}Sr were only obtained for samples grown on heath and varied with the species. The heads of the fungi contained more ^{137}Cs but less ^{90}Sr than the stems. J. B. WOOF.

Vegetable protein food product resembling meat. Worthington Foods Inc. (B.P. 1,061,637, 26.5.54. U.S., 27.5.63).—The food

product consists of a coherent body of molecularly-orientated man-made fibres of natural vegetable protein (preferably soyabean protein fibres, 0·25–5 in. long and containing 50–70 wt.-% of water) held together with an edible binder [preferably aq. dispersion of albumin and a metal (alkali or alkaline earth) soya proteinate], and containing meat-like flavouring and having zones coloured to simulate in appearance lean portions of meat and other zones simulating non-lean portions. The product, in which the fibres are disposed randomly, is preferably shaped in the form of a strip, a bacon-shaped slice, a ribbon, or a sliceable slab, and is substantially fat-free and smoked. F. R. BASFORD.

Non-alcoholic beverages

Detection of adulteration of fruit juices by thin-layer chromatography. B. M. Alvarez (*Analyst, Lond.*, 1967, **92,** 176–179).—For non-citrus juices, coloured extracts (prepared as described) are spotted, together with pure juices, on to cellulose chromatoplates (400 μ) for development with propanol-H_2O (7 : 3). Adulteration is detected by observing the fluorescent spots revealed by u.v. light and the coloured spots formed by spraying the chromatogram with 1% vanillin in 96% EtOH-1% H_2SO_4. For citrus juices, an ethanolic solution is centrifuged and the clear liquid spotted, together with a similar extract of pure juice, on to a SiO_2-gel chromatoplate for development with propanol-H_2O. Adulteration with glycine is detected by spraying with 0·5% ninhydrin in butanol and drying the plate at 110°. Method detects 10% of apple juice in another juice, 25% of one foreign juice in another, and 0·1 μg of glycine in 5 μl of citrus-juice extract. (11 references.)
W. J. BAKER.

Vegetable juices and pickled fodder. F. G. Keitel (B.P. 1,062,988, 22.2.66. Switz., 1.4.65).—A vegetable mash is subjected to lactic acid fermentation until the pH has dropped to 4·5, when the juice is squeezed out of the mash and is pasteurised before or after decanting, the lactic-acidic residues being recovered. Thus a raw mash of carrots at 35°, to which 0·2% protective colloids may be added, is fermented until a pH of 3·7–3·8 is attained after 14 h. The mash is then pumped off into a press and the juice squeezed out, either for storage or for bottling. Pasteurisation is then carried out at 72° and the remaining mash re-used with an unfermented mash, not injected with lactic acid bacteria.
S. D. HUGGINS.

Tea, coffee, cocoa

Investigation of aromas. Coffee aroma. (I). M. Stoll, M. Winter, F. Gautschi, I. Flament and B. Willhalm (*Helv. chim. Acta*, 1967, **50,** 628–694).—A comprehensive review describes the analysis of a coffee concentrate in which 202 constituents were identified, 154 for the first time. Tables of constituents are given, including hydrocarbons, alcohols, ethers, aldehydes, ketones, acids and anhydrides, esters and lactones, mercaptans, phenols, furans, thiophens, pyrroles and thiazoles. The methods of separation such as fractional distillation, column and gas chromatography, procedures of identification based upon mass spectrometry combined with i.r. spectrometry, and detailed examination of fractions are presented. The synthesis of new compounds found is also described. A large number of minor constituents could not be identified. (132 references.) (In French.) M. SULZBACHER.

Investigation of aromas. Coffee aroma. (II). Pyrazines and pyridines. I. M. Goldman, J. Seibl, I. Flament, F. Gautschi, M. Winter, B. Willhalm and M. Stoll (*Helv. chim. Acta*, 1967, **50.** 694–705).—Pyrazines and pyridines present in a coffee concentrate, are tabulated. Gas and column chromatography were used for separation and isolation, mass and i.r. spectral data for identification; 24 compounds were identified. 10 more were probably present. A number of substituted pyrazines were synthetised. (14 references.) (In French.) M. SULZBACHER.

Milk, Dairy Products, Eggs

The dye-binding of milk proteins. N. P. Tarassuk, N. Abe and W. A. Moats (*Tech. Bull. U.S. Dep. Agric.*, 1967, 1369).—Amido black 10B was shown to give a greater change in optical density per unit of protein than orange G. The dye-binding test, using amido black 10B, was significantly affected by the presence of $K_2Cr_2O_7$ and HCHO but not by $HgCl_2$. The dye-binding capacity (*DBC*) of milk protein was not affected by homogenisation, condensing, or heating to 90° for 15 min. Extensive proteolysis increased *DBC* whereas heating to browning reduced it. Because of variations in the *DBC* of various protein fractions the dye-binding protein test is considered unsuitable for atypical milk such as colostrum, mastitic, and very late lactation milks. Mass protein analysis by the amido black method of milk from three areas of California revealed standard errors of estimate of 0·077, 0·082, and 0·064% protein compared with Kjeldahl protein. (38 references.) M. O'LEARY.

Distribution of lipase in milk proteins. II. Dissociation from κ-casein with dimethylformamide. P. F. Fox, M. Yaguchi and N. P. Tarassuk (*J. Dairy Sci.*, 1967, **50,** 309–312).—By means of DEAE cellulose chromatography, Sephadex G-200 filtration, and starch gel (containing >M urea) electrophoresis the lipase (**I**) of milk was shown to be normally associated with κ-casein, and it is concluded that **I** is a discrete minor component of the casein system. (12 references.) M. O'LEARY.

Determination of major cations in milk by atomic absorption spectrophotometry. G. K. Murthy and U. Rhea (*J. Dairy Sci.*, 1967, **50,** 313–317).—The following standard deviations for the major cations were obtained, Ca, 1·6%; Mg, 1·6%; K, 1·9%; and Na, 2·4%. Recoveries of added elements were quant. Results obtained by this method compared favourably with those obtained by flame photometry, EDTA titration, and oxalate–permanganate titration. (10 references.) M. O'LEARY.

Fluorescence of milk and some preservatives in ultraviolet light. E. Elmossalami (*J. Arab vet. med. Ass.*, 1966, **26,** 119–130).—The use of u.v. light to ascertain the source and condition of milk and the presence of preservatives therein has been investigated. Milk from cows, buffaloes and goats could be distinguished by its fluorescence, (*F*). Boiling the milk did not alter the intensity of *F*, but the addition of water did. Addition of cow milk to buffalo milk in the ratio 1 : 5 or more was easily detected. Buffalo and cow milk containing salicylic acid up to 0·01% showed blue *F* but lower concn. could not be detected. Benzoic acid, H_2O_2, borax and formalin showed no distinct *F*. (38 references.)
S. A. BROOKS.

A modified qualitative test for rapid detection of phosphatase enzyme in milk. S. Nasr, H. El-Sawah and M. H. Youssef (*J. Arab vet. med. Ass.*, 1966, **26,** 131–135).—A sensitive, rapid and reliable qual. test for detection of phosphatase enzyme in milk is described. Two ml of a solution containing 0·5 g Na_2Ph phosphate and 4·5 g $NaHCO_3$ in 100 ml water and 1 ml of 50% aq. Na_2CO_3 are added to 1 ml milk. After 1½ min. 0·5 ml. of 0·1% 2,6-dibromoquinine-4-chlorimide in Pr^iOH is added. Raw milk gives a green-blue, and pasteurised a brown colour. The presence of 1·56% raw milk in boiled milk could be detected. S. A. BROOKS.

Thioctic acid in milk. R. J. Bingham, J. D. Huber, and L. W. Auranol (*J. Dairy Sci.*, 1967, **50,** 318–323).—A description is given of a procedure by means of which thioctic acid (α-lipoic acid) was isolated from milk. This compound was shown to be associated with the fat globule membrane. (16 references.) M. O'LEARY.

Comparison of two milk fat extraction methods for pesticide residue analysis. M. Kroger and S. Patton (*J. Dairy Sci.*, 1967, **50,** 324–326).—A surfactant method, employing a solution of alkyl aryl polyether alcohol and Na tetraphosphate, was shown to be more convenient and cheaper than a modified Roese-Gottlieb procedure for extraction of milk fat prior to GLC analysis of heptachlor-epoxide and dieldrin residues. (10 references.)
M. O'LEARY.

Effect of hexane and *N,N*-dimethyl formamide on pancreatic lipolysis of milk fat. J. Sampugna and R. G. Jensen (*J. Dairy Sci.*, 1967, **50,** 386–388).—Experiments with butter oil showed that neither *N,N*-dimethylformamide nor hexane eliminated preferential digestion of milk fat triglycerides by pancreatic lipase. (13 references.) M. O'LEARY.

Value of multiple reading reazurin tests for estimating bacterial content count of milk in warm countries. H. Lück, N. N. Hermann and S. A. Fellingham (*S. Afr. J. agric. Sci.*, 1966, **9,** 661–683).—An approx. rectilinear relationship between bacterial counts (z) and disc readings (y) for 260 samples was shown on application of the equation $y = \sqrt{z}$. The coeff. of correlation were 0·71–0·76. Double readings gave greater accuracy than did single ones, but the latter generally sufficed for practical use. Readings taken after 1 and 3 h gave satisfactory accuracy. (11 references.)
P. S. ARUP.

Studies on milk lipase activation. L. L. Claypool (*Diss. Abstr.*, B, 1967, **27,** 4438–4439).—Studies were made of the mechanisms whereby activation treatments, particularly temp. activation, promote lipolysis (*L*) in milk. Also, investigations were made by GLC to determine if the method of promoting *L* and season of the year affected the end products of lipase action. The susceptibility

of milk to *L* after only an initial cooling (spontaneous *L*) was due to some factor in skim milk obtained from uncooled milk (probably 'membrane' lipase). The milk fat did not contribute to the susceptibility of milk to spontaneous *L*. The development of rancidity in ice-milk mix sterilised by ultra-high temp. (295°F for 5 sec) appears to be due to the action of 'reactivated' lipase. The activity of the enzyme phosphatase was demonstrable in the sterile ice-milk mixes. Fat hydrolysis was inhibited by addition of *N*-ethylmaleimide and hexametaphosphate, storage at cold temp. and post-sterilisation heat treatment of the mix. F. C. SUTTON.

Nutritional evaluation of milk processed for removal of cationic radionucleides. [A] **Chemical analyses.** [B] **Feeding studies.** R. E. Isaacks, D. G. Hazzard, J. Barth, J. H. Foohs, L. F. Edmondson (and J. P. Walker [B]) (*J. agric. Fd Chem.*, 1967, **15,** 295–299; 300–304).—[A] Treatment of milk (pasteurised and homogenised) with Amberlite IR 120 resin in the Ca/Mg/K/Na cycle caused no significant changes in total solids, fat, protein and flavour quality. Losses of vitamins were limited to thiamine (50%) niacin (27%), and B_6 (15%). The contents of ash, K and citric acid were increased by 14%, 80%, and 100%, respectively. The content of Cu was decreased by ~23%. (31 references.)

[B] Rats and young pigs fed with the Amberlite-treated milk, as milk powder, showed the same growth rates and blood-serum cation concn. as did those fed with control milk powder. (19 references.) P. S. ARUP.

Fluorimetric method for microgram quantities of ethylenediamine residues in milk. N. R. Pasarela and C. Waldron (*J. agric. Fd Chem.*, 1967, **15,** 221–226).—The whey obtained after the digestion of the milk with cytase is treated with EtOH and centrifuged. Ethylenediamine (I) is removed from the aq. EtOH solution by adsorption on Amberlite resin XE 89 from which it is eluted with 15% CCl_3CO_2H, and determined by spectrofluorimetric measurement of the product formed by I with adrenchrome in aq. solution at pH 10. The activation and emission λ are at 460 mμ and 510 mμ respectively. The calibration graph is rectilinear for 0–10 μg of I. Recoveries were 88–140%. The average apparent content of I in control milk was 7·7 ppb. Min. detectable amounts were 5–6 ppb. (13 references.) P. S. ARUP.

Cause and nature of the residue adhering to milk tanks after spray cleaning. G. Wildbrett, K. v. Grundherr and F. Kiermeier (*Fette Seifen AnstrMittel.*, 1967, **69,** 281–285).—The formation of scale in milk storage tanks which have been cleaned out by automated spray methods using two different phosphate-containing detergent solutions is examined. It is found that more coating is formed when spraying with steam and detergent than when spraying with detergent alone, using only the pressure of the pump. The high temp. of the detergent solution and the Ca content of the rinsing water cause the resultant scale to assume the consistency of hydroxyapatite. Only trace quantities of Mg, Na, carbonate and silicate are found in the scale. G. R. WHALLEY.

Modern emulsifiers: the basis of improved form retention by ice cream. K. G. Ludwig and W. C. Gakenheimer (*Fette Seifen AnstrMittel.*, 1967, **69,** 285–291).—The properties of modern emulsifiers, e.g., Span, Tween, glycerol dioleate and distearate and their effects upon the ice creams produced, are generally discussed. Production of ice creams which are stable or 'dry', in which the ice cream retains its solid form, and does not drip before consumption is specially desirable. This 'dried' form is primarily due to the agglomeration of dispersed fat particles being achieved by choice of the correct emulsifier. G. R. WHALLEY.

Development of infant foods based on soyabean. M. R. Chandrasekhara, S. R. Shurpalekar, B. H. S. Rau, S. Kurien and K. S. Shurpalekar (*J. Fd Sci. Technol.*, 1966, **3,** 94–97).—Laboratory and pilot plant trials of a spray-dried infant food based on soya dhal, skim milk powder and barley malt are described. The product has a protein efficiency ratio of 2·7 but this can be raised to that of skim milk (3·0) by the addition of *dl*-methionine. S. A. BROOKS.

Octadecadienoic acids in butter fat. I. Precursors of 4-*cis*-heptenal. II. Identification of some non-conjugated fatty acids. K. de Jong (**I** and **II**) and H. van der Wel (**II**) (*Fette Seifen AnstrMittel.*, 1967, **69,** 277–279, 279–281).—**I.** The substance contributing a cream-like odour to butterfat (*BF*) was isolated from 100 kg butter. It was found to be a type of unsaturated aliphatic aldehyde. Further examination of the *BF* fatty acids likely to produce such a product upon autoxidation was carried out. A method is also described for the determination of points of unsaturation by means of oxidation with OsO_4 in pyridine. Special attention is paid to compounds yielding 4-*cis*-heptenal.

II. Using this oxidation process, the products of unsaturated *BF* acids were monitored by both TLC and GLC and the following iso-linoleic acids were identified: *cis-trans* or *trans-cis* 11,16 and/or 11,15; 10,16 and/or 10,15; 9,15 and/or 9,16; 8,16 and/or 8,15 and/or 8,12. Also *trans-trans* 12,16; 11,16 and/or 11,15; 10,16 and/or 10,15; 9,16 and/or 9,15 and/or 9,13. G. R. WHALLEY.

Improved technique for analysis of free fatty acids in butteroil and Provolone cheese. M. Iyer, T. Richardson, C. H. Amundson and A. Boudreau (*J. Dairy Sci.*, 1967, **50,** 285–291).—A description is given of a modification and extension of the technique of McCarthy and Duthie (*J. Lipid Res.*, 1962, **3,** 117) for isolating the free fatty acids (*FFA*). The concentration of short-chain *FFA* in butteroil was found to be low while that of the long chain acids was high. The reverse was found in Provolone cheese. (13 references.) M. O'LEARY.

Inter-relationships of flavour and chemical changes in cheese. T. Kristoffersen (*J. Dairy Sci.*, 1967, **50,** 279–284).—Desirable flavour of Cheddar (*C*) cheese was shown to be dependent upon the presence of free fatty acids and H_2S in definite interdependent concn. The formation of active sulphydryl (-SH) groups in *C* and Swiss (*S*) cheese during curing is suggested as a cause of characteristic flavour. In *C* cheese manufacture formation of active -SH groups was shown to be impaired by heat treatment of the milk, relatively high redox potential of the cheese, and the presence of Cu. The concn. of active -SH groups in *S* cheese were not affected by redox potential or Cu content but it appears that they may be affected by the rate of acidity development during manufacture. Consumer-packaging of cheese was shown to result in loss of flavour, probably due to the exclusion of air from the cheese surface. M. O'LEARY.

Cheddar cheese flavour. IV. Directed and accelerated ripening process. T. Kristoffersen, E. M. Mikolajcik and I. A. Gould (*J. Dairy Sci.*, 1967, **50,** 292–297).—A description is given of a process in which a liquid cheese product, having the characteristic flavour of Cheddar (**I**), Brick (**II**) or Romano (**III**) cheeses, may be produced from fresh curd in 4 to 5 days. The addition of reduced glutathione (10–100 ppm) (**IV**) to the **I** curd slurry gives a fuller flavour, which upon prolonged storage changes to the **II** type; addition of porcine lipase + reduced **IV** yields the **III** type. Using reduced **IV** an increased formation of C_4 is found together with longer chain fatty acids and sol. protein. There is also accelerated bacterial growth. M. O'LEARY.

Major free fatty acids in Gouda cheese. M. Iyer, T. Richardson, C. H. Amundson and R. C. Tripp (*J. Dairy Sci.*, 1967, **50,** 385).—Four samples of American Gouda cheese were analysed for the major free fatty acids. Total values obtained were 1,949, 3,454, 2,939, and 2,190 mg acid/kg cheese. M. O'LEARY.

Identification and evaluation of selected compounds in Swiss cheese flavour. J. E. Langler, L. M. Libbey and E. A. Day (*J. agric. Fd Chem.*, 1967, **15,** 386–391).—Neutral volatiles obtained from low-temp. low pressure distillates from cheese fat by the method of Langler and Day were identified by GLC and mass spectrometry. Further identification by these methods was carried out by gas-entrainment of the volatiles and on-column trapping by the method of Morgan and Day. Selected volatiles were determined quant.; a mixture of these volatiles with selected amino-acids and fatty acids had a flavour and aroma resembling but not duplicating that of the best Swiss cheese. (22 references.) P. S. ARUP.

Degradation of sulphur-35-methionine to methanethiol in surface-ripened cheese. H. Grill, S. Patton and J. F. Cone (*J. agric. Fd Chem.*, 1967, **15,** 392–393).—After the injection of ^{35}S-methionine into Trappist (or Limburger) cheese and subsequent ripening of the cheese, the methanethiol obtained by gas-entrainment and selective trapping showed radio-activity. (10 references.) P. S. ARUP.

Enumeration of *Staphylococcus aureus* in cheese. M. E. Stiles, H. Hinch, E.-C. Flockemann (*S. Afr. J. agric. Sci.*, 1966, **9,** 725–726).—Survival values in Cheddar cheese kept at 13° varied with the composition of the nutrient media used for making counts. Differences in resistance to the conditions in the cheese were also observed as between two strains of *Staphylococcus*. P. S. ARUP.

Natural β-carotene content of fresh, dried and frozen duck egg yolk. E. Benk, R. Dietl and L. Brixius (*Dt. LebensmittRdsch.*, 1967, **63,** 110–112).—Egg samples from different sources have been analysed for total carotenoid and β-carotene content. The carotenoids in light petroleum (**I**) were fractionated on Al_2O_3 containing 8% water, and eluted with **I**, **I**–benzene, benzene, acetone and acetone–NH_3. After removal of solvent the fractions

were redissolved in I and the light absorption of each determined. The identity of each fraction was checked by its spectrum and R_F after TLC on silica gel G with AcOEt–CH_2Cl_2 (2 : 8) as solvent. The carotenoid content varied from 2·6 to 13·4 mg/% and 1·3 to 20·6% of this was β-carotene; dried and frozen yolks gave similar results. On average the total carotenoid extract contained 14·1% β-carotene, 69·5% xanthophyll and 5·2% zeaxanthin.
J. B. WOOF.

Rapid method for determining alkaline phosphatase in hen egg yolk. F. Günther and O. Burckhart (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 305–309).—A rapid enzyme assay based on hydrolysis of *p*-nitrophenyl phosphate is described and compared with the procedures of Morton, of Bamann and Meisenheimer and with the $CHCl_3$ method. The enzyme solution (1 ml) is mixed with an equal vol. of 0·1 M glycine buffer, pH 10·4, containing 2·5 mg of Na_2 *p*-nitrophenyl phosphate; if the pH of the mixture is < 10·2, a buffer of rather higher initial pH must be used. The mixture is incubated for 1 h at 37° and 5 ml 0·02 N NaOH is added. The absorption of the solution at 400 mμ is measured against a reagent blank, to determine the amount of liberated *p*-nitrophenol. The method agrees well with the modified Bamann and Meisenheimer method and is better than the other two for the study of egg yolk. (10 references.)
J. B. WOOF.

Conditioning cheese curd. National Dairy Products Corp. (B.P. 1,060,518, 6.8.65. Austral., 14.8.64).—The discrete curd granules are pressed, so as to cause partial knitting into a flowable curd mass which is delivered, without substantial rupturing, into a receptacle. Further pressing, with heat application, is then carried out, followed by salting and hooping or packing.
S. D. HUGGINS.

Rapid cheese manufacture. Beatrice Foods Co. (B.P. 1,076,669, 18.10.65. U.S., 29.10.64).—Unripe cheese is ripened by agitating a 9–50% by wt. solids aq. suspension, under anaerobic, aerobic or intermediate conditions for 1–10 days at the temp. appropriate for the cheese-forming organism. Thus, a 30 day old Cheddar cheese is macerated and fluidised with water to a 30% solids suspension. After incubation under N_2 at 85°F for 2 days, the product is spray dried to give a highly flavoured cheese powder equiv. in flavour to a Cheddar cheese aged for 12 months. Alternatively, the cheese may be formed *in situ* by agitation of a cheese inoculum with whole milk, or milk solids, and maintained in suspension until ripening has taken place.
S. D. HUGGINS.

Edible Oils and Fats

Non-glyceridic substances of vegetable oils. G. Jacini, E. Fedeli and A. Lanzani (*J. Ass. off. analyt. Chem.*, 1967, **50**, 84–90).—For olive oil, 20% of the non-glyceridic substances present are 'unsaponifiable matter' as determined by the usual analytical procedures. The oil contains terpenic alcohols and sterols and their esters; especially noteworthy are those of 20-carbon acids. Ethyl esters of fatty acids are also present, but phytol is not found as such in the non-glyceridic fraction.
A. A. ELDRIDGE.

Classification and evaluation of margarine in relation to food law requirements. R. Ristrow (*Dt. LebensmittRdsch.*, 1967, **63**, 115–117).—Data and a discussion are presented on the chemical composition of margarine samples from Summer 1962 and 1965 and Winter 1964/65. The relationship between classification according to price and the content of essential fatty acids, tocopherol etc. is discussed.
J. B. WOOF.

Continuous rendering operations. Geo. A. Hormel and Co. (B.P. 1,062,798, 3.12.64. U.S., 9.12.63).—Fat is rendered continuously from adipose tissue by placing the animal matter in an accumulating tank to form a uniform mixture which is then discharged in a continuous stream, under gravity, through interconnected heating chambers at successively higher temp. (max. 210–280°F). The liquid fat is separated from the solid material and collected.
S. D. HUGGINS.

Meat and Poultry

Experimental canning of edible offals of slaughtered animals. M. H. Yousef, M. El Hidik, I. H. Sherif and H. M. El Sawah (*J. Arab vet. med. Ass.*, 1966, **26**, 113–118).—Legs, rumen, heads, tongues, lungs, hearts, livers and spleens of Somalian cattle were cooked in sauce and canned in ½ kilo cans. Cans were stored at 37° for 2, 6 and 12 months and controls at room temp. Others were stored at 55° for 10 days. All cans showed a negative pressure of 24–27 cmg. The contents were sterile and had an acceptable taste.
S. A. BROOKS.

Nitrogen factor for tongue. Analytical Methods Ctee., Meat Products Sub-Ctee. (*Analyst, Lond.*, 1967, **92**, 326–327).—Results for the N-contents of ox- and pig-tongue (uncured) are summarised. It is recommended that an average N-factor of 3·0 be used in the analysis of comminuted tongue products.
W. L. BAKER.

Contour curves for determining added water in sausages. M. H. S. Radwan and I. M. Sadek (*J. vet. Sci. U.A.R.*, 1966, **3**, 7–13).—A procedure which avoids calculation is suggested. Moisture and protein are determined in the finished product and two sets of contour curves are drawn. The first set is based on protein % dry wt., the second set on protein % wet basis. Examples of the application of these contour curves and analytical data of five sausage samples are demonstrated. (17 references.)
I. DICKINSON.

Tenderising meat. Baxter Laboratories Inc. (B.P. 1,061,450, 27.12.63. U.S., 9.1.63) —Meat is tenderised by injecting into the carcass of a freshly killed animal 1–4 wt -% of an enzyme solution (e.g., papain) having an activity of 0·01–0·05 M.C. (milk clotting) units per g.
F. R. BASFORD.

Fish

Investigation of volatile compounds in codfish by gas chromatography and mass spectrometry. N. P. Wong, J. N. Damico and H. Salwin (*J. Ass. off. analyt. Chem.*, 1967, **50**, 8–15).—Volatile compounds vacuum distilled in N_2 at 35° were condensed with solid CO_2–EtOH and liquid N_2, and separated on a β,β′-oxydipropionitrile column by programmed cryogenic temp. gas chromatography before recording the mass spectra. Fresh fish contained H_2S,CS_2, dimethylsulphide, acetaldehyde, CH_2Cl_2, $CHCl_3$, EtOH, acetone, benzene, toluene, and diethyl ether. Stored fish also contained propionaldehyde, butyraldehyde, methyl ethyl ketone, diethyl ketone, methyl propyl ketone, methyl vinyl ketone, and Me_3N. (27 references.)
A. A. ELDRIDGE.

Fish muscle purine and pyrimidine nucleoside phosphorylases. H. L. A. Tarr and J. E. Roy (*Can. J. Biochem.*, 1967, **45**, 409–419).—Three purine nucleoside phosphorylase prep. have been obtained from lingcod muscle by $(NH_4)_2SO_4$ fractionation and DEAE-cellulose chromatography. The most active prep. utilised hypoxanthine (I), 6-mercaptopurine, guanine, 8-azaguanine, xanthine, adenine, 2,6-diaminopurine and 6-methylpurine in presence of ribose 1-phosphate or deoxyribose 1-phosphate. Several substituted purines were not utilised and did not inhibit the reaction between I and the pentose phosphates. A pyrimidine nucleoside phosphorylase with much lower activity was also isolated; the best substrates for this were uridine and thymidine; cytidine and deoxycytidine were not utilised. (24 references.)
S. A. BROOKS.

Preservation of fresh whitefish with gamma radiation. K. Ostovar, M. Slusar and M. Vaisey (*J. Fish. Res. Bd Can.*, 1967, **24**, 9–19).—The storage life of fresh whitefish (*Coregonus clupeaformis*) was considerably extended by low levels of γ-radiation. Samples exposed to 150,000 rad were bacteriologically and organoleptically acceptable after 29 days storage at 0°. Samples exposed to 75,000 rad remained acceptable for 22 days but those exposed to 300,000 rad were organoleptically unacceptable. Unirradiated fish samples were dominated bacteriologically by Gram-negative micro-organisms of the genus *Pseudomonus*, *Achromobacter* and *Flavobacterium*. Gram-positive organisms belonging to the genera *Corynebacterium* and *Micrococcus* survived 150,000 rad but were replaced by Gram-negative micro-organisms after 29 days storage. (16 references.)
S. A. BROOKS.

Mineral content of aquatic foods. R. Viswanathan, Y. M. Bhatt, C. Sreekumaran, G. R. Doshi, S. S. Gogate, A. M. Bhagwat and C. K. Unni (*Proc. Indian Acad. Sci.*, B, 1966, **64**, 301–313)—Contents of Ca, K, P, Cu and Fe are reported for 53 samples of freshwater fishes and prawns (including 20 different species) from the various Indian States. Seven samples from Bihar were also analysed for U. The relevant water supplies were examined to determine the concn. factors (concn. of element in the organism/concn. of element in the water) and these are reported for each species. The findings are briefly discussed in relation to prescribed max. permissible concn. of radio-isotopes of the elements in water. (25 references.)
E. C. APLING.

Temperature measurement and fish. J. Graham (*Fd Technol. Aust.*, 1967, **19**, 216–223).—Torry Advisory Note No. 27 deals

with temp. measurement of wet and frozen fish during processing and distribution, the measurement of temp. in freezing plants, and the selection and use of instruments to give the most accurate results. (19 references.) G. W. FLINN.

Nucleotide degradation and quality in ordinary and red muscle of iced and frozen swordfish (*Xiphias gladius*). W. J. Dyer, D. I. Fraser and D. P. Lohnes (*J. Fish. Res. Bd Can.*, 1966, **23**, 1821–1833).—Changes in nucleotide composition, especially the rate of hypoxanthine formation, and in quality as assessed by taste panel are examined in swordfish held in ice for various periods; slow- and fast-frozen steaks were chosen. In iced swordfish, inosine phosphate was dephosphorylated to inosine after 19 days storage and hypoxanthine (**I**) increased very slowly; quality did not decrease significantly until after 15 days. These changes occurred much more rapidly in red muscle. Rapid freezing and storage at −26° for four to five months inhibited nucleotide enzymic activity, quality remaining unchanged. Slow freezing and storage for one week at −4° significantly reduced quality but only slightly affected nucleotide degradation; dephosphorylation and **I** accumulation continued during further storage at −4°. (31 references.) S. A. BROOKS.

Effects of water and dielectric thawing processes on shelf life of double-frozen cod and redfish. W. A. MacCallum, D. A. Chalker, W. J. Dyer and D. R. Idler (*J. Fish. Res. Bd Can.*, 1967, **24**, 127–144).—Immersion thawing in tap water at 7·2 and 15·5° and dielectric thawing at 38 MH_z were shown by taste panel testing to have similar effects on the cold-storage life of processed, re-frozen fillets of cod (*Gadus morhua*). Grand Bank cod caught in March, June or July and frozen twice had an acceptable quality for at least 28 weeks but the quality was not as good as once-frozen samples. Grand Bank redfish frozen twice maintained the same quality as samples frozen once. (29 references.) S. A. BROOKS.

Cholesterol content of herring oil for evaluating herrings and herring products. J. Wurziger, G. Hensel and B. Dagbjartsson (*Dt. Lebensmitt Rdsch.*, 1967, **63**, 309–312).—Samples of herring fillets have been analysed to determine water content, fat and the level of cholesterol (**I**) in the fat and fat-free residue. The distribution of **I** varied but the total was always close to 0·06%, decreasing by 11·1–14·2% of this figure on cooking. J. B. WOOF.

Spices, Flavours, etc.

Packaging and storage of dried ginger. K. C. Richardson (*Fd Technol. Aust.*, 1967, **19**, 165–166).—Green ginger was dried at 120°F to constant wt. in a cross draught tray dehydrator. Multi-wall paper bag packages made of seven different materials were filled with dried sliced ginger and ground ginger; the oleoresin (**I**) and volatile oil (**II**) concn. being determined at monthly intervals for five months. At the commencement **I** was 22·8% and **II** 3·1%, the **II** content of the ground ginger dropping by 50%. It was unchanged in the sliced ginger experiments. **I** content did not alter. G. W. FLINN.

High resolution gas chromatography in aroma research. R. Teranishi (*Perfum. essent. Oil Rec.*, 1967 **58**, 172–178).—The detection limits of gas chromatographic procedures are discussed in relation to the limiting odour threshold values of various odorants, together with the new significance of these levels on 'purity'. Some experimental data are discussed, using programmed temp. resolution on 0·01 in. internal dia. open columns to demonstrate the complexity of odour extracts. (11 references.) G. R. WHALLEY.

Pyromeconic acid derivatives. C. Pfizer and Co. Inc. (B.P. 1,057,446, 5.8.64. U.S., 19.9. and 23.9.63).—2-Alkyl- and 2-aryl-pyromeconic acids are prepared by treating pyromeconic acid (**I**) with an aldehyde containing 2–18 C at pH > 5 to form 2-(1-hydroxyalkyl) pyromeconic acid or 2-(1-aryl-1-hydroxy)methylpyromeconic acid and reducing these acids with Zn and HCl, or dehydrating them to obtain 2-(1-alkenyl)pyromeconic acids. Thus, aq. **I**, adjusted to pH 10·5 with NaOH, is cooled to 17° and reacted with MeCHO in ice-water. The solution is acidified with 50% H_2SO_4 and cooled to give a 79% yield of 2-(1-hydroxyethyl)pyromeconic acid, m.p. 131–132° (from Pr^iOH). Conc. HCl is added to a stirred mixture of this acid, Zn and water, while the temp. is raised to 60°. After stirring for 1 h at 60–65°, the filtered solution is cooled to give a 54% yield of 2-ethyl-pyromeconic acid, m.p. 90–91° (from water). The product is effective against, e.g., *Salmonella typhosa*, *E. coli* and *A. aerogenes*. The compounds can also be used as flavour and aroma enhancers for food, and in perfumed compositions. S. D. HUGGINS.

[A] **Chlorinated amino-acids,** [B] **Amino derivatives of amino-acids.** Merck & Co. Inc. (B.P. 1,057,651–2, 7.1.64. U.S., 16.1., 5.7., and 18.12.63).—[A] Amino-acids containing a chain of ≮3 C, also N-R-N-R^I ester, acid halide, amide, or cyclic anhydride derivatives thereof (R and R^I are C_{1-5}-alkyl or acyl, or R is H) are C-chlorinated in high yield by interaction with Cl_2 in strongly acid medium (≮1 mol. of acid per mol. of amino-acid) in presence of free-radicals. E.g., Cl_2 is passed for 6 h at 85–90° through a solution of α-aminobutyric acid in 98% H_2SO_4–20% oleum with intermittent addition of $(:N\cdot CMe_2CN)_2$. After degassing/< 1 atm., the mixture is then boiled with water and Ag_2SO_4, and the filtered liquor is freed from Ag^+ by pptn. as AgCl; filtrate is purified on Dowex 100 (H^+ form) and eluted with 2 N-NaOH to give crude homoserine, m.p. 155–157°. Further purification may be effected by lactonisation with HBr, to give homoserine of final m.p. 185–186°. [B] γ-Chloro derivatives of amino-acids, e.g., of [A] are reacted with NH_3 to give the corresponding γ-amino derivatives. E.g., a mixture of *β-chloroglutamic acid hydrochloride* and conc. aq. HN_3 is heated at 100°/> 1 atm. for 4 h, then evaporated. An aq. solution of the residue is passed through I.R.120 resin (H^+ form) which is then eluted with water, N-HCl, and water again. Product is eluted with aq. NH_3, this eluate is evaporated, and the residue is re-dissolved in water, heated, adjusted to pH 5, and diluted with 1 vol. of EtOH, to give 2,3-*diaminoglutaric acid*, m.p. 250° (decomp.) after further purification; the *l*-isomer, m.p. 235° (decomp.) is also claimed. These products are useful as food flavours. F. R. BASFORD.

Flavouring materials. Ajinomoto Co. Inc. (B.P. 1,061,406, 9.4.64. Jap., 9.4. and 20.9.63).—A synergistic flavouring material is produced by coating Na *l*-glutamate crystals with a solution of one or more Na_2 5′-ribonucleotides. F. R. BASFORD.

Pyrazine derivatives. Firmenich et Cie and R. Firmenich, G. Firmenich and R. E. Firmenich (B.P., 1,061,734, 21.2.64. Switz., 26.2.63).—Useful as flavouring agents, the claimed compounds are obtained by treating the appropriate monohalogenated (Cl or Br) methyl pyrazines with MeONa or MeOK or with Na- or K-Me-mercaptide to replace the Cl or Br by MeO or MeS. Thus, Me-mercaptan in EtOH is added to NaOEt followed by a mixture of isomeric monochlorinated Me-pyrazines, the total mixture being refluxed for 3 h after the exothermic reaction has subsided. The filtered solution is concentrated and distilled, the fraction distilling at 84–88°/10 Torr. consisting of 2-methyl-3-methylmercapto-pyrazine (70%) and 2-methyl-5- and -6-methylmercaptopyrazines. The isomeric mixture is used to give a roasted hazelnut, peanut or almond flavour to foods. S. D. HUGGINS.

Seasoning compositions. Ajinomoto Co. Inc. (B.P. 1,076,948, 5.4.66. Japan, 5.4.65).—A meat-flavoured composition for seasoning food comprises a mixture of amino-acids (glutamic acid, alanine, histidine, arginine, threonine, methionine, leucine). (> 30 wt.-%, excluding glutamic acid); at least one non-toxic edible org. acid (lactic, succinic, tartaric, malic, fumaric, citric, gluconic, pyruvic, and/or an α-keto-glutaric acid) (0·1–2 pt. per pt. of amino-acids exclusive of glutamic acid); at least one phosphate (inorg. phosphate, phosphoethanolamine, phosphoserine, Na glycerophosphates) (≯50 wt.-%, as P_2O_5, on amino-acids excluding glutamic acid); and at least one 5′-nucleotide (5′-inosinic, 5′-guanylic, 5′-adenylic, or 5′-xanthylic acid) (> 1 wt.-% on amino-acids excluding glutamic acid.). F. R. BASFORD.

Colouring matters

Isocryptoxanthin and echinenone. F. Hoffmann-La Roche & Co. A.-G. (B.P., 1,076,306, 22.4.66. U.S., 28.4.65).—A polyene aldehyde, viz., R·CH:CH·CMe:CH·CH:CH·CMe:CH·CHO (R is 3-X-2,6,6-trimethylcyclohexen-1-yl) is reacted with $R^{IV}\cdot(CH{:}CH\cdot CMe{:}CH)_2\cdot CH_2PR^IR^{II}R^{III}Z$ (Z is anion; R^{IV} is 3-Y-2,6,6-trimethylcyclohexenyl; X is H and Y is low-mol. alkanoyloxy of 1–8 C or aroyl of 6–16 C, or *vice versa*; R^I–R^{III} are alkyl, aryl, or aralkyl) in presence of an **alkali metal**-hydride -amide, -alkoxide, or hydroxide (in alkanol of 1–7 C), or C_{1-7}-alkyl-group IA metallo-org. compound, or an optionally C_{1-7}-alkyl-substituted Ph-group IA metallo-org. compound. The product of the reaction is isocryptoxanthin (**I**) which can be converted by oxidation (Al alkoxide in acetone, COMeEt, or cyclohexanone) into echinenone (**II**) (both products being colouring substances of use in the food and pharmaceutical industries). Thus, 4-acetoxyretinene (prep. described), dissolved in Pr^iOH, is reacted at 15–20° with a triphenyl phosphonium salt (prepared

from vitamin-A acetate and Ph_3P) in presence of NaOMe in MeOH, to give **I**, m.p. 136–139° (from Me_2CO–MeOH). **I** is oxidised with $Al(OPr^i)_3$ to give **II**, m.p. 179° (from CH_2Cl_2).
F. R. BASFORD.

Preservatives

Compositions for the preservation of foodstuffs, for the sterilisation and hygiene of air in confined spaces and for cosmetic and pharmaceutical purposes. Maple Leaf Trust (B.P. 1,060,447, 1.5.64. Switz., 14.5.63).—The composition comprises acetic or citric acid; EtOH or Pr^iOH; and at least one bactericidal and/or bacteriostatic essential oil (or suitable extract therefrom). e.g., thymol and/or oil of cloves, cinnamon, peppermint, rosemary, mustard or thyme. F. R. BASFORD.

Foodstuff treatment for long periods of storage. Farbwerke Hoechst A.-G. (B.P. 1,061,014, 30.10.63. Ger., 2.11.62).—The foodstuff is dipped into an aq. suspension of Ca sorbate containing a thickening agent and dried. Thus, Provolone cheese is compacted in forms and salted and then dipped for 10–15 sec. into a homogeneous suspension of 10·0% Ca sorbate and 1·5% carboxymethyl cellulose in water. The suspension is allowed to drip off and the cheese dried for ripening (3 months). On comparing with controls, either untreated, or treated with sorbic acid or K sorbate, the Ca sorbate treated cheeses have much less mould.
S. D. HUGGINS.

Pesticides in Foods

Effects of washing, trimming, and cooking on levels of DDT derivatives in green beans. D. D. Hemphill, R. E. Baldwin, A. Deguzman and H. K. Deloach (*J. agric. Fd Chem.*, 1967, **15**, 290–294).—The three treatments reduced the mean total of pesticide residues by 47–63%, the max. losses being caused by pressure-cooking. Increases of ~12% occurred in the content of DDD and *o,p*-DDD. The cooking liquids contained only traces of pesticides. (15 references.) P. S. ARUP.

Determination of residual amounts of barium-containing fungicides [in fruit]. E. Kröller (*Dt. LebensmittRdsch.*, 1967, **63**, 112–114).—The fruit (grapes, gooseberries) is soaked in 0·1 N-HCl for 15 min. before filtration and re-extraction with more acid. The extracts are evaporated to low bulk and transferred quant. to a Pt dish. Org. material is carbonised by evaporation to dryness and i.r. heating; this is repeated after addition of $(NH_4)_2CO_3$, more HCl and finally $HClO_4$. The residue is stirred with aq. EtOH, filtered, and EtOH is evaporated off. A portion of the filtrate is chromatographed on filter paper together with 1 μg of Ba standard; the solvent system is 25% HCl–MeOH–tetrahydrofuran (7: 2 : 1). After development the paper is air dried and sprayed with 1% sodium rhodizonate followed by 25% ammonia when the Ba is revealed as a spot 2 cm from the origin; <0·01 ppm of Ba can be detected. (24 references.) J. B. WOOF.

Food Processing, Refrigeration

Botulism. R. A. Gordon and W. G. Murrell (*C.S.I.R.O. Fd Preserv. Q.*, 1967, **27**, 6–18).—A review of the present situation. (35 references.) P. S. ARUP.

Freeze-drying of foods. P. N. Rao, P. Rajagopal and P. K. Ramanathan (*J. Fd Sci. Technol.*, 1966, **3**, 98–100).—Freeze drying of potatoes, cooked mutton and cooked fish fillets has been studied using (1) a conventional freeze drier (*FD*) (Stoke's laboratory model), (2) an accelerated *FD* of the contact plate type, and (3) an accelerated *FD* of the radiant type. The time–temp. cycles in all three types of unit have been compared to that of air-drying. Controlling mechanisms appear to be similar for both freeze-drying and air-drying. S. A. BROOKS.

Food processing. J. Cording, jun. (B.P. 1,062,485, 3.6.64. U.S., 3.6.63).—An apparatus for the continuous production of puffed food pieces (e.g., rehydratable fruit or vegetable) is figured and claimed. F. R. BASFORD.

Preservation of foodstuffs by drying. Unilever Ltd. (Inventors: J. P. Savage and R. Chenneour) (B.P. 1,076,923) 22.4.64).—Meat (animal, fish, poultry) is dehydrated in presence of a starch subjected to or capable of retrogradation (2–15 wt.-% on water content of meat), to give a product of improved properties on rehydration. F. R. BASFORD.

Apparatus for the storage of perishable animal and plant materials [including foods]. Whirlpool Corp. (B.P. 1,076,584, 5 and 6, 5.2.65. U.S., 9.3.64).—The materials subject to respiratory deterioration changes on storage in air containing normal quantities of O_2 and CO_2 are stored in apparatus [A] consisting of a storage container, atmosphere control means to retard deterioration, a temp. control means, humidity control means and an air circulation means. [B] Consisting of storage space, cooling means and thermoresponsive means (for interior and surface temp. control), and [C] consisting of storage space, O_2 detection means and a means activated by the latter for maintaining a preselected O_2 quantity. S. D. HUGGINS.

Packaging

Plastics in the dairy industry: a critical study. I. Related problems and legal specifications. G. Wildbrett (*Fette Seifen Anstr-Mittel*, 1967, **69**, 269–277).—The historical development of plastics and their general application is reviewed, with particular reference to medical and agricultural uses, including their uses for wrapping materials, containers, utensils and machinery and equipment. The food regulations dealing with special dairy laws in relation to the use of plastics are discussed, especially in their application to factory hygiene. (69 references.) G. R. WHALLEY.

Protection of edible oils from light by transparent plastic bottles. E. Hadorn and K. Zürcher (*Dt. LebensmittRdsch.*, 1967, **63**, 99–106).—Light absorption by PVC foil was studied photometrically and compared with that of clear bottle glass. The glass transmits about 80% of all light above 320 mμ whereas PVC only transmits 50% even up to 420 mμ. By including 0·2% u.v. absorber and yellow dye, transmission below 460 mμ can be almost eliminated. Samples of refined groundnut oil were exposed to up to 225 h of sunlight and the protection offered by glass, PVC and PVC with additives compared; u.v. differential spectrometry, peroxide number, oxidation sensitivity, the Kreis reaction and flavour were used to detect changes on storage. It is concluded that radiation in the 200–420 mμ region is most injurious and that PVC which contains additives, by not transmitting light in this range, affords protection against triene degradation and resultant flavour changes. (16 references.) J B. WOOF.

Oxygen permeability of plastic bottles. K. Wucherpfennig, G. Bretthauer and D. Ratzka (*Brauwissenschaft*, 1967, **20**, 275–279).—A standard curve is obtained by the reduction of a 0·1% indigo-carmine (5,5′-indigo sulphonic acid) solution with nascent H in presence of 0·2 ml/l of colloidal Pd solution. The colour intensity, which increases linearly with increasing concn. of absorbed O_2, is measured at 680 mμ. A reduced test solution is poured into the containers to be tested (previously filled with CO_2) and closed with a rubber stopper. The blue colour, which depends on the O_2 permeability, is measured at intervals against a water blank. Another method in which a solution of 300 mg ascorbic acid, 85 mg Cu^{2+} and 7·4 mg Fe^{2+} is dissolved in 1 l of water is also described. Results show that PVC bottles have a smaller O_2 permeability than polyethylene and polystyrene bottles. The results are relevant to the packaging of fruit juice, wine, etc. in plastic containers. I. DICKINSON.

Glass closures. Anon. (*Fd Technol. Aust.*, 1967, **19**, 227).—The use of metal screw, lug, vacuum, thermosetting and thermoplastic, crown, and twist metal screw caps for glass containers is reviewed. G. W. FLYNN.

Current problems of hygenic and sanitary testing of plastics used in food packaging. A. Sporn and A. Hobincu (*Materiale plastice România*, 1966, **3**, 327–329).—Roumanian and E. European standards and practice are reviewed. (18 references.) C. A. FINCH.

Science in food packaging. J. F. Kefford (*Fd Technol. Aust.*, 1967, **19**, 204–213, 241).—Problems in food packaging encountered in Australia are surveyed. Abnormal detinning due to polyphosphates in canned meats, pitting corrosion in canned pears and apples, sulphur staining in low-grade mutton, and the measurement of the susceptibility of canned food to corrosion were investigated. Off-flavours in bottled citrus fruit drinks and bottled beer were investigated by gas chromatography. Rigid plastic and flexible plastic films must have good mechanical properties, high temp. stability and freedom from taint. Flexible packaging films of different permeabilities are required for various foods. Data on permeabilities of packaging films and on the way they are influenced by R.H. and temp. and by freezing temp. are filed by C.S.I.R.O. Bruising and mould in exported fruit can be prevented by suitable

packaging. Water damage to exported, packaged food due to condensation can be prevented by sealing off the hold completely at loading time or by warming cans to a temp. above the expected dew points. Packaging of prunes and peanuts was examined.
G. W. FLINN.

Sausage skins. Wolff & Co. K.-G. a. A.- (B.P. 1,060,403, 22.5.64. Ger., 22.5.63).—The improved sausage casings have an outer layer in form of a tube of shrinkable material [long fibre paper coated or impregnated with cellulose hydrate (**I**) or a cellulose ether, or consisting of **I** alone] and a seamless inner layer of a material (**II**) of substantially lower permeability to gas and water carried by (and bonded) to the layer of **I**. **II** is preferably an impermeable material such as a vinylidene chloride polymer, polyolefine, or nitrocellulose. The casings are made by applying a coating of **II** to a tube of **I**, and turning the coated tube inside out.
H. L. WHITEHEAD.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Interaction of alkaline earth metal ions with polyphosphates and citrate in presence and absence of casein. I. Vujičić, S. C. Batra and J. M. Deman (*J. agric. Fd Chem.*, 1967, **15**, 403–407).—Measurements in 0·002 M solutions with a Beckman specific ion electrode showed the cations Ca, Mg, Ba, and Sr to be capable of forming complexes with pyro-, tripoly-, tetra- and hexametaphosphate and citrate, which results in the displacement of Na and H ions. Casein behaves as a multivalent cation; this results in almost complete displacement of Na from polyphosphates and citrate. (13 references.) P. S. ARUP.

Protein quality of feeding-stuffs. IV. Progress report on collaborative studies on the microbiological assay of available amino-acids. A. W. Boyne S. A. Price, G. D. Rosen and J. A. Stott (*Br. J. Nutr.*, 1967, **21**, 181–206).—Samples of whale, meat, fish, groundnut and soya-bean meals and skim milk powders were assayed microbiologically using *Tetrahymena pyriformis* (**I**) and *Streptococcus zymogenes* (**II**) for measurement of available methionine (*M*) and lysine (*L*). Detailed studies on the assay of *M* with **I** were conducted to ascertain the effect of dry- or wet-grinding of the test meals and crude and purified papain was used to predigest the ground meals. Optical density and titratable acidity were the criteria of bacterial growth response. In the preliminary studies wide differences in *M* and *L* were found but the assay of available *M* by **II** gave promising results. In this work, dry grinding and predigestion with papain gave the optimum results but no general recommendation was possible concerning the use of optical density measurements or titratable acidity. (43 references.) C.V.

Unclassified

Air-oven methods for moisture determination. J. C. Halverson (*Cereal Sci. Today*, 1967, **12**, 93–94, 142).—Comments are requested on changes in these methods proposed by the Proximate Analysis Committee of the American Association of Cereal Chemists. The proposals include a one-stage drying process applicable to samples containing $< 16\%$ of moisture and samples of soya-beans or rough rice containing $< 10\%$ or 13% of moisture, respectively, and a two-stage process for samples containing moisture above these limits. Procedures for corn, beans, flax and bread are given. (11 references.) P. S. ARUP.

Determination of potassium in food using the Technicon Autoanalyser Flame Photometer and Solid Prep. Sampler. R. V. Smith, L. L. Ciaccio and R. L. Lipichus (*J. agric. Fd Chem.*, 1967, **15**, 408–411).—The construction and operation of the apparatus are described. With the use of a modified homogeniser blade K is quant. extracted from solid samples by 0·5 N-NH_4 acetate within 1 min., 20 sec. The results for K in solid vegetable products were, on the average, $1{\cdot}2\%$ greater than those obtained by ashing, varying between $+4{\cdot}0$ to $-1{\cdot}6\%$. The apparatus was capable of dealing with 20 solid samples per h. P. S. ARUP.

Rapid method for quantitative estimation of microbial lipases. R. C. Lawrence, T. F. Fryer and B. Reiter (*Nature, Lond.*, 1967, **213**, 1264–1265).—The assay, based on thin-layer diffusion, consists in measuring the dia. (x) of zones of clearing (due to hydrolysis) when the lipase is grown on $0{\cdot}1\%$ tributyrin-agar at pH 8 and 30° for ~48 h. An emulsion of any other synthetic triglyceride can be used. Hydrolysis rate is proportional to incubation period, and linear graphs of log %-concn. of diluted lipase *vs.* x after e.g. 19 h at 30°, are parallel and permit determination of min. lipase concn. giving discernible zones on a specific substrate. The sensitivity of this assay (limit of dilution 1 in 17500) is ~90 times that of the butterfat-assay; the method is suitable for screening microorganisms for lipolytic activity, for determining activities of lipases from biological samples, and for following the purification of lipases by column procedures. Lipolytic activity against butterfat is detected by adding the lipase to agar gel (pH 8) and placing on it a lens tissue painted with melted butterfat saturated with Victoria blue. Any hydrolysis is revealed by a blue zone against the red background of unchanged dye. W. J. BAKER.

Concept of carbohydrates and 'standardisability' of the content of digestible materials, in section 4a of the food regulations. Thümmel and Schäuble (*Dt. LebensmittRdsch.*, 1967, **63**, 117–118).—A discussion of the relevant section of the German food regulations.
J. B. WOOF.

***Streptomyces griseus* (Krainsky) Waksman and Henrici. A taxonomic study of some strains.** A. J. Lyons, jun. and T. G. Pridham (*Tech. Bull. U.S. Dep. Agric. Res. Serv.* 1966, 1360, 31 pp).—Study was made of 53 streptomycete strains exhibiting spore chains that were straight to flexuous, sporulating aerial mycelium coloured in tints and shades of yellowish grey, inability to darken peptone–iron agar or to form brown or black diffusible pigments, and ability to utilise D-xylose but not L-arabinose or L-rhamnose in a chemically defined agar medium. Additional characteristics were determined and results suggest that these strains comprise several subspecies of *Streptomyces griseus* (Krainsky) Waksman and Henrici. E. G. BRICKELL.

Multiplication of yeast on prehydrolysates of olive wood. V. Continuous fermentation in two phases with single flow. I. Schnabel, L. Deheza and J. M. Garrido (*Revta Ciencia apl.*, 1967, **21**, 24–28).—The multiplication of *Hansenula anomala* 925 using two fermentation vessels in series has been studied. Performance at the first stage (assimilation of hexoses) was satisfactory, with a yield of dry material formed of 50% on the reducing matter in the medium, but much less so at the second stage (assimilation of pentoses).
L. A. O'NEILL.

Improvement of yields in anaerobic cultures. K. Sargeant (*Chemy Ind.*, 1968, 85–88).—Yields of anaerobic bacteria can be much increased by bacterial growth in stirred culture vessels in which variables (pH, nutrient concn., energy sources, etc.) are suitably controlled. Production of *Thiobacillus denitrificans* and *Clostridium pasteurianum* was almost trebled when the pH was kept const. by automatic addition of $NaHCO_3$ or NaOH. Other methods include (*i*) use of a dialysis membrane to ensure removal of spent medium and supply of further nutrient, (*ii*) use of dialysis fermentor, in which the fermentor, medium reservoir, and dialysis are separated, (*iii*) use of biphasic systems, e.g., dextran and polyethylene glycol, to grow *Clostridium tetani* in protein-free medium, (*iv*) continuous culture in preference to batch culture. All methods of refinement must be based on measurement and control of culture variables. (24 references.) W. J. BAKER.

3.—SANITATION, WATER, etc.

Insecticidal action of *dl*-allethrolone *trans*-chrysanthemate on *Musca domestica*. L. J. Lhoste, J. Lambert and F. Rauch (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 686–691).—*dl*-Allethrolone *trans*-chrysanthemate (**I**) produced similar knock-down and killing effects as did the natural pyrethrins. *dl cis*, *trans*-allethrolone (allethrine) was far less effective than **I**. **I** (but not allethrine) was synergised by piperonyl butoxide. P. S. ARUP.

Area treatment to combat mosquitoes. J. E. Simpson and R. H. Wright (*Nature, Lond.*, 1967, **214**, 113–114).—An area treatment with very low concn. of a chemical is proposed to supplement the usual protective measures, especially where resistance to insecticides has developed. The mosquitoes are thus rendered less capable of responding to emanations of CO_2 from nearby hosts. Continuous exposure, at 27° and 50% R.H., to outdoor-air containing vapour of 2-ethylhexane-1,3-diol (Rutgers 612) (0·5–5 ppm) inhibited the normal response of female *Aedes aegypti* and *Anopheles quadrimaculatus* to a rise in concn. of CO_2 to ~$0{\cdot}1\%$. Practical application is discussed briefly. W. J. BAKER.

Symposium on chemical disinfection. (*J. appl. Bact.*, 1967, **30**, 1–158).—**Disinfection—How, why, when, where?** G. Sykes (1–5). **Types and characteristics of disinfectants.** H. S. Bean (32 references) (6–16). **Mode of action of antibacterial agents.** W. B. Hugo (143 references) (17–50). **Legal implications of using disinfectants in relation to food.** J. M. Ross (51–55). **Hard surface**

disinfection and its evaluation. A. H. Walters (17 references) (56–65). **Evaluation of skin germicides.** B. M. Gibbs and L. W. Stuttard (42 references) (66–77). **Biocidal activities of glutaraldehyde and related compounds.** S. D. Rubbo, J. F. Gardner and R. L. Webb (78–87). **Use of gaseous antimicrobial agents with special reference to ethylene oxide.** J. C. Kelsey (38 references) (92–100). **Disinfectants in the soft drinks industry.** F. G. R. Rice (101–105). **Sanitation practices in egg handling and breaking plants and the application of several disinfectants for sanitising eggs.** J. C. Ayres, A. A. Kraft, G. S. Torrey and S. S. Rizk (32 references) (106–116). **Disinfectants in the dairy industry.** L. F. L. Clegg (114 references) (117–140). **Use of disinfectants in the food industry.** N. Goldenberg and C. J. Relf (141–147). ***Salmonella* infection in a meat products factory: Rôle of the medical officer of health.** N. S. Galbraith (27 references) (148–158). C.V.

Field methods for determining organomercurial vapours in air. A. A. Christie, A. J. Dunsdon and B. S. Marshall (*Analyst, Lond.*, 1967, **92**, 185–191).—Certain organo-Hg compounds in concn. ~10 μg Hg/m^3 are determined by collection on either (*i*) a glass fibre pad impregnated with ~2% CdS or (*ii*) a fluidised bed of active C (or iodised C for Hg-vapour alone). The mercurial is then decomposed by heating and the evolved Hg determined colorimetrically or by SeS test-papers. Both methods of collection are applicable to EtHg chloride, EtHg phosphate, $HgPh_2$ and MeHg dicyandiamide, but (*i*) should be used for mercurial dusts and (*ii*) for diethylmercury and Hg-vapour. Neither method is affected by R.H. unless this is very high. The apparatus for collection of the vapours and for recovery of Hg is shown in diagrams. A result can be obtained in ~30 min. but the results are approx. (10 references.) R.J.M.

Sterilisation. American Sterilizer Co. (B.P. 1,060,529, 30.11.65. U.S., 30.11.64).—Sterilisation is accomplished by introducing a load of material into a closed chamber, exhausting the air from and introducing steam into the chamber until the pressure is 50–350 mm Hg at 35–80° (10–30 min.) and then simultaneously introducing further steam and a sterilising agent (e.g. ethylene- and/or propylene-oxide) for 30 min. or longer. The sterile gas is then exhausted from the chamber and sterile air is introduced. The method destroys spores enclosed in e.g. salt crystals or org. material.
S. D. HUGGINS.

4.—APPARATUS AND UNCLASSIFIED

Nitrogen analysis according to Analytica- EBC. P. Diedering (*Mschr. Brau.*, 1967, **20**, 268–269).—The recommended digestion of 0·1 g acetanilide in the presence of 1 g sucrose for use as a control in N analysis has been found unsatisfactory. Only 95–96% recovery of N is obtained under standard conditions because of formation of intermediate products which are difficult to digest. It is recommended that sucrose be eliminated and that the distillation and titration steps should be checked by means of $(NH_4)_2SO_4$. J. B. WOOF.

Detection of nitrosamines in tobacco smoke and foods. E. Kröller (*Dt. LebensmittRdsch.*, 1967, **63**, 303–305.)—The carcinogenic compounds *N*-nitroso-di-n-butylnitrosamine and *N*-nitrosopiperidine were identified in a condensate of tobacco smoke. Nitroso compounds could be detected in CH_2Cl_2 extracts of food by similar treatments, thus *N*-nitrosodiethylamine was found in bleached wheat flour and *N*-nitrosodi-n-propylamine in cheese. These substances could be detected by gas chromatography of a hexane concentrate on a column of 5% SE 32 on Celite with helium carrier, an operating temp. of 200° and a flame ionisation detector, or by TLC on silica gel G from the same solvent. R_F values of a number of reference compounds are given. (27 references.) J. B. WOOF.

Measurement of bacterial dry weight using an infra-red oven. J. W. S. Ford (*Chemy Ind.*, 1967, 1556–1557).—An i.r. oven permits a centrifuged and re-suspended bacterial deposit, e.g., 0·05 g in 10 ml, to be dried to const. wt. in ~60 min. instead of the normal 18–30 h at ~110°. A const. temp. of 160 ± 3° is maintained continuously over long periods (2–3 years) and the results are only very slightly higher than those for normal drying. Construction of the oven is explained and the entire operational procedure is described. W. J. BAKER.

Simplified method for determining radio-active calcium-45 in biological material by gel scintillation counting. R. A. Turpin and J. E. Bethune (*Analyt. Chem.*, 1967, **39**, 362–364).—The method, applicable to protein- or non-protein-containing samples (e.g., urine, plasma, etc.), consists in counting a very fine suspension in a SiO_2-gel (Cab-o-Sil) scintillator. The suspension is obtained by addition of acetone dimethyl acetal and conc. HCl together with carrier Ca and sufficient Cab-o-Sil to give a firm gel. A scintillation mixture of 1% 2,5-diphenyloxazole in toluene–EtOH (1 : 1) gives max. counting efficiency. There is relatively little quenching, all operations are done in one counting cell, and method is applicable to material of low sp. activities. Even when ratios of P and Mg to Ca are very high there is no effect on Ca counts.
W. J. BAKER.

Separation of muscle lipids into classes by non-chromatographic techniques. I. Hornstein, P. F. Crowe and J. B. Ruck (*Analyt. Chem.*, 1967, **39**, 352–354).—The separation is made on a total-lipid extract in $CHCl_3$. Phospholipids (**I**) are removed by adsorption, from $CHCl_3$-hexane-ether (2 : 1 : 1), on activated silicic acid and free fatty acids in the supernatant are then adsorbed on Dowex 1-X8 resin. The percolate is saponified, the mixture is made just acid with HCl, and the glyceride fatty acids plus cholesterol (**II**) are extracted into hexane. These fatty acids are also adsorbed on Dowex 1-X8 resin, on which they are directly converted into Me esters (recovery ≮95%). The total **II** remains in solution, the resin being washed with $CHCl_3$–MeOH–hexane (1 : 1 : 6) before the esterification. Adsorbed **I** are transmethylated to form Me esters of phospholipid fatty acids, which are submitted to gas chromatography. Most of the recoveries are approx. quant. W. J. BAKER.

Determination of warfarin in animal relicta. F. B. Fishwick and A. Taylor (*Analyst, Lond.*, 1967, **92**, 192–195).—The procedure consists in extraction of relicta from HCl solution by ether, clean-up of the extract by column chromatography on activated SiO_2-gel, purification of the eluate by extraction with 1% aq. $Na_4P_2O_7$ and partition into $CHCl_3$, separation of warfarin by TLC (the band is located by u.v. light), elution and subsequent spectrophotometric determination at 305 nm. Recovery of 18–128 μg ranges from 78–97%. (11 references.) W. J. BAKER.

A portable step-logarithmic sprayer for field research work. S. S. Szabo and L. E. Limpel (*Contr. Boyce Thompson Inst. Pl. Res.*, 1967, **23**, 327–329).—This consists, basically, of a one gal. stainless steel spray tank, serving as reservoir for both diluent and air, plus an attached concentrate container. It is highly versatile and can be used as a conventional logarithmic sprayer. A serial dilution of a given chemical formulation can be applied without changing concentrate containers. S. A. BROOKS.

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ABSTRACTS

APRIL 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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