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STORAGE OF HAY. IV.—Effect of Storage in Nitrogen on the Soluble Sugar and Dry Matter Contents of Ryegrass

By JEAN F. MELVIN

Ground ryegrass hay was stored at 25° and about 76% R.H. in nitrogen and also in air to simulate normal storage conditions, and the soluble sugar and dry matter contents were determined at intervals for about one year.

Fructosans and oligosaccharides were not affected by these storage conditions.

Sucrose was almost completely hydrolysed to glucose and fructose in 21 weeks. This reaction was independent of the storage conditions and could have been due to enzymic activity.

The hexoses did not change appreciably in nitrogen, but in air both glucose and fructose began to decrease after 21 weeks and only a trace remained after 45 weeks. These losses appeared to be mainly due to mould. Losses of dry matter followed the same pattern as those of hexose but were consistently less.

Introduction

In previous papers concerned with the storage of hay,[1, 2] the effects of temperature and moisture on the loss of dry matter and the changes in the soluble sugar contents of ryegrass were described. Losses in dry matter increased with increase in both temperature and moisture. Within the ranges of −18° to 7° temperature and 7 to 12% moisture, the losses were small, but when the ranges were extended to 36° and 18%, respectively, to include normal storage conditions, the losses increased appreciably and reached a maximum of about 8% under the extreme conditions after 9 months. Losses of sugar accounted for losses of dry matter and, in fact, were usually higher, indicating that some of the decomposition products were retained in the hay. Sucrose was hydrolysed to glucose and fructose, and the hexoses gradually disappeared. At the high moisture content, fructosans also decreased.

The changes outlined above would be expected to affect the nutritive properties as the sugars are a useful source of energy. Restricting losses by control of temperature and/or moisture content may well present difficulties on a large scale, but if the mechanism of the chemical changes involved were known, alternative methods of preservation might be devised. To this end the rôle of oxygen as a contributing factor has been investigated by comparing the effect of storage in nitrogen on the contents of soluble sugar and dry matter with that in air. The possibility of enzymic activity being responsible for hydrolysis of sucrose during the early part of storage has also been examined. Evidence of invertase in hay has recently been reported by Thomas.[3]

Experimental

About 1 kg. of freshly cut short rotation ryegrass (*Lolium* sp. N.Z. H1 strain) with heads present on 15% of the stems was air-dried in the open, but brought indoors at night. When its moisture content was between 20 and 30%, the drying was completed in a ventilated dryer at 45° for 16 h. The material was then cut into short lengths and ground in a Wiley mill using a $\frac{1}{2}$ mm. screen. Grinding facilitated sampling during the storage period and was considered legitimate for comparative purposes. The ground material, however, may have been more quickly affected by the storage conditions than if intact hay was stored.

The ground hay, 60–70 g. in glass dishes (6 in. dia.) and 3–4 g. in weighing bottles (for determining losses of dry matter), was placed in each of two desiccators over saturated sodium chloride solution, one of which was evacuated and filled with oxygen-free nitrogen. In a room at 21°, the samples were kept at about 25° by means of a lamp (100 W) above the desiccators. The desiccators were usually opened three times per week in order to stir the samples. (The R.H. of saturated sodium chloride solution is 75·7% at 25°—see [4].)

Tests of invertase activity were made on a mixture of fresh hay (1·0 g.), sucrose (50 mg.) and water (10 ml.). The hay used was the same variety and of similar maturity but of different composition to that in the storage experiments.

The soluble sugars were determined by the methods described previously,[1] and moisture contents by drying at 80° for 16 h.

Results and discussion

The results are shown in Tables I and II and Fig. 1.

Table I

Moisture content of hay stored in nitrogen and air

Storage period, weeks	Moisture, % of test weight		Storage period, weeks	Moisture, % of test weight	
	Nitrogen	Air		Nitrogen	Air
0	7·3	7·3	21	13·9	14·9
4	14·0	14·0	29	13·9	14·2
8	13·7	14·7	37	12·7	13·0
13	13·6	14·8	45	13·1	12·1
			53	12·2	

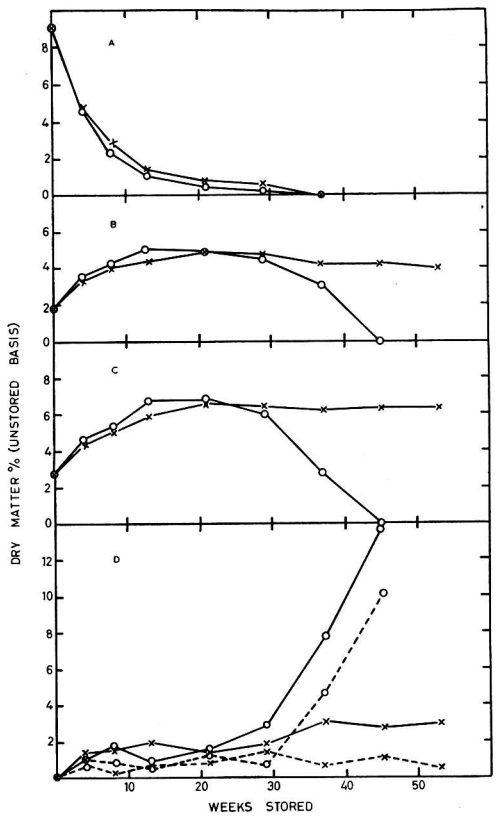

FIG. 1.—*Changes in soluble sugars and dry matter in stored hay*

× stored in nitrogen ○ stored in air
——— sugar – – – dry matter
A sucrose B glucose C fructose D losses of total sugar and dry matter

Table II

*Effect of incubation at 38° on the sugar contents of mixtures of hay, sucrose and water**

(results in mg. of sugar)

Pre-treatment	Incubation period, h.	Sucrose			Glucose	Fructose	Total sugars
		Present in hay	Added	Total			
None	0	54	50	104	15	15	134
None	2			42	48	48	138
None	5			3	70	69	142
100°†	2			100	14	13	127
100°†	5			103	12	13	128

* Hay, 892 mg. dry wt., sucrose 50 mg., water, 10 ml. † 15 min. in boiling water-bath

Fructosans (4·2%) and oligosaccharides (1·2%) were not affected by the length of their storage or by the presence of oxygen. In both nitrogen and air, sucrose was almost completely converted to glucose and fructose after 21 weeks.

The possibility of invertase activity, which could account for this, was tested by incubating sucrose and water mixtures with hay at 38°. The results showed that 60% of the total sucrose was hydrolysed in 2 h. and 97% in 5 h., but no hydrolysis occurred in mixtures heated at 100° for 15 min. before incubation (Table II). As enzymes are usually inactivated by heating at 100°, this provided almost certain proof of enzymic activity in the hay.

From the sample stored in nitrogen, losses of hexose were small even after 1 year, whereas, in air, both glucose and fructose began to disappear after about 21 weeks, slowly at first and then more rapidly, only a trace remaining after 45 weeks. Small balls, in which mould was detected, formed in the latter sample providing a possible explanation of these losses. Mould deterioration of feeding-stuffs, including dried grass, has been studied by Snow *et al.*[5a] who showed that the main factors controlling growth were relative humidity, storage period, balance and type of nutrients provided, temperature, and type of mould species present. Safe maximum humidities of 72% and 65% for short (3 months) and long (2–3 years) term storage periods respectively at temperatures in the range 15–21° were recommended.[5b]

The material which had been stored in nitrogen was subsequently stored, under the same conditions, in air. In a period of 40 weeks no mould developed and the hexose content was unchanged. Failure to develop mould in this case may have been due to the change in balance of nutrients caused by hydrolysis of the sucrose. Certain moulds, for example, those of the *Aspergillus glaucus* group which are common in drying plant products, often grow more readily on media which contain a high content of sucrose.[6]

Losses of dry matter were not appreciable in nitrogen; during the subsequent storage in air, they were small for the first 29 weeks, after which a sharp increase occurred, following the same pattern as the losses in hexose. Losses of dry matter were consistently less than the total sugar losses, confirming that some degradation products were retained in the samples.

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ISOLATION OF ENDOSPERM PROTEIN AND ALEURONE CELL CONTENTS FROM WHEAT, AND DETERMINATION OF THEIR AMINO-ACID COMPOSITION

By D. J. STEVENS, E. E. McDERMOTT and J. PACE

The techniques of air classification, and differential centrifugation in non-aqueous media, have been combined to isolate from flour (*a*) endosperm protein and (*b*) aleurone cell contents. The amino-acid composition of the isolated protein has been determined and compared with that of the total protein complex in the parent flour. The results show that in the isolated protein fraction the proportion of the glutenin/gliadin group of proteins is higher than in the parent flour. This was observed in the material obtained from both a strong and a weak flour. It is suggested that this partial segregation occurs in the air classification process. The protein fractions isolated from the weak and strong flours were closely similar in their overall amino-acid composition. The amino-acid composition of the protein contained in the isolated aleurone cell contents has also been determined. The composition is quite different from that of the main endosperm proteins and resembles, in this respect, some of the soluble protein fractions which have been previously isolated from flour. A notable feature of the composition is a relatively high content of arginine.

Introduction

Hess[1] has shown that the interstitial protein of wheat endosperm can be largely separated, in the native dry state, from the starch by differential centrifugation of finely ground flour in mixtures of benzene and carbon tetrachloride of density intermediate between that of starch (density 1·5) and that of the protein (density 1·3). This technique provides the possibility of isolating endosperm protein in a state more closely approximating to the state in which it occurs in the grain than is possible when conventional extraction procedures using aqueous solutions are used. Furthermore there is the additional advantage that the amino-acid composition may then be determined on preparations which are free from starch, thus eliminating the uncertainty about the degree of destruction of certain amino-acids which arises when carbohydrate is present during the hydrolytic procedure.

In the present work the method has been applied not to flour but to the fractions relatively rich in endosperm protein prepared from flour by air classification.[2] These fractions contain none of the larger starch granules and have a protein content about twice that of the flours from which they are derived. By using this combination of these two ' dry state ' fractionation techniques, material has been prepared from flour which, with a nitrogen content of 16·4%, is predominately protein and free from starch. The amino-acid composition of the protein isolated in this way has been determined and compared with that of the protein in the flour from which it was derived. The procedure has been carried out with two flours which differ markedly in their baking characteristics.

While applying these procedures to the concentration of endosperm protein it was also observed that a fraction could be obtained which was substantially the uncontaminated contents of aleurone cells. This material has been isolated and the amino-acid composition of the protein contained in it has been determined.

Materials and methods

Wheat

Samples of Manitoba wheat and of the variety Hybrid 46 grown in the U.K. were used. They were selected as examples of wheats which yield, respectively, strong and weak bread-making flours.

Flour

The wheats were milled on a laboratory Buhler mill. The extraction rate of flour from the Manitoba wheat was about 68%, from the Hybrid 46 about 62%. The nitrogen contents of the flours (dry weight basis) were: Manitoba, 2·65%, Hybrid 46, 1·55%.

Isolation of interstitial endosperm protein

The flour was further disintegrated by grinding in a special mill (Kek pinned-disc), which shatters much of the endosperm cell structure into a debris of free particles of protein and starch. This material was then fractionated, on the basis of particle size, by air classification in a Mikroplex air classifier.[2] Only the fractions containing particles of size less than 17 μ were retained for further purification.

The fine fractions (particles <17 μ) from the Manitoba and Hybrid 46 flours contained N contents of 4·55% and 3·65% (dry wt. basis) respectively. These fractions were fractionated further on the basis of the difference in density between the endosperm protein and the starch. Batches of 150 g. were dispersed, using a Waring Blendor, in 500 ml. of a mixture of benzene and carbon tetrachloride of density 1·35. The suspension was then centrifuged at 500 *g* for 30 min. The upper coherent layer of material rich in protein, P1, was taken off and the liquid decanted from the starchy sediment, S1. The latter was dispersed in more of the liquid medium and centrifuged to give a further yield of protein-rich material, P2, and starchy sediment, S2. P1 and P2 were bulked, dispersed in medium of density 1·34, centrifuged as before to give a final product, P3. The nitrogen content of P3 from the Manitoba flour was 16·5% (dry wt. basis) and from the Hybrid 46 flour 16·4%.

Isolation of aleurone cell contents

While attempting to isolate starch from a soft wheat flour by the differential centrifugation method, it was observed that a layer of yellowish material separated in the lower part of the centrifuge tube. Microscopical examination of this layer showed that approximately half of it was starch ; the remainder appeared to be composed of the contents of aleurone cells, free from the cell walls. A ' fourth break ' flour was next used as starting material, since this flour stream is comparatively rich in aleurone layer, and was given a preliminary grind in a pestle and mortar so as to break down the layer structure and release the cell contents from the cell walls. A medium of slightly higher density was used so as to improve the separation, and a product was obtained which, though still containing some starch, had a nicotinic acid content of 800 μg./g. (air-dry basis). This may be compared with the figure of 613 μg./g., obtained by Heathcote *et al.*[3] for the intact aleurone layer dissected from soft wheat, and confirms the aleurone origin of this comparatively dense material. It has also recently been found by Jones, Fraser & Moran,[4] that the fine fraction (<17 μ) prepared by air classification of hard wheat flour after treatment in a pinned-disc grinder, has a much higher nicotinic acid content than that of the original flour. Presumably the special grinding releases the content of the aleurone cells from the cell walls and reduces them to fragments smaller than 17 μ (the size of the intact aleurone cell varies from 25–75 μ).

Following these preliminary observations, the fine fraction from the air classification of the Manitoba flour was used to isolate the material from the aleurone cells. Batches of 125 g. of the starchy sediment S2, from the endosperm protein separation, were dispersed in 500 ml. of a benzene/carbon tetrachloride mixture of density 1·5. After being centrifuged at 500 *g* for 30 min., the upper layer containing most of the starch and any residual endosperm protein was poured off and the sediment removed and allowed to dry. Microscopical examination showed that the sediment contained a small amount of starch. The material was dispersed in 100 ml. of medium, density 1·51, and the dispersion was centrifuged at 1000 *g* for 15 min. This procedure gave a floating layer of starch which was poured off with the supernatant liquid. The sediment was allowed to dry in the centrifuge tube and was then removed, care being taken to reject a small amount of dark material at the bottom of the tube. The yield of air-dried product, which was free from starch, was about 1% of the high protein fraction from the air classifier. The material had (on dry weight basis) nitrogen 5·45%, nicotinic acid 1450 μg./g., vitamin B_6 57 μg./g. and ash 36%. These figures confirm the identification, made initially by microscopy, that the isolated fraction is the material contained in the aleurone cells. Conversion to a dry-weight basis of published results, by Hinton and co-workers, for the whole aleurone layer (i.e., cell walls + contents) obtained by hand dissection from Manitoba wheat, gives the following figures: nicotonic acid 840 μg./g.,[5] vitamin B_6 41 μg./g.,[6] ash 20%,[7] and fat about 20%.[8]

The fraction isolated in this work is material without the cell walls and the fat is dissolved out in the solvent used in the sedimentation procedure.

Amino-acids

These were determined on the hydrolysates (hydrolysis for 24 h. with 6N-hydrochloric acid), by ion-exchange chromatography by the methods of Moore & Stein,[9] with operating conditions as described by McDermott & Pace.[10, 11]

Results

The contents of amino-acids found in the hydrolysates of the parent flours and of the fractions derived from them are given in Table I. This Table shows that both samples of the

Table I

Amino-acid N, g./100 g. of total N

Amino-acid	Manitoba		Hybrid 46		Aleurone cell contents (ex Manitoba)
	Parent flour	Isolated endosperm protein	Parent flour	Isolated endosperm protein	
Aspartic acid	2·4	2·0	2·7	2·1	4·8
Threonine	1·8	1·8	2·0	2·1	2·1
Serine	3·9	4·0	3·8	4·3	3·4
Glutamic acid	20·0	21·3	20·3	23·1	8·9
Proline	9·05	10·2	9·0	10·6	2·7
Alanine	2·8	3·1	3·1	3·0	4·6
Valine	3·1	2·9	3·3	3·2	3·7
Methionine	1·0	1·0	1·1	1·05	0·8
Isoleucine	2·2	2·5	2·6	2·5	1·9
Leucine	4·6	4·6	5·0	4·8	3·65
Tyrosine	1·4	1·7	1·3	1·6	1·3
Phenylalanine	2·7	2·9	2·9	3·0	1·95
Lysine	2·2	1·8	2·45	1·45	5·0
Histidine	3·4	3·6	3·5	3·2	6·1
Arginine	6·8	6·4	7·2	6·3	21·1
Cystine					1·2

isolated endosperm protein follow the same trend of variation in their amino-acid composition when compared with the composition of the protein in the parent flours. In the isolated protein the content of glutamic acid and proline is higher and that of aspartic acid, lysine and arginine is lower than in the protein of the parent flour. These variations imply that, in the isolated protein, there has been some segregation of the ' gliadin/glutenin ' type of proteins (with their high glutamic acid and proline content) from the mixture of these and the group of soluble proteins (with lower glutamic acid and higher lysine content) which makes up the total protein in the parent flour. The ' soluble ' protein has presumably tended to associate preferentially with the high starch fraction in the fractionation procedure, with the overall result that the isolated endosperm protein has an amino-acid composition intermediate between that of gluten and that of the *total* flour protein complex. It thus appears that there has been, in the isolation procedure, some slight enrichment of the characteristic main gluten proteins with a corresponding diminution in the ' soluble ' proteins. It seems likely that this partial segregation occurs in the air-classification step of the fractionation procedure. This view is supported by the recent work of Jones & Dimler[12] on the fractionation of the protein in air-classified flours. They have found that the protein of the high-protein fractions contains a lower proportion of water-soluble protein than does the protein of the low-protein fractions.

If the protein isolated from the Manitoba flour is compared with that from the Hybrid 46, it will be seen from Table I that, while there are some small variations, the overall composition of the two is closely similar. Thus the amino-acid composition of this fraction of the total protein provides no obvious indication of why the protein systems in the two flours exhibit such very different rheological behaviour. In this respect the results resemble those found by Pence *et al.*[13] on the composition of glutens from 17 different flours with a wide range of

protein content and baking characteristics. They found all the glutens were essentially uniform in their amino-acid composition. More illuminating evidence may perhaps be found in the variations in the composition of the total protein complex in different flours, which may be ascribed to variation in the proportion and type of ' soluble ' protein associated with the main ' insoluble ' gluten proteins (cf. McDermott & Pace[11]). This possibility has been discussed in greater detail elsewhere.[14]

The overall amino-acid composition of the protein in the aleurone cells, as shown in Table I, is quite different from that of the main gluten proteins. It is much closer to the composition of some of the soluble fractions which have been extracted from flour (Coates & Simmonds[15]). A notable feature is the relatively high arginine content. This is also a distinctive feature of some of the soluble components in the protein of flour, as noted by Pence & Elder[16] and shown in the results given by Coates & Simmonds.[15] Some of the soluble protein in flour may be derived from the aleurone cells. The amount would clearly be very small, but would be of interest if some of it were enzyme protein.

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THE COMPONENT FATTY ACIDS OF THE GLYCERIDE AND PHOSPHOLIPID FRACTIONS OF THE BAOBAB SEED *(ADANSONIA DIGITATA)*

By S. H. W. CMELIK*

Glycerides from the decorticated seed of the baobab tree (*Adansonia digitata*) have been extracted with acetone and the fatty acid composition investigated by reversed-phase paper chromatography. Pure lecithin and cephalin have been isolated from the alcohol–ether (3 : 1) extract and their fatty acids studied. All fractions contain palmitic acid as the predominant saturated and oleic acid as the main unsaturated acid. Linoleic acid is present in all fractions to the extent of 21·7 to 30·6, but no other polyunsaturated acids have been found.

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Introduction

The original idea of this paper was to present information on the composition of the baobab seed oil, which has not been described previously for *Adansonia digitata*. In the course of the work it has been realised that an investigation of various phospholipid fractions might yield interesting results, as information on seed phosphatides is generally rather scarce and in the case of tree seeds practically non-existent.

Reports referring to the content of lecithin, cephalin and similar phosphatides in seed phospholipids or in total lipids are rather conflicting. In some cases,[1–3] lecithin is considered as the predominant fraction, comprising up to 97% of the total phospholipids, while Rewald[4] found that four out of five species contained cephalin as a major fraction.

There are also inconsistencies in reports on the ratio of saturated and unsaturated acids and in the fatty acids composition of various fractions.[5, 6] The reason for such discrepancies might be in the use of not sufficiently purified substances, characterised in some cases only as ' alcohol soluble ' or ' alcohol insoluble '.[7, 8]

In this paper it has been shown possible to isolate lecithin and phosphatidyl ethanolamine fractions of a fairly high purity. An attempt was made also to isolate phosphatidic acids from the alcohol-insoluble part, but the substance obtained did not correspond in composition to phosphatidic acids.

Experimental

Preparation of material

The content of dry baobab fruits was crushed in large mortars to free the seed from the adherent pulp. A remaining layer of pulp was removed by washing with water and subsequent drying in the sun. The very hard, kidney-shaped seed was ground in a Christy & Norris laboratory-type hammer mill and the seeds separated from the shells by screening through a system of sieves. From 8 kg. of seed were obtained 2 kg. of a greyish powder (25%).

Extraction and isolation of various fractions

To avoid contamination of the seed glycerides with phospholipids, the extraction of the glycerides was effected with acetone. The powdered material (total fat content 17%) was extracted exhaustively with acetone in 2-kg. portions. After the acetone had been distilled off under vacuum in a stream of CO_2, the remaining glycerides were washed with hot water to remove the gums and finally dried over sodium sulphate. This oil contained only negligible quantities of organic phosphorus.

After having expelled the remaining acetone from the extracted powder, the extraction was repeated with alcohol–ether (3 : 1). The solvent was removed by distillation in vacuum in a CO_2 current and the orange coloured residue dissolved in light petroleum and centrifuged. The solution was concentrated in vacuum and the phospholipids precipitated with 8 volumes of acetone. After having settled overnight in a refrigerator the phospholipids were centrifuged and dissolved again in light petroleum. The precipitation was repeated four times and the slightly yellowish product finally dried in a desiccator over phosphorus pentoxide. After the removal of the solvent, a further amount of phospholipids was precipitated with acetone from light petroleum. A total yield of 26 g. (0·13%) of phospholipids was obtained from 2 kg. of powdered seed.

The first fractionation of the phospholipids was carried out on the basis of different solubilities in alcohol. The purified product (13 g.) was treated with 150 ml. of 96% ethanol, cooled to 5° and centrifuged. The residue was treated again with 50 ml. of ethanol at 5°, centrifuged and the combined alcoholic solutions evaporated under vacuum. Yield : 4·9 g. (37%) of crude lecithin.

A further purification of lecithin was effected by precipitation with cadmium chloride according to Pangborne.[9] The final product contained nitrogen and phosphorus in the molar ratio 1·2 : 1. There was no free amino-nitrogen in the substance.

Acidic components were separated from the cephalin fraction by the precipitation technique with lead acetate in chloroform–methanol as described by Scholfield & Dutton.[10] The purified cephalin contained nitrogen and phosphorus in the theoretical molar ratio 1 : 1.

From the lead acetate precipitate a substance was isolated which did not correspond in composition to inositol phosphatidic acids. No attempt was made to identify the water-soluble phosphoric component of the fraction.

For the determination and isolation of fully saturated glycerides the permanganate oxidation method of Hilditch & Lea was adopted,[11] with a chromatographic purification in the last stage. A solution of the fully saturated glycerides in ether was passed through a column of aluminium oxide (B.D.H., for chromatography) to retain all the acidic products of the oxidation.[12, 13]

Analytical methods

The physical constants of the glycerides were determined in the usual way (see Table I). In phospholipid fractions, nitrogen was determined by a semi-micro Kjeldahl method and phosphorus by Elek's method[14] (see Table II). Unsaponifiable matter was determined according to the Official Method of the Society of Public Analysts.[15] The determination of squalene was carried out on the hydrocarbon fraction obtained by filtration of the unsaponifiable dissolved in light petroleum through a column of aluminium oxide according to Grossfeld & Timm.[16] The AOCS Official Methods[17] were used for the determination of the acid value, saponification value and ester value and the iodine value was determined by Yasuda's method.[18]

Table I

Physical and chemical properties of baobab seed oil

Specific gravity at 20°	0·922	Squalene	1·3 mg./100 g.
Refractive index at 20°	1·4680	Total fatty acids	93·7%
Acid value	4·6	Saturated fatty acids	42·7%
Saponification value	192	Unsaturated fatty acids	57·3%
Ester value	187·4	Fully saturated glycerides	1·5%
Iodine value	76		

Table II

Characteristics of various phospholipid fractions

	Lecithin	Cephalin	Acidic fraction
P	4·06	4·20	2·71
N	1·51	1·94	2·46
P : N ratio	1 : 1·2	1 : 1	2 : 1
Fatty acids, %	71·6	66·8	67·0
Saturated acids, % of total acids	34	43	41
Unsaturated acids, % of total acids	66	57	59

All lipid fractions were hydrolysed with 5% potassium hydroxide in methanol. The higher fatty acids were recovered from the acidified soap solutions by ether extraction and separated into solid and liquid acids by the lead acetate method.[19]

Higher fatty acids were determined by reversed-phase chromatography on Schleicher & Schüll No. 2043b filter paper impregnated with undecane fractions (b.p. 180–220°) saturated previously with 95% acetic acid.[20] The mobile phase was 95% acetic acid saturated with the undecane fraction. For qualitative purposes the fractions were revealed by immersion in 2% mercuric acetate solution and subsequent spraying with a 0·1% alcoholic solution of diphenylcarbazone[21] which is far more sensitive than the cupric acetate–potassium ferrocyanide technique. However, for quantitative studies this method was not found to be very satisfactory, because of the unevenly stained background. The cupric acetate–potassium ferrocyanide method was therefore preferred for quantitative work. All measurements were carried out on a Zeiss type II extinction recorder.

Unsaturated acids were identified and determined by a simultaneous chromatography of the original acids and acids hydrogenated directly on the filter paper with a paladium catalyst according to Kaufmann & Karabatur.[22] Conjugated and non-conjugated polyunsaturated acids were determined spectrophotometrically as described by Holman & Hayes.[23]

Choline was detected as its Reinecke salt in the alkaline hydrolysate of the lecithin.[24] For the detection of ethanolamine the water-soluble part of an acid hydrolysate was examined chromatographically on Whatman No. 3 paper in ethyl methyl ketone/propionic acid/water (6 : 2 : 2) mixture against pure ethanolamine hydrochloride.

To identify the glycerophosphoric acid, chromatograms were run on Whatman No. 54 paper in n-propanol/ammonia/water (6 : 3 : 1) and sprayed with the ammonium molybdate–perchloric acid reagent.[25] For the detection of inositol the test by Scherer was used.

Results and discussion

The baobab seed oil from *Adansonia grandidieri* was investigated by Thomas & Boiry[26] who found palmitic 37%, stearic 2%, oleic 42% and linoleic acid 10%. These figures are quite well in agreement with the figures obtained on the oil from *Adansonia digitata* (Table III), with the exception that the content of linoleic acid was considerably higher.

Table III

Component fatty acids of Adansonia digitata *seed glycerides and phosphatides* (% by wt. of fraction)

Acid	Glycerides	Lecithin	Cephalin	Acidic fraction
Myristic	1·4	—	1	—
Palmitic	34·0	30·3	37	34·8
Stearic	6·2	3·7	4·8	6·1
Oleic	36·7	40·6	33·3	28·5
Linoleic	21·7	25·4	23·9	30·6
Linolenic	—	Traces	—	—

As is seen from Table I the squalene content of the oil is only 1·3 mg. per 100 g. Values of 5–28 mg. have been reported by Fitelson[27] in various vegetable oils while a value of 156 mg. per 100 g. has been found recently in olive oil.[28] No other hydrocarbon seems to be present in the light petroleum eluate of the unsaponifiable matter from the chromatogram on alumina.

The amount of fully saturated glycerides is low. Since the quantity of saturated acids does not exceed 42·7% this conforms to the pattern of ' even ' distribution. The acids from the fully saturated glycerides consist of palmitic 73·6% and stearic acid 26·4% (mol. ratio 3 : 1). This means that at least two different glycerides must be present in this fraction.

From the phospholipids, 37% of crude lecithin and 63% of crude cephalin were isolated. The same proportion was found in phospholipids from groundnuts, linseed and sunflower,[4] while other authors[1–3] found the lecithin to be the major phospholipid in seed phosphatides. The alcohol-insoluble part seems to contain only phosphatidyl ethanolamine, as no serine was detected in the hydrolysate of the crude product. Precipitation with lead acetate[10] yielded only 10% of a fraction containing fatty acids in the same proportion as cephalin. In this fraction ethanolamine and a number of amino-acids were found after hydrolysis, but no inositol or carbohydrates. This precludes the presence of more complex phosphatides, such as are found in soya-beans.[29, 30]

The proportion of saturated acids in various phospholipid fractions from baobab seed is somewhat higher than in the glycerides. Although not investigated by the same authors and by identical methods, the phosphatides of various Gramineae seeds[1–5] also show a larger proportion of saturated acids than the corresponding glycerides.[31–34] It should be stressed that in all seed phosphatides investigated the proportion of saturated acids never exceeds 25%. In this connexion, it would be interesting to elucidate the composition of some other tree seed phosphatides, since data used for comparison have been obtained only for phosphatides from annual plants.

As far as individual acids are concerned, Table III is self-explanatory. In all fractions isolated the most prominent saturated acid is palmitic and the main unsaturated acid is oleic acid. There are not very significant variations in the fatty acid composition of various lipid fractions, a fact which has also been observed in some seed glycerides and phosphatides[7, 8] and also in glycerides and phospholipids from cocksfoot grass.[35]

The complete absence of linolenic acid from all fractions investigated should be noted, except for a very slight absorption in the triene region with the lecithin. Polyunsaturated acids have not been found even in traces. About 10% of the total linoleic acid in the glycerides is present in the conjugated form. The phospholipid fractions contain only traces of this acid. From all lipid fractions linoleic acid was isolated as a tetrabromide with a sharp melting point at 114°.

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SOME POSSIBLE GLUCOSE/GLYCINE BROWNING INTERMEDIATES AND THEIR REACTIONS WITH SULPHITES

By D. J. McWEENY and H. S. BURTON

In near neutral aldose/glycine systems, hydroxymethylfurfural is not an important intermediate in browning. The possible intermediates studied all require glycine (or a similar compound) for rapid browning; glucose is not required. In retarding browning, bisulphites do not react directly with any of the intermediates tested. Bisulphite addition to unsaturated aldehydes (such as 3,4-unsaturated 3,4-dideoxyhexosone), derived from a cycle of reactions involving glucose dehydration, would explain the observed results. Conversion of bisulphite to sugar sulphates or to elemental sulphur does not appear to be important in retarding non-enzymic browning under these pH conditions.

Introduction

The group of reactions often collectively referred to as the non-enzymic browning reaction is of great importance to the food industry in the production of desirable qualities in certain foods, and in deterioration leading to undesirable appearance and flavour in some others. As food processing has developed, methods of predicting and to some extent controlling the browning potentialities of foodstuffs have developed on an empirical or semi-empirical basis. There is still no detailed understanding of the reactions which lead to the coloured and highly flavoured products of non-enzymic browning. The field was reviewed and some mechanisms suggested by Hodge[1] and a further review made by Ellis.[2] Recently Anet and co-workers have collaborated in detailed studies of the reducing sugar/amino-acid system with particular reference to the operation of this system in apricot purees.[3] Some studies of the field of non-enzymic browning on a more general basis have recently been reported by Burton and co-workers.[4]

Sulphur dioxide in the form of alkali metal sulphites or bisulphites or as sulphurous acid solutions has been used for many years as a means of retarding browning, without there being any real understanding of its mode of action. The actions of sulphites as reported by Ingles are (i) formation of carbonyl bisulphite compounds,[5a] (ii) formation of sugar sulphates,[5c] (iii) oxidation of sugars with liberation of elemental sulphur,[5b] (iv) formation of β-sulphopropionic acid by an unknown route.[5d] With the possible exception of the last, none of these reactions appear to explain the effect of small quantities of sulphites in retarding browning. Recent experiments by Burton *et al.*[4c] implicate $\alpha\beta$-unsaturated aldehydes as important browning intermediates and demonstrate that interaction of these compounds with bisulphites can result in an addition reaction at the olefinic bond, with the production of stable compounds of low browning potentiality.[6]

The experiments reported here represent an investigation into the importance in browning reactions of some compounds isolated directly or indirectly from glucose/glycine systems and some studies of the mode of action of sulphites and bisulphites in retarding browning of these materials.

Experimental

Methods

Monofructose glycine (MFG), difructose glycine (DFG) and 3-deoxyhexosone (3DH) were prepared according to the method of Anet.[7]

Colour determinations were made visually within the range: colourless, straw, yellow, golden yellow, light brown, red brown, cherry red, dark brown. Because of the variation in absorption maximum observed at various stages of browning, a measurement of the absorption at any particular wavelength does not necessarily give a good indication of the colour as a whole (cf. Hodge & Rist[8]).

Unless otherwise stated, incubations were carried out at 50° in screw-cap glass tubes with a metal foil liner in the cap. In the cases where the solution volume was 0·5 ml. and 1·0 ml. the headspace (air) was 2·5 ml. and 2·0 ml. respectively.

Paper chromatography was carried out on Whatman No. 1 and No. 4 paper using as solvents (i) isopropanol/water (4 : 1); (ii) butanol/acetic acid/water (120 : 30 : 50); (iii) ethyl acetate/pyridine/water (120 : 50 : 40); (iv) phenol/water/ammonia (160 : 40 : 2) and (v) butanol/pyridine/water (80 : 80 : 40). The papers were sprayed with *p*-anisidine (3% solution in butanol) as a general spray. $\alpha\beta$-Unsaturated carbonyl compounds were detected with *m*-phenylenediamine (1% in 85% ethanol containing 1% oxalic acid), which gives a positive brown colour within 2 min. at room temperature with $\alpha\beta$-unsaturated carbonyls, whilst saturated carbonyls require several days to produce maximum coloration (McWeeny & Burton, 1961, unpublished).

Results

Incubation of monofructose glycine and difructose glycine

Monofructose glycine (100 mg.) was dissolved in 0·5 ml. of distilled water with the following additives:

Sample M1, no additive; sample M2, 100 mg. of glucose; sample M3, 100 mg. of glycine; sample M4, 100 mg. of glucose + 100 mg. of glycine.

Samples D1–D4 were made up as M1–M4 but containing difructose glycine in place of monofructose glycine. In parallel series of incubations tubes M1s–M4s and D1s–D4s contained the same materials as M1–M4 and D1–D4 respectively but with the addition of 2·8 mg. of sodium ^{35}S-sulphite to each tube. Colour observations and chromatograms were made at intervals. The observed colours are shown in Table I.

Table I

*Colour development in monofructose glycine and difructose glycine solutions at 50° **

Sample	Additive	Time of incubation, h. 18	42	76	140	210	350	500
M1	—	—	v.l. brown	v.l. brown	v.l. brown	l. brown	brown	red brown
M2	Glucose (100 mg.)	—	v.l. brown	v.l. brown	v.l. brown	l. brown	brown	red brown
M3	Glycine (100 mg.)	l. red brown	deep red brown	cherry red	v. dark brown	v. dark brown	v. dark brown	v. dark brown
M4	Glucose + glycine (100 mg. each)	red brown	deep red brown	dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown
D1	—	pale straw	pale straw	straw	straw	l. brown	l. red brown	red brown
D2	Glucose (100 mg.)	pale straw	pale straw	pale straw	straw	l. brown	l. red brown	red brown
D3	Glycine (100 mg.)†	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown
D4	Glucose + glycine† (100 mg. each)	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown
M1s	—	—	l. brown	l brown	l. red brown	l. red brown	red brown	dark brown
M2s	Glucose (100 mg.)	—	l. brown	l. brown	l. red brown	l. red brown	dark brown	dark brown
M3s	Glycine (100 mg.)	golden brown	red brown	deep red brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown
M4s	Glucose + glycine (100 mg. each)	golden brown	deep red brown	dark brown	dark brown	v. dark brown	v. dark brown	v. dark brown
D1s	—	—	v. pale straw	pale straw	l. brown	l. red brown	l. red brown	red brown
D2s	Glucose (100 mg.)	—	v. pale straw	v. pale straw	pale straw	l. brown	l. red brown	red brown
D3s	Glycine (100 mg.)‡	l. brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown
D4s	Glucose + glycine‡ (100 mg. each)	l. brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown	v. dark brown

s = sulphite present v = very l = light

* 100 mg. of MFG or DFG in 0·5 ml. of water
† light brown after 0·5 h. and red brown after 3 h.
‡ straw " " and light " " "

Chromatogams in the isopropanol/water solvent showed monofructose glycine (MFG) and difructose glycine (DFG) to have R_G values of 28–33 and 22–25 respectively (glucose R_G = 100). In severely browned systems large numbers of compounds were obtained which after development gave blue, violet, turquoise and yellow fluorescence under ultra-violet light. These were predominantly slow-moving compounds of R_G <30 but in severely browned systems three fluorescent spots appeared in positions between those of glycine (R_G 40) and glucose (R_G 100). Streaks of a blue fluorescent compound appeared at R_G 100–150. The systems based on MFG showed only traces of DFG even on incubation with glucose, whereas decomposition of DFG to MFG in solutions based on DFG took place readily and was noticeable after 1½ h. incubation; DFG was no longer readily detectable 140 h. after incubation commenced. A spot of low intensity was just detectable in the glycine position after only 1½ h. incubation of the M1 and M2 systems. The intensity of this spot remained low until at 140 h. incubation it appeared in larger amounts and thereafter was readily detected in the M1 and M2 and also in the D1 and D2 systems. It is noteworthy that browning in these systems did not become pronounced until after this time.

Scanning both sides of the chromatograms for radioactivity with a twin Geiger–Müller (G.M.) tube assembly[9] revealed that prior to the appearance of browning the distribution of ^{35}S compounds on the chromatograms was substantially the same in all the systems, with small peaks at R_G 8 and $R_G \simeq$ 60 corresponding to sulphate and an unknown compound respectively. ^{35}S-sulphites do not give reliably a peak in this system or in butanol/acetic acid/water, presumably due to liberation and loss of SO_2 during development of the chromatograms.

As incubation proceeded, the R_G 8 area increased in activity at a rate related to the rate of browning, while the faster-moving radioactive area disappeared slowly in all systems. As browning developed, an increasing amount of ^{35}S was located at the origin along with the brown polymer. When browning had become severe the ^{35}S activity at R_G 0 in systems M3, M4, D3 and D4 was greater than that at R_G 8 whereas in systems M1, M2, D1 and D2, which were initially glycine free, this relationship was reversed.

Incubation of MFG or DFG with sodium sulphite or sodium metabisulphite

Sample tubes containing MFG (samples M'1–M'4) and DFG (D'1–D'4) with glucose and glycine additives in similar quantities and combinations to those described above were incubated with sodium sulphite or sodium metabisulphite. In this case the level of SO_2 addition was much higher and was calculated as being the molar equivalent of the MFG or DFG present. As controls, similar mixtures were incubated without the addition of a sulphur dioxide source. The pH was not adjusted in any way. The colour observations were consistent and showed that at this level sodium metabisulphite gave a very good measure of protection against browning whilst sodium sulphite caused a significant increase in browning after an initial retarding effect. In Table II the observations after 70 h. incubation are given in full to demonstrate this effect.

Table II

Effect of sodium sulphite and metabisulphite on browning of mono- and di-fructose glycine (70 h. at 50°)*

Sample	Additive	Sodium metabisulphite	Sodium sulphite	Control
M'1	—	—	l. brown	—
M'2	Glucose (100 mg.)	—	l. brown	—
M'3	Glycine (100 mg.)	—	l. red brown	dark cherry red
M'4	Glucose + glycine (100 mg. each)	—	v. dark brown	v. dark brown
D'1	—	—	golden yellow	yellow
D'2	Glucose (100 mg.)	—	golden yellow	yellow
D'3	Glycine (100 mg.)	golden yellow	v. dark brown	v. dark brown
D'4	Glucose + glycine (100 mg. each)	golden yellow	v. dark brown	v. dark brown
Glucose–glycine	—	—	l. brown	deep golden yellow

* 100 mg. of MFG or DFG in 0·5 ml. of water
l = light v = very

Effect of pH on monofructose glycine production in glucose–glycine mixtures

Four solutions containing glucose (36 g., 0·20 mole) and glycine (3·75 g., 0·05 mole) in 20 ml. of water were prepared. Two of the solutions contained sodium hydroxide (2·0 g., 0·05 mole) and to one of these hydrated sodium sulphite (12·7 g., 0·05 mole) was added. The pH of these two mixtures was adjusted to 9·0. The remaining pair of solutions were adjusted to pH 4·5 after the addition of sodium metabisulphite (4·75 g., 0·05 mole SO_2) to one of them. The solutions were heated at 95° in glass boiling-tubes, and samples were withdrawn at intervals between 3 and 150 min. Measured aliquots were chromatographed in isopropanol/water. Examination of the chromatograms showed that at each of the two pH values, the addition of an SO_2 source caused a reduction in the MFG concentration. Two characteristic fluorescent spots were noted on the chromatograms. Amongst many other fluorescent areas appearing on the chromatograms, a fluorescent area at R_G 12–13 in the samples from the sulphited solution at pH 9·0 and a similar fluorescent area at R_G 17–18 in samples from the sulphited solution at pH 4·5 were consistently noted. These spots were absent from chromatograms from the corresponding sulphite-free solutions. A further observation was that at pH 9·0 the MFG concentration rose to a maximum value and then decreased markedly despite the continued presence of relatively large quantities of glucose and glycine. The maximum concentration was found after 20 min. incubation at 95°. After 150 min. the concentration was estimated at less than 20% of the maximum.

Colour development in 3-deoxyhexosone incubations

(*a*) 3-Deoxyhexosone (3DH) (20 mg.) was incubated at 50° in 0·5 ml. of water. (i) alone; (ii) with 100 mg. of glucose; (iii) with 100 mg. of glycine; (iv) with 100 mg. of glucose + 100 mg. of glycine. The results are shown in Table III. The solutions were initially slightly coloured due to a trace of coloured 3DH decomposition products in the 3-deoxyhexosone used.

(*b*) The addition of 1-M quantities of SO_2 as sodium sulphite (calculated on 3DH present) to systems made up as described in the previous experiment had a pronounced effect upon colour development rates as indicated in Table IV.

(*c*) Sodium sulphite was added in graded amounts to 3DH/glycine incubations ; on a molar basis, relative to 3DH present, the sulphite levels were 0, 0·5, 1·0, 1·5, 2·0 and 2·5. Each incubation mixture contained 10 mg. of 3DH and 100 mg. of glycine in 0·5 ml. of water. The observations of colour are given in Table V.

Table III

Effect of glucose and glycine on colour development in the incubation of 3-deoxyhexosone at 50°*

Additives	Time of incubation, h.							
	0	1	2	5	21	96	240	840
Nil	straw	pale straw	pale straw	v. pale straw	v. pale straw	v. pale straw	v. pale straw	l. brown
Glycose (100 mg.)	straw	pale straw	pale straw	v. pale straw	v. pale straw	v. pale straw	v. pale straw	l. brown
Glycine (100 mg.)	straw	l. brown	red brown	cherry red	v. dark brown	v.v. dark brown	v.v. dark brown	v.v. dark brown
Glucose + glycine (100 mg. each)	straw	l. brown	red brown	cherry red	v. dark brown	v.v. dark brown	v.v. dark brown	v.v. dark brown

l = light v = very * 20 mg. of 3DH in 0·5 ml. of water

Table IV

*Colour development in sulphited 3-deoxyhexosone**

Addition	Time of incubation, days					
	0	1	2	5	8	22
Nil	pale straw	pale straw	pale straw	pale straw	slight pink	l. brown
Glucose (100 mg.)	pale straw	pale straw	pale straw	pale straw	pale straw	golden yellow
Glycine (100 mg.)	pale straw	pale straw	yellow	l. brown	l. brown	cherry red
Glucose + glycine (100 mg. of each)	pale straw	straw	l. red brown	dark cherry red	dark brown	v. dark brown

* 20 mg. of 3DH + 1 molar equivalent of sodium sulphite in 0·5 ml. of water

v = very l = light

Table V

Effect of SO_2 level on the browning of 3-deoxyhexosone-glycine mixtures at 50°*

Sulphite level, moles	Time of incubation, h.							
	0	1	2	4	21	96	150	850
0	straw	l. brown	l. red brown	red brown	v. dark brown	v.v. dark brown	v.v. dark brown	v.v. dark brown
0·5	straw	golden yellow	golden yellow	golden yellow	cherry red	dark cherry red	v.v. dark cherry red	v.v. dark brown
1·0	straw	straw	straw	deep straw	golden yellow	golden yellow	l. brown	cherry red
1·5	straw	straw	straw	straw	deep straw	deep straw	deep straw	deep golden yellow
2·0	straw	straw	straw	straw	deep straw	deep straw	deep straw	deep golden yellow
2·5	straw	straw	straw	straw	deep straw	deep straw	deep straw	deep golden yellow

* 3DH 10 mg. and glycine 100 mg. in water 0·5 ml. v = very l = light

Comparison of colour development of DFG, 3DH, hydroxymethylfurfural (HMF) and pyruvaldehyde

Solutions were made up containing glycine (100 mg.) and 0·1 millimole of the test substance in 1·0 ml. water. Sodium metabisulphite was added to these solutions of each of the test materials in quantities required to give SO_2 molar equivalents of 1·0, 0·5, 0·1, 0·2 and 0 of the test material present. The mixtures were incubated at 50° for examination of colour development. The observations are recorded in Table VI.

Incubation of glucose 6-sulphate

Glucose-6-sulphate (sodium salt) was incubated with glycine and with glucose + glycine and colour development compared with that in a simple solution of glucose and glycine. Glycine (100 mg.) was incubated with (i) glucose (100 mg.) ; (ii) glucose-6-sulphate sodium salt (100 mg.) ; (iii) glucose-6-sulphate sodium salt (10 mg.) and glucose (100 mg.). Colour observations are recorded in Table VII.

Carbon disulphide extraction of severely browned DFG/glycine/sodium ^{35}S-sulphite solution

A solution containing DFG (6 g.), sodium metabisulphite (1 molar equiv. of SO_2), glycine (8 g.) and sodium ^{35}S-sulphite (200 μc ; 62 mg.) in 15 ml. of water was incubated for 1 month at 50°. A 1-ml. aliquot was extracted with carbon disulphide and the extract washed twice

with water. When a portion (0·3 ml.) of the washed carbon disulphide extract was dried on to a planchet and the radioactivity present measured with an end-window G.M. tube, a net count of 10 c.p.m. was obtained. This corresponds to less than 0·05% of the radioactivity in the aliquot extracted being present in the total carbon disulphide extract. Conversion of sulphite to elemental sulphur was apparently negligible in this system.

Table VI

*Colour development in incubations of difructose glycine, 3-deoxyhexosone, hydroxymethylfurfural or pyruvaldehyde with glycine and various bisulphite concentrations**

Sulphite level, mmole	Time of incubation, h. 0·50	1·5	3	5	6	22	46	120	500
					Difructose glycine				
0	—	l. brown	l. brown	brown	l. cherry red	v. dark cherry red	v. dark brown	v. dark brown	v. dark brown
0·01	—	v.v. l. brown	pale red brown	red brown	red brown	dark cherry red	v. dark cherry red	v. dark brown	v. dark brown
0·02	—	pale straw	pale straw	v.l. brown	l. brown	cherry red	dark cherry red	v. dark brown	v. dark brown
0·05	—	—	v.v. pale straw	v. pale straw	v. pale straw	orange brown	cherry red	v. dark brown	v. dark brown
0·10	—	—	v.v. pale straw	—	—	—	—	orange brown	dark cherry red
					3-Deoxyhexosone				
0	straw	yellow	orange yellow	orange yellow	orange brown	dark cherry red	v. dark brown	v. dark brown	v. dark brown
0·01	straw	l. yellow	yellow	l. orange brown	l. orange brown	cherry red	dark brown	v. dark brown	v. dark brown
0·02	pale straw	straw	straw	golden yellow	orange yellow	red brown	dark cherry red	v. dark brown	v. dark brown
0·05	v. pale straw	pale straw	pale straw	pale straw	pale straw	yellow	orange-brown	v. dark cherry red	dark brown
0·10	v.v. pale straw	v.v. pale straw	v.v. pale straw	v. pale straw	v. pale straw	v. pale straw	pale straw	straw	orange brown
					Hydroxymethylfurfuraldehyde				
0	pale straw	pale straw	pale straw	yellow	golden yellow	l. brown	red brown	dark cherry red	v. dark brown
0·01	v. pale straw	v.v. pale straw	v.v. pale straw	pale straw	pale straw	yellow	golden yellow	orange brown	v. dark brown
0·02	v.v. pale straw	—	—	v. pale straw	v. pale straw	pale straw	yellow	golden yellow	v. dark brown
0·05	v.v. pale straw	—	—	v.v. pale straw	v.v. pale straw	v. pale straw	straw	yellow	orange brown
0·10	—	—	—	—	—	—	—	v. pale straw	straw
					Pyruvaldehyde				
0	v.v. pale straw	pale yellow	golden yellow	l. orange brown	l. orange brown	red brown	dark red brown	dark cherry red	dark cherry red
0·01	v.v. pale straw	straw	yellow	golden yellow	orange yellow	orange yellow	brown	cherry red	cherry red
0·02	—	pale straw	straw	yellow	yellow	golden yellow	orange-brown	orange brown	orange brown
0·05	—	—	—	—	—	v.v. pale straw	v.v. pale straw	pale straw	straw
0·10	—	—	—	—	—	—	—	—	—

l = light v = very

* Each solution contains 0·1 mmole of test material + 100 mg. of glycine + 1 ml. of water

Table VII

Colour development in incubations of glucose 6-sulphate at 50°*

Components	Duration of incubation, h. 1	4	7	24	48
Glucose 6-sulphate (100 mg.)	golden yellow	light red brown	red brown	cherry red	dark red brown
Glucose 6-sulphate (10 mg.) + Glucose (100 mg.)	—	—	pale yellow	light brown	red brown
Glucose (100 mg.)	—	—	—	straw	deep golden yellow

* All solutions contain glycine (100 mg.) and water (1 ml.)

Chromatograms of ^{35}S-sulphited model browning systems

One-dimensional chromatograms in each of the five solvent systems described earlier were prepared at intervals from incubations of glycine (100 mg.) sodium, ^{35}S-sulphite (4·0 mg.), sodium metabisulphite (6·9 mg.) and water (1 ml.) with 0·1 millimole of (i) DFG; (ii) 3DH; (iii) hydroxymethylfurfural (HMF); (iv) pyruvaldehyde or with (v) 100 mg. of glucose. As standards, mixtures of glucose + sulphites and of glycine + sulphites were studied. The chromatogram strips were examined for fluorescence and then sprayed with *p*-anisidine before re-examining for visible and fluorescent spots. Finally the chromatograms were scanned for

radioactivity. Determinations of the ^{35}S radioactivity on chromatograms of samples taken from the incubations of DFG, 3DH, HMF and pyruvaldehyde with glycine and labelled bisulphite may be summarised by the following observations:

(i) In those solvent systems in which a bisulphite spot was detectable, the radioactivity of the area around the spot decreased with time in all samples.
(ii) In all systems radioactivity in the area to which sulphate ion migrated increased with time in all samples.
(iii) In all systems ^{35}S-activity at or near the chromatographic origin was associated with chromophore development in the incubation material and with the amount of coloured material appearing in this part of the chromatogram.
(iv) No other parts of the chromatograms showed a progressive increase in radioactivity during incubation.
(v) The radioactivity of some parts of the chromatograms showed a temporary increase which was followed later by a decrease.
(vi) In severely browned systems the radioactivity was almost exclusively confined to positions at or near the origin and the position associated with the sulphate ion.
(vii) In severely browned samples from the DFG and 3DH systems the amount of radioactivity near the origin was greater than that at the sulphate position. This relative distribution was reversed in similar samples from HMF and pyruvaldehyde incubations.

Production of unsaturated carbonyls from difructose glycine

When difructose glycine (100 mg.) is heated with glucose (100 mg.) at 90° in 1·0 ml. of water, browning developed slowly. Chromatograms in solvents (i)–(iv) were prepared in duplicate. One set was sprayed with *m*-phenylenediamine (to detect $\alpha\beta$-unsaturated carbonyls) and the other with 2,4-dinitrophenylhydrazine (to detect total carbonyls). Evidence for the existence of at least six $\alpha\beta$-unsaturated carbonyls as products of interaction of DFG with glucose was obtained.

Discussion

The first experiments, the results of which are given in Table I, demonstrate quite clearly that browning of mono- and di-fructose glycine is very much accelerated by the addition of glycine. It might even be true to state that these compounds will only brown at an observable rate under the condition of this experiment when glycine is present. Certainly browning did not proceed at any great rate in M1 and M2 and D1 and D2 until appreciable amounts of glycine had been made available by decomposition reactions. Decomposition of difructose glycine was rapid as might be expected from the work of Anet,[7] and led to formation of monofructose glycine and other products. It seems likely that under these conditions a cycle of reactions may operate in the manner shown in Fig. 1. The net effect of these reactions is to cause conversion of glucose to 3-deoxyhexosone by removal of the elements of water. In the presence of large excesses of glucose this cycle could operate to produce considerable quantities of 3-deoxyhexosone and unsaturated carbonyl decomposition products. However the findings reported here point to the conclusion that there would still be a requirement for amino-nitrogen at a later stage in the reactions leading to rapid brown colour production. Any browning reactions which are not dependent on amino-nitrogen are probably slow under the conditions of moderate temperature and near-neutral pH used in these experiments. Under other conditions where the later stages of browning are not necessarily amino-nitrogen dependent, however, this cycle could explain colour development in systems containing only traces of amino-compounds. It could be important, for instance, in glucose syrups derived from starch.

Hydroxymethylfurfural may be produced by decomposition of 3-deoxyhexosone[10] and has long been regarded as an intermediate in melanoidin production. The evidence obtained in the studies on the relative rates of browning of DFG, 3DH, HMF and pyruvaldehyde is in strong opposition to the view that HMF is an important intermediate in browning under these conditions.

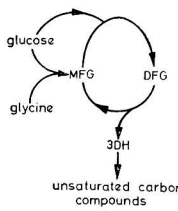

FIG. 1.—*Reaction scheme for glucose dehydration by a cyclic process*

The rate of browning of HMF/glycine mixtures was several times less than that of DFG or 3DH (Table VI, unsulphited samples), and thus it is difficult to sustain the view that browning of DFG and 3DH proceeds via formation of HMF. This conclusion is supported by the work of Yoshihiro *et al.*,[11] who showed that addition of HMF did not markedly accelerate the browning of glucose/glycine solutions. Furthermore, the difference in distribution of ^{35}S observed in chromatograms from browned mixtures containing DFG or 3DH as compared with HMF and pyruvaldehyde supports the view that different routes are involved.

A further observation from this comparison of browning rates was that in solutions containing equimolar amounts of the two compounds difructose glycine browned at a greater rate than 3-deoxyhexosone. If the route suggested by Anet as being important in browning is correct, one would expect that 3DH would have browned at least as rapidly as DFG. By taking account of the cycle of reactions referred to above, it is now possible to propose a mechanism by which both of the hexose moieties in DFG could be liberated as 3DH and lead to a higher rate of browning than would be observed if decomposition to 3DH was not followed by re-cycling of the other decomposition product (MFG). The availability of both hexose moieties for 3DH production would adequately account for the higher rate of browning observed with DFG. In recent studies Burton *et al.*[4c] have shown that α-unsubstituted αβ-unsaturated aldehydes, as a class, brown rapidly with glycine but that this behaviour is not shown by αβ-unsaturated ketones or by saturated aldehydes and ketones. These observations were made following the preliminary observation of αβ-unsaturated carbonyls in browning mixtures and correlation of their appearance with colour development and sulphite utilisation.[4a, 12] In view of this observation of the general property of conjugated unsaturated aldehydes in promoting browning, it is interesting that Anet[13] has isolated 3,4-unsaturated 3,4-dideoxyhexosones from model browning systems. These compounds have a conjugated unsaturated aldehyde structure similar to that found to be associated with browning activity. In view of the wide difference between the browning rates of HMF and of 3DH (from which the unsaturated osone is readily derived) it is now suggested that, at intermediate pH values, browning of 3DH does not proceed primarily via the formation of HMF but follows the suggested route only as far as dehydration to the unsaturated hexosone. From this point the evidence suggests that browning proceeds in the manner typical of αβ-unsaturated aldehydes. The route via HMF may be important in highly acid solutions in which HMF production is enhanced. However, since sugar/amine browning is accelerated by increasing the pH whilst HMF production is favoured by low pH conditions, it seems unlikely that the normal route of sugar/amine browning involves HMF as an intermediate. It would appear from the present experiments and those of Yoshihiro *et al.*[11] that, other than in strongly acid conditions, HMF is not an intermediate in the principal browning reaction, but is a reaction by-product which accumulates in detectable amounts only because of its relatively low reactivity in browning reactions.

Unsaturated carbonyls have been reported as occurring in browning systems[4a, 14] and, as mentioned above, an unsaturated osone was isolated by Anet. When it was observed that difructose glycine browning is glycine-dependent, an incubation of DFG with glycine was carried out in the hope of detecting the formation of unsaturated aldehydic intermediates (McWeeny & Burton, 1962, unpublished). Presumably because of the high reactivity of these compounds and the excess of glycine present any such intermediates attained only very low concentrations. However, on considering the implications of the cycle of reactions, now proposed as operating in aldose/amino-acid systems, it became apparent that, in the absence of side reactions, incubation of DFG with glucose should lead to continuous production of 3-deoxyhexosone and other breakdown products, while limiting the utilisation of these products in mechanisms leading to the rapid development of chromophores. The concentrations of 3DH and the other breakdown products might be expected to reach appreciable values under these conditions. This prediction was verified experimentally by the detection of at least six αβ-unsaturated carbonyl compounds on chromatograms from DFG/glucose incubations.

In the experiments in which sodium sulphite or sodium metabisulphite was added to solutions containing MFG or DFG (Table II) it seems likely that addition of the former caused an increase in pH with a resultant increase in browning reaction and that the increase in reaction velocity more than outweighed the browning-retardant action of the sodium sulphite added. If this is so it may be concluded that at least one of the major sulphite effects operates subsequent to a stage in browning which is accelerated by increasing the pH of the system, or alternatively that the mode of action of sulphite is itself pH-dependent. Some evidence to the effect that the action of sulphite is pH-dependent may be derived from the experiment in which glucose and glycine were heated at pH 4·5 and pH 9·0 in the presence and absence of sulphites and reference has already been made to the action of sulphites in multifunctional systems.[4b] In the sulphited solution at pH 9·0 a fluorescent area was found at R_G 12–13 whereas at pH 4·5 this fluorescent spot was absent and was replaced by one at R_G 17–18. No fluorescence was observed in either of these positions in the unsulphited mixtures.

The observation that the concentration of MFG decreased markedly when browning was well advanced, despite the continued presence of large amounts of glucose and glycine, indicated the possibility of a reaction between MFG (or a compound in equilibrium with MFG) and the product of one of the later reactions. This would be consistent with the observation made elsewhere (Burton *et al.*, 1962, unpublished) that when a heavily sulphited glucose-glycine system does eventually begin to brown it does so at a very rapid rate.

The rate of colour development in model systems containing 3-deoxyhexosone as shown in Table III appears to be very slow in the absence of glycine. Glucose has no accelerating effect, but addition of glycine to the system produced an increase in browning velocity which was even more marked than in the cases of MFG and DFG. It is possible that for rapid colour development these three compounds are all dependent upon the presence of glycine (or a similar compound) to the same degree, but that it is not so marked with MFG and DFG in the present experiments due to liberation of glycine from the Amadori compounds with a consequential increase in browning rate.

The addition of one molar equivalent of SO_2 as sodium metabisulphite (Table IV) to incubations of 3DH showed a very high degree of protection against colour development. This would indicate that one molecule of sodium bisulphite per molecule of the dicarbonyl compound, 3-deoxyhexosone, was sufficient to prevent browning between this compound (and/or its decomposition products) and glycine. This was confirmed by the results shown in Table V where 3DH was incubated with glycine in the presence of amounts of sodium sulphite varying from 0 to 2·5 times the molar equivalent of 3DH. It is clear that the addition of more than one mole of sulphite gave virtually complete protection and that one mole was almost adequate. On the other hand, 0·5 mole of SO_2 gave a good protection but only for a relatively short time. Whereas in the absence of sulphite, browning commenced virtually immediately, there was little colour development for over 4 h. with 0·5 mole of SO_2, and the time required to reach a cherry red colour was extended from 5 h. to 21 h.

If 3-deoxyhexosone was the compound with which bisulphite reacts in retarding browning in this system, then addition of a 0·5 molar quantity of bisulphite would reduce by a half the amount of 3DH available for browning reactions, and browning of the remainder should commence immediately. The rate of colour development would be reduced, due to the lower concentration of 3DH, by an amount dependent upon the apparent order of reaction of the browning process. The experimental observations were that colour development did not commence immediately, that there was a considerable time lapse before colour development occurred and that colour development then proceeded at a rapid rate.

These observations were interpreted as indicating that 3-deoxyhexosone itself was not the compound with which bisulphite reacted in retarding non-enzymic browning in this system and that bisulphites act upon a compound derived from 3DH. If these conjectures are correct, then little colour development would occur until the new intermediate had been formed in a quantity sufficient to react with all the bisulphite available. Subsequently any further decomposition of 3DH to the new intermediate would lead to rapid colour development. In view of the known reactivity of $\alpha\beta$-unsaturated aldehydes in browning reactions and the ability of bisulphites to react readily with these compounds at the olefinic bond to form compounds

of low browning activity,[6] it seems likely that one of the intermediates which could be responsible for this behaviour with bisulphite is the 3,4-unsaturated 3,4-dideoxyhexosone prepared by Anet.[13]

Further evidence in favour of this view may be derived from the experiment in which DFG, 3DH, HMF and pyruvaldehyde were each heated with glycine in the presence of various amounts of bisulphite (Table VI).

It was noticeable with all the compounds tested, as with 3DH in the previous experiment, that the addition of a small amount of bisulphite was sufficient to delay considerably the onset of visible browning. As an example, the addition of 0·2 molar equivalent of bisulphite greatly lengthened the time interval before browning became significant. If the sulphite had reacted with any of the substances to give a stable compound, it would have been quantitatively inadequate to react stoicheiometrically and 0·8 of the original browning intermediate would still be available for reaction. Consequently it would be expected that browning would proceed at a rate only slightly less than that found for the unsulphited sample ; this was by no means the case, and it was concluded that the reaction of bisulphites with DFG, 3-deoxyhexosone, HMF and pyruvaldehyde is not their main action in retarding the browning in the system. However, due to the ease with which β-hydroxy carbonyl compounds dehydrate to produce $\alpha\beta$-unsaturated carbonyls (viz., preparation of crotonaldehyde and mesityl oxide), it is likely that 3DH–glycine browning is retarded by bisulphite addition at the olefinic bond in the 3,4-unsaturated 3,4-dideoxyhexosone derived from 3DH.

The bisulphite bleached the slight discoloration initially present in 3-deoxyhexosone, HMF and pyruvaldehyde, most noticeably in the more heavily sulphited samples. The observation that the chromophores in browning systems are subject to a certain amount of bleaching on the addition of sulphite has also been made by Burton *et al.*[4a] in sulphited glucose/glycine systems. The reaction by which this bleaching is effected is not fully understood but is being investigated.

The possible reactions of sulphites or bisulphites in browning systems as suggested by Ingles include the formation of sulphate esters of sugars, and the production of elemental sulphur.[5b] Incubation of glucose 6-sulphate (sodium salt) with glycine and with glucose and glycine led to the conclusion that glucose 6-sulphate was not active in retarding aldose/amino-acid browning under our conditions and that bisulphite did not exert any retarding effect on browning via the formation of this compound.

The production of elemental sulphur and sulphate by heating sugars with bisulphite solutions was investigated by Hägglund,[15] who found that this reaction took place with glucose, mannose, xylose, arabinose and fructose, but under the conditions used by Ingles, not mannose. In unpublished experiments we have found sulphur production from fructose and glucose as reported by Ingles. Extraction with CS_2 of a mixture of DFG, glycine and sodium ^{35}S-sulphite after prolonged incubation failed to yield any evidence that this reaction takes place in systems involving DFG/glycine browning. In browned dehydrated potato strips, treated with sodium ^{35}S-sulphite during preparation, no evidence for the production of significant quantities of elemental sulphur during browning was obtained (McWeeny & Burton, 1960, unpublished). Less than 1 part in 5000 of the sulphite added was in a form soluble in carbon disulphide after browning. It seems therefore that, although heating of concentrated sugar solutions at 100° with bisulphite can lead to production of elemental sulphur, the reaction is of limited significance in sugar/amino-acid browning at lower temperatures.

The fate of the SO_2 in the ^{35}S-sulphited series of incubations involving DFG, 3DH, HMF and pyruvaldehyde remains somewhat uncertain. A number of ^{35}S-compounds were formed as intermediates during browning, but the ^{35}S finally appeared in two forms, one of which appeared to be sulphate, and the other on chromatography in five solvent systems seemed to be associated with the brown polymer. It has been demonstrated previously[4c] that, although saturated aldehydes do not brown readily with glycine, they will do so if incubated over longer periods. It is concluded that β-sulphoaldehydes, which are derived from $\alpha\beta$-unsaturated aldehydes by addition of bisulphite at the olefinic bond, can behave in the same manner as simple saturated aldehydes. In view of this, one interpretation of the transient ^{35}S peaks obtained on the incubation chromatograms is that ^{35}S-sulphonated aldehydes are formed from

the unsaturated carbonyl compounds which exist in browning systems, and that these are subsequently incorporated into the brown polymer by the slow reaction typical of saturated aldehydes. The possibility that the sulphite becomes attached to other polyfunctional molecules which themselves are quite active in polymer formation should not be neglected. There is also evidence from unpublished experiments (McWeeny & Burton, 1961) on incubations of sodium ^{35}S-sulphite with the water-soluble components of potatoes, that the ^{35}S-activity in the browned products which remain at the chromatographic origin is not entirely associated with high molecular weight compounds. Separations on Sephadex G25 showed that the ^{35}S activity with R_G values of 0·1 was heterogeneous and comprised both high- and low-molecular weight components. It is apparent that sulphites exert their action in retarding non-enzymic browning in a complex manner and the efficiency of these compounds as browning retarders may well be a consequence of this diversity of action.

Conclusions

From the results of observations of comparative rates of browning it is concluded that, at 50° and initial pH 5·5–6·0, browning of aldose/amino systems via the formation and reaction of hydroxymethylfurfural is very slow. Production of Amadori compounds and their unsaturated carbonyl decomposition products appears to be followed by rapid reaction of these compounds with amino groups rather than dehydration to and further reaction of hydroxymethylfurfural.

The glycine-dependence of browning reactions of monofructose glycine, difructose glycine and 3-deoxyhexosone has been demonstrated. These reactions are not accelerated by adding glucose.

Addition of limited amounts of bisulphite to model mixtures containing difructose glycine, 3-deoxyhexosone, hydroxymethylfurfural or pyruvaldehyde demonstrated that bisulphites do not react directly with these compounds in retarding browning. It is concluded that one of the compounds with which bisulphites react in retarding browning occurring by this route may be 3,4-unsaturated 3,4-dideoxyhexosone which is readily derived from 3-deoxyhexosone.

In confirmation of other work it is apparent from incubations of difructose glycine with glucose that unsaturated aldehydes other than the osone referred to above may be produced in considerable numbers during browning. It is probable that bisulphite exert their action in retarding this stage of non-enzymic browning by reaction with conjugated unsaturated aldehydes in general, rather than with this one compound in particular.

At moderate temperatures and near neutral pH formation of sugar sulphate ester and sulphite oxidation of sugars with production of elemental sulphur are not important reactions in the retarding of browning by sulphites.

The operation of a cycle of reactions which could effect continuous dehydration of glucose to 3-deoxyhexosone is suggested as being probable; subsequent reactions could lead to the formation of a number of aldehydes.

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CHEMICAL COMPOSITION OF SOME NATURAL AND PROCESSED ORANGE JUICES

By R. SAWYER

The composition of juice from fresh oranges of a type used in the preparation of processed juice, is compared with that of prepared ' pulp extracts ' and manufactured concentrates of varied origin. Some indication of the means of the detection of adulteration in trade concentrates from one particular growing area is given together with a basis for assessment of general quality.

Introduction

During the post-war years a large world trade has grown up in the manufacturing and marketing of citrus fruit products for use in the soft drinks industry. Recently doubts have been cast on the quality of the juice used in their preparation and also on their freedom from adulteration.

Whilst many of the schemes of analysis involving rather more sophisticated determinations are satisfactory when applied to fresh juices, they become of a somewhat restricted value when attempts are made to use them in assessment of processed and preserved concentrates.[1–4] Values which may be affected in varying degrees, by processing and preserving, include such determinations as acid–base balance, cation-exchange capacity,[4] chloramine number and alkalinity of the ash.[5]

In commercial practice the degree of concentration of a processed juice is determined from the refractive index and specific gravity followed by the use of conversion tables approved by the Citrus Fruit Juices Control Board. The addition of sugars enhances the refractive index and specific gravity and hence may falsify the apparent degree of concentration obtained from conventional tables. Examination of the juices, by paper chromatographic techniques, has yielded useful information on the nature and relative quantity of various sugars present.

The waste products, obtained after normal commercial production of best quality (rosehead) juice, yield on extraction the so-called ' peel juice ' or ' rag extract ', which may be used with the true juice to obtain increased yields of a lower grade product.

Analytical values such as peel number,[6] flavone number[7] and hesperidin content[8] have been used to show evidence of the presence of peel constituents, in order to gain some indication of the quality of the juice. In the scheme of analysis described below the evidence of the presence of peel has been derived from the determination of the products of acid hydrolysis

of pectin. A scheme of analysis has been devised after consideration of the results of analysis of juices extracted from fresh oranges and of concentrates of guaranteed quality.

Preparation of fresh juices

In order that some assessment of processed juices could be made and in the face of rather scanty and variable data on fresh juices, a series of samples of fresh oranges of the type used in preparation of commercial concentrates was obtained, and these were extracted in the laboratory.

Three batches of oranges from the early, mid- and late season crops were obtained from the Central Mediterranean area (subsequently designated Area 'A'). The fruits were drawn from bulk factory supply in the country of origin, and carried into this country by air in crates. Samples of the first two batches were subdivided by visual inspection into ripe and unripe fruits; the third batch was uniform. Samples approx. 20 lb. in weight were taken and the fruits cut into halves at right angles to the axis. The normal first-quality juice was extracted with a domestic rosehead extractor and the volume of juice so obtained was noted.

The combined peel and pulp residue was then macerated with a volume of water equivalent to twice that of the first-quality juice to prepare a rag extract. To obtain a product similar to that obtained by pressing, the macerated pulp was centrifuged through a layer of butter muslin in a basket centrifuge. The aqueous extract, so prepared, was collected and the specific gravity determined. All analytical operations were carried out immediately after preparation and a reserve was kept in deep-freeze during the period of the analysis. All analytical results obtained on the extracts were calculated back to a specific gravity equivalent to that of the parent first-quality juice so that a direct comparison could be made.

Methods of analysis

Specific gravity.—This was measured at 20° by means of a standardised glass hydrometer in the range of 1·000–1·100 or by specific gravity bottle; directly on the fresh juices or on suitable dilutions of the concentrates.

Refractive index was determined at 20° by means of an Abbe instrument.

Sugar content.—The reducing value, before and after total inversion by acid at 68°, was determined on solutions previously clarified by the use of dialysed iron. The titrations were carried out under the constant-volume conditions of the Government Laboratory modification of the Lane & Eynon procedure.[9] Results are expressed in terms of invert sugar. Polarimetric readings before and after inversion were made on clarified solutions under the conditions of the Clerget determination of sucrose.[10]

Paper chromatography was carried out on 5% w/v solutions of the concentrates by the method of Buchan & Savage.[11]

Acidity.—The acidity of the juices was determined by direct titration of a 10-ml. aliquot, with 0·5N-sodium hydroxide to a phenolphthalein end-point; the concentrates were similarly titrated after suitable dilution of a weighed amount with boiled distilled water. Use was made of comparison solutions without indicator to which equivalent amounts of sodium hydroxide were added from a second burette. Results are expressed in terms of citric acid monohydrate.

Ash.—The ash was determined by heating a 50-ml. aliquot of the single-strength juice, or 10 g. of the concentrate after drying, in a muffle furnace at 520° ± 20° in a platinum dish. The process of combustion was considerably speeded up and rendered easier to control by the preliminary evaporation and charring of the samples under infra-red lamps. The combustion period was governed by the time necessary to produce a carbon-free ash.

After being weighed, the ash was dissolved in 50 ml. of 0·1N-sulphuric acid and the solution was made to a volume of 100 ml. The excess acidity of an aliquot was back-titrated with 0·1N-sodium hydroxide to a phenolphthalein end-point and the alkalinity of the ash is expressed in terms of ml. of N alkali equivalent to 1000 ml. of juice.

Calcium.—The calcium was precipitated as oxalate from an aliquot portion of the ash solution adjusted to pH 4·6. The precipitate was filtered off, dissolved in hydrochloric acid and reprecipitated as oxalate. The washed precipitate was then dissolved in sulphuric acid

and the oxalic acid titrated with 0·05N-potassium permanganate.[12] Results are expressed in terms of CaO per 100 ml. of juice.

Potassium.—Ten ml. of juice or 5 g. of concentrate were diluted with distilled water through a series of dilutions to give a solution containing 5–10 μg. of potassium per ml., the final dilution being in 0·1N-hydrochloric acid. This solution was then compared with a series of standard potassium solutions on an EEL flame photometer. The potash content is expressed in terms of K_2O per 100 ml. of juice.

Sodium.—A procedure similar to that for potassium was used and results are expressed as Na_2O per 100 ml. of juice.

Phosphorus.—The colorimetric molybdenum blue method as described in A.O.A.C. ' Official Methods of Analysis '[13] was employed on a suitable aliquot portion of the ash solution, or on a separate acid digest from the original solution. The results are expressed as P_2O_5 per 100 ml. of juice.

Total nitrogen.—25 ml. of juice or 5 g. of concentrate were digested with 25 ml. of conc. sulphuric acid and a catalyst consisting of 0·7 g. of mercuric oxide plus 15 g. of potassium sulphate. A small quantity of silicone Antifoam A (Hopkin & Williams Ltd.) was added with the catalyst to reduce foaming. After being made alkaline with sodium hydroxide and sodium thiosulphate[14] the liberated ammonia was distilled into boric acid (three-quarters saturated) and finally titrated with 0·1N-sulphuric acid to methylene blue–methyl red indicator. The results are expressed as N per 100 ml. of juice.

' Albuminoid ammonia value.'—The albuminoid ammonia was defined as the combined organic nitrogen capable of liberation as ammonia by distillation from alkaline permanganate. The ammonia so liberated from 5 ml. of juice or 1 g. of concentrate was determined colorimetrically with Nessler reagent[15] and reported in terms of albuminoid ammonia expressed as N per 100 ml. of juice.

Pentose equivalent.—An indication of the amount of peel and peel extractives was obtained by destructive distillation of the juices and concentrates from 12% w/v hydrochloric acid. One g. of concentrate or 5 ml. of juice was distilled with a constant volume of acid maintained at 100 ml. by means of a dropping funnel, a total distillate of 360 ml. being collected and then diluted to 500 ml.

The furfural produced[10, 16] in the distillate was assayed colorimetrically by use of an orcinol and ferric chloride solution in hydrochloric acid ; various modifications of this technique have been described.[17–19] From the 500 ml. of distillate, 1 and 2 ml. aliquots were taken in stoppered test tubes, and diluted to 3 ml. with 12% hydrochloric acid ; 9 ml. of orcinol solution [orcinol 0·4 g. ; ferric chloride 0·067 g. ; hydrochloric acid (conc.) 160 ml., diluted to 200 ml. with water] were added to the tubes and the mixed solutions were heated in a boiling water bath for 20 min. The intensity of the colour developed was measured at 670 mμ in a 1-cm. cell. The furfural content of the solution was calculated from a reference curve obtained by treating 10 mg. of xylose in the manner of a sample and by using 0, 0·5, 1·0, 1·5, 2·0 and 2·5 ml. of the acid distillate for the colour reaction. The final result is expressed in terms of equivalent xylose content per 100 ml. of juice. It is necessary to correct for the hydroxymethylfurfural produced from sucrose and invert sugar in the samples, 1 g. of sucrose or invert sugar being equivalent to 0·004 g. of xylose under the conditions of test.

Formol titre.—This was determined on 10 ml. of fresh juice or 2 g. of concentrate ; the pH was first adjusted to 7·0 with caustic soda solution (pH meter). The free amino-groups were blocked by the addition of 5 ml. of neutralised 36% formalin and the mixed solution was then titrated with 0·1N-sodium hydroxide to an end-point at pH 8·5 observed with a pH meter.[20] The results are expressed in terms of ml. of N-alkali/litre of juice.

Ascorbic acid.—This was determined on 20 ml. of fresh juice or 2 g. of concentrate dissolved in 20 ml. of boiled distilled water to which 5 ml. of acetone had been added. An equal volume of metaphosphoric acid–acetic acid mixture[21] was added and the mixed solution was titrated with 2,6-dichlorophenolindophenol. The results are expressed in terms of mg. of ascorbic acid per 100 ml. of juice.

Nicotinic acid.—The microbiological assay described by the Analytical Methods Committee of the Society for Analytical Chemistry[22] was used on 20-ml. aliquots of fresh juice or 5 g. of

concentrate. The results are expressed in terms of mg. of nicotinic acid per 100 ml. of juice.

Sulphur dioxide.—This was measured as sulphuric acid by titration with 0·1N-sodium hydroxide after distillation from acidified solution into hydrogen peroxide.[23] The results are expressed in terms of g. of SO_2 per 100 ml. of juice.

Results

Results of the above analysis are shown in Tables I–III. The results on concentrated juices have been calculated to the 'equivalent natural strength juice' by the use of apparent concentration factors obtained from the refractive index vs concentration tables of the Citrus Fruit Juices Control Board.

Table I

Composition of the juice from fresh oranges from area 'A', prepared in laboratory

	Early season		Mid season		Late season	Range	Mean
	Unripe	Ripe	Unripe	Ripe	Ripe		
Specific gravity at 20°	1·040	1·043	1·042	1·043	1·043	1·040–1·043	1·042
Refractive index at 20°	1·3481	1·3488	1·3480	1·3490	1·3499	1·340–1·3499	1·3488
Soluble solids from refractive index, % w/v	10·6	11·1	10·6	11·3	11·8	10·6–11·8	11·1
Reducing sugar as invert, % w/v	3·11	3·47	2·83	3·64	4·41	2·83–4·41	3·49
Total sugar as invert, % w/v	7·39	7·70	6·89	7·57	8·33	6·89–8·33	7·58
Sucrose, % w/v	4·07	4·02	3·86	3·73	3·72	3·72–4·07	3·88
Citric acid + $1H_2O$, % w/v	1·62	1·39	1·55	1·23	0·94	0·94–1·62	1·35
Ash, % w/v	0·35	0·34	0·38	0·38	0·43	0·34–0·43	0·38
Calcium oxide, % w/v	0·017	0·018	0·020	0·022	0·027	0·017–0·027	0·021
Potassium oxide, % w/v	0·193	0·176	0·245	0·236	0·221	0·176–0·245	0·214
Sodium oxide, % w/v	0·0011	0·0011	0·0009	0·0009	0·0019	0·0009–0·0019	0·0012
Phosphorus pentoxide, % w/v	0·028	0·030	0·033	0·036	0·043	0·028–0·043	0·034
Nitrogen, % w/v	0·059	0·074	0·074	0·095	0·095	0·059–0·095	0·079
Albuminoid N, % w/v	0·036	0·047	0·049	0·062	0·064	0·036–0·064	0·052
Pentose equivalent, % w/v	0·05	0·07	0·06	0·05	0·10	0·05–0·10	0·07
Ascorbic acid, mg./100 ml.	52	50	53	54	54	50–54	53
Nicotinic acid, mg./100 ml.	0·22	0·22	0·22	0·28	0·41	0·22–0·41	0·27
Formol titre, ml. N-alkali/litre	12	15	20	20	21	12–21	17
Alkalinity of ash, ml. N-alkali/litre	43	41	47	44	39	39–47	43
Specific rotation before inversion	+2·00	+1·87	+1·80	+1·80	+1·80	+1·80–+2·00	+1·86
Specific rotation after inversion	−1·47	−1·47	−1·40	−1·32	−1·30	−1·30–−1·47	−1·39
pH	2·98	3·08	2·77	2·90	3·60	2·77–3·60	3·07
Total invert/total soluble solids	0·697	0·694	0·650	0·668	0·706	0·650–0·706	0·68
Total sugar/citric acid	4·56	5·53	4·45	6·15	8·86	4·45–8·86	5·91
Sucrose/reducing sugar	1·31	1·16	1·36	1·03	0·84	0·84–1·36	1·14
Albuminoid N/total N	0·61	0·64	0·66	0·65	0·67	0·61–0·67	0·65
Ash/P_2O_5	12·5	11·3	11·5	10·6	10·0	10·0–12·5	11·2
Ash/CaO	20·6	18·9	19·0	17·3	15·9	15·9–20·6	18·3
Ash/K_2O	1·81	1·93	1·55	1·61	1·95	1·55–1·95	1·77
Ash/Na_2O	318	309	422	422	226	226–422	340

Discussion

Examination of results in Tables I and III show that the results of analysis are in general agreement with those reported for juices of varying origin.[24–28] The most significant figures from the point of view of analysis for detection of any adulteration are likely to be those less commonly determined and reported, and hence less commonly accounted for in any serious sophistication of a juice.

(I) *Fresh juices* (results reported on w/v basis)

The ascorbic acid content of the fresh juices is remarkably consistent. Trends in variation of composition during ripening and over the season are indicated in that the gradual increases

of total sugar content, nicotinic acid and pH are coupled with a reduction in citric acid content. Graded increases over the season are also apparent in the P_2O_5 and nitrogen contents and in the formol titres.

(II) *'Rag' extracts* (results calculated to specific gravity equivalent to parent juice and reported on w/v basis)

The most obvious differences between the rag juices and the fresh juices are the generally lower citric acid and ascorbic acid and high nicotinic acid and nitrogen contents. Differences in ash composition are reflected in the ratios of ash/alkali metal oxide. An increase in the pectin constituents is also indicated by the significantly higher pentose equivalent figures. General evidence of nature of a juice may be built up from results of this type.

Table II

Composition of rag extract expressed from fresh oranges adjusted to a specific gravity equivalent to that of the parent fruit juice (fruit from area 'A')

	Early season		Mid season		Late season	Range	Mean
	Unripe	Ripe	Unripe	Ripe	Ripe		
Specific gravity 20° (adjusted)	1·040	1·043	1·042	1·043	1·043	1·040–1·043	1·042
Reducing sugars (as invert), % w/v	4·4	4·6	4·9	5·7	6·5	4·4–6·5	5·2
Total sugar as invert, % w/v	6·4	6·4	6·9	7·7	8·0	6·4–8·0	7·1
Sucrose, % w/v	1·9	1·7	1·9	1·9	2·4	1·7–2·4	2·0
Citric acid $+1H_2O$, % w/v	0·73	0·43	0·53	0·46	0·45	0·43–0·73	0·52
Ash, % w/v	0·67	0·50	0·40	0·43	0·31	0·31–0·67	0·46
Calcium oxide, % w/v	0·108	0·082	0·065	0·083	0·056	0·056–0·108	0·079
Potassium oxide, % w/v	0·192	0·152	0·172	0·183	0·118	0·118–0·192	0·163
Sodium oxide, % w/v	0·0054	0·0045	0·0048	0·0047	0·0039	0·0039–0·0054	0·0046
Phosphorus pentoxide, % w/v	0·049	0·036	0·032	0·037	0·028	0·028–0·049	0·036
Nitrogen, % w/v	0·120	0·091	0·071	0·102	0·076	0·071–0·120	0·092
Albuminoid N, % w/v	0·071	0·063	0·053	0·078	0·051	0·051–0·078	0·063
Pentose equivalent, % w/v	0·33	0·20	0·17	0·28	0·32	0·17–0·33	0·26
Ascorbic acid, mg./100 g.	15	19	14	46	19	14–46	23
Nicotinic acid, mg./100 g.	0·44	0·50	0·38	0·67	0·52	0·38–0·67	0·50
Formol titre, ml. N-alkali/litre	27	12	17	24	15	12–27	19
Specific rotation after inversion	−0·9	−0·9	−1·0	−1·0	−0·9	−0·9–−1·0	−0·96
Total sugar/citric acid	8·8	14·9	13·0	16·7	17·8	8·8–17·8	14·2
Sucrose/reducing sugar	0·42	0·36	0·39	0·33	0·37	0·33–0·42	0·37
Albuminoid N/total N	0·59	0·69	0·75	0·76	0·67	0·59–0·76	0·69
Ash/P_2O_5	13·7	13·9	12·5	11·6	11·1	11·1–13·9	12·4
Ash/CaO	6·2	6·1	6·2	5·2	5·5	5·2–6·2	5·8
Ash/K_2O	3·5	3·3	2·3	2·4	2·6	2·3–3·5	2·8
Ash/Na_2O	124	111	83	92	80	80–124	98

(III) *Concentrated juices* (results calculated to 'equivalent of original juice' and reported on w/v basis)

A marked general agreement between the figures for processed juices from other countries and those for the fresh juices from area 'A' may be observed. The main point of difference is that the contents of sugar and soluble solids in the fresh juices are lower than those calculated for the parent juices of the guaranteed concentrates. Nevertheless the figures obtained on the fresh juices are in good agreement with those published by Cananzi[2] for juices from this area. Since all the observed figures fall within the generally accepted limits for all juices,[28, 29] the observed variations are attributed to the varied origin of the parent fruits. The similarity of ash composition is indicated by the ash/K_2O and ash/P_2O_5 ratios, the slight decrease in CaO being probably due to the loss of calcium pectate in processing and the high sodium figures being due to the use of sodium metabisulphite as a sulphiting agent. (Corrections for the excess Na_2O have been applied to calculation of ash/oxide ratios in Table III.)

Table III

Country of origin	Israel			Spain		W. Indies		S. Africa		Range	Mean
Specific gravity 20°/[a]	1·275	1·280	1·274	1·284	1·290	1·288	1·300	1·295	1·292	1·274 –1·300	
Refractive index 20°/[a]	1·4398	1·4429	1·4407	1·4409	1·4432	1·4411	1·4423	1·4414	1·4404	1·4398–1·4432	
Apparent concentration[a]	6	6	6	6	6¼	6	6	6	6	6–6¼	
Total soluble solids from refractive index, % w/v	12·58	12·92	12·63	12·78	12·50	12·83	13·05	12·92	12·79	12·50–13·05	12·78
Reducing sugar as invert, % w/v	7·26	7·95	7·70	7·00	5·86	8·81	7·15	8·36	7·91	5·86–8·81	7·55
Total sugar as invert, % w/v	9·15	9·80	9·56	8·84	9·16	9·35	9·80	9·40	8·98	8·84–9·80	9·34
Sucrose, % w/v	1·78	1·75	1·76	1·75	3·14	0·52	2·53	0·97	1·02	0·52–3·14	1·69
Citric acid + $1H_2O$, % w/v	1·21	1·07	1·14	1·57	1·27	1·46	1·03	1·38	1·70	1·03–1·70	1·31
Ash, % w/v	0·33	0·33	0·33	0·37	0·33	0·46	0·52	0·45	0·46	0·33–0·52	0·40
Calcium oxide, % w/v	0·014	0·012	0·010	0·013	0·012	0·017	0·016	0·015	0·012	0·010–0·017	0·013
Potassium oxide, % w/v	0·173	0·168	0·168	0·211	0·192	0·269	0·312	0·244	0·258	0·168–0·312	0·222
Sodium oxide, % w/v	0·023	0·025	0·022	0·001	0·002	0·001	0·001	0·022	0·025	0·001–0·025	
Phosphorus pentoxide, % w/v	0·034	0·033	0·034	0·033	0·029	0·038	0·042	0·035	0·036	0·029–0·042	0·035
Nitrogen, % w/v	0·123	0·115	0·113	0·109	0·111	0·073	0·104	0·091	0·101	0·073–0·123	0·104
Albuminoid N, % w/v	0·062	0·067	0·067	0·068	0·065	0·042	0·063	0·045	0·062	0·042–0·068	0·060
Pentose equivalent, % w/v	0·07	0·07	0·06	0·10	0·10	0·10	0·10	0·07	0·07	0·06–0·10	0·08
Sulphur dioxide % w/v	0·022	0·031	0·028	0·021	0·023	0·022	0·019	0·025	0·034	0·019–0·034	0·025
Ascorbic acid, mg./100 ml.	43	35	36	54	52	43	37	56	55	35–56	46
Nicotinic acid, mg./100 ml.	0·37	0·32	0·29	0·36	0·43	0·22	0·21	0·24	0·24	0·21–0·43	0·30
Formol titre, ml. N-alkali/litre	13	15	16	17	17	11	17	13	11	11–17	14
Alkalinity of ash, ml. N-alkali/litre	34	35	35	42	36	53	54	56	55	34–56	44
Specific rotation before inversion	−0·14	−0·46	−0·43	−0·18	+0·79	−1·37	+0·25	−0·99	−0·50	−1·37– +0·79	
Specific rotation after inversion	−1·93	−2·00	−1·99	−1·79	−1·94	−2·04	−2·06	−2·05	−1·95	−2·06– −1·79	−1·97
Total invert/total soluble solids	0·729	0·759	0·754	0·692	0·703	0·731	0·752	0·727	0·702	0·692–0·759	0·728
Total sugar/citric acid	7·56	9·16	8·40	5·63	7·22	6·41	9·52	6·82	5·28	5·28–9·52	7·33
Sucrose/reducing sugar	0·25	0·22	0·23	0·25	0·54	0·06	0·35	0·12	0·13	0·06–0·54	0·24
Albuminoid N/total N	0·50	0·58	0·59	0·62	0·59	0·58	0·61	0·49	0·61	0·49–0·62	0·57
Ash/P_2O_5*	9·1	9·1	9·1	11·2	11·4	12·1	12·4	12·3	11·9	9·1–12·4	11·0
Ash/CaO*	22·1	25·0	31·0	28·5	27·5	27·1	32·5	28·7	35·8	22·1–35·8	28·7
Ash/K_2O*	1·79	1·79	1·84	1·75	1·72	1·71	1·67	1·76	1·66	1·66–1·84	1·74
Ash/Na_2O	14	13	15	370	165	460	520	21	18	13–21 165–520	16 380

[a] Figures on the concentrate as received
* Ash figure corrected for excess Na_2O in this calculation

Detection of sophistication in some concentrated juices

During the course of this investigation 112 samples of concentrated juice from the same growing region as the fresh fruits under 'A' were also examined. All samples were submitted to routine examination by the paper chromatographic technique for the determination of sugars, as a result they were given a broad preliminary classification as follows:

(a) *Samples shown to contain starch hydrolysis products.*—Of the first 33 samples examined, 11 contained amounts of starch hydrolysis products varying from 15 to 25% w/v expressed as liquid glucose. These amounts approximated to 40% of the total sugar found in the individual sample. Furthermore all samples in this group were found to have a positive specific rotation after inversion, this being contrary to experience on all the *bona fide* samples of juice and pulp extracts. In general physical character, these samples resembled juices containing a high proportion of peel extractives. This character was emphasised by comparison of the results shown in Table IV with those in Tables I–III; in particular the pentose values were of a similar order and the ash/alkali-oxide ratios resembled those of the rag juices rather than those of true juice when allowance was made for loss of calcium salts during processing. Owing to the relatively large amounts of sodium oxide present in the juices (up to 0·11% w/v of single-strength juice) corrections were applied to the ash figures in calculating the ratios shown. The amounts of sodium oxide found also greatly exceeded, by a factor of five to ten, that which would have been equivalent to the sulphur dioxide present if the sulphiting had been carried out by additions of sodium metabisulphite. The general picture built up of this group of samples suggested that they were composite articles manufactured from low-grade juice and liquid glucose.

This assessment was also supported by the presence of quantities of orange oil in approximately tenfold excess of that normally found in rosehead juices.

Table IV

Ranges of results obtained on various groups of concentrated juices, from area 'A'

(figures expressed on the basis of single-strength juice)

Type classification†	Samples with liquid glucose		Samples TI/TSS† under 0·74		Samples with TI TSS† over 0·74		Samples with pentose equivalent greater than 0·15	
No. of samples	11		17		73		9	
	Range	Average	Range	Average	Range	Average	Range	Average
Total soluble solids, % w/v	11·4–14·8	13·0	12·2–14·4	13·4	12·3–14·8	13·1	12·3–14·2	13·3
Total sugar as invert, % w/v	6·16–10·00	8·13	8·35–10·30	9·42	9·32–11·50	10·10	7·80–10·13	9·46
Citric acid, % w/v	1·28–1·80	1·49	1·43–2·20	1·81	1·08–1·86	1·55	1·21–1·69	1·47
Calcium oxide, % w/v	0·023–0·034	0·029	0·014–0·025	0·019	0·008–0·048	0·020	0·009–0·034	0·021
Potassium oxide, % w/v	0·147–0·248	0·200	0·222–0·340	0·264	0·094–0·405	0·220	0·184–0·290	0·225
Phosphorus pentoxide, % w/v	0·026–0·074	0·044	0·029–0·075	0·043	0·027–0·080	0·049	0·028–0·071	0·048
Nitrogen, % w/v	0·070–0·086	0·079	0·063–0·113	0·084	0·020–0·093	0·058	0·065–0·088	0·073
Pentose equivalent, % w/v	0·17–0·29	0·21	0·05–0·15	0·11	0·04–0·15	0·09	0·16–0·33	0·19
Ascorbic acid, mg./100 ml.	27–45	39	25–63	45	8–69	33	22–56	36
Nicotinic acid, mg./100 ml.	0·22–0·29	0·25	0·13–0·33	0·22	0·05–0·40	0·17	0·16–0·42	0·25
Formol titre, ml. N-alkali/litre	5–12	10	8–18	12	2–16	8	5–13	11
Specific rotation after inversion	+1·6–+5·1	+2·8	All negative		All negative		All negative	
Total invert/total soluble solids	0·511–0·750	0·64	0·660–0·740	0·70	0·741–0·880	0·77	0·634–0·766	0·73
Ash/P_2O_5*	7·4–14·3	10·6	7·8–17·1	12·4	4·0–17·0	9·4	7·1–13·4	10·2
Ash CaO*	13·3–19·0	15·0	19·0–31·9	27·1	7·2–55·3	21·9	15·3–43·7	24·7
Ash K_2O*	2·10–2·46	2·26	1·76–2·70	2·04	1·62–2·88	2·07	1·93–2·39	2·45

† See text

* Ash figure corrected for excess of Na_2O in this calculation

(b) *Samples containing apparently excessive quantities of sucrose.*—By examination of the sugar chromatograms, two other concentrates were found to contain excess of sucrose: the maximum ratio of sucrose to invert sugar found in the examination of the fresh juices was 1·3 : 1 which agreed with that reported elsewhere.[2, 28, 30] However, the two in question were found with sucrose/invert ratios of 1·5 : 1 and 2·8 : 1 respectively, which corresponded to additions of sucrose of 10 and 40% respectively of the total sugar found in the samples. Correspondingly low values for ascorbic acid, nitrogen, nicotinic acid and formol titration were obtained, whilst the ash/alkali metal oxide ratios were normal and the total invert/total soluble solids ratios were 0·77 : 1 and 0·81 : 1 respectively. Both samples appeared to be good-quality juice diluted with sucrose or partially inverted sucrose.

(c) *Others not shown by chromatography to have added sugar.*—The remaining samples were further classified after the preliminary screening by paper chromatography, by subsequent consideration of the following figures: (i) total invert sugar (TI); (ii) total soluble solids from refractive index (TSS); (iii) pentose equivalent. By the use of the range values obtained on the fresh juices, the 95% limits for values of TI/TSS and pentose equivalent were calculated from the figures for the fresh juices in Table I by the method of Grubbs & Weaver.[31] The upper individual 95% confidence limit for TI/TSS obtained was 0·74 and for pentose equivalent 0·15; and as a result the results for the remaining samples of concentrate were further subdivided as follows: (1) ratios of TI/TSS less than 0·74 : 1, pentose equivalent less than 0·15 : 1; (2) ratio of TI/TSS greater than 0·74 : 1, pentose equivalent less than 0·15 : 1; (3) pentose equivalent greater than 0·15 : 1. The remaining results in Table IV are presented as a result of this breakdown. All the samples in group c(1) with the exception of one sample were apparently satisfactory from all points of view, the exception being atypical in having the lowest ascorbic acid, nicotinic acid and nitrogen contents of the range, but with extremely high ash-content and mineral figures at the upper extremities of the ranges quoted.

Group c(2) was by far the largest group with 73 samples in all. The results quoted show extremely wide ranges for nicotinic acid, ascorbic acid, formol titre, nitrogen, phosphorus pentoxide and potassium oxide. In many cases the levels of nicotinic acid, ascorbic acid and nitrogen fell to below one half of those found on normal juices whilst mineral figures fluctuated from one extreme to the other. This group appeared to consist, in part, of apparently good quality samples and, also, of samples which had been sophisticated to a major or minor degree by addition of invert sugar, mineral salts, and probably citric acid.

The remaining samples group c(3) appeared to be comprised of juices with the physical appearance and chemical indications of concentrates prepared from low-grade fruit and pulp juice.

Conclusions

Consideration of results obtained from all the samples of the concentrates from area 'A', other than those which could be shown by paper chromatography to have foreign sugar (liquid glucose) present, showed that the mineral figures and citric acid contents were most likely to indicate sophistication; this was made particularly obvious when sodium salts were found in amounts far in excess of those expected from sulphiting agents. Further consideration of the results showed a relationship between nicotinic acid and the TI/TSS ratio, although the values for these two parameters on fresh juices and the *bona fide* concentrates showed an apparently random relationship.

By statistical analysis of the plot of nicotinic acid against log (TI/TSS) for the 101 concentrates from area 'A' not shown to contain liquid glucose, a significant negative correlation was obtained over the range of values experienced in our examination.

The data showed a correlation coefficient $r = -0.60$ for 101 samples which proved to be highly significant at the 0·1% probability level by Student's t test. Calculation of the equation for the regression of nicotinic acid on log (TI/TSS) gave:

$$\text{Nicotinic acid, mg./100 ml.} = -1.80 \log (\text{TI/TSS}) - 0.0298$$

The mean values for the two ranges being 0·184 mg./100 ml. for nicotinic acid and 0·761 : 1 for TI/TSS ratio, distribution data for these two parameters are shown in Tables V and VI.

Table V

Distribution of results for nicotinic acid on 101 trade samples of concentrate from area 'A'

Nicotinic acid, mg./100 ml. (original juice basis)	No. of samples	% of total
0·06 and under	4	3·96
0·08 ,,	7	6·93
0·10 ,,	10	9·90
0·12 ,,	13	12·87
0·14 ,,	24	23·76
0·15 ,,	32	31·68
0·16 ,,	39	38·61
0·18 ,,	58	57·42
0·20 ,,	76	75·25
0·22 ,,	81	80·20
0·24 ,,	88	87·13
0·26 ,,	92	91·09
0·28 ,,	92	91·09
0·30 ,,	94	93·07
0·32 ,,	96	95·05
0·34 ,,	97	96·04
0·36 ,,	97	96·04
0·38 ,,	99	98·02
0·40 ,,	100	99·01
0·42 ,,	101	100·00

Table VI

Distribution of results for TI/TSS on 101 trade samples of concentrate from area 'A'

TI/TSS	No. of samples	% of total
0·63 and under	1	0·99
0·64 ,,	1	0·99
0·65 ,,	1	0·99
0·66 ,,	4	3·96
0·70 ,,	4	3·96
0·71 ,,	5	4·95
0·72 ,,	10	9·90
0·73 ,,	16	15·84
0·74 ,,	22	21·78
0·75 ,,	45	44·55
0·76 ,,	65	64·36
0·77 ,,	79	78·21
0·78 ,,	85	84·16
0·79 ,,	86	85·15
0·80 ,,	86	85·15
0·81 ,,	89	88·12
0·82 ,,	90	89·11
0·83 ,,	94	93·07
0·84 ,,	96	95·05
0·85 ,,	98	97·03
0·86 ,,	99	98·02
0·87 ,,	100	99·01
0·88 ,,	101	100·00

Analysis of the pooled results obtained on the 14 *bona fide* samples of fresh juice and concentrates, by random selection into groups of 2×7 results, gave the following 95% confidence limits for the means.[31]

$$\text{Mean nicotinic acid} = 0.29 \pm 0.04 \text{ mg./100 ml.}$$
$$\text{Mean TI/TSS} = 0.71 \pm 0.02$$

The 95% confidence limits for individual values were also obtained as follows:

$$\text{Nicotinic acid} = 0.29 \pm 0.14 \text{ mg./100 ml.}$$
$$\text{TI/TSS} = 0.71 \pm 0.05$$

Comparison of the ranges obtained from the pooled results with the figures obtained on the area 'A' concentrates showed that some 30% of the concentrates fell below the lower 95% limit for nicotinic acid and above the upper 95% limit for TI/TSS.

In view of the negative correlation between nicotinic acid and log (TI/TSS) obtained for the samples of concentrates from area ' A ' and the non-correlation for the others, the conclusions to be drawn are as follows :

(*a*) the relation between TI/TSS and nicotinic acid becomes significantly correlated by dilution of juices with sucrose or partially inverted sucrose ;
(*b*) samples of concentrate giving TI/TSS of 0·76 and over, and having nicotinic acid of 0·15 mg./100 ml. of original juice and under, warrant a detailed investigation.

An indication of general quality may be obtained from the pentose equivalent figure.

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RELATIONSHIP BETWEEN ISOTOPICALLY EXCHANGEABLE CALCIUM AND ABSORPTION BY PLANTS*

By P. NEWBOULD

When soils are suspended in solutions of calcium chloride labelled with radioactive tracer, isotopic exchange with the labile soil-calcium occurs rapidly. This may be followed by a slow secondary exchange reaction, but its magnitude is not great and equilibrium is nearly, if not completely, attained within 7 days.

When, however, plants are grown in soil throughout which carrier-free calcium-45 (^{45}Ca) has been thoroughly mixed, the ^{45}Ca absorbed by the plants equilibrates with a quantity of soil-calcium larger than that which undergoes isotopic exchange when soils are suspended in solutions of labelled calcium chloride. The analysis of plants grown for varying periods shows that equilibration can continue for several weeks, and that the quantity of soil-calcium with which the ^{45}Ca is associated can be increased by growing plants under ' exhaustion ' conditions. In five soils, the ' extra ' calcium which equilibrated with ^{45}Ca in this way never exceeded 3·5% of the total soil-calcium, and was usually considerably lower.

The continued equilibration of ^{45}Ca with soil-calcium causes the specific activity (^{45}Ca/stable Ca) of the calcium entering plants to decrease. Because the calcium in plant roots has, on average, been absorbed more recently than that in shoots, the latter show higher specific activities.

The causes of these effects are discussed, and their significance in the interpretation of results of experiments which involve the use of ^{45}Ca as a tracer in research work on soil/plant relations is considered.

Introduction

It is well known that in most soils the exchange complex is largely saturated with calcium ions, and that there is a small concentration of soluble calcium salts in the soil solution. The calcium ions in these two categories, as distinct from those present in insoluble minerals, have been described as the ' labile pool '.[1–3] In other words, while it is impossible to say which particular ion will be absorbed by plants, it is generally accepted that only ions from this pool may be absorbed at any time. Extraction of a soil with N-ammonium acetate at pH 7, the method used for many years for assessing the quantity of calcium available to plants, measures the size of this labile pool to a good approximation. Values determined by this procedure correlate reasonably closely with the availability of calcium as determined by the yield of plants and/or their content of calcium. Thus this procedure has been of great practical value.

More recently it has become possible to measure the quantity of labile calcium ions in a soil by isotopic exchange. This quantity (the *E*-value) is closely similar, in most comparisons, to the quantity of calcium extracted by N-ammonium acetate. It is only in very acid soils and in soils containing calcium carbonate that any major divergences occur. In the latter case for example the *E*-value is greater than the value determined by N-ammonium acetate extraction.[3, 4]

This suggests that the relationship between isotopically exchangeable calcium and absorption by plants is a simple one. However, as emphasised by Scott Russell,[5] plants absorb ions only from the soil solution, and the most important single factor which controls uptake is the thermodynamic potential or activity of the ions in the soil solution. Thus, the size of the labile pool is only of importance when considering the long-term situation. This suggests a less direct relationship between the isotopically exchangeable calcium (calcium ions both in the solution and on surfaces in the soil) and the calcium absorbed by plants. On this basis, it is expected that there will be a good fit between the two quantities only when there is a constant relationship between the quantity of ions in the labile pool and their chemical potential. Variations in the availability of calcium in the labile pool may occur, however ; Allaway[6] and Mehlich & Colwell[7] reported that all the calcium ions held on clay surfaces were not equally available to plants, although they could all be displaced with N-ammonium acetate.

The desirability of a closer study of the reactions of calcium in soil arose from experiments in which carrier-free ^{45}Ca was incorporated into the soil in which plants were subsequently

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grown. If the labile pool of calcium is a simple physico-chemical entity, a constant specific activity of calcium (^{45}Ca/ordinary Ca) should be absorbed by the plants. This however was not so. There was evidence that calcium which was not isotopically exchangeable when soils were shaken with a solution of labelled calcium chloride was part of the labile pool utilised by plants. The present investigation was therefore undertaken.

The size of the labile pool of calcium in a series of soils was characterised by measurements of isotopically exchangeable calcium and by extraction with N-ammonium acetate. The values obtained by these methods were compared with each other and with estimates of the size of the labile pool which plants growing in the soils appear to utilise. The latter estimate can be made by determining the amount of calcium from the soil with which added radioactive calcium is diluted when absorbed by plants[8] or by following changes with time in the specific activity of calcium absorbed from a labelled soil.

Experimental

Soils.—Five soils which had a wide range in calcium content were used (Table I).

Table I

Origin and calcium content of the soils investigated

(All calcium values in mequiv./100 g. of soil)

Soil No.	1	2	3	4	5
Place of origin	Harwell, Berks	Larne, N. Ireland	Banbury, Oxon	Sandford, Oxon	Dorchester, Oxon
Geological description	Greensand	Basalt	Middle Lias	Kimmeridge	Gault
pH (in 0·1N-KCl)	5·8	4·7	7·2	7·4	7·3
Isotopically exchangeable calcium[1]	10·4	15·3	26·0	26·9	39·8
Calcium extracted by N-ammonium acetate[2]	11·0	14·7	22·1	24·0	37·1
5N-HCl[2]	11·7	16·2	38·4	330·0	50·3
Total calcium by alkali fusion	26·9	69·9	57·1	335·0	62·7
Calcium present as $CaCO_3$[3]	0	0	12	240	8

[1] Mean values from four or more experiments carried out over a period of 18 months are shown. The maximum deviation from the mean in any single experiment never exceeded 5%.

[2] By the method of Peech *et al.*[12]

[3] By estimation of the CO_2 released when the soil was treated with 11N-HCl.

Measurement of isotopically exchangeable calcium

Five-g. portions of soil were suspended in 60 ml. of labelled 0·004N-calcium chloride solution and shaken for varying periods on an end-over-end shaker. The suspensions were then centrifuged and calcium was determined in the solution by titration with Versene.[9, 10] Calcium-45 was measured by precipitation of the oxalate which was slurried on to aluminium dishes and counted beneath an end-window Geiger-Müller counter.

On the basis that the ratio of ^{45}Ca/Ca in the solution was the same as that in the labile pool of calcium in the soil at equilibrium, the amount of isotopically exchangeable calcium could be calculated from the equation

$$E = y(x_t/y_t) - x$$

where x and y are the amounts of Ca and ^{45}Ca in the initial solution and x_t and y_t the amounts after shaking with the soil for time t^2.

Plant culture

Barley, cabbage and ryegrass were grown in pot culture. The soil (210 g.) together with acid-washed silica sand (420 g.) was mixed with carrier-free ^{45}Ca in a planetary mixing machine. The pots were set out at random in a greenhouse. Shoots and roots were harvested separately, except for ryegrass where the shoots only were sampled at successive intervals of time.

Determination of Larsen values

The plant material was wet-ashed in nitric and perchloric acids. Calcium was determined by precipitation as oxalate and titration with potassium permanganate,[11] and ^{45}Ca by the method already described.

The extent (L) to which the added ^{45}Ca had been diluted by soil calcium on absorption by the plant was estimated by the Larsen procedure, using the equation

$$L = (X_p - D)Y_f/Y_p - X_f$$

where X_f and Y_f are the quantities of calcium and ^{45}Ca added to the soil, X_p and Y_p the total plant content and D is the calcium content of the seed.[8]

Results

Isotopically exchangeable calcium

The time course of isotopic exchange in Soils 1–5 was measured for periods up to 14 days (Fig. 1). In all soils, an initial fast exchange reaction occurred within 1 day; thereafter there were only small changes except in Soil 4 which contained the largest total amount of calcium as well as calcium carbonate.

In a further experiment, the extent of isotopic exchange was measured over a period of 42 days in Soils 1 and 4. In Soil 1 no increase in isotopic exchange was observed, but in Soil 4 exchange continued at a slow rate. Since the secondary exchange reaction only occurred in a soil which contained calcium carbonate, it is possible that it was due to the slow penetration of ^{45}Ca into the crystal lattice of calcium carbonate.

In subsequent discussion for the purpose of characterising each soil, the E values after shaking for 7 days, when the initial fast exchange reaction was complete, are alone used.

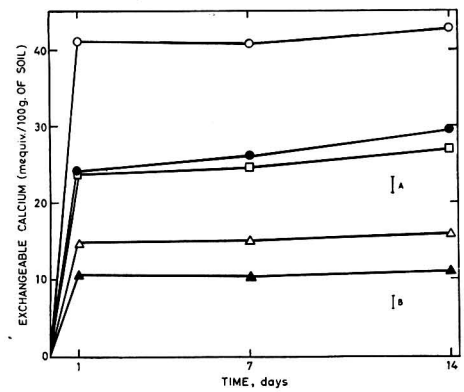

FIG. 1.—*Effect of the duration of equilibration on isotopically exchangeable calcium in Soils 1–5*

Soil 1 ▲ Soil 2 △ Soil 3 □ Soil 4 ● Soil 5 ○

A Soils 3–5 S.D. = 0·05, L.S.D. = 1·91
B Soils 1–2 S.D. = 0·06, L.S.D. = 1·03

Effect on the E *value of the addition of lime to air-dry soil*

Lime (CaO) was added to the soil at a rate of 5·36 mequiv. of Ca/100 g. of soil, equivalent to 1½ tons incorporated to the depth of 6 in. per acre of land. The limed soil was kept air-dry for 20 days and the E value was then determined. Expressed as percentages of the quantity of calcium added, the increments in E value caused by the addition of lime were 63, 89, 36, 4 and 0% respectively for Soils 1 to 5. Thus only a fraction of the added calcium remained isotopically exchangeable. This may have been due to the precipitation of calcium as carbonate especially in Soils 3, 4 and 5 which were of highest pH and contained the largest amount of isotopically exchangeable calcium.

Comparison of the E *value with chemical measurements of soil-calcium*

The quantities of calcium extracted from soil by N-ammonium acetate are compared with the amount of isotopically exchangeable calcium (Table I). The results for Soils 1, 2 and 5 by the two methods are within 8%. For Soils 3 and 4 the E values were 18% and 12% higher than the quantity of calcium extracted with ammonium acetate. Other workers have also found higher E values in soils containing calcium carbonate.[4]

The calcium content of the soil determined by extraction with 5N-hydrochloric acid,[12] the total calcium measured by fusion analysis, and the quantity of calcium present in the form of carbonate are also shown in Table I. The results show that in all the soils there is a considerable amount of calcium which is not part of the labile pool as measured by either isotopic exchange or extraction with ammonium acetate.

Absorption of soil-calcium and calcium-45 by plants

In one of the early experiments, barley and cabbage were grown in Soils 1–5 which had been labelled 49 days previously with carrier-free ^{45}Ca (3·64 μc per pot). The soil was kept moist from the time the tracer was added until the conclusion of the experiment. A standard nutrient, consisting of an initial application of 63 mg. of phosphate as potassium dihydrogen phosphate added with the tracer and 10 ml. of 1% KNO_3 applied weekly, was given to all soils (5 replicates). Barley was harvested after 35 days; because of the slower initial growth, cabbage was harvested after 42 days.

The L values were measured for each crop in each soil. The specific activity of calcium was determined in the roots and shoots separately and also in ammonium acetate extracts of the soils at the conclusion of the experiment. The results are shown in Table II. The E values, which had been previously determined for the soils, are shown for comparison. In each soil the two crops gave similar L values; these exceeded the E values by 15% (Soil 3)–35% (Soil 1). Thus an appreciable quantity of soil-calcium, which did not undergo isotopic exchange with ^{45}Ca in solution, was accessible to the plants.

Table II

Comparison of isotopically exchangeable soil-calcium in Soils 1–5 with Larsen values found when barley and cabbage plants were grown, for 35 and 42 days respectively

(The specific activities of calcium in shoots and roots and in ammonium acetate extracts of the soil at the end of the experiments are also shown)

Crop		Soil 1	2	3	4	5	S.D. (P = 0·05)
Isotopically exchangeable calcium		10·4	15·3	26·0	26·9	39·8	
Larsen value (mequiv. of Ca/100 g. of soil)							
Barley		14·10	19·27	29·80	32·09	48·73	2·39
Cabbage		14·11	18·45	30·26	32·01	48·56	
Specific activity of calcium (mμc of ^{45}Ca/mg. of Ca)							
Barley	Shoot	6·37	4·89	2·94	3·02	1·94	0·56
	Root	5·78	3·21	2·61	1·65	1·48	
Ratio :	shoot/root	1·05	1·52	1·12	1·83	1·31	
Cabbage	Shoot	6·34	5·11	2·96	2·84	1·78	0·56
	Root	3·28	4·06	2·29	2·21	1·94	
Ratio :	shoot/root	1·93	1·25	1·29	1·28	0·91	
Ammonium acetate extract of soil at end of experiment		6·07	4·49	2·50	1·72	1·66	

The specific activity of the calcium in roots was always significantly lower than that in shoots. Because all the soils contained calcium which was not isotopically exchangeable (Table I), the possibility was examined that soil particles, which contain unlabelled calcium, may have remained attached to the roots. It was concluded, however, that the lower specific activity in the roots found in this (Table II) and other experiments could not be attributed to contamination with soil-calcium.

An alternative explanation was suggested since calcium in plant roots has, on average,

been absorbed more recently than that in shoots; calcium moves only unidirectionally from the root to the shoot.[13] The specific activity in the shoots would, therefore, be higher than that in the roots, if the equilibrium of ^{45}Ca with soil-calcium had continued throughout the growth of the plants, thus progressively lowering the specific activity of the soil-calcium available to the plants. An experiment was therefore undertaken to study how the specific activity of calcium absorbed by plants changes with time.

Changes with time in the specific activity of calcium absorbed by plants and the effect of depletion of calcium in the soil

Ryegrass was grown in pots of different sizes so that the soil became depleted of calcium to varying extents. The use of ryegrass had the advantage that the shoots could be sampled at successive intervals so that the changing ratio of ^{45}Ca to calcium absorbed by the roots of the same plant could be followed. Soils 1 and 5 were used in pots containing 20 or 210 g. of soil mixed with sand. A solution of nutrients containing 55 mg. of magnesium sulphate, 106 mg. of potassium nitrate, 38 mg. of sodium nitrate and 20 mg. of potassium dihydrogen phosphate, was added once weekly to all the pots, irrespective of size. The effect of the addition of nutrients to the soil on the size of the labile pool of calcium will be described elsewhere.[3]

Samples of the shoots were taken after 32, 60, 88 and 122 days; the last three samples contained little tissue which had developed before the previous sampling. The soil was sampled on all harvesting occasions, except that at 60 days. The experiment was carried out in quadruplicate.

The quantity of calcium absorbed by plants at the end of the experiment represented the following percentages of the exchangeable calcium originally present in the soil:

Soil No. 1	small pots	31	large pots	6
Soil No. 5	,, ,,	16	,, ,,	2

The depletion of calcium was thus considerably greater in the small than in the large pots. Moreover, the figures for the small pots underestimate the depletion of the soil in which the majority of the roots were growing because the application of water and nutrients at the base of the pots led to the greatest root development in the lower 2 cm. of soil. At the end of the experiment young secondary roots were proliferating in the upper few cm. of the small pots, but not of the large ones. This was attributed to exhaustion of the soil in which the primary roots had developed.

The specific activity of calcium in plants and in ammonium acetate extracts of the roots and the soil is shown in Figs. 2 and 3 for Soils 1 and 5 respectively. In the small pots, the specific activity of calcium in the shoots reached a minimum value at the 80-day sampling and then rose at the final harvest; this increase may have been due to active root development in the relatively undepleted upper layers of soil. The values both for the roots and the soil in which the plants had grown decreased throughout the experiment and were considerably lower than those for the shoots at the final harvest. In the large pots, the specific activity of calcium in the plant roots was again lower than that in the shoots but there was little difference between the values for the shoots and for the soil.

It is concluded that the much lower specific activity of calcium in roots than in the soil, on all but one occasion, in both sizes of pot was due to local depletion of the soil in the neighbourhood of the active roots.

The horizontal broken lines on Figs. 2 and 3 show the ratio of the ^{45}Ca added to the total soil-calcium estimated by alkali fusion. This is the lowest possible value for the specific activity of calcium either in the soil solution or in plants unless calcium of a considerably higher specific activity has been previously absorbed. In the small pots the specific activity of the calcium in extracts of the roots sank below this value, as did also that in soil extracts for the small pots of Soil 1.

These results show clearly that the action of plant roots caused the specific activity of labile calcium in soil to fall progressively and that the depletion of the soil enhanced this effect. Therefore it might be expected that L values would increase according to the quantity of calcium absorbed by the plants. To investigate this possibility the results from a number of experiments were examined.

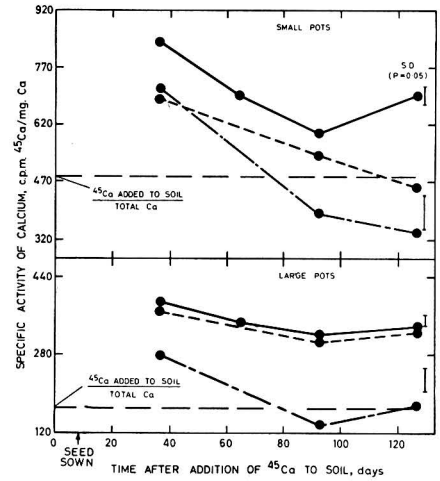

FIG. 2.—*Specific activity of calcium in the shoots of ryegrass grown in Soil* 1 *and in the* N*-ammonium acetate extracts both of the soil and of the roots*

Plants were grown for varying periods in two sizes of pots
Significant differences are shown (P = 0·05)
Plant shoots ——— Soil - - - - - - - Roots — · — · —

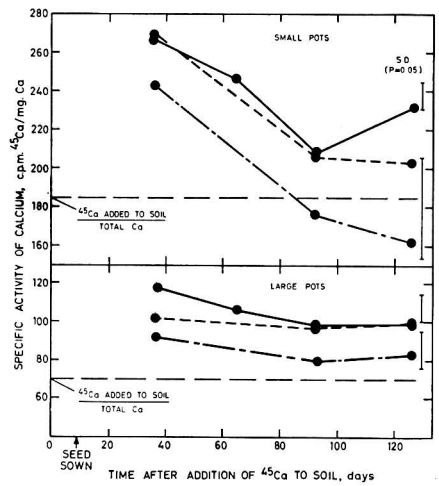

FIG. 3.—*Specific activity of calcium in the shoots of ryegrass grown in Soil* 5 *and in the* N*-ammonium acetate extracts both of the soil and of the roots*

Significant differences are shown (P = 0·05)
Legend as Fig. 2

Effect of the total quantity of calcium absorbed by plants on the L *value*

The results were available for four experiments (12 comparisons because of the different ages of the plants) with Soil 1 in which the quantity of calcium absorbed ranged from 2·61 to 21·81 mg. per 210 g. of soil, and the L values were 10·52–15·13 mequiv. of Ca/100 g. of soil. The E value for this soil was 10·4 mequiv. of Ca/100 g. of soil. The relationship between the quantity of calcium absorbed (x) and the L value (y) is shown in Fig. 4. The linear regression of L on the quantity of calcium absorbed was calculated. The regression equation was $y = 0{\cdot}177x + 11{\cdot}2$ with a correlation coefficient (r) of 0·70. This was significant at the 0·01% probability level. Sufficient results are not available for the other soils to be examined in the same way, although a general relationship of this type was apparent.

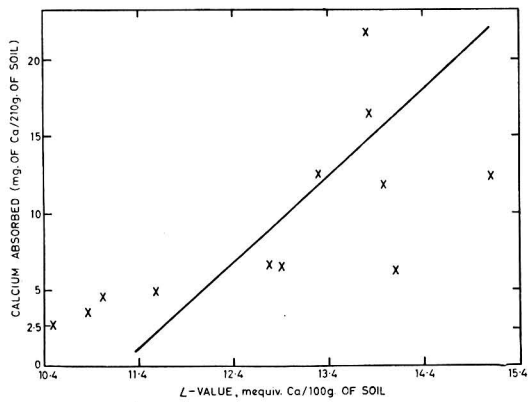

FIG. 4.—*Relationship between the quantity of calcium absorbed by barley plants grown in Soil* 1 *and the* L *value*

Discussion

Measurements of isotopically exchangeable calcium made on suspensions of soils in labelled solutions (E values) suggest that added ^{45}Ca equilibrates in the soil in a relatively simple manner and rapidly, except in soils where the slow secondary exchange could be attributed to the presence of calcium carbonate (Fig. 1). The total amount of isotopically exchangeable calcium was very similar to the amount extracted by N-ammonium acetate (Table I). These results accord readily with the view that the calcium immediately accessible to plants in soils can be regarded as a simple homogenous source or labile pool.

When plants were grown in the soil, however, this interpretation of the behaviour of labile calcium proved inadequate. The specific activity of calcium which plants absorbed was lower than that which could be expected from measurements of isotopically exchangeable calcium or ammonium acetate extraction. Moreover, the specific activity of the calcium entering plants decreased with time (Figs. 2 and 3), the extent of this effect being enhanced by the exhaustion of the soil (Figs. 2–4). In the extreme case it caused the specific activity of calcium both in extracts of soil and in plant roots to fall below the ratio of the ^{45}Ca added to the total calcium as measured by alkali fusion.

At first sight the results of measurements of the E value appear irreconcilable with observations made when plants were grown in the soil. The E values changed little after 7 days, whereas the specific activity of calcium absorbed by plants decreased with time. It appears probable that this difference could be due to the contrasting concentrations of calcium in the solution phase in the two types of experiments. For the determination of E values, the concentration of calcium in the solution was raised considerably above that in the soil solution

to permit accurate measurement. When plants were grown for determinations of L value, no calcium was added to the soil, and the continued equilibration of calcium was accelerated by the depletion of the soil-calcium through absorption by the plants. Since this continued equilibration appears to be dependent on a low concentration of calcium in the solution phase, the measurement of exchangeable calcium by the method here described appears to provide incomplete information on the behaviour of calcium under normal conditions.

Estimates of how much of the soil-calcium which is not initially in an exchangeable form may eventually be absorbed by plants can be made in the following way. If no ' extra ' calcium became exchangeable while plants were growing, the mean specific activity of the calcium absorbed by plants would be the ratio of the added ^{45}Ca to the exchangeable soil-calcium as measured by the E value technique. The ' extra ' calcium which became exchangeable can, therefore, be calculated from the total quantities of ^{45}Ca and stable calcium absorbed by the plants, the quantities of ^{45}Ca added to the soil in which they were grown and the content of isotopically exchangeable calcium in the soil. Calculations of the ' extra ' calcium which became exchangeable have been made on this basis for the experiments illustrated in Table II and Figs. 2–4. When the quantity of ' extra ' calcium is expressed as a percentage of the total calcium in the soil (as measured by alkali fusion), it is found that, except under the extreme exhaustion conditions when plants were grown in small pots for prolonged periods, the ' extra ' soil-calcium in no case exceeded 0·8% of the total soil-calcium (average 0·2%). Under conditions of extreme exhaustion the values rose to 3·1 and 1·7% for Soils 1 and 5 respectively. It is thus apparent that only a very small fraction of the non-exchangeable calcium in soils was absorbed by plants.

Although the experiments described here provide no direct information on the origin of the ' extra ' calcium, it is possibly derived from sparingly soluble componnds. The precipitation of compounds of this type probably explains the failure of lime added to Soils 1–5 to increase the E value in direct proportion to the quantity of calcium added (see above) ; the reverse effect would account for the present results. The relatively slow rate of equilibration of calcium present in these sparingly soluble forms will depend on the rates of solution and diffusion of the calcium ions. It has been suggested recently that the action of 2-ketogluconic acid, which is produced by micro-organisms in the rhizosphere, may release calcium from such compounds ;[14] investigations on this aspect are now in progress.

This investigation indicates the importance of assessing the rate at which calcium can enter plants from the soil relative to that at which labile calcium adjacent to the root can be replenished by diffusion. If the latter process is slower, depletion will rapidly occur. The fact that under exhaustion conditions the specific activity in plant roots was considerably lower than that of samples of soil, suggests that diffusion of calcium was the limiting factor ; it points to the fact that equilibria observed in even quite a small volume of soil in which plants are grown may not reflect at all closely the conditions at the soil/plant interface. In this connexion it should be noted that Wiersum[15] has concluded that 5% or less of the total volume of soil is usually in direct contact with plant roots and functions as a source of plant nutrients. The ' extra ' calcium which was rendered exchangeable as a result of the activity of plant roots in the present experiments was always a considerably smaller fraction of the total calcium in the soil.

If the rate of migration of calcium to the plant/soil interface exerts a considerable effect on the equilibration of labile calcium with sparingly soluble forms, the ratio in which ^{45}Ca and stable calcium are absorbed by plants could vary depending on the quantity of calcium which enters per unit area of root surface. Interspecific variations could therefore arise. Significant differences in the specific activity of absorbed calcium were on occasion observed between barley and cabbage during the present investigation ; there is, however, insufficient information on the pattern of absorption in their roots to show whether this explanation is applicable.

Conclusions

Although the simple concept that plants absorb calcium from a single homogenous source in the soil is inadequate to explain the results obtained in the present experiments, there is no reason to believe that the acceptance of this concept will lead to erroneous interpretations

of practical situations. Under field conditions it appears that either the amount of calcium extracted by N-ammonium acetate or the *E* value will be an equally good measure of the size of the labile pool of calcium in soils. The secondary equilibration of ^{45}Ca with sources of calcium over and above that described as exchangeable is too small to invalidate such assessments. It is mainly of importance in assessing the results of tracer studies, especially if the object is to compare changes in the availability of calcium with that of other ions which have been added to the soil.

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BEHAVIOUR OF CARRIER-FREE PHOSPHORUS-32 IN NATURAL SOILS IN RELATION TO THE MEASUREMENT OF LABILE SOIL PHOSPHORUS*

By D. GUNARY†

The process of isotopic equilibration in perfused crumbs from five soils has been studied by following changes in the specific activity of the perfusates. The soils vary considerably in the times required for isotopic equilibrium with phosphate ions in the perfusate and in the penetration of labelled phosphate into the soil crumbs.

A 55% drop in isotopic dilution was brought about by a reduction from 23° to 3° of the temperature at which crumbs from one soil type were equilibrated in a static system. This temperature drop doubled the time required to achieve uniform distribution of ^{32}P.

It is concluded that the rate of isotopic equilibration in pot experiments will be slow and may be limited in certain soils by the slow rate of redistribution on a micro scale of unevenly applied ^{32}P.

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Introduction

When labelled phosphate is added to a soil suspension, isotopic exchange will take place according to the scheme first applied to soil by McAuliffe *et al.*[1]

$$^{32}P \text{ (solution)} \rightleftharpoons {}^{31}P \text{ (solid)}$$

Under these conditions isotopic exchange consists of at least two processes: (1) a fast reaction which may be completed within a few hours; (2) a slow reaction which may take weeks to reach equilibrium. The above process may be used to calculate the size of the labile pool of phosphorus in the soil by isotopic dilution; this is often referred to as the E value.[2]

The labelled phosphate may also be added to intact soil possessing a crumb structure as in pot experiments for L value determinations,[3] where isotopic dilution is measured from a sample of a plant growing in the treated soil. In this case the process described by McAuliffe may be further complicated as follows.

$$^{32}P \text{ (solution)} \rightleftharpoons {}^{31}P \text{ (crumb surface)} \rightleftharpoons {}^{31}P \text{ (crumb centre)}$$

Thus for complete isotopic exchange ^{32}P must migrate from the surface to the centre of soil crumbs. In this way isotopic equilibrium may be delayed or even prevented from occurring during the growing period, the L value thus being dependent upon the time of sampling.

The experiments to be described are an attempt to develop and employ a technique which allows the process of isotopic exchange to be studied in soil with an intact structure.

Materials and methods

Soils

Five soil types were used: their principal properties were as follows.

	Soil No.				
	219	144	1R	191	157
pH	7·2	7·1	6·0	5·4	5·0
Organic carbon, %	3·0	3·4	6·8	2·4	29·6
Clay, %	29·7	34·5	23·2	29·9	21·8

Treatment of soil with labelled phosphate

Soil crumbs were treated with carrier-free $H_3{}^{32}PO_4$ either by spraying with a chromatography spray or by immersion of the crumbs in a labelled phosphate solution. Radioautographs of sections of labelled crumbs showed that the latter method provided a more even distribution of ^{32}P on the surface of the crumbs.

Perfusion

^{32}P-treated soil crumbs (20 g.) were perfused in 400 ml. of well aerated 10^{-2}M-calcium chloride solution in the apparatus of Simms & Collins.[4] The perfusate was sampled at intervals, each sample being replaced by a similar quantity of 10^{-2}M-calcium chloride. The isotopically diluted phosphate was then calculated from the relationship:

$$\text{Isotopically diluted phosphate} = \frac{\text{Activity added}}{\text{Specific activity of sample}}$$

$$\text{Where specific activity sample} = \frac{\text{Activity in sample}}{\text{Total P in sample}}$$

Phosphate determinations were carried out by the method of Fogg & Wilkinson[5] and radioassay was by liquid counting using a 20th Century Electronics G.M. tube type M.6.H. At the end of the perfusion, crumbs were air-dried for radioautography.

Incubation

^{32}P-treated soil crumbs were placed for the appropriate period in Petri dishes together with sufficient 10^{-2}M-calcium chloride just to cover them. At sampling the specific activity of the supernatant liquid was determined before crumbs were air-dried for radioassay.

Air-drying of soil crumbs

Ideally, after incubation or perfusion the distribution of ^{32}P would be examined within normally moist crumbs, but in order to obtain a radioautograph of the face of a section of a soil crumb, it was necessary first to air-dry the crumbs and then impregnate them with resin. During air-drying a certain amount of bulk flow of soil solution towards the outside of the soil crumbs will take place. As a very high proportion of the labelled phosphate within the crumbs will, during the course of isotopic exchange, have become adsorbed on to the soil particles, the movement of the soil solution will not markedly change the distribution of ^{32}P.

Preparation of radioautographs

Single layers of air-dried soil crumbs were carefully impregnated with Bakelite resin in $2 \times \frac{3}{4}$ in. specimen tubes and sections were cut from the impregnated crumbs with a resinoid-bonded diamond cutting wheel so that, as far as possible, an equatorial slice was taken from each crumb. If a slice was taken $1{\cdot}5 \pm 0{\cdot}1$ mm. thick variations in radioactivity at a section face were within $\pm 4\%$ of the value for a section 1·5 mm. thick. In some instances sections were mounted on to microscope slides and ground down to a thickness of about 0·1 mm. with emery paper mounted on a lapping wheel. Kodirex *X*-ray film was exposed to a cleaned flat face of all sections ensuring the best possible contact between section face and *X*-ray film. Exposures, which varied from 30 min. to 120 h., were adjusted to allow for radioactive decay.

Experimental treatments

A summary of the experimental treatments is given in Table I.

Table I

Summary of experimental treatments

Expt. No.	Soil type	Crumb size (dia.), mm.	Labelling method	^{32}P level, μc per g. of soil	Temperature, °c	Duration, days	Equilibration method
1	219	0·5–2·0† 2·0–3·3†	Spray	1·0	Laboratory	31	Perfusion
2A	219*	3·0–4·0	Spray	1·1	3°, 23°	42	Incubation
2B	219*	3·0–4·0	Spray	1·1	Laboratory	42	Perfusion
3	219, 144 1R, 191 157	4·0–6·3*	Immersion	7·9	23°	42–84	Perfusion
4	219, 144 1R, 191 Artificial and natural crumbs‡	4·0–7·0	Immersion	2·6	23°	28	Incubation

* Natural soil crumbs
† All crumbs produced by regranulation of fine soil
‡ Artificial crumbs produced by regranulation of fine soil

Results

Influence of crumb size on rate of isotopic dilution

There was little apparent effect of crumb size on rate of isotopic dilution and in neither case was isotopic equilibrium reached during the 31 days' perfusion.

Influence of temperature on ^{32}P movement and isotopic dilution

The results of this experiment are shown in Fig. 1. Temperature had a large influence on both the degree of isotopic dilution and on rate of movement of ^{32}P : the isotopically diluted phosphate at 3° was only 55% of that at 23°. A radioautograph of sections of the crumbs showed that ^{32}P initially concentrated on the surface of the crumbs attained uniform distribution after $1\frac{1}{2}$ weeks at 23° and $3\frac{1}{2}$ weeks at 3°.

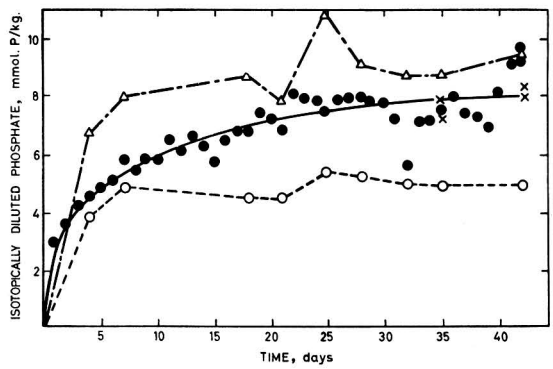

FIG. 1.—*Isotopic dilution during perfusion and incubation of soil 219*

●●● ^{32}P-tagged crumbs, perfused : daily sampling, room temp.
××× ^{32}P-tagged crumbs, perfused : sampled twice, room temp.
○○○ ^{32}P-tagged crumbs incubated in Petri dishes at 3°.
△△△ ^{32}P-tagged crumbs incubated in Petri dishes at 23°

Influence of phosphate removal on isotopic dilution

In this experiment the soil was perfused in two apparatuses. Samples were taken daily from one, but only one sample was taken from the other during the 6 week's perfusion. The amount of phosphate removed in the former case was 0·417 mmole of P per kg. of soil and in the latter case was 0·023 mmole of P per kg. This difference had little effect on the amount of isotopic dilution (Fig. 1).

Isotopic dilution and ^{32}P movement in five soils

Results from this experiment are shown in Table II and Fig. 2. At the beginning of the experiment ^{32}P was distributed on the surface of the crumbs of all mineral soils, while some penetration of ^{32}P into the crumbs of the organic soil occurred during the 1 h. tagging period. After perfusion, ^{32}P was uniformly distributed within the crumbs of soils 219 and 157, whereas in soils 1R and 191, there was little, if any, movement of ^{32}P. Isotopic equilibrium had apparently been reached in soil 219 after 60 days' perfusion and in soil 157 after 70 days'. In the other soils, which were perfused for a shorter period, isotopic equilibrium was not reached.

^{32}P mobility in natural and artificial crumbs

To test whether differences in porosity between crumbs of different soils might influence the mobility of phosphate, a comparison was made between natural and artificial soil crumbs. Resulting radioautographs after 4 weeks' incubation showed no influence of crumb type on ^{32}P mobility.

Table II

Information obtained during perfusion of five soils

	Soil No.				
	219	144	1R	191	157
Duration of perfusion, days	84	66	57	41	84
Phosphate desorbed during perfusion, mmol. of P/kg.	0·90	0·14	0·05	0·02	2·91
P concn. in perfusate, p.p.m. of P					
3 days	0·37	0·07	0·04	0·02	0·50
41 days	0·32	0·06	0·02	0·01	0·37
P isotopically diluted day 1 as % of that diluted day 41	29·1	18·6	10·9	11·2	52·8

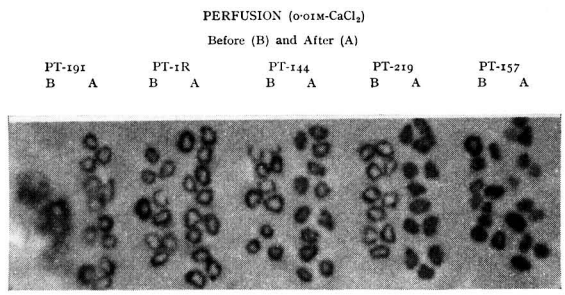

FIG. 2.—*Distribution of ^{32}P before (B) and after (A) perfusion of soils with* 10^{-2}M-$CaCl_2$

Soil	191	1R	144	219	157
Time of perfusion (days)	41	57	66	84	84

Discussion

The process of isotopic dilution during perfusion of intact soil crumbs is very slow. In no soil was isotopic equilibrium obtained in less than 7 weeks. By perfusing surface-tagged small crumbs in comparison with large crumbs, the importance during isotopic dilution of ^{32}P movement within soil crumbs should become apparent. There was, however, no marked effect of crumb size on rate of isotopic dilution. The soil used in this experiment was one in which uniform distribution of ^{32}P, even in large crumbs, occurs in 11 days so this conclusion may not be extended to soils in which ^{32}P movement is slower.

For complete isotopic equilibrium, ^{32}P must become distributed uniformly throughout the soil. The rate at which this redistribution occurs varies greatly from soil to soil. In soils 1R and 191, ^{32}P movement is so slow that little significant change in position can be detected after 6 week's perfusion. In spite of this the specific activity of the perfusate continued to fall throughout the experiment. That is, the process of isotopic dilution continued in spite of apparent lack of mobility of ^{32}P within the crumbs. It is difficult to suggest a simple explanation for this and further investigations are required.

Reasons for differences in mobility of ^{32}P in different soils are not immediately apparent from a study of the soil properties. The porosity of the natural soil crumbs is not responsible, since artificial soil crumbs behave in much the same way as do natural crumbs. The phosphate concentration in the perfusate (Table II) is qualitatively related to the differences in phosphate mobility. The degree of saturation with phosphate of the phosphate sorbing sites in a soil is thought to relate to phosphate fertility.[6, 7] Rennie & McKercher[6] have shown with a few soils that percentage saturation of the soil with phosphate up to 100% saturation is linearly related to phosphate in solution. An expression for the degree of phosphate saturation derived, in a similar way,[8] for the soils used in the present experiment shows that this is related both to phosphate concentration in solution and to ^{32}P mobility.

These experimental findings have a bearing on the determination of L values. Isotopic equilibrium may be attained in many soils, but from this evidence some soils exist which will not reach isotopic equilibrium during the course of a normal L value experiment. An average L value may be calculated (as is usual practice in some places) from a number of successive cuts of the experimental crop, but in most cases this will not compensate for lack of equilibrium.

Some indication has been obtained in these experiments of the relative mobility of phosphate in soil crumbs. This mobility is important with respect both to the supply of soil phosphate to plant roots and to the fate in soil of applied phosphate. Some labile phosphate might thus exist within soil crumbs and, because of lack of mobility, may not be available to the growing plant. On the other hand movement of applied phosphate from the surface to the centre of soil crumbs may considerably reduce its availability.

Conclusions

From the above experiments on isotopic exchange carried out in soil with an intact crumb structure, it is concluded that the process of isotopic dilution is slow and may be limited in certain soils by the slow rate at which ^{32}P, initially distributed on the surface of soil crumbs, becomes uniformly distributed throughout those crumbs.

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NOTE ON THE AVAILABILITY OF MAGNESIUM IN BASIC SLAGS

By S. G. HEINTZE

1. Pot experiments, designed to measure effects produced solely by Mg, showed that two basic slags of similar Mg content and made by the same process differed in the extent to which the Mg was available to grass, and also in the yields of grass obtained, while a slag with much lower Mg content had negligible effect on yield on an acid soil and released no Mg to either acid or basic soil.

2. Additions of calcium carbonate or magnesium sulphate increased yields of grass from an acid, magnesium-deficient soil containing little exchangeable Mg and each increased the uptake of Mg.

3. The amount of magnesium released when soils were incubated with slags agrees with results from pot experiments.

Introduction

The magnesium status of soils and plants has received less attention than the status of some of the minor elements. On the Continent[1, 2] and Scandinavia,[3, 4] magnesium-deficiency has long been recognised in agricultural crops on certain soils, but in England until recently it has been mainly associated with horticultural crops grown in glasshouses where large applications of potassium fertilisers may lessen uptake of magnesium. However, since the incidence of hypomagnesaemic tetany in cattle and sheep appears to be increasing and since there is evidence that this disorder is related to the magnesium status of the herbage,[5, 6] there is obvious need for more information on supplies of magnesium for crops. As basic slags are commonly used on grassland, it was of practical interest to estimate the availability to grass of magnesium contained in slags.

Results of other workers have not been consistent. Thus Gericke,[7] who worked with basic slags artificially enriched with or impoverished in magnesium, claimed that on magnesium-deficient soils, the magnesium of slag was as effective as that of magnesium sulphate. Hasler & Pulver[8] in contrast concluded, from pot experiments with oats and lucerne, that effects on yield from slag applied to acid soils could be ascribed to liming rather than to increased availability of magnesium; they further showed that magnesium sulphate increased the yield of lucerne on alkaline soils more than slag. Both crops also took up more magnesium from both types of soils when given magnesium sulphate. From several years of field trials comparing effects on the yields of a range of crops from slag or magnesium sulphate applications, Sluijsman[9] concluded that only about 20% of the total magnesium contained in slag was available to crops.

The following experiments differ from those described above by having a design which allows the availability of magnesium to be estimated without interference from effects of the phosphate and calcium in the slags.

Experimental

The four soils, all sandy loams, contained little exchangeable magnesium. Field crops with signs of magnesium-deficiency had been observed on all except Gloucestershire 2. Table I gives some relevant soil data. One Bessemer-process and two open-hearth process slags were used, with total and 2% citric-acid-soluble contents of phosphorus, calcium and magnesium shown in Table II.

Table I

Characteristics of soils

	pH	Exchangeable magnesium, mequiv.-%
Bagley (Oxon)	4·00	0·07
Gloucestershire		
1	5·06	0·26
2	6·81	0·37
Hockwold (Cambs)	7·40	0·14

In the pot experiments 400 g. of soil was mixed with 200 g. of quartz. A basal dressing of 50 mg. of N and 50 mg. of K as ammonium nitrate and potassium sulphate respectively was mixed with the soil before sowing and this dressing was repeated after the second cut. Slags and monocalcium phosphate were added to supply 15 mg. of P or, in one experiment, 30 mg. of P per pot. Calcium carbonate and magnesium sulphate were added at three levels, alone and in all combinations: Ca_0, no added $CaCO_3$; Ca_1, 0·160 g. of $CaCO_3$ per pot; and Ca_2, 0·320 g. of $CaCO_3$ per pot. Mg_0, no added magnesium; Mg_1, 10 mg. of Mg per pot, and Mg_2, 20 mg. of Mg per pot. The pots were placed in saucers to which water was added daily. The test crop was perennial ryegrass. Three experiments were made on Bagley soil and one each on the other soils. The treatments were replicated four times. In the incubation experiment, amounts of slags equivalent to 10 mg. of P per 100 g. of soil were mixed with the soil and the mixture was held at 50% water-holding capacity at about 20° for 1 and 2 months. Calcium was determined by flame photometer and magnesium by the Scott & Ure[10] direct photometric method.

Table II

Total calcium and magnesium and citric-acid-soluble magnesium in basic slags

Slag no.	Process	Total Ca, %	Total Mg, %	Mg soluble in 2% citric acid solution, %
1	Open hearth	31	5·4	44
2	Open hearth	27	5·9	31
3	Bessemer	33	1·2	55

Results

(1) *Pot experiments*

On the very acid Bagley soil, germination was very sparse in pots without added calcium carbonate. Magnesium-deficiency symptoms were soon pronounced, and persisted throughout the growing season at all calcium levels without additional magnesium and in pots given Bessemer slag. Slight but distinct deficiency symptoms were visible for similar treatments on the alkaline soil, Hockwold, whereas the two soils from Gloucestershire carried healthy crops. Table III, which gives average yields for the three cuts on Bagley soil, and Figs. 1 and 2, which show 5 weeks' growth of ryegrass on Bagley soil, indicate that ryegrass responded both to calcium carbonate and to magnesium sulphate. Detailed results for other soils are not given. Substantial increases were obtained with calcium carbonate at all magnesium levels, whereas only small further increases were obtained from Mg applied at the higher rate at any Ca level. Slag 1

Table III

Average yields of grass on Bagley soil
(g. of dry matter/pot)

Calcium level	Magnesium levels				Slags		
	0	1	2	Mean	1	2	3
		±0·104		±0·060		±0·104	
0	0·01	0·35	0·36	0·24	4·32	3·72	0·13
1	0·15	3·50	3·84	2·49	—	—	—
2	0·76	5·16	5·40	3·74	—	—	—
Mean (±0·035)	0·30	3·00	3·20	2·14	—	—	—

was superior to Slag 2 and Slag 3 had negligible effect on yield. The yield from Slag 1 was significantly below that of treatment Ca_2Mg_1, whereas the increase from Slag 2 was not significantly above that of Ca_1Mg_1. There was no interaction between Ca and Mg on yields at the Mg_1 and Mg_2 levels. The effects on yield with the other soils were smaller, but there was a consistent increase from magnesium at all calcium levels with the alkaline Hockwold soil.

Magnesium uptake

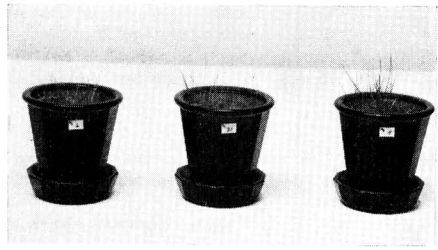
Basic slag may act as a liming material, particularly on acid soils, so it was necessary to allow for such an effect when estimating the availability of magnesium in slags from the amount taken up by grass. The soil pH values for all treatments were therefore determined at the end of the experiment. The magnesium taken up by grass from the slags was estimated from curves relating uptake of magnesium from magnesium sulphate in the presence of dressings of calcium carbonate that raised the pH of the soil to the same value as did the slag. The results with Bagley soil (1st + 2nd cut) are shown in Fig. 3, where the magnesium uptakes from Slags 1 and 2 are plotted on the appropriate calcium carbonate, magnesium sulphate curves. These values thus corresponded to 6·3 and 8·3 mg. of Mg/pot for Slags 1 and 2 respectively, indicating that 28 and 53% of the total magnesium of the respective slags was available to the grass. The average percentage ' magnesium sulphate equivalents ' (amounts of Mg given as sulphate that would have given the same Mg uptake as the basic slags and expressed as percentage of total Mg supplied by the slags) from all experiments were :

	Cut 1	Cut 2
Slag 1	23	23
Slag 2	40	54

On the acid soil (Bagley), Slag 2 continued to release magnesium, whereas on the alkaline soil (Hockwold) the magnesium uptake decreased after the first cut. Increases in calcium levels caused small but consistent decreases in magnesium uptake on the two Gloucestershire and Hockwold soils.

(2) *Incubation experiment*

To obtain independent evidence of the release of magnesium from slags added to soils, two soils were incubated with amounts of slags supplying equal amounts of phosphorus. The



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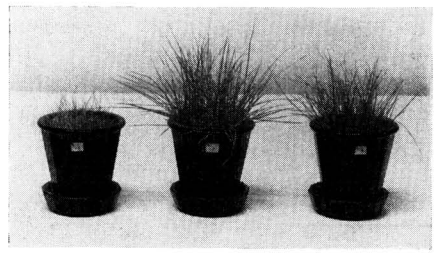

B

C

FIG. 1.—*Ryegrass (5 weeks old) on Bagley soil*

A From left to right Ca_0Mg_0, Ca_0Mg_1, Ca_0Mg_2
B ,, ,, ,, ,, Ca_1Mg_0, Ca_1Mg_1, Ca_1Mg_2
C ,, ,, ,, ,, Ca_2Mg_0, Ca_2Mg_1, Ca_2Mg_2

FIG. 2.—*Ryegrass (5 weeks old) on Bagley soil*
From left to right Slag 3, Slag 1, Slag 2

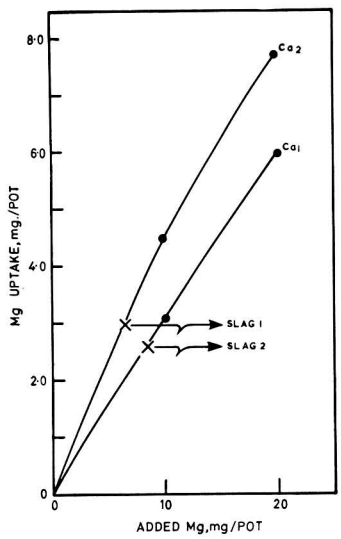

FIG. 3.—*Mg uptake from Slags* 1 *and* 2 *by ryegrass on Bagley soil compared with that from magnesium sulphate in the presence of calcium carbonate producing the same liming effect as the Slags* 1 *and* 2 *respectively*

soils were equilibrated for 1 h. with 0·01M-calcium chloride solution and the amounts of calcium and magnesium determined in the filtrate. The calcium concentration may have varied, especially in the acid soil where calcium may have exchanged with aluminium ions, so the results in Table IV are presented as Mg/(Ca + Mg) ratios instead of Mg concentrations.

Table IV

Ratio of Mg/(Mg + Ca) dissolved in 0·01M-*calcium chloride solution equilibrated with soils which had been incubated with basic slags*

	Slag	[Mg]/[Ca + Mg]	
		1 month	2 months
Hockwold (alkaline)	None	0·0210	0·0195
	1	0·0144	0·0132
	2	0·0377	0·0378
	3	0·0196	0·0191
Bagley (acid)	None	0·0113	0·0139
	1	0·0393	0·0412
	2	0·0739	0·0742
	3	0·0138	0·0151

Comparisons of releases are valid only within each soil because the time of contact of the solution with the soil was 1 h. only, and hence no true equilibrium was reached. Slag 3 released no magnesium in either soil; Slag 1 released none in the alkaline soil but some in the acid one. Slag 2 was a much more effective source of Mg, but there was no gain from doubling the 1 month incubation time. The decrease of magnesium concentration for the alkaline soil after addition of Slag 1 may be associated with the rise in pH from 7·7 to 8·3 and the increase in calcium concentration. The results are in general agreement with those of the pot experiment.

Discussion

Although the results show that part of the magnesium in some basic slags is available to grass, they do not allow such general conclusions on the value of the Mg in raising yields or magnesium uptakes as some authors have drawn. Basic slags made by the same process and with similar total magnesium contents may differ considerably both in the amount of magnesium released to plants and also the rate of release; they also differ in the way they release magnesium soluble in calcium chloride solution when incubated with soils. Adding calcium carbonate alone greatly influenced yield and magnesium uptake of grass on a magnesium-deficient and acid

soil ; this emphasises the need for more extensive work on the interaction between lime dressings and native or added magnesium before any valid general conclusions can be formulated on the effects on the magnesium status of soils and of crops from application of basic slags which can, potentially, supply both calcium and magnesium.

Acknowledgments

Grateful acknowledgements are due to Dr. A. Weir for magnesium estimations and to Miss B. Close for help with the pot experiments.

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AVAILABILITY OF SOIL AND FERTILISER PHOSPHATES TO GROWING CROPS*

By S. McCONAGHY and J. W. B. STEWART

Results are presented of two groups of experiments involving radish and ryegrass crops in different soil types in the greenhouse.

Several extractants were used to assess the 'plant available' phosphate contents of soils. Results by the Olsen bicarbonate and Egnér type extractants correlated well with phosphate uptake by the crops, although Egnér values had previously been found not to correlate well with yields of dry matter when results for basaltic soils were included. Phosphate contents of water extracts classified the soils in much the same order as Olsen soil-P values. Total phosphate uptake was highly correlated with the percentage phosphate saturation of the soils. Phosphate uptake was also highly correlated with the 'isotopic dilution factor'.

The effect of fertiliser phosphate on the uptake of fertiliser P at two levels was studied in three soils using ^{32}P also at two levels. Radioactive phosphate had no effect on total phosphate content of ryegrass but at the highest level (100μC of ^{32}P/pot) it significantly reduced the uptake of fertiliser phosphate.

The *L* value of the soils was increased significantly both by increasing phosphate fertiliser and by increasing the level of ^{32}P incorporated in the fertiliser, although the utilisation of soil phosphate was increased in only one soil—the basalt soil—by the application of phosphate fertiliser. *L* values of the same order were obtained for two soils which differed noticeably in their capacity to supply phosphate for ryegrass growth.

* Paper read at Agriculture Group Symposium, 3–5 April, 1963

Introduction

The phosphate status of a soil may be defined simply as the capacity of the soil to supply phosphate to a growing crop. It would seem straightforward to measure with precision the 'status' as so defined, but in fact it is difficult if not impossible to do so, and, since one crop may not react in the same way as another to a given concentration of phosphate in the soil solution, it is sometimes difficult in practice to even make a reasonable assessment of soil phosphate status. The determination of total phosphate is of little or no value for this purpose and the normal use of chemical extractants, although of considerable value, may give no indication of the rate at which the soil solution is replenished as phosphate is removed from solution by growing plants. In recent years the introduction of radioactive phosphorus compounds has given renewed impetus to the study of soil phosphates and by their use the 'reactive' phosphates in the soil—the so-called 'labile phosphate pool'—is now being measured as isotopically exchangeable phosphate by methods like those of Talibudeen[1, 2] and by determining the uptake of fertiliser and of soil phosphates by growing crops as in the *A* value determinations of Fried & Dean[3] or the *L* value determinations of Larsen.[4, 5] This paper records the results of several methods of assessing soil phosphate status and briefly considers the limitations of radiochemical techniques.

Results of two groups of pot experiments are included. In the first of these, nine arable soils from different localities in Northern Ireland were used to assess their 'phosphate status' and the responses of radish and ryegrass to applied phosphate under standard glasshouse conditions. In the second group of experiments the labile phosphate 'pool' of a similar series of arable soils was determined by use of ^{32}P and the effect of the level of ^{32}P on fertiliser phosphate uptake was also studied.

(1) Group I experiments

Experimental

Each of nine soils was air-dried, sieved through a ¼ in. mesh sieve, and thoroughly mixed. Nine polythene pots 5 in. in diameter were filled, each with 900 g. of each of these soils. Six of the pots received monoammonium phosphate, in solution, three at 61 mg. of P and three at 124 mg. of P per pot. Three received no phosphate. The fertiliser was intimately mixed throughout the soil in shallow aluminium trays. Ammonium sulphate, also in solution, was added up to a standard level. The soil was then replaced in its pot and moistened to field capacity. After 24 h. ten radish seeds were planted in each pot, and on germination these were thinned to five plants. Yields of dry matter of radish leaves and roots were separately recorded. When the radishes were harvested, each soil received 0·2 g. of ammonium nitrate, in solution, and 20 ryegrass seeds were sown. At germination these were thinned to ten plants per pot. The crop was harvested after 7 weeks, dried, weighed and analysed.

Representative samples of the untreated soils were used in laboratory studies of their phosphate status and phosphate sorption capacity. Analyses were as follows:

(i) *Determination of Egnér (modified) phosphate values.* Two g. of soil were shaken for 2 h. at 35 r.p.m. with 100 ml. of a solution of 0·02N-hydrochloric acid and 0·02N-calcium lactate; the extract was filtered and the phosphate content of the filtrate determined by the method of Williams & Stewart.[6]

(ii) *Determination of Olsen soil-P values.*[7] Five g. of soil were shaken with 100 ml. of M/2-sodium bicarbonate solution (pH 8·5) for 30 min., filtered and the phosphate content determined as described by Jackson.[8]

(iii) Determination of total P content after fusion with sodium carbonate.

(iv) Determination of phosphate bound with aluminium, iron and calcium by methods essentially those of Bray & Kurtz[9] and of Williams,[10] and described by Chang & Jackson.[11]

(v) *Water-soluble phosphate contents of the soils.* One g. of soil was shaken with 50 ml. of water for 2 h. The suspension was centrifuged and P was determined colorimetrically in the clear supernatant solution.

(vi) *Phosphate contents of radish roots and foliage and of ryegrass.* The oven-dry sample (0·5 g.) containing a little magnesium acetate was ignited at 500°. The ash was dissolved in

10 ml. of hot N-hydrochloric acid, diluted, filtered and made up to 50 ml. The phosphate concentrations of aliquots of the solutions were determined by the molybdenum blue technique similar to that described by Williams & Stewart.[6]

Results

The soils used in these studies varied in texture from medium-light to medium-heavy loams with pH values between 5·5 and 7·1, there being no free carbonate present.

Analytical results are given in Table I.

Egnér (acid lactate) extractions and Olsen-bicarbonate extractions of the experimental soils both indicated a fairly wide range of available phosphate and both methods classified the soils in roughly the same order of phosphate status. Water-soluble phosphate contents also classified the soils in much the same order as Olsen values. Yields of dry matter and responses of radish and ryegrass to applied phosphate are given in Table II.

Table I

Characteristics of soils used

No.	Soil origin	pH (water)	Texture	Egnér soil value* as P_2O_5, p.p.m.	Olsen soil value as P, p.p.m.	Water-soluble P, p.p.m.	Total P, p.p.m.
1	Interbasaltic laterite	6·0	Medium loam	1	0	4·4	940
2	O.R.S.	6·6	,, ,,	15	10	7·0	560
3	Basalt (a)	7·1	,, ,,	14	12	3·4	1560
4	Glacial sands (a)	5·5	Sandy loam	19	13	4·0	800
5	Basalt (b)	7·1	Medium loam	13	16	4·8	1320
6	Carb. limestone	6·0	,, ,,	40	10	5·8	800
7	Glacial sands (b)	5·5	Medium light loam	49	23	6·4	1100
8	Garden soil	6·3	Medium loam	63	29	16·0	860
9	Silurian slate	6·5	,, ,,	65	18	8·0	860

* Note that Egnér values are expressed as P_2O_5, p.p.m.

Table II

Average yields and responses of radish and ryegrass to applied phosphate

Soil	Olsen soil value	Radish			Ryegrass		
		Dry matter, g./pot (no P added)	Average response to applied P, g./pot	Response to applied P, %	Dry matter, g./pot (no P added)	Average response to applied P, g./pot	Response to applied P, %
1	0	0·90	2·60	300	0·09	0·30	300
2	10	2·90	1·20	42	0·99	−0·14	−14
3	12	3·16	0·41	13	0·56	0·64	114
4	13	3·20	1·16	36	1·11	0·53	48
5	16	3·75	0·44	12	1·29	0·38	30
6	10	3·37	0·93	28	0·72	0·71	99
7	23	5·09	−0·08	−2	1·24	0·51	41
8	29	3·20	0·71	22	1·31	0·05	4
9	18	2·76	−0·50	−19	0·50	−0·01	−2

The poorest yields of dry matter were obtained on those soils with the lowest Olsen and Egnér soil values but there was no significant correlation between control yields and either of these values. When all nine results were considered there was no significant correlation between responses of radish dry matter and the Egnér soil values ($r = -0.636$) although, when results for the two basaltic soils were omitted, the correlation ($r = -0.891$) was significant at the 5% probability level. The correlation between Olsen soil-P values for all nine soils and responses of radish dry matter was significant at this level ($r = -0.716$). The correlation between responses of radish to fertiliser phosphate and of ryegrass to residual phosphate was no better than those between either Egnér or Olsen soil-P values and percentage responses (i.e., responses expressed as percentages of control yields).

Table III records the phosphate contents of herbage and the phosphate uptake values.

Table III

Average phosphate contents of herbage and uptake of P by radish

No.	Radish							Ryegrass					
	Soil-P value		Average % P in dry matter (foliage)			Uptake of P, mg./pot (no P added)	Average increase in P uptake by	% P in dry matter			Uptake of P, mg. P/pot		
	Egnér	Olsen	No P	P1	P2	(Whole plant)	phosphate, mg. P/pot	No P	P1	P2	No P	P1	P2
1	1	0	0·16	0·21	0·30	1·27	7·0	0·16	0·35	0·35	0·15	0·81	1·30
2	15	10	0·19	0·25	0·32	4·95	5·3	0·20	0·28	0·32	1·0	2·30	1·98
3	14	12	0·19	0·22	0·26	6·83	2·9	0·27	0·30	0·31	1·5	3·18	4·12
4	19	13	0·21	0·29	0·34	5·93	6·5	0·26	0·32	0·33	2·89	5·54	5·11
5	13	16	0·22	0·28	0·31	8·90	4·0	0·27	0·32	0·34	3·48	5·38	5·64
6	40	10	0·25	0·35	0·44	8·03	8·5	0·24	0·31	0·38	1·73	4·12	5·78
7	49	23	0·27	0·36	0·37	12·58	4·7	0·32	0·38	0·47	3·97	6·35	8·60
8	63	29	0·39	0·51	(0·39)	11·38	6·0	0·45	0·51	0·51	5·90	6·94	6·89
9	65	18	(0·27)	0·40	0·55	6·78	3·2	0·32	0·29	0·47	1·60	2·49	1·41

There was a highly significant correlation (r = 0·90) between the average phosphate contents of radish foliage receiving no phosphate and the Egnér soil-P values. There was also a similarly good correlation (r = 0·898 ; significant at P = 0·01) between the phosphate uptake by radish in the absence of phosphate and Olsen soil-P values ; between phosphate uptake by radish and by grass the correlation was significant at P = 0·01. These are illustrated in Figs. 1–3.

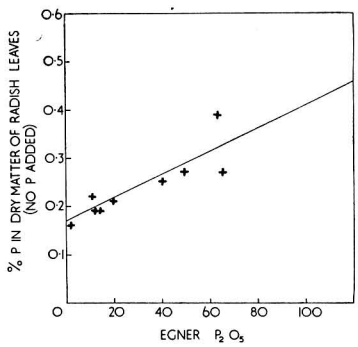

FIG. 1.—*Correlations between Egnér P_2O_5 (x) and P content (%) of dry matter in radish leaves (y)*

line fits equation $y = 0{\cdot}0024x + 0{\cdot}17$

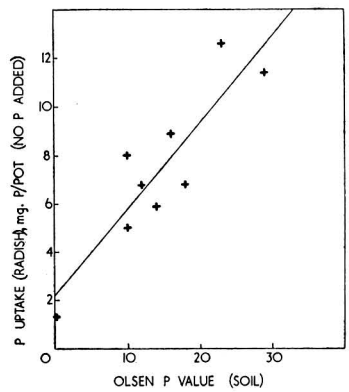

FIG. 2.—*Correlations between Olsen P value of soil (x) and P uptake (y) by radish (no P added)*

r = 0·898** line fits equation $y = 0{\cdot}36x + 2{\cdot}2$

The results in general indicate that Egnér- and Olsen-extractable P contents of the soils correlate rather better with phosphate contents of plants than with yields of dry matter or yield responses to applied phosphate. These extractant methods of assessment must be regarded as superior to assessments based on phosphate contents of plants, since the chemical methods are rapid and predict results (with a fair degree of accuracy), whereas phosphate contents of plants can only be determined after the plants have grown.

The phosphate extracted by a chemical extractant must be affected both by the amount and by the nature of the phosphate present in the soil and it must also be affected by the capacity of the soil to sorb or fix phosphate from solution. Attempts were made to measure these quantities for the nine soils in this experiment.

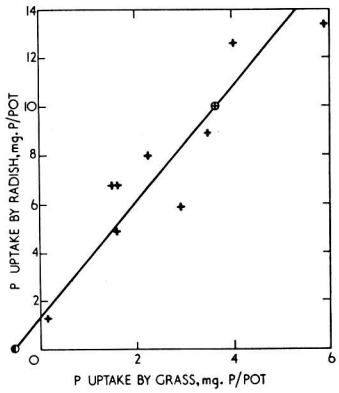

FIG. 3.—*Correlation between P-uptake by rye-grass* (x) *and P-uptake by radish* (y)
r = 0·836** line fits equation $x = 0{\cdot}419y - 0{\cdot}56$

Determinations were made of the contents of phosphate bound to aluminium, iron and calcium in the soils and the sum of these values was taken to represent the inorganic phosphate present in the soil. The extra phosphate which each soil can sorb before saturation was measured by shaking 2-g. samples of soil with 100 ml. of solutions containing 500, 1000 and 2000 μg. of P for 120 h. at 35 r.p.m. The phosphate sorbed was determined from the loss of P in the filtrates. These values were plotted for each soil against the P concentration used, and by extrapolation to the point where the curve levelled off, the amount of P still needed by the soil to reach the maximum P sorption state was estimated (Fig. 4). It may be noted that the sorption capacity of the basaltic soils was so high that this maximum could not be measured with accuracy even when the soils were shaken with concentrations of up to 10,000 μg. of P per g. of soil. The % P saturation of the soils was calculated as the sum of aluminium-, iron- and calcium-bound P expressed as percentage of the maximum P sorption capacity (Table IV).

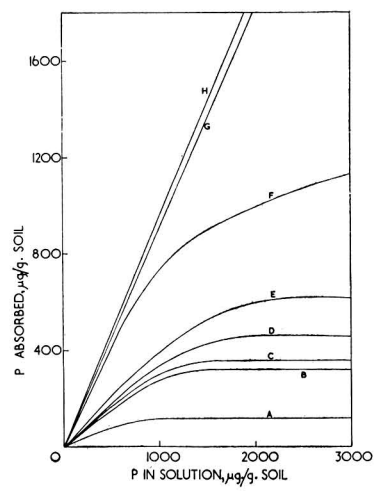

FIG. 4.—*Relation between P in solution and P absorbed by soil*
A (soil 7) E468 B (soil 2) E466 and 469 C (soil 8) E471
D (soil 1) E465 E (soil 3) E473 F (soil 6) E467
G (soils 9 and 4) E481 H (soil 5) E482

Table IV

Egnér and Olsen values and relations to sorption capacity and % P saturation

Soil	Egnér soil value, P_2O_5 p.p.m.	Olsen value, P p.p.m.	Phosphate in soil, p.p.m. Phosphorus combined with Aluminium A	iron B	calcium C	Total A+B+C, p.p.m.	Extra P sorbed to saturate D	Total P sorption capacity, p.p.m. A+B+C+D	P saturation, % (A+B+C) 100 / A+B+C+D
1	1	0	50	138	35	223	1100	1323	16·9
2	15	10	20	75	30	125	620	745	16·8
3	14	12	140	137	95	372	>7500	>7872	<4·7
4	19	13	75	100	35	210	300	510	41·2
5	13	16	258	200	138	596	7500	8096	7·4
6	40	10	50	113	102	265	350	615	43·1
7	49	23	130	188	108	426	460	886	48·1
8	63	29	320	188	175	683	120	803	85·1
9	65	18	85	115	100	300	300	600	50·0

The results reveal the wide differences in the capacity of the soils to remove phosphate from solution and the varying extent to which the phosphate in the soils satisfies the full sorption capacity. It is reasonable to assume that the amount of phosphate extracted from such soils by a dilute solution should be related to the degree of phosphate saturation, and that the ability of plants to obtain phosphate from the soil would be similarly affected by the degree of phosphate saturation.

There is obviously a reasonably good correlation between the % phosphate saturation values and the Olsen-extractable P values, although the Olsen reagent extracted more phosphate from the basaltic soils than the degree of phosphate saturation would predict. The relatively high quantities of calcium-bound phosphate in the two basaltic soils are presumably an important factor in spite of the low degree of P-saturation. There was very good correlation between the Egnér-extractable P values and the % P-saturation (Fig. 5), but it must be emphasised that while the Egnér (and the Olsen) values are useful reflectors of the degree of phosphate saturation, neither method accurately reflected for all the soils the crop responses to applied phosphate or the yields of plants grown in the soils without added phosphates. The two basaltic soils gave better yields of herbage (and better P uptake) without applied phosphate than the Olsen or Egnér values would predict. On the other hand swedes grown on these two soils showed very poor growth and low phosphate uptake in the absence of fertiliser phosphates.

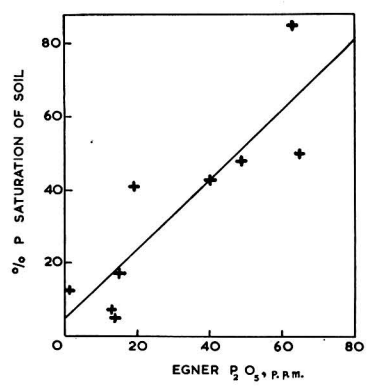

FIG. 5.—*Relation between Egnér value* (x) *and % P saturation of soil* (y)

$r = 0{\cdot}853^{**}$ line fits equation $y = 0{\cdot}929x + 6{\cdot}0$

Isotopic dilution factor

The determination of isotopically exchangeable phosphate values by a variety of techniques gave quite unsatisfactory results for some of these soils, particularly for the interbasaltic and the basaltic soils. Erroneously high values were obtained, some higher than the total phosphate contents of the soils. This was due to the rapid decrease in ^{32}P content relative to ^{31}P of phosphate in solutions with which the soils were being shaken to attain equilibrium. In one case there was a complete disappearance of ^{32}P from solution within a short time. The isotopically exchangeable P value is determined from the formula

$$P_e = {}^{32}P_0/{}^{32}P_t \cdot {}^{31}P_t - {}^{31}P_0$$

where $^{32}P_0$ and $^{32}P_t$ represent the activities of solutions initially and at time t respectively and $^{31}P_0$ and $^{31}P_t$ represent the concentrations of ^{31}P initially and at time t, so if $^{32}P_t$ rapidly decreases to minute values the P_e value becomes very large and obviously erroneous. High values are likely with soils having a high P sorption capacity and Scott Russell *et al.*[12] record an appreciable P_e value for a basic igneous soil which barely supported growth of barley plants without phosphate fertiliser. Amer[13] reports very high values for high P-fixing soils even though it would appear that equilibrium may not have been attained in the short shaking time used by him. It is suggested that such high values are suspect if the ^{31}P content remains measurable.

For this reason determinations were made of what are termed ' isotopic dilution factors ' for each of the experimental soils. This was done by shaking 2 g. of soil for 120 h. with 100 ml. of solution containing 1000 μg. of P as potassium dihydrogen phosphate to which ^{32}P had been added. The concentration of phosphate in solution after this time was determined (*a*) by normal colorimetric methods and (*b*) by radiochemical assay, the difference between the two values being recorded as the isotopic dilution factor (Table V). Tests, previously made by withdrawing small quantities of solution at given time intervals, suggested that the differences in the solution P values as determined by methods (*a*) and (*b*) above were constant after about 4 days' shaking. The radiochemical assay determines the added P still in solution.

Table V

' Available ' phosphate assessments

Soil	Egnér value, P_2O_5 p.p.m.	Olsen value, soil-P p.p.m.	P saturation of untreated soil, %	Uptake of phosphorus without P fertiliser, mg. P/pot			' Isotopic dilution factor ', p.p.m.
				Radish crop	Ryegrass	Total of radish+ryegrass	
1	1	0	16·9	1·27	0·15	1·42	17
2	15	10	16·8	4·95	1·60	6·55	22
3	14	12	<4·7	6·83	1·50	8·33	28
4	19	13	41·2	5·93	2·89	8·82	34
5	13	16	7·4	8·88	3·48	12·36	35
6	40	10	43·1	8·03	1·73	9·76	46
7	49	23	48·1	12·58	3·97	16·55	54
8	63	29	85·1	11·38	5·90	17·28	81
9	65	18	50·0	6·78	1·60	8·38	40

The ' isotopic dilution factor ' shows good correlation with total phosphate uptake (Table V) and appears to provide a useful indication of the ' available ' phosphate status of these soils. For the nine results, the correlation coefficient $r \equiv 0{\cdot}88$ indicates significance at 1% levels (Fig. 6). The isotopic factors (I.D.F.) also showed a similarly good correlation with Olsen soil values (Fig. 7) which in turn reflected accurately the total phosphate uptake of radish and ryegrass in this experiment (Fig. 8).

Effects of applied P on phosphate uptake

The phosphate uptake by the crops (radish and ryegrass) with and without added P are given in Table VI with the % of this phosphate apparently coming from fertiliser sources. The values range from 20 to 80% at the P1 level (62 mg. of P per pot) and from about 28 to 88% at the higher level of applied phosphate. They suggest that fertiliser-P percentage apparently accounts for most of the plant phosphate in the crop grown on the most P-deficient soil, and that an increase in the level of application of fertiliser-P increases, as expected, the percentage

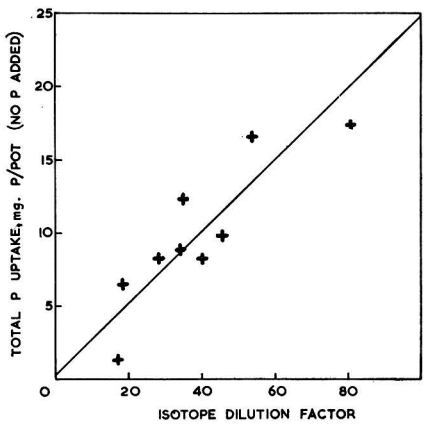

FIG. 6.—*Relation between isotope dilution factor* (x) *and total P uptake from soil* (y)
r = 0·88** line fits equation $y = 0{\cdot}245x + 0{\cdot}35$

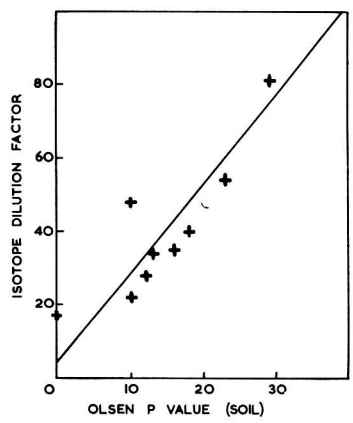

FIG. 7.—*Correlation between Olsen P value for soil* (x) *and isotope dilution factor* (y)
r = 0·902** line fits equation $y = 2{\cdot}44x + 3{\cdot}66$

of plant phosphate assumed to be derived from the fertiliser. This of course does not mean that the recovery of applied fertiliser increases with the level of applied P. The percentage recovery usually falls with increasing rate.

The average recovery rates in these experiments were 8·8% and 7·0% at P1 and P2 level respectively with an overall mean of 7·9% recovery. This average is almost exactly the same as that calculated (8·0%) from results of seventeen field experiments on potatoes in Northern Ireland in post-war years,[14] and although it seems small it is large in comparison with the average percentage of native soil phosphate apparently taken up by the crops in the same experiments. In fact however it may be that even these apparent recovery values are higher than they should be and that the uptake of soil phosphate is underestimated. The values are based on the assumption that the increased P uptake as a result of application of fertiliser-P

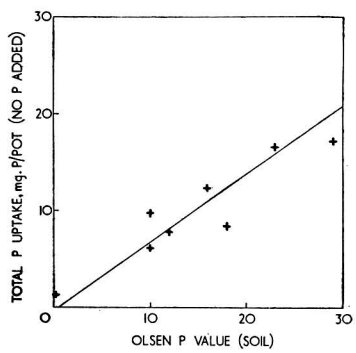

FIG. 8.—*Correlation between Olsen P value for soil* (x) *and total P-uptake by radish and ryegrass* (y)
= 0·909** line fits equation $y = 0{\cdot}7x + 0{\cdot}35$

Table VI

Apparent recovery of applied phosphate

Soil	Total P in soil, mg./pot	Phosphate uptake, mg. P/pot			Total uptake apparent from fertiliser, %		Apparent recovery of applied P, %	
		without added P	with P added at 62 mg./pot (P1)	with P added at 124 mg./pot (P2)	at P1	at P2	at P1	at P2
1	846	1·42	7·12	11·51	80·02	87·65	9·2	8·8
2	504	6·55	9·34	15·36	29·9	46·28	4·5	5·8
3	1188	8·33	11·59	15·07	28·1	44·72	5·3	5·4
4	720	8·82	17·31	18·20	49·0	51·54	13·7	7·6
5	1404	12·38	17·12	19·73	27·7	37·25	7·6	5·9
6	720	9·76	17·96	24·91	45·6	60·81	13·2	12·2
7	990	16·55	22·98	26·54	28·0	37·63	10·4	8·1
8	774	17·28	24·65	24·02	30·0	28·06	11·9	5·4
9	774	8·38	10·45	12·56	19·8	33·26	3·3	3·4

is entirely from fertiliser sources, but this of course is not necessarily the case, since a little fertiliser-P may increase the growth of roots sufficiently to enable them to make better utilisation of native soil phosphate. The tendency therefore is for the contribution that fertiliser-P makes to the plant phosphate to be overestimated by this method of assessment. On the other hand the use of ^{32}P-labelled phosphates to assess the uptake of fertiliser-P (phosphate) by crops may tend to underestimate the fertiliser phosphate contribution, particularly if the presence of ^{32}P isotope has any deleterious effect on the absorption of labelled fertiliser phosphate.

The second part of this paper briefly considers these points.

(2) Group II experiments

Experimental

Italian ryegrass was grown in 5-in. pots in the greenhouse using three different soil types, without added phosphate and at two levels of applied phosphate (*a*) without and (*b*) with ^{32}P which was incorporated at two levels. Monoammonium phosphate was used in solutions to which carrier-free ^{32}P was added where required. Ammonium sulphate and muriate of potash were incorporated to ensure uniform N- and K-levels at all levels of P. The fertilisers were mixed as uniformly as possible with the soil from each pot in shallow dishes. There were four replicates of each treatment. Details of treatments are shown in Table VII. Fifteen ryegrass seeds were planted in each pot and immediately after germination these were thinned to ten seedlings. Moisture contents were carefully controlled and plants were grown for 7 weeks before the first of three harvests was taken. After the first harvest, uniform applications of sulphate of ammonia were given in solution and the plants were grown for a further 8 weeks. Fresh and oven-dry weights of ryegrass were recorded for each pot. The ^{32}P-contents of dry matter were determined by ashing 0·5 g. oven-dry weight at 500°, dissolving the ash in 2 ml. of hydrochloric acid, 50/50, heating, diluting, centrifuging and making up to 10 ml. Five ml. were used for counting ^{32}P in a liquid counter. Similar counts were made of fertiliser solutions (diluted as necessary) at the time of counting the ^{32}P contents of the plant materials. Another aliquot of the 10-ml. solution (= 0·5 g. plant dry matter) was used for the colorimetric determination of phosphate. Counts per minute with necessary background and dead-time corrections were converted into counts per 100 g. of dry matter and, knowing the P content of the dry matter, into counts per 0·1 g. of P in the plant material. Corresponding counts were calculated per 0·1 g. of P in the fertiliser. From these values were determined the proportions of plant phosphate derived from fertiliser and also the *L* values of the soils.[4]

Results

Table VII records the plant phosphate contents from two cuts (bulked) and the quantities of this phosphate derived from the fertiliser in the experiment. The fertiliser-P-uptake values are calculated from the formula $100C_p/C_f$ where C_p and C_f are counts per 0·1 g. of P in the plant and fertiliser respectively. In all three soils the application of phosphate increased the uptake of phosphate by the plants, although significant increases in yield of dry matter at both phosphate levels were obtained only with the phosphate-deficient basaltic soil. The presence of ^{32}P

had no significant effect on the total phosphate content of plants, and at the low level of applied phosphate, ^{32}P had no significant effect on the total amount of fertiliser phosphate taken up in the two cuts of ryegrass. At the higher level of fertiliser phosphate, however, an increase in the level of ^{32}P from 50 to 100 μc. per pot caused a significant decrease in the uptake of fertiliser phosphate.

Table VII

Total phosphate contents of ryegrass (mg. P/pot) *with fertiliser-P and soil-P contents of the plants*

(averages of four replicates; 2 cwt. bulked)

Soil and treatment			Total P in plant	Fertiliser-P in plant	Soil-P in plant	Increase in soil-P due to treatment
	mg. ^{31}P/pot	μc. ^{32}P/pot				
I. *Peat soil*						
A	0	0	0·516		0·516	
B	62	0	0·761			
D	62	50	0·729	0·267	0·462	−0·054
F	62	100	0·794	0·241	0·553	+0·037
C	124	0	0·980			
E	124	50	1·107	0·558	0·549	+0·033
G	124	100	1·071	0·386	0·685	+0·169
II. *Silurian soil*						
A	0	0	0·968		0·968	
B	62	0	1·094			
D	62	50	1·053	0·217	0·836	−0·132
F	62	100	0·979	0·233	0·746	−0·222
C	124	0	1·578			
E	124	50	1·478	0·592	0·886	−0·082
G	124	100	1·597	0·413	1·184	+0·216
III. *Basalt soil*						
A	0	0	0·096		0·096	
B	62	0	0·299			
D	62	50	0·207	0·072	0·135	+0·039
F	62	100	0·295	0·068	0·227	+0·131
C	124	0	0·651			
E	124	50	0·517	0·215	0·302	+0·206
G	124	100	0·563	0·139	0·424	+0·328

Discussion

Many workers[5, 15–17] have found the fertiliser phosphate uptake of plants to be affected by the level of ^{32}P incorporated with a fertiliser and the results in Table VII support these findings. But it is surprising that the effect of ^{32}P is not shown at the lower level of fertiliser although it is significant at the higher level of fertiliser phosphate. It may be mentioned here that one of the authors (J. W. B. S.) in a further series of experiments (in which labelled phosphate was banded in the surface layer of the experimental pots) has found no decrease in the uptake of fertiliser phosphate as the level of radioactive phosphate increased.

It must be realised, however, that of the phosphate in a plant the percentage due to fertiliser-P may decrease even though the actual quantity of fertiliser phosphate in the plant does not change. This could occur where there was an increase in soil phosphate uptake due to the application of fertiliser phosphate, i.e., on phosphate-deficient soils such as the basaltic soil used in this radiochemical experiment. It has been shown, by Spinks & Barber[18] and by Mattingley,[17] that, after the application of fertiliser phosphate, the uptake of soil phosphate does not increase when the crop does not show any further increase in yield. This of course must assume that incorporated ^{32}P does not result in a significant reduction of fertiliser phosphate without affecting total plant phosphate content. The results given in Table VII support such findings, as there was no significant increase in uptake of soil phosphate after applications of fertiliser phosphate on the two soils on which there was little or no yield response to applied phosphate.

In the first part of this paper the increases in phosphate contents of plants due to fertiliser phosphate treatments were expressed as percentages of total phosphate uptake to give values representing the percentages of total phosphate contents apparently due to fertiliser phosphate.

The results in Table VII indicate how erroneous such assumptions may sometimes prove and Table VIII further illustrates this. The increases in phosphate contents of plants grown in the peat soil corresponded fairly closely with the fertiliser phosphate contents, i.e., the uptake of soil phosphate was about maximum. In the other two soils on the other hand there were appreciable discrepancies in the two sets of results (Table VIII). In the phosphate-deficient basaltic soil, the increase in plant phosphate due to fertiliser was much greater than the contribution of fertiliser phosphate assessed radiochemically, confirming the statement made earlier that a little phosphate in P-deficient soils may encourage the growth of roots sufficiently to enable them to utilise more fully the native soil phosphate. In the silurian soil (with the ' highest phosphate status ' of the three experimental soils) fertiliser phosphates appeared actually to decrease the utilisation of soil phosphate (Table VII) except where ^{32}P reduced absorption of the fertiliser phosphate with which it was incorporated.

Table VIII

Fertiliser-P uptake in relation to the increase in phosphate uptake by ryegrass

Soil and treatment mg. ^{31}P/pot	mg. ^{32}P/pot	Total P in plant, mg./pot	Increase in plant P due to fertiliser treatment	Fertiliser-P in plant, mg. P/pot as assessed by ^{32}P
I. *Peat soil*				
A 0	0	0·516		
B 62	0	0·761	0·245	
D 62	50	0·729	0·213	0·267
F 62	100	0·794	0·278	0·241
C 124	0	0·980	0·464	
E 124	50	1·107	0·591	0·558
G 124	100	1·071	0·555	0·386
II. *Silurian soil*				
A 0	0	0·968		
B 62	0			
D 62	50	1·053	0·085	0·217
F 62	100	0·979	0·011	0·233
C 124	0	1·578	0·610	
E 124	50	1·478	0·510	0·592
G 124	100	1·597	0·629	0·413
III. *Basalt soil*				
A 0	0	0·096		
B 62	0	0·299	0·203	
D 62	50	0·207	0·111	0·072
F 62	100	0·295	0·199	0·068
C 124	0	0·651	0·555	
E 124	50	0·517	0·421	0·215
G 124	100	0·503	0·467	0·139

A and L values

The methods introduced by Fried & Dean[3] and by Larsen[4] have been used extensively in recent years to measure the ' labile soil phosphate pool '. It has generally been found that the A or L values tend to increase with increasing level of application of phosphate and also where high levels of ^{32}P are incorporated. The results given in Table IX certainly show substantial increases in L values both with increasing level of fertiliser phosphate and with increasing ^{32}P level. The increases arise from the decrease in ^{32}P content of the plant relative to that in the fertiliser, the L value being calculated from the formula

$$L = \frac{^{32}P \text{ (fertiliser)}}{^{32}P \text{ (plant)}} \cdot (\text{plant P} - \text{seed P}) - {}^{31}P \text{ (fertiliser)}$$

An increase in L value can arise by an increase in uptake of soil phosphate or by a decrease in uptake of fertiliser phosphate, or by a combination of the two. Perhaps this is more readily appreciated where the A rather than the essentially similar L values are calculated, from the formula $A \equiv B$ (uptake of soil-P)/(uptake of fertiliser-P), where B represents the quantity of fertiliser-P applied.

Table IX

Soil L values

(Each is the average of four values)

Soil and treatment			P mg.-atoms/pot			
^{31}P mg.-atoms/pot	^{32}P per pot, μc.		Cut I	Significance P = 0·05	Cut II	Significance P = 0·05
I. *Peat soil*						
D 2	50		2·6		4·6	
F 2	100		3·9	Significant	6·1	Significant
E 4	50		3·2		5·1	
G 4	100		6·0	Significant	9·2	Significant
II. *Silurian soil*						
D 2	50		(5·6)		7·2	
F 2	100		5·0		8·6	Significant
E 4	50		5·0		8·6	
G 4	100		9·1	Significant	17·9	
III. *Basalt soil*						
D 2	50		4·0		3·4	
F 2	100		(4·6)		6·7	Significant
E 4	50		5·1		4·4	
G 4	100		12·4	Significant	12·6	Significant

1 mg.-atom of P = 31 mg.

Increases in *A* (or corresponding increases in *L*) values obtained by the application of phosphate to soils may in some cases genuinely reflect an increased utilisation of soil phosphate where an actual increase in uptake of soil-P occurs without a decrease in the uptake of fertiliser-P due to such factors as radiochemical injury. Such increases are certainly found in the case of the basaltic soil (Table VII) and have been found by other workers, e.g., Spinks & Barber,[18] although these workers could not confirm such results in later work. Mitchell[19] found a steady decline in uptake of soil-P as the application of fertiliser was increased, although in his work the labelled P fertiliser was banded with the seed. The results for the basaltic soil recorded in Table IX show that an increase in fertiliser from 62 to 124 mg. of P per pot at the lower level of ^{32}P resulted in a substantial increase in the uptake of soil-P and at the same time a substantial increase in the uptake of fertiliser phosphate by the plants. Incidentally the increase in uptake of soil-P was 167 mg. of P/pot while the mean *A* value (Fried & Dean) increased by 112 mg. of P per pot. Results in Table IX indicate that *L* values for the basaltic soil are of the same order as for the peat soil. Yet in this experiment the basaltic soil produced little growth without fertiliser phosphate and gave good responses of dry matter to applied phosphate, while the peat soil gave reasonable growth without phosphate and little growth response to fertiliser phosphate. The *L* values obtained at the highest levels of ^{31}P and ^{32}P in this experiment (Tr. G.) are at least double those obtained at the lower levels. This applies for all three soils, although with the peat and silurian soils fertiliser phosphate had no positive effect on the uptake of soil phosphate by the plant. In this experiment the phosphates were mixed throughout the soil. Rennie & Spratt[20] suggest that such a method of application of P is more likely to result in increases in *L* or *A* values than their method of applying their labelled P fertiliser in a band near the seed, i.e., in a small volume of soil, to minimise the effects of phosphate fixation and of isotopic exchange reactions. Recent unpublished results by one of us (J. W. B. S.) seem to give some support to this claim.

The results presented in this paper fully support the findings of Scott Russell *et al.*[12] that *L* values cannot be regarded as a measure of phosphate available to plants. Very high values were obtained for isotopically exchangeable P contents of the interbasaltic and basaltic soils used in these studies, even where a wide soil-solution ratio was used (2 g. of soil + 800 ml. of solution), and as these could not be accepted as real they have not been presented. Results of isotopic dilution experiments are presented however (isotopic dilution factors) which appear to be of some value in assessing availability of soil phosphates. They can be obtained by using a reasonably narrow soil-solution ratio (8 g. of soil/800 ml. of solution) and a reasonably high

concentration of phosphate (^{31}P) labelled with ^{32}P. The results correlate well with values indicating the extent to which the phosphate sorption capacity of the soils is satisfied. Such measures are considered to be an improvement on the reciprocals of sorption capacity. They may be regarded as an indication of what Schofield termed the ' phosphate potential '. They may also be considered as an indication of the probable rate at which the soil can supply phosphate for the needs of plants, while the ' labile phosphate ', determined either by *A*- or *L*-value or as the total isotopically exchangeable phosphate content, indicates the quantity of phosphate in the soil ' pool '. This quantity (in the pool) is by itself of little value in predicting uptake of soil phosphate by plants especially in soils whose sorption capacities vary widely or where there are wide differences in the degree of phosphate saturation. This has been clearly shown by many workers.[12, 21, 22] Thompson and co-workers[21] used % phosphorus saturation as an index of the probable uptake of phosphate by plants, but their method of determining this value was different from the method described in the present paper. Their formula was % P saturation $\equiv 100P_s/S$ where P_s is determined by isotopic exchange and S = P-sorption capacity, but it is doubtful if their P_s values can be accepted, as their method involved a narrow ratio of soil to labelled P solution and a short shaking time which would not be likely to ensure equilibrium conditions. Incidentally these workers found that a simple extraction of soils with water was the most effective of the methods used by them to estimate phosphorus uptake in greenhouse plant studies.

Mattingley[22] (like Russell and others) found that total isotopically exchangeable phosphate and plant uptake of phosphate were highly correlated only for soils with the same ' phosphate capacity ' which he measured by the gradient of the curves relating the negative logarithm of P concentration in 0·01M-calcium chloride to isotopically exchangeable phosphate. Mattingley did note however that on continued cropping the growth and P-uptake of plants became almost independent of soil-P concentration (in calcium chloride solutions) and closely correlated with total isotopically exchangeable phosphate.

The results presented above show that the Olsen bicarbonate extractant is an effective method of assessing the probable growth and phosphate uptake of plants grown in a wide range of soil types. The main difficulty with this method is in accurately and rapidly determining the concentration of phosphate in the bicarbonate extracts which are often dark in colour. The value of water-extractants in predicting crop performances of soils is also limited by practical difficulties in phosphate determinations.

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PHOSPHORUS TRANSFORMATION IN TEJGAON CLAY SOIL, EAST PAKISTAN, UNDER DIFFERENT CULTURAL PRACTICES

By D. H. KHAN* and S. I. CHOWDHURY

The distribution of forms of phosphorus in an acid ferruginous soil after application of superphosphate is studied. Of the P_2O_5 added as superphosphate, 88% was retained by the soil 3 days after application. Most of the superphosphate added was converted into sesquioxide-bound P_2O_5 and adsorbed P_2O_5. Liming the soil increased the Truog's sulphuric acid-soluble P_2O_5 by about 10%. Adsorbed P_2O_5 (hydroxyl-replaceable P_2O_5) rather than Truog's soluble P_2O_5 appeared to be utilised by the jute crop.

Introduction

Soil fertility trials on Tejgaon clay (a soil type of Madhupur red soil tract, East Pakistan) have shown a poor response to phosphate application by the jute crop, which implies either a low phosphate requirement of jute or the unavailability of soil and fertiliser phosphate. Plant analysis shows that jute removes a substantial amount of phosphate from soil, varying from about 57·3 lb. of P_2O_5 per acre (East Pakistan soil condition)[1] to 38·4 lb. of P_2O_5 per acre (Taiwan soil condition).[2] The nature of phosphate fixation in Tejgaon clay appeared, therefore, to be worth studying. Phosphorus transformations in soil and changes in phosphate availability due to chemical processes have not been thoroughly studied in East Pakistan except in a few isolated cases.[3, 4] This paper describes some chemical changes that take place when phosphate is added to soil, which may account for the poor crop response to soil and fertiliser phosphate in the heavy clay soils of East Pakistan.

Experimental

Soil type and experimental layout

The experiment was carried out on Tejgaon clay soil on the Dacca Farm, East Pakistan. Morphological and chemical properties of the soil are shown in Table I. The heavy ferruginous soil has poor nutrient status and a distinctly acid reaction.

Transformations of soils and fertiliser phosphate were studied under different cultural practices as follows:

(*a*) Fallow (with no lime and superphosphate application),
(*b*) Fallow + superphosphate at 60 lb. of P_2O_5/acre,
(*c*) Fallow + superphosphate at 60 lb. of P_2O_5/acre + lime at 1640 lb./acre,
(*d*) Cropped + superphosphate at 60 lb. P_2O_5/acre + lime at 1640 lb./acre.

The treatments are denoted '*a*', '*b*', '*c*' and '*d*' in the Tables and text. The treatments were replicated thrice, plot size being 20 ft. × 15 ft. Cropped plots were laid out in a separate block. A basal application of ammonium sulphate at 60 lb. of N/acre and potassium chloride at 75 lb. of K_2O/acre was given to all the plots. Lime was applied to the specific plots one month before sowing plots in treatment (*d*). NPK fertilisers were applied 3 days before sowing. All the cultural operations (weeding, hand hoeing, etc.) were common to all the plots. *Corchorus capsularis* (variety D-154), a widely cultivated species of jute, was sown in lines 1 ft. apart and 3 in. between plants.

Analytical

Composite soil samples from the three replicated plots were mixed thoroughly before taking a representative sample for chemical study. Phosphorus transformations in soil were studied at intervals of 0, 3, 14, 35 and 56 days after application of fertiliser by measuring the following forms: available, adsorbed, sesquioxide-bound and total P_2O_5. Available P_2O_5 was determined by Truog's 0·002N-sulphuric acid extraction method;[5] adsorbed P_2O_5 by the hydroxyl replacement method of Piper;[6] sesquioxide bound P_2O_5 by Tamm's acid oxalate extraction

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method ;[7] total P_2O_5 by sodium carbonate fusion followed by acid extraction ;[6] organic carbon by Walkley & Black's rapid titration method ;[8] available and total nitrogen as described by Piper ;[6] total calcium on an aliquot of the hydrochloric acid extract by double oxalate precipitation (Piper[6]) ; total iron on an aliquot of the hydrochloric acid extract by the sensitive colorimetric method of Sandell ;[9] and pH by a comparator.

Results

The results in Tables II and III are self-explanatory. Values for phosphate retention shown in Table IV were calculated from the difference between treatments (*a*) and (*b*) expressed as a % of added P_2O_5.

Discussion

The results show that 88% of the added P_2O_5 has been retained by the soil 3 days after applying superphosphate. This high retention of phosphate continued up to 8 weeks after application (Tables II and IV). Heck[10] stated that most of the phosphate added to soils is

Table I

Morphological and chemical properties of the soil

A. Morphology

I. Field description

Locality	Dacca Farm, Tejgaon, Dacca
Genetic type	Podzolic (?)
Soil type	Tejgaon clay
Parent material	Red argillaceous deposits (old alluvium)
Geographic landscape	High land
Vegetation	Cropped with jute, paddy, etc.
Flood level	Above flood level

II. Profile data

Horizon	Depth, in.	Description
A	0–9	Reddish grey clay ; nuts and granules, hard when dry, sticky when moist, red ferruginous concretions present ; drainage medium
B	12–21	Greyish brown clay ; texture getting heavier ; nuts and granules ; sticky ; concretions increasing ; drainage tends to be impeded
	23–26	Reddish brown clay ; nuts and granules ; very sticky ; ferruginous concretion abundant ; drainage slightly impeded

B. Composition

Depth, in.	Moisture, %	Mechanical composition (on air-dry soil) Sand, %	Silt, %	Clay, %	pH	Chemical composition (oven-dry basis) Organic C, %	Available N, mg.-%	Available K_2O, mg.-%	Available P_2O_5, mg.-%	Total N, %	Total P_2O_5, %	Total CaO, %	Total Fe_2O_3, %
0–6	1·6	41·8	18·0	40·2	5·8	0·42	0·70	12·8	5·1	0·059	0·084	0·47	4·89
0–12	0·8	43·8	19·0	37·2	5·3	0·27	0·20	10·0	4·0	0·048	0·077	0·40	7·04

Table II

Distribution of different forms of phosphorus

(Results expressed as mg. of P_2O_5/100 g. of soil)

	Treatment *a*	*b*	*c*	*d*
Just before fertiliser application				
Available	6·1	6·1	6·1	6·1
Sesquioxide-bound	12·7	12·7	12·2	12·2
Adsorbed	17·1	17·1	16·1	16·1
Total	81·8	81·8	80·9	80·9

After fertiliser application

	3 days *a*	*b*	*c*	*d*	14 days *a*	*b*	*c*	*d*
Available	6·0	6·6	7·0	7·3	6·2	6·6	7·1	7·8
Sesquioxide-bound	13·6	15·9	16·6	16·9	14·6	16·0	15·4	16·7
Adsorbed	17·0	19·5	18·6	18·6	19·6	21·7	23·3	23·3
Total	80·1	84·4	86·4	86·4	79·3	84·7	85·3	86·8
	35 days				56 days			
Available	6·0	6·5	6·8	8·6	5·6	6·0	6·5	8·6
Sesquioxide-bound	13·1	14·9	14·3	14·5	12·8	14·5	13·1	13·9
Adsorbed	21·7	24·2	24·6	23·9	19·3	22·8	22·5	21·7
Total	81·3	86·0	85·6	85·4	80·4	85·3	86·3	86·1

' fixed ' within a few hours and that the relationship between the amount ' fixed ' and the time of contact of phosphate and soil was asymptotic. Hibbard[11] also found that pronounced ' fixation ' occurs within a short time and it may continue for a year or more, the rate being influenced by the proportion of water to soil.

Phosphate retention by tropical red earths has been attributed to chemical reactions of phosphate ions with sesquioxides.[3] The podzolic belt of Madhupur tract, which contains Tejgaon clay soil as a genetic type, has been found to contain appreciable amount of free sesquioxide-bound phosphorus.[4] Field examination of the Tejgaon clay showed an abundance of ferruginous concretions and soft gel-like material which increased with depth (Table I). Chemical analyses also show appreciable amounts of Fe_2O_3 in this soil (Table I). Retention of phosphate by sesquioxides has been noted by many investigators, notably Midgley,[12] Coleman,[13] Chu & Sherman,[14] and Kanwar.[15] The results of the present investigation show that 46% of the added phosphorus had been converted into R_2O_3-bound phosphorus (Table II) 3 days after application of phosphate. The R_2O_3-bound phosphorus decreased and adsorbed P_2O_5 increased with time (Table IV). The phosphorus soluble in 0·002N-sulphuric acid (Truog's ' available ' P) also decreased with time (Table II). It seems possible that sesquioxides control the solubility of fertiliser phosphorus and release phosphorus in so-called ' available ' or acid-soluble forms. According to Black[16] the free oxides control the solubility of phosphates after the source of soluble phosphates has been exhausted. Several workers[17] have found that Fe and Al phosphates could be available for crop use in course of time.

Table III

Effect of time on pH values

Treatment	Just before fertiliser application	Days after application of fertiliser	
		3	14
a	6·3	6·1	6·0
b	6·3	6·0	5·8
c	6·9	6·7	6·4
d	6·9	6·7	6·4

Table IV

% of added phosphate retained in different forms

(Results are the differences between treatments *b* and *a*)

Days after fertiliser application	Adsorbed	R_2O_3-bound	Total retained by soil
3	50	46	88
14	42	32	92
35	50	36	90
56	70	34	92

The retention of phosphorus by the exchange or substitution of phosphate ions for hydroxyl ions has been suggested by Mattson,[18] Dean[19] and others. Table IV shows that 50% of the added phosphorus was held as adsorbed P_2O_5 3 days after application of the fertiliser. Adsorption of phosphate increased up to 35 days after application but subsequently decreased. Adsorbed phosphorus, considered as % of added P_2O_5, showed a progressive increase. Black[16] stated that a long period of contact with a concentrated solution produces increased fixation of phosphate by hydroxyl replacement because of slow penetration of phosphate between the lattice layers of the clay minerals and by replacements of hydroxyl ions directly exposed to the solution.

Table II further shows that liming increased the Truog's ' available ' P_2O_5 by about 10%, and soil pH by 0·6–0·7 unit. The increase in soil pH was small because of the highly buffered nature of Tejgaon clay. Mattson *et al.*[20] also observed that liming increases soluble P_2O_5 in sulphuric acid. According to them, the mobilisation of PO_4^{3-} ions by liming takes place by (*a*) displacement of R_2O_3-bound PO_4^{3-} by OH^- ions, (*b*) conversion of organic phosphorus to inorganic phosphorus from the decomposition of organic matter at a higher pH and (*c*) displacement of R_2O_3-bound phosphates by β-humate which is formed by autoxidation of soil organic matter.

Liming steadily reduced the sesquioxide-bound phosphorus 2 weeks after phosphorus application (Table II), but did not immediately increase the amount of adsorbed P_2O_5. Increase in adsorbed P_2O_5 was observed 2 weeks after phosphorus application, but again decreased after 7 weeks. Decrease in adsorbed P_2O_5 immediately after phosphorus application might be due to an increase in the OH^- ion concentration. Bray & Kurtz[21] reported that above pH 6 adsorbed P_2O_5 forms a smaller proportion of total P than it does below pH 6. Karim & Khan[4] also showed that above pH 5·3 adsorbed P_2O_5 steadily decreases. The increase in adsorbed

P_2O_5, 2 weeks after application of phosphorus, might be due to the change in soil pH from 6·7 to 6·4, probably because of intensive leaching by heavy rain.

Cropping *increased* the Truog ' available ' P_2O_5 (Table II) which suggests that the 0·002N acid does not measure ' available ' P_2O_5 in this soil (cf. Rubins[22]). However, under cropping, organic matter may increase because of leaf fall and the crop would continuously remove phosphorus from the soil. Cropping also reduced adsorbed P_2O_5 which suggests that phosphate ions in exchange positions on soil colloids are used up by crops—a finding which is supported by other workers.[4, 23]

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RELATIONSHIP BETWEEN THE SULPHUR/NITROGEN RATIO AND THE PROTEIN VALUE OF DIETS

By D. S. MILLER and G. DONOSO*

Evidence is presented that the protein values of most human diets are limited by their content of the sulphur amino-acids and a chemical method is proposed for the measurement of nutritive value based on sulphur (S) and nitrogen (N) contents. It is shown that for practical purposes the protein scores of mixed diets are equal to 1000 S/N and thus net dietary-protein values may be estimated for natural mixed diets by simple chemical procedures.

Introduction

The factors influencing the protein value of diets have been studied by Miller & Payne[1–3] who give equations for calculating net dietary-protein values from chemical data. The most difficult variable to measure is the protein ' score ' which depends upon the amino-acid composition of the diet: a number of methods are available for its determination:

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(a) *Complete amino-acid analysis* by column chromatography. The method has much to commend it theoretically but is tedious and requires expensive apparatus: the values are influenced by the conditions of hydrolysis, and it is not certain that amino-acids so determined are biologically available.

(b) *Amino-acid tables.*—Many workers use data from tables giving the amino-acid composition of foods and compute the protein score of mixed diets, rather than making direct chemical determinations because of the analytical problems involved: however the range of values reported in these tables is many times wider than for most nutrients.[4]

(c) *Determination of specific amino-acids.*—For scoring practical diets it is only necessary to know their content of a few of the essential amino-acids. Microbiological assay of methionine, cystine, lysine and tryptophan would be adequate but the accuracy of the technique is open to criticism:[5] an improved method using proteolytic bacteria[5a] is more satisfactory but cannot at present be used for the estimation of cystine or lysine. Chromatographic methods which have been abbreviated to yield data for a few amino-acids show more promise. A chemical method for 'available lysine' has been proposed,[6] but, although most diets normally eaten by man and animals are limited by the sulphur amino-acids,[7–9] methods for the determination of methionine and cystine are at present not suitable for routine use.

(d) *Determination of total S.*—Miller & Naismith[10, 11] have suggested that the total sulphur content of mixed diets could indicate their content of sulphur amino-acids and so provide a simple chemical method for estimating protein value. However in correlating the sulphur content directly with net protein values, these authors made no allowance for the metabolic wastage of protein occurring at high levels of protein intake. In the present work this difficulty has been overcome by deriving a 'score' for the protein of each diet from its sulphur/nitrogen (S/N) ratio, and applying the equation of Miller & Payne.[2]

Experimental

Methods

Net Protein Utilisation (NPU) was determined on rats by the methods of Miller & Bender.[12] The diets were prepared either according to food survey data or according to traditional recipes, freeze dried, and fed unmodified; the results are therefore designated NPU operative (NPU_{op}).[2] Metabolisable energy was determined by the method of Miller & Payne[13] and the percentage of energy supplied by protein (protein cal. %) was calculated from

$$\frac{25 \times \%\ \text{N}}{\text{Metabolisable energy (kcal. per g.)}}$$

Net dietary-protein values (NDpCal. %) were expressed as the product of NPU_{op} and protein cal. %. Sulphur was determined by combustion of samples in a bomb and estimation of the sulphate formed.[14]

An alternative method for the estimation of the sulphur content of food, suggested to us by our colleague Dr. P. R. Pellet, is to burn the sample in a glass flask filled with oxygen at atmospheric pressure.

Weigh 150–180 mg. of the food into a small piece of cigarette paper, clip on to a spiral of platinum wire held in a glass rod fixed into a plastic stopper. Ignite the sample by touching the twisted paper into a flame and plunge it into a 500-ml. conical flask containing 10 ml. of water, 3 drops of conc. hydrogen peroxide and filled with oxygen by displacement of air. Set aside for 30 min. with occasional shaking and pour the liquid down a drained column (5 cm. × 1·5 cm. dia.) of Dowex 50 (WX8; 20–50 mesh; H form). Reject the first 1–2 ml. and collect the remainder in a graduated test-tube. Add an equal volume of buffer (sodium acetate 10·5 g., glacial acetic acid 2 ml., water 50 ml. per litre of ethanol); and add knife-point (~20 mg.) of barium chloranilate. Shake the tube for 10 min., centrifuge or filter (Whatman No. 5) and compare the optical density of the solution at 530 mμ with that of standard potassium sulphate solutions or methionine/sucrose mixtures treated in a similar way.

This method gives substantially the same values, does not require specialised apparatus, and is a suitable routine laboratory method when applied to batches of samples treated concurrently.

Calculation of results

Protein 'scores' were calculated by comparing the S/N ratio of the diets with that given by whole egg powder, a foodstuff known to contain proteins that are completely utilisable.[15]

Egg that had been carefully freeze-dried with a NPU standardised to 100% was shown to contain S 0·72%, N 7·35%, a S/N ratio of 0·098 (approximately 0·10) : protein scores of the diets were therefore taken as 1000 S/N. The calculated protein values were then estimated from the equation given by Miller & Payne.[2]

$$\text{NDpCal.}\% = \text{'score'} \times P\ (54 - P)/(54 - P_m)$$

where P = protein cal.% and $P_m = 400/\text{score}$.

Results

Further evidence that most human diets are limited by the sulphur amino-acids is presented in Table I which shows the protein value of 25 human diets based on a number of staples. Each diet has been fed with and without a supplement of methionine and it will be seen that the protein value has been raised in all cases, 70% of which have been improved by more than 5 NPU units. However, the importance of these increments in terms of human feeding should be examined in relation to protein requirements and it is evident that only 8% of the diets are improved by more than 2 NDpCal. units.

Table I

Protein value of diets with and without supplementation with methionine

Origin	Staple[1]	Additional effective sources of protein[2]	Total no. of components	Protein calories %	NPU_{op}[3]		NDpCal. %	
					Alone	With DL-methionine	Alone	With DL-methionine
Nyasaland	Cassava	Fish	10	6·7	50	65	3·3	4·4
Nigeria	,,	Yam	17	7·4	44·3±2·0	55·5±2·5	3·3	4·1
	,,	Fish	13	15·7	52·0±3·5	59·0±4·0	8·2	9·3
Gambia	,,	Fish & pulses	9	7·3	66·0±2·0	69	4·8	5·0
	,,	Meat	11	10·8	48·5±3·5	56·5±2·5	5·2	6·1
	,,	Pulses	5	4·7	33·0±2·0	56	1·6	2·6
	,,	,,	4	2·9	45	53	1·3	1·5
Papua	Sago	Fish	3	3·1	75·0±3·0	83	2·5	2·6
Nigeria	Yam		19	8·0	53·0±2·0	64·0±1·0	4·2	5·1
Gambia	Rice		4	8·9	63	65	4·6	5·8
	,,	Pulses	7	13·3	43	44	5·7	5·9
	,,	,,	10	12·4	45·0±2·0	51·3±1·9	5·6	6·4
	,,	Fish	8	9·0	55	61	5·0	5·5
	,,	,,	8	24·9	37	41	9·2	10·2
	,,	Meat & pulses	9	14·5	58	72	8·4	10·4
Nigeria	Sorghum	Fish	14	15·0	59·3±3·9	60·0±1·0	8·9	9·0
	,,		19	10·5	46·6±3·7	48·6±3·4	4·9	5·1
Gambia	,,	Meat	9	13·5	76	78	10·3	10·5
	Pennisetum	,,	9	19·1	60	67	11·5	12·8
Nyasaland	Maize	Pulses	10	9·8	55·5±0·5	59·5±1·5	5·4	5·8
Poland	Wheat	Meat	4	15·3	79	86	12·1	13·2
Britain	Potato	,,	3	17·3	55·2±2·2	63·0±1·1	9·6	10·9
Gambia	Pulse		6	22·6	41	52	9·3	11·8
	,,		7	17·7	35	50	6·2	8·9
	,,		3	17·8	38	48	6·8	8·5

[1] Staple = chief source of calories
[2] Foods (excluding the staple) contributing more than 20% of the protein in the diet
[3] The figures given are means and the limits are standard errors

In the second series of experiments a further 43 diets were examined. In addition to the biological measurements of protein value, NDpCal. % was calculated from sulphur and nitrogen contents. Table II shows protein scores estimated as 1000 S/N, and the values for NDpCal. % obtained in the two ways. Two-thirds of the calculated values fall within 0·6 NDpCal. units of the values obtained by bioassay, and 95% within 1·4 units. The differences between 'score' and NPU_{op} depend upon the protein concentration,[1] and when allowance is made for this factor, the calculated protein values are in good agreement with the results of bioassay.

Table II

Comparison of net dietary-protein values determined by biological assay and those predicted from the S/N ratio

Origin	Protein cal. %	1000 S/N[1]	NPU_{op} %	NDpCal. % Found	NDpCal. % Calc.[2]	Origin	Protein cal. %	1000 S/N[1]	NPU_{op} %	NDpCal. % Found	NDpCal. % Calc.[2]
Jamaica	8·4	69	67	5·6	5·6	Gambia	11·8	68	58	6·8	7·0
	13·9	65	57	7·9	7·5		7·4	68	40	3·0	4·7
	10·9	59	59	6·4	5·8		11·9	75	63	7·5	7·7
	13·2	76	59	7·8	8·4		13·9	74	57	8·0	8·5
Britain	15·7	61	51	8·0	7·7		4·7	81	33	1·6	3·8
	12·5	80	67	8·3	8·5		8·4	56	47	4·0	4·5
	13·1	73	63	8·3	8·3		8·9	78	63	5·6	6·4
	13·8	71	63	8·7	8·1	Ghana	11·4	81	65	7·4	8·1
	15·4	70	59	9·1	8·6	Nigeria	14·6	75	59	8·6	8·8
	13·9	70	59	8·2	8·1	Nyasaland	10·2	85	66	6·7	7·7
	13·5	69	62	8·4	7·8		11·4	76	71	8·1	7·6
	9·6	74	78	7·5	6·5	Pakistan	11·3	67	62	7·0	6·8
	14·5	83	69	10·0	9·7		11·5	61	57	6·5	6·2
	17·5	65	47	8·2	8·6		9·0	74	60	5·4	6·1
Turkey	12·3	53	41	5·1	5·8		13·1	65	58	7·6	7·2
	18·8	63	46	8·7	8·7	Poland	10·7	57	45	7·5	7·5
	14·9	70	56	8·3	8·3		12·6	71	49	6·2	7·5
	15·5	56	48	7·4	7·1	Persia	15·9	64	50	8·0	8·1
	13·3	61	48	6·4	6·9		14·7	67	59	8·7	7·9
Iraq	14·6	66	43	6·3	7·7		15·1	61	54	8·2	7·6
South Africa	13·2	63	53	7·0	7·1		17·2	65	51	8·7	8·6
Guatemala	23·7	75	42	10·0	10·9						

[1] Taken as protein score
[2] Calculated by the method of Miller & Payne[2]

Discussion

Both series of experiments confirm the opinion of Rose *et al.*[7] that ' the diets consumed by a large part of the human race are relatively low in methionine '. Whereas the protein value of some staple foods (e.g., the cereals) is limited by lysine, the sulphur amino-acids are frequently the second factor restricting nitrogen retention, and in the mixed diets consumed by man and animals this lysine deficit is normally filled by small quantities of animal protein. As a result, such diets are limited by cystine and methionine.

It is therefore of practical importance whether the measurement of the total sulphur content of mixed diets provides a valid indication of their combined methionine and cystine contents. Three sources of error are possible :

(*a*) Diets may contain variable amounts of non-amino-acid sulphur. In particular no account is taken of the level of inorganic sulphur in the diets, but the error will be negligible if the amount is either small or constant. To minimise the effect, an intact foodstuff was chosen as a standard rather than a theoretical amino-acid mixture.

(*b*) The relative proportions of methionine and cystine may vary. As methionine is the essential sulphur amino-acid and it cannot be replaced entirely by cystine, diets very low in methionine and rich in cystine could give false values, but such diets are rare : Rose & Wixom[16] have shown that cystine can replace 80–89% of the methionine requirement. The percentages of sulphur in methionine and cystine are similar (21·5 and 26·5% respectively).

(*c*) The amino-acids may be biologically unavailable. Its been shown that some of the sulphur of proteins damaged by heat treatment is not retained by growing animals ;[14] during severe processing of foods, especially drying at high temperatures, a little sulphur is lost but much is converted to unavailable forms. The method proposed in this paper should therefore be considered in conjunction with tests for heat damage[6] when applied to animal foodstuffs. One possibility is to multiply the score by the availability of lysine.[14] Normal cooking processes do not however alter protein values.

It should be stated that it is proposed to apply the technique described only to mixed diets : clearly anomalous results may result if individual foodstuffs are examined, especially those limited by lysine. In addition, false values could be obtained with synthetic diets, particularly those containing salt mixtures with amounts of sulphate larger than those usually found in mixed diets as ordinarily eaten.

Methods for the prediction of the protein values of diets should be considered in relation to the accuracy required to assess or prescribe diets with reference to requirements: food composition tables appear to be sufficiently accurate for the evaluation of dietary data based on records of group intake, but, in more precise nutritional investigations on individual human or animal subjects, direct analytical control is advantageous. The method described in this paper provides a simple chemical procedure for this purpose.

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THE SESQUITERPENE FRACTION OF THE ESSENTIAL OIL OF *OLEARIA PANICULATA*

By R. E. CORBETT, G. A. JAMIESON and (the late) J. MURRAY

The sesquiterpene fraction of the oil of *Olearia paniculata* has been separated by distillation into three fractions. The first of these appears to be aromadendrene, while the second and third are shown to be (±)-*ar*-curcumene and (±)-γ-curcumene respectively.

Introduction

Olearia paniculata is a small evergreen tree endemic to both the North and South Islands of New Zealand. It makes an excellent hedge and is much used for this purpose. The essential oil from this tree has already been examined by McLean & Slater,[1] who obtained a yield of 0·1% by steam distillation of the leaves and terminal branchlets. They isolated a terpene fraction (10%) containing (−)-β-pinene and limonene, and a sesquiterpene fraction (48%). The high-boiling fraction yielded a crystalline oxide, olearyl oxide. This paper describes an investigation of the monocyclic sesquiterpene described by McLean & Slater.[1]

Efficient fractionation of the sesquiterpene fraction of the essential oil has revealed the presence of three sesquiterpenes. The lowest boiling was not obtained pure, but the physical constants for the pure compound estimated[2] from an *n/d* diagram (Fig. 1) were close to those of aromadendrene and its presence was confirmed by the production of *apo*-aromadendrone

on ozonolysis. The remaining two sesquiterpenes were identified as (±)-*ar*-curcumene[3] and (±)-γ-curcumene[4] by ultra-violet spectra, and by the production of (±)-γ-*p*-tolyl-n-valeric acid on ozonolysis.[4] The ultra-violet spectrum of γ-curcumene has a maximum at 267 mμ. Optically inactive *ar*- and γ-curcumenes have not previously been reported to occur naturally, although both have been synthesised by Birch & Mukherji.[3] In fact this is only the second reported occurrence of γ-curcumene. It is very probable that the *ar*-curcumene is an artefact, since γ-curcumene is readily oxidised on exposure to air. Thus, although γ-curcumene is the major constituent of a fresh sample of the essential oil, it was found that a sample of *Olearia paniculata* sesquiterpenes which had been stored for several years contained only *ar*-curcumene together with some polymeric and oxidised material. The *ar*-curcumene in this sample was isolated and purified through the monohydrochloride.

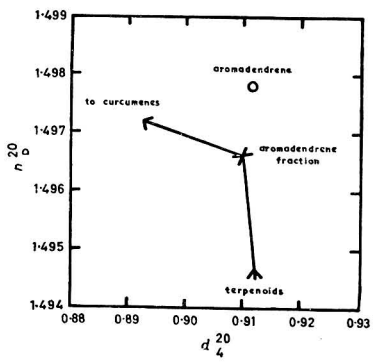

FIG. 1. n/d *diagram of portions of distillate preceding and following the aromadendrene fraction*

Experimental

Leaves and terminal branchlets of *Olearia paniculata* were collected in February from trees growing in Dunedin. These were steam-distilled in 250-lb. batches, extraction being complete after 20–24 h. The autumn oil (yield 440 g., 0·11%) was lemon coloured and had the following constants: n_D^{20} 1·4978, d_4^{20} 0·9334. It was separated by fractional distillation in a Claisen flask into three fractions: (*a*) b.p. to 90°/4 mm. (30%); (*b*) b.p. 90–148°/4 mm. (60%); (*c*) semi-crystalline residue (10%). With a column of 48 theoretical plates, after the pattern of Lecky & Ewell,[5] the middle fraction (*b*), backed with paraffin, was fractionated at 3 mm. The separation was followed by plotting the refractive index and density of successive distillate aliquots (2·5 c.c.) against the take-off volume. The plateau in the graphs revealed the presence of three sesquiterpenes (23% of the total oil).

Aromadendrene fraction (1% of total oil) had b.p. 92°/3 mm., n_D^{20} 1·4966, d_4^{20} 0·9100, $[R_L]^D$ 65·5. This fraction was clearly contaminated by its neighbours. Ozonolysis of the fraction (3 c.c.) in glacial acetic acid gave a neutral fraction (100 mg.), b.p. 125–135°/3 mm., with the typical musty odour of *apo*-aromadendrone. This compound (40 mg.) furnished a 2,4-dinitrophenylhydrazone (10 mg.), m.p. 160–180°, which gave after chromatography on alumina (Brockmann grade III) in heptane/benzene (25 : 75) a fraction (2 mg.), m.p. 217–219°, after crystallisation from ethanol. This specimen had m.p. 219–225°, on admixture with an authentic specimen of *apo*-aromadendrone-2,4-dinitrophenylhydrazone, m.p. 235°.

(±)-*ar-Curcumene* (2·5% of total oil) had b.p. 92°/3 mm., n_D^{20} 1·4979, d_4^{20} 0·8712, $[R_L]^D$ 68·1. Birch & Mukherji[3] report b.p. 140°/19 mm., n_D^{20} 1·5014, and Simonsen *et al.*[6] give b.p. 134°/16 mm., n_D^{20} 1·5002, d_4^{20} 0·8786, for this compound. The fraction in glacial acetic acid gave with amyl nitrite and nitric acid, (±)-*ar*-curcumene nitrosate, m.p. 114° after crystallisation from methanol/chloroform, undepressed on admixture with an authentic specimen.

(±)-*γ-curcumene* (19.5% of total oil) had b.p. 94°/3 mm., n_D^{20} 1·4979, d_4^{20} 0·8807, $[R_L]^D$ 67·4, $\lambda_{max.}$ 267 mμ, $\varepsilon_{max.}$ 3600 (ethanol). Batt & Slater[4] report n_D^{25} 1·4975, d_4^{20} 0·8810, $\lambda_{max.}$ 267 mμ, $\varepsilon_{max.}$ 3500 (cyclohexane) for (+)-γ-curcumene, while Birch & Mukherji[3] give n_D^{20} 1·4956, $\lambda_{max.}$ 265 mμ, $\varepsilon_{max.}$ 2900 (ethanol) for (±)-γ-curcumene. Ozonolysis of γ-curcumene (9 g.) in ethyl acetate (60 c.c.) gave a colourless viscous ozonide which was decomposed by gentle warming with water under reflux.

The volatile product of ozonolysis was identified as acetone by the preparation of the 2,4-dinitrophenylhydrazone, m.p. and mixed m.p. 124°. The neutral and acid products were isolated in the usual way.

The acid fraction (1·25 g.) was characterised as the *p*-phenylphenacyl ester, m.p. 69–69·5°, undepressed on admixture with an authentic specimen of *p*-phenylphenacyl (±)-*p*-tolyl-n-valerate, m.p. 69–69·5°.[4]

The neutral fraction (1·26 g.) furnished a resinous product with ethanolic 2,4-dinitrophenylhydrazine which gave a small amount of solid from ethanol, m.p. 83°, probably the racemic form of the (+)-γ-*p*-tolyl-n-valeraldehyde-2,4-dinitrophenylhydrazone, m.p. 85–86°, reported by Batt & Slater.[4] Dehydrogenation of (±)-γ-curcumene with sulphur gave cadalene, b.p. 135°/5 mm., n_D^{20} 1·5885, which gave a picrate, m.p. and mixed m.p. 114°, with an authentic specimen.

The sesquiterpene fraction of an oil sample isolated 5 years previously had become dark brown and somewhat viscous. This fraction when distilled gave a light yellow oil, b.p. 146°/20 mm., n_D^{20} 1·5034, d_4^{20} 0·9110; light absorption at 267 mμ, ε 400. Ozonolysis gave acetone and (±)-γ-*p*-tolyl-n-valeric acid, which were characterised as above. The sesquiterpene in dry ether, treated with hydrogen chloride, gave (±)-*ar*-curcumene monohydrochloride, b.p. 138–143°/5·5 mm., n_D^{20} 1·5053 (Found: C, 75·1; H, 9·71; Cl, 14·62. Calc. for $C_{15}H_{23}Cl$: C, 75·48; H, 9·64; Cl, 14·88%). The monohydrochloride (3·8 g.) was heated with dimethylaniline (25 c.c.) under gentle reflux for 2 h. The product was dissolved in ether (50 c.c.) and washed with diluted hydrochloric acid (3 × 50 c.c. of 50%) and water (3 × 50 c.c.). After being dried over anhydrous sodium sulphate, the ether was removed *in vacuo*, and the product distilled to give (±)-*ar*-curcumene, b.p. 94–98°/3 mm., n_D^{20} 1·5028, d_4^{20} 0·8960, characterised as the nitrosate, m.p. 114° (chloroform/methanol) alone and on admixture with an authentic specimen (Found: C, 61·37; H, 7·54; N, 9·23. Calc. for $C_{15}H_{22}O_4N_2$: C, 61·22; H, 7·48; H, 9·53%).

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EFFECT OF IRON/PHOSPHORUS RATIO AND ACID CONCENTRATION ON THE PRECIPITATION OF FERRIC INOSITOL HEXAPHOSPHATE

By GEORGE ANDERSON

The precipitation of ferric inositol hexaphosphate has been measured under a variety of experimental conditions.

The salt can be precipitated in a wide range of concentrations of both hydrochloric and sulphuric acids, but in the presence of excess iron soluble complexes are formed. The ratio of iron to phosphorus which causes maximum precipitation depends upon acid concentration. In presence of excess iron precipitation is minimum in hydrochloric acid at pH 1. Precipitation in sulphuric acid is greater than in hydrochloric acid, but can be increased in the latter by the presence of sodium chloride or a small amount of sulphate ion, or by raising the temperature of the system. If ferric ammonium sulphate instead of ferric chloride is used for precipitating inositol hexaphosphate from hydrochloric acid solution, then quantitative precipitation will be obtained with a wider range of ester concentrations.

The presence of relatively high amounts of soluble iron in plant material can interfere with phytate extraction by 0·5N-hydrochloric acid. In the estimation of phytate by ferric precipitation other inositol phosphates will be included if present in sufficient concentration.

Introduction

Salts of inositol hexaphosphoric acid (phytic acid) are common constituents of many plant tissues and related foodstuffs. The most widely used method of estimating phytate in these materials involves extraction with 0·5N-hydrochloric acid and precipitation of ferric phytate in N/6 acid.[1] The ester also occurs in soils but cannot readily be extracted with acid and this is likely to be due in part to adsorption by clay minerals,[2, 3] and in part to the formation of insoluble iron and aluminium salts. Jackman & Black[4] have shown that iron and aluminium phytates can be precipitated at pH values between 1 and 10, and there is evidence that ferric phytate can be precipitated even in 2N-hydrochloric acid.[3] However, difficulty has been experienced in the precipitation of ferric phytate from some plant sources and manures.[5]

The following investigation was carried out to determine the conditions under which precipitation of ferric phytate is likely to take place and to establish whether aluminium phytate would behave in a similar fashion in highly acid media. In addition a study was made of the effect of varying iron concentration on the determination of phytate by precipitation and of the presence of iron on its extraction from plant material. The effect of the presence of other inositol phosphates on the estimation of phytate has been noted.

Experimental

Materials

Ferric reagents.—Standard solutions of ferric chloride were prepared containing 0·04–10 g. of Fe/l. in several concentrations of hydrochloric acid. Similar solutions were prepared containing ferric sulphate and ferric ammonium sulphate in sulphuric acid, and ferric ammonium sulphate in hydrochloric acid. The iron concentrations were confirmed by colorimetric analysis with $\alpha\alpha'$-bipyridyl.

Inositol hexaphosphate.—A commercial sample of phytin (calcium magnesium inositol hexaphosphate) was converted to sodium phytate as follows: 6 g. of the salt were dissolved in 200 ml. of N-hydrochloric acid and a concentrated solution of ferric chloride added with vigorous stirring until the suspension became slightly yellow. The ferric inositol hexaphosphate was washed once with N-acid, and twice with water, and stirred with excess of a 10% solution of sodium hydroxide until it had been completely converted to the sodium salt. Ferric hydroxide was removed by centrifuging and sodium inositol hexaphosphate was precipitated by the addition of 95% ethanol. The aqueous ethanol was decanted and the hexaphosphate redissolved in water and reprecipitated with ethanol. It was then triturated with absolute ethanol until it had solidified and then the ethanol was decanted, the last traces being removed by warming

the hexaphosphate gently on the water bath. The ester was dissolved in water. Chromatographic examination[6] confirmed that it consisted of inositol hexaphosphate with a trace of orthophosphate present. The concentration of the ester was determined by phosphate analysis.

Lower phosphate esters of inositol.—Approximately 22 g. of commercial phytin were converted to the sodium salt and dissolved in 1 litre of water in a 2-litre flask. The pH of the solution was adjusted to 5·6 with dilute nitric acid, 10 g. of wheat bran and 1 ml. of toluene were added, and the whole was incubated at 30° for 14 days. The liquid was filtered, decolorised with charcoal, made alkaline with aq. ammonia and an excess of a 10% solution of barium acetate added with stirring. The white precipitate formed was centrifuged, washed with water and redissolved in the minimum volume of 3N-hydrochloric acid. An equal volume of 95% ethanol was added slowly with vigorous mechanical stirring and the precipitate centrifuged, washed with ethanol, and dried *in vacuo* at 40°. Chromatographic examination of the product showed that it consisted of a mixture of inositol tetra-, tri- and di-phosphates and orthophosphate. The yield was 12 g.

Phosphate analysis

Orthophosphate was determined by a modification[7] of the procedure of Dickman & Bray. Total phosphate in solution was determined by the same colorimetric procedure after evaporation with magnesium nitrate and ignition.

Precipitation of ferric inositol hexaphosphate in acid systems

(a) *Precipitation in high acid concentrations.*—Suitable aliquots of a solution of sodium inositol hexaphosphate, containing 0·83 g. of P/l., were transferred to volumetric flasks and diluted with acid to give a series of solutions containing 0·166 g. P/l. in different acid concentrations. To 5-ml. aliquots of these solutions were added 5-ml. aliquots of iron solutions in the same concentration of acid, and containing 0·04–10 g. of Fe/l. On the assumption that 1 molecule of inositol hexaphosphate is equivalent to 4 atoms of ferric iron, these additions would represent 0·2–50 equivalents of iron. The solutions were quickly mixed in small glass tubes which were stoppered and stored at 20° for 24 h. The suspensions were transferred to 15-ml. tapered centrifuge tubes, centrifuged at 2500 r.p.m. for 20 min., and 2 ml. of the supernatant liquids diluted to 100 ml. in volumetric flasks. Total phosphate was estimated in suitable aliquots, and from this was calculated the amount of phosphate precipitated.

(b) *Effect of high salt concentration on the precipitation.*—The precipitations in 0·5N-hydrochloric acid at 20° were also carried out in the presence of sodium chloride. This was added during the preparation of the ferric chloride solutions, the final normality in the ferric phytate systems being 0·25.

(c) *Effect of heat on the precipitation.*—The precipitation of inositol hexaphosphate in 0·1 and 0·5N-hydrochloric acid was repeated, but after the iron and phosphate solutions had been mixed, the glass tubes were immersed in a boiling-water bath for 15 min. and then cooled in running cold water for 15 min. The phosphate content of the solution was not in this case measured, but instead the suspensions were centrifuged, the precipitates treated with 2% sodium hydroxide[8] and total phosphate in the resultant solution of sodium inositol hexaphosphate was determined.

(d) *Precipitation at pH 1–5.*—About 10 ml. of water were added to 5 ml. of an aqueous solution of sodium inositol hexaphosphate containing 0·415 g. of P/l. After adjustment of the pH with HCl, the solution was transferred to a 25-ml. volumetric flask, an aliquot (1 or 5 ml.) of a freshly prepared aqueous solution of ferric chloride containing 5 g. of Fe/l. was added, and the volume was made to 25 ml. with water. The contents of the flasks were mixed thoroughly and stored for 8 days at about 20°. A portion was centrifuged and 2 ml. of the supernatant liquid removed for analysis. The pH value of the remaining portion was measured.

Examination of aluminium inositol hexaphosphate systems

Aliquots of a standard solution of aluminium chloride or aluminium potassium sulphate were diluted with acid to give a range of solutions containing 0·1, 0·2, 1·0 and 5·0 g. of Al/l.

in 0·1, 0·5 and 1·0N-hydrochloric or -sulphuric acid. Five-ml. portions of the inositol hexaphosphate solutions (0·170 g. of P/l.) were mixed with 5 ml. of each of the aluminium solutions, and stored at 20°. No precipitates appeared within a week and the solutions were discarded.

Precipitation of the ferric salts of other inositol phosphates

The mixed barium salts of inositol di-, tri- and tetra-phosphate, and orthophosphate (0·2 g.) were treated with 1 ml. of Amberlite IR-120H cation-exchange resin and 1 ml. of water in a test-tube. The suspension was stirred until the white particles had dissolved and then decanted with washing into a burette containing a column of resin (7 × 1 cm.). The solution was run through at a rate of 0·5 ml./min. and the resin washed with small quantities of water until the volume of eluate was 12 ml. Analysis showed that the organic phosphate and orthophosphate contents were, respectively, 1·00 and 0·78 mg. of P/ml. To 1 ml. of the solution were added 1 ml. of 2N-hydrochloric acid and 2 ml. of a solution of ferric chloride in N-hydrochloric acid, containing 2 mg. of Fe—more than equivalent to the organic phosphate present but less than equivalent to the total phosphate. By varying the acid concentrations, systems were obtained which were 0·1, 0·5 and 1·0N with respect to hydrochloric acid. Similar systems were prepared with sulphuric acid and ferric ammonium sulphate. After 2 days, where precipitates had appeared, the suspensions were centrifuged and the supernatant liquids analysed for inorganic and total phosphate. The precipitates were treated with up to 100 μl. of 4N-sodium hydroxide, the volume added depending on their bulk. They were broken up and the suspensions heated for a short time in a boiling-water bath and centrifuged. About 1–5 μl. of the solutions were applied to Whatman No. 1 paper, and chromatograms were developed downwards for 18 h. with n-propanol, aq. ammonia, water, 5 : 4 : 1 by volume.[6] The papers were sprayed with the ferric chloride–sulphosalicylic acid reagent of Wade & Morgan[9] (Table I).

Table I

Precipitation of the ferric salts of inositol tetra-, tri- and di-phosphates at 20°

A mixture of esters and orthophosphate in acid solution was treated with a solution of a ferric salt in the same normality of acid. Concentrations of organic and inorganic phosphates in the systems were, respectively, 1·00 and 0·78 mg. of P/4 ml.

Precipitant	Medium	Phosphate precipitated from 4 ml. (mg. P)		Phosphates identified in precipitate
		Organic	Inorganic	
Ferric chloride	0·1N-HCl	0·04	0·04	—
	0·5N-HCl	0	0	—
	1·0N-HCl	0	0	—
Ferric ammonium sulphate	0·1N-H_2SO_4	0·94	0·16	Inositol tetra-, tri- and di-phosphates; orthophosphate
	0·5N-H_2SO_4	0·61	0·01	Inositol tetra-, tri- and di-phosphates
	1·0N-H_2SO_4	0·26	0·02	Inositol tetraphosphate

Estimation of phytate by precipitation of its ferric salt

There are several established procedures for the estimation of phytate by this means, based on the method of McCance & Widdowson.[1] The method was established for the estimation of phytic acid in foodstuffs from which it was extracted with hydrochloric acid. In view of the ease with which soluble ferric phytate systems appear to be formed, an investigation was made of the effect of varying iron concentration on the estimation.

The procedure was first applied to an aqueous solution of inositol hexaphosphate. A solution of the sodium salt was prepared containing approximately 0·2 mg. of P/ml., the exact concentration being confirmed by analysis. Aliquots of the solution, ranging from 1 to 5 ml., were diluted to 20 ml. in 50-ml. centrifuge tubes and made slightly acid with 0·5N-hydrochloric acid. To the solutions were added 4 ml. of a solution of ferric chloride or ferric ammonium sulphate in N-hydrochloric acid, containing 1 g. of Fe/l. The mixtures were further treated as described by Common[8] (Table II).

The amount of iron added to the phytate solution in this experiment was several times that required for equivalence and, since excess iron can favour the formation of soluble complexes (Fig. 1), the experiment was repeated with smaller additions of iron. The precipitation was

also carried out in the presence of 1 ml. of 5N-sodium chloride to simulate the conditions used by McCance & Widdowson who analysed solutions of phytate in hydrochloric acid, neutralising with sodium hydroxide prior to addition of the ferric reagent (Table III).

Table II

*Precipitation of inositol hexaphosphate at 100° by addition of solutions of ferric salts in hydrochloric acid**

Total P in test solution 0·211 mg./ml. (4 mg. of Fe in precipitant)

Aliquot analysed, ml.	Phosphate precipitated (mg. P/ml. of test solution)	
	By ferric chloride	By ferric ammonium sulphate
1	0	0·127
2	0·145	0·192
3	0·181	0·203
4	0·204	0·208
5	0·206	0·209

* Values are means of analyses of at least 4 precipitations

Table III

Effect of iron concentration and the presence of sodium chloride on the precipitation of inositol hexaphosphate at 100°

(Total P in test solution was 0·204 mg./ml.)

Aliquot analysed, ml.	Phosphate precipitated (mg. P/ml.)*					
	No sodium chloride added				Sodium chloride added	
	Ferric chloride		Ferric ammonium sulphate		Ferric chloride	Ferric ammonium sulphate
	4 mg. Fe	1·5 mg. Fe	4 mg. Fe	1·5 mg. Fe	4 mg. Fe	4 mg. Fe
1	—	—	—	—	0·189	0·194
2	0·133	0·192	0·180	0·191	0·201	0·200
5	0·186	0·197	0·201	0·199	0·203	0·202
6	0·188	0·202	0·197	0·200	—	—

* Values are means of analyses of at least 4 precipitations

An experiment was also carried out to determine to what extent other inositol phosphates might be precipitated during the estimation of phytate. A weighed sample of the mixed barium salts of inositol di-, tri- and tetra-phosphate and orthophosphate (0·1 or 0·2 g.) was dissolved in 100 ml. of 0·5N-hydrochloric acid. An aliquot of the solution (10 ml.) was neutralised with 25% sodium hydroxide, the volume diluted to 20 ml. with water, and the apparent phytate content estimated. With 0·1 g. of the salts no precipitate was obtained with ferric chloride, but with 0·2 g. a flocculent precipitate appeared. Another sample of the mixed barium salts of the esters was converted into the acid form by ion-exchange. Aliquots of the solution, containing 30·7 μg. of P, were chromatographed as before, and the phosphates detected with the Wade & Morgan reagent. The spots corresponding to inositol di-, tri- and tetra-phosphates, and orthophosphate, with corresponding blanks, were cut out and ashed with 0·5 ml. of 12% aqueous magnesium acetate solution, and phosphate in the residue determined colorimetrically (Table IV).

Effect of iron on the extraction and estimation of phytate in plant material

In the methods based on that of McCance & Widdowson, phytate is extracted from plant material with 0·5N-hydrochloric acid prior to estimation. If an extract is to be made with a sufficiently high concentration of phytate to ensure quantitative precipitation, then with some plant materials an appreciable amount of iron may also be dissolved. An extraction of barley meal was therefore made in the presence of added iron to test its effect on the apparent phytate content of the meal.

Samples of barley meal (2·5 g.) were extracted with 50 ml. of 0·5N-hydrochloric acid containing 0, 0·7, 1·4 or 2·1 mg. of Fe as ferric chloride. The phytate content of the extracts was determined by the method of Common[8] (Table V). The iron content of the control extract was also estimated.

Table IV

Analysis of a mixture of the barium salts of orthophosphate and inositol di-, tri- and tetra-phosphates

Component	Method of analysis	P content (% of total P)
Orthophosphate	Paper chromatography	36·5
Inositol diphosphate	,, ,,	31·0
,, triphosphate	,, ,,	17·3
,, tetraphosphate	,, ,,	12·4
,, hexaphosphate	,, ,,	0
'Phytate'	Precipitation	63·1

Table V

Effect of iron on the extraction of phytate from barley meal

Iron added to extractant (mg. Fe/l.)	Apparent phytate content of meal (mg. P/100 g.)	Iron extracted (mg. Fe/l.)	Ratio Initial soluble Fe / Total phytate P
0	274	3·7	0·03
14	272	—	0·13
28	254	—	0·23
42	218	—	0·33

Results and discussion

The precipitation of ferric inositol hexaphosphate in acid solution at 20° was markedly affected by the ratio of iron to phosphorus, the acid concentration and the nature of the anions present (Fig. 1). As the ratio of iron to phosphorus increased towards unity, the precipitation of phosphate increased also. The ratio at which maximum precipitation occurred depended on the acid concentration and in most cases increased with it. It appears that, above this optimum ratio, soluble complexes were formed, particularly in hydrochloric acid. The effect was marked in 0·1 and 0·5N-hydrochloric acid, especially in the former where 1 equivalent of iron gave almost complete precipitation, while 2 gave virtually none. Precipitation of the phosphate was more complete in sulphuric acid or in hydrochloric acid containing a small amount of sulphate. Similar values were obtained whether ferric sulphate or ferric ammonium

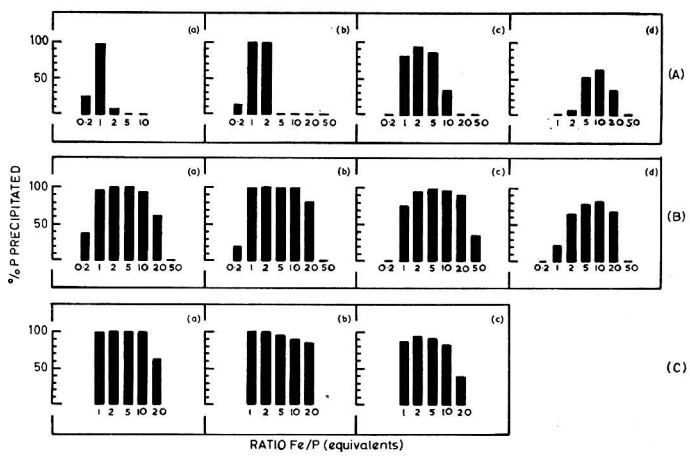

FIG. 1.—*Precipitation of ferric inositol hexaphosphate from acid solution at 20°*

Precipitant A Ferric chloride, from HCl (*a*) 0·1N, (*b*) 0·5N, (*c*) 1·0N, (*d*) 2·0N
,, B Ferric ammonium sulphate, from H_2SO_4 (*a*) 0·1N, (*b*) 0·5N, (*c*) 1·0N, (*d*) 2·0N
,, C Ferric ammonium sulphate, from HCl (*a*) 0·1N, (*b*) 0·5N, (*c*) 1·0N

sulphate were used as precipitants and no values for the former have been included here. Precipitation in 0·5N-hydrochloric acid was improved somewhat if the salt concentration was high (Fig. 2), but if 10 or more equivalents of iron to one of phosphorus were present, the phosphate remained in solution. With excess iron present in hydrochloric acid systems, there is a minimum in the precipitation of inositol hexaphosphate occurring in the region of pH 1 (Fig. 3). No such minimum occurs when the iron is equivalent to the phosphorus.

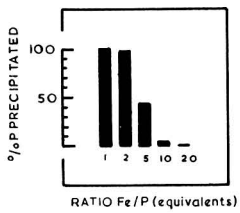

FIG. 2.—*Precipitation of ferric inositol hexaphosphate from 0·5N-HCl at 20° in presence of 0·25N-NaCl*

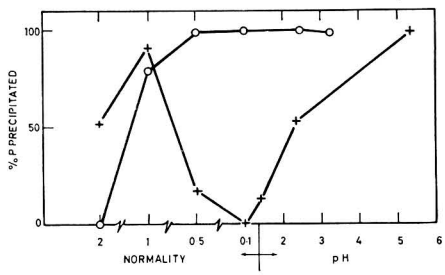

FIG. 3.—*Effect of Fe/P ratio and degree of acidity on the precipitation of inositol hexaphosphate from hydrochloric acid*

○——○ Ratio Fe/P = 1 (equivalents)
+——+ „ „ = 5 („)

When the mixtures were heated to 100° and cooled, precipitation of ferric inositol hexaphosphate took place over a wider range of Fe/P ratio, but excess iron still produced soluble complexes (Fig. 4).

No precipitation of aluminium inositol hexaphosphate could be obtained in 0·1, 0·5 or 1·0N-hydrochloric or sulphuric acid. It has previously been shown that certain hydrated sesquioxides, such as boehmite and ferric oxide gel, can remove inositol hexaphosphate from solution in high concentrations of acid.[3] The appearance of the systems containing ferric oxide gel indicated that dissolution of the oxide was taking place, followed by reprecipitation of insoluble ferric salts, and the possibility of a similar mechanism was suggested for the aluminium systems. The results now obtained with aluminium salts indicate that precipitation of aluminium inositol hexaphosphate from high concentrations of acid is unlikely and that the removal of the phosphate from solution by boehmite was due to adsorption.

When a mixture of lower phosphate esters of inositol was treated with slightly more than an equivalent amount of ferric chloride in hydrochloric acid solution at 20°, no precipitation was obtained. With ferric ammonium sulphate in sulphuric acid, however, precipitation of the three esters was almost quantitative in 0·1N-acid (Table I), and all three were precipitated to some extent in 0·5N-acid. In 1N-acid only the tetraphosphate was precipitated.

The effect of iron in producing soluble complexes with phytate was also demonstrated in the estimation of the latter by methods based on that of McCance & Widdowson. In the original method, 2 mg. of iron as ferric chloride were added to precipitate phytate from solutions of widely varying concentration. Common[8] added 4 mg. of Fe to precipitate 0·8–1·0 mg. of P as phytate, and Pringle & Moran[10] added the same amount to precipitate 0·7–1·2 mg. of P.

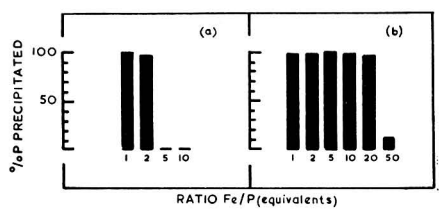

FIG. 4.—*Precipitation of ferric inositol hexaphosphate from acid solution at 100°*
(*a*) 0·1N-HCl (*b*) 0·5N-HCl

In these methods, and that of McCance & Widdowson, sodium chloride was present, since the acid extracts were neutralised before the precipitant was added. The results in Table II show that, if no salt is added to the system, 0·6 mg. of phytate phosphorus or below cannot be quantitatively precipitated by this amount of ferric chloride. With ferric ammonium sulphate as precipitant, however, precipitation of 0·6 mg. of P was virtually quantitative and with 0·4 mg. of P over 90% was recovered. Values obtained with 0·8 and 1·0 mg. of P tended to be slightly higher with the sulphate. Sulphate in very small amount is a normal constituent of hydrochloric acid extracts of plant material, but it is noteworthy that in the method of Pons *et al.*[11] the plant material is extracted with hydrochloric acid containing a high concentration of sodium sulphate which is therefore present during subsequent precipitation of ferric phytate. Analysis of another preparation of inositol phosphate showed the same trend (Table III) but, if the iron addition was reduced until it was only slightly more than equivalent to the highest phosphate level, then the efficiency of precipitation in several cases increased. The efficiency was also increased by the inclusion of sodium chloride in the system which gave quantitative precipitation of 0·4 mg. of phytate phosphorus, and about 95% precipitation of 0·2 mg.

It was found that the fraction estimated as phytate by the above procedure was not specific for inositol hexaphosphate alone. When a mixture of lower phosphate esters of inositol and orthophosphate was analysed by ferric precipitation, the apparent phytate content was 63·1% of the total phosphate (Table IV). Chromatographic analysis showed that the combined di-, tri- and tetra-phosphates of inositol made up 60·7% of the total phosphate and confirmed the absence of inositol hexaphosphate.

The amount of iron present in grain is very small relative to the phytate content, and is unlikely to have any noticeable effect on the extraction of phytate with 0·5N-hydrochloric acid. In some common foodstuffs, however, for example carrots, beans and cornflakes, the ratio of iron to phytate phosphorus is about 0·2 : 1,[12] and no doubt in other cases will be very much higher. When increasing amounts of iron were added to hydrochloric acid prior to extraction of phytate from barley, it was found that the apparent phytate content of the barley decreased (Table V), the recovery being only 80% when the ratio of soluble iron to total phytate phosphorus was 0·33 : 1.

Conclusions

The analytical results obtained here indicate that the standard techniques for estimating phytate, which depend on acid extraction and precipitation of ferric phytate, must be used with caution and are not of general application. Above a certain (undetermined) level of iron in the material to be analysed, extraction will be incomplete. In grains and similar phytate-rich sources, iron is not likely to influence extraction. If the hexaphosphate is the only phosphate ester of inositol present in an extract, then quantitative precipitation should be obtained by the methods of, for example, Common or Pringle & Moran. If other inositol phosphates are present in sufficient concentration, they will also be precipitated in what might be called the ' phytate group '. Evidence that this occurs with a variety of plant materials has been presented by Marrese *et al.*[13] who found that precipitation of phytate from plant extracts gave higher values than an ion-exchange method specific for inositol hexaphosphate.

The precipitation of ferric phytate from soil extracts is likely to be complicated by the fact that considerable amounts of iron will already be present and the formation of soluble complexes is then possible

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LEAF ANALYSIS AS A GUIDE TO THE NUTRITION OF FRUIT CROPS. II.*—Distribution of Total N, P, K, Ca and Mg in the Laminae and Petioles of Raspberry *(Rubus idaeus L.)* as influenced by Soil Treatments

By C. BOULD, E. G. BRADFIELD and G. M. CLARKE

A study has been made of the concentrations of the major nutrient elements in the leaves and petioles from non-fruiting raspberry canes, in relation to position on the cane and to manurial treatment. Laminae have a higher nitrogen concentration, and are more sensitive to nitrogen supply, than petioles; the lower-third position best reflects soil nitrogen treatment. Laminae and petioles, from any position on the cane, reflect soil potassium treatment. The petiole is more sensitive to changes in potassium supply than is the lamina, but the values are more variable. Phosphorus concentration in the lamina is significantly greater than that in the petiole for all treatments, but in the absence of any response to soil phosphate it is not possible to decide which organ best reflects phosphorus supply. Calcium and magnesium increase basipetally.

Introduction

A previous paper[1] dealt with the general principles of leaf analysis, sampling techniques, errors of sampling and analytical methods. It was shown, for black currant (*Ribes nigrum*), that the nutrient composition of the leaves changes with position and season, and that there are large differences between laminae and petioles with respect to total nitrogen, phosphorus and potassium. Furthermore, leaf samples taken from the mid-third position of extension shoots, at the fruit ripening stage, are most suitable for reflecting the nutritional status of the bush and for predicting the response to fertilisers. The present paper deals with the concentrations of the major nutrient elements in the leaves and petioles from non-fruiting raspberry canes, as affected by position on the cane and by manurial treatment, and the sensitivity with which the composition of samples from different cane positions reflects the soil treatments.

Experimental

The samples were taken from young plants, variety Lloyd George, growing on one of the long-term fertiliser plots at Long Ashton. There were six soil treatments, each in triplicate,

* Part I: *J. Sci. Fd Agric.*, 1960, **11,** 229

arranged in three randomised blocks. They were: no manure or fertilisers (Nil); farmyard manure (FYM), at 10 tons per acre annually; complete fertiliser (NPK); PK; NK and NP. The fertiliser rates and the effect of treatments on soil fertility are given in Table I.

Leaf sampling procedure

On July 29, 1958 (fruit ripening stage), one non-fruiting cane, representative of the general vigour of the canes on the plot, was selected from each of 20 stools per plot and removed at ground level, thus giving a total of 20 canes per sample per plot. Each cane was then divided into three equal parts, upper, middle and lower. The leaves were removed from each part, separated into petiole and lamina and combined according to position and treatment, thus making a total of 108 samples for the whole experiment. The samples were dried at 90–100° and analysed for total N, P, K and Ca according to the methods described by Bould *et al.*,[1] and for Mg by the method of Bradfield.[2]

Table I

Chemical composition of soil, February, 1954
(Mean values as % of air-dry soil)

Soil constituent	Annual treatments from 1927 onwards						Significant differences	
	Nil	FYM	NPK	PK	NK	NP	1%	5%
Organic carbon (C)	1·97	2·58	2·00	2·14	2·28	2·04	0·32	0·23
Exchangeable potassium (K)	0·006	0·012	0·009	0·012	0·012	0·008	0·003	0·002
Phosphorus (P) sol. in 1% citric acid	0·015	0·036	0·030	0·029	0·017	0·030	0·009	0·007
Phosphorus (P) sol. in 0·5N-acetic acid	0·006	0·015	0·012	0·012	0·006	0·010	0·007	0·005
pH (Soil/water = 1 : 2·5)	7·8	7·8	7·8	7·6	7·9	7·9	—	—

FYM = 10 tons per acre
N = 3 cwt. ammonium sulphate (21% N) per acre
P = 4 cwt. calcium superphosphate (19% P_2O_5) per acre
K = 1 cwt. potassium sulphate (48% K_2O) per acre

Statistical methods

There were 10 sets of analyses: N, P, K, Ca and Mg in lamina and in petiole. Each set contained 54 values, being the determinations made from the three sampling positions (upper, middle and lower third of shoots) for each of the 18 field plots (3 replicates of 6 treatments). An analysis of variance was carried out for each set, with sampling positions treated as a systematic factor.[3] The error variances (each with 20 d.f.) for determination of the same element in lamina and petiole were then compared; where these were not different (P, Ca, Mg), a pooled variance (40 d.f.) was used and only one set of significant differences is required in Tables IV–VI. These Tables enable comparisons to be made between any two treatment-position combinations within lamina or within petiole, and between the corresponding treatment-position combination in lamina and in petiole. The latter comparisons in Tables II and III (N and K) have been made using an average variance from the two sets of data (lamina and petiole).

For an assessment of the size of differences shown up in particular positions, relative to their precision (Table VII), a finer analysis of the data is necessary, in groups of 18 figures (3 replicates of 6 treatments within an element, either lamina or petiole, and either upper or middle or lower third of shoot). Error variances used in calculating significant differences for this table have 10 d.f.

Results

The analytical results are given in Tables II–VI. The values in each case are the means of three replicates. Table VII summarises the sensitivity to soil treatments of lamina and petiole with respect to position on the cane.

Nitrogen

For all treatments, nitrogen in the lamina is significantly higher (about 3 times) than nitrogen

in the petiole and, in both lamina and petiole, nitrogen decreases from the upper to lower third of the shoot (Table II). These results are in agreement with those obtained by Ramig & Vandecaveye[4] for raspberries grown in water culture. Since the lamina has a much higher N content than the petiole, it would seem a better organ to use for indicating the nitrogen status of the plant. The effect of nitrogen, as judged by the difference in % N between plants on NPK and PK plots, is best shown in the lamina from the lower-third region of the shoot (Table VII). For both lamina and petiole, the upper and middle third of the shoot show no significant differences due to soil nitrogen treatments.

Table II

Total nitrogen

(Mean values as % of dry matter)

Treatment	Lamina			Petiole		
	Upper third	Middle third	Lower third	Upper third	Middle third	Lower third
Nil	3·52	2·86	2·24	1·27	0·94	0·82
FYM	3·27	2·99	2·42	1·27	1·00	0·87
NPK	3·35	2·92	2·45	1·21	0·95	0·85
PK	3·39	2·76	2·13	1·15	0·86	0·73
NK	3·34	3·06	2·54	1·20	0·97	0·88
NP	3·64	3·15	2·54	1·41	1·15	1·06
Sig. diff. between any two means in each section	0·31***	0·23**	0·17*	0·14***	0·10**	0·07*
Sig. diff. between lamina and petiole means for corresponding positions and treatments		0·22***		0·17**		0·13*

* Significant at 5% level
** ,, ,, 1% ,,
*** ,, ,, 0·1% ,,

Potassium

On plots receiving K, potassium levels in the petiole from the upper and middle regions are significantly higher than those of the lamina from corresponding positions, but the reverse is true for plots deficient in K (i.e., when the level of K in lamina and petiole is low). This is in agreement with the findings of Ramig & Vandecaveye (*loc. cit.*), who found petiole-K < lamina-K until a level of about 1·1% was reached, when reversal occurred. In the lower-third region of growth, no significant differences are observed between petiole- and lamina-K (Table III).

Table III

Total potassium

(Mean values as % of dry matter)

Treatment	Lamina			Petiole		
	Upper third	Middle third	Lower third	Upper third	Middle third	Lower third
Nil	0·96	0·61	0·63	0·89	0·60	0·50
FYM	1·93	1·92	2·10	2·48	2·33	2·28
NPK	1·59	1·42	1·55	1·97	1·72	1·52
PK	1·99	1·89	2·10	2·27	2·14	1·99
NK	1·81	1·70	1·95	2·09	2·00	1·94
NP	0·74	0·45	0·46	0·48	0·38	0·34
Sig. diff. between any two means in each section	0·17***	0·13**	0·09*	0·34***	0·25**	0·18*
Sig. diff. between lamina and petiole means for corresponding positions and treatments		0·27***		0·20**		0·14*

In the petiole, the potassium decreases from the upper to the lower third of the shoot whereas, in the lamina, a decrease from upper to middle is followed by a slight increase from middle to lower.

Ramig & Vandecaveye (*loc. cit.*), who sampled from the middle third region of the shoot (first six physiologically mature leaves beginning with the third or fourth leaf from the tip), preferred the petiole for establishing the K status of the plant since it had a lower K content than the lamina at low levels of K and a higher one at high levels of K. However, Table VII indicates that although bigger differences (NPK — NP) are shown by the petiole, the standard deviation of the determination (as indicated by the differences necessary for significance) is also greater. Laminae or petioles sampled from any position on the shoot appear to reflect the soil treatments adequately.

Phosphorus

The phosphorus content of the petiole is significantly less than that of the lamina for all treatments (Table IV). Ramig & Vandecaveye,[4] using the middle-third region of shoot growth, found that at low levels of P nutrition, petiole-P was less than lamina-P until a concentration of about 0·3% was reached, when reversal took place. In our experiment, the level of P for either tissue in the middle-third region did not exceed 0·3% for any treatment and no indication of reversal was obtained. Variation in % P down the shoot is dependent on fertiliser treatment for both lamina and petiole. P % in the lamina decreases from the upper to lower third for all treatments except FYM and PK, where a decrease from upper to middle is followed by an increase from middle to lower.

Table IV

Total phosphorus

(Mean values as % of dry matter)

Treatment	Lamina			Petiole		
	Upper third	Middle third	Lower third	Upper third	Middle third	Lower third
Nil	0·324	0·298	0·295	0·238	0·251	0·251
FYM	0·270	0·255	0·279	0·191	0·195	0·212
NPK	0·266	0·216	0·203	0·191	0·171	0·172
PK	0·301	0·275	0·314	0·216	0·213	0·222
NK	0·258	0·215	0·200	0·184	0·168	0·157
NP	0·336	0·257	0·227	0·211	0·193	0·179
Sig. diff. between any two means in above table		0·034***		0·026**		0·019*

From this experiment, it is not possible to assess which organ best reflects P treatments since omission of fertiliser phosphate has no effect on the P status of the plants. For the petiole and lamina from all positions, the differences between NPK and NK are not significant (Table VII). The high values for % P in the Nil and PK plots are probably due to restriction of growth by the omission of nitrogen, with a consequent concentrating effect on phosphorus.

Calcium and magnesium

Petiole-calcium is higher than lamina-calcium in the upper third of the shoot. In the middle third region, petiole-Ca is higher than lamina-Ca at the lower levels of Ca, but reversal takes place at about 2·5% (Table V). This effect may well be associated with potassium reversal which also takes place in this part of the shoot. Ramig & Vandecaveye[4] found petiole-Ca was always higher than lamina-Ca, but the figures they obtained for % Ca seem extremely low (e.g., a highest level in the lamina of 0·16% and a suggested critical level of 0·2%). For all treatments, % Ca in the lamina and petiole increases from upper third to the lower third of the shoot.

Lamina-Mg in the upper third of the shoot is significantly greater than petiole-Mg except on the two treatments (Nil and NP) not receiving K where reversal occurs (Table VI). In the mid-third region, lamina-Mg is always higher than petiole-Mg, whereas in the lower third

Table V

Total calcium

(Mean values as % of dry matter)

Treatment	Lamina			Petiole		
	Upper third	Middle third	Lower third	Upper third	Middle third	Lower third
Nil	1·73	2·94	3·89	2·19	2·81	3·29
FYM	1·03	1·71	2·35	1·36	1·87	2·44
NPK	1·24	2·18	3·19	1·60	2·38	3·25
PK	1·15	1·84	2·55	1·58	2·27	2·71
NK	1·16	1·90	2·75	1·52	2·20	2·78
NP	1·94	3·15	4·27	2·14	2·81	3·10
Sig. diff. between any two means in above table		0·32***	0·24**	0·18*		

Table VI

Total magnesium

(Mean values as % of dry matter)

Treatment	Lamina			Petiole		
	Upper third	Middle third	Lower third	Upper third	Middle third	Lower third
Nil	0·367	0·564	0·648	0·377	0·528	0·722
FYM	0·288	0·334	0·370	0·242	0·278	0·381
NPK	0·245	0·297	0·377	0·193	0·246	0·352
PK	0·245	0·267	0·291	0·206	0·238	0·300
NK	0·250	0·287	0·343	0·208	0·250	0·342
NP	0·328	0·504	0·665	0·334	0·491	0·669
Sig. diff. between any two means in above table		0·057***	0·043**	0·032*		

Table VII

Significance of the effects of treatments on the composition of lamina and petiole from different positions on the shoot

	Total nitrogen (as % of dry matter)		Total potassium (as % of dry matter)		Total phosphorus (as % of dry matter)	
	Treatment effect NPK—PK	Size of effect needed for significance	Treatment effect NPK—NP	Size of effect needed for significance	Treatment effect NPK—NK	Size of effect needed for significance
Lamina						
Upper third	−0·043	0·440*** 0·304** 0·214*	0·856***	0·468***	0·0080	0·0193*
Middle third	0·157	0·663*** 0·458** 0·322*	0·970***	0·569***	0·0013	0·0286*
Lower third	0·317**	0·423*** 0·292** 0·206*	1·096***	0·730***	0·0030	0·0300*
Petiole						
Upper third	0·060	0·229*** 0·158** 0·111*	1·493***	0·813***	0·0070	0·0247*
Middle third	0·093	0·236*** 0·163** 0·115*	1·334***	0·798***	0·0030	0·0258*
Lower third	0·123*	0·202*** 0·140** 0·098*	1·177***	0·835***	0·0153	0·0348*

there is no significant difference between them, apart from the Nil plot in which petiole-Mg is significantly greater than lamina-Mg. For all treatments, % Mg in the lamina and petiole increases from the upper to the lower third of the shoot.

It is not possible from this experiment to assess which organ best reflects magnesium and calcium status of the plant since neither of these elements was included in the soil treatments.

Conclusions

It is clear from the analytical results in Tables II–VI that the chemical composition of the lamina and petiole is considerably affected both by position and soil treatment. For samples taken at the fruit ripening stage from non-fruiting canes, the lamina has a higher nitrogen concentration, and is more sensitive to nitrogen supply, than the petiole, and of all positions the lower third best reflects soil nitrogen treatment. Lamina and petiole, from any position on the cane, reflect soil potassium treatment. The petiole is more sensitive than the lamina to changes in potassium supply, but the values are more variable and a greater difference is required for the same level of significance.

The phosphorus concentration in the lamina is significantly greater than that in the petiole for all treatments, and all positions, but in the absence of any response to soil phosphate it is not possible to decide which organ best reflects phosphorus supply.

Bearing in mind the sensitivity to nutrient supply, the variability between samples, and the introduction of errors in sub-sampling,[1] it would appear that the lamina from the middle to lower third region of non-fruiting canes best reflects N and K supply and may be the most suitable organ for relating nutrient supply and crop yield.

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ERRATUM

In the paper by Hemingway and Ritchie, ' Hypomagnesaemia in sheep. Some inconsistencies ' (*J. Sci. Fd Agric.*, 1963, **17**, 162–171), the points on the ordinate (% of ewes) should read 10, 20, 30 instead of 1·0, 2·0 and 3·0.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

MAY, 1963

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.

INDEX OF AUTHORS' NAMES

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

MAY, 1963

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Micromorphological implications of time as a soil-forming factor, illustrated from sites in South-Eastern England. J. B. Dalrymple (*Z. PflErnähr. Düng.*, 1962, **98**, 232—239).—South-Eastern England soils formed during the post-glacial period from brickearths are characterised by clay transformations and the formation of B_t horizons. This results in the addition of iron-stained clay to the 'erde' fabric. These B_t fabrics are differentiated from other relic features found in some other brickearths. M. LONG.

Characteristics of Brown Grumusols of Arizona. W. M. Johnson, J. G. Cady and M. S. James (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 389—393).—Characteristics of the soils are described. A. H. CORNFIELD.

Soil morphology and genesis at higher elevations of the Great Smoky Mountains. R. J. McCracken, R. E. Shanks and E. E. C. Clebsch (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 384—388).—Characteristics and genesis of soils of the area are described and discussed. They are classified as podsols and Sol Brun Acide. A. H. CORNFIELD.

Contribution of molecular sized particles to the morphology of soil. W. Flaig, H. Beutelspacher and H. Söchtig (*Z. PflErnähr. Düng.*, 1962, **98**, 225—231).—Water dipoles are responsible for the retention of cations on clay minerals. Linear polymers cause flocculation or dispersion, according to their chain length, charge and the presence of cations. M. LONG.

Micromorphological differentiation of grey-brown podsols, brown podsols and humus podsols. H.-J. Altemüller (*Z. PflErnähr. Düng.*, 1962, **98**, 247—258).—Both the fabric of the B horizons of grey-brown podsols and the orstein fabric in podsols possess the characteristics of chlamydomorphic fabrics, whilst brown podsols have 'erde' fabrics. M. LONG.

The term 'lessivé' based on micromorphological observations. G. Manil (*Z. PflErnähr. Düng.*, 1962, **98**, 214—218).—The term is defined and an explanation given for the formation of 'sols lessivés' which takes into account the effect of climatic changes and human influences. An attempt is made to relate 'sols lessivés' to podsolic soils, 'sols brauns lessivés' and pseudo-gleys. M. LONG.

Lessivé-brown earth interferences in glacial till. G. Reuter (*Z. PflErnähr. Düng.*, 1962, **98**, 240—246).—Several lessivé (brown earth interferences on glacial deposits) on the upper Pleistocene, due to disharmonious and non-repeatable processes, are demonstrated. M. LONG.

Micromorphological contribution to the knowledge of the acid brown earths of the Belgian Ardennes. F. Delecour and G. Manil (*Z. PflErnähr. Düng.*, 1962, **98**, 219—224).—The A_1 horizons of these soils generally contain mull-type humus in association with *Festuca silvatica* and moder-type humus in association with *Vaccinium myrtillus* or similar vegetation. The usefulness of micromorphological considerations are discussed with regard to interpretation of analytical data. M. LONG.

Formation, morphology and micromorphology of hillside peat soils lying on limestone in Croatia. Z. Gračanin (*Z. PflErnähr. Düng.*, 1962, **98**, 264—272).—The hillside peat soils are related to the high moor soils, described by Kubiena as 'sphagnum peat moor of the podsol zone'. M. LONG.

Extractable iron in relation to soil classification. G. G. C. Claridge (*N.Z. J. Sci.*, 1962, **5**, 269—278).—Results show that the proportion of Fe oxides extractable with Na dithionate in a citrate buffer at pH 7 to the total Fe oxide content of a soil derived from igneous rocks gives a good indication of the weathering that the soil has undergone. The test is less suitable for soils from non-igneous rocks. (12 references.) W. ELSTOW.

Taxonomic significance of the type and formation of iron hydroxide minerals in tropical soils. W. Kubiena (*Z. PflErnähr. Düng.*, 1962, **98**, 205—213).—Many newly-formed mineral formations, especially those with Fe hydroxide minerals, are of important taxonomic significance. Peptised amorphous Fe hydroxide (**I**) is found in brown loams, a preponderance of flocculated **I** in brown earths, peptised amorphous **I** mottles and crystal aggregates of goethite (**II**) and lepidocrocite in pseudo-gleys, very fine **II** and haematite (**III**) crystallites in red loams and flocculated **II** and **III** in red earths. Coarse crystal aggregates of **II** and **III**, tending to fuse together, are found in laterites. These have an important influence on scoria formation. M. LONG.

Shape of hydrated ferric oxide deposits in gleys and pseudo-gleys. F. Blümel (*Z. PflErnähr. Düng.*, 1962, **98**, 258—264).—Hydrated Fe_2O_3 deposits (**I**) in gleys are generally tubular or exist as flakes, whilst in pseudo-gleys they tend to become concretions in the leached G-horizons. An attempt is made to correlate shape of **I** with the degree of waterlogging. M. LONG.

Temperature effect on the equilibrium energy status of water held by porous media. G. E. Wilkinson and A. Klute (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 326—329).—Desorption curves were determined for two sand fractions and a silt fraction at 48° and 44°. Temp. coeff. of the potential calculated on the basis that surface tension–radius of curvature was the principal water-holding mechanism were compared with the experimental observations. The results indicated that in the fine sand (53—74 μ) and silt (13·5—18·0 μ) other mechanisms besides surface tension–radius of curvature may be involved in holding water in the porous medium. A. H. CORNFIELD.

Retention of water in soil and imprisonment of air. Effect of non-wettability and rôle of organic matter. A. Feodoroff (*C. R. Acad. Agric. Fr.*, 1962, **48**, 533—536).—Two soils (one with org. matter content 3·4% and 33% of mineral matter smaller than 20 μ and the other with org. matter content 1·55% and >70% of fine sand between 50 and 200 μ) were subjected to high and low intensity watering at atm. pressure and under vac. Max. water retention was obtained by high intensity watering at atm. pressure and min. by low intensity at atm. pressure. Distribution of water in the soil was most regular by low intensity watering. Removal of org. matter by treatment with H_2O_2 leads to lower water retention and more even distribution. J. V. RUSSO.

Drying patterns of a sandy clay loam in relation to optimal depths of seeding. C. L. Wiegand (*Agron. J.*, 1962, **54**, 473—476).—Within two days of irrigation of a sandy clay loam a parabolic moisture distribution arose in the zone where seed is normally planted. The drying rate could be expressed in a simple analytic expression, which could be used for estimating moisture content at any period and for calculating the rate at which the moisture content of the soil at any depth is changing. A. H. CORNFIELD.

Field determination of hydraulic conductivity above a water table with the double-tube method. H. Bouwer (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 330—335).—Equipment and procedures for field application of the double-tube method are described. A. H. CORNFIELD.

Accuracy and source strength in soil moisture neutron probes. C. H. M. van Bavel (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 405).—In routine work satisfactory accuracy may be obtained with current neutron equipment, but using neutron sources of less strength and consequently less radiation hazard. In addition, lower soil moisture does not require greater source strength or longer counting time for equal accuracy. A. H. CORNFIELD.

Soil aggregates and their colloid chemistry. I. N. Antipov-Karataev and V. V. Kellermann (*Z. PflErnähr. Düng.*, 1962, **98**, 193—204).—No granulometric and micromorphological differences exist between the various sizes of water-stable aggregates. Aggregates are stabilised by colloidal binding substances during soil development. Different types of grain binding can be differentiated by successive treatment with various extractants. M. LONG.

Water stability of aggregates of two soils as influenced by incorporation of lucerne. D. E. Miller and W. D. Kemper (*Agron. J.*, 1962, **54**, 494—496).—In incubation studies water stability of the 1—2 mm. size aggregates of a fine sandy loam increased with time, after a lag period of about one week, when lucerne was added and also increased with rate of addition of lucerne (0·2—0·8% dry matter). In a clay loam aggregate water stability increased for a few weeks after addition of lucerne and then decreased. In the field aggregate water stability of a clay loam was increased for one growing season following the ploughing in of lucerne which had grown for 3 years. A. H. CORNFIELD.

4-t-Butylpyrocatechol as a fracturing aid in crusting soils. J. B. Hemwall and H. H. Scott (*Agron. J.*, 1962, **54**, 535—538).—The amount of 4-t-butylpyrocatechol required by soils to reduce their modulus of rupture (a measure of crusting) to zero increased with base exchange capacity and $CaCO_3$ contents and decreased with org. matter content. Most of the 14 soils studied required 150—250 p.p.m. of the chemical to reduce modulus of rupture to zero, and 400 p.p.m. of the chemical was sufficient for all soils. Radish emergence was greatly improved by the treatment of a sandy loam with the chemical. A. H. CORNFIELD.

Organic materials which stabilise natural soil aggregates. D. J. Greenland, G. R. Lindstrom and J. P. Quirk (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 366—371).—Aggregate stability (measured by permeability and wet sieving procedures) in cropped soils and those under pasture for 4 years or less was primarily due to polysaccharides and polyuronides, since destruction of these by treatment with aq. $NaIO_4$ followed by aq. $Na_2B_4O_7$, resulted in much reduced aggregate stability. In old pasture soils and those containing $CaCO_3$ aggregate stability was due to materials other than polysaccharides and polyuronides. A. H. CORNFIELD.

Evaluation of air-to-water permeability ratio for measuring differences in soil structure under different cropping systems. O. P. Cohen and E. Strickling (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 323—326).—The water stability of aggregates of 2·00—4·76 mm. dia. in a silt loam determined by a wet sieving method (*Soil Sci.*, 1948, **65**, 341) showed significant difference due to type of cropping. The highest aggregate stability occurred under continuous bluegrass, wheat and orchardgrass–ladino clover pasture and the lowest under continuous maize, soya-beans and fallow and a maize–wheat rotation. The air-to-water permeability ratio method (*Proc. Soil Sci. Soc. Amer.*, 1953, **17**, 324) showed no significant differences in the soil due to type of cropping system. A. H. CORNFIELD.

Soil moisture loss method for characterising simulated tillage treatments. W. H. Johnson (*Dissert. Abstr.*, 1962, **22**, 4300).—The effect of varying clod sizes in the tilled profile on soil moisture loss was evaluated. As clod size increased and compactive effort decreased the rate of soil drying increased and the total emergence of maize was reduced. A seedbed profile built up by separating and placing clods by size rather than subjecting a soil to a continual size reduction process appears a potentially practical proposition. F. C. SUTTON.

Effects of additions of pumice and expanded perlite on the physical properties of Taita clay loam soil. D. C. McDonald (*N.Z. J. Sci.*, 1962, **5**, 279—294).—Addition of pumice or perlite to certain clay soils produces a soil of an improved physical state for plant growth. Economically its use would be restricted to greenhouses and home and market gardens where at least 20% by vol. of the amendment should be used. The particle size of the amendment should be >0·84 mm. (15 references.) W. ELSTOW.

Precision of estimates of evapotranspiration in Missouri. W. L. Decker (*Agron. J.*, 1962, **54**, 529—531).—Both the Penman and Thornthwaite methods of estimating evapotranspiration approximated the measured values, with the former method giving a lower variability. A. H. CORNFIELD.

Effects of soil aeration and compaction upon yield, nutrient uptake and variability in a greenhouse fertility experiment. A. van Diest (*Agron. J.*, 1962, **54**, 515—518).—Dry-matter yields, root production and distribution, and total nutrient uptake by maize in pot tests using a sandy loam were little affected by compaction and aeration, although compaction increased soil bulk density and reduced the O_2-diffusion rate. Variability between replicates was increased by compaction and fertilisation. A. H. CORNFIELD.

Properties of intergradient chlorite-expansible layer silicates of soils. J. B. Dixon and M. L. Jackson (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 358—362).—The extent of interlayer deposition of 'gibbsite islands' that are differentially sol. in aq. NaOH from clay heated to 400° increased with increasing heat-stability of 14 Å spacing. The amount of interlayer Al removed from three soil clays was small (1—2% Al_2O_3) but the effect of its removal on the collapse of interlayer spaces was very marked. A. H. CORNFIELD.

Occurrence of chlorite-like intergrade clay minerals in Coastal Plain, Piedmont, and Mountain Soils of North Carolina. S. B. Weed and L. A. Nelson (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 393—398).—Chlorite-like intergrade clay minerals occurred commonly in the soils examined, ranging from trace to large amounts. The degree of interlayering usually decreased with depth. Chemical and mineralogical studies suggested that the intergrade clay minerals originated from mica precursors. A. H. CORNFIELD.

Problems in clay mineral identification by X-ray diffraction. M. E. Harward and A. A. Thdisen (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 335—341).—Clay minerals identified in a given sample depended on the specimen carrier, methods of Fe removal, dispersion reagent used, cation saturation of the clay, and in some cases, peeling of the specimens. These effects varied with different soils and size fractions of the same soil. This raises the question of whether clay minerals identified following sample prep. for analysis are really always those which occur in the soil. A. H. CORNFIELD.

Adsorptive behaviour of D-glucose with clay minerals. S. K. Re and R. C. Rastogi (*Z. PflErnähr. Düng.*, 1962, **98**, 121—125).—The Fe and Al contents of bentonite, biotite, muscovite, kaolinite and glauconite greatly influence the adsorption of glucose. In kaolinite OH^- ions are as important as Al. Ca and Mg depress glucose adsorption. M. LONG.

pH of Puerto Rican soils used for principal crops. G. Samuels (*J. Agric. Puerto Rico*, 1962, **46**, 107—119).—Most of the soils planted to bananas and coconut had pH >6, whilst pineapple, plantain and tobacco soils usually had pH <6 and 63% of the coffee soils had pH 4—6. Maize soils had a wide pH range, whilst cotton soils had a pH 5·0—5·9. 57% of the grapefruit soils had pH 4·0—4·9 and 29% pH 5·0—5·9. Most of the natural pasture soils had pH <6, whereas most of the improved and rotational pastures had pH >6. Most of the sugarcane soils had pH 5—8. The relationship between soil pH and soil series is presented. A. H. CORNFIELD.

Rapid method of measuring lime requirement of red-yellow podsolic soils. F. Adams and C. E. Evans (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 355—357).—The change in pH of a buffer solution (*p*-nitrophenol–H_3BO_3–KCl–KOH, pH 8·00) after shaking with soil was highly correlated with soil-exchangeable H^+ (exchange capacity minus total exchangeable bases by the NH_4 OAc method) for 348 soil samples from profiles of red-yellow podsolic soils. Thus the lime requirement of these soils can be estimated fairly accurately from a knowledge of their pH in water and their pH in the buffer solution. A table showing actual lime requirements derived from these two values is presented. A. H. CORNFIELD.

Effect of soil treatment on the accumulation of ions by several crops. K. McA. Pretty (*Dissert. Abstr.*, 1962, **22**, 4150—4151).—Field studies were conducted over a 2-year period to determine the effect of unbalanced soil fertility conditions on the growth and chemical composition of several crops grown on an infertile sandy loam soil. Changes in the composition of various plant species as a result of soil treatment can be caused by the relative activity of ions on the soil colloids, the varying cation-exchange capacities of the roots, the presence of specialised absorption sites on ion-carrier mechanisms, and the effect of climate on the physiological processes of the plants. F. C. SUTTON.

Fixation of added ammonium by the clay and organic fractions of soil. T. H. McIntosh (*Dissert. Abstr.*, 1962, **23**, 372—373).—A method for quant. estimating the non-biological fixation of NH_4^+ added to mineral surface soils was used in studies of NH_4^+ fixation by the org. and clay fractions of soil, lignin and vermiculite. Residual and fixed N contents of paired samples were determined after treatment with a measured amount of NH_4^+ in a closed system. A linear relationship was found between the soil C content and the amount of added NH_4^+ fixed by the soil org. matter. F. C. SUTTON.

The phosphorus-fixing capacity of organic soils. C. I. Harris and G. F. Warren (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 381—383).—Yields of tomato seedlings in a greenhouse were highly correlated with water-extractable P from 15 org. soils treated with various levels of P. The amount of P adsorbed from aq. PO_4^{3-} was a better indicator of the PO_4^{3-}-fixing capacity of the soils than was extraction with 0·75N-HCl. The PO_4^{3-}-fixing capacity of the soils were poorly related to their total P, total Fe + Al, or total Fe + Al + Ca contents. A. H. CORNFIELD.

Interconversions of inorganic phosphates as function of calcium potential. B. Ulrich (*Z. PflErnähr. Düng.*, 1963, **100**, 97—102).—The interconversions occurring between monocalcium, dicalcium, tricalcium and octacalcium phosphate, hydroxyapatite and Fe^{III} phosphate are investigated on the basis of their standard free energies and given as a function of the Ca potential (pH—0·5pCa). M. LONG.

Comparison of a number of chemical methods for extracting phosphorus from soils. H. L. Breland and F. A. Sierra (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 348—350).—The amounts of P extracted by 8 chemical methods from 7 different soils are reported. There were considerable differences in the amounts of P extracted due to extract and soil. The effect of time of shaking was also studied. A. H. CORNFIELD.

Rapid method for the estimation of total phosphorus in soils. R. S. Beckwith and I. P. Little (*J. Sci. Fd Agric.*, 1963, **14**, 15—19).—

If samples of soil are ignited (1 h. at 500—550°) before extraction, boiling HCl (100 ml. for 4 h. to 20 g. of soil) can extract the total P. Pre-ignition removes interference by Ti oxides; the soil-Ti is rendered less sol. The P is then determined by the molybdovanadophosphoric acid method after fuming off with $HClO_4$. Any interference caused by even a pale yellow colour can be removed by reduction with Fe, Zn or Mg. (10 references.) E. M. J.

Fluctuations in available potassium in soils of paddy fields where wet and dry cultivations are being practised. P. K. Zachariah (*J. Instn Chem. India*, 1962, **34**, 226—229).—Exchangeable K was determined in 26 samples of laterite soils and one sample of black soil from paddy fields cultivated under wet and dry conditions. A sharp rise in available K occurred on air-drying the samples and this was further increased by sun-drying. (10 references.) S. A. BROOKS.

Possible fluctuations in available phosphorus in soils of paddy fields where wet and dry cultivations are being practised. P. K. Zachariah (*J. Instn. Chem. India*, 1962, **34**, 230—232).—The available-P level in 26 samples of laterite soils and one sample of black soil was raised to a measurable amount and then determined after 3 months in a submerged state. The effect of drying on available P was found to be negligible. S. A. BROOKS.

Biological and chemical changes following scrub burning on a New Zealand hill soil. IV. Changes in some chemical properties. R. B. Miller (*N.Z. J. Sci.*, 1962, **5**, 259—268).—By burning the scrub, consisting mainly of gorse, and giving annual applications of lime (5 cwt./acre) and superphosphate (3 cwt./acre) the top inch of the original poor soil improved to a good soil and within 2 years most of the ground was covered by grass. W. ELSTOW.

Sorption and leaching of nutrient ions in horticultural composts. II. Potassium ions. J. Soukup (*Z. PflErnähr. Düng.*, 1962, **98**, 55—57).—As with NH_4^+ ions, the fraction of K held on columns of bentonite alone or mixed with peat as a whole and in the lower half rises with increasing bentonite content. That held is in an a table showing actual lime requirements derived from these two exchangeable form. M. LONG.

Nutrient content of Egyptian soils with special reference to the trace elements copper, zinc and boron. H. Kick (*Z. PflErnähr. Düng.*, 1963, **100**, 102—114).—The soils examined contain more B than do European soils—water-sol. B averaging 15% of the total B. The heavy coastal soils of the Nile Delta and Valley contain very high amounts of B, Zn and Cu. The application of composted town refuse markedly enriches those sandy soils which, initially, have very low nutrient and org. matter contents. Irrigation water from Nile-dependent canals and from artesian wells in two oases have high B contents (0·26—0·47 g./m.^{3}). The Ca, Na, Mg and K contents of these water supplies are generally lower than those of the lower Rhine. M. LONG.

Solubility of gibbsite in aqueous solutions and soil extracts. C. R. Frink and M. Peech (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 346—347).—The thermodynamic solubility product of pure, synthetic gibbsite expressed as pK_{sp} was 33·5 at 25° as determined in undersaturated and supersaturated $AlCl_3$ solutions and in 0·01M-$CaCl_2$ soil extracts. The rate of dissolution of gibbsite in undersaturated solutions and in synthetic soil extracts was extremely low. A. H. CORNFIELD.

Prediction of the concentration of solutes in soil solutions for soil systems containing gypsum and exchangeable calcium and magnesium. G. R. Dutt (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 341—343).—A method is described for calculating the equilibrium concn. of adsorbed and dissolved ions when a soil containing adsorbed Ca and Mg and an excess of $CaSO_4$ is mixed with water or a solution of Ca and Mg salts. Theoretical and experimental values for the ionic concn. of the equilibrium solution were obtained and compared for mixtures of $CaSO_4$, Ca–Mg–soil (with different ratios of Ca and Mg) and water or 0·05M-$MgCl_2$. A. H. CORNFIELD.

Effect of soil pH and texture on the availability of water-soluble boron in the soil. J. I. Wear and R. M. Patterson (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 344—346).—Pot tests with lucerne showed that B % in the plant was significantly correlated with water-sol. soil B for each of three soils of different texture receiving varying levels of applied B. However, B % in the plant per unit of water-sol. B in soil decreased in the order sandy loam, fine sandy loam, silty clay. In all three soils plant-B % decreased with increasing soil pH (from about 5·5 to 7·5). Differences due to pH were usually greater at high than at low water-sol. B levels in soil. A. H. CORNFIELD.

Determination of aluminium in plant materials and soil extracts. A. L. Page and F. T. Bingham (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 351—355).—An aluminon method, preceded by an ion-exchange technique for removing interfering substances, is described for determining Al in ashed plant material and in N-NH_4OAc or N-KCl (both at pH 4·8) extracts of soils. A. H. CORNFIELD.

Preparation of plant samples for *X*-ray emission spectrography. L. Chesnin and A. H. Beavers (*Agron. J.*, 1962, **54**, 487—489).—The prep. of briquettes of ground plant material, using 'Permount' as a cementing agent, for use in the *X*-ray emission spectrograph is described. The application of the method for the determination of Zn in maize leaf samples is presented. A. H. CORNFIELD.

Molecular and equivalent weights of the organic matter of a podsol. M. Schnitzer and J. G. Desjardins (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 362—365).—The mol. wt. of the org. matter extracted (NaOH) from The A_0 and B_h horizons of a podsol were 1684 and 669 respectively. From these values together with ultimate and functional group analyses values the calculated mol. formulae were $C_{75}H_{33}O_{17}N_3(COOH)_3(OH)_{12}(CO)_2$ for the A_0 and $C_{21}H_{12}(COOH)_6(OH)_5(CO)_2$ for the B_h org. matter. The equiv. wt. of the two materials were 165 for the A_0 and 76 for the B_h org. matter. A. H. CORNFIELD.

Sterilisation of soil by irradiation and observations on soil enzyme activity. A. D. McLaren, R. A. Luse and J. J. Skujins (*Proc. Soil. Sci. Soc. Amer.*, 1962, **26**, 371—377).—The extent of reduction in no. of aerobic bacteria by an electron beam (5 or 9 MeV), γ-radiation, and hard *X*-rays was very similar with increasing dose for a given soil, but differed among soils. A dose of 4 Mrep was necessary for complete sterility. Even sterilised soil showed phosphatase and urease activity in the presence of suitable substrates. Sterilised soil was not toxic to tomato plants nor did it provide extra nutrient to the plant as a result of radiation. A. H. CORNFIELD.

Viability of *Escherichia coli* in various soil types. H. Glathe, K. H. Knoll and A. A. M. Makawi (*Z. PflErnähr. Düng.*, 1963, **100**, 142—150).—Twenty days after inoculation of the soil with *Esch. coli communis* there is a marked increase in the bacterial count, the counts are higher in a loam than in a sandy soil and decrease immediately in the latter. At an inoculation rate of 100 bacteria per ml. *Esch. coli* can no longer be detected after 90 days or at a rate of 10,000 per ml. after 120 days. M. LONG.

Peroxide production by *Rhizobium meliloti*. F. D. Cook and C. Quadling (*Canad. J. Microbiol.*, 1962, **8**, 933—935).—The production of H_2O_2 by this and other organisms is briefly reviewed. The biological significance is considered; there is evidence that low O_2-tensions occur in root nodules. Possibly the production of traces of H_2O_2 contributes to the ineffectiveness of *Rhizobium* spp. C. V.

Determining the saccharase activity of soil. N. V. Peterson and E. V. Astaf'eva (*Mikrobiologiya*, 1962, **31**, 918—922).—Soil (passing a 2-mm. sieve) (20 g.) and toluene (10 ml.) are placed in a stoppered 100-ml. flask in a thermostat and stored for 3 h. at 50°. Then 10 ml. of phosphate buffer (pH 5·5) and 10 ml. of 20% sucrose are introduced and the mixture stored for 24 h. at 37°. The contents of the flask are diluted to the mark and the reduction of sugar determined. Pretreatment with toluene decreases the no. of micro-organisms in the sample. (15 references.) R. A. KEEN.

Suspension fertilisers. Anon. (*Farm Chem.*, 1962, **125**, No. 7, 14, 48).—Suspension fertilisers are defined as 'fluid fertilisers which contain crystals of fertiliser materials suspended in saturated fertiliser solution'. A short résumé is given of current problems in this field and of present means of solving them. A. G. POLLARD.

Automatic sampling device for improved reproducibility in testing granular materials. E. L. Gooden (*J. agric. Fd Chem.*, 1962, **10**, 397—399).—The apparatus consists of a small cup placed towards the circumference of a larger circular container which is mounted centrally on a turntable. The material to be sampled is placed in a funnel delivering into the large container in such a way that as this rotates the small cup continually passes beneath the stream of material thus collecting continuous regular cuts of the whole delivery of the sample. The mean deviation in analyses for % dust in materials of normal quality was seldom more than 0·2% of the sample wt., which was a great improvement on analyses of samples drawn by conventional methods. W. ELSTOW.

Application of the solubility-product principle to the dissolution of phosphate rock. J. G. Chaverri (*Dissert. Abstr.*, 1962, **23**, 371—372).—The solubility-product principle was applied in an investigation of the dissolution of a sample of finely ground phosphate rock in dil. aq. HCl under various conditions of wt. of solid material, concn. of acid, period of equilibration, and procedural techniques. The hypothesis was proposed that H^+ ions in solution react with OH^- supplied by the hydroxyfluorapatite to form water, thus keeping the OH^- activity at a low level. The solution and solid interact, raising the ratio of fluoride to hydroxyl in the solid. Experimental data are given in proof. F. C. SUTTON.

Particle size effects of water-soluble phosphate fertiliser. G. R. Burns (*Dissert. Abstr.*, 1962, **23**, 371).—A laboratory method was

developed for estimating the relative uptake of fertiliser P by plants from soil fertilised with NaH_2PO_4 in particles of different sizes. Measurements were made of fertiliser P absorbed during incubation by particles of anion-exchange resin strategically placed on the surface of moist soil with an appropriate quantity of ^{32}P-tagged NaH_2PO_4. The P absorbed by resin particles for individual soil segments provides an index of availability of the P supplied by a single particle of fertiliser. Field and greenhouse experiments are described and solubilities under various particle sizes are given. F. C. SUTTON.

High-analysis fertilisers from phosphoric acid and conventional ammoniating materials. M. R. Siegel, R. S. Meline and T. M. Kelso (*J. agric. Fd Chem.*, 1962, **10**, 350—361).—Processes for the prep. of granulated fertilisers by the reaction of H_3PO_4 and standard ammoniating materials are described both on laboratory and pilot-plant scales. Typical products are described. K incorporated in the product is fed as KCl to the ammoniator. (15 references.) W. ELSTOW.

Influence of ammonia and nitrate fertilisers on the amount of mineral nitrogen in the soil. II. Pot trials. G. Maass (*Z. PflErnähr. Düng.*, 1962, **98**, 146—154).—Applications of NO_3^--N to soils brings about more positive deviations from expected mineral N content than do applications of NH_4^+-N. M. LONG.

Pilot plant verification of the course of the ammonification of nitrosulphate fertilisers. V. Lakota (*Chem. Prům.*, 1962, **12**, 113—116).—Reactions occurring during ammonification in the production of nitrosulphate fertilisers from $Ca_5F(PO_4)_3$,$1/2SiO_2$ are examined and conditions under which a max. amount of SO_4^{2-} ions may be utilised for the formation of water-sol. P_2O_5 (as $NH_4H_2PO_4$) with lowest viscosities of processed liquors are established. Max. viscosities appear at pH between 2 and 3 and are caused by separation of $Ca(H_2PO_4)_2$. Absorption of NH_3 (at 85 ± 5°) is complete at pH 4·6. Above this value $NH_4H_2PO_4$ is converted into $CaHPO_4$. S. MALACHTA.

Effect of particle size on the action of crotonylidenediurea (CD-Harnstoff). J. Jung (*Z. PflErnähr. Düng.*, 1963, **100**, 115—120).—Among particles of CD-Harnstoff (**I**) <1 mm. there was no difference in release of N from the fertiliser in soil due to particle size. A granulation effect (slower release of N) appears with particles of **I**, 2—4 mm., and is accentuated if a binder is used. **I** is only very slowly mineralised when in tablet or briquette form and may persist over a period of years in these forms. M. LONG.

Effect of 2-chloro-6-(trichloromethyl)pyridine for the control of nitrification of ammonium and urea fertilisers. A. W. Swezey and G. O. Turner (*Agron. J.*, 1962, **54**, 532—535).—The effect of treating urea and NH_4 fertilisers with 2-chloro-6-(trichloromethyl)pyridine (0·125—2·000% of the N in the fertiliser) before application to soil, as compared with normal fertilisers was tested on a no. of crops grown on soils with texture ranging from sandy loam to clay. The treated fertiliser increased cotton yields compared with untreated fertiliser where $(NH_4)_2SO_4$, anhyd. and aq. NH_3 and urea were used, increased maize yields where $(NH_4)_2SO_4$ and anhyd. NH_3 were used, and sugar beet where $(NH_4)_2SO_4$ was used. The material functioned by inhibiting nitrification of fertiliser in the soil, thus preventing losses of fertiliser-N through leaching as NO_3^-. A. H. CORNFIELD.

Solid and liquid phosphorus fertilisers in the field, greenhouse and laboratory. K. N. Satyapal (*Dissert. Abstr.*, 1962, **22**, 4151).—Field and greenhouse experiments were conducted to compare the effectiveness of solid and liquid fertilisers in different placements on the yield and P absorption of several crops. $NH_4H_2PO_4$ labelled with ^{32}P was mixed with KCl and NH_4NO_3 to give a material of approximately 1—2—2 ratio. Maize yields were highest when liquid was dribbled followed by those obtained by the banded application. The vertical migration of P exceeded that of lateral distribution, although this disproportion appeared to decrease with distance and time. F. C. SUTTON.

Responses of crops to very high rates of superphosphate. B. A. Brown (*Agron. J.*, 1962, **54**, 547).—The effects of banded and mixed-in additions of superphosphate (46% P_2O_5) at rates ranging from 400 to 102,400 lb./acre on the performance of four crops was studied in pot tests. Although the 6400-lb. rate stimulated the greatest early growth and largest yields with cabbage, tomato, lucerne and ryegrass in general, ryegrass produced the largest yields at the second cutting with the 102,400-lb. mixed-in treatment and the 25,600-lb. banded treatment. Banded superphosphate was usually toxic at the 25,600 and 102,400 rates. A. H. CORNFIELD.

Uptake of band- and broadcast-applied potassium and phosphorus by maize roots. E. H. Vasey (*Dissert. Abstr.*, 1962, **23**, 374).—A technique using both autoradiography and counting was used to study the efficiency of maize roots in absorbing Rb and P from labelled band and mixed application. Apparent diffusion coeff. for Rb and P were calculated from densitometer tracings of the autoradiographs. Greater movement of K out of a band and a greater efficiency per unit area of root for absorption of K play a rôle in the observed differences between band and broadcast applications of high rates of K and P. F. C. SUTTON.

Solubility status of zinc carriers intermixed with N–P–K fertilisers. W. A. Jackson, N. A. Heinly and J. H. Caro (*J. agric. Fd Chem.*, 1962, **10**, 361—364).—Application of trace quantities of Zn to Zn-deficient soils is conveniently carried out by adding the Zn salt to a conventional mixed fertiliser. Reaction of the Zn salt with the components of the fertiliser may make the Zn insol. Added as basic $ZnSO_4$ or zinc sulphate monohydrate the degree of water solubility of the Zn depends mainly on the pH of the fertiliser system. Ammoniated mixed fertilisers of pH 4·6—6·1 fixed more Zn than did non-ammoniated fertilisers of pH 3·6—4·2. Water-sol. Zn recovery was greatest at pH 6·1—6·6 due possibly to the formation of sol. zincates. Chelated Zn ($Na_2ZnEDTA$) remained water-sol. in N–P–K fertilisers which normally had a large capacity for immobilising free Zn^{2+}. (12 references.) W. ELSTOW.

Effect of nitrogen from six liquid stable manures on yield of oats and on uptake of nitrogen in pot trials. C. Tietjen (*Z. PflErnähr. Düng.*, 1962, **98**, 137—145).—Varying amounts of N, up to half the total in the sample, depending on the proportion of straw present are available to plants. The uptake and physiologically effective N (**I**) are virtually identical. A negative correlation exists between the C/N ratio of the manure and amount of **I**. M. LONG.

Correlation studies with maize using seasonal soil and tissue tests. H. M. Singh (*Dissert. Abstr.*, 1962, **22**, 4139).—The usefulness of nutritional indices derived for maize from seasonal soil tests for NO_3^-, PO_4^{3-} and K^+ was compared with those from seasonal tissue tests for the same nutrients. Soil and maize midrib samples were collected periodically. The time of appearance of visible N-deficiency symptoms was noted, and final yields of maize were taken. The best multiple correlations with both soil and tissue tests were obtained near the end of the grand period of growth and physiological development in maize, i.e., at about silking time. F. C. SUTTON.

Treatment of polymethyl methacrylate-containing materials. Röhm & Haas G.m.b.H. (B.P. 883,555, 23.3.59. Ger., 23.3.58).—A soil-improving agent is produced by interaction of polymethyl methacrylate with aq. NH_3 under pressure at 180—300°. H. S. R.

Plant Physiology, Nutrition and Biochemistry

Radiant energy utilised in evapotranspiration. C. B. Tanner and E. R. Lemon (*Agron. J.*, 1962, **54**, 207—212).—An evaluation of the amount of radiant energy utilised in evapotranspiration under field conditions briefly considers the radiation balance, the various components of the energy balance and their relative magnitudes, and the crop and soil factors influencing the amount of the net radiation exchange utilised in evapotranspiration. Data presented show that when soil moisture is available and a substantial crop cover shades the ground most of the net radiation is used in the evapotranspiration process. A. H. CORNFIELD.

Influence of soil moisture stress, localised in the fertilised zone, on the uptake and translocation of phosphate by soya-beans. J. N. Marais (*Dissert. Abstr.*, 1962, **23**, 386).—Greenhouse studies with soya-beans were undertaken to determine the reasons for the variable effect of soil moisture stress on the P uptake by plants grown for extended periods. A technique was employed by which labelled-P content could be measured with 93% accuracy during the growing period. Plants which had been subjected to high moisture stress in the fertilised zone resumed P-uptake at a greatly enhanced rate when the soil moisture stress was alleviated. The rate of P-uptake by these plants temporarily exceeded that of the well-watered plants. Moisture stress appeared to affect P-uptake through its influence on diffusion rather than on mass flow. F. C. SUTTON.

Effect of hexadecanol and octadecanol on efficiency of water use and growth of maize. S. R. Olsen, F. S. Watanabe, W. D. Kemper and F. E. Clark (*Agron. J.*, 1962, **54**, 544—545).—Addition of $C_{16}H_{33}$·OH or $C_{18}H_{37}$·OH (1—25 g. per 3 kg. soil) to soil in pot tests had no effect on water used per unit of dry matter production of maize over 52 days whether the chemicals were mixed with the soil or placed in bands. The treatments reduced yields when mixed at 25 g., but not at 5 g., per 3 kg. of soil. Band placement even at 25 g. had little effect on yields. Much of the added alcohols had decomposed by the end of the test. A. H. CORNFIELD.

Apparent free space [in plant tissue]. H. Marschner and K.

Mengel (*Z. PflErnähr. Düng.*, 1962, **98**, 40—44).—A review with 114 references. M. Long.

Absorption of labile phosphate by plants. O. Machold (*Z. PflErnähr. Düng.*, 1962, **98**, 99—113).—Drying soil increases its labile P content. PO_4^{3-} ions on the surface of fissures are presumably exchangeable isotopically and to a smaller extent extractable. The correlation coeff. for the relationship between plant absorption of P and labile P depends on the time taken to achieve isotopic equilibrium during the analysis, longer times of contact giving lower values of correlation coeff. M. Long.

Accumulation and redistribution of potassium and calcium in young apple trees. N. S. Ghosheh (*Dissert. Abstr.*, 1962, **23**, 385).—The uptake and redistribution of K and Ca in young apple trees growing under three different environmental conditions were studied by measuring the rate of uptake of both elements from nutrient solutions. Linear relationships were found between the K and the Ca content of the trees and the concn. of these elements in the nutrient solutions applied. F. C. Sutton.

Influence of the metabolism on the uptake and distribution of labelled potassium in sunflowers. K. Mengel (*Z. PflErnähr. Düng.*, 1962, **98**, 57—63).—Glucose in the nutrient solution does not affect the uptake of K by roots, but does alter the distribution within the plants. Foliar applications of glucose increase root uptake of K, although this effect is depressed by the addition of KCl to the spray. 2,4-Dinitrophenol (10^{-5}-M) in the nutrient solution halves the K uptake. This is most noticeable in the centres of active metabolism. M. Long.

Relation between root carbohydrate concentration and the potassium and calcium uptake of plants. K. Mengel (*Z. PflErnähr. Düng.*, 1962, **98**, 44—54).—The uptake of Ca and K by roots with low carbohydrate content (**I**) is low and transport of Ca and K within the plant is reduced. **I** has no influence on the concn. of Ca and K in the free space of the root. M. Long.

Soil salinity studies. I. Effect of calcium sulphate on the correlation between plant growth and electrical conductivity of soil extracts. G. W. Winsor, J. N. Davies and D. M. Massey (*J. Sci. Fd Agric.*, 1963, **14**, 42—48).—Lettuce were grown in pots given five levels of a mixture of KNO_3 and $(NH_4)_2HPO_4$ and four levels of $CaSO_4$ in factorial combination. Soil salinity was measured by the electrical conductivity of displaced soil solutions (**I**), saturation extracts (**II**), and soil suspensions prepared at water/soil (air-dry) ratios of 1 : 1, 2·5 : 1 and 5 : 1. With regard to correlation between fresh wt. of lettuce and conductivity measurements, that at a water/soil ratio of 1·1 was best. Highly significant correlations of plant wt. with measurements in **II** and **I** were obtained. The highest correlations of all were obtained in soil suspensions saturated with $CaSO_4$. E. M. J.

Physiological studies of salt tolerance of crop plants. VIII. Influence of IAA spraying on the deleterious effect of sodium sulphate on growth and maturity of wheat. M. N. Sarin and I. M. Rao. **XII. Influence of sodium sulphate on early seedling growth of wheat and gram varieties.** M. N. Sarin (*Agra Univ. J. Res.*, 1961, **10**, ii, 7—16, 41—60).—VIII. Wheat was grown in pots of soil containing 0·15% of Na_2SO_4. Four weeks after sowing the plants were sprayed with IAA (5 p.p.m.); control plants in normal soil were similarly treated. Na_2SO_4 retarded the growth and maturation of the plants, grain yields being affected more than the dry wt. of the shoot. Treatment of plants in normal soil with IAA increased dry wt. of shoots without affecting grain yields; in the saline soil IAA increased both wt. of shoot and yield of grain.

XII. Varieties of wheat and gram were grown in test-tube cultures to which toxic concn. (0·8%) of Na_2SO_4 were added. In the first 96 h. root growth (total length, no. of laterals) of wheat was restricted. Varietal differences were apparent and were probably dependent on the normal growth rates, rapidly growing varieties being the most affected. In corresponding tests with grain, differences in tolerance were shown, but in this case the slower-growing variety showed the greater damage. Plumule growth was adversely affected in both varieties without appreciable varietal difference. A. G. Pollard.

Submicroscopical aspects of mineral deficiencies. I. Calcium deficiency in the shoot apex of barley. N. G. Marinos (*Amer. J. Bot.*, 1962, **49**, 834—841).—Changes described in the structure of meristematic cells in the apical dome of the barley shoot due to Ca deficiency appear very quickly. Ca is essential for the maintenance of cell membrane systems. A. G. Pollard.

Symptoms of calcium deficiency in higher plants. W. Bussler (*Z. PflErnähr. Düng.*, 1963, **100**, 129—142).—In all species some Ca-deficiency symptoms are the same, developing from the same cause. The general symptoms are browning of the veins, softening, cracking and final death of the younger parts of the stem and, where applicable, blossom end rot. Troubles associated with Ca deficiency are quite different from those arising from high soil acidity. M. Long.

Resistance to aluminium and manganese toxicities in plants related to variety and cation-exchange capacity. P. B. Vose and P. J. Randall (*Nature, Lond.*, 1962, **196**, 85—86).—Twenty varieties of *Lolium perenne* and *L. multiflorum* were screened for resistance to Al; some ryegrass varieties were also investigated for resistance to Mn toxicity. Resistance to Al toxicity was independent of geographical region; annual and biennial varieties showed a high resistance. Resistance to both Al and Mn toxicities was associated with low cation-exchange capacity. S. A. Brooks.

Nutrient solutions for sugar beet grown in water and sand culture. C. Horovitz (*Z. PflErnähr. Düng.*, 1963, **100**, 127—129).—The most satisfactory nutrient solution, of six tested, is that of Ukradyga and Oleksijuk (*Dokl. Akad. Sci. SSSR*, 1937, **17**, 483) to which the following micronutrients are added: Zn, Cu, Mo, Al, Co, Sr, F, I, Br, Sn. M. Long.

Acid-soluble compounds in rape and sugar beet, grown at different soil temperatures, and with different mineral feeding. V. P. Dadykin and R. P. Ivanova (*Dokl. Akad. Nauk SSSR*, 1962, **146**, 229—232).—Rape and sugar beet were grown under laboratory conditions with soil temp. 6—8°, and controls at 20—25°. Plants were also grown with and without the application of a fertiliser particularly rich in phosphates. The foliage of the plants was cut off, extracted with trichloroacetic acid, and the extracts, after suitable treatment, examined in a spectrophotometer. The foliage of plants grown in low-temp. soil, and of plants which had received applications of fertiliser, contained larger quantities of acid-sol. derivatives (not identified) than did controls. Since the foliage of plants, grown under conditions similar to those described, absorbs increased light energy it is concluded that this energy contributes to the formation of increased quantities of acid-sol. deriv., which play an important part in the accumulation and distribution of energy in the plant. (16 references.) A. S. Levesley.

Distribution of nitrogen in the growing apple shoot. N. A. Turner (*N.Z. J. agric. Res.*, 1962, **5**, 368—372).—The results given suggest that during growth the basal leaves of the shoots lose and regain a considerate portion of the N_2. Sol. N at all times is $<10\%$ of the total N. W. Elstow.

Isolation of poly-β-hydroxybutyric acid from root nodules of legumes. H. G. Schlegel (*Flora*, 1962, **152**, 236—240).—Nodules of all legume species examined contained poly-β-hydroxybutyric acid (analyses recorded). A. G. Pollard.

Occurrence and characteristics of invertase in the fruit of green plantain, *Musa parasidiaca*. B. G. Garcia (*J. Agric. Puerto Rico*, 1962, **46**, 120—126).—The crude-protein fraction isolated from the green plantain caused inversion of sucrose solution by the invertase of the green plantain at rates proportional to the concn. of the enzyme. The inversion followed a first-order reaction when concn. of the substrate was below 6%. pH 4·15 and temp. 44·4° were the optimum conditions for the activity of the invertase. A. H. Cornfield.

Water-soluble vitamins in the sieve tubes of some trees. H. Ziegler and I. Ziegler (*Flora*, 1962, **152**, 257—278).—Data for about 30 species are recorded. In general there were considerable proportions of thiamine (mainly free), nicotinic acid (largely in vitamin mol.) and vitamin C (with dehydroascorbic and diketogulonic acids). Riboflavin, pantothenic acid (partly bound), vitamin B_6 (mostly free; absent only from *Robinia* and possibly *Populus nigra*), biotin and folic acid were present in smaller amounts, and vitamin B_{12} was not definitely detected. A. G. Pollard.

Effects of kinetin and other plant-growth regulators on starch degradation. D. Boothby and S. T. C. Wright (*Nature, Lond.*, 1962, **196**, 389—390).—Treatment of wheat coleoptiles with gibberellic acid and kinetin caused an increased growth response due to release of D-glucose from starch. In seed halves from which the embryo had been removed, gibberellic acid released more reducing sugar than did kinetin; indol-3-ylacetic acid caused little, if any, breakdown of starch. S. A. Brooks.

Influence of dichlorophenoxyacetic acid on the seedling respiration of maize T.41. S. K. Sinha and I. M. Rao (*Agra Univ. J. Res.*, 1961, **10**, ii, 91—97).—The maize seeds were planted in contact with filter paper wetted with 2,4-D solution (0·1, 10·0 and 100 p.p.m.). After 24 h. the plants were transferred to water. As compared with controls, the lowest concn. of 2,4-D stimulated respiration for 2 days and subsequently depressed it; at 10 p.p.m., 2,4-D increased respiration for only 24 h. before restricting it and at 100 p.p.m. depressed respiration throughout the experimental period. A. G. Pollard.

Characterisation of an indole-type compound similar to indol-3-ylacetic acid, from Golden Delicious apples. A. L. Kenworthy and N. Harris (*Mich. agric. Exp. Sta., quart. Bull.*, 1961, **44**, 67—69).—Fractionation of the liquid from pulped flesh and skin of the apple yielded a substance exhibiting many of the properties of indol-3-ylacetic acid but some differences were established. The substance had growth-promoting properties (tomato ovary test). Other varieties of apple did not produce the substance. A. G. POLLARD.

Growth substances in developing peach fruit. Pyung Kyung Yu (*Dissert. Abstr.*, 1962, **22**, 4152—4153).—Fruit from three varieties of peach were collected at one-week intervals from two weeks after fruit set until ripening. The flesh, kernel and non-embryonic portion of the kernel of each fruit were extracted with ether, analysed by ascending paper chromatography with Bu^{t}OH–MeOH–water (80 : 5 : 15) as solvent and the fractions examined by the *Avena* first internode test. Bands were obtained at R_F values corresponding to the auxins, indole-pyruvic acid, -3-acetic acid, -3-butyric acid (**I**) and -3-acetonitrile and ethyl indol-3-ylacetate. Only **I** was detected chemically. The auxin content appeared to parallel the growth rate of the peach, rising to a max. after fruit set, falling to a min. during the retarded growth stage and rising to a further max. during accelerated growth before ripening. During the retarded growth period there was a marked thickening of the cell walls. R. J. M.

Change of chemical composition of plants through adsorption of phenol and coumarin derivatives from both nutrient solutions and soil. F. Schönbeck (*Z. PflErnähr. Düng.*, 1962, **98**, 126—136; cf. Winter *et al.*, Symposium on metal chelates, Seattle, 1959).—Coumarin deriv. are absorbed from nutrient solutions by the roots of young wheat and bean plants, are partly transported to the aerial parts of the plant and changed chemically. Some absorption of foliar sprays also takes place. Phenol and coumarin deriv. are absorbed from soils of low absorptive power and biological activity. M. LONG.

Effect of β-hydroxyethylhydrazine on the flowering of pineapple. H. R. Cibes and H. Gandia (*J. Agric. Puerto Rico*, 1962, **46**, 65—67).—Application of 0·06—0·12% β-hydroxyethylhydrazine (50 ml. applied to the centre of the plant) to pineapple plants resulted in uniform flowering in a single flush 48 days after treatment, whilst untreated plants flowered 2 months later. The treatment resulted in earlier and more uniform harvesting of the fruit. A. H. CORNFIELD.

Experimental control of flowering in *Lemna*. IV. Inhibition of photoperiodic sensitivity by copper. W. S. Hillman (*Amer. J. Bot.*, 1962, **49**, 892—897).—*L. perpusilla*, normally a short-day plant, was grown in a Hoagland medium containing Cu (0·5—10·0 μM/l.) and flowered under long- or short-day conditions. In the Cu-containing medium *L. gibba*, a long-day species failed to flower. The effect of Cu was modified but not obscured by some macro-nutrients, notably Ca^{2+} and PO_4^{3-}. Other micronutrients tested (Cd, Co, Cr, Mn, Ni, Pb, Zn) had no effect on the photoperiodic responses of the plants. A. G. POLLARD.

Determination of naphthylacetic (NAA) acid in apples. C. A. Bache, L. J. Edgerton and D. J. Lisk (*J. agric. Fd Chem.*, 1962, **10**, 365—366).—The NAA residue is extracted from the apples with 0·1N-aq. NH_3 and separated from interfering substances by chromatography on a silicic acid column. The NAA may then be determined by its u.v. absorption at 283 mμ in $CHCl_3$ solution or colorimetrically after nitration with a 0·1% solution of $NaNO_2$ in H_3PO_4, the yellow colour produced being measured at 370 mμ. A suitable standard curve covers the range 0—75 μg. NAA. One h. after being sprayed with NAA (20 p.p.m.), apples showed a residue of <0·1 p.p.m. W. ELSTOW.

Crops and Cropping

Nitrogen and phosphorus effects on the properties of the stems of selected varieties of winter wheat (*Triticum aestivum*, L.). F. L. Millen (*Dissert. Abstr.*, 1962, **22**, 4149—4150).—The effects of N and P fertilisation on the properties of the stems of winter wheat were studied, using five N and three P treatments. The effects of the fertilisers were assessed by both physical and microchemical techniques. Load-bearing capacity (L.B.C.) had little direct application in the prediction of lodging. Both L.B.C. and breaking strength of internodes were affected by applications of N. Lodging was increased by applying higher N levels and by autumn applications of fertiliser. P increased lodging, decreased breaking strength and had no effect on L.B.C. F. C. SUTTON.

Effects of autumn-applied nitrogen and winter rainfall on wheat yields. E. C. Doll (*Agron. J.*, 1962, **54**, 471—473).—In seven experiments carried out at two locations over 3—4 years yields of wheat were significantly higher in four cases with spring than with autumn-applied N (40—80 lb./acre). In long-term (23—26 years) rotation experiments at three locations 20—23% of the deviations from expected yields of wheat could be attributed to variations in winter rainfall. A. H. CORNFIELD.

Effect of cathode ray irradiation of wheat on surface moulds, respiration, flour flavour and odour, and germination. M. G. Cropsey, C. M. Leach, T. M. Ching, L. A. Sather and D. E. Wiant (*Ann. appl. Biol.*, 1962, **50**, 487—505).—Viable spores of *Aspergillus repens* in wheat kernels decreased with increasing dose of cathode ray irradiation (10,600—2,000,000 rep). Control was best when irradiation was done with high-moisture grain. A dosage applied on two sides was more effective than an equal dosage applied on one side. Dosages exceeding 42,400 rep (total dosage of 600,000 V) significantly reduced the total no. of normal plants germinating from irradiated kernels. Similar results were obtained with 23,000 rep and a total dosage of 1,000,000 V. No off-flavours or off-odours occurred with dosages of up to 169,600 rep (600,000 V) or 23,000 rep (1,000,000 V), regardless of moisture content or whether the dosage was applied on one or both sides. The respiration of high-moisture wheat inoculated with *A. repens* was significantly reduced with dosages exceeding 11,500 rep (1,000,000 V) or 19,500 rep (600,000 V), whether irradiation was applied to one or both sides. The respiration rate of high moisture kernels was not reduced to that of the low moisture kernels until the 500,000 rep dosage. A. H. CORNFIELD.

Influence of soil oxygen on growth and mineral concentration of barley. J. Letey, L. H. Stolzy, N. Valoras and T. E. Szuszkiewicz (*Agron. J.*, 1962, **54**, 538—540).—A soil-O_2 diffusion rate of approx. 15×10^{-8} g. cm.$^{-2}$ min.$^{-1}$ was limiting for root growth of barley; that for barley shoot growth was less than 40×10^{-8} g. cm.$^{-2}$ min.$^{-1}$. Vegetative growth of barley was relatively insensitive to low soil O_2. Low soil O_2 reduced shoot K % and P % but had little effect on Na% or (Ca + Mg) %. A. H. CORNFIELD.

Influence of leaf-blade removal on seed weight of oats. K. J. Frey (*Iowa J. Sci.*, 1962, **37**, 17—22).—Removal of all leaf blades reduced groat wt. to 55—88% compared with unclipped checks. Removal of the fourth and fifth leaf blades contributed little to seed wt. but the sixth and seventh (flag) leaf blades did increase the seed wt. Removal of half of the spikelets from oat panicles caused an approx 20% increase in seed wt. E. G. BRICKELL.

Evapotranspiration by irrigated maize. B. D. Doss, O. L. Bennett and D. A. Ashley (*Agron. J.*, 1962, **54**, 497—498).—Evapotranspiration by irrigated maize increased with plant development to a max. of 0·3 in. per day at the dough stage and decreased thereafter. The ratio of evapotranspiration by maize to open pan evaporation increased from 0·38 at emergence to 1·12 during the early dough stage and then decreased to 0·95 at grain maturity. A. H. CORNFIELD.

Relationship of nitrogen in maize leaves to yield. B. L. Baird, J. W. Fitts and D. D. Mason (*Proc. Soil Sci. Soc. Amer.*, 1962, **26**, 378—381).—The N % in maize leaves (first leaf below the lowest ear collected at silking) from plants growing on many sandy loam to loamy sand soils was usually highly correlated with yields where no or little N was applied, but was poorly correlated with yields at the higher rate of N application. N % of the leaf was a poor indicator of the amount of N fertiliser which should be applied; this held with respect to both max. possible yields and economic returns. A. H. CORNFIELD.

Effect of varying leaf area by partial defoliation and plant density on dry matter production in maize. P. Hoyt and R. Bradfield (*Agron. J.*, 1962, **54**, 523—525).—The net assimilation rate of maize increased linearly with leaf area index (LAI, leaf area per unit area of land) up to 2·7, but at higher LAI values it declined rapidly. Dry matter produced per unit of leaf area from grain initiation to maturity showed that top leaves were much more productive than bottom leaves. A. H. CORNFIELD.

Maize root distribution and moisture extraction in relation to nitrogen fertilisation and soil properties. D. L. Linscott, R. L. Fox and R. C. Lipps (*Agron. J.*, 1962, **54**, 185—189).—N-fertilised maize in a loamy sand produced deeper and more extensive roots during the early part of the growing system than did unfertilised maize, but by the end of the season root distribution was essentially the same under both conditions. The roots reached a depth of 71 in. in the loamy sand and 65 in. in a silty clay loam. Moisture utilisation was related to root growth, the period of greatest moisture use occurring 45—65 days after sowing. Water use increased with N fertilisation. Efficiency of water use, as measured by grain production, was increased 1·4 times by N fertilisation. The O_2 % in the soil air in the silty clay loam varied with soil moisture, depth and stage of growth of maize. The lowest O_2 % (about 12%) occurred in the B horizon during July and Aug. In the loamy sand O_2 % did not fall below 19%. A. H. CORNFIELD.

Maize growth and composition in relation to soil fertility. II. Uptake of nitrogen, phosphorus and potassium and their distribution in different plant parts during the growing season. III. Percentages of nitrogen, phosphorus and potassium in different plant parts in relation to stage of growth. J. J. Hanway (*Agron. J.*, 1962, **54**, 217—222, 222—229).—II. Differences in soil fertility influenced the amounts of N, P and K taken up by maize plants, but did not markedly change the seasonal pattern of uptake and distribution of these elements in the plants. Large proportions of N and P and a small proportion of the K were translocated from other plant parts to the grain. Late in the season K was lost from the leaves to the stalks.

III. Nutrient deficiencies were reflected in differences in N, P and K % in the leaves and leaf sheath more than in other plant parts. In early season samples, differences in NO_3^- % in the plant due to differences in N-availability were greater than those of total N. Within each plant part the water-sol. P % was highly correlated with the total P %. A. H. CORNFIELD.

Spatial distribution of net radiation in a maize field. O. T. Denmead, L. J. Fritschen and R. H. Shaw (*Agron. J.*, 1962, **54**, 505—510).—On clear days after max. leaf area development the net radiation at the ground constituted only 25% of the total net radiation measured above the crop, the rest being expended within the crop canopy. 73% of the energy expenditure within the crop occurred in its upper half. Row spacing closer than 40 in. could increase the energy available to the crop for photosynthesis by 15—20%. A. H. CORNFIELD.

Production of maize and soya-beans in alternate pairs of rows. M. W. Alexander and C. F. Genter (*Agron. J.*, 1962, **54**, 233—234).—Maize intercropped with soya-beans (both in alternate double rows) yielded approx. 30% more (for area actually in maize) than when planted alone. Degree of lodging was no different between intercropped maize and maize planted alone. On the area basis soya-bean yields obtained by intercropping were about the same as would be expected when planted alone in 36-in. rows. A. H. CORNFIELD.

Effects of high nitrogen fertilisation and lodging on rice yields. M. N. Basak, S. K. Sen and P. K. Bhattacharjee (*Agron. J.*, 1962, **54**, 477—480).—Results from many field tests in West Bengal showed that providing the crop did not lodge yield responses increased with level of applied N, with optimum yields at about 100 lb. applied N per acre. Where lodging occurred N uptake was much reduced and losses in grain yields increased with rate of application of N. Lodging seems to be a physiological phenomenon rather than a varietal characteristic. It originates from structural weakness developing in culm tissue caused primarily by deeper submergence of plants during vegetative growth aided by high N in the soil. A. H. CORNFIELD.

Effect of placement and time of incorporation of vetch on rice yields. W. A. Williams and D. C. Finfrock (*Agron. J.*, 1962, **54**, 547—449).—Placement of vetch tops at a 4—6 in. depth in the soil (clay) gave higher yields of rice (flooded) than did placement nearer the surface. Yields from the deeper placement of vetch were ⩾ those from the same amount of N (30—40 lb./acre) applied as $(NH_4)_2SO_4$ at 2—4 in. depth over 3 years. When vetch was buried at the 4 in. depth the highest yields of rice in one year were obtained when the soil was flooded on the day of incorporation of the vetch and yields became progressively lower as flooding was delayed for up to 30 days. In two other years time of flooding had no effect on increases in yield due to vetch additions. A. H. CORNFIELD.

Accumulation and elimination of ethanol in roots of sugar beet plants. D. G. Kenefick (*Dissert. Abstr.*, 1962, **22**, 4149).—The effect of an anaerobic environment upon the metabolism of intact sugar beet plants was primarily the accumulation of ethanol. It was further demonstrated that ethanol was transpired from the top portions of the plant when the taproot was placed under anaerobic stress. F. C. SUTTON.

Soil moisture regime effect on yield and evapotranspiration from warm-season perennial forage species. B. D. Doss, O. L. Bennett, D. A. Ashley and H. A. Weaver (*Agron. J.*, 1962, **54**, 239—242).—Average yields of dallis, common and Coastal Bermuda, and *Pensacola bahia* grasses increased as soil moisture increased (irrigating to field capacity when 30%, 65% and 85% of the available soil moisture had been removed from the top 24 in.). *Lespedeza sericea* yielded highest at the intermediate moisture regime. Total water used by the grasses depended more on the amount of available moisture in the soil than on plant species. Average daily evapotranspiration rates varied with moisture regime but there was little difference due to species. A. H. CORNFIELD.

Influence of time and rate of nitrogen application on production and botanical composition of forages. C. W. Alexander and D. E. McCloud (*Agron. J.*, 1962, **54**, 521—522).—Application of N (40—160 lb./acre) to an orchardgrass–lucerne pasture had little effect on forage yields or proportion of lucerne in the forage over 4 years. Orchardgrass–ladino clover and tall fescue–ladino clover pastures showed little differences in yields due to N applications initially but with time the clover virtually disappeared and high yields of forage were obtained only with the heavy N applications. Pure stands of orchardgrass and tall fescue gave increasing yields of forage with increasing rates of N. 80 lb. of N per acre was required to produce yields equal to those given by orchardgrass– and tall fescue–clover mixtures, and 240 lb. of N were needed to give yields equal to those of orchardgrass–lucerne. Earlier than normal and split applications of N did not increase total forage yields compared with single annual applications. A. H. CORNFIELD.

Wear resistance of cool season turf grasses. Effects of previous mowing practices. V. B. Youngner (*Agron. J.*, 1962, **54**, 198—199).—Studies with an 'accelerated wear' machine showed significant differences in wear resistance among several grass mixtures. Clipping at 2 in. for 3 years prior to testing resulted in better wear resistance than did clipping at 0·5 in. height. All the mixtures were affected to the same extent by the short clipping. A. H. CORNFIELD.

Root weight and distribution of blue panicgrass, *Panicum antidotale* Retz., as affected by fertilisers, cutting height and soil moisture stress. N. Wright (*Agron. J.*, 1962, **54**, 200—202).—Although roots were found even at 12 ft. depth in a sandy loam, 70% of the total root wt. occurred in the top 2 ft. Root wt. increased somewhat with rate of application of N with max. wt. at 525 lb. of N per acre. Root wt. was significantly increased by application of 525 lb. P per acre only when 175 lb. N per acre was applied. Application of K had no effect on root wt. Root wt. increased with clipping height (3—12 in.) and decreased with increasing depth of soil moisture stress. A. H. CORNFIELD.

Effects of five nitrogen sources on yield and composition of Napier grass, *Pennisetum purpureum*. J. Vicente-Chandler and J. Figarella (*J. Agric. Puerto Rico.*, 1962, **46**, 102—106).—The effects of applying 600 lb. of N/acre/annum from five sources on yield and composition of Napier grass growing on a latosol over a 3-year period were studied. Average dry-matter yields and % of P, K, Ca and Mn in the forage were no different between the five sources of N. However, protein % was less with urea and aq. NH_3 than with $(NH_4)_2SO_4$, $NaNO_3$ or NH_4NO_3. Forage yields were lower but protein % was higher during the 'winter' months of low rainfall, short days and cool weather. A. H. CORNFIELD.

Relationship between potassium fertilisation and the alanine, aspartate, asparagine and glutamate content of orchardgrass (*Dactylis glomerata*, L.) herbage. A. F. Gohlke (*Dissert. Abstr.*, 1962, **23**, 385—386).—Orchardgrass was fertilised with a factorial set of treatments including three N levels, each with three K levels. The effect on protein, and on the ratios of the constituents of the sol. N pool, was investigated in 4-week-old herbage. Chromatographic separation of the constituents in an ethanol extract revealed that asparagine was associated with the total N content, irrespective of K level, while alanine was correlated with K content at all N levels. F. C. SUTTON.

Estimation of pasture production by means of a measuring plate. W. D. Jagtenberb (*Landbouwvoorlichting*, 1962, **19**, 576—583).—An appliance is described by means of which an Al plate (dia. 50 cm., wt. 840 g.) can be lowered by a pulley and counterwt. (590 g.) device to rest on top of the herbage; the height of the plate from the ground is measured by a scale on the supporting structure. The appliance has the following advantages over the measuring board: (*a*) the readings are independent of personal experience; (*b*) the degree of resistance of the herbage to the pressure of the plate is to some extent dependent on the crop density; (*c*) repeated measurements can be made in the same place without injury to the herbage, in order to study the effect of climatic factors on the growth. (14 references.) P. S. ARUP.

Response of lucerne to irrigation and fertilisers. D. J. Lathwell and M. T. Vittum (*Agron. J.*, 1962, **54**, 195—198).—Irrigation of a silt loam increased dry-matter yields of lucerne by more than 1200 lb./acre/annum in 5 of 8 years. Over the 8 years 200 lb. of dry matter/acre was produced/in. of irrigation water applied. High-level fertilisation (average P 33 and K 62 lb./acre/annum) increased dry-matter yields by an average of 240 lb./acre/annum over low-level fertilisation (P 6 and K 12 lb./acre/annum). The highest yields, averaging 10,500 lb. of dry matter/acre/annum, were obtained on irrigated plots receiving the high level of fertilisers. A. H. CORNFIELD.

Vegetative development of lucerne seedlings under varying levels of shading and potassium fertilisation. A. G. Matches, G. O. Mott and R. J. Bula (*Agron. J.*, 1962, **54**, 541—543).—The effects of light treatment (full sunlight, 46% and 69% light reduction) and K applications (0—250 lb./acre) on the development of three varieties of

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lucerne during the initial season of establishment were studied. Light reductions imposed when the plants were 6 weeks old had much more influence upon root dry wt. accumulation than upon top dry wt. accumulation. The lowest dry wt. accumulation occurred under the 69% light-reduction treatment. K applications increased root dry wt. accumulation, but had no effect on top dry wt. accumulation. Top dry wt. showed K × variety × light interaction, whilst root dry wt. showed dates × varieties and dates × light interactions. A. H. CORNFIELD.

Lucerne fractionation. Effects of various procedures in laboratory processing of fresh lucerne on separation of nitrogen and solids from fibre. A. W. Halverson (*J. agric. Fd Chem.*, 1962, **10,** 419—422).—Variations in grinding, temp. of grinding, method of extraction of the juice from the macerate and similar variables are examined in the water extraction of lucerne. Of the N, 66%, and of the solids, 45%, in lucerne may be extracted in one aq. extraction if the grinding temp. is kept between 15° and 18° and water ratio is 2 : 1. With larger-scale extractions the % extraction was not so good due possibly to an uncontrollable rise in temp. during a grinding that was also less efficient. The chemical composition of the extract dry matter is compared with that of lucerne dry matter. W. ELSTOW.

Stand and nodulation of lucerne from seeds pelleted with high dosages of Arasan. M. V. Desai and W. F. Buchholtz (*Iowa S. Sci.*, 1962, **37,** 79—85).—Pelleting with Arasan (40%) yielded better stands of lucerne and lespedesa in the field than did Arasan slurry or Ceresan M seed treatments. Nodulation was not adversely affected. (21 references.) E. G. BRICKELL.

Ladino clover–tall fescue association as affected by soil treatment and grass population variables. W. R. Paden (*Agron. J.*, 1962, **54,** 190—192).—Planting tall fescue at 12 in. spacing with ladino clover produced better yields and quality of forage (as measured by clipping to simulate rotational grazing) than did 6 in. or 18 in. spacing. Increasing rates of N application and reduction in spacing of fescue resulted in reduction of clover stand. K applications stimulated growth of both grass and clover and maintained the proportion of clover in the stand. Best results with respect to forage yields and stand maintenance were obtained with high K and medium N applications. A. H. CORNFIELD.

Magnesium deficiency in white clover (*Trifolium repens*, L.) on a pumice soil. F. D. Dorofaeff and K. J. McNaught (*N.Z. J. agric. Res.*, 1962, **5,** 310—317).—White clover presenting symptoms of Mg deficiency whilst growing in a N.Z. soil containing 0·06 mequiv. % exchangeable Mg responded to applications of $MgSO_4$ and $CaCO_3$. Response to the $MgSO_4$ was due to correction of the Mg deficiency, response to the $CaCO_3$ was possibly due to a correction of Mn toxicity. Mg levels in Mg-deficient foliage ranged from 0·03 to 0·07%, leaves from healthy plants contained 0·16 to 0·40% Mg. (14 references.) W. ELSTOW.

Yield response, residual nitrogen and clover content of an irrigated grass–clover pasture as affected by various rates and frequencies of nitrogen application. E. F. Maas, G. R. Webster, E. H. Gardner and R. H. Turley (*Agron. J.*, 1962, **54,** 212—214).—Forage yields over 2 years from a ladino clover–ryegrass–orchardgrass sward on a fine sandy loam increased with rate of application of N (75—225 lb./acre/annum). Splitting the N into 2—5 applications gave more uniform forage yields but had no effect on total yields as compared with a single spring application. The proportion of clover in the stand decreased with increasing rate of N irrespective of whether the N was split or not. N % in the forage increased in the clipping following an N application. The average N % in the six clippings made through the season increased with rate of N applied, to the same extent irrespective of the frequency of N application. A. H. CORNFIELD.

Feeding value losses in haymaking incurred by use of ventilation. N. D. Dijkstra and P. J. J. Philipsen (*Versl. landbouwk. Onderz.*, 1962, 68.4, 64 pp.).—In these experiments (made during 1957—59) the hay was first dried by spreading on the fields with daily shuffling and then stacked in barns and ventilated by air through slatted ducts at the base and through ducts from one or two vertical shafts. The average losses in this procedure were dry matter 11·4, digestible protein 20·4 and starch equiv. 31·3%, viz., ~50, 58 and 71%, respectively, of the losses previously reported for the conventional methods of haymaking (cf. Dijkstra, *ibid.*, 1947, 53.3; Brandsma and Dijkstra, *ibid.*, 62.14). The relationships between the composition and feeding value in these hays were the same as those in ordinary hays. For ventilation with unheated air the hay must contain <60% of dry matter before stacking; the permissible moisture content can probably be increased with the use of heated air. The use of heated instead of unheated air decreased the loss of carotene in a poor hay by ~50%. (21 references.) P. S. ARUP.

Effects of several foliarly-applied nutrients and plant growth-substances on a 'shelling' disorder of Concord grapes. R. P. Larsen and M. J. Bukovac (*Mich. agric. Exp. Sta. quart. Bull.*, 1962, **44,** 608—618).—The 'shelling' disorder (excessive fall of berries within a few days after flowering, and poor growth of vines) was not attributable to deficiency of 12 nutrient elements examined. Application of growth-substances produced no improvement. Gibberellin (20—250 p.p.m.), applied at full bloom or after, increased the set of fruit somewhat producing smaller (50—80% normal) parthenocarpic berries. A. G. POLLARD.

Increasing sugarcane yield by fertilisation and irrigation. Anon. (*Fertil. News*, 1962, **7,** 7—12).—Efforts to promote larger and better quality yields in peninsular India are described. The applications of farmyard manure and oil cake, inorg. N, P and K are discussed. C. A. P.

Relationships between leaf-potassium percentage and maturity of sugarcane. G. Acevedo-Ramos (*J. Agric. Puerto Rico*, 1962, **46,** 15—22).—Changes in the K % of the sugarcane leaf as related to time, level of K application and irrigation are reported. The K % in the leaf showed a marked decrease, especially where available K was low, initially, followed by a slow increase. Leaf K % increased with rate of application of K, particularly with the young leaf. Irrigation increased the leaf K %. A. H. CORNFIELD.

Comparison of phosphate fertilisers for tobacco. G. Samuels E. G. Beneta-Garcia and F. González-Vélez (*J. Agric. Puerto Rico*, 1962, **46,** 48—54).—The value of the tobacco crop per acre was increased to the greatest extent by applying P as powdered $Ca(PO_3)_2$ (**I**). Use of the granular form of **I** did not increase the value of the crop over the control. $CaHPO_4$ and $K_2CaP_2O_7$ (**II**) were the only other forms of P which increased the value of the crop/acre over that of the control. The highest quality tobacco was obtained where **II** was used. A. H. CORNFIELD.

Influence of applications of urea, sucrose and hormones on the carbon–nitrogen relation in cotton plants; its bearing on boll-shedding. S. N. Mathur (*Agra Univ. J. Res.*, 1961, **10,** ii, 99—101).—The treatments were applied to cotton seedlings as sprays. Urea produced shorter plants with shorter internodes than did controls. Sucrose increased the rate of growth and accelerated flowering and fruiting although the no. of flowers and bolls produced was generally lowered. Growth-substances (2,4-D, IAA) produced short bushy plants showing epinasty and, frequently, leaf deformation, with increased boll-formation and -shedding. The wt. of seed per plant was increased by 2,4-D in one variety and by sucrose and growth-substance in a second variety. A. G. POLLARD.

Reversal of non-dehiscence of anthers in cotton by foliar application of choline chloride and urea. R. A. Scott, jun. (*Agron. J.*, 1962, **54,** 499—505).—Non-dehiscent anther disorders (acromania and hollow-boll) of cotton were associated with restricted water movement, poor aeration and high NO_2^- in the soil. Foliar sprays of urea and choline chloride (0·5—1·0 lb./acre) prevented the conditions and greatly increased cotton yields. A. H. CORNFIELD.

Effect of clipping on yield and certain agronomic characteristics of irrigated grain sorghum. S. S. Singh and W. L. Colville (*Agron. J.*, 1962, **54,** 484—486).—Hybrid grain sorghum RS 610 was injured by clipping at several positions on the plant at six different stages of growth. Clipping increased side branching. Head wt., total dry matter per plant and grain yields were reduced by clipping. Test wt. was reduced by clipping, except where half of the tips of the heads were removed. A. H. CORNFIELD.

Chemical composition, growth habit and yield of grain sorghum as affected by clipping treatments. S. S. Singh (*Dissert. Abstr.*, 1962, **23,** 387—388).—The effects of inflicting certain defined injuries on the hydrid sorghum, R.S.610 are described. The % crude protein in grain sorghum leaves and stem decreased with the increasing age of the plant. Clipping increased the protein content of leaves and stem. Clipping in general reduced the % of total available carbohydrate in seed. The HCN content in grain sorghum leaves was influenced by environment, age of the plant and clipping treatment. F. C. SUTTON.

Soya-bean response to molybdenum and lime and the relationship between yield and chemical composition. M. B. Parker and H. B. Harris (*Agron. J.*, 1962, **54,** 480—483).—Soya-bean leaf N %, seed yields, seed wt. and seed protein % were increased by the application of Mo or lime to moderately acid soils (pH 5·5—5·6). There were significant positive correlations between seed yields and soil pH (where no Mo was applied), seed yield and leaf N %, leaf N % and seed protein %, and seed yield and seed wt. When Mo was applied seed yields were independent of soil pH. Seed protein % was negatively correlated with oil %. A. H. CORNFIELD.

Response of slash pine to nitrogen and phosphorus fertilisation. L. C. Walker and C. T. Youngberg (*Proc. Soil Sci. Soc. Amer.*, 1962,

26, 399—401).—Application of NH_4NO_3 (200 lb. N per acre) to 9-year-old slash pine on a deep sand resulted in increased dia. and basal area growth, particularly in the year after application, but had no effect on height growth. Application of superphosphate (44 lb. P per acre) had no effect. Needle-N % was increased by the N treatment during the year after treatment. Needle-N, -P, -K and -Ca % and N, P and K % in the A_p and B_2 soil horizons were not affected by the treatments after 3 years. A. H. CORNFIELD.

Deposition of nutrien ts in poplars. W. Schulze and K. Lehmann (*Flora*, 1962, **152,** 253—256).—The distribution of water and mineral nutrients in black poplar is examined. In comparison with 1-year wood, older wood has less water, lower nutrient content (dry basis) and smaller seasonal variation in contents of individual nutrients (greatest in Na and K, very little in P and N). In the young wood the Ca and Mg contents were double those in old wood; the K content was exceptionally high (3·5%) in June–Sept. and low (0·7%) in Dec.–April; the P and N contents declined progressively from June throughout the following year. A. G. POLLARD.

Effect of soil saturation upon the dry weight, ash content and nutrient absorption of various bottomland tree seedlings. J. F. Hosner and A. L. Leaf (*Proc. Soil Sci. Soc. Amer.*, 1962, **26,** 401—404).—Fourteen bottomland tree species are arranged in order of tolerance to waterlogged conditions based on differences in root and shoot growth and nutrient absorption between seedlings waterlogged for 60 days and those grown with adequate moisture but good drainage. A. H. CORNFIELD.

Pest Control

Relationships between structure and activity of maleic hydrazide analogues and related compounds. E. V. Parups, I. Hoffman and H. V. Morley (*Canad. J. Biochem. Physiol.*, 1962, **40,** 1159—1165).—The growth-inhibiting activities of compounds structurally related to maleic anhydride, examined by the technique of bud growth inhibition, depended on the ease with which the plant can split off substituents. Straight-chain compounds were not as active as the parent compound and ring-closure was necessary for full activity. Activity failure was not due to lack of uptake and translocation. (16 references.) S. A. BROOKS.

Persistence of Parathion residues on fresh West Indian cherries (acerola) and in the canned juice. G. G. Monge M. E. Pérez and R. A. Canals (*J. Agric. Puerto Rico*, 1962, **46,** 9—14).—Parathion residues were present to the extent of <1 p.p.m. in the fruit the day after the last spray and had virtually disappeared by the fourth day; the canned juice from treated trees contained less than 0·15 p.p.m. A. H. CORNFIELD.

Bioassay of soil containing residues of chlorinated hydrocarbon insecticides. W. E. Fleming, L. B. Parker, W. W. Maines, E. L. Plasket and P. J. McCabe (*U.S. Dep. Agric., agric. Res. Ser., Tech. Bull.*, 1962, No. 1266, 44 pp).—*Drosophilia melanogaster*, Meigen is used as the test insect for assaying the toxicity of soil containing residues of aldrin, chlordane, DDT, dieldrin, endrin, heptachlor and toxaphene resulting from the control of Japanese beetle grubs (*Popillia japonica*, Newman). When 50% of the flies are killed with an exposure of 24 h. the toxicity of the soil is adequate to prevent the development of newly hatched grubs. The toxicity may be expressed as equivalent lb. per acre of any one of the insecticides. (136 references.) E. G. BRICKELL.

Effects of DDT in the diet of Japanese quail. D. L. Cross, H. L. King and D. L. Haynes (*Mich. agric. Exp. Sta. quart. Bull.*, 1962, **44,** 688—696).—Feeding DDT (up to 300 p.p.m. of the diet) to the quail did not affect the no. or wt. of the eggs laid. With 500 p.p.m. in the diet, none of the eggs hatched and with 700 p.p.m. no eggs were obtained. Food consumption was reduced by concn. ≮500 p.p.m. when introduced during the period 25—80 days of age, and resulted in lowered body wt. and increased mortality. Field application of DDT in oil (1 lb. actual DDT/acre) as insecticide on forage crops produced a residue of DDT on the crops (15 p.p.m.). It is unlikely that normal field application of DDT involves a risk of poisoning the birds. A. G. POLLARD.

Adsorption of monuron and diuron by Hawaiian sugarcane soils. Q. H. Yuen and H. W. Hilton (*J. agric. Fd Chem.*, 1962, **10,** 386—392).—Experience has shown that the considerable variation in weed control and crop injury in sugar plantations is not related to variation in the residues of monuron and diuron present in the soil, but appears to be related to soil factors. Experiments to measure the variation in equilibrium adsorption and desorption of these herbicides from a variety of Hawaiian sugarcane soils, which are mostly clays of volcanic origin, are described. Resistance to desorption was directly related to adsorptive capacity. W. ELSTOW

Resistance of conifers to bark beetle infestation. J. P. Vité and J. A. Rudinsky (*Int. Kongr. Entom., Wien, 1960*, 1962, **2,** 219—225).—The pressure of exudation of oleoresins (measurement described) affords a measure of the resistance of ponderosa pine to attack by bark beetles. This does not apply to Douglas fir. A. G. POLLARD.

Root excretions of *Erogrostis curvula*, (Shrad.) Nees, and their influence on root nodule nematodes. II. F. Scheffer, R. Kickuth and J. H. Visser (*Z. PflErnähr. Düng.*, 1962, **98,** 114—120).—Catechol is the causative factor in nematode resistance and is effective in dilutions as low as $1 : 10^8$. Catechol and probably its derivatives are genuine anthelminthics. M. LONG.

Synthesis of fluorobenzoates as possible pesticides. K. C. Joshi and S. Giri (*J. Indian chem. Soc.*, 1962, **39,** 495—496).—4-Fluorobenzoates were prepared by refluxing the appropriate benzoyl chloride with the required alcohol or phenol. Physical properties are given for 15 compounds but no pesticidal tests were made. J. I. M. JONES.

Action of derivatives of benzimidazole on growth and development of seed cultures. A. M. Efros and M. P. Fedoseeva (*Dokl. Akad. Nauk SSSR*, 1962, **146,** 236—237).—Seeds of spring wheat, variety 'Diamond', were soaked in a 0·01% solution of benzimidazole deriv. before germination. The yield of grain and straw from the plants was determined, and also the general development of the plant. 2-Methyl-5-amino- and 4,7-diamino-benzimidazole increased the yield of grain by up to 24%. 4-Aminobenzimidazole increased the quantity of grain on the stalk, and 2-methyl-4-amino-7-nitrobenzimidazole increased the bushiness of the plants. 5-Amino- and 2-methyl-5-nitro-benzimidazoles had only a weak action on grain production but increased the amount of green matter. *o*-Phenylenediamine was used as control. A. S. LEVESLEY.

Effects of calcium and other minerals on incidence of bitter pit in Cox's Orange apples. D. I. Jackson (*N.Z. J. agric. Res.*, 1962, **5,** 302—309).—The incidence of bitter pit in stored apples was significantly reduced by both pre- and post-harvest spraying with $Ca(NO_3)_2$ (optimum 6 lb./100 gal.) The later sprays were most effective. Sprays containing Mg, K, P, N, B, Fe, Sr, W and V were ineffective. W. ELSTOW.

***Pythium* in established lucerne.** D. C. Norton (*Iowa J. Sci.*, 1962, **37,** 1—5).—Colonisation of fibrous roots by *Pythium* may be considerable. *P. debaryanum* reduced dry wt. of the foliage in greenhouse tests and delayed and reduced flowering in established lucerne. *P. rostratum* reduced flowering only slightly even though plants often contained an abundance of oospores and sporangia. (12 references.) E. G. BRICKELL.

Inhibitory and lethal effects of three seed protectants against *Pythium debaryanum*. W. L. Staudinger and W. F. Buchholtz (*Iowa J. Sci.*, 1962, **37,** 67—78).—Ceresan M is inhibitory and lethal to the mycelium of *P. debaryanum* and other fungi in the soil at some distance from the treated seed. Arasan and Spergon function almost exclusively at the zone of contact between the treated seed and the surrounding soil.(12 references.) E. G. BRICKELL.

Effect of pesticides on the yield of vegetable crops. R. Abrams, D. Walker and M. R. Ballester (*J. Agric. Puerto Rico*, 1962, **46,** 1—8).—Parathion or Guthion sprays had no effect on yields of cucumbers. DDT (2 lb.) and Guthion (6 lb./acre) increased the yields of snap beans. DDT (4 lb./acre) applied weekly increased plant growth and the no. and quality of ears. DDT, parathion and Di-Syston had no effect on tomato yields. A. H. CORNFIELD.

Chemotherapy of silver leaf disease, *Stereum purpureum*, (Fr.) Fr., of plum trees. M. Bennett (*Ann. appl. Biol.*, 1962, **50,** 515—524).—Injection of infected 3- or 4-year old plum trees with 0·5% 8-hydroxyquinoline K sulphate (Oxine KS) into the trunk at different times in the season increased the vol. of diseased wood, but there was evidence that it reduced the density of active fungal mycelium in the wood. This treatment as well as griseofulvin injections did not permanently reduce disease symptoms. A. H. CORNFIELD.

Post-harvest treatment with 2,6-dichloro-4-nitroaniline for fruit rot control on fresh market peaches. D. H. Dewey and D. C. MacLean (*Mich. agric. Exp. Sta., quart. Bull.*, 1962, **44,** 679—683).—Fruit rot in peaches stored at 60°F for 13 days was greatly reduced by dipping for 1 min. in 2,6-dichloro-4-nitroaniline (Botran) in concn. 1000 and 2000 p.p.m. Both concn. prevented *Rhizopus* rot even when the fruit was rinsed in water after treatment. At concn. 2000 p.p.m. Botran effectively delayed the development of brown rot (*Sclerotinia fructicola*). A. G. POLLARD.

Effect of 2-chloro-4,6-bis(ethylamino)-*s*-triazine and 3-(3,4-dichlorophenyl)-1,1-dimethylurea on healthy and mosaic-infected sugarcane. J. Adsuar (*J. Agric. Puerto Rico*, 1962, **46,** 156—157).—

Application of 2-chloro-4,6-bis(ethylamino)-*s*-triazine(simazine) (**I**) (4—8 lb./acre), for weed control, 40 days after the planting of cuttings had no effect on growth after 3 months. Growth was slightly retarded by 12 lb. of **I**/acre. Application of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) even at 4 lb./acre slightly retarded growth of canes and at 12 lb./acre had a marked toxic effect on both normal and mosaic-affected plants. This last treatment also increased the incidence of brown strip disease on both healthy and mosaic-infected plants. A. H. CORNFIELD.

Effect of antibiotic compounds on tomato tumours caused by *Pseudomonas tumefaciens*. A. D. Koveshnikov (*Mikrobiologiya*, 1962, **31**, 827—831).—Antibiotics such as streptomycin, hydroxytetracycline, chloramphenicol, chlortetracycline and canamycin were used to treat tomato tumours. Treatment for 2 h. with concn. of 1 mg. per ml., applied 10 days after infection did not produce an anti-tumour effect. With the max. safe dose (5—25 mg. per ml.) 30-day tumours were not destroyed although growth was retarded or stopped. The anti-tumour effect is not identical with antimicrobial action. R. A. KEEN.

Photometric microdetermination of malathion. B. J. Kallman (*Chemist Analyst*, 1962, **51**, [3—4], 75—76).—The observation that malathion interferes with the Fe^{III} hydroxamate colorimetric determination of acetylcholine has led to the use of this colour reaction for the determination of malathion. As the reaction depends on the carboxylic ester groups of malathion, compounds containing these linkages occurring in the extract will interfere. Parathion and Phosdrin do not interfere seriously. W. ELSTOW.

Electrochemical determination of organophosphorus compounds. G. G. Guilbault, D. N. Kramer and P. L. Cannon, jun. (*Analyt. Chem.*, 1962, **34**, 1437—1439).—A recently published method (*ibid.*, p. 842) is extended to give rapid, sensitive and highly accurate determinations of anticholinesterase organophosphorus compounds such as Systox, Sarin, parathion and malathion. A constant current is applied across two Pt electrodes, and the change of potential on enzymic hydrolysis of butyrylthiocholine iodide is recorded vs. time. Incubation for 3—10 min. permits the determination of Sarin, 2×10^{-4}—3×10^{-3}, Systox, 0·01—0·20, parathion, 0·18—1·8 and malathion, 1·8—18 μg./ml. C. B. BAINES.

Technique for inoculating wheat with rusts for glasshouse and test-tube culture. M. L. Gattani (*Nature, Lond.*, 1962, **196**, 190—191).—When the plumule of a germinated wheat seed has grown about ½ in. a spore suspension of rust containing a little detergent is injected slightly above the point of differentiation between the plumule and radical. The seedling can then be transplanted either to the glasshouse or test-tubes containing 0·5 % agar. S. A. BROOKS.

Weed control studies with nursery crops. H. Davidson and S. K. Ries (*Mich. agric. Exp. Sta., quart. Bull.*, 1962, **44**, 751—758).—Control of quack grass in forest nursery stock was obtained by spraying in early spring at ~3 in. height with amitrole 1 + simazine 3 lb./acre. With the grass ~2 ft. high double this concn. gave fairly good control. Preplanting application (autumn) of dalapon 10—20 lb./acre was also effective. Summer weed was controlled by simazine 2—4 or Casoron 4 lb./acre. A. G. POLLARD.

Allyl alcohol as a pre-emergent weedkiller in tobacco seedbeds. C. L. González-Molina and A. Acosta-Matienzo (*J. Agric. Puerto Rico*, 1962, **46**, 97—101).—Application of 1—3 gal. of allyl alcohol (diluted to 50 gal./acre) together with DD (for nematode control) to tobacco seedbeds 5—15 days before sowing tobacco gave good control of weeds and resulted in the production of healthy seedlings. A. H. CORNFIELD.

Treatment of grains and seeds. Produits Chimiques Industriels & Agricoles Procida S.A. (B.P. 882, 910, 21.5.58. Fr., 23.5.57).—Grain or seed is rendered resistant to external cryptogamic diseases and damping-off, without need for immediate sowing, by treating the material with a very small amount (<1%) of water, and either previously or subsequently with a powder mixture of an anti-cryptogamic substance (e.g., a fungicide), a moisture-distributing agent (e.g., condensation product of ethylene oxide, polyethylene glycol or an ether or ester thereof, etc.), a water-fixing agent (especially a compound capable of forming with a small amount of water a pseudo-solution or gel, e.g., Na alginate, Na caseinate, dextrin, powdered Irish moss, fine Senegal gum or tragacanth gum) and optionally a carrier. F. R. BASFORD.

4-Alkoxymetanilamides and compositions containing them. E. I. Du Pont de Nemours & Co. (B.P. 884,206, 2.4.58. U.S., 17.6 and 21.8.57).—The prep. is described by conventional methods of 4-*propoxy*-3-*aminobenzenesulphonamide*, m.p. 91—91·5°, useful *inter alia* as fungicidal agent for treatment of early blight due to *Alternaria solani* on tomatoes. H. S. R.

Insecticides. VEB Farbenfabrik Wolfen (Inventor: W. Faatz) (B.P. 884,168, 25.7.60).—A syngergistic insecticidal composition contains a dialkyl 2,2-dichlorovinyl phosphate (0·5—9·5) and a dialkyl (e.g. Me_2) (2,2,2-trichloro-1-hydroxyethyl)phosphonate (0·5—99·5%). F. R. BASFORD.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 883,566, 6.11.59. Ger., 7.11.58).—Compounds of the general formula RR′·PX·S·CH_2·S·CH_2R″ are claimed, also their use as active ingredients on insecticidal compositions [R and R′ are alkyl or alkoxy of 1—6 C, cycloalkyl, alkenyl of >10 C, Ph optionally substituted, or R may be NH_2, alkyl- or dialkylamino (alkyl of 1—4 C) or cycloamino; R″ is substituted or unsubstituted Ph; X is O or S]. Details are given for the prep. of OO-*Et*$_2$S-*benzylthiomethyl phosphorothiolothionate*, b.p. 138—140°/0·01 mm. F. R. BASFORD.

Acylaminotriazole compounds and herbicidal compositions containing the same. Amchem Products Inc. (Inventor: J. Norris) (B.P. 883, 732, 9.2.59).—The herbicides claimed comprise products obtained by acylation of 3-amino-1,2,4-triazole with CO_2H·X·OR (or a functional derivative thereof) (X is CH_2, CHMe or $[CH_2]_3$; R is Ph substituted by halogen and/or alkyl, preferably by Cl). The prep. is detailed of 3-(p-*chlorophenoxyacetamido*)-1,2,4-*triazole*. F. R. BASFORD.

Thionophosphonic acid fluoroamides. Farbenfabriken Bayer A.-G. (B.P. 883,982, 3.3.60. Ger., 3.3.59).—Compounds R·PS(F)·NR′R″, useful as insecticides, are obtained by interaction of R·PSF_2 with NHR′R″ at 20—40° in presence of an acid-binding agent in a solvent (R–R″ are aliphatic radicals of 6 C or are cycloaliphatic radicals, or R′ is H, or R′ and R″ together with N form a heterocyclic residue). The prep. is described of NN-*dimethylmethylphosphonamidothioic fluoride* (41%), b.p. 72°/2 mm. The product is water-sol., and at 0·1% concn. is 100% lethal to aphids. F. R. BASFORD.

Animal Husbandry

Nutritive value of timothy hay as affected by date of harvest. T. N. Mellin, B. R. Poulton and M. J. Anderson (*J. Anim. Sci.*, 1962, **21**, 123—126).—Samples from a practically pure stand of timothy were examined weekly over the period, May 27 to Aug. 5. Until July 22 there was a steady decline in content and digestibility of protein in the grass, subsequent changes being small. The digestibility of energy in the forage diminished considerably as maturity was approached. A linear relationship is demonstrated between digestibility of dry matter and time after May 17. A. G. POLLARD.

Forage digestion by rabbits compared with crude fibre, methoxyl and crude protein contents as indicators of digestion by ruminants. C. R. Richards, G. F. W. Haenlein, J. D. Connolly and M. C. Calhoun (*J. Anim. Sci.*, 1962, **21**, 73—77).—Comparison is made of the digestibility coeff. of legumes, grasses and mixtures of these by rabbits and sheep and also with the methoxyl, crude fibre and crude protein levels in the diet. When the forage consisted of legume (lucerne) + grasses the methoxyl content afforded the best indication of ruminant digestibility: it was followed, in decreasing order, by fibre content and rabbit digestibility. With legume only the relative indicator values were, rabbit digestibility > crude fibre (methoxyl content showing significant relation); with grasses alone the order was, methoxyl content > rabbit digestibility > crude fibre. A. G. POLLARD.

Relation of certain end products of rumen fermentation to forage feeding value. R. W. Rice (*Dissert. Abstr.*, 1962, **22**, 4143).—Oat straw and lucerne–bromegrass hay were compared by conventional total digestible nutrient determinations, by *in vitro* volatile fatty acid production and cellulose digestibility. The proportions of volatile fatty acids produced after 8 h. or longer of *in vitro* fermentation appear to be more characteristic of the fermentation systems than of the substrate being fermented. A short-term *in vitro* fermentation of less than 8 h. appears to be necessary for a differentiation between forages on the basis of proportions of volatile fatty acids. F. C. SUTTON.

Measurement of feed intake by grazing cattle and sheep. VIII. Accuracy of the chromic oxide technique for the estimation of faeces output of dairy cattle. A. E. Stevenson (*N.Z. J. agric. Res.*, 1962, **5**, 339—345).—The administration of Cr_2O_3 capsules as an indirect method of estimating faecal output is shown to give an over-estimate due to a loss in the recovery of Cr_2O_3 possibly occurring during grinding of the dried faeces. It is suggested that a correction factor, according to the specific conditions of an experiment, be developed and applied if absolute measurements are required. (12 references.) W. ELSTOW.

Effects of various rations, methods of preparation, enzymes and

cobalt on volatile fatty acid production in beef cattle. J. K. Ward (*Dissert. Abstr.*, 1962, **22,** 4144).—In addition to the above-mentioned studies other data on volatile fatty acids (VFA) levels and proportions in various portions of the gastrointestinal tract of beef cattle were collected. VFA proportions in samples from the tract of full-fed heifers showed substantial levels of formic and lactic acids in the abomasum and colon samples. Slight amounts of formic and substantial amounts of lactic acids were observed in the caecum and colon samples. F. C. SUTTON.

Value of hay pellets for beef cattle. R. L. Park (*Dissert. Abstr.*, 1962, **22,** 4142).—The effects of the addition of hay pellets to variations of normal diets, e.g., mixed hay and mixed hay silage and/or maize silage for wintering Hereford steer calves are described, and the effect of lucerne hay pellets, straw bedding and Dynafac on rumination and bloat was studied. Rumination was fairly regular as to frequency and duration for individual steers on each ration. F. C. SUTTON.

Effect of pelleting Coastal Bermudagrass on livestock gains. W. H. Hogan, O. L. Brooks, E. R. Beaty and R. A. McCreery (*Agron. J.*, 1962, **54,** 193—195).—Weight gains of steers to 60—100 days were as high with pelleted Bermudagrass as with pelleted lucerne. These treatments gave better wt. gains than did Bermudagrass supplied as green forage, dried hay or grazing. Feed efficiency was much better with the pelleted feeds than with the other forms of Bermudagrass. A. H. CORNFIELD.

Laboratory studies on ensiling. J. M. Yoder (*Dissert. Abstr.*, 1962, **23,** 381—382).—Over a period of 3 years, 40 different cuttings of various crops were ensiled in small plastic laboratory silos. The silages were allowed to ferment for 1·5—7 months before opening. Compared with chopping, mincing before ensiling lowered the pH. Ethanol, formaldehyde, $Na_2S_2O_5$ and wilting also resulted in better quality silages although the initial fall in pH and acid production was delayed. Best results were obtained from prompt ensiling after cropping. F. C. SUTTON.

Grass silage as sole roughage for dairy cows. N. D. Dijkstra (*Versl. landbouwk. Onderz.*, 1962, 68.11, 47 pp.).—Previous observations (cf. *ibid.*, 1959, 65.14) that grass silages are inferior to hay for milk production are confirmed. It is now shown that in this respect, and on an equiv. dry-matter basis, autumn silage is not inferior to spring-silage. P. S. ARUP.

Protein quality of a lucerne concentrate. R. L. Larson and A. W. Halverson (*J. agric. Fd Chem.*, 1962, **10,** 422—425).—Lucerne protein concentrate is inadequate for the growth of rats when unsupplemented by other proteins. Marked improvement in growth is exhibited when methionine and lysine are added but growth was still less than that obtained when casein was the source of protein indicating that there was a further amino-acid deficiency. Addition of cholesterol to diets containing the concentrate gave a small improvement in growth. (16 references.) W. ELSTOW.

Evaluation of forage crops by chemical analysis. J. T. Sullivan (*Agron. J.*, 1962, **54,** 511—515).—A critical review and discussion of chemical methods used for predicting the feeding quality of forages, particularly as to their digestibility to ruminants. A. H. CORNFIELD.

Determination of the diallyl derivatives of diethylstilboestrol and hexoestrol in feeds and other materials. J. B. Himes, L. D. Metcalfe and R. Hubata (*J. agric. Fd Chem.*, 1962, **10,** 404—406).—The compounds are extracted from the dried feed in a Butt extractor with 7% ethanol in $CHCl_3$. The extract is extracted with dil. alkali. The pH of the alkaline extract is adjusted to $9·0 \pm 0·1$ with dil. H_3PO_4 and the additives are re-extracted into $CHCl_3$. The residue on evaporation is taken up in methanol and aliquots chromatographed, with 30% aq. pyridine on paper previously treated with 3% soyabean oil in ether and dried. Similar aliquots of standard solutions of the additives are developed alongside on the same sheet. The spots are detected with Folin–Ciocalteau reagent followed by 10% Na_2CO_3 and the density of the spots measured on a densitometer-recorder. Concn. vs. absorbance is linear over the range 1—5 μg. The method provides an accurate means of analysing animal feeds for these compounds even in the presence of phenolic compounds. (10 references.) W. ELSTOW.

Artefacts of ornithine and αγ-diaminobutyric acid found during chromatographic investigation of rumen liquid. C. J. G. van der Horst (*Nature, Lond.*, 1962, **196,** 147—148).—A new compound, found in rumen liquid by paper electrophoresis and chromatography, was shown to be β-aminopiperidone; this is an artifact produced by decomposition of ornithine. Another compound present, β-aminopyrrolidone, is formed in the same way from αγ-diaminobutyric acid. S. A. BROOKS.

Biuret and bicarbonate in the rations of ruminants—their influence on general nitrogen metabolism. T. C. Campbell (*Dissert. Abstr.*, 1962, **22,** 4139—4140).—Biuret was compared with urea and soya-bean oil-meal as N source in several types of trials. Although biuret appeared to be slightly inferior to urea in feeding value, there were several demonstrations of bacterial adaptation within the rumen to this form of N. It was shown that there was an increased post-prandial concn. of ruminal NH_3 with time over a 5-week period of feeding biuret to sheep. The effects of Na- and K-HCO_3 on the utilisation of urea by ruminants were studied also. F. C. SUTTON.

Production factors and carcass characteristics of fattening calves influenced by rate of grain feeding. L. G. Young (*Dissert. Abstr.*, 1962, **22,** 4144—4145).—An experiment was conducted (1957—58) to secure additional information to unpublished data (1940—42) regarding the effect of feeding a similar total amount of grain to weanling beef calves at varying rates and the effect on the carcasses produced. The final wt., slaughter dates, carcass grades and carcass analyses did not differ significantly between treatments. F. C. SUTTON.

Effect of heat treatment on the nutritive value of milk for the young calf. III. IV. Effect of preheating spray-dried skim milk and a study of the effect of ultra-high-temperature treatment of separated milk. K. W. G. Shillam, J. H. B. Roy and P. L. Ingram (*Brit. J. Nutr.*, 1962, **16,** 585—591; 593–601).—New-born calves, under conditions of moderate 'infection', were fed spray-dried skim milk preheated to 77° for 15 sec. before drying (milk B), or that preheated at 74° for 30 min. (milk A) or milk B subjected to ultra-high temp. treatment at 135° for 1—3 sec. (UHT) after reconstitution. Addition of whey fraction of milk B to milk A, gave a growth response unlikely to be due entirely to the extra caloric intake. The UHT treatment of milk denatured ~55% of the whey proteins but the mortality rates of the various experiments were not significantly different. C. V.

High fat milk replacers for veal production. N. J. Drouliscos (*Dissert. Abstr.*, 1962, **22,** 4164—4165).—A high-fat milk replacer containing 22% of fat (sweet butter, tallow, choice white grease, coconut fat) on an air-dry basis was formulated and its efficacy in terms of growth and quality of veal from Holstein bull calves was tested. Fat in the spray-dried milk replacer was digested to about the same extent as when a fat homogenate was prepared and incorporated with the milk ingredients at feeding time. The batched milk replacer was inferior in fat digestibility except in the case of coconut fat. F. C. SUTTON.

Factors influencing the utilisation of lipid materials by the dairy calf. W. A. Olson (*Dissert. Abstr.*, 1962, **23,** 378).—The following effects were studied on calves in long-term digestion trials: antibiotic supplementation of the ration, age of calf, quantity of feed consumed, fat content of diet, lipase addition to diet and incidence of diarrhoea. The most important factor influencing the utilisation of lipid material was the occurrence of diarrhoea. Aureomycin was not effective in increasing lipid digestion by young calves. 'Pregastric esterase' supplementation of milk rations did not increase the apparent digestibility of milk fat. Attempts to maintain calves on liquid diets were unsuccessful. F. C. SUTTON.

Evaluation of diammonium phosphate as a nitrogen source for ruminants. C. A. Lassiter, L. D. Brown and D. Keyser (*Mich. agric. Exp. Sta., quart. Bull.*, 1962, **44,** 763–771).—In trials with dairy cattle $(NH_4)_2HPO_4$ (**I**) proved a satisfactory N supplement when incorporated in customary types of ruminant ration; in proportions providing 35% of the total dietary N, **I** maintained growth rates comparable with those obtained with equivalent amounts of N as urea or soya-bean meal. When >2% of **I** was added to a grain ration there was a risk of impaired palatability. A. G. POLLARD.

Sprouted oats as feed for dairy cows. J. W. Thomas and B. S. Reddy (*Mich. agric. Exp. Sta., quart. Bull.*, 1962, **44,** 654—665).—Changes in the composition of oats during sprouting and the value of sprouted oats for cows are examined. The loss of nutrients during sprouting and the diminution in digestibility of the product, coupled with the cost of sprouting and doubtful increase in milk production, render the sprouting uneconomical. A. G. POLLARD.

Estimation of empty body weight of beef cattle. G. P. Lofgreen, J. L. Hull and K. K. Otagaki (*J. Anim. Sci.*, 1962, **21,** 20—24).—The empty body wt. of beef cattle (Y lb.) and the warm carcass wt. (X lb.) are related by the equation $Y = 70 + 1·45X$. The standard error of the estimate was 2·9% of the empty body wt. as determined in practice. The % of carcass fat, in the range 4—30% did not affect the relationship. A. G. POLLARD.

Substitution of maize for forage in the fattening ration for steers. W. Woods and J. M. Scholl (*J. Anim. Sci.*, 1962, **21,** 69—72).—Steers were fed rations of bromegrass–lucerne soilage and maize in proportions 20—2 : 1 or with soilage only, all animals being finished on a full maize–hay ration supplemented with concentrate, minerals and stilboestrol. Prior to the finishing stage rates of gain

in wt. increased linearly with the proportion of maize given, 1 lb. of maize replacing 8·9 lb. of soilage. Carcass grading also rose with the level of maize in the diet. A. G. POLLARD.

Residue studies in beef tissues following oral administration of methimazole. N. S. Raun, W. Burroughs, S. Balloun and P. Homeyer (*J. Anim. Sci.*, 1962, **21**, 98—100).—Methimazole (**I**) (400—800 mg./head, daily) was added to finishing rations for steers for 79 days to increase rates of gain in wt. and feed efficiency. No goitrogenic residues were detected in tissues (lean, fat, liver, kidney, tripe) by chick tests. Simultaneous feeding of **I** and stilboestrol produced no evidence of oestrogenic activity (mouse test) in lean or fat tissues. A. G. POLLARD.

Removing sources of error in lamb feeding experiments. J. H. Meyer (*J. Anim. Sci.*, 1962, **21**, 127—131).—Variations in results of feeding experiments are occasioned by differences in body composition and wt. of contents of the gastrointestinal tract. Determinations of carcass wt. and sp. gr. permit correction of the wt. to a standard equiv. in energy and protein content. The corrected equiv. wt. gives a better measure of the response to nutritional factors than does comparative or increased wt. coupled with carcass grading and dressing %. Appropriate regression equations and details of experimental method are given. A. G. POLLARD.

Influence of frequent feeding on the performance of growing and fattening lambs. R. W. Rhodes and W. Woods (*J. Anim. Sci.*, 1962, **21**, 108—111).—With rations varying widely in composition and form (ground, pelleted and conventional) feeding more than twice daily (4—6 feeds in 12—24 h.) did not affect growth rates, feed efficiency, digestibility or rate of N retention. A. G. POLLARD.

Levels of volatile fatty acids [in rations] for growing-fattening lambs. H. W. Essig, U. S. Garrigus and B. C. Johnson (*J. Anim. Sci.*, 1962, **21**, 37—40).—Feeding salts of volatile fatty acids (**I**) to sheep lowered the feed intake and the resulting gain in live-wt. The effect was still greater when the free acids were gievn. When acetic (**II**) and propionic (**III**) acids were included in the diet in proportions 2·5 : 1·0 (as free acids), butyric acid (**IV**) was not an essential component of the **I** supplied. The proportions of **II**, **III** and **IV** in the ration were reflected in those present in the rumen. A. G. POLLARD.

Purified diets and magnesium requirement as affected by calcium and phosphorus level, for growing lambs. N. Gay (*Dissert. Abstr.*, 1962, **23**, 376).—Diets containing pre-formed protein or non-protein N sources (urea and biuret), cellulosic and non-cellulosic energy sources (free fatty acids), physiologically inert polyethylene, minerals and vitamins, were fed to growing lambs. The diets fed did not support normal growth. Three levels of Mg, two of Ca and two of P were added. The requirement for Mg in the diet was 600—900 p.p.m. on an air-dry basis. F. C. SUTTON.

Molybdenum studies with sheep. G. M. Sheriha, R. J. Sirny and A. D. Tillman (*J. Anim. Sci.*, 1962, **21**, 53—56).—Partially or wholly purified diets containing various levels of Mo were fed to lambs. The Mo requirement thus determined was 0·01 p.p.m. of the diet. Addition of W to give a ratio W/Mo = 1000 : 1 slightly reduced growth rates. The Mo requirement was not increased by addition of KNO_3 (1% in the ration). A. G. POLLARD.

Relation of selenium, vitamin E and an unidentified factor to muscular dystrophy (stiff-lamb or white muscle disease). D. E. Hogue, J. F. Proctor, R. G. Warner and J. K. Loosli (*J. Anim. Sci.*, 1962, **21**, 25—29).—Naturally occurring muscular dystrophy in lambs was prevented equally satisfactorily by inclusion in the diet of vitamin E (50 i.u. daily) or of Se (1 p.p.m.). Neither of these supplements separately was fully effective when fed to the ewe during later gestation or during early lactation, but vitamin E (100 i.u. daily) and Se (1 p.p.m. in the diet), given simultaneously, completely protected the lambs against dystrophy. The dystrophy was also prevented by cooking the basal ration for the ewes but not by addition of an antioxidant. A. G. POLLARD.

Groundnut meal as a source of protein in pig starter and grower rations. G. E. Combs and H. D. Wallace (*J. Anim. Sci.*, 1962, **21**, 95—97).—Use of groundnut meal (55% protein) as protein supplement in starter rations for pigs weaned at 15 days, and later in grower rations is examined. The meal did not produce satisfactory growth and addition of various amino-acids did not increase growth rates to equal that obtained with soya-bean meal. In starter rations ≯25% of the customary soya-bean meal can be replaced satisfactorily by groundnut meal. A. G. POLLARD.

Effect of isolated soya-bean protein and casein on the gastric pH and rate of passage of food residues in baby pigs. J. H. Maner, W. G. Pond, J. K. Loosli and R. S. Lowrey (*J. Anim. Sci.*, 1962, **21**, 49—52).—A liquid diet containing casein, fed to pigs aged 4—10 weeks, lowered the pH (initially, 1·6—1·8) of the stomach contents (measured by gastric fistula) rapidly from 5·6, 5 min. after feeding, to 1·7 in 2 h. When soya-bean protein was given the initial pH was regained only after 4 h. In 8-week pigs given corresponding dry diets, the gastric pH returned to pre-feeding level in ≯2 h. regardless of the protein used. Rates of passage of food residues through the digestive tract in 4-week pigs were 14—24 h. on the casein and 36—48 h. on the soya-bean protein ration. In 8-week pigs the period was 40—50 h. with either ration. A. G. POLLARD.

Phosphorus requirement of young pigs. G. E. Combs, J. M. Vandepopuliere, H. D. Wallace and M. Koger (*J. Anim. Sci.*, 1962, **21**, 3—8).—Piglings weaned at 2 weeks of age were fed dry rations based on maize and soya-bean but containing various proportions of Ca and P. From 2 to 7 weeks the optimum P content of the ration was 0·44% with an optimum Ca/P ratio of 0·9 : 1·0. From 7 to 22 weeks with the same level of P the optimum Ca/P ratio was 1·2 : 1 for growth and skeletal development (bone ash and density). A. G. POLLARD.

Calcium and phosphorus requirements of growing-finishing swine. H. L. Chapman, jun., J. Kastelic, G. C. Ashton, P. G. Homeyer, C. Y. Roberts, D. V. Catron, V. W. Hays and V. C. Speer (*J. Anim, Sci.*, 1962, **21**, 112—118).—Responses of pigs to differences in dietary Ca and P levels were measured in terms of gain in wt. and in breaking strength and ash of femurs. Levels of P had a greater effect on these responses than had those of Ca ; the Ca/P ratio had greater influence when dietary P levels were min. Optimum levels of Ca and P for growth and skeletal development were, respectively, 0·8 and 0·6% for pigs from 25 to 100 lb. and 0·7 and 0·5% from 25 to 200 lb. live wt. A. G. POLLARD.

Influence of energy-protein ratio on performance and carcass characteristics of swine. A. J. Clawson, T. N. Blumer, W. W. G. Smart, jun. and E. R. Barrick (*J. Anim. Sci.*, 1962, **21**, 62—68).—Rations containing 10—18% of protein but the same calorie/protein ratio (**I**), were fed to pigs averaging 38 lb. live-wt. Both **I** and the fat content of the ration affected feed consumption but not the gross energy intake. During the first 28 days of the trial the narrower **I** produced the higher rate of growth ; the difference had disappeared when the animals reached market wt. Rates of gain in wt. and of feed consumption per unit gain increased with rise in fat content of the diet. Neither the **I** nor the fat content of the ration affected the energy utilisation or carcass characteristics. A. G. POLLARD.

Pig diets containing different amounts of linoleic acid. W. M. F. Leat (*Brit. J. Nutr.*, 1962, **16**, 559—568).—The essential fatty acid (EFA) requirement was studied in pigs from 10 to 200 lb. live wt. on diets containing various amounts of linoleic acid (**I**) given as olive oil. Linoleate was included to give 0·07—3·50% of the calories but no difference in growth rate or efficiency of food conversion was noted ; the condition of the skin was favourably affected. By use of the trienoic/tetraenoic acids ratio as an index of EFA status, it is shown that 1% of the calories should be given as **I**, the requirement increasing to a max. at 12–16 weeks. Of the fatty acids from depot fat 95% had 16—18 C-atoms ; only 2% had longer chains and fatty acids with 15 or 17 C-atoms were detected. C. V.

Effects of phytic acid on zinc availability and parakeratosis in swine. D. Oberleas, M. E. Muhrer and B. L. O'Dell (*J. Anim. Sci.*, 1962, **21**, 57—61).—Phytic acid (**I**) added to a semi-purified diet based predominantly on casein-glucose, depressed growth and produced symptoms of parakeratosis similar to those occurring with plant-protein diets. Zn supplements completely counteracted this effect. No evidence of parakeratosis resulted from the casein ration without **I** or from a corresponding purified diet based on soya-bean protein. Ca salts (Ca 0·8 and 1·5%) fed without **I** caused no difference in growth rates but when given with **I** lowered growth rates especially with the higher level of dietary Ca. A. G. POLLARD.

Measurement of feeding activity in chickens to eight weeks of age. P. B. Siegel, W. L. Beane and C. Y. Kramer (*Poultry Sci.*, 1962, **41** 1419—1422).—Photoelectric systems were used successfully to measure the feeding activity of chicks from 4 weeks of age. Diurnal rhythms were found for activity about the feed trough and followed a pattern similar to that for feed consumption. A. H. CORNFIELD.

Eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. L. S. Jensen, L. H. Merrill, C. V. Reddy and J. McGinnis (*Poultry Sci.*, 1962, **41**, 1414—1419).—Poults fed an unpelleted (mash) diet used 18·8% of a 12-h. day eating, whilst those fed pellets using only 2·2% of the time. Chicks fed mash used 14·3% and those fed pellets used 4·7% of the time eating. There were no great differences in total food consumed or no. of appearances at the feeder between mash and pellets. There was

little difference in the rate of food passage through chicks between mash, pellets and ground pellets. A. H. CORNFIELD.

Floor space studies using slat floors and litter floors for laying hens. Q. B. Kinder and A. B. Stephenson (*Poultry Sci.*, 1962, **41**, 1394—1400).—After birds had been in production about 10 months egg production, mortality and culling rate were little different with floor-space density ranging from 1·5 to 3·0 sq. ft. per bird in a laying shelter. Egg production was about the same from birds on slat floor and litter floors with 1·5 sq. ft. of floor space per bird. A. H. CORNFIELD.

Comparison of the requirements of battery- and floor-reared chicks for calcium and phosphorus. P. W. Waldroup, C. B. Ammerman and R. H. Harms (*Poultry Sci.*, 1962, **41**, 1433—1436).—The Ca and P requirements of chicks to 4 weeks of age were similar whether grown in batteries or in floor pens. The significant Ca × P interaction was due to the detrimental effects of high Ca at low P levels. The diet with P 0·59% was just adequate for females, but not for males. The Ca requirement of the chicks was 0·60—0·74% in the diet. A. H. CORNFIELD.

Growth-stimulating effects of antibiotics on chicks raised in old and new batteries. L. M. Potter, L. D. Matterson, J. J. Tlustohowicz and E. P. Singsen (*Poultry Sci.*, 1962, **41**, 1602—1611).—Addition of antibiotics (procaine penicillin, Zn bacitracin, spiramycin and erythromycin thiocyanate at 10—20 g. per ton of feed, aspartocin at 5—10 g. and PSQ, a mixed antibiotic, at 30—100 g. per ton of feed) to chick diets resulted in increased wt. gains of about 2—3% to 4–8 weeks of age and increased feed efficiency by about 2%. Differences were usually insignificant. Chicks raised in new batteries showed higher wt. gains and poorer feed efficiency than those raised in old batteries, but responses to antibiotics were similar in both environments. A. H. CORNFIELD.

Effects of debeaking at eight weeks of age on grit consumption, weight gains and feed efficiency of growing pullets. S. J. Slinger, W. F. Pepper and I. R. Sibbald (*Poultry Sci.*, 1962, **41**, 1614—1615).—Debeaking pullets at 8 weeks of age reduced wt. gains and feed efficiency to 20 weeks of age and virtually prevented grit consumption. A. H. CORNFIELD.

Dietary requirements for maximum gains of chicks fed purified-type diets containing adequate quantities of all recognised essential nutrients. T. R. Zeigler (*Dissert. Abstr.*, 1962, **22**, 4145).—Various purified experimental diets were used to study the different facets of this problem. When the diet contained isolated soya-bean protein, the Zn requirement of chicks was high and was reduced by various dietary supplements. There were indications that the crude feedstuffs may contain natural chelating agents responsible for the Zn-sparing effects. The effect of unidentified growth factors sources were small in comparison with that of the known recognised nutrients. F. C. SUTTON.

Metabolisable energy of materials fed to growing chickens. I. R. Sibbald and S. J. Slinger (*Poultry Sci.*, 1962, **41**, 1612—1613).—The ranges and mean values for metabolisable energy (kcal. per lb. of dry matter) of a wide range of feed materials are presented. A. H. CORNFIELD.

Free amino-acid diet for the growing chick. J. S. Adkins, M. L. Sunde and A. E. Harper (*Poultry Sci.*, 1962, **41**, 1382—1388).—A free amino-acid diet containing N equiv. to 20% of protein supplied by the indispensable amino-acids, L-tyrosine and L-glutamic acid supported a growth rate of 7—12 g. per day for 2—4 weeks. A mixture of the five dispensable amino-acids increased wt. gains by 1 g. per day for 2 weeks. After the second week chick growth on the amino-acid diet was considerably poorer than that on a purified protein diet. A. H. CORNFIELD.

Effects of pelleting on the utilisation of feed by the growing chicken. N. Hussar and A. R. Robblee (*Poultry Sci.*, 1962, **41**, 1489—1493).—Pelleting had no effect on the lysine content of wheat, oats and barley grain. Chicks fed a pelleted wheat–oats–barley ration consumed 15% more feed, gained 25% more wt. and showed higher feed efficiency than did those fed the same ration in mash form. The performance of chicks fed pellets which were reground was usually superior to those fed mash but inferior to those fed pellets. Pelleting did not influence apparent metabolisability of the feed, % retention of energy or metabolisable energy value of the ration, although there were indications that pelleting may have resulted in a slight increase in N retention. A. H. CORNFIELD.

Energy level of a 16%-protein diet for layers in a semi-arid, subtropical climate. B. W. Heywang and M. G. Vavich (*Poultry Sci.*, 1962, **41**, 1389—1393).—Variation of the metabolisable energy (M.E.) of a 16% protein diet from 1150 to 1550 kcal. per lb. had no significant effect on average egg production in three experiments (280—336 days). Feed consumption decreased and feed efficiency with respect to egg production increased with the M.E. level of the diets. Body wt. was highest at the higher level of M.E., and increased during cool and decreased during hot weather. Hatchability of fertile eggs and egg wt. were little effected by the M.E. level of the diet. Mortality increased with the M.E. level of the diet and was greater during hot than during cool weather. A. H. CORNFIELD.

Methods for measuring the 'calcium-metabolising ability' of laying hens. T. G. Taylor and F. Hertelendy (*Poultry Sci.*, 1962, **41**, 1509—1512).—The three measures of Ca-metabolising ability studied in 17 individual birds of highly uniform genetic constitution were: (*A*) shell-thickness of the last egg of a clutch laid on a normal-Ca diet, (*B*) shell thickness of the second egg laid on a low-Ca diet and (*C*) total plasma-Ca level shortly after oviposition of the latter egg. Measures *B* and *C* were significantly correlated and other correlations were non-significant. Probably all three criteria might be of use in the selection of birds for the breeding stock. A. H. CORNFIELD.

Effect of nutrient density and protein–energy interrelationships on reproductive performance of the hen. S. P. Touchburn and E. C. Naber (*Poultry Sci.*, 1962, **41**, 1481—1488).—The rate of egg production by laying hens was not significantly affected by the nutrient density (concn. of known nutrients per lb.; 12–20% protein, 720—1200 kcal. per lb., kcal/protein ratio constant at 60) of the rations. A ration with 12% protein and a kcal./protein ratio of 80 decreased egg production. Feed efficiency with respect to egg production increased with nutrient density of the rations. Energy values assigned to the major ingredients adequately predicted the efficiency with which the nutrients were used for egg production. A. H. CORNFIELD.

Water requirements of broilers. H. Patrick and A. Ferrise (*Poultry Sci.*, 1962, **41**, 1363—1367).—Water consumption per chick per week increased from 0·2 lb. in the first to 0·8 lb. in the third week and 2·4 lb. in the ninth week. Over the 9 weeks birds consumed an average of 1·5 lb. of water per lb. of feed consumed. Water consumption was higher where a high- than where a low-protein feed was supplied. A. H. CORNFIELD.

Phosphorus in the nutrition of the adult hen. I. Minimum phosphorus requirements. E. P. Singsen, A. H. Spandorf, L. D. Matterson, J. A. Serafin and J. J. Tlustohowicz (*Poultry Sci.*, 1962, **41**, 1401—1414).—Laying hens received a basal diet containing 0·2% of total P (0·1% phytin P) supplemented with $CaHPO_4$ to provide up to 0·7% total P (Ca was at 2·25% for all P levels). Optimum egg production occurred with dietary total P ranging from 0·4% to 0·6%, with the calculated point of max. response at 0·53% total (0·48% available) P. Birds on litter required slightly less P in the diet for optimum performance compared with those on wire floors because of the accessibility of the former birds to accumulated P in the litter. Hatchability and adult body wt. were lower at the 0·2% P level than at the higher levels. Birds fed 0·2—0·3% of P had lower bone ash than had those fed higher levels. Wt., shell thickness and sp. gr. of eggs were not affected by the treatments. A. H. CORNFIELD.

Use of antibiotics and oestrogens in rearing of broiler chickens in the Netherlands. A. R. Kuit (*Versl. landbouwk. Onderz.*, 1961, 67.14, 57 pp.).—A report of comparative experiments made with 25,600 chickens. Supplements of 5 mg. of procaine penicillin or 10 mg. of Aureomycin per kg. of feed gave, after 9—13 weeks, gains in wt. representing profits of 1—3 cents (Dutch) per chicken, and reduced mortality by 1·6%; no detrimental effects were detected as regards the composition and general quality of the muscular tissue, or the condition of the thyroid gland. The subcutaneous administration of hexoestrol (15 mg.) 1·5—5 weeks before slaughtering gave gains in wt. (by 45—93 g. per chicken) and improved carcass quality, but increased the necessary consumption of feed and doubled the mortality. The use of this and other hormones has now been prohibited in the Netherlands. (16 references.) P. S. ARUP.

Effects of progesterone and a progestational compound on the production of laying pullets. R. E. Cook and A. C. Warnick (*Poultry Sci.*, 1962, **41**, 1545—1550).—Injecting laying pullets with progesterone (0·002—0·064 g. per bird) in single or multiple doses depressed egg production during the 15-day treatment and 28-day post-treatment period. The progestational compound, 6-methyl-17-acetoxyprogesterone, administered orally or in pellet form at levels up to 0·16 g. had no significant effect on production of pullets during either the treatment or post-treatment period. A. H. CORNFIELD.

Influence of riboflavin and extended black pigmentation on a defective down condition in the domestic fowl. R. O. Hawes and T. W. Fox (*Poultry Sci.*, 1962, **41**, 1504—1508).—The defective down condition (ranging from shortening of the down to extensive clubbing) in the female progeny from mating Rhode Island Red males with Barred Plymouth Rock females was no different in

extent whether the breeder diet was deficient or sufficient in riboflavin. The defective down condition occurred only in chicks with non-barred extended black down colour. A. H. CORNFIELD.

Weight, ascorbic acid and cholesterol levels of the adrenal gland and differential leucocytes as physiological indicators of 'stressor' agents in laying hens. J. H. Wolford and R. K. Ringer (*Poultry Sci.*, 1962, **41,** 1521—1529).—Single intravenous injections of ACTH (10 i.u.) had no effect on the cholesterol or ascorbic acid levels of the adrenal gland. Intramuscular injections twice daily for 4 days reduced adrenal cholesterol level by 68%. In general the % of lymphocytes decreased and/or the % of heterophiles increased following the various stress treatments (ACTH, handling, deprivation of food, and exposure to cold), but differences were significant only for the last three treatments. A. H. CORNFIELD.

Sources of xanthophyll for pigmentation in broilers. R. G. Ratcliff, E. J. Day, C. O. Grogan and J. E. Hill (*Poultry Sci.*, 1962, **41,** 1529—1532).—When a no. of supplements were added to the basal diet at rates such as to supply the same level of added xanthophyll (0·0030—0·0055 g. per lb. of feed) the extent of toe web pigmentation showed that xanthophyll in normal maize was as available as that in three high-xanthophyll maize strains. Xanthophyll in clover meal was 86% as available and in lucerne meal 74% as available as that in maize. A. H. CORNFIELD.

Influence of Triparanol on growth, egg production and cholesterol metabolism of chickens. E. L. Nichols, W. W. Marion and S. L. Balloun (*Poultry Sci.*, 1962, **41,** 1494—1499).—Addition of Triparanol (0·025—0·100 g. per lb. of feed) to the diet of chicks and hens retarded chick growth and increased mortality and resulted in reduced production and size of eggs. The treatment had no effect on either serum- or egg yolk-cholesterol levels. A. H. CORNFIELD.

Variation in initial quality of chicken eggs. I. Physical measurements of albumin and yolks. II. Chemical properties of the albumin. J. H. Skala and M. H. Swanson (*Poultry Sci.*, 1962, **41,** 1533—1536, 1537—1545).—I. Higher quality eggs (as measured by Haugh units) were heavier and contained a larger amount of total white than did the lower quality eggs. There were no differences in yolk wt. between the two classes. The total white of the higher-quality eggs contained a significantly higher % of middle thick white and a significantly lower % of outer thin white than did the total white of lower quality eggs.

II. The whole whites and the middle thick and inner thin white layers, but not the outer thin layer, of high-quality eggs had higher total solids % and total N % than had those of low-quality eggs. Total ash % and Na % in the whole whites were the same for the two qualities as also were the composition of major proteins in the whole white or white layers. The higher quality whole and middle thick whites had a higher apparent ovomucin % than had the lower quality whites. A. H. CORNFIELD.

Effect of dietary components on egg yolk colour. T. W. Sullivan and K. A. Holleman (*Poultry Sci.*, 1962, **41,** 1474—1478).—With a diet containing mainly yellow maize, 10—12% of dehydrated lucerne meal or about 10% of maize gluten meal was needed to produce good egg yolk colour. It was calculated that 0·010—0·012 g. of 'available' xanthophyll per lb. of diet was required to produce this coloration. When milo replaced yellow maize in the diet 15% lucerne meal was needed to produce good egg colour; 4% of animal fat in the hen's diet did not influence egg yolk colour. Addition of xanthophyll (0·01—0·04 g. per lb. of feed) to the hen's diet had no consistent effect on egg yolk colour. A. H. CORNFIELD.

Effect of feeding achiote (*Bixa orellana*) seed on egg-yolk pigmentation. F. T. Landagora (*J. Agric. Puerto Rico*, 1962, **46,** 91—96).—Addition of 1—2% whole achiote seed to the diet of laying hens increased egg production and produced deep red-orange coloured yolks. The taste of eggs from treated birds was preferred to those from untreated birds. A. H. CORNFIELD.

Effect of new carotenoid pigment on egg-yolk colour. J. Edmonson (*N.Z. J. Agric.*, 1962, **104,** 299—300).—Incorporation of the Et ester of β-apo-8-carotenoic acid in chicken mash (20—30 g./ton) produced eggs of good yolk colour. Used similarly, β-apo-carotenal (30 g./ton) was less effective. A. G. POLLARD.

Effect of dietary calcium level, calcium lactate and ascorbic acid on egg production of hens. T. W. Sullivan and J. R. Kingan (*Poultry Sci.*, 1962, **41,** 1596—1602).—Addition of ascorbic acid (0·025 g. per lb. of feed) to a maize-soya-bean oil-meal-meat scraps diet had no effect on egg production but increased slightly egg sp. gr. and shell thickness. Addition of 1% of Ca lactate to the diet increased egg production when the diet contained 2·8% of Ca, but not with higher or lower levels of Ca; the treatment tended to decrease egg sp. gr. and shell thickness. Ca (2·8%) in the diet was adequate for max. egg production. Higher levels increased slightly egg sp. gr. and shell thickness. A. H. CORNFIELD.

Egg production of chickens raised and kept in darkness. D. F. King (*Poultry Sci.*, 1962, **41,** 1499—1503).—Birds were given the following treatments: (*A*) raised and kept during the laying year in continuous darkness (*B*) raised in continuous darkness and in the laying year given 1 h. of light plus weekly increases of 15 min. per day and (*C*) raised with 6 h. light and during the laying year given 6 h. light plus weekly increases of 15 min. per day. Pullets raised with treatment *A* were more docile, slightly lighter in wt. at sexual maturity, experienced slightly higher mortality and matured sexually about 12 days later than did birds raised under treatment *C*. Egg production averaged 59, 66 and 73% for treatments *A*, *B* and *C* respectively. Pullets under treatment *A* laid larger eggs with thicker shells than did those under treatment *C*. The former group also showed the highest mortality during the laying year. A. H. CORNFIELD.

Taste preferences of the chick. C. W. Deyoe, R. E. Davies, R. Krishnan, R. Khaund and J. R. Couch (*Poultry Sci.*, 1962, **41,** 781—784).—Of many flavouring materials tested in the drinking water E.B.P.A. (mixture of esters, org. acids and essential oils) was the most preferred by chicks. The flavour of butter alone and in combination with that of molasses, orange, quince, chocolate, onion and coconut were also well accepted. Eugenol and neralin were offensive to the chick. A. H. CORNFIELD.

Animal vs vegetable protein in high-energy laying rations. L. M. Thuan Komkris (*J. nat. Res. Counc. Thailand*, 1961, **2,** No. 3, 21—36).—The principles of 'high energy' layer rations are reviewed. The optimum requirement of breeding hens is 900 kcal. per lb. The ration most widely used in Thailand, based on a Kasetsart University formulation is 812 kcal. per lb. In the present study an all-vegetable protein ration of 885 kcal. per lb. (diet I) is compared with a mixed vegetable and animal protein ratio of 945 kcal. per lb. (diet II). Soya-bean oil meal was the protein source for the vegetable protein ration, which was supplemented with 225 g. of methionine per ton to cover its deficiency in the diet. Diet II was the most economical for egg production, both in no. and size of eggs, but in the times of scarcity of animal protein the replacement of animal protein by vegetable protein is justified. Use of high-energy rations does not result in overweight birds in warm countries. W. ELSTOW.

Influence of fast and slow drops in ambient temperature on egg production traits. A. C. Campos, F. H. Wilcox and C. S. Shaffner (*Poultry Sci.*, 1962, **41,** 856—865).—When air temp. was reduced from 21·1—26·7° to −12·2° egg production, shell thickness, feed consumption and body wt. were reduced, whilst egg wt. and Haugh units were hardly affected. Feed consumption and body wt. were reduced to a greater extent with the fast (1·1° per h.) than with the slow (0·27° per h.) drop in temp., but there were no differences in other production traits between the two rates of temp. fall. Heavy breeds were less affected than were White Leghorns. A. H. CORNFIELD.

Effect of 17-α-ethyl-17-hydroxynorandrostenone on rate of lay in the domestic fowl. C. M. Winget, E. L. Griffin and J. P. Walker (*Poultry Sci.*, 1962, **41,** 788—794).—Administration of the drug to laying hens resulted in a rapid reduction or cessation of rate of laying depending on dose level, route of administration and frequency of administration. Cessation of laying within 5 days of the start of administration was caused by 0·008 g. per bird per day. Rate of lay returned to normal within a few days of termination of drug administration. A. H. CORNFIELD.

Effects of dietary protein and changes in energy levels on the laying house performance of egg production stocks. J. H. Quisenberry and J. W. Bradley (*Poultry Sci.*, 1962, **41,** 717—724).—Hen day production, egg wt. and feed efficiency increased with the level of the hen's dietary protein (13—17%) over 331 days. Although final body wt. was not significantly different due to protein level of the diet, body wt. was higher earlier in production period with the higher level of protein. Increasing dietary fat from 1 to 5% tended to decrease egg production during the following 56 days. Highest final body wt. occurred when dietary fat was increased from 1 to 5% early in the production period. A. H. CORNFIELD.

Physical changes in eggs produced by hens receiving *Sterculia foetida* oil supplements. A. R. Doberenz, D. L. Schneider, A. A. Kurnick, M. G. Vavich and A. R. Kemmerer (*Poultry Sci.*, 1962, **41,** 700—705).—Eggs from hens receiving *Sterculia foetida* oil (0·15 g. per bird daily) showed pink discoloration after 2 weeks of storage, and the pH of the yolks increased to about the same as that of the whites after 4 weeks of storage. The eggs had more viscous yolks than had eggs from hens receiving maize oil. Holding the eggs at 7° overnight gave a putty-like consistency to the yolks. A. H. CORNFIELD.

Influence of reserpine on late growth of turkeys. F. R. Craig, W. L. Blow, R. J. Monroe and C. W. Barber (*Poultry Sci.*, 1962, **41,** 711—713).—Addition of reserpine (0·1—0·5 p.p.m.) to the diet of turkeys from 24 to 26 weeks of age reduced wt. gains except for males receiving 0·1 p.p.m. and also reduced feed efficiency. There was no evidence of any tranquillising effect of the drug on the birds. A. H. CORNFIELD.

Treatment and prophylaxis of cannibalism in poultry with Haloanisone-R 2028. R. Marsboon and G. Sierens (*Poultry Sci.*, 1962, **41,** 776—780).—Addition of Haloanisone-R 2028 (0·004 g. per kg. body wt. per day) to the drinking water of chicks controlled cannibalism. A. H. CORNFIELD.

Crystalline amino-acid diets for the turkey poult. D. C. Snetsinger, P. E. Waibel and R. C. Fitzsimmons (*Poultry Sci.*, 1962, **41,** 1428—1433).—A cryst. amino-acid mixture (based on chicken egg-white protein) added to a purified starch–maize oil diet produced wt. gains of 6 g. per bird per day when fed to poults. This rate of growth occurred only when all essential amino-acids, except methionine, were present as the L-isomers. Substitution of the L-isomers of tryptophan, valine, isoleucine, phenylalanine and threonine by twice the level of a mixture of the DL-isomers resulted in reduced wt. gains. This depression appeared to be due specifically to one or more of the five D-isomers, as opposed merely to an excess of amino-acid N, since L-isomers at equiv. levels had no growth-retarding effect. A. H. CORNFIELD.

Light regulation in turkey management. I. Effect on body weight. II. Female reproductive performance. III. Male reproductive performance. R. N. Shoffner, C. R. Polley, R. E. Burger and E. L. Johnson (*Poultry Sci.*, 1962, **41,** 1560—1562, 1563—1569, 1570–1573).—I. The comparative effects of continuous light, an intermediate day-length (increasing with season) and a 6-h. day on the performance of turkeys were studied. The best wt. gains to both 14 and 24 weeks of age occurred with the 6-h. day in one season, whilst there was little difference in performance due to type of lighting in another season. Mortality was consistently lowest with the 6-h. day, whilst feather quality was consistently poorest with continuous lighting.

II. The effects of various light treatments for 3 weeks (conditioning) followed by different light treatments for 1 week (stimulatory) on reproductive performance of female turkeys, which had been subjected to the above different light treatments to 24 weeks of age, were studied. In general the larger the amount and the greater the rate of change of light from the conditioning period to the stimulatory light period the faster was the onset of egg production. The treatments had little effect on natural mating fertility or hatchability of fertile eggs.

III. Males produced semen under a wide range of lighting conditions; those receiving the earliest and longest stimulatory light started first. A. H. CORNFIELD.

Influence of dietary ascorbic acid on the severity of β-aminopropionitrile (BAPN) toxicity in turkeys. P. A. Thornton, D. Brownrigg and F. B. Mather (*Poultry Sci.*, 1962, **41,** 1586—1589).—Addition of BAPN (0·1%) to the diet of turkeys to 21 days of age decreased wt. gains and caused hock and toe abnormalities and aortic haemorrhage. Addition of ascorbic acid (0·02 g. per lb. of feed) together with BAPN reduced wt. gains even further; hock and toe abnormalities were also increased further, but aortic haemorrhage was not. BAPN reduced skeletal ash per unit body wt. and retarded the biological turnover of Ca. Addition of ascorbic acid nullified these effects. A. H. CORNFIELD.

Effect of pre-incubation warming of chicken eggs on hatchability. J. Kan, B. N. McPherson and N. R. Gyles (*Poultry Sci.*, 1962, **41,** 1478—1480).—The effect of varying length of storage (up to 4 weeks) and pre-incubation warming (5 h. at 37·5°) given once weekly on hatchability of fertile eggs was studied. The pre-incubation tended to increase hatchability of eggs stored up to 3 weeks, but decreased hatchability for those stored for 4 weeks. The pre-incubation treatment tended to increase the % of chicks hatching within 12 h. after the first appearance of hatched chicks, although differences were not significant. A. H. CORNFIELD.

Diseases of free-living wild animals. A. McDiarmid (*F.A.O. agric. Stud.*, 1962, No. 57, 119 pp.).—Diseases of free-living mammals and birds (particularly those communicable to man and/or domestic animals) caused by bacteria, viruses, fungi, protozoa and rickettsiae are reviewed. (21 pages of references.) M. O'LEARY.

Relationship of magnesium ammonium phosphate to froth production in ruminant bloat. R. A. Phelps (*Dissert. Abstr.*, 1962, **22,** 4142).—A colourless, cryst. material spontaneously formed and artificially precipitated from rumen fluid was identified as $MgNH_4$ phosphate hexahydrate. Frothy rumen ingesta exhibited an average of three times more of the phosphate-containing ppt. and 1·4 times more H^+ than did non-frothy ingesta. F. C. SUTTON.

Bloat in cattle. XXIII. Suitability of three possible prophylactic agents for administration to milking cows. A. T. Johns and F. H. McDowall (*N.Z. J. agric. Res.*, 1962, **5,** 1—7).—Deep-frying fat available as discarded waste product from a local fish shop was administered as an emulsion to milking cows at four times the dose rate for tallow, in treatment of bloat. The fat was in a highly oxidised condition but the flavour of the milk, cream and butter was not affected and the oxidisability of the butter fat was not increased. Drenching of cows with detergent L62 did not affect milk, cream or butter quality or cause change in the characteristics of the butter fat. L62 could be used safely as an anti-foaming agent for the control of bloat in cows. Drenching of a cow with Rawleigh's 'colic ease' (designed for treatment of horses) resulted in tainted milk, cream and butter. E. M. J.

Concentrations and percentage recovery of furacin in milk following intramammary infusions. G. E. Hawkins, G. E. Paar and R. Y. Cannon (*J. Dairy Sci.*, 1961, **44,** 2212—2217).—Milk from 11 cows taken 14 and 24 h. after infusion of furacin (**I**) (60 mg.) contained <0·5 p.p.m. of **I**. The concn. of **I** in milk reached max. 1—2 h. after the infusion. The apparent disappearance of **I** from milk after infusion probably results from absorption of the drug or its metabolites into the blood stream or to the formation of an inactive complex with milk proteins. No definite evidence of the metabolism of **I** was obtained. A. G. POLLARD.

Fly control in faeces from cattle fed Co-Ral. J. S. Skaptason and C. W. Pitts (*J. econ. Ent.*, 1962, **55,** 404—405).—Co-Ral was incorporated into cattle rations and the emergence of fly larvae placed in the faeces was compared. At 5, 10 or 50 p.p.m. emergence was significantly reduced. C. M. HARDWICK.

Studies with ^{32}P-labelled Bayer 22408 in steers and guinea pigs. P. E. Gatterdan, W. F. Chamberlain and D. E. Hopkins (*J. econ. Ent.*, 1962, **55,** 326—332).—^{32}P-labelled *OO*-diethyl *O*-naphthalimido phosphorothioate was given dermally and orally to steers at 12 mg./kg. body wt. and subcutaneously to guinea pigs at 95—117 mg./kg. The level of radioactivity in the blood, the amount excreted in the urine and faeces, residues in other tissues and the hydrolytic products are described. The identification of Bayer 22408 and related compounds by two paper-chromatographic systems is described. (17 references.) C. M. HARDWICK.

Removal of embedded Lone Star ticks, *Amblyomma americanum*. K. L. Knight, D. E. Bryan and C. W. Taylor (*J. econ. Ent.*, 1962, **55,** 273—276).—Of 31 compounds tested, deodorised kerosene and camphorated phenol caused voluntary detachment of 25% of the ticks from rabbits' ears and together with pyrethrins and light petroleum greatly decreased the pull required to detach the rest. Greater forces were required to remove ticks from a rabbit's back. C. M. HARDWICK.

Control of the face fly in Virginia. J. B. Wallace and E. C. Turner (*J. econ. Ent.*, 1962, **55,** 415—416).—In an area of heavy infestation, none of eight insecticides applied as sprays or dusts reduced populations of adult *Musca autumnalis*. When the cattle were allowed access to a ronnel–salt mixture there was no reduction in the no. of adult flies but fly larvae breeding in the manure were reduced by 58—90%. C. M. HARDWICK.

Face-fly control studies in West Virginia in 1960 and 1961. C. K. Dorsey, H. E. Kidder and C. J. Cunningham (*J. econ. Ent.*, 1962, **55,** 369—374).—Various (22) sprays, smears or dusts were tested on various herds over a period of 2 years and the results are tabulated. After 7 and 14 days smears were marginally more effective but after 21 days sprays were better. The only products meriting a 'good' rating were diazinon spray and smear, dimethoate smear and Ruelene or Sevin dust. C. M. HARDWICK.

Effect of vitamin A on the course of *Mycoplasma* infection in chicks. F. M. Boyd and H. M. Edwards, jun. (*Poultry Sci.*, 1962, **41,** 750—754).—Addition of vitamin A (50—3200 i.u. per lb. of feed) to the diet of chicks infected with *Mycoplasma gallisepticum* increased wt. gains and decreased mortality. Wt. gains of infected birds were less than those of uninfected birds even where vitamin A was supplied. There was no correlation between level of vitamin supplied and serological reaction, extent of lesions or liver vitamin content. A. H. CORNFIELD.

Systemic acaricidal effects of Sevin in poultry. D. P. Farman and G. R. Pieper (*J. econ. Ent.*, 1962, **55,** 355—357).—A bioassay technique using the mite *Haemogamasus liponyssoides hesperus* is described. This showed a toxic substance in the blood for 48—72 h. The percentage recovery in various tissues varied between 75—100%. The max. residue 24 h. after a 150 mg./kg. dose of Sevin was in the gizzard. C. M. HARDWICK.

Influence of reserpine and antibiotics on incidence of dissecting aneurism in turkeys as induced by β-aminopropionitrile. P. E. Waibel, R. E. Burger and L. M. Krista (*Poultry Sci.*, 1962, **41**, 1554—1559).—Supplying turkeys with 1 p.p.m. of reserpine between 7 and 28 days of age depressed wt. gains in the absence of antibiotics but supported growth at about the same level as the control in the presence of chlortetracycline hydrochloride (220 p.p.m.) or procaine penicillin (110 p.p.m.). Reserpine delayed the onset of dissecting aneurism (aortic rupture) induced by feeding 0·045% of β-aminopropionitrile, the effect being similar in the absence or presence of antibiotics. The antibiotics themselves had no effect on the onset of dissecting aneurism. A. H. CORNFIELD.

Effect of ventilation rate on the response of chicks inoculated with infectious bronchitis virus and housed at 9·4°. R. P. Prince, L. D. Matterson, R. E. Luginbuhl and T. W. Chomiak (*Poultry Sci.*, 1962, **41**, 1512—1516).—Gains in wt. of chicks, from 4 to 8 weeks of age, which had been inoculated with infectious bronchitis were significantly lower than those of uninoculated birds. Varying the ventilation rate (from 0·75 to 2·00 cu. ft. per min. per bird) had no effect on wt. gains. Infection resulted in lower feed consumption and feed efficiency ; rate of ventilation had no effect on these factors. There were no significant differences in mortality due to infection or ventilation rate. A. H. CORNFIELD.

Nutritionally balanced animal feed composition. American Cyanamid Co. (B.P. 883,927, 29.1.60. U.S., 12.2.59).—A feed composition, especially adapted for the feeding of chickens, contains a broad-spectrum tetracycline-type antibiotic (chlortetracycline), $CaSO_4$ (as a source of Ca), and a source of P comprising a phosphoric acid compound substantially free from Ca, e.g., H_3PO_4 or an alkali metal salt thereof. If desired, terephthalic acid may be included (to increase blood level). F. R. BASFORD.

Proteinaceous feed material from mustard, rape and similar seeds. Oil Seed Products Inc. (B.P. 883,836, 20.4.59. U.S., 10.6.58 and 23.1.59).—The seed is extracted with solvent (for removal of oil) without heat, so that the enzyme myrosin is preserved, then the residual meal is heated with water at 45—55°, whereby the enzyme effects hydrolysis of the thioglucosides responsible for unpalatable flavour. The S-containing compounds thus released are distilled off in a current of steam, to leave a meal suitable for use as animal feedstuff. F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Rice quality factors. VII. Screening tests based on ageing : organoleptic and physico-chemical properties of the grain. E. Primo, A. Casas, S. Barber and C. Benedito de Barber (*Rev. Agroquim. Technol. Aliment.*, 1962, **2**, 241—246).—Cohesion of the rice grains on cooking reduces with ageing of the grain, and results in improved consumer acceptance. Physico-chemical changes during ageing include increased water absorption and paste η and reduction in solids dissolved in residual cooking liquor and in the I colour after hot-water extraction. The extent of the changes differs with variety, but no single factor correlated well with the organoleptic assessment of quality. (14 references.) E. C. APLING.

Development and evaluation of maize carbohydrate coatings for foods. L. Allen (*Dissert. Abstr.*, 1962, **23**, 595).—Maize carbohydrates, because of their edibility and economical availability, would be advantageous to use as coatings to be applied to various products and thus benefit the production and distribution of foods. These studies led to the formulation of coatings composed of regular maize starch or an oxidised maize starch compounded with Na alginate. These coatings required the application of a cation to convert the starch slurry to a plastic-like coating. Ca was the most effective cation and $CaCl_2$ the best salt. The preservative effect of coatings on a variety of foods is described. F. C. SUTTON.

Determination of the heats of combustion of proteins of wheat grain during thermal denaturation. Y. V. Ponomarev, T. A. Alekseeva, N. I. Sosedov and Z. B. Drozdova (*Dokl. Akad. Nauk SSSR*, 1962, **146**, 213—214).—The heats of combustion of gluten, gliadin and glutenin from the wheat 'Liutectsens 062' were measured in a microcalorimeter during denaturing at temp. 20—110°. Changes in the heats of combustion were taken to indicate a change in the mol. structure of the proteins as a result of thermal denaturing. Additivity in the heats of combustion of gliadin and glutenin began at 35°, indicating the beginning of the decomposition of gluten into these two proteins. This change was complete at a denaturing temp. of 70°. Above 70° the heats of combustion of gliadin and glutenin increased, indicating the formation of less stable bonds in these proteins. The max. change in the heat of combustion during denaturing occurred with gliadin (133·5 cal./g.), showing that this protein undergoes fundamental structural changes under the influence of heat. A. S. LEVESLEY.

Phenolic compounds of commercial wheat germ. H. G. C. King (*J. Fd Sci.*, 1962, **27**, 446—454).—Two related glycoflavones (flavonoids A and B) occurring at 0·2—0·3% were isolated. Each consists of an apigenin nucleus to which is attached a highly hydroxylated glycosyl-type side chain which evidence suggests is in the 8-position. Short hydrolysis of flavonoid A splits off a mol. of sinapic acid, leaving flavonoid B ; a third glycoflavone is partially formed by treating A and B with more prolonged mineral acid hydrolysis. Ferulic and vanillic acids were also found in the germ in the free form. The presence of a methoxyquinol glycoside was indicated. (19 references.) E. M. J.

Effect of some soluble chemicals on liquid ferments. P. E. Snell and K. L. Fortmann (*Baker's Dig.*, 1962, **36**, No. 5, 58—62).—A variable factor affecting the fermentation of yeast in breadmaking was the mineral content of the water supply. Water analysis data from 62 cities were obtained and summarised. The effects of 67 common chemicals on the fermentation of liquid ferments was measured. Of those significantly affecting fermentation, usually only Cl^-, Ca^{2+} and HCO_3^- ions and $CuSO_4$ are in sufficient amounts to cause variation. The effects of these substances, and of other chemicals often used as ingredients, can be balanced by varying the amounts of yeast and salt used. S. G. AYERST.

Use of sugars in continuous dough processing. E. G. Snyder and G. W. Trum (*Baker's Dig.*, 1962, **36**, No. 5, 76—78).—Maize sugar, which is chemically dextrose, and cane and beet sugar, chemically sucrose, are compared on the basis of cost, and as substrates for yeast fermentation. The process of preparing preferments is discussed. The by-products which provide flavour and are substrates for the browning reaction are produced without using large amounts of sugar. The advantages and disadvantages of using liquid sugars are considered. The type and amount of sugar used does not affect bread softness or staling. S. G. AYERST.

Dough structure, its basis and implications. I. Hlynka (*Baker's Dig.*, 1962, **36**, No. 5, 44—47).—The structural elements of flour can be classified by their particle size and by their protein and starch content. The mobility of dough, measured in reciprocal Brabender units, is directly related to its water content. When water is added to flour the water-sol. proteins eventually form a continuous phase coating the starch granules. This phase changes in character during prolonged mixing. The viscous and elastic properties of the dough are measured by the Brabender Extensograph. The sulphydryl–disulphide linkages are thought to play an important part in dough behaviour. S. G. AYERST.

Comparison of data obtained in different laboratories from the Alveograph, Farinograph and Extensograph. A. H. Bloksma, B. Francis and J. C. A. Zaat (*Cereal Sci. Today*, 1962, **7**, 308—310, 312—313, 336).—A sample of French flour and a sample of English flour were sent to 13 laboratories in Europe. In general, each laboratory tested its Chopin Alveograph, Brabender Farinograph and Brabender Extensograph with three doughs on each of 2 days. A statistical analysis of the results was made and variations between laboratories are discussed and explanations made on the basis of data about condition of instruments, temp. of laboratories, cabinets and doughs, and motor and chart speeds. S. G. AYERST.

Quality fruit cakes with gelatinised wheat starch. D. K. Dubois and N. R. Brown (*Baker's Dig.*, 1962, **36**, No. 5, 66—69).—The addition of 4—6% of gelatinised wheat starch to the flour when baking fruit cake gave a more moist and tender cake and improved slicing, fruit dispersion and batter mixing tolerance. To obtain optimum results the amounts added should be varied according to the % of fruit. S. G. AYERST.

Effects of electronic method of cookery on the quality of shortened cakes. M. Neuzil and R. E. Baldwin (*Food Technol.*, 1962, **16**, No. 11, 110—112).—In tests on plain, white and devil's food cakes, all cakes cooked electronically tended to be less tender and less moist than conventional cakes, although cell structure and flavour were not significantly affected. E. M. J.

Quick-cooking rice product. General Foods Corp. (Inventors : F. V. Rosseau and J. Rossen) (B.P. 884,315, 30.10.59).—A quick-cooking rice product is prepared by cooking fissured rice grains (obtained by heating the grains) in a moisture-permeable container

by submerging the latter in hot water (or hot aq. cooking fluid) under such conditions as to hydrate and gelatinise the starch content, e.g., 20—30 min. to a final temp. of 140—225°F at 11% moisture in the rice or 140—200°F at 18% moisture. F. R. BASFORD.

Carbon-containing organo-silicon resin coating compositions. Ekco Products Co. (B.P. 884,208, 19.5.58. U.S., 6.8.57).—The coating compositions consist of a polyorganosilane and/or a polyorganosiloxane and containing 0·05—5·0% by vol. of finely divided carbon particles having a particle size in the mμ range. The products are useful for coating of baking pans. E. ENOS JONES.

Sugars and confectionery

Carob bean sugars. VI. Influence of aluminium salts on the defecation of carob diffusion liquors with calcium hydroxide–phosphoric acid and barium hydroxide–phosphoric acid. V. Cortés, B. Lafuente and E. Primo (*Rev. Agroquím. Technol. Aliment.*, 1962, **2**, 247—252).—Defecation of carob bean musts with $Al_2(SO_4)_3$ and $Ca(OH)_2$ to pH 11, followed by H_3PO_4 to pH 7·5 to 8·5 eliminates 85% of the colour, 66% of the tannins and 24% of the salts. Use of $Ba(OH)_2$ in place of $Ca(OH)_2$ results in greater elimination of salts, but is less efficient in removal of tannins (46%) and colour (76%). E. C. APLING.

Quantitative determination of sucrose, starch syrup and invert sugar. V. Rošper (*Listy cukrovar.*, 1962, **78**, 277—280).—When the double polarisation method is applied to hard candies and fondant, account must be made of the change in rotation of the starch syrup during acid inversion (HCl). It is suggested that calculations should be based on dextrose equiv. of the starch syrup of 34, 38 and 40 according to the degree of conversion of the starch. It is claimed that the method suggested allows determination of sucrose, invert sugar and starch syrup to within 0·3%. J. S. B.

Effect of sucrose and cyclamate on the gel strength of gelatin, carrageenan and algin in the preparation of jellied custard. M. E. Zabik, G. A. Miller and P. J. Aldrich (*Food Technol.*, 1962, **16**, No. 12, 87—91).—Custards prepared with gelatin or algin exhibited greater gel strength than those prepared with carrageenan (**I**). **I** custards with 40% sucrose were less rigid than those with 20% sucrose or the corresponding control. Ca cyclamate increased gel strength in gelatin custards (**II**), but decreased it in **I** and algin custards (**III**). Na cyclamate decreased gel strength of **III** and **II**. (19 references.) E. M. J.

Detection and identification of carbohydrate thickeners by chromatography and electrophoresis. S. Stoll and Y. Prat (*Ann. Falsif., Paris*, 1962, **54**, 109—125; **55**, 159—176).—A review including descriptions of methods applicable to gums, mucilages, pectins, alginates, carragheens, agar, starches, and deriv. of cellulose. (26 references.) P. S. ARUP.

Infra-red spectra of some pectic substances. M. Reintjes, D. D. Musco and G. H. Joseph (*J. Fd Sci.*, 1962, **27**, 441—445).—The i.r. spectra of current commercially available pectins, citrus and apple, slow and rapid set, and some commercial deriv. of pectin are presented. Distinction can be made between pectins with high and with low methoxyl and polygalacturonic acid. Results reported should be of use in general qual. identification of pectin substances. E. M. J.

Compatibility and manipulation of guar gum. W. A. Carlson, E. M. Ziegenfuss and J. D. Overton (*Food Technol.*, 1962, **16**, No. 10, 50—54).—A discussion of the high purity galactomannan separated from the guar bean *Cyamopsis tetragonaloba*. E. M. J.

Refining of crude sugar-juices. Pfeifer & Langen K.-G. (B.P. 884,460, 1.4.58. Ger., 9.4.57).—Crude sugar juice is refined by effecting preliminary liming in several stages with opaque juice and/or slime free from (or containing only traces of) suspended $Ca(OH)_2$, under such conditions that the optimum pptn. point is achieved in the last stage at pH 10·9. F. R. BASFORD.

Fermentation and Alcoholic Beverages

Functions of reducing agents and their practical evaluation in production of Austrian wines. F. Paul (*Mitt. Wein u. Obstbau, Wien*, 1962, **12A**, 275—287).—A review and discussion of recent research on the respective functions of H_2SO_3 and ascorbic acid (**I**). Objectives achieved by H_2SO_3 (but not by **I**) are (*a*) the suppression of biological infections during fermentation, (*b*) the inhibition of polyphenoloxidases which cause discoloration, (*c*) the preservation of flavour-producing reductones during maceration, and (*d*) the binding of MeCHO (which is desirable in the production of still wines, but not in that of sparkling wines). **I**, in virtue of its autoxidative character, is more efficient than H_2SO_3 in regulating the atm. oxidation of wine during maturing. The specific effects of the two treatments on the aroma and flavour of typical wines are considered. (10 references.) P. S. ARUP.

Application of sorbic acid for stabilising wine. E. Minárik (*Kvasný prùmysl*, 1962, **9**, 253—257).—The results of experiments aimed at determining the efficiency of sorbic acid and K sorbate as stabilising agents in sweet wines indicate that their antiseptic effect starts at concn. as low as 50 mg./l. For practical application concn. in the range of 50—200 mg./l. can be recommended. The appropriate concn. depends on the content of alcohol, degree of sulphuration and previous treatment of wine. If the doses of chemicals are held within the indicated limits, the organoleptic qualities are not harmed. Application of sorbic acid permits reduction of the doses of H_2SO_3 by 30—50%, but not complete omission. Sorbic acid is not suitable as preserving agent for grape must. (19 references.) J. S. B.

Constitution and anticrystallisation effect of 'metatartaric acid'. E. Peynaud and G. Guimberteau (*Industr. aliment. agricoles*, 1961, **78**, 131—135, 413—418).—The prevention of the formation of tartar deposits in wine by the addition of 'metatartaric acid' (**I**) (≯10 g./hl.) was permitted by a 1956 French regulation. **I** is prepared by heating tartaric acid to 170° (its m.p.). Previous suggestions as to the composition of the product are criticised (formation of dilactide, a bimol. triacid monoester, etc.), and it is shown that it is a mixture of substances with lactide structure, probably macromolecular, the composition depending on the duration of heating. Pyruvic acid (5—6%) is always present. The crystallisation-inhibiting activity depends on the proportion of lactide groups present, i.e., the size of the macromolecule. **I** is transformed to tartaric acid by alkaline hydrolysis. H. S. R.

Determination of fusel oil in fermentation products. H. Burmeister (*Z. LebensmittUntersuch.*, 1962, **118**, 233—244).—Available methods are critically examined. A procedure is developed in which the distilled sample is first freed from aldehyde by treatment with aq. H_2SO_3 followed by passage through a column of Permutit ES; the determination is then made by spectrophotometric measurement of the colour formed on the addition of *p*-dimethylaminobenzaldehyde and H_2SO_4; the mixture must be well cooled during the addition of the acid, after which it is heated during 20 min. in boiling water, cooled, and set aside for 25 min. The colour is then measured at 529 mμ at which λ the absorptions of isoamyl and isobutyl alcohols are equal. Determinations should be made in triplicate. A blank value is determined with pure 40% EtOH. A calibration graph is given which shows consistent results for mixtures of the two alcohols containing 10—35% of isobutanol. (24 references.) P. S. ARUP.

Influence of polyphenols and tannins on physiology of wine yeasts, and value of the pH 7 test in choice of wines for production of dessert and sparkling wines. H. Schanderl (*Mitt. Wein u. Obstbau, Wien*, 1962, **12A**, 265—274).—Wine yeasts are not affected by naturally occurring polyphenols or tannins (or by 2—4 mg. per l. of tannin in a synthetic medium) before the concn. of EtOH in the must (or medium) has passed ~8%. High concn. of polyphenols and/or tannins cause injury to the yeast and discoloration of the wine when the concn. reaches 10—14%. An approx. titration of the wine to 7·0—7·3 reveals the presence of undesirable concn. of polyphenols and/or tannins by the formation of a black, dark-violet, or red-brown colour; such wines containing very little or no Fe give a yellow or deep yellow colour which darkens on the addition of a trace of Fe^{3+}. Gallic acid is indicated by a dark violet, and polyphenols by a red-brown colour. Wild yeasts of the *apiculatus* type are injured by the polyphenols or tannins in the presence even of low concn. of EtOH. (10 references.) P. S. ARUP.

Present knowledge of grape and wine flavours. A. D. Webb (*Food Technol.*, 1962, **16**, No. 11, 56—59).—In general, the no. of compounds found in wines is greater than that in grapes. These are listed and discussed. E. M. J.

Confusion in sensory scoring [in wines] induced by experimental design. M. A. Amerine, G. A. Baker and C. S. Ough (*J. Fd Sci.*, 1962, **27**, 489—494).—Replicated scorings of four series of wines were made by eight tasters with two different designs of presentation (i) different wines with additives (each wine with four concn. of additives was tasted as a block); (ii) randomisation of tastings over all wines and additives. In any one design, tasters did not react the same for any given series. The differences showed in mean scorings and in the variability of the scorings. When the design was broadened to include more diverse categories in a randomised fashion, the variability of the scorings increased in general in some cases. E. M. J.

Respiration and phosphorylation in water-sensitive barley. G. Jansson (*Ark. Kemi*, 1962, **19**, 141—148).—Respiration rates of intact seeds in the early stages of germination, and of uptake of O_2

and P by mitochondria prepared from seeds germinated for 18 h., were substantially the same for normal and water-sensitive barley. Oxidative phosphorylation was unchanged in mitochondria from barley in which water-sensitivity was induced by treatment with indol-3-ylacetic acid (**I**) or hot water, but mitochondria from normal barley were sensitive to **I**. It is suggested that water sensitivity is due to uncoupling of phosphorylation from oxidation following the development of high concn. of **I** in the grain. (16 references.) (In English.) E. C. APLING.

Study of indol-3-ylacetic acid in barley seeds in relation to water-sensitivity. G. Jansson (*Ark. Kemi*, 1962, **19**, 149—159).—Water-sensitivity is induced in normal barley by indol-3-ylacetic acid (**I**) and also by *p*-chlorophenoxyisobutyric acid; and dormancy is relieved by uranyl acetate. Determination of free **I** in maturing barley seeds showed a max. value approx. 2 weeks before harvest (when moisture content is high and risk of germination in the ear is max.), subsequently falling to zero at full maturation. The presence of a lag-factor (unidentified), which delays enzymic oxidation of **I** was demonstrated in embryos of water-sensitive grains, and determinations of the lag-factor showed positive correlation with germinative capacity under water-sensitive conditions. (20 references.) (In English.) E. C. APLING.

Some properties of a substance in barley seeds which induces a lag period in the enzymic oxidation of indol-3-ylacetic acid. G. Jansson (*Ark. Kemi*, 1962, **19**, 161—171).—Barley seeds contain a substance (**I**) which induces a lag phase on the oxidation of indol-3-ylacetic acid, and which can be removed from extracts by dialysis, by activated C or by *p*-chloromercuribenzoic acid-treated ion-exchange resin. **I** is precipitated by Ag^+ and Hg^{2+} solutions, is co-precipitated with indolylacetic acid oxidase by $(NH_4)_2SO_4$, but is not precipitated by acetone. Cysteine, reduced glutathione and mercaptoacetic acid can induce the lag phase, but do not correspond in properties with **I** and have little effect in inducing water-sensitivity in normal grain. (30 references.) (In English.) E. C. APLING.

Methods for determining nitrogen [in agricultural products]. H. Hecht and A. Fritz (*Brauwissenschaft*, 1962, **15**, 347—353).—As regards freedom from experimental error, the Kjeldahl (distillation and titration) method for evaluating the NH_4^+ produced by the digestion with H_2SO_4 is somewhat superior to the Nessler spectrophotometric method and much superior to the formol titration method. Taking the Kjeldahl method as a standard, it is shown by comparative analyses of barleys and the 20 amino-acids of barley protein that the rapid Nessler method can well be used by trained workers, but that the formol titration method gives comparatively rough results. (49 references.) P. S. ARUP.

Differences in composition of non-volatile aliphatic acids formed by yeasts (*Candida robusta*, *C. pulcherrima* and *C. albicans*) with different forms of dormant cells. V. I. Kudryavtsev, M. V. Fateeva and T. N. Nikitina (*Mikrobiologiya*, 1962, **31**, 582—585).—Yeasts were cultivated on improved Wickerham (1951) medium with 5% glucose. Detailed analysis is given. Acids were separated at max. yield when 75—80% of glucose was fermented after 2—4 days at 24—25°. Details of extraction by Et_2O and identification of 14 acids by chromatography are given. Four systems of mixed solvents found suitable for chromatographic separation of named acids are described, together with developers used. Behaviour of three yeasts differed both on new medium and on earlier Rider medium as regards: (i) morphology of organisms after cessation of vegetative growth, especially morphology of dormant cell-arthrospores on malt agar; (ii) dynamics of fermentation of glucose; (iii) amount of products formed at end of fermentation: alcohol, glycerol, volatile and non-volatile aliphatic acids; (iv) qual. composition of non-volatile aliphatic acids. (15 references.) P. W. B. HARRISON.

Determination and presence of chromium in beer. A. R. Deschreider and R. Meaux (*Rev. Ferment.*, 1962, **17**, 73—76).—Chromium is determined in beer, after ashing, by a colorimetric method based on the colour formed by the Cr–S–diphenylcarbazide complex. Cr contents of 0—13·75 μg./l. are found in various beers. These are well below the limits for drinking water. J. V. RUSSO.

Production of spirit vinegar by quick process with a pure culture of *Acetobacter rancens*. H. Suomalainen (*Brauwissenschaft*, 1962, **15**, 356—357).—Recent investigation has shown that the culture previously described as *Acetobacter suboxydans* (cf. *ibid.*, 1961, **14**, 95) should be classified as *Acetobacter rancens*. The culture, which has been in use for many years, continues to function satisfactorily in spite of the presence of some mutant cells. P. S. ARUP.

Fruits, Vegetables, etc.

Enzymic browning of apple fruit. J. R. L. Walker (*N.Z. J. Sci.*, 1962, **5**, 316—324).—The actual browning produced under optimum oxidation conditions, the total potential browning, determined by adding a suitable substrate (catechol), and the ascorbic acid, chlorogenic acid and total phenolic contents of Cox's Orange and Sturmer apples were determined during tree ripening and at periods during cold storage. Results indicate that chlorogenic acid is the major substrate for apple polyphenol oxidase and that the degree of browning bore some relationship to the chlorogenic acid content of the apples. (13 references.) W. ELSTOW.

Malic enzyme activity in apple fruit. D. R. Dilley (*Nature, Lond.*, 1962, **196**, 387—388).—The onset of the malate effect in McIntosh apple peel was found to coincide with the beginning of the respiratory climacteric. The apple fruit cortical tissue contained ~50 units of malic enzyme activity per g. The specific activity was higher in post-climacteric than in pre-climacteric tissue. (11 references.) S. A. BROOKS.

Separation of the enzymes present in the mitochondrial fraction from apple peel. A. C. Hulme and L. S. C. Wooltorton (*Nature, Lond.*, 1962, **196**, 388—389).—The activities of the malic enzyme from various prep. obtained from apple fruits were estimated. The carboxylase activity of whole and sonicated mitochondria has also been determined. Sonication is suggested as the first step in the isolation of the enzymes in the mitochondrial fraction. S. A. BROOKS.

Method proposed for the determination of an index of quality of oranges. J. Royo Iranzo (*Fruits, Paris*, 1962, **17**, 457—464).—By summing the following qualities or values, degrees Brix, % acid as citric, formol titre (ml. of 0·1N-NaOH per 10 ml.), vitamin C content, flavours and fragrance, edibility and % juice, each value multiplied by its own coeff., a figure or index of quality is obtained by which the fruit may be judged. The organoleptic qualities are assessed on a scale 1 to 10 by a panel of five experts. In the examples given the index varies from 35 to 40 for fruit of bad quality to 66 for fruit of exceptionally high quality. W. ELSTOW.

Index of quality of orange varieties. I. Statement of the problem and comparison between the 'Navelate' and Washington Navel varieties. E. Primo Yúfera, J. Royo Iranzo, J. M. Sala Gomis and F. Gasque Pastor (*Rev. Agroquím. Technol. Aliment.*, 1962, **2**, 235—240).—Factors affecting quality assessment of oranges are briefly discussed. The new variety 'Navelate' compares favourably with Washington Navel in respect of proportions of juice and peel, juice strength, edibility of the pulp and time of ripening. E. C. APLING.

Biochemistry of fruit maturation. J. B. Biale and R. E. Young (*Endeavour*, 1962, **21**, 164—174).—Physiology and biochemistry of fruit ripening are described. Rates of oxidation in plant cells, the hormone-like substance that promotes fruit formation and effects of light and gases on rate of maturation of cells were studied and the results applied to modern storage methods. Data, diagrams and photographs for climacteric and non-climacteric type fruits are given. C. A. P.

Quality of frozen fruit from retail markets. J. P. Sweeney, V. J. Chapman, M. E. Martin and E. H. Dawson (*Food Technol.*, 1962, **16**, No. 10, 136—143).—The results of a study of six frozen fruits from local retail markets, selected brands from selected stores, four times each season for four consecutive seasons showed that a relatively high proportion of samples were of high table quality. There was considerable variation in quality of all fruits, e.g., in ascorbic acid oxidation ratios, solids to liquids ratios of ascorbic acid, titratable acids and sol. solids and panel scores for colour, texture and flavour. Strawberries, raspberries and peaches tended to be more variable than grapefruit sections, orange juice or pineapple. A constant and sufficiently low storage temp. is very important to quality but this is not the only problem in supplying high-quality frozen fruit. (20 references.) E. M. J.

Effect of γ-radiation on grapes and strawberries. M. Tomaňa and O. Švábenský (*Prům. potravin*, 1963, **14**, 43—45).—Results of experiments with fruit in the highest stage of ripeness exposed to γ-radiation after a long lasting transport are reported. The batch to be processed must be in sound condition, since γ-rays speed up spoilage of bruised or damaged fruits. Better results are achieved with fruit not yet quite ripe and in general, with strawberries than grapes. The irradiation method is promising for prolonging the storage life of the fruit. (14 references.) J. S. B.

Physico-chemical studies on ground olive pastes. XV. Effect of surface-active agents on the waste liquors (alpechins), foots and pastes. Industrial trials. J. M. Martinez Moreno and J. M. Martinez Suárez (*Grasas y Aceites*, 1962, **13**, 155—160).—Some of the previously reported laboratory experiments on the breaking of oil emulsions in the alpechins, the recovery of oil from foots and the improvement of oil yield in pressing of the pastes have been carried out on an industrial scale and the economics considered. It was

again found that incorporation of Na dialkyl sulphosuccinate in the pastes sometimes but not always improved the oil yield.
L. A. O'NEILL.

Effects of low-dose irradiation and storage on acceptability of broccoli, sweet corn and strawberries. D. C. Martin and D. A. Tickenor (*Food Technol.*, 1962, **16,** No. 11, 96—100).—Broccoli, sweet corn and strawberries were given γ-radiation doses of $0{\cdot}25 \times 10^6$ to $1{\cdot}0 \times 10^6$ rads and stored up to 305 days at 35°F. Reflectance readings were taken on a Bausch and Lomb colour analyser. All N_2-packed irradiated sweet corn was acceptable after 305 days. Control unirradiated samples at 35°F were too spoiled to be presented. One set of N_2-packed irradiated broccoli samples was acceptable after 270 days at 35°F. All of the irradiated strawberries were less acceptable than 35°F controls at all three time periods. Dominant wavelength, purity or brightness was significantly related to colour score for all products tested. (12 references.) E. M. J.

Textural quality of potatoes. I. Comparison of three organoleptic methods. M. V. Zaehringer and D. Le Tourneau. **II. An objective method for evaluating texture.** D. Le Tourneau, M. V. Zaehringer and A. L. Potter (*Food Technol.*, 1962, **16,** No. 10, 131—134, 135—138).—I. The study included three varieties, two dates of harvest, three sp. gr. groups in two areas for two years. The most sensitive method of evaluating potato texture was mild mashing. In all series the Early Gems were less mealy than the Kennebecs and Russets. There was a highly significant correlation between sp. gr. and mealiness. (24 references.)

II. A rapid-cooking method, based on the amount of sloughing of slices of tuber tissue mechanically agitated during cooking, was used on 69 lots of experimental tubers. There was a high degree of correlation (−0·77 to −0·92) between cooked potato wt. (after sloughing) and subjective taste panel appraisals of texture; and between cooked potato wt. and sp. gr. of the tubers, viz., (−0·88 to −0·95). (15 references.) E. M. J.

Dehydrated diced sweet potatoes: a pilot-plant process and product evaluation. L. J. Molaison, J. J. Spadaro, M. T. Roby and F. H. Lee (*Food Technol.*, 1962, **16,** No. 11, 101—104).—The product had a residual sulphite content of 200—500 p.p.m. and a moisture content of <4%. When canned under N_2 in an atm. containing <2% of O_2 it had good keeping qualities at 70°F. Dehydrated sweet potato dice have a storage stability of at least 12 months at 100°F. E. M. J.

Chemical evolution of non-nitrogenous substances in the nuts of *Juglans regia* L. during growth and senescence. J. K. Pazarinčević and A. F. Damanster (*Bull. Soc. chim. Beograd*, 1962, **27,** 24—35).—Determinations are made of moisture, ascorbic (**I**) and dehydroascorbic acids, reductones, strong reducing substances (**II**), sugars, pentosans and cellulose on samples of the nuts at various stages of development. In the initial stages of growth **II** and enediol compounds were present and diminished with increase in cellulose and pentosans which were max. at max. wt. and vol. of the nuts. Max. **I** was found in fruits with max. water content. Two types of reductones were present. Max. content of sugars were found in the max. period of growth, but only traces were present in the ripe nuts, except in the kernel. (From French summary.) H. S. R.

Non-alcoholic beverages

Production of fruit and vegetable nectars. J. Kolek (*Prům. potravin*, 1963, **14,** 12—16).—Problems involved in the design of machinery for fruit beverages production, especially of units for disintegration of various kinds of fruit and for deaeration of the pulp are discussed; a newly designed production line is described. J. S. B.

Soft drink flavours, their history and characteristics. XXIV. Passionfruit and its variants. G. B. Beattie (*Perfum. essent. Oil Rec.*, 1962, **53,** 549—558).—The problems are generally discussed and several formulae are given. (48 references.) C. V.

Research experiments on the nutritional properties of canned orange juice. J. Lavollay, J. Kayser, J. Saubobert and J. Neumann (*Fruits, Paris*, 1962, **17,** 395—401).—Experiments with rats show that the addition of 1 ml. of orange juice per day to a diet deficient in all vitamins gives a marked improvement in survival and growth rate. Although the B vitamins present in the juice form only a fraction of the normal requirement estimated from the literature the amount present would appear to exert a marked effect. The 1 ml. of juice assured that a large fraction of the requirement of the vitamins A and E was supplied. The possibility that other constituents of the juice contributed the beneficial effects noted cannot be excluded. W. ELSTOW.

Influence of light on orange juice and orange drinks. P. Dupaigne (*Fruits, Paris*, 1962, **17,** 407—409).—In general, exposure to light does not seriously affect the colour of orange drinks. Measured by the system described there is a slight alteration in the orange component of the colour which is in part compensated by an increase in the yellow component. The blue component may also increase but this may be due to oxidation rather than the effect of light. Flavour suffers severely on exposure to light, cooked and overheated rubber flavours develop and for this reason alone orange juices should be kept away from light. W. ELSTOW.

Stability of frozen concentrated citrus juices following adverse storage. T. J. Kew and M. K. Veldhuis (*Food Technol.*, 1962, **16,** No. 10, 119—122).—Various lots were collected from commercial processors, 1957—60 inclusive and tested for stability at 40°F before and after storage at selected temp. Cloud stability as received varied from 4 to >340 days at 40°F and this stability was not materially affected by extended storage at 0°F. At temp. >0°F cloud stability was reduced and separate periods of such storage had additive effects. In 1958 season, concentrates produced after a major freeze had cloud stability comparable with that of other concentrates; in 1957 concentrates exhibited flavour stability equal to or greater than cloud stability; in 1960 concentrates, cloud stability greatly exceeded flavour stability. Heat stabilisation of concentrate produced by high-yield juice-recovery methods was more effective with cloud than with flavour. (17 references.) E. M. J.

Effect of processing and storage on stability of concentrated orange juice. E. L. Moore, A. H. Rouse and C. D. Atkins (*Food Technol.*, 1962, **16,** No. 12, 91—95).—In this study Pineapple and Valencia oranges were used to determine at what point in the concentration process of the juice the heat treatment should be applied. If water-sol. pectin is being converted into the insol. pectinates and pectates (determined as NH_4 oxalate-sol.) with accompanying loss of cloud in concentrates prepared from juices heat-treated at intermediate concn., as illustrated by the Pineapple orange concentrates, then the single strength juice should be heat-treated after extraction and finishing. If no loss occurs in water-sol. pectin and cloud, then heat treatment at intermediate concn. should give adequate cloud stability in Valencia orange concentrates. (15 references.) E. M. J.

Residual pulp from the industrial production of citrus juices as a clouding agent for orange drinks. E. Primo Yúfera, J. Koen Mosse and J. Royo Iranzo (*Rev. Agroquím. Technol. Aliment.*, 1962, **2,** 229—234).—Residual pulp, pasteurised at 100° for 15 min., homogenised to a particle size range of from 1·5 to 5μ, and preserved with or without the addition of β-carotene (730 p.p.m.), by autoclaving for 1 min. at 15 lb./sq. in., or by addition of SO_2, benzoic acid or sorbic acid (1000 p.p.m.), showed no enzymic or microbiological deterioration on storage for 6 months at ambient temp. When added to carbonated orange drink (9·5° Brix) at the 0·5 to 1·0% level, the pulp materially increased turbidity and improved suspension stability. Organoleptic tests showed no influence on flavour or acceptability, but pulp prepared with the addition of β-carotene improved the colour of the product. The prepared pulp, added either to the syrup or to the orange concentrate, is an effective clouding agent, which at the same time increases the nutritional value of the product (vitamin C, flavonoids, pectin, and where added β-carotene). E. C. APLING.

Tea, coffee, cocoa

Fractionation of colouring and flavouring substances of roasted coffee by Sephadex G25. H. Streuli (*Chimia*, 1962, **16,** 371—372).—Conc. coffee extracts were separated on Sephadex G25 columns into fractions showing different spectral types, viz., melanoidins, trigonelline/caffeine, and chlorogenic acid. The retention times did not correspond to mol. wt. M. SULZBACHER.

Caffeine sublimation in vacuum-packed coffee. P. Klein (*Food Technol.*, 1962, **16,** No. 12, 96—98).—Since CO_2 has been measured as the largest single gas emitted by roasted and ground coffee (measured at 95% of total volatiles and 0·4% of total coffee wt.), shelf-life studies indicate that entrainer sublimation of caffeine occurs under conditions of reduced pressure in a vac.-packed coffee can. Caffeine is present in the range of 1·0—1·5% in average blends of coffee. E. M. J.

Quantitative paper chromatography exemplified by chlorogenic acid. K. Täufel and J. Voigt (*Z. LebensmittUntersuch.*, 1963, **118,** 481—485).—The spots obtained from parallel runs (on the same chromatogram) of five known amounts (10—50 μg.) of the acid and from the sample are located in u.v. light, and the aq. eluates are measured spectrophotometrically at 324 mμ. Results obtained by reference to a calibration graph based on the five reference runs show a scatter of ±5·9% (with 95% security) as against ±16·9% with reference to a graph based on the average of results from eight separate chromatograms. P. S. ARUP.

Occurrence of polyhydroxyphenols in chocolate and its behaviour during the kneading (conching) process. W. Mohr (*Fette Seif. Anstrichm.*, 1962, **64,** 831—840).—A method is described whereby the polyhydroxyphenols occurring in chocolate, either before or after conching, can be isolated and quant. determined, using a powdered Perlon chromatographic column and eluted with an aq. methanol solution. Similar separations of known catechols and various chocolate extracts are also resolved. Spectrophotometric, and thin-layer chromatographic procedures are also described for determining the % catechols and pro-anthocyanidin L_I in milk-free chocolate. (14 references.) G. R. WHALLEY.

Milk, Dairy Products, Eggs

Increase in sensitivity for the assay of penicillin in milk. H. M. Mei and L. F. L. Clegg (*Nature, Lond.*, 1962, **196,** 691—693).—The sensitivity of tests for antibiotics in milk was increased by a method which involves separation of the whey by rennet coagulation and concn. by rotary film evaporation. By this method 0·003 units/ml. of penicillin in milk can be detected; this min. concn. could be halved by further concn. of the whey S. A. BROOKS.

Influence of organic insecticides on enzymes. I. Problems and experimental technique. F. Kiermeier, R. Kern and G. Wildbrett (*Z. LebensmittUntersuch.*, 1962, **118,** 201—214).—Published data on the subject are tabulated. Peroxidase activity in milk or in extracts of peroxidase from milk is appreciably inhibited by diazinon and moderately inhibited by Sevin in concn. $>10^{-4}$M, but is not affected by schradan or Dipterex in concn. M10^{-2}. The peroxidase of horseradish is appreciably inhibited by Sevin at 10^{-4}M, by diazinon and Schradan at 10^{-2}M, but not by Dipterix. The activity of horseradish peroxidase shows a distinct max. at pH 5·5. (78 references.) P. S. ARUP.

Content of nitrous oxide in [mechanically] whipped cream. S. W. Souci and E. Mergenthaler (*Z. LebensmittUntersuch.*, 1962, **118,** 522—527).—An apparatus for charging cream with a mixture of compressed $N_2O + CO_2$ is described. For the determination, the sample is drawn into an evacuated two-necked flask; sufficient saturated aq. $Al_2(SO_4)_3$ is then drawn in to equalise the pressure while the mixture is being (magnetically) stirred. The $N_2O + CO_2$ are determined by absorption in $CHCl_3$ which has been saturated with air, and (in a second sample), the CO_2 is determined by absorption in aq. KOH which has been saturated with N_2O. The mechanically prepared product is comparatively more voluminous than hand-whipped cream but it is structurally less stable. (12 references.) P. S. ARUP.

Model leavening system illustrates some functional properties of calcium and protein in non-fat dry milk used in food products. R. M. Lauck and J. W. Tucker (*Cereal Sci. Today*, 1962, **7,** 314, 316, 322).—The part played by non-fat dry milk in the production of cold milk gels, the control of reactivity of Na acid pyrophosphate, and the binding of meat in sausages, is discussed with particular reference to the action of the Ca^{2+} in the milk. A method for measuring the reactivity of Na acid pyrophosphate, based on foam expansion time, is described. S. G. AYERST.

Gas-chromatographic study of fatty acids of butter made in Italy and in other countries. Application to detection of adulterations in commercial butter. L. Boniforti (*Ann. Falsif., Paris*, 1962, **55,** 255—263).—Previously described techniques (cf. *Anal. Abstr.*, 1961, **8,** 2148) applied to 15 samples of butter gave values for the ratio of acids C_{12}/C_{10} >1—1·2; this ratio can be determined from the chromatogram of the Et esters of the total acids, even if the absolute amounts are not known. For the detection of isovaleric acid (due to adulteration with hydrogenated dolphin oil) use must be made of the chromatogram of the Et esters of the acids below C_{12}. Adulteration with 10% of a 'synthetic butter' which has often escaped detection by the usual methods, could be detected by a C_{12}/C_{10} ratio of 1·4; this adulterant contained abnormally high amounts of lauric and butyric esters, a low content of caproic ester, and showed a lack of several normal minor constituents of butter. P. S. ARUP.

Gas–liquid chromatographic analysis of the triglyceride composition of molecular distillates of butter oil. M. J. McCarthy, A. Kuksis and J. M. R. Beveridge (*Canad. J. Biochem. Physiol.*, 1962, **40,** 1693—1703).—Gas–liquid chromatographic analysis of the triglycerides of butter oil and its distillates showed that mol. distillation could effect fractionation of butterfat. The distillates differed greatly from each other and from the original oil in their glyceride composition. Tables given include distillation conditions and fatty acid and triglyceride composition of the butter oil and its distillates, together with gas–liquid chromatographic elution patterns for the various fractions. (13 references.) A. S. CARMICHAEL.

Determination of fatty matter in cheeses. Critical study of method used in the Municipal Laboratory [of Paris]. Comparison with the international method of Schmid, Bondzynski and Ratzlaff. L. Martelli and I. Saenz-Lascano-Ruiz (with J. Helie and J. Monvoisin) (*Ann. Falsif., Paris*, 1962, **55,** 245—254).—In the Municipal (MP) method the fat is separated by filtration from the mixture obtained by heating the sample with HCl; after washing with hot water and then drying the filter and the fat at 20—28°, the fat is extracted (continuously) by Et_2O and weighed after evaporation of the solvent and drying. In the analysis of cheeses not containing large amounts of free fatty acids the losses of these acids by dissolution in the HCl and washing water are shown to be ⩽0·1—0·2% of the total fatty matter; it is, however, pointed out that similar losses must occur in the procedure of Schmid *et al.* (SBR method) during the oven-drying of the fat; the results given by the two methods agree within the limits of experimental error. The following modification to the MP method is recommended for the analysis of matured cheeses containing large amounts (~1—1·7%) of free fatty acids: the acids are extracted with Et_2O from the acid filtrate and washings, and this extract is added to the Et_2O solution of the fat; the mixed solution is now titrated to neutrality with 0·1N-KOH, after which the fat and K salts obtained after evaporation of the solvent are oven-dried and weighed together; a factor is given to compensate for the conversion of the acids into K salts. This modification to include the acids cannot be applied to the SBR method. P. S. ARUP.

Ultra-high-temperature processing of dairy products. II. Skim milk and the resulting cottage cheese. T. I. Hedrick and E. Kondrup (*Mich. agric. Exp. Sta., quart. Bull.*, 1962, **44,** 731—735).—Pasteurisation of skim-milk at 162—165°F for 20 sec. with additional 3 sec. at a temp. between 186° and 220°F caused more rapid acid production during setting, as compared with that at 162°F for 23 sec. Cottage cheeses from skim milk pasteurised at 162°, 190° and 220°F averaged, respectively, 1·79, 1·91 and 1·92 lb. per lb. of skim milk solids. No differences in flavour, body or texture of the fresh, creamed cheeses were apparent, but after 14 days the keeping quality of the high temp.-pasteurised samples was equal or superior to that of the control (pasteurised at 162°F). A. G. POLLARD.

Cheese-making properties of milk produced from herd fed dried beet leaves and tops (Troblako). O. Bolliger and E. Siegenthaler (*Schweiz. landw. Forsch.*, 1962, **1,** 92—104).—The cheese-making qualities of milk produced from herds fed Troblako and from cows on a control feed were compared. No significant differences in cheese-making suitability were found and the cheese produced showed no differences when evaluated after storage for 6½ months. No butyric acid bacteria were found in either milk. J. V. RUSSO.

Effect of sodium chloride on the quality of Nalžov type cheese. J. Doležálek and D. Jandová (*Prům. potravin*, 1963, **14,** 10—11).—Salting is one of the principal factors determining the result of curing and consequently of the quality of Nalžov cheese. In view of the specific bacterial flora higher amounts of salt employed with other sorts of mould-coated cheese cannot be applied with Nalžov cheese. It is recommended to salt this cheese with boiled brine and to maintain the salt content in the whole cheese at 1·5%. In this way favourable conditions are created for vigorous growth of the mould *Penicillium nalgiovensis*, strain Dk. A fine white to light pink coat forms soon on the cheeses, and the consistency of cheese thus obtained permits spreading; the product is of excellent taste, having its specific mushroom flavour. J. S. B.

Factors affecting alkaline coagulation of egg white. F. E. Cunningham and O. J. Cotterill (*Poultry Sci.*, 1962, **41,** 1453—1461).—A study is reported of factors affecting the gelation of egg white in the alkaline pH range and of the utilisation of derivatives of the resultant product as an additive to normal egg white. A. H. CORNFIELD.

Haugh unit as a measure of egg albumin quality. E. J. Eisen, B. B. Bohren and H. E. McKean (*Poultry Sci.*, 1962, **41,** 1461—1468).—Detailed studies of egg wt. and albumin height for large samples of eggs from two genetically related strains of pullets at 40 weeks were made. Statistical analysis of the results raised a question as to the validity and desirability of using the Haugh unit score as a measure of albumin quality. A. H. CORNFIELD.

Rapid method for evaluating the strength of the vitelline membrane of the hen's egg yolk. D. Fromm and G. Matrone (*Poultry Sci.*, 1962, **41,** 1516—1521).—The method involves placing a 2-mm. capillary tube against the membrane, applying a vac. and measuring the time required for the membrane to rupture. A. H. CORNFIELD.

Penetration of the egg shell membranes by *Pseudomonas fluorescens*. T. E. Hartung (*Dissert. Abstr.*, 1962, **23,** 399—400).—Factors were studied which might influence the bacterial penetration of the shell membranes and the capacity of shell membranes to permit pH equilibium *in vitro*. An *in vitro* system was developed which per-

mitted the mounting of selected shell membranes between two cells. The positive side represented the outside of the shell membranes, and the negative side the inside of the egg. Aseptic assembly permitted controlled inoculation of the positive side with known quantities of the primary egg spoilage organism. Susceptibility of the shell membranes to bacterial penetration was influenced by changes in the membranes and the micro-environment in contact with the membranes. F. C. SUTTON.

Influence of metallic cations on the penetration of the egg shell membranes of *Pseudomonas fluorescens*. T. E. Hartung and W. J. Stadelman (*Poultry Sci.*, 1962, **41**, 1590—1596).—*In vitro* studies showed that the shell membrane was penetrated more easily by *Pseudomonas fluorescens* when the contact liquid contained Fe (10—20) or Mg (10 p.p.m.). 1% $ZnSO_4,7H_2O$ completely destroyed *P. fluorescens*. Penetration of the shell membrane + egg white was increased by Fe (10—20) or Na-EDTA (500 p.p.m.); with the combined treatments increased penetration even further. The growth rate of *P. fluorescens* in culture was increased by Fe or Mg 10 or Na-EDTA (500 p.p.m.). A. H. CORNFIELD.

Factors related to egg spoilage. A. W. Brant and P. B. Starr (*Poultry Sci.*, 1962, **41**, 1468—1473).—The effects of variation in temp. of the immersion water, time of immersion, initial egg temp. and extent of inoculation of the immersion water with *Pseudomonas polycolor* on subsequent infection of eggs were studied. Results are discussed in relation to egg washing techniques. The washing water temp. should be considerably higher than the egg temp. and initial egg temp. should be as low as possible. Concn. of spoilage organisms should be as low as possible (this is related to efficiency of sanitation). Immersion time (1—5 min.) had little effect. A. H. CORNFIELD.

Composition of egg lipid. O. S. Privett, M. L. Blank and J. A. Schmit (*J. Fd Sci.*, 1962, **27**, 463—468).—The fractionation and analysis of egg lipid by methods employing thin-layer chromatography are described. The fatty acid composition of the dietary fat and the crude egg lipid was determined by both alkali isomerisation and gas–liquid chromatography. The positional arrangements of the fatty acids in the glycerides and lecithin fraction were determined. The fatty acids in lecithin were distributed mainly in one type, the α-saturated β-unsaturated type, in contrast with the fatty acids of triglycerides. (25 references.) E. M. J.

Cheese manufacture. National Research Development Corp. (Inventor: N. J. Berridge) (B.P. 884,762, 11.7. and 8.9.58).—A cheese-manufacturing process, in which the curd is obtained in its final form without cutting and laborious techniques in subsequent stages are obviated, comprises keeping a curd-forming mixture of milk, rennet and acidifying material (if desired) under such conditions (e.g., at >15° during 10—240 min.) that enzymic action of the rennet is nearing completion (or is complete) but coagulation has not commenced, then quickly heating the mixture (to, e.g., 50—70°) in a form in which its surface area is large compared with its volume, so that rapid coagulation followed by rapid exudation of whey can occur. F. R. BASFORD.

Edible Oils and Fats

Inter-esterification of fats. V. Detection of methanol in fats by inter-esterification with sodium methylate. H. P. Kaufmann and B. Grothues (*Fette Seif. Anstrichm.*, 1962, **64**, 805—807).—The determination of the Me esters of fatty acids in the presence of fats is impossible using the method saponification, steam distillation followed by oxidation and the detection of formaldehyde with chromotropic acid. A direct paper chromatographic method is employed, using paper impregnated with undecane and a mobile phase of 87 : 13 acetone/water mixture. For the detection of small quantities of Me esters in fats, a preliminary distillation at 140° is carried out prior to chromatographic separation. G. R. WHALLEY.

α-Glyceryl ethers of cod-liver oil. A. Emmerie and C. Engel (*Fette Seif. Anstrichm.*, 1962, **64**, 813—816).—The unsaponifiable matter in cod-liver oil is separated from the saponifiable matter, by dissolving the liberated fatty acids in light petroleum and filtering through a silica gel column. The unsaponifiable fraction is eluted from the column with ethanol. Microbiological methods are used to purify and separate the constituents, and it is shown that the biological activity is confined to the unsaturated α-glyceryl ethers, some of which are unknown. Some i.r. and u.v. spectra are given. G. R. WHALLEY.

Molecular weight determinations of fats and fat polymers by ultracentrifuge. II. Molecular weight determination of fats from the approximation of the sedimentation equilibrium. H. Lück and E. Rickerl (*Fette Seif. Anstrichm.*, 1962, **64**, 825—831).—The sedimentation equilibrium is determined with a Spinco ultracentrifuge and used to calculate the mol. wt. of groundnut oil with I.V. of 88. The determinations are carried out with 0·004 to 0·02 g. of oil per ml. of solvent, and the results calculated according to a modified Archibald method. The results, using polar and non-polar solvents, show significant differences; and only at oil concn. of 5 and 10% are abnormal results obtained, which may be due to association phenomena. G. R. WHALLEY.

Isolation of methyl ketones from fats and differentiation of the 2,4-dinitrophenylhydrazones by paper chromatography. K. Taüfel, K. Schmidt and Cl. Franzke (*Fette Seif. Anstrichm.*, 1962, **64**, 957—961).—The occurrence, detection and significance of methyl ketones in fat products are discussed. It is shown that 2,4-dinitrophenylhydrazones of ketones with odd numbers of C-atoms can be separated very effectively on paper chromatograms in about 10 h. with an heptane/ethanolic H_3PO_4 eluant. On the basis of the model systems, heat-treated butter fat is examined. All odd-numbered ketones from C_3 to C_{15} are present and additional spots indicate the presence of even-numbered and branched-chain ketones. (14 references.) J. B. WOOF.

Esterified oils. G. Bigoni (*Riv. ital. Sostanze grasse*, 1962, **39**, 428—431).—The *trans*-isomer content of olive oils has been determined by bromination of the oil (in $CHCl_3$), conversion to Me esters and gas chromatographic analysis (on a succinate polyester column). Peaks due to the bromo derivatives of the *cis* (oleic) and *trans* (elaidic) acids are clearly separated. The *trans*-contents of a range of olive, sansa, rectified and esterified oils have been compared. All but the virgin olive oils had a significant content of *trans*-acids (1–4%). It was no greater for esterified than the other oils and in the sansa oils increased with the acidity. L. A. O'NEILL.

Gas chromatographic examination of the minor fatty acid components of olive oil. D. Grieco (*Riv. ital. Sostanze grasse*, 1962, **39**, 432—438).—The fatty acid composition of a range of sansa oils, olive oils and related products has been examined. In addition to the main acids, linolenic, arachidic and eicosenoic acids are always present, while traces of lauric, myristic, behenic or lignoceric may be found. Two C_{17} acids, one saturated and the other unsaturated, are usually found in minor or trace amounts. One acid of high equivalent carbon number has not yet been identified. (22 references.) L. A. O'NEILL.

Variation of lipid composition during ripening of Tunisian olives. O. T. Rotini, S. Baragli and M. Gentili (*Chim. e Industr.*, 1962, **44**, 1126—1129).—Composition and acidic characteristics of oils from Tunisian olives are studied, during different periods of ripening, in four regions of different climate. During ripening, I val., thermosulphuric acid value and refractive index increase, whilst quality and Bellier's index decrease differently in relation to latitude. The amounts of palmitic and stearic acids decrease during ripening, whilst the amounts of palmitoleic, oleic and linoleic acid increase. Variations of acid composition are wider in oils from the northern region, and are less evident in those from central and southern regions. Acid contents are measured by gas chromatography. C. A. FINCH.

Ultra-violet spectra of antioxidants. II. New direct ultra-violet-spectrophotometric method for determing antioxidants in fats. B. A. J. Sedláček (*Fette Seif. Anstrichm.*, 1962, **64**, 962—967).—Effects of concentration, solvent and sample size on a direct spectrophotometric method for determining antioxidants have been evaluated. With $CHCl_3$ as fat solvent and the technique described, reliable results may be obtained. These are evaluated statistically and compared with colorimetric and compleximetric methods. (12 references.) J. B. WOOF.

Application of acetous perchloric acid to cosmetic analysis. J. S. Hopwood (*Perfum. Essent. Oil Rec.*, 1962, **53**, 692—694).—The technique is described and examples are given. Salts of fatty acids (Zn, Mg, Na, etc. stearates or oleates) can be titrated as well as quaternary ammonium halides (**I**) (cetrimide or benzalkonium chloride). Creams containing **I** as emulsifying agents can also be assayed using a suitable technique and triethanolamine, tri-iso-propanolamine, KOH and NaOH occurring in creams in combination with fatty acids can be readily determined. C. V.

Stabilisation of unsaturated fatty acids by conversion to carbamide complexes. J. Žalud, H. Daníčková and J. Pokorný (*Prům. potravin*, 1962, **13**, 660—661).—Fatty acids and esters thereof containing monovalent alcohols can be protected against oxidation by atm. O_2 by converting them into carbamide addition compounds. The method is effective only for oleic and linoleic acids, whereas linolenic acid undergoes oxidation even in complex compounds. Esters are protected less effectively than free acids. Carbamide addition compounds must be stored at low temp., since at 40—60° their

oxidation is very pronounced. Air moisture has no adverse effect, unless linolenic acid is present. J. S. B.

Meat and Poultry

Nutritional properties of unrefrigerated animal products. D. H. Calloway (*Food Technol.*, 1962, **16,** No. 10, 102, 104—106).—The discussion deals with the preservation and storage stability of nutrients in the flesh of animals, poultry and fish. E. M. J.

Two mechanical devices compared with taste panel evaluation for measuring tenderness. L. M. Burrill, D. Deethardt and R. L. Saffle (*Food Technol.*, 1962, **16,** No. 10, 145—146).—Various comparisons were made among Kramer shear, Warner-Bratzler shear, panel scores and panel evaluation by counting the no. of chews. Highly significant correlation coeff. were found in every case for all the various combinations used to test for tenderness when all muscles were combined. E.g., r = 0·65 (panel chews × Kramer max. force); r = −0·91 (panel scores × panel chews). E. M. J.

Tenderness and chemical composition of beef. I. Variation among animals treated alike. II. Variations due to animal treatment and to extent of heating. P. C. Paul (*Food Technol.*, 1962, **16,** No. 11, 115—117, 117—119).—I. Variability in chemical composition, cooking losses and tenderness of roasts from four groups (each of eight animals) given four different treatments was determined. Data were obtained on the *semitendinosus* muscles for raw meat, roasts cooked to rare (60°) and well-done (77°). There were significant differences among animals assumed to be similar, but no appreciable variation between matched cuts from the right and left sides of the same animal. More significant differences were found in raw than in cooked meat. Total moisture and total N were the most stable figures. The cross-sectional areas of raw muscle fibres varied significantly among animals within each group and among sections from different parts of the same muscle within each animal.

II. The effect of stilboestrol in the feed increased wt. of the *semitendinosus*, area of the muscle fibres, N content of the lean and losses during cooking, but did not significantly change the other chemical measures or meat tenderness. E. M. J.

Tenderness of beef. I. Connective-tissue component of tenderness. II. Juiciness and the softness components of tenderness. III. Muscle-fibre components of tenderness. S. Cover, S. J. Ritchey and R. L. Hostetter (*J. Fd Sci.*, 1962, **27,** 469—475, 476—482, 483—488).—I. One-in. steaks from *longissimus dorsi* (**I**) and *biceps femoris* (**II**) from 180 young cattle in nine lots were cooked to 61° (rare) and to 80° (well done) by dry heat and to 100° by moist heat. Connective tissue in **I** was scored tender at 61° and became only slightly more tender at 80 and 100°; in **II** it was scored tough at 61° and became more tender with increase in temp. Losses of collagen in **I** and **II** increased with increasing meat temp.

II. Changes in **I** and **II** occurring between 61 and 80° and 80 to 100° regarding juiciness and two kinds of softness, to tongue and cheek, and to tooth pressure are reported. These changes were studied in relation to cooking times and wt. losses during cooking; to prevent loss of water from raw meat; and to the size and possible hydration of muscle fibres. Juiciness was not closely associated with any of the six components of tenderness. The closest correlation was softness to tongue and cheek which accounted for ~35% of the variation in **II** at 80°. (10 references.)

III. With increase in temp. (61, 80 and 100°), scores for ease of fragmentation and lack of adhesion between muscle fibres trended towards greater toughness in **I** and towards greater tenderness in **II**. **II** was more easily fragmented than was **I**, had less adhesion between muscle fibres at 80 and 100° and was more mealy. Changes in tenderness are considered in relation to chemical and histological changes during ageing. E. M. J.

Effect of heat on the water-soluble proteins of beef skeletal muscle. C. K. Davies, jun. (*Dissert. Abstr.*, 1962, **23,** 418).—The thermal denaturation of myoglobin and other water-sol. protein fractions of bovine skeletal muscle was studied to examine the mechanism of protein denaturation during heating. Myoglobin denaturation seemed to follow two separate reactions at temp. of 65° or higher. A rapid loss occurred during the first 15 min. of heating, followed by slow loss of myoglobin for times up to 60 min. Loss of colour was due to the denaturation of the myoglobin alone or with co-pptn. with the other proteins. F. C. Sutton.

Nutrient content of variety meats. I. Vitamin A, vitamin C, iron and proximate composition. L. Kizlaitis, M. I. Steinfeld and A. J. Siedler (*J. Fd Sci.*, 1962, **27,** 459—462).—Vitamin A, vitamin C and Fe contents of raw, variety meats from beef, calf, lamb and pork were determined. Proximate composition was also obtained on all the samples analysed. Vitamin A content of liver varied considerably from values reported in the literature. They were lower, probably reflecting differences in feeding practices. Beef, calf and pork liver varied considerably in vitamin A content between individual livers from the same type of meat animal. For lamb liver values were high. Vitamin C content was greatest in calf and beef thymus. (10 references.) E. M. J.

Nitrate reductase of different micro-organisms participating in ripening of raw sausage meat. J. Schormüller and M. Schilling (*Z. LebensmittUntersuch.*, 1962, **118,** 492—508).—In continuation of previous work (cf. *Nahrung*, 1961, **5,** 18) freeze-dried prep. were made of 18 micro-organisms; by far the most active in reducing NO_3^- to NO_2^- were nine micrococci (especially *M. epidermis* var. *albus*) and *Sarcina flava*. The activity of the prep. was promoted by adding traces of Mo to the culture solution and by anaerobic cultivation. The activity of test solutions was greatly increased by the addition of traces of Mo, Na lactate (in concn. 10^{-2}M) and by providing anaerobic conditions. A stable prep. of *M. epidermis* var. *albus* showed max. activity at 45° and pH 7 and the general characteristics of other nitrate reductases. The activity of the prep. was concentrated in the insol. matter. Na lactate was much more active than Na citrate, tartrate or acetate in promoting enzymic activity in sausage meat. (42 references.) P. S. Arup.

Rapid butyrometric method of fat determination in meat products. J. Daněk and J. Barvíř (*Prům. potravin*, 1963, **14,** 30—32).—A new rapid method for determining fat in meat products with a cream butyrometer is described. Samples (5 g.) with H_2SO_4 *d* 1·818 (10 ml.), water (10 ml.) and amyl alcohol (1 ml.), are centrifuged in a Gerber apparatus for 5 min. The amount of fat thus determined is multiplied by the reduction factor 0·97 to bring the results close to those obtained by extraction. J. S. B.

Comparison of natural and synthetic coating materials used in the meat industry. B. Doležal and I. Vognarová (*Prům. potravin*, 1962, **13,** 628—630).—Natural and synthetic casings for meat products are discussed and compared in regard to their operational and economical significance; experimental results are given in tables. Materials considered are: paper, collagen, viscose, amylose acetate, alginates, polyamide (Rilsan) and PVC. J. S. B.

Heat treatment of meat and meat products by electric current. J. Štálík, K. Plichtová and R. Sládek (*Prům. potravin*, 1962, **13,** 630—633).—Methods for the heat treatment of meat and meat products by electric current, comprising i.r., dielectric and resistance heating, are discussed and compared. Resistance heating in general is more economical, allowing reduction of the standard heating time of 3—4 h. to 1 h., presenting min. heat losses, securing uniform heating of homogeneous products, and offering facilities for mechanisation and automatisation. J. S. B.

New possibilities of determining the nutritional value of meat and meat products. S. Klein, J. Hrdlička and J. Hlaváček (*Prům. potravin*, 1962, **13,** 599—601).—By determination of tryptophan and hydroxyproline in raw meat material the index Try/Hypro can be established in finished meat products, and this in connexion with other indices reflects the relative distribution of plasma and tissue proteins. The determination of the index offers a method of observing the technological standards in grading and mixing the meat components and the nutritive value of the finished products. (11 references.) J. S. B.

Cooking losses, acceptability and edible yield of U.S.-Graded turkey hens. G. E. Goertz, B. Weathers and J. L. Fry (*Food Technol.*, 1962, **16,** No. 10, 128—130).—Grade A turkeys had higher ether extracts for light meat and more edible cooked meat, smaller total cooking losses and shorter cooking times than had Brade B. Flavour, juiciness, tenderness and general acceptability scores were similar. When turkeys were evaluated for general appearance, before and after roasting, scores were higher for Grade A, intermediate for B and lowest for C. Differences attributable to Grade were greater before than after roasting. Edible yield of cooked turkey was 34·4—39·1% of the ready-to-cook wt.; approx. 60% was light meat and 40% dark meat. E. M. J.

Compositions for tenderising meat. Miles Laboratories Inc. (B.P. 884,277, 23.1.59. U.S., 27.1.58).—A meat tenderising composition comprises an enzyme system having collagenase and elastase activity; an enzyme system having destructive activity on the muscle fibre; and a diluent (viz., lactose, glucose, maltose or sucrose, to stabilise the enzyme systems against loss of activity due to high temp.). F. R. Basford.

Fish

Significance of the variations in the nucleotide, free amino-acid and carbohydrate content of fish muscle in quality assessment. F.

Bramstedt (*Fette Seif. Anstrichm.*, 1962, **64,** 820—825).—Variation in the quality of fish muscle, derived from cod, sea salmon and shell fish, is related to its amino-acid (leucine, lysine and isolysine), glycogen and nucleotide content. The seasonal variations are also tabulated. G. R. WHALLEY.

Direct vertical starch gel electrophoresis of cod muscle tissue. P. H. Odense and C. W. Shinners (*Canad. J. Biochem. Physiol.*, 1962, **40,** 1842—1843).—A clear solution of cod muscle albumins was prepared at 2° and used immediately for an electrophoresis run, together with small pieces of cod muscle fitted into starch gel sample slots. Results showed 10 protein bands in the electrophoresis pattern of the tissue but only nine in that of the extract. After the run the tissue samples were sectioned, showing water-insol. structural proteins still visible. Direct electrophoresis thus eliminates prep. of extracts with protein denaturation, and may also be used to elute tissue components. A. S. CARMICHAEL.

Isolation of and direct analysis of uncontaminated muscle cell contents. R. M. Love (*Nature, Lond.*, 1962, **196,** 593—594).—A method is described for the clean removal of the contents of single cod muscle cells. The amount of Na and K in the cell contents has been determined. S. A. BROOKS.

Spices, Flavours, etc.

Use of gas chromatography to identify geographical origin of some spices. P. R. Datta, H. Susi, H. C. Higman and V. J. Filipic (*Food Technol.*, 1962, **16,** No. 10, 116—119).—Suitable conditions for gas chromatographic separation of the components of cassia, black pepper, nutmeg and ginger oils are described. Comparison of the peak heights of some of the major components of the spice oils showed marked and consistent differences between spices from different geographical sources. The characteristic components were identified by i.r. micro-spectroscopy. (10 references.) E. M. J.

Application of physical methods to the analysis of essential oils. III. Polarography. S. S. Nigam and G. L. Kumari (*Perfum. essent. Oil Rec.*, 1962, **53,** 752—756).—Examination of the polarograms of the higher (hexanal to dodecanal) saturated aliphatic aldehydes shows that their reduction at the dropping Hg-electrode takes place in steps; this probably involves the formation of alcohol through an intermediate. The no. of steps increases as the acidity increases. The half-wave potentials of the reduction steps of the 2,4-dinitrophenylhydrazones of these aldehydes are almost similar and thus cannot be used for this polarographic estimation. The behaviour of 3,5-dinitrobenzoates of a few alcohols and phenols was studied in various buffers; generally reduction occurs in two stages; the first involves one electron and the second is irreversible. The polarograms of heliotropin, cyclamen aldehyde, hydroxy citronellal, cineol (1,8) and citral were studied to identify the presence of these in essential oils and cineol and citral have been quant. estimated in *Saccopetalum tormentosum* and *Cythocline lyrata*, etc., by this method. (11 references.) C. V.

Semimicro and micro steam distillation. Estimation of the essential oil content of small plant samples. W. J. Franklin and H. Keyzer (*Analyt. Chem.*, 1962, **34,** 1650—1653).—Two micro- and one semimicro stills are described and illustrated. The results obtained are of an accuracy comparable with those of conventional micromethods, recoveries of added oils being >90%. In some cases a single, e.g., *Eucalyptus*, leaf provides the necessary sample. The microstills can be used for oil quantities of 2—50 μl. G. P. COOK.

Terpenoids. XXVIII. Gas–liquid chromatography of monoterpenes and its application to essential oils. T. C. Jain, K. R. Varma and S. C. Bhattacharyya (*Perfum. Essent. Oil Rec.*, 1962, **53,** 678—684).—The corrected retention vol. data for several monoterpenes and allied compounds (22) on silicone and Carbowax columns were successfully used for the identification of monoterpenes of nine essential oils. (15 references.) C. V.

Monoterpene hydrocarbon composition of some essential oils. R. M. Ikeda, W. L. Stanley, S. H. Vannier and E. M. Spitler (*J. Fd Sci.*, 1962, **27,** 455—458).—The monoterpene hydrocarbon content of 29 non-citrus essential oils was determined with silicic acid chromatostrips and gas–liquid chromatography. Data are presented showing the total monoterpene hydrocarbon content and the relative proportions of 19 of these compounds in each oil. E. M. J.

Detection of adulterants in vanilla extracts. P. Horst and J. H. McGlumphy (*Ann. Falsif., Paris*, 1962, **55,** 264—273).—A review giving descriptions of recent analytical methods (chiefly paper chromatographic) with brief comments. (22 references.) P. S. ARUP.

(Mandarin) orange peel oil. II. Physico-chemical characteristics of Sikkim (mandarin) orange oil. S. K. Mukherjee and A. N. Bose (*J. Instn Chem. India*, 1962, **34,** 233—238).—The cold-pressed oil from Sikkim mandarins contained a larger proportion of flavouring components than did the distilled oil. The oil resembles Florida tangerine oil in colour and in physico-chemical properties. S. A. BROOKS.

Flavours. IV. Isolation of volatile constituents from strawberry and raspberry. V. Analysis of raspberry flavour. i. Volatile carbonyl constituents. M. Winter, E. Palling, M. Hinden and B. Willhalm. **VI. ii, Volatile alcohols.** E. Sundt and M. Winter (*Helv. chim. Acta*, 1962, **45,** 2186—2195, 2195—2211, 2212—2218).—IV. A process for the removal of volatile flavour constituents directly from the fruits by distillation and further concentration by extraction of the distillate is described and illustrated. The efficiency of the procedure is evaluated by processing model solutions of known concn. of volatile substances and by organoleptic panel tests. Concentrates of the volatile part of fresh strawberry and raspberry flavour have been prepared.

V. The most characteristic constituents of raspberry oil, prepared by distillation and extraction, are carbonyl compounds and alcohols. The functional analysis of the carbonyls by pptn. of the 2,4-dinitrophenylhydrazones indicated the presence of 14 carbonyl constituents: diacetyl, acetoin, acetaldehyde, acrolein, acetone, propanal, ββ-dimethylacrolein, 2-pentenal, *cis*-3-hexenal, 2-hexenal, 2-pentanone, hexanal, (+)-α-ionone and β-ionone. The hydrazones were separated by paper chromatography.

VI. The 3,5-dinitrobenzoates obtained from the neutral part of the vacuum distillates of 610 kg. of fresh raspberries have been separated by column and paper chromatography. Eleven alcohols were identified: four previously known to be present, viz. *cis*-3-hexen-1-ol, 1-hexanol, ethanol and methanol; and seven newly found: 3-methyl-3-buten-1-ol, 1-penten-3-ol, 1-pentanol, *trans*-2-buten-1-ol, 1-butanol, 3-methyl-3-buten-1-ol and geraniol. M. SULZBACHER.

Taste interrelationships. III. Suprathreshold solutions of sucrose and sodium chloride. R. M. Pangborn (*J. Fd Sci.*, 1962, **27,** 495—500).—As determined by highly trained subjects using single and paired-sample presentations, the apparent saltiness of 0·12—3·24% NaCl was reduced by sucrose. In general the sweetness of 0·75, 2·25 and 6·75% sucrose was enhanced by the lower and depressed by higher salt additions. All levels of salt depressed the sweetness of 20·25% sucrose. Reproducibility of judgement was much greater in the paired than in the single-stimulus method. (18 references.) E. M. J.

Direct gas chromatographic analysis as an objective method of flavour measurement. W. W. Nawar and I. S. Fagerson (*Food Technol.*, 1962, **16,** No. 11, 107—109).—Analyses by gas chromatography of volatiles from Roquefort cheese by direct and enrichment methods are shown. The chromatogram obtained by enrichment techniques provides considerably more qual. information. When a food product contains a relatively large concn. of lower-boiling compounds, direct transfer of the headspace sample to the instrument gives a fairly informative gas chromatographic pattern, but does not provide adequately for analysis of higher-boiling components especially when present in trace concn. Some means of concn. or enrichment is necessary. Evidence presented also shows that in some cases compounds individually present at sub-threshold concn. show a synergistic effect contributing to aroma. E. M. J.

Colouring matters

β-Apo-8′-carotenal—a new food colour. J. C. Bauernfeind and R. H. Bunnell (*Food Technol.*, 1962, **16,** No. 12, 76—82).—β-Apo-8′-carotenal, a synthesised aldehydic carotenoid with vitamin A value, and occurring widely in nature was prepared in oil solutions, oil suspensions, emulsions and beadlets. Used to colour such foods as cheese, carbonated beverages, cakes, etc., the colour was stable in the prepared forms and in cheese, etc. The purplish-black crystals give orange to red solutions in vegetable oil and aq. dispersions are orange to orange-red. (13 references.) E. M. J.

Carotenoids of fruits. G. Mackinney (*Fruits, Paris*, 1962, **17,** 341—350).—The colouring matter of plants consists of flavonoids, chlorophylls and carotenoids. Flavonoids are soluble in water and the others in non-polar solvents. The chlorophylls are then removed by saponification. Carotenoids are separated into their various classes (hydrocarbons, monols, diols, ketocarotenoids and epoxyols) by counter-current extraction and then the individual components of each class are separated by adsorption column chromatography. All carotenoids have a central core based on isoprene and some have open and some cyclic end groups. Colour depends on the no. of conjugated double bonds and on *cis–trans* isomerism of the side groups about these double bonds. Colours vary from pale yellow to red. Biologically the function of caro-

tenoids depends on their ability to form complexes with proteins. (21 references.) J. V. Russo.

Preservatives

Influence of chemical compounds on antiseptic action of preservatives. I. Influence of different groups of substances on preservatives against *Aspergillus niger*. H.-J. Rehm (*Z. LebensmittUntersuch.*, 1962, **118**, 508—522).—Graduated amounts (generally 0·01—0·25%) of the substances are added to the nutrient solution and the amounts of sorbic acid, BzOH, Na formate or Et *p*-hydroxybenzoate (**I**) required to inhibit completely growth of the mould at 30° and pH 5 for 7 days are determined and stated as % of the threshold concn. found without any added substance. The effects of four sugars, KCl, NaCl and $MgCl_2$ (at 0·25%) are negligible, but $CaCl_2$ (at 0·1%) is antagonistic to all four preservatives. Synergistic effects are shown by isobutyric and gluconic acids and antagonistic effects (towards sorbic acid and BzOH, only) by malonic and malic acids. Riboflavin phosphate and pyridoxine hydrochloride (at 0·1%) are antagonistic towards sorbic acid. Out of 13 amino-acids, cysteine hydrochloride (at 0·1%) is synergistic to the preservatives (not including **I**), and aspartic acid is synergistic towards Na formate. Edible fats and oleic acid (at 5—15%) are very strongly antagonistic towards **I** and, in some cases, moderately so towards sorbic acid and BzOH. (54 references.) P. S. Arup.

Rapid spectrophotometric determination of preservatives in foods. W. Lorenzen and R. Sieh (*Z. LebensmittUntersuch.*, 1962, **118**, 223—233).—An analytical scheme is given based on the steam-distillation of the (homogenised) sample (0·5—1 g. acidified with a tartrate buffer at pH 3) (cf. *Anal. Abstr.*, 1961, **8**, 2216), and the spectrophotometric measurement (in the range 215—320 mμ) of the volatile (or partly volatile) products in the distillate (acidified with H_2SO_4 to 0·01N). A table of max. and min. values for *E* at the appropriate λ is given for the determination of benzoic acid (**I**), sorbic acid (**II**), *p*-hydroxy-benzoic acid (**III**), salicylic acid (**IV**), *p*-chlorobenzoic acid (**V**) and the Me and Et esters of **III**. **I, II** and **V** can be determined directly in the distillate. **III** and **IV** and the esters are determined partly in the distillate and partly in the distillation residue from which they are extracted with Et_2O; details are given. A procedure can also be applied to the distillate for the removal of suspected volatile interfering substances, e.g., hydroxymethylfurfural which has two absorption max. in the range 200—300 mμ. A method is given for the separate determination of **I** and **II** or **II** and **V** (the absorption spectra of which nearly coincide) which is based on the selective destruction of **II** by acid $KMnO_4$. Hexamethylenetetramine is hydrolysed during the steam-distillation to and determined as HCHO. By this scheme it is possible to detect as little as 20 mg./kg. of **II** or 40 mg./kg. of the other preservatives. (16 references.) P. S. Arup.

Antibiotics in food canning. A Bardsley (*Food Technol. Aust.*, 1962, **14**, 532—533, 535, 537, 572, 606—607, 609—611).—The use of subtilin is reviewed; it seems to have possibilities for the prevention of certain types of spoilage in peas and tomato juice after mild heat treatment. Nisin is also discussed. Of the large no. of antibiotics studied, only subtilin and nisin gave a reduction in D-value (the time required, under particular conditions of temp., medium and many spores, to kill 90% of the spores) of >45%. Approval of the use of nisin in foods in Australia, England and in Europe is discussed. (26 references.) E. M. J.

Food Processing, Refrigeration

Gas storage of bananas. K. Sarveswara Rao, N. S. Kapur, H. Subramanyam, S. D'Souza and H. C. Srivastava (*J. sci. industr. Res.*, 1962, **21D**, 331—335).—Physical and chemical changes occurring in waxed and unwaxed bananas, stored under varying conditions of temp. and CO_2 concn., are reported. Gas storage prolonged the storage life by 150% at room temp. (78—92°F) and by 60% at 52—55°F, whereas waxing the fruit only gave increases of 50 and 33% respectively. Increases in reducing and non-reducing sugars, reduction in vitamin C loss and physiological loss in wt. and increased moisture retention are among the factors favourably affected by gas storage and to a varied extent by waxing. (32 references.) A. S. Carmichael.

Effects of pre- and post-harvest treatments on storage of fruits. D. K. Salunkhe, G. M. Cooper, A. S. Dhaliwal, A. A. Boe and A. L. Rivers (*Food Technol.*, 1962, **16**, No. 11, 119—123).—The effects of pre-harvest chemical sprays, post-harvest dips, hydro-cooling and packaging films on the storage life of strawberries, sweet cherries and peaches are discussed. E. M. J.

Effect of drying conditions on initial colour, colour retention and pungency of red peppers. J. G. Lease and E. J. Lease (*Food Technol.*, 1962, **16**, No. 11, 104—106).—Drying or curing sliced pods at 150°F was optimum for quality. Added BHA markedly improved colour retention of cured pods and, to a lesser extent, freshly harvested pods. At 120 and 150°F extended drying was permissible, but at 175°F led to lower initial colour, colour retention and pungency. Cured peppers retained more colour when stored whole than when ground following curing. Autoclaving pepper samples before drying increased the rate of colour loss. Colour breakdown could not be attributed directly to enzyme activity. E. M. J.

Drying of seaweeds and other plants. V. Through-circulation drying of *Ascophyllum nodosum* in a semi-continuous dryer. J. H. Merritt and E. Gordon Young (*J. Sci. Fd Agric.*, 1963, **14**, 39—42; cf. J.S.F.A. Abstr., 1962, i, 41).—The experiments described were designed to test the efficacy of the special, semi-continuous dryer (see J.S.F.A. Abstr., 1962, i, 41) for rockweed and to provide data as a basis for the design of dryers of commercial size. A feasible loading for fresh rockweed of 80% initial moisture content was approx. 6·0 lb./sq. ft./tray with air flow up to 80 lb. of dry air/min./sq. ft. At temp. from 120 to 210°F heat consumptions of 1200—2000 B.Th.U./lb. of water evaporated were recorded. The yield of alginate was not affected by these temp., but discoloration of the powdered weed and of the extracted product occurred in direct proportion. The coeff. of η of the alginate was approx. inversely proportional to the temp. of drying. (11 references.) E. M. J.

Influence of chemical changes in dried food mixtures with respect to storage stability. V. Analytical determination of colour changes and their preliminary indications in tomato products. J. Schormüller and H.-J. Lange. **VI. Food mixtures with dried separated milk as main component.** J. Schormüller and K.-H. Müller. (*Z. Lebensmitt-Untersuch.*, 1962, **118**, 214—223; 1963, **118**, 485—491).—V. Previously described analytical methods (cf. *ibid.*, 1962, **117**, 379) were used in connexion with storage experiments (cf. *ibid.*, 1962, **118**, 112). In tomato powders ~60% of the lycopene had disappeared after 1 year at 30° and R.H. 70%, whilst samples stored at 20° showed some discoloration, but lost relatively small amounts of lycopene. Powders containing 7% of moisture stored in tins during 6 months at 38° lost no lycopene if kept under N_2; in similar experiments admixture with anhyd. SiO_2-gel. (whether under N_2 or air) gave protection against discoloration and completely inhibited increases in the content of 5-hydroxymethylfurfural (HMF) above 300 mg. per 100 g. of dry matter. A high content of HMF indicates either thermal damage during manufacture or the onset of changes due to bad storage conditions which lead to subsequent browning. Experiments with soup powders indicated the necessity for packaging in material impervious to moisture. (16 references.)

VI. Comparisons are made between the changes in composition undergone at 3, 20, and 30° during 18 months by mixtures containing dried separated milk and Na glutamate with (*a*) dried green beans and (*b*) salt meat extract. The moisture content of the former remained practically constant, whilst that of the latter increased continuously. Mixture *b* was more subject to change than mixture *a*. The losses of lysine and arginine at 30° in both mixtures were 25—40%, whilst those of glutamic acid were ~6—16%. Both mixtures increased in acidity, especially at 30°. The (apparently) greater losses of glucose at 30° (determined by the Bertrand method) in *a* (~20%) than in *b* (~16%) were probably due to the greater production of reducing non-sugars in *a* than in *b*; for the same reason, the losses of glucose indicated by the Bertrand method were smaller than those indicated by the (specific) enzymic method of determination. (13 references.) P. S. Arup.

Smoking of foods. IV. Acids in smoke and smoked products. P. Spanyár and I. Szeredy (*Z. LebensmittUntersuch.*, 1962, **118**, 293—299).—The following scheme is applied to the analysis of the smoke solution obtained as previously described (cf. J.S.F.A. Abstr., 1961, i. 48; ii. 285). The solution (50 ml. in 5-ml. portions) is extracted with ethyl ether; the aq. phase contains the non-volatile acids. The ethereal solution containing the carbonyl compounds is extracted with NaOH, saturated with CO_2 and the phenols extracted with ether; the aq. phase is acidified with HCl and the volatile acids extracted with ether. Known methods of paper-chromatographic analysis are applied to concentrates of each of the four fractions: non-volatile acids, volatile acids, phenols and carbonyls. The compounds identified include acetic and formic acids (constituting 40 and 30% of the total volatile acids), with recognisable amounts of malonic, succinic, syringic, vanillic and β-resorcylic acids, vanillin and ethylvanillin. Several of these compounds were found in smoked products. P. S. Arup.

Dissolved and dispersed gas content of certain commercially processed foods. E. H. Sheets and A. Lopez (*Food Technol.*, 1962, **16**, No. 10, 143—144).—Test products (15) comprised applesauce, strained vegetable and beef baby food, tomato juice, pasteurised milk, etc. Total dissolved and dispersed gas content was deter-

mined in the commercially packed food in cans or glass containers. O_2 and CO_2 were analysed quant. by absorption and the residual gas, presumed to be N_2, was determined by difference. There was a significant difference in the gas content of the cream layer (**I**) and the ' milk ' layer of pasteurised milk. The greater gas content of **I** was due to increased residual gas, mainly N_2 caused by denaturation of the protein on the surface of the fat globules ; there was also a greater O_2 content. E. M. J.

Pasteurisation and sterilisation of meat with ionising radiations. V. Preininger (*Prům. potravin*, 1963, **14**, 49—55).—Preservation of beef and pork in lump and in the form of forcemeat was studied in 16 series of trials, five thereof in a nuclear reactor, the other 11 were irradiated by a ^{60}Co source of 5000°, under application of pasteurising and sterilising doses in the range of 50,000 to 2·1 millions rep. Attention was paid to the influence of packing material (tin, polyethylene) and of additives preventing organoleptic deterioration, such as ascorbic acid. Different kinds of radiation caused different organoleptic changes, the most active being pure γ-rays. By irradiation of raw meat H_2S and methyl mercaptan, unfavourably influencing the flavour, taste and colour, are formed, while lysine, glutathione, glycogen and thiamine, are decreased and glutamic and aspartic acids and alanine are increased. The extent of changes depends further on numerous factors, especially composition of meat, meat ripeness, and on the character of the radiation. For storage at room temp. of beef and pork, pasteurising doses up to 1 million rep are insufficient ; the sterilisation dose varies in a range of 1·2 to 2·1 millions rep. Meat packed in polyethylene bags was susceptible to colour change. Of preventive additives ascorbic acid in concn. of 0·1—0·5% was best, quercetin was unsuitable. (14 references.) J. S. B.

Effect of ice/water coolant ratios upon moisture absorption and rate of chilling of eviscerated chicken carcasses. W. C. Mickelberry, D. V. Schwall and W. J. Stadelman (*Poultry Sci.*, 1962, **41**, 1550—1553).—When the % of ice in the ice–water coolant ranged from nil to 100 the highest moisture absorption by the carcasses occurred with 33% ice and the most rapid cooling with 66% ice, although there was not much difference in rate of cooling with 33% to 66% ice. Carcasses having higher eviscerated wt. were associated with a lower degree of moisture absorption. A. H. CORNFIELD.

Freezer burn as a limiting factor in the storage of animal tissue. IV. Dipping treatments to control freezer burn. G. Kaess and J. F. Weidemann (*Food Technol.*, 1962, **16**, No. 12, 83—86).—Substantial reduction of freezer burn by dipping unfrozen liver in aq. solutions of substance of comparatively low mol. wt. (alcohols, hexoses, etc.,) which readily penetrate the tissue is reported. (18 references.) E. M. J.

Time-temperature tolerance of frozen foods. XXIV. Quality changes in cauliflower. W. C. Dietrich, M.-D. F. Nutting, M. M. Boggs and N. E. Weinstein (*Food Technol.*, 1962, **16**, No. 10, 123—128).—Quality changes in commercial retail packs of frozen cauliflowers were determined by measurement of the optical density of an acetone extract of the cauliflower, reflectance colour of floret surfaces, ascorbic acid content, and flavour and colour changes as evaluated by judges. Rates of deterioration increased about fourfold for each 10°F rise in temp. in the range 0—25°. Cauliflowers were considered acceptable, but not of high quality after 3—6 months at 10°F and 5—11 weeks. (13 references.) E. M. J.

Dehydrated potatoes. F.M.S. (Farm Products) Ltd. (B.P. 884,267, 2.2.60. U.S., 3.2.59).—Dehydrated potatoes are produced by precooking raw potatoes below 100° but above the gelatinisation temp. (viz., at 60—82° during 10—60 min. according to the total solids content). They are then cooled with water until soft enough to mash, mashed, and dehydrated (to give flakes or granules).
F. R. BASFORD.

Packaging

Wrapping or packing material having a preserving action. Farbwerke Hoechst A.-G. (B.P. 884,253, 12.1.59. Ger., 11.1.58).—Wrapping or packing material, of basis of paper sheet or Al foil etc., and having preserving action on packaged foodstuffs etc., contains or is coated (at rate 0·5—20 g./m.²) with Ca sorbate as the preserving agent, formed *in situ* on the paper. H. L. WHITEHEAD.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Place of food technology in combating hunger and malnutrition. V. Subrahmanyan (*Food Technol.*, 1962, **16**, No. 10, 23—27).—An address stressing the need to prevent loss of food through spoilage and the value of conservation, e.g., protecting stored grains, improving protein quality, use of protein isolates, use of enzymes in making milk products, etc. E. M. J.

Evaluation of protein in foods. VII. Factors influencing net protein utilisation as determined by carcass analysis. A. B. Morrison, Z. I. Sabry and J. A. Campbell (*Canad. J. Biochem. Physiol.*, 1962, **40**, 1663—1670).—The effects of vitamin and mineral deficiency on net protein utilisation (N.P.U.), at different protein (casein) levels, were studied in male rats. The animals lost wt. and had reduced N.P.U. values ; carcass analysis indicated N retention as well as loss of body water and lipid. It was further shown that N.P.U. values were dependent on dietary lysine at concn. of 0·32—0·72%. Three different procedures for estimating endogenous and metabolic N losses, gave similar N.P.U. values. (17 references.)
A. S. CARMICHAEL.

Ion-exchange of amino-acids on resins with carboxylic active groups in acid-salt form. III. Chromatographic equilibria and mechanism. I. R. Bellobono (*Chim e. Industr.*, 1962, **44**, 984—989).—Ion-exchange of arginine, lysine and histidine on resins with carboxylic active groups, buffered at pH between 3·5 and 8·0, is studied by elution chromatography ; analysis of the mechanism of mass transfer is applied. Distribution coeff. are measured as a function of the ratio of $[Na^+]$ in resin phase and in solution, to obtain equilibrium quotients. Isotherms at 25° are linear only for low Na^+ concn. (0·12—0·2M) in the buffer. Exchange capacity as a function of pH is also studied with 1,2-diaminopropionic and 1,4-diamino-n-valeric acids, lysyl-lysine, and β-alanylhistidine. For all compounds examined (which have $7·5 < pI < 10·5$; $6 < pK_2 < 9·7$) exchange capacities as a function of pH pass through a max. value, connected with the equilibrium between bipolar ions and ions with positive unitary charge of amino-acids in solution. pH of max. exchange capacity is simply related to iso-ionic points of amino-acids. Molal selectivity coeff. measured by chromatographic methods, from parameters of metathetic equations, agree excellently with results of equilibrium methods.
C. A. FINCH.

***O* → *N*-Acetyl shift of *O*-acetylserine and *O*-acetylthreonine. An approach to the separation of serine and threonine fraction from amino-acid mixture.** S. Fujiwara, S. Morinaga and K. Narita (*Bull. chem. Soc., Japan*, 1962, **35**, 438—442).—*O*-Acetylthreonine (**I**) is found to be more labile than *O*-acetylserine (**II**) and shifts to the *N*-acetyl deriv. at pH 7. The acid hydrolysate of casein was treated with HCl and AcOH, **I** and **II** were converted to the *N*-acetyl deriv. by incubation at pH 8 and these were separated from the other amino-acids by an ion-exchange resin. The shift reaction is affected by temp., pH and the ionic concn. of inorg. salts. Attempts to separate serine and threonine using the difference in shift reaction rates have been unsuccessful. (22 references.) E. C. DOLTON.

Mineral contents of a few cooked foods. S. P. Roychowdhury, J. A. Khan, K. N. Bose and M. N. Habibi (*J. Instn Chem. India*, 1962, **34**, 186—192).—The effect of cooking on the mineral content of 11 common foods was investigated. It was raised by tap water and metallic utensils and by the addition of NaCl for flavouring but, in all cases, the content exceeded min. required. Tables give the methods of prep. and mineral contents of the foods (with and without salt) and of the raw materials. C. A. P.

Bacterial infection in feeding-yeast production. A. Čejková, H. Němčanská and J. Vintika (*Kvasný průmysl*, 1962, **9**, 272—281).—Results of analyses of mashes and final products at industrial plants producing *Torula* yeast are reported. Bacterial infection can be suppressed by providing optimum conditions for yeast propagation, supposing the substrate is not infected beyond certain limits, and the strain of yeast is physiologically sound and vigorous. Hot method of preparing mash is recommended when heavily infected substrates are to be processed. (15 references.) J. S. B.

Stability of vitamin B_{12} in biological concentrates. E. Buntová (*Prům. potravin*, 1962, **13**, 597—599).—The stability of vitamin B_{12} in biological prep. used as feed components was studied. In prep. consisting of the biological mass of *Actinomyces olivaceus* yeast the losses after storage for 6 months under laboratory conditions ranged from 12 to 21%. In another prep. based on the biological substance of *Propionibacterium shermanii* the losses after 12 months amounted to 48—64%. (13 references.) J. S. B.

Paper-chromatographic determination of ascorbic acid, dehydroascorbic acid and ascorbigen in heated biological material. IV. Improvement of quantitative evaluation of chromatograms. M. Zobel and A. Teutloff (*Z. LebensmittUntersuch.*, 1962, **118**, 299—304).—To each of the eluates obtained as previously described (cf. *ibid.*, 1962, **116**, 477), containing $\gtrsim 15\mu$g. of ascorbic acid, is added 2 ml. of 10^{-4}N-2,6-dichlorophenolindophenol ; after centrifuging, the

absorption of the solution is measured at 510 mμ in a 2-cm. cell in comparison with the same vol. of the buffer solution containing 2 ml. of the titrant. This improvement avoids reliance on visual judgement. (11 references.) P. S. ARUP.

Protein hydrolysates [for dietary use]. Merck & Co. Inc. (B.P. 877,727, 13.1.58. U.S., 25.1.57).—A protein (casein) hydrolysate is freed from phenylalanine, also aspartic and glutamic acid (to give a dietary prep. suitable for use in the treatment of phenylketonuria), by passing it through an anion-exchange resin (on the OH cycle) simultaneously to neutralise it and adsorb aspartic and glutamic acid; bringing the effluent into contact with C (to adsorb tyrosine and phenylalanine); then converting residual glutamic acid in the treated effluent into pyrrolidonedicarboxylic acid. F. R. BASFORD.

Unclassified

Application of centrifugal chromatography for routine analyses in the food industry. Z. Deyl, J. Rosmus and M. Pavlíček (*Prům. potravin*, 1962, **13,** 601—605).—A review and description of methods using chromatography in centrifugal field for separation and determination of alkaloids, dyestuffs and colouring materials, amino-acids, proteins, antibiotics, growth factors, vitamins, wood distillation products significant for smoking foods, aminoplast semicondensates, and sugars. Selected methods can be used advantageously in routine analyses in the food industry as in pharmaceutical and clinical analyses. (33 references.) J. S. B.

Gas chromatographic analysis of head space gas of dilute aqueous solutions. R. Bassette, S. Özeris and C. H. Whitnah (*Analyt. Chem.*, 1962, **34,** 1540—1543).—Enrichment of the head space gas in sulphides, carbonyls, esters and alcohols was achieved by adding anhyd. Na_2SO_4 to the dil. aq. solution. An analysis of a solution containing 0·01 p.p.m. of carbonyls was possible by this means. Procedures for the removal of individual groups from the head space gas are given. G. P. COOK.

Formation of hydrogen sulphide from substrates by groups of bacteria frequently encountered in perishable food materials. D. A. A. Mossel (*Ann. Inst. Pasteur*, 1962, **103,** 108—111, Reprint).—Two techniques are used to indicate the formation of H_2S by the action of various bacteria on cysteine hydrolysates and on Na thiosulphate and sulphite. In the first a piece of filter paper impregnated with lead sub-acetate is placed in the growth medium and in the second a ferrous salt is placed in the medium. Results by both techniques agree well although the first is more sensitive. All *Enterobacteriaceae* produce H_2S from cysteine, thiosulphate and sulphite. Most *Streptococcus faecalis* and *faecium* and *Aeromonas* produce H_2S from cysteine. *Pseudomonas* and *Achromobacter anitratus* do not produce H_2S. (14 references.) J. V. RUSSO.

***Clostridium perfringens* in foods.** R. Angelotte, H. E. Hall, M. J. Foter and K. H. Lewis (*Apptl. Microbiol.*, 1962, **10,** 193—199).—A procedure is described for the identification and enumeration of this organism. As few as 10 organisms/g. were recovered without interference from associated flora or food constituents. Two food poisoning outbreaks are discussed in which this was the causative organism. (25 references.) C. V.

Radiation survival curves of *Clostridium botulinum* spores. E. Wheaton and G. B. Pratt (*J. Fd Sci.*, 1962, **27,** 327—334).—Survival curves were obtained for concentrations of spores (37,000—64,800,000,000 per ampoule) of *Cl. botulinum* strain 12885A, subjected to a wide range of γ-radiation doses under various conditions with no recovery at >5 Mrad. Freeze-dried spores exhibited less radioresistance in the dry state than when resuspended in neutral phosphate buffer, and these suspensions exhibited less radioresistance when irradiated under air than under N_2. Spores exhibited less radioresistance suspended in phosphate buffer than in a nutrient medium (pork–pea infusion). Regardless of spore concn. a so-called 'tailing off' of surviving spores was observed at the higher dose levels employed for varying spore concn. (16 references.) E. M. J.

Salmonella in food commodities. — Willems, — Van Oye, — Granville and — Paix (*Rev. Ferment.*, 1962, **17,** 39—42).—The following are covered: fundamental characteristics of the organism, method of culture, isolation, identification, tests for salmonella in fresh and frozen meat, in milk, butter, cheese, eggs and meat and fish flour. Recipes for recommended culture media are given. E. M. J.

Simple test of foods for *Salmonella, Shigella, Proteus,* and *coli* bacteria. H. Eschmann (*Mitt. Lebensm. Hyg., Bern*, 1962, **53,** 149—153).—The two enrichment media, selenite broth followed by Bi-sulphite-agar, should be pasteurised, but not sterilised. The absence of colonies on the agar after 48 h. can be taken as evidence of the absence of pathogenic organisms. Colonies appearing on the agar are inoculated on to Difco Kligler $FeSO_4$-agar slopes. Characteristic reactions in the aerobic or anaerobic sections (or both) of the Kligler agar are described (with illustrations) by which seven different pathogenic organisms can readily be identified. The indications include acid formation (yellow colour), gas formation (bubbles in the agar), H_2S formation (blackening) and characteristic odours. P. S. ARUP.

Determination of the number of coliform organisms and detection of salmonella. H. Forster and H. Gasser (*Mitt. Lebensm. Hyg., Bern*, 1962, **53,** 230—234).—Plate counts of infected yoghourt and milk powder are investigated on Endo agar, Eosin–methylene blue agar and Violet-red-bile agar. The first is insufficiently selective and the last, in conjunction with formate–ricinoleate broth, is recommended. Enrichment media for detecting salmonella and possible difficulties are mentioned. J. B. WOOF.

Bactericidal properties of compounds which protect living cells against freezing damage. T. Nash (*J. Hyg., Lond.*, 1962, **60,** 353—358).—Most air disinfectants fail at low R.H. values. Those which remain active can protect living cells against lethal damage caused by freezing to low temp. and then thawing. The compounds are either the simple glycols or the weaker Lewis bases but the air-disinfectant properties of these have not hitherto been examined. It is suggested that both freezing protection and bactericidal power are due to the special solvent properties of these compounds which come into play when most of the water usually present has been removed by freezing or drying. (17 references.) C. V.

Maintenance of microbiological organisms as freeze-dried cultures. P. M. Scholes (*Analyst*, 1961, **86,** 714—719).—The possibility of the use of freeze-dried organisms as inocula for microbiological assay was investigated with six organisms, viz., *Lactobacillus arabinosus* 17/5 (**I**), *L. fermenti* 36 (**II**), *L. viridescens*, Strain S 38A (**III**), *Streptococcus faecalis* (**IV**), *Pediococcus cerevisiae* 8081 (**V**) and *L. helveticus* (**VI**). The organisms were freeze-dried and stored at 2° to 4° for 1—16 months. The organisms were grown in suitable media and used for the assay of nicotinic acid and pantothenic acid (organism **I**), thiamine (**II** and **III**), folic acid (**IV**), folinic acid (**V**) and riboflavin (**VI**) control assays being made with organisms maintained in the standard way. All the organisms gave satisfactory dose–response graphs up to 12 months after freeze-drying. In assays of thiamine with freeze-dried cultures of *L. fermenti* that had been subcultured once, results were less precise than those with unsubcultured freeze-dried inoculum. Direct use of washed suspensions of assay organisms maintained as freeze-dried cultures is suggested as a useful microbiological technique. A. O. JONES.

Use of tissue culture for the bioassay of staphylococcal enterotoxin. N. A. Milone (*J. Fd Sci.*, 1962, **27,** 501—507).—An assay procedure based on the possible induction of cytopathogenic effects on several strains of tissue culture cells with enterotoxin prep. of varying degrees of purity produced in a laboratory medium is described. No observable cytopathogenic effect could be attributed directly to the enterotoxin. Cruder prep. contained a thermolabile cytotoxic material, that is removed in progressive purification. (11 references.) E. M. J.

Thermophile bacteria as incitants for the spoiling of preserves. L. P. Naidenova (*Mikrobiologiya*, 1962, **31,** 910—917).—Seven species of sporogenous bacteria, related either to *Bacillus aerothermophilus* or *B. coagulans* were isolated from peas, tomato soup, spinach and other tinned foodstuffs. The temp. ranges for growth were generally 37 or 42—72°. R. A. KEEN.

Some biological properties of specifically formed silica gel. V. V. Patrikeev, Z. S. Smirnova and G. I. Maksimova (*Dokl. Akad. Nauk SSSR*, 1962, **146,** 707—709).—The heat stability of *Pseudomas fluorescens*, at 55°, was increased by the presence of silica gel. Silica gel, treated with H_2O_2 to remove org. matter, possessed stereospecificity. Silica gel formed in the presence of *Bacillus mycoides* (dextro-form) adsorbed D-linalol 5·12 times more than L-linalol. With formation in the presence of the L-form the adsorption of the L-linalol was eight times greater than that of the D-isomer. (12 references.) R. A. KEEN.

Barium in bones and foodstuffs. E. H. Henderson, A. Parker and M. S. W. Webb (*U.K. Atomic Energy Authority A.E.R.E. Rep.*, 1962, R4035, 10 pp.).—Spectrographic analysis of Ba in 60 bone samples showed a content/age correlation which was negative and not significant even at the 10% level; the corresponding Sr/age relationship gave a positive value significant at the 0·1% level. In this respect Ba resembles Ra rather than Sr. Little information could be found on the Ba contents of foodstuffs, but a discrimination factor of approx. 13 was deduced for Ba relative to Ca between the level in the diet and that in the bones. J. W. TAYLOR.

Direct spectrographic determination of trace metals in food products by the rotating disk technique. A. Paolini and R. M. Kennedy (*Appl. Spectroscopy*, 1962, **16**, 15—17).—The food is blended with water to a smooth paste, which is then spectrographed directly by means of a rotating disk assembly. By using Bi as an internal standard, Ca, Cu, Fe, P and Sn can be determined in a variety of foods. The results are in good agreement with those obtained chemically. R. M. ROWLEY.

Recommended methods of analysis of pesticide residues in foodstuffs. Determination of small amounts of BHC in flour and edible oils. B.H.C. Panel set up jointly by the Scientific Sub-Commee of the Advisory Commee on Poisonous Substances used in Agriculture and Food Storage, the Analytical Methods Commee of the Society for Analytical Chemistry and the Ass. of British Manufacturers of Agricultural Chemicals (*Analyst*, 1962, **87**, 220—227).—The BHC is extracted by specified methods and after removal of the solvent the residue is dechlorinated by heating with Zn dust and AcOH in presence of malonic acid as internal source of CO_2. The benzene so produced is passed into a nitration mixture from which the nitrated product is subsequently extracted with dichloromethane. After removal of the solvent the residue is dissolved in ethyl methyl ketone, and the extinction of the violet solution after addition of KOH is measured at 565 mμ and referred to a calibration graph prepared with standard solution of lindane. Two forms of apparatus are recommended, and methods are given for the purification of light petroleum and n-hexane which are used as solvents.
A. O. JONES.

3.—SANITATION, WATER, etc.

Metabolism of Methaphoxide in mosquitoes, houseflies and mice. F. W. Plapp, jun., W. S. Bigley, G. A. Chapman and G. W. Eddy (*J. econ. Ent.*, 1962, **55**, 607—613).—Topically applied Methaphoxide [tris-(2-methyl-1-aziridinyl)phosphine oxide] (**I**) was rapidly absorbed and detoxified by houseflies. When injected, degradation was faster. The amount of ^{32}P in the ovaries increased although the % of **I** declined and there was no selective absorption. *Culex tarsalis* adults and larvae also degraded **I** rapidly. Mosquitoes which fed on mice treated with **I** degraded it within 24 h. Blood of mice treated intraperitoneally contained enough **I** to produce sterility in mosquitoes for 2—6 h. Residues were greatest in liver and kidney. There was no difference in reaction to **I** between org. P-susceptible and -resistant flies. C. M. HARDWICK.

Effect of Apholate on ovarian development of houseflies. P. B. Morgan and G. C. LaBrecque (*J. econ. Ent.*, 1962, **55**, 626—628).—Apholate [2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis-(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine] (1%), fed to newly emerged flies, did not stop the growth of the oöcyte and adjacent cells that had already passed a certain stage in development in the first egg chamber. The cells in the second chamber did not enlarge. Apholate also inhibited germarium development as indicated by delayed formation of the third egg chamber. (12 references.)
C. M. HARDWICK.

Effects of selection on cross resistance in diazinon-resistant *Musca domestica*. A. J. Forgash and E. J. Hansens (*J. econ. Ent.*, 1962, **55**, 679—682).—Flies were selected by topical application of diazinon. A max. resistance against diazinon was reached after 24 generations. The max. resistance and time taken to reach it is given for Isolan, lindane, ronnel, dimethoate, Dibrom and Dimetilan. No further resistance to diazinon was obtained over 15 generations. Repression of resistance after 12 generations' selection took up to 30 generations and resulted in a level above normal susceptibility. Slight tolerance to pyrethrins and Lethane 384 was probably due to vigour tolerance.
C. M. HARDWICK.

Field studies of housefly resistance to diazinon, ronnel and other insecticides. E. J. Hansens and A. P. Morris (*J. econ. Ent.*, 1962, **55**, 702—708).—The level of resistance of flies in 30 barns was determined. Barns in the area had been sprayed with diazinon or ronnel for 5 years. Levels of resistance to diazinon, ronnel, malathion, lindane and DDT in relation to treatments used are given. Lower levels of resistance were found in barns where good sanitation was maintained. C. M. HARDWICK.

Effect of 5-fluorouracil on the viability of house-fly eggs. W. W. Kilgore and R. R. Painter (*J. econ. Ent.*, 1962, **55**, 710—712).—Labelled 5-fluorouracil was mixed with fly diet and consumed within 36—48 h. and was followed by a normal diet. Most of the radioactivity was excreted but eggs laid during the first 4 days oviposition incorporated a detectable amount. The % hatch from these eggs was only 20% and the no. of eggs was reduced to half.
C. M. HARDWICK.

Carbamate insecticides: toxic action of synergised carbamates against twelve resistant strains of the housefly. G. P. Georghiou (*J. econ. Ent.*, 1962, **55**, 768—772).—The relationship between different types of carbamates and their reaction to different strains is discussed. Isolan caused 100% mortality in all strains. Zectran (*m*-isopropylphenyl *N*-methylcarbamate) and *o*-isopropoxyphenyl *N*-methylcarbamate without piperonyl butoxide were ineffective against all resistant strains. The addition of piperonyl butoxide produced some toxicity to all the resistant strains. There was no evidence of absorption limits. The use of Zectran + piperonyl butoxide for 45 generations did not appreciably alter the tolerance to it. (12 references.) C. M. HARDWICK.

Effectiveness of WARF antiresistant as an additive to DDT for control of resistant houseflies. B. H. Wilson (*J. econ. Ent.*, 1962, **55**, 792—793).—Topical application of DDT + WARF (*N*-di-n-butyl-*p*-chlorobenzenesulphonamide) produced only a slightly greater mortality of resistant houseflies than did DDT alone.
C. M. HARDWICK.

Control of houseflies on poultry ranches with antiresistant/DDT. D. Bell and R. H. Daehnert (*J. econ. Ent.*, 1962, **55**, 817—819).—Graphs show the level of fly populations during July–Nov. A 5% A.R./DDT gave 2—3 weeks' fly control compared with 1 week for 5% DDT suspension. Topical application showed that at one location the flies were >200 times as resistant as the laboratory strain. C. M. HARDWICK.

Control of the cocoa moth, *Ephestia cautella* in Ghana, W. Africa. H. A. Mould and J. Rawnsley (*Pyrethrum Post*, 1962, **6**, No. 4, 7—10).—Space treatments are extremely effective in killing the adults. There was little difference between the effect of a particular concn. of straight pyrethrins (**I**) and the effect of **I** with 10 times as much synergist. *E. cautella* infestations may be controlled by daily fogging directed against adults. The % kill increased with the concn. of pyrethrins used, up to 0·4%. The addition of the synergist had no effect. The observation that the basis of synergism with pyrethrins is probably physiological supports the findings that different insects may respond differently to the addition of synergist to pyrethrins. E. M. J.

Applications of the 'sulphur colour' test in the estimation of pyrethrins. N. C. Brown and M. C. Wood (*Pyrethrum Post*, 1962, **6**, No. 4, 11—15).—By suitable modifications of the 'sulphur colour' test (Brown *et al.*, J.S.F.A. Abstr., 1956, ii, 70) by which pyrethrins could be determined down to a min. of 5 mg. with error <2%, amounts of ~0·5 mg. can be estimated, but there is an increase in potential error up to ~10%. The method is based on measurement of the brown colour produced by heating pyrethrins with solutions of S and LiOH. Methods for removal of interfering materials are discussed, viz., chromatography on Al_2O_3, on activated silica gel, treatment of solutions in light petroleum with H_3PO_4, cautious washing of solutions with dil. alkali. E. M. J.

Behaviour of pyrethrins during thermal fogging. D. R. Maciver (*Pyrethrum Post*, 1962, **6**, No. 4, 16—19).—The extent of damage to pyrethrins during normal thermal fogging of unsynergised pyrethrum in light liquid paraffin with an apparatus such as the Swingfog SN7 portable fogging machine is >5% of the input pyrethrins. Since piperonyl butoxide protects pyrethrins during fogging the difference in activity due to fogging synergised pyrethrum is insignificant from a practical standpoint. E. M. J.

Stability of pyrethrins in aqueous emulsion. D. R. Maciver (*Pyrethrum Post*, 1962, **6**, No. 4, 20—21).—The stability of a prepared concentrate: oleoresin 23·65% wt./wt. pyrethrins 4·0% (= 0·946% pyrethrins); piperonyl butoxide 4·0; Ethylan B.C.P. 4·0; Mannoxol O.T. 1·0; solvents 13·0%; water to 100 vol. was examined. The extent of hydrolysis likely to occur on storage was determined by an accelerated heat storage test. The emulsion was heated under reflux at 92·5° for 10 days and the pyrethrins were determined at regular intervals by dinitrophenylhydrazone chromatographic assay. A second sample of emulsion was placed in a brown-tinted glass bottle with screw cap, on a shelf in an Equatorial laboratory. Samples were tested by the chromatographic procedure, over a year. A biological check on the emulsion stored for 1 year was compared with that of a fresh emulsion. The pyrethrins in the prepared concentrate proved to have a high degree of stability after shelf storage for 1 year. (10 references.) E. M. J.

Insecticidal activity of pyrethrum extract and its four insecticidal constituents against houseflies. V. Knock-down activity of the four constituents with piperonyl butoxide. R. M. Sawicki (*J. Sci. Fd Agric.*, 1962, **13**, 591—598).—Female houseflies were treated by a measured drop method with each of the four active constituents of pyrethrum extract alone and in presence of piperonyl butoxide. (1 part of pyrethroid + 10 parts of piperonyl butoxide) in n-dodecane at 20°. The synergist increased the overall efficiency of the constituents and the effect was least in the first few min., increasing most rapidly in the first 2 h. after treatment. The synergist changed

the order and magnitude of the relative toxicities from those obtained with constituents alone. At first the order with the mixtures (as with the constituents alone) was pyrethrin II, cinerin II, pyrethrin I, cinerin I. After 3 h. the order was (constituents alone in brackets) pyrethrin I (pyrethrin II), cinerin I (pyrethrin I), pyrethrin II (cinerin I), cinerin II (cinerin II), and it was the same at death (24 h. after treatment). Cinerin I was synergised most, pyrethrin II least; pyrethrin I and cinerin I were synergised better than pyrethrin II and cinerin II, and the cinerins better than the corresponding pyrethrins. (12 references.) E. M. J.

Development of male melon fly attractants. B. H. Alexander, M. Beroza, T. A. Oda, L. F. Steiner, D. H. Miyashita and W. C. Mitchell (*J. agric. Fd Chem.*, 1962, **10,** 270—276).—The examination of 119 compounds related to anisylacetone (**I**) [4-(*p*-methoxyphenyl)-2-butanone], a known effective lure for the male melon fly (*Dacus cucurbitae*, Coq.), in a search for a better lure is reported. The relationship between structure and the ability to lure is discussed. The best attractant discovered, 4-(*p*-acetoxyphenyl)-2-butanone, known now as Cue-lure, attracted 20 times as many flies as **I**. (35 references.) W. Elstow.

Field evaluation of malathion dust for control of body lice. W. W. Barnes, B. F. Eldridge, J. H. Greenberg and S. Vivona (*J. econ. Ent.*, 1962, **55,** 591—594).—The experiment was conducted at a prison farm in Korea with four groups of 100 prisoners each housed in iron buildings. A simple application of 1% malathion dust controlled lice for 4 weeks by power-duster, and 2 weeks by hand duster. Lindane 1% gave only a small temporary reduction of lice. Malathion was also ovicidal in action. Malathion produced no toxic symptoms. Initially the lice were resistant to DDT. (11 references.) C. M. Hardwick.

Effects of various chemicals on eggs of the yellow-fever mosquito *Aedes aegypti*. C. L. Judson, Y. Hokama and A. D. Bray (*J. econ. Ent.*, 1962, **55,** 805—807).—Of 28 compounds tested only five were lethal to at least 90% of exposed eggs, after 24-h. exposure at 0·001 ml./quart. These compounds were all relatively volatile. Some relationships between structure and toxicity were found for various groups. The most effective compounds were DDVP, 1,2-dichloro- and 1,2-dibromo-propane, Nemagon, chloropicrin, Niagara 5961, ethylene dibromide acrylonitrile and Phosdrin at the concn. tested. C. M. Hardwick.

Bromine residues in wheat and milled wheat fractions fumigated with methyl bromide. D. L. Lindgren, F. A. Gunther and L. E. Vincent (*J. econ. Ent.*, 1962, **55,** 773—776).—The residual Br in wheat fumigated at 15% moisture content was three times that of grain fumigated at 9% moisture. A reduction of ~25% occurred after 6-h. aeration but there was little further reduction. The higher the temp. the greater was the residue. This increase was greater from 70—90°F than from 50—70°F. The increase from 2—48-h. exposure increased the residues eight-fold. A second fumigation gave a 60—75% increase. Residues were in the order wheat bran > shoots > flour > middlings whether fumigation occurred before or after milling. Br residues were determined by instrumental neutron activation analysis. C. M. Hardwick.

Thiophosphoric acid derivatives of ethylamine, DL-methionine and L-proline ethyl esters. III. Biological activities on fruit flies. F. C. Klee and E. R. Kirch (*J. pharm. Sci.*, 1962, **51,** 540—543).—*Drosophila melanogaster* were exposed to 0·1, 0·01 and 0·001% w/v aq. solutions or suspensions of 17 deriv. having structures similar to those of known acetylcholinesterase inhibitors. Neither sex of mature fly survived 0·1% wt/vol. suspensions of most of the deriv., 0·01% solutions were more toxic to males than to females, and both sexes were unaffected by 0·001% solutions except those of five ethylamine esters and one methionine deriv. which were toxic to males. Concn.-toxicity and structure-toxicity relationships are discussed. D. M. Benoliel.

Bulk fumigation of apples with ethylene dibromide under plastic tarpaulins for apple maggot. K. H. Sanford (*J. econ. Ent.*, 1962, **55,** 659—661).—A 2-h. exposure to ethylene dibromide at 6 oz./1000 cu. ft. killed all larvae of *Rhagoletis pomonella* in 1960 and only one emerged from 600 infested apples in 1961 and this probably survived as an egg. The apples were not injured by the fumigant. Fumigation should not be carried out below 60°F. (11 references.) C. M. Hardwick.

Use of pyrethrum powders in the tomato canning industry in the U.S.A. C. N. Watts (*Pyrethrum Post*, 1962, **6,** No. 4, 3—6).—The control of fruit flies of the genus *Drosophila* is discussed. Dusts were superior to liquids and 0·75% piperonyl butoxide and 0·075% pyrethrins as well as the 1·0% piperonyl butoxide and 0·1% pyrethrins formulations were both superior to 0·11% dust without synergist. Dusts as applied during picking (to reduce egg contamination) and at farm or field level gave only partial protection, but at the receiving station were more skilfully applied. Good coverage is normally realised and no additional treatment is made at the cannery. E. M. J.

Water, wastes and sewage

Procedure for the determination of the rate of destruction of organic matter in benthic sediments of deep reservoirs. M. E. Gambaryan (*Mikrobiologiya*, 1962, **31,** 895—898).—A device is described, with a diagram, for sampling soil and benthic water in reservoirs. The apparatus consists essentially of metal encased glass tubes, 2·15 cm. in dia. and 40 cm. long, mounted on the base of a Perfiliev stratometer. Four samples can be taken simultaneously. Sand and sludge samples from the Sevan lake, taken during the summer months by this method, showed that the O_2 consumption reached 114·47 and 130·26 mg. O_2 per sq. m. per day. R. A. Keen.

Colorimetric determination of traces of copper in drinking water. D. C. Abbott and J. R. Harris (*Analyst*, 1962, **87,** 497—499).—The sample of water (50 ml. or a portion containing ≯40 μg. of Cu diluted to 50 ml.) is acidified with 5 ml. of 50% v/v H_2SO_4 and 10 ml of Zn dibenzyldithiocarbamate solution (0·05% in CCl_4) are added in a separating funnel. The extinction of the separated and filtered org. layer is measured at 430 mμ against CCl_4 and referred to a calibration graph prepared with standard solution of $CuSO_4$ covering the range 0 to 40 μg. of Cu per 50 ml. Recovery of Cu added to various waters was satisfactory. Added Fe, Pb, and Zn caused no interference. A. O. Jones.

Determination of traces of lead in drinking water. D. C. Abbott and J. R. Harris (*Analyst*, 1962, **87,** 387—389).—To such a vol. of the sample as will contain ≯25 μg. of Pb when diluted to 50 ml are added 10% w/v $(NaPO_3)_6$ and 1% (w/v) hydroxylamine hydrochloride and, after mixing, alkaline cyanide solution containing aq. NH_3 and Na_2SO_3. The liquid is extracted with dithizone in $CHCl_3$ and the extinction of the $CHCl_3$ is measured at 520 mμ. Recovery of known amounts of Pb in various waters was satisfactory. A. O. Jones.

4.—APPARATUS AND UNCLASSIFIED

Fouling inside vertical evaporator tubes. R. A. Carlson and A. I. Morgan, jun. (*Food Technol.*, 1962, **16,** No. 11, 112—114).—A diagram of experimental equipment is given. In general conclusions relating to long-tube evaporator design for materials that foul regardless of direction of flow are presented: ample tube area must be provided; sufficient feed preheaters must be supplied to ensure that boiling occurs throughout the tubes; recirculation provided must be sufficient to reduce the vapour fraction in the tubes below ~20% by wt.; heat exchange should be to a liquid of as low a solids content and as high a temp. as possible to keep η at a min.; flow should be up through the tubes for two-phase mixtures, or boiling should be entirely suppressed inside the heat-exchange tubes, all recommendations being based on fouling considerations without respect to cost of construction. E. M. J.

Simultaneous spectrophotometric determination of fructose and glucose mixtures by differential reaction rates. Application to blood serum analysis. L. J. Papa, H. B. Mark, jun., and C. N. Reilley (*Analyt. Chem.*, 1962, **34,** 1443—1446).—The method described is based on the differential reaction rates of fructose and glucose towards acidic NH_4 molybdate. Both a single- and double-point method are presented. C. B. Baines.

Effect of sulphate content of several anionic polymers on *in vitro* activity of pepsin. L. J. Ravin, J. G. Baldinus and M. L. Mazur (*J. pharm. Sci.*, 1962, **51,** 857—860).—The inhibitory action of degraded carrageenan and other sulphated natural and synthetic polymers on the proteolytic action of pepsin on human plasma was studied in relation to the sulphate content of the polymer. A linear relationship was found between sulphate content of hydrolysed carrageenan prep. and inhibitory activity. Tests on a series of sulphate-containing anionic polymers, natural and synthetic, compared with commercial carrageenan prep. C16 as a standard showed there was also a correlation between the sulphate content of the polymer and its inhibitory activity, the latter increasing with the former. The carrageenan prep. and the anionic polymers all precipitated protein in the test. Inhibitory substances that did not precipitate protein, e.g., Na lauryl sluphate or Na *m*-xylenesulphonate, showed no such correlation. (23 references.) J. I. M. Jones.

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