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THE QUALITY OF CANADIAN AMBER DURUM WHEAT GRADES AND THE ROLE OF A PENTOSAN-RICH FRACTION IN MACARONI DOUGH QUALITY

By G. S. BAINS* and G. N. IRVINE

Apart from differences in constituents, such as ash, protein, gluten, yellow pigment, and farinograph curve characteristics of semolina milled from various grades of Canadian Amber Durum wheat, the importance of a pentosan-rich fraction recovered during the washing of gluten is indicated. The pentosan content of the crude fraction ranged from 27·7 to 39·6% and appears to be associated with protein and polyglucosan. Its significance in determining dough quality and its relation to grade are discussed. The mixing time and the stability of macaroni doughs are increased when this fraction is added at levels of 2 to 3% to semolinas of the higher grades, No. 2 C.W. and No. 3 C.W. Semolinas from the higher grades contained relatively lower amounts of the crude fraction than the lower grades. Under similar conditions of gluten washing, semolinas of the Grades No. 2 C.W., No. 3 C.W., Extra No. 4 C.W., No. 4 C.W., No. 5 C.W., and No. 6 C.W. were found to contain 1·59, 1·69, 2·15, 2·25, 3·08, and 3·77%, respectively, of the pentosan-rich fraction.

Introduction

Since 1941, annual studies on the quality of various grades of Amber Durum wheat grown in Canada have been conducted by the Grain Research Laboratory and are reported in their periodical Crop Bulletins. Factors such as vitreous kernels, ' vulgare kernels ', yield of semolina, protein content, yellow pigment, and colour measurements of macaroni,[1] were originally the main criteria of quality. Subsequently, lipoxidase activity,[2] ash and gluten content of semolina have been added. These tests, however, do not reflect the physical quality of dough, which is important in the modern continuous processing of macaroni. The use of a farinograph technique for the study of the stiff macaroni doughs has therefore been developed[3] to provide information on the rheological properties of semolina doughs. This technique has indicated that significant differences exist between grades, and such data are now provided in routine assessment of wheat quality. Concurrently, with the exploitation of the farinograph technique, it was found that glutens washed from semolinas left variable amounts of a gelatinous residue on the gluten washing sieve.[4] The amount of residue appeared to be related to grade and to the rheological properties of the dough as indicated by the farinograph. A systematic study has been made to ascertain the quantitative relationship of the crude residue to grade, and particularly the amount of pentosan present in the residue in order to elucidate its effect on the rheological properties of the dough.

The results outlined in this paper indicate that semolinas milled from the lower grades of Durum wheat contain relatively higher amounts of the crude residue, and that the addition of the isolated material to the higher grades produces rheological effects on those doughs similar to those normally observed with the lower grades.

Experimental

The principal experimental material consisted of Grades No. 2 C.W., No. 3 C.W., Extra No. 4 C.W., No. 4 C.W., and No. 5 C.W. Amber Durum representing the average of these respective grades exported from Canada during the third and fourth quarters of the 1957–58 crop year. In addition to these, composite samples of No. 5 C.W. and No. 6 C.W. from the general run of the 1957–58 crop year were used and, in one experiment, an individual low protein sample of No. 2 C.W. Three varieties of bread wheats, Marquis, Beard and Marquis × Exchange from the 1958 crop, also formed a part of the study for comparison of pentosan content with those of the durum wheat semolinas.

All the wheats were tempered overnight to 16·5% moisture, and milled into semolina on an Allis Chalmers laboratory mill following the standard procedure employed in this laboratory. The yield of purified semolina is expressed as a percentage of clean wheat (14% moisture basis).

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Analytical methods

Ash was determined by incineration of the weighed samples at 585°, protein (N × 5·7) according to the A.A.C.C. Kjeldahl method and in the pentosan-rich fractions by the micro-Kjeldahl A.O.A.C. procedure; lipoxidase activity according to the method of Irvine & Anderson,[2] and the wet gluten by washing freshly made dough (10 g. of semolina plus 5 ml. of distilled water) with salt–phosphate buffer of pH 6·7 in a Theby Gluten Washer. Yellow pigment (p.p.m.) was determined colorimetrically after extraction with water-saturated n-butyl alcohol.[5]

The amount of pentosan was calculated from the furfural content of the hydrochloric acid distillates using potassium bromate–potassium bromide reagent[6] adopting the modification suggested by Vernon & Metzner,[7] and following the procedure outlined by Loska & Shellenberger.[8]

Total hydrolysable carbohydrates of the crude fraction expressed as dextrose were determined by hydrolysing a weighed aliquot (100 mg. plus 5 ml. of 1 N-acid) with sulphuric acid on a boiling water bath for six hours and determining the reducing sugars by the A.O.A.C. potassium ferricyanide method.[9]

Farinograms

The Brabender farinograph was set at 1 : 1 sensitivity before mixing 50-g. lots of semolina in the small stainless steel bowl using appropriate water absorptions which conformed to the optimum dough conditions considered suitable for processing into macaroni by the micro method of Martin *et al.*[10]

Yield of pentosan-rich crude fraction

In order to obtain consistent yields, a number of preliminary experiments comprising dough treatment, use of different gluten washing sieves, and mechanical versus hand washing of gluten, were carried out. The dough treatments consisted of immediate washing of gluten after the dough was made and after resting the dough under distilled water for an hour prior to washing the gluten. In another series, the dough was prepared using a solution of 0·2% papain, keeping the dough under distilled water for 1–2 h. respectively and washing the gluten mechanically. In all cases the gluten was washed for 10 min., the residues on the sieves washed with distilled water, then transferred to a weighed crucible, dried at 100°, and weighed. The yield is expressed on the basis of semolina (14% moisture). The dried residues were used for the determination of pentosan contents.

Composition of the crude fraction

Sufficient quantity of the residues from semolina of each grade were recovered using the Theby gluten washer, washed with distilled water, dried at 40° in a vacuum oven and analysed for ash, protein, and total hydrolysable carbohydrates.

The pentosan content of various fractions obtained during the gluten washing operation was studied; i.e., soluble pentosan in washing solution and pentosan content of the starch an gluten separated. The washing solution and the starch passing through the gluten washing screen (11XX silk) were collected and centrifuged for 15 min. at 2000 r.p.m. Pentosans were determined in the supernatant and in the gluten; the amount associated with the starch was calculated by difference.

Nature of the pentosan-rich fraction

The material was examined microscopically after it was stained with iodine solution. Dispersion in 4% sodium hydroxide at room temperature was also studied. The component sugars in the hydrolysate were detected by qualitative paper partition chromatography, using ethyl acetate–pyridine–water as the irrigant.[11] The sugar spots were developed by spraying with aniline phosphoric acid.[12]

Effect of pentosan-rich fraction on the rheological properties of dough

The crude fraction was separated from a low-grade durum, No. 5 C.W., to study the effect of its incorporation on the rheological properties of doughs made from semolinas of the higher grades, No. 2 and No. 3 C.W. In addition to the normal 2 C.W., a selected sample of this grade of lower protein was tested, as very low protein often has similar rheological properties to those exhibited by low grades. Additions of the crude extract of 0 to 3% were made.

Results

Yield and composition of semolina

The general characteristics of the semolinas milled from five grades of durum wheat are given in Table I together with the water absorption and the dough development time. The

Table I

Yield, water absorption, dough development time and composition of semolina of various amber durum grades

Grade	Quarterly cargo	Yield of semolina, %	Water absorption, %	Dough development time, min.	Ash, %	Protein (N × 5·7), %	Wet gluten, %	Lipoxidase units μl.O_2/g./min.	Yellow pigment* p.p.m.,
Amber durum									
No. 2 C.W.	3rd	55·0	31·5	4·0	0·57	12·0	36·1	17·0	4·90
	4th	54·0	31·5	4·5	0·58	11·8	35·7	16·0	4·43
No. 3 C.W.	3rd	54·4	31·5	4·5	0·57	11·2	32·8	17·0	4·55
	4th	52·8	31·5	5·25	0·58	10·7	33·5	17·0	4·15
Extn. No. 4 C.W.	3rd	54·5	32·5	6·0	0·64	11·1	31·5	20·0	4·44
	4th	54·9	32·5	5·25	0·62	11·4	33·2	20·0	4·12
No. 4 C.W.	3rd	54·0	33·5	5·75	0·60	10·5	29·6	20·0	4·23
	4th	53·4	33·5	5·00	0·62	10·4	30·0	20·0	4·02
No. 5 C.W.	3rd	51·4	33·5	8·75	0·64	10·1	24·6	18·0	4·22
	4th	51·4	33·5	8·00	0·68	9·7	25·1	20·0	4·01

* As β-carotene

data are typical for the grades, and of principal interest in this study is the increase in water absorption, together with a parallel increase in dough development time from the higher to the lower grades.

Effect of milling on the pentosan content of semolina

The pentosan of the durum and bread wheats, together with the pentosan levels for the respective semolinas, are given in Table II. The durum wheats show a slight tendency to higher pentosan content with decreasing grade, the bread wheats seem slightly higher on the average than the durum grades. Semolinas, however, show a rather stronger trend towards higher pentosan content in the lower grades, and the bread-wheat semolinas are slightly higher than the lowest grade durum. These latter differences could be accounted for by differences in semolina milling quality.

Content and composition of the pentosan-rich fraction

Recovery of the residues remaining on the gluten washing screen following the washing of the gluten from semolina doughs yielded a water-insoluble gelatinous material which appears to be related to the grade of wheat. Maintaining the doughs of semolinas prepared from the various grades under water yielded residues whose amounts and constituents were closely related to the grade. The mechanical method of washing gluten furnished residues which contained significantly higher percentage of pentosans as compared with the pentosan content of residues obtained by the process of washing gluten by hand (Table III). However, in the mechanical method the type and the mesh size of the gluten screen constitute an important factor which influences the recovery of the crude fraction. It is interesting to note that the

Table II

Effect of milling on the pentosan content of semolina

Grade	Quarterly cargo	Pentosans in Wheat %	Pentosans in Semolina %	% proportions in semolina
Amber durum				
No. 2 C.W.	3rd	5·85	2·25	38·5
	4th	6·26	2·53	40·4
No. 3 C.W.	3rd	5·90	2·33	39·5
	4th	6·24	2·49	39·9
Ext. No. 4 C.W.	3rd	5·90	2·44	41·4
	4th	6·21	2·70	43·5
No. 4 C.W.	3rd	5·85	2·44	41·7
	4th	6·31	2·78	44·1
No. 5 C.W.	3rd	6·72	2·90	45·5
	4th	6·68	2·98	44·6
No. 6 C.W.	—	7·46	3·02	40·5
Bread wheats				
Marquis	—	6·93	3·15	45·5
Marquis × Exchange	—	6·72	3·08	45·7
Beard	—	7·05	3·21	45·7

total amount of pentosans in respective fractions, whether obtained mechanically or by hand washing of gluten, is about the same. As the mechanical technique resulted in residues richer in pentosans, it was adopted for securing detailed information on the yield and composition of the crude fractions as reported in Tables V and VI.

When a papain-treated dough was kept under water for 1 h. it gave values comparable with those of the control, despite the fact that the enzyme destroyed the gluten which passed through the sieve during the washing operation (Table IV). Extending the time to 2 h. reduced the amount of the residues, but the pentosan content was increased, the total pentosans accounted for being about the same.

Statistical examination of the amount and the pentosan content data of the gummy residues (Table V) revealed significant differences, the lower the grade the higher the percentage of gummy residues and lower the content of pentosans in the crude fractions. However, the respective crude fractions of lower grades, e.g., No. 4 C.W. and No. 5 C.W., yielded much higher amounts of pentosans, ranging from 0·87 to 1·11 g. as compared with 0·69 g. of No. 2 C.W. and 0·72 g. of No. 3 C.W. respectively. The protein content of the fractions appeared to increase with inferior grades, but the ash and total hydrolysable carbohydrates do not bear any relationship to the grade (Table V). The results of the study on the grade samples of the fourth quarterly cargoes (Table VI) support the observations made on the third quarterly cargoes. The amount of crude fraction determined from washing gluten by the hand technique was found to be generally higher, but at the same time the percentage of pentosans was decreased, as shown in Table VII.

On the basis of total pentosan content of the semolina, about 30·4 to 32·5% appears in the crude fraction of grade No. 2 C.W. and No. 3 C.W. respectively, whereas the corresponding values for grade No. 5 C.W. and No. 6 C.W. ranged from 39·3 to 40·1% respectively (Table VI).

Table III

*Effect of mechanical versus hand washing of gluten on yield and pentosan content of crude fraction**

Mesh size	Recovery of pentosan-rich fraction (Theby) %	Recovery of pentosan-rich fraction (Hand) %	Pentosan content (Theby) %	Pentosan content (Hand) %	Amount of pentosans in the fraction (Theby) g.	Amount of pentosans in the fraction (Hand) g.
IIXX Silk	2·25	3·39	33·3	22·5	0·87	0·89
13XX Silk	2·13	3·55	36·0	22·9	0·89	0·94
14XX Silk	2·14	3·41	35·3	22·7	0·88	0·90
10XX Nylon	1·56	3·45	36·1	22·0	0·66	0·88

* Semolina of No. 4 C.W.

Table IV

Effect of papain on the recovery of pentosan-rich fraction of semolina of No. 5 C.W. amber durum wheat

Treatment	Dough rest period* h.	Weight of fraction %	Pentosan content %	Amount of pentosans in the fraction g.
Control (no papain)	1	3·15	28·2	1·03
	2	2·98	29·4	1·04
Papain 0·2%	1	3·00	29·7	1·02
	2	2·72	36·8	1·16

* The dough was allowed to rest under distilled water

Table V

Effect of grade on weight[1] *and composition*[2] *of pentosan-rich crude fraction recovered during washing of gluten*

Grade	Weight of the fraction %	Ash %	Protein (N × 5·7) %	Pentosans %	Hydrolysable carbohydrates as dextrose %	Pentosans in the fraction g.
Amber Durum						
No. 2 C.W.	1·59	0·87	7·8	37·5	72·7	0·69
No. 3 C.W.	1·69	0·86	7·6	36·8	71·7	0·72
Ext. No. 4 C.W.	2·15	0·79	8·7	35·1	72·7	0·88
No. 4 C.W.	2·25	0·75	9·2	33·3	73·3	0·87
No. 5 C.W.	3·08	0·84	9·8	31·0	72·9	1·11

[1] Average of quadruplicate determinations using the Theby gluten washer

[2] Analysis of composited fractions

Expressed as percentage of the actual pentosans contained in residues of No. 2 C.W., viz., 0·77 g., the residues of lower grades represent 52 to 57% more pentosans. The crude fraction of the semolina of Marquis wheat represented 43·5% of the total pentosans, which exceeded even the value for the lowermost grade No. 6 C.W. of durum wheat. The centrifugate of No. 2 C.W. accounted for about 21% of the total pentosans, as against 17·2–17·7% in respect of grades No. 5 and No. 6 C.W. Whether this difference can be attributed to pentosanase activity or is an artefact of hydration or both is difficult to explain on the basis of the data obtained in this study. The existence of pentosanase activity in wheat flour has been reported by Pence *et al.*,[13] but no such study has been reported in the case of durum semolina so far.

Table VI

Distribution of pentosans in various fractions obtained during the washing of gluten

Grade	Weight of crude fraction %	Pentosan content %	Amount of pentosans in various fractions*			
			Crude fraction g.	Solution g.	Gluten g.	Starch (by difference) g.
Amber Durum						
No. 2 C.W.	1·68	39·6	0·77	0·53	0·22	1·02
No. 3 C.W.	1·78	39·0	0·81	0·56	0·20	0·92
Ext. No. 4 C.W.	2·07	39·3	0·95	0·57	0·14	1·04
No. 4 C.W.	2·25	36·8	0·96	0·56	0·17	1·09
No. 5 C.W.	3·24	31·1	1·17	0·52	0·16	1·13
No. 6 C.W.	3·77	27·7	1·21	0·52	0·13	1·16
Bread wheats						
Marquis	3·44	34·3	1·37	0·52	0·31	0·95
Marquis × Exchange	2·56	35·3	1·05	0·64	0·21	1·18
Beard	2·93	35·8	1·21	0·55	0·25	1·20

* Pentosans in various fractions based on 100 g. (14% moisture) of semolina

Table VII

Effect of grade on the recovery of pentosan-rich fraction by hand washing of gluten

Grade	Weight of crude fraction %	Pentosan content %	Amount of pentosans in the fraction g.
Amber durum			
No. 2 C.W.	2·44	24·5	0·70
No. 3 C.W.	2·51	25·7	0·75
Ext. No. 4 C.W.	3·15	26·6	0·97
No. 4 C.W.	3·47	22·4	0·91
No. 6 C.W.	5·44	18·6	1·18

Effect of pentosan-rich fraction on the rheological properties of dough

The replacement of a part of the semolina with the pentosan-rich fraction (Table VIII) significantly altered the rheological properties of the dough. The mixing times increased and the doughs were relatively more stable to mixing. The normal protein semolina of grade No. 2 C.W. having 3% of incorporated gummy residues developed higher consistency, the maximum being 720 B.U. as against 680 B.U. of the control. The mixing time increased from 4 to 7½ min. The consistency of the former dough at 4½ min. after attaining the peak was still higher (600 B.U.) as compared with 550 B.U. of the control. The low-protein semolina of the same grade behaved similarly, but at 3% level of incorporation for the same water absorption the farinogram became rough and scattered, the development of dough seemed to be inhibited, a phenomenon observed in the case of No. 5 C.W. semolina. The latter at comparable water absorption produced a very rough farinogram, taking inordinately long to mix.

Properties of the pentosan-rich fractions

The crude fraction swells in water and on acid hydrolysis revealed the presence of glucose, xylose and arabinose, which confirms the results of Simpson.[14] Tiny granules of starch which stained deep blue with iodine appear enmeshed in a jelly-like mass when moistened with water. A major fraction of the residue is dispersible in 4% sodium hydroxide, the remaining portion on washing and drying stained blue with iodine, and on acid hydrolysis glucose, arabinose and xylose were detected. The indispersible fraction probably consists of a highly polymerised pentosan and an associated polyglucosan.

Discussion

The results presented in this paper offer evidence for the existence of substantial amounts of pentosan-rich comparatively insoluble fractions in the experimentally milled semolina from

Table VIII

Effect of incorporating pentosan-rich fraction on the farinogram characteristics of macaroni doughs

Grade	Water absorption ml.%	Crude fraction incorporated %	Dough development time, min.	Dough consistency Maximum B.U.	Dough consistency after 4½ min. B.U.
Amber durum					
No. 2 C.W.*	30·0	0·0	8·0	500	440
	30·0	2·0	15·5	500	480
	31·5	0·0	5·0	480	410
	31·5	2·0	11·0	500	450
	31·5	3·0	15·0	480	480
No. 2 C.W.**	31·5	0·0	4·0	680	550
	31·5	3·0	7·25	720	600
No. 3 C.W.	31·5	0·0	6·75	630	550
	31·5	3·0	11·00	640	600
No. 5 C.W.	31·5	0·0	21·5	480	480

* 9·5% protein in the semolina (low-protein)
** 11·8% protein in the semolina (high-protein)

durum and bread wheats. This fraction can be recovered on the sieve in the process of washing glutens from semolinas. Literature on the significance of pentosans in durum wheats is scanty, whereas a number of studies have already been reported on the composition, structure[15–21] and the role of pentosans in wheat flour,[8, 22] doughs,[13, 23] milling,[17, 24–27] baking,[28, 29] and in the manufacture of starch from wheat flours.[14, 30] Earlier work on the pentosans in wheat has been reviewed by Bailey.[31] The probable rôle of pentosans in bread doughs has been emphasised by Hlynka.[32] The results of this study indicate the probable rôle of a pentosan-rich fraction, associated with protein and very small starch granules, in modifying the rheological properties of stiff semolina doughs as evaluated by the farinograph technique. The fact that the gummy residues recovered from semolina of the lower grades have a higher protein content may have significance in the mixing characteristics of the semolina, especially in view of the interaction of gluten proteins with the gummy polysaccharides reported by Udy.[33] This might also account, to some extent, for the low yields of wet gluten recovered from the No. 4 and No. 5 C.W. semolinas. The mutual contamination of soluble proteins and pentosan preparations based on flour extracts[13, 14, 20, 34] seems to indicate the possibility of protein-pentosan complexes in flour. Our observations have shown that the incorporation of crude pentosan-rich residues recovered during the washing of gluten from semolina altered the farinograph pattern of the dough as compared with the control. The extension in mixing time as a result of employing water-extracted flour instead of the water-soluble fractions has been reported by Mattern & Sandstedt.[35] The residual flour still contains all the relatively insoluble pentosan-rich fractions with associated protein.

The principal factor responsible for the degrading of Canadian durum wheat into the lower grades is frost damage. This type of damage usually arrests the normal maturing of the kernel; it seems apparent from this work that the amount of insoluble pentosan (probably associated with the cell walls) is higher in immature kernels and decreases upon normal maturation of the kernel. The insoluble pentosan is capable of rapidly absorbing a large amount of water, proportional to its weight, and this seems to account for the marked change in rheological properties of doughs which contain higher amounts of this material, or to which it is added. The bread wheats examined in this study appear to contain a higher percentage of insoluble pentosan in the normal mature state than do the durum wheats, and this may be one of the factors responsible for the higher water absorption normally associated with red spring wheats. The low grades of Canadian hard red spring wheat normally contain quite high percentages of frost-damaged kernels, and these invariably show a markedly higher water absorption than the grades containing only normally matured wheat. Durum wheats from North Africa often exhibit rheological properties similar to those induced in other durums by heavy additions of the insoluble pentosan material. As these wheats are often forced to maturity by hot dry winds, it seems likely that their rheological behaviour can be attributed, in part at least, to a similar phenomenon as postulated for low-grade Canadian durums, i.e., abnormally high percentages of insoluble pentosan resulting from the arresting of the normal maturing process.

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THE AMINO-ACIDS OF SUGAR CANE. I.—The amino-acids of cane-juice and the effect of nitrogenous fertilisation on the levels of these substances

By D. H. PARISH

Twenty-three amino-acids have been detected in cane juice, pipecolic acid, methionine, tryptophan and β-alanine for the first time, and the presence of a hydroxypipecolic acid is suggested; the presence of arginine is confirmed.

Increasing the supply of nitrogen increases markedly the amides asparagine and glutamine and less markedly the basic amino-acids lysine, histidine, arginine and tryptophan. The neutral and acidic amino-acids tend to increase and then decrease in level with increasing nitrogen supply.

Introduction

Several workers have studied the amino-acid composition of cane juice, notably Pratt & Wiggins,[1] Brinkley & Wolfrom[2] and Martin.[3]

The efficiency of recovery of the sugar contained in the cane-juice is low for an industrial process, being only around 93%, because of losses in the molasses. Many propositions have

been put forward to account for 'melassogenism' and amino-acids have been held to exert some important influence on the limitation of crystallisation of sucrose from molasses.

Kelly[4] postulated that crystallisation of sucrose ceases when a second organic solute reaches saturation concentration in the molasses, and, as some amino-acids have low solubilities, then they may be exerting a controlling influence in stopping sucrose crystallisation.

During a study of the effect of nitrogen fertilisation on the amino-acids of cane leaf laminae, some 32 ninhydrin-reacting spots have been found to occur in all varieties.[5] The amino-acids isolated from cane-juice so far number only 18, and such a common amino-acid as pipecolic acid, which occurs generally in the plant kingdom, had not yet been detected. Moreover, with the interest now shown in arginine as a growth stimulator for germinating sugar cane[6] and the doubts raised by these workers as to the actual presence of arginine in cane juice, it was thought that a re-examination of the amino-acid composition of cane juice using a reliable chromatographic method would produce results both of industrial and physiological interest.

Parish[7] has shown that nitrogen fertilisation influences markedly the total amino-acid content of cane-juice and the effect of this nutrient on the distribution of the individual amino-acids isolated was therefore studied.

Thompson *et al.*[8a] gave a method for purifying extracts of plants and isolating the constituent amino-acids which is well suited to cane-juice studies, where because of the low levels of amino-acids which occur, and the preponderance of asparagine, detection of some of the trace constituents is difficult.

Methods and materials

Mature canes of the variety M.147/44 grown under three levels of nitrogen fertilisation, viz. 0, 30 and 60 kg. of nitrogen per acre were passed through a cane chipper and the juice was extracted by hydraulic press, filtered through cotton wool, brought to 80% v/v ethyl alcohol content and set aside overnight. The precipitated protein was filtered off and the alcoholic solution evaporated to a small volume under reduced pressure below 40°. The liquid was again filtered and diluted to volume with water and isopropyl alcohol to give a solution of the free amino-acids of the cane juices in 10% v/v isopropanol.

The method of Thompson *et al.*[8b] was used for the analyses of the amino-acids.

Results and discussion

Line diagrams of typical chromatograms obtained during this work are shown in Fig. 1. Table I gives the levels of 20 amino-acids which occur in measurable amounts in cane juice; three other amino-acids were present in trace amounts.

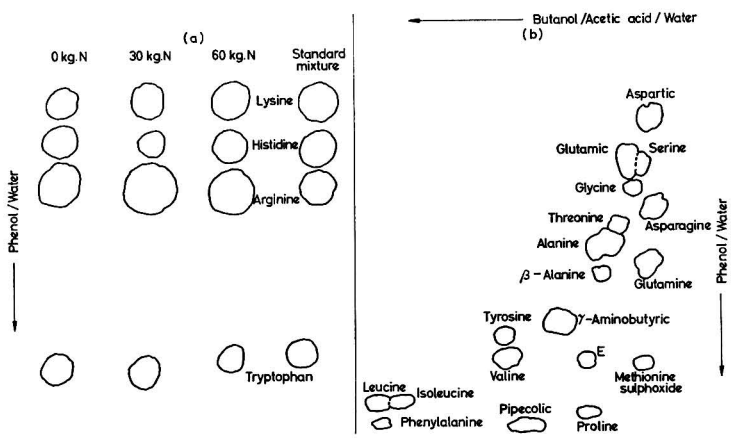

FIG. 1.—*Amino-acids in 80% alcohol-soluble fraction of sugar-cane juice*
(*a*) Basic amino-acids (variety M.147/44)
(*b*) Neutral and acidic amino-acids (variety M.147/44, 30 kg. of N/acre)

Table I

Effect of nitrogen fertilisation on the amino-acids of cane juice

Level of N, kg./acre	0	30	60	Level of N, kg./acre	0	30	60
Acid	Amino-acid, mg./100 ml. of juice			Acid	Amino-acid, mg./100 ml. of juice		
Aspartic	2·4	2·9	4·1	Spot E	trace	trace	trace
Glutamic	4·2	5·7	4·7	Methionine	0·2	0·2	0·2
Serine	0·8	1·8	1·8	γ-Aminobutyric	2·6	3·7	2·7
Glycine	0·2	0·4	0·4	Tyrosine	0·1	0·6	0·4
Threonine	0·3	0·9	0·7	Valine	0·4	1·0	0·8
Alanine	1·6	3·5	3·2	Pipecolic	0·1	0·2	0·3
Asparagine	6·6	14·2	50·9	Proline	trace	0·07	0·09
β-Alanine	trace	trace	0·1	Isoleucine	0·2	0·6	0·5
Glutamine	2·9	4·8	8·6	Leucine	0·1	0·3	0·2
				Phenylalanine	0·2	0·5	0·6
	Level of N, kg./acre			Lysine	0·3	0·4	1·2
	0	30	60	Histidine	0·3	0·9	2·0
Juice Brix	19·4	19·5	19·0	Arginine	1·2	2·8	2·8
Density	1·078	1·079	1·077	Tryptophan	trace	trace	trace

The results show, for the first time, that pipecolic acid and methionine (measured as methionine sulphoxide) occur in comparatively important amounts in cane juice, whilst arginine, which was detected as a trace constituent by Martin[3] but not by other workers, also occurs in important amounts. Three other substances not before described as being present in cane-juice have been detected, substance E, which is possibly a hydroxypipecolic acid,[9] β-alanine and tryptophan, thus bringing the number of amino-acids known to occur in cane-juice to 23. This is 10 less than the number so far detected in cane leaves, but non-detection does not necessarily mean that they are absent. The predominance of asparagine and glutamine and their associated amino-acids at high rates of nitrogen fertilisation is typical of many plants.

The results also indicate accumulation of the basic amino-acids lysine, histidine and arginine, whilst the contents of the neutral and acidic components increase with the 30-kg. addition of nitrogen and then level off or even fall slightly.

The confirmation of the presence of arginine in the cane juices studied, accounts for the failure of the author to obtain a growth-stimulating effect following the addition of arginine to germinating cane setts, whereas Nickell & Kortschak[6] demonstrated such an effect.

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THE NITROGENOUS CONSTITUENTS OF THE DEHYDRATED MUSHROOM, *BOLETUS EDULIS*, AND THEIR RELATION TO FLAVOUR

By J. D. CRASKE* and F. H. REUTER†

Steam distillation of dehydrated *Boletus edulis* gave a ' flavour essence ' which contained only the off-flavour components of the sample and was reminiscent of town gas and/or rubber. The true mushroom flavour was found to be non-volatile, the residue after distillation being of undiminished flavour intensity.

About half of the sample was water soluble and this extract contained all of the flavour components. These were concentrated into the basic fraction by an ion-exchange technique, and further separated into fractions containing amino-acids of progressively increasing basicity by a displacement development ion-exchange chromatographic technique. The most basic compounds tasted most characteristically of mushrooms, but it was clear that all the nitrogenous constituents contributed something to the overall flavour profile. The contribution of the highly basic amino-acids is considered to be especially important as it was found that their flavours lingered in the mouth and were therefore savoured more strongly than would be expected simply from a consideration of their taste intensity as measured by threshold dilution technique.

Introduction

The mushroom, *Agaricus campestris*, is usually either canned or sold for consumption in the fresh state. Until the advent of freeze-drying, no commercial attempt was made to preserve the fungus by dehydration, as it was reputed[1] to lose its flavour during the drying process. Now that the freeze-drying process is being used more widely, it has become evident that *Agaricus campestris* can be dehydrated without incurring gross flavour loss, providing good drying techniques are adopted.

On the other hand *Boletus edulis*, a mushroom which grows wild in a number of European countries, is extensively preserved on a commercial scale using quite unsophisticated drying techniques. The flavour of the reconstituted dehydrated *Boletus edulis* is similar to that of the fresh *Agaricus campestris* and, as the two fungi are closely related taxonomically, it is perhaps surprising that an apparent difference in ease of drying should exist.

Discussion of the chemical composition of mushrooms and the relation to flavour is complicated by the very large number of fungi which are available for study, even when only considering the edible fungi. Yet, in comparison to other foodstuffs, the volume of research which has been carried out on the flavour components is small.

Essex & Shelton[2] published a brief description of several English and Continental mushrooms and likened the flavour of the *Heterophylla* or Blue Cap to that of cray fish and of *Agaricus deliciosus* to that of kidney. They gave no indication of the nature of the flavour components. Bernhard & Simone[3] investigated the locus of the aroma in *Agaricus campestris* L., but again no comment was made on the identity of the flavourous substances.

The carbohydrates of *Agaricus campestris* were found by McConnell & Esselen[4] to be principally mannitol, hemicellulose, glycogen and reducing sugars. Ratcliffe[5] found trehalose in *Boletus edulis*. Frère-Jacque[6] isolated D-arabitol from *Boletus bovinus* and mannitol from *Boletus luteus* L. Birkinshaw *et al.*[7] detected D-threitol in the wood-rotting fungus, *Armillaria mellea*. No comment is made in any of the above studies of the influence of the various sugars on flavour, but it seems unlikely that they could contribute anything but a slightly sweet background.

A number of workers have investigated the amino-acids and nitrogenous constituents of mushrooms. Reuter[8] demonstrated the presence in *Boletus edulis* of glycine, alanine, leucine, valine, aspartic acid, glutamic acid, proline, phenylalanine, trimethylhistidine, arginine, histidinebetaine, lysine, tetramethylenediamine, adenine, and small amounts of guanine and

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hypoxanthine. The main volatile bases were ammonia and trimethylamine. Keil & Bartmann[9] found phenylethylamine in the alkaline distillate from *Boletus luteus*. Also isolated were choline, putrescine and fumaric acid. Again no comments on flavour are included in the above work.

Yamashita & Yamanishi[10] examined the flavour constituents of dried *Cortinellus shiitake* and reported the presence of a number of amino-acids and acetamide. In addition they isolated two steroid-like substances and a strong smelling wax which was shown to contain aldehyde and sulphydryl or disulphide groups.

Further studies of the amino-acid composition of various mushrooms have been made by a number of authors.[11–19] Botticher *et al.*[20] showed that a high flavour level was related to a high protein content.

Aye[21] isolated from *Helvella esculenta* a volatile alkaloid with an odour resembling mouse urine. The structure was not determined.

Anisaldehyde was shown by Birkinshaw *et al.*[22] to be present in *Polyporus benzoinus* and by the same school[23] to be present together with methyl anisate in *Trametes suavolens*, L.

Other compounds which have been isolated include benzyl isothiocyanate, phenylethyl thiocyanate and benzaldehyde cyanhydrin,[24] choline,[14, 16] methyl cinnamate,[25] furfural[26] and matsutake alcohol.[25, 26]

More recently, Hoffman[27] isolated matsutake alcohol from oxidising soya-bean oil and identified it as oct-1-en-3-ol. Stark & Forss[28] isolated a mushroom-flavoured compound from butterfat and washed cream and showed that it was invariably accompanied by oct-1-en-3-one. They also showed that oct-1-en-3-ol, from which the authentic sample of the corresponding ketone was prepared, had a mushroom odour and it was suggested that this alcohol might have been responsible for the odour. However, Hoffman[29] has demonstrated that pure oct-1-en-3-ol has a pine-like odour when freshly prepared and only develops a mushroom odour when kept, possibly due to the formation of a dimeric cyclic acetal.

In summary, the above work gives no clear indication of the nature of the chemical constituents primarily responsible for mushroom flavour, although it is clear that many of the compounds which have been isolated might contribute to a greater or lesser degree.

Experimental

Boletus edulis mushrooms were imported as the dehydrated product by Unilever Australia Pty. Ltd. As received they were in the form of kibbled pieces, which were ground to pass a 60-mesh screen before examination. To inhibit deterioration, the ground product was stored under nitrogen by packing the product into screw-top jars, evacuating in a vacuum oven and then breaking the vacuum with dry nitrogen.

Flavour intensity of the various extracts and fractions was assessed by a threshold dilution technique in which the sample in question is progressively diluted with water until the solution is only just distinguishable in flavour from that of the water used for the dilution.

The nitrogenous components in solution were determined quantitatively by spectrophotometric estimation of the blue colour developed with ninhydrin, essentially according to the method of Moore & Stein.[30] They recommended that the ninhydrin be recrystallised from water to reduce the blank value due to trace quantities of nitrogenous contaminants and to store the made-up reagent under nitrogen to inhibit oxidation. In the present work, the blank could not be reduced to a satisfactory level by recrystallisation, and instead the reagent solution was shaken for about 1 h. with, and then stored over, a few g. of Zeocarb-225 ion-exchange resin in the hydrogen cycle. This effectively removed any basic impurities and reduced the blank to a very low level. Since the reagent was not stored for any great length of time before use, it was not found necessary to de-aerate the solution or to store it under nitrogen.

Moore & Stein showed that the reaction between ninhydrin and amino-acids, although reproducible, is not stoicheiometric. The amino-acids shown to be present in major amounts in *Boletus edulis* have reported colour yields[30] within the range 82 to 98% This is considered to be sufficiently accurate for correlations of ' ninhydrin blue value ' with threshold dilution,

as flavour analysis is inherently inaccurate. For computation of a materials balance of ninhydrin-reactive material (the main use of the analysis) no error is introduced by this varying yield.

Volatile components were isolated by vacuum steam-distillation using the apparatus of Guadagni & Dimick.[31] Since the powdered mushroom sample contained a large volume of air, the still was evacuated continuously throughout the distillation and volatile components were trapped in two receivers connected in series and cooled in a solid carbon dioxide/acetone bath.

An aqueous extract was obtained by boiling a sample of powdered mushrooms with 10 times its volume of water and then filtering after addition of a weight of Hyflo-supercel equal in weight to that of the mushroom sample taken. The extraction was repeated twice with the addition of Supercel each time: this extracted about 90% of the water-soluble components quickly and easily. This massive dosage of Supercel was found to be necessary to obtain a reasonable filtration rate.

The combined extract obtained as above from 5 g. of mushrooms was fractionated by allowing it to percolate successively through columns (15 mm. × 200 mm.) of Zeocarb-225 resin (strongly acidic cation-exchanger) and Deacidite-FF resin (strongly basic anion-exchanger). The columns were washed with water and the exchanged ions recovered from the columns by displacement respectively with solutions of 2N-aqueous ammonia and 2N-hydrochloric acid, to afford three fractions (one retained on each column and one passing all columns), which were titled respectively the ' basic ', ' acidic ' and ' neutral ' fractions.

The addition of a column of Zeocarb-226 resin (weakly acidic cation-exchanger) at the beginning of the train allowed separation into four fractions, the additional fraction being named the ' highly basic ' fraction. These components were recovered from the resin by elution with 2N-acetic acid.

The amino-acids in the ' highly basic ' and ' basic ' fractions were separated by two-dimensional ascending paper chromatography, using the following conditions: Whatman No. 20 paper; first solvent n-butanol/acetic acid/water (120 : 30 : 50 by vol.) (' butanol solvent '), second solvent either phenol/water/15N-aqueous ammonia (80 : 20 : 0·5 (w/w/v) (' ammoniacal phenol solvent ') or phenol/water (80 : 20 (w/w) (' phenol solvent '). The chromatogram was developed so that the first solvent ran across the machine direction of the paper and second solvent ran with the machine direction. This sequence of solvent usage gave a clearer chromatogram than when the reverse order was utilised.

Ninhydrin was used as a general location reagent for amino-acids. The chromatograms were dipped in a solution of 0·5% ninhydrin in acetone containing 2% pyridine and the colour developed by incubating at 30° for 2 h. β-Amino-acids were revealed by further development at 105° for 2–3 min.

Other location reagents used for the types of compounds mentioned were as follows:

Isatin[32] to detect prolines, phenylalanines, sulphur amino-acids and as a general location reagent of lower sensitivity than ninhydrin, but giving more characteristic colours; *Ehrlich reagent*[32] to detect indoles, hydroxyindoles, aromatic amines and ureides; *Pauly reagent*[32] to detect imidazoles and phenolic compounds; *Sakaguchi reagent*[32] to detect mono-substituted guanidines; *Phosphate reagent*[32] to detect compounds such as phosphoethanolamine; *Isatin followed by Ehrlich reagent*[32] to detect hydroxyproline.

4-Benzoylamino-2, 5-dimethoxyaniline diazotate zinc salt[33] to detect phenolic compounds.

Large-scale fractionation of the amino-acid components was effected according to the displacement development ion-exchange technique of Reynolds,[34] with three columns of the following dimensions: 330 mm. × 20 mm.; 290 mm. × 10 mm.; and 100 mm. × 6 mm., packed with Zeocarb-225 ion-exchange resin and connected in series. Potassium hydroxide (0·1N) was the displacing base.

Results and discussion

(1) *Examination of the volatile components*

An overall ' flavour ' sensation may conveniently be divided into sensations due to ' taste '

and ' aroma '. In the present case, since a dehydrated product was under consideration, it was felt that the contribution of aromatic substances would be small in comparison with that due to the non-volatile taste-bearing components. However, as a number of volatile compounds have been identified in mushrooms, a sample was submitted to vacuum steam-distillation in the apparatus of Guadagni & Dimick.[31] The distillate obtained was reminiscent of rubber and/or town gas and carried no aroma that might be classed as typical of mushrooms. Furthermore, it was of sufficiently low intensity that the change in the flavour intensity of the aqueous residue, due to removal of the volatile compounds, could not be detected by measurement of threshold dilutions before and after distillation (10^3). It is, of course, evident that the threshold dilution technique is of limited accuracy and that a small change would be easily lost in experimental error, but the unusual flavour character of the distillate lends weight to the conclusion that the fraction isolated consisted of off-flavour components only and that any aromatic compounds which might have been present in the original mushroom had disappeared during the drying process. Confirmation of this hypothesis was obtained by the observation that the flavour of the distillation residue was superior to that of the undistilled aqueous slurry, being cleaner and more pleasant.

(2) *Fractionation of the water-soluble extract*

When the water extract was first submitted to ion-exchange fractionation, a two-column train was used—viz., Zeocarb-225 and Deacidite-FF—thus dividing the extract into three fractions. Elution of the columns with aqueous ammonia and hydrochloric acid, respectively, gave only 77% recovery of solute. The loss was found to be due to retention by the cation-exchange resin of amino-acids more strongly basic than the eluting ammonia solution. Accordingly, a column of weak cation resin (Zeocarb-226) was inserted into the beginning of the train and elution of this column with dilute acetic acid resulted in a virtually quantitative recovery of added solute. These results are shown in Table I.

Table I

Comparison of two-column and three-column ion-exchange separation of the aqueous extract

Fraction	Solute recovered, %	
	Two-column fractionation	Three-column fractionation
' Highly basic '	—	25·0
' Basic '	24·7	22·9
' Acidic '	14·8	18·4
' Neutral '	37·4	32·0
Unrecovered	23·1	1·7

The flavour intensity of each of the fractions of the two-column separation was determined by measurement of threshold dilution and the result obtained was rather unexpected in that the total flavour recovery was approximately twice that applied to the columns.

It was readily demonstrated that the extraneous flavour was derived from the resins, but no method of preparing them could be devised to avoid this. This emphasises the difficulty of working with a product such as mushrooms, the flavour of which is subtle and easily masked by contaminating influences. The only information on flavour that could be derived from these experiments was qualitative in nature and was to the effect that the mushroom flavour resided in the basic fractions.

(3) *Identification of the basic components*

The constituents of the ' basic ' and ' highly basic ' fractions were resolved by two-dimensional chromatography. The separated components were identified by reaction of the resultant chromatograms with a variety of location reagents, by co-chromatography in the presence of authentic samples and by noting the changes to the chromatogram due to chemical modification of the original extract. Results are shown in Table II. The presence of neutral

Table II

Identification of the components of the ' Basic fraction ' and ' Highly basic fraction ' resolved by two-dimensional paper chromatography. (Arranged in approximate decreasing order of concentration)

' Basic fraction '[a]	*' Highly basic fraction '*[b]
α-Alanine	Arginine
Phosphoethanolamine	Histidine
γ-Amino-n-butyric acid	Lysine
Glycine	α-Alanine
Serine	Glycine
Glutamine	γ-Amino-n-butyric acid
Arginine	Glutamic acid
Histidine	Ethanolamine
Leucine	Unknown No. 1[c]
Isoleucine	Aspartic acid
Glutamic acid	
Threonine	
Lysine	
Tryptophan	
Valine	
Methionine sulphoxide	
Aspartic acid	
Tyrosine	
Phenylalanine	
Ethanolamine	
Proline	
Asparagine	
α-Amino-n-butyric acid	
Cysteic acid	
Cysteine	
Cystine	
αβ-Diaminopimelic acid	
α-Aminoadipic acid	
Iso-asparagine	
Unknown No. 1[c]	
Unknown No. 2[d]	

[a] Two-column fractionation.
[b] Three-column fractionation.
[c] R_F values as phosphoethanolamine, but gave no phosphate test and gave a reaction with isatin.
[d] R_F values as leucine, but shown to be a β-amino-acid.

and acidic amino-acids in the ' highly basic fraction ' was unexpected, but as their concentration was very small it is assumed that they were retained by the weak cation resin largely by adsorption forces rather than by an ion-exchange mechanism. The phenomenon of adsorption of organic compounds on ion exchange resins is discussed by Lederer & Lederer.[35]

(4) *Displacement development ion-exchange fractionation of the amino-acids*

As the unwanted flavour introduced by the resin could not be reduced in intensity sufficiently to allow meaningful evaluation of the flavour of fractions obtained by the techniques discussed in section (2) above, the converse approach was adopted, viz., that of increasing the concentration of mushroom flavour components without increasing that of the unwanted flavours.

This was achieved by separating the basic components by a displacement development ion-exchange chromatographic technique which allowed the ratio of mushroom extract to weight of resin to be increased very markedly (sample size approximately equivalent to one half the exchange capacity of the resin). This gave fractions of a much higher flavour intensity and, as the unwanted flavour did not increase, they were suitable for organoleptic evaluation.

The extract from 50 g. of mushrooms was processed through the three series-connected column train using 0·1N potassium hydroxide as displacing base. The nitrogenous constituents were distributed through 87 10-ml. fractions. Later fractions were strongly alkaline and contained negligible amounts of ninhydrin-reactive material.

Each of the fractions was examined by single-dimensional paper chromatography using the ' ammoniacal phenol ' solvent; it could be seen that whilst the resolution of the components was by no means complete, a considerable separation was achieved, the acidic amino-acids being concentrated in the early fractions and the basic amino-acids towards the end of the series.

Selected fractions were submitted to two-dimensional paper chromatography and the components identified. The fractions selected represented major changes in composition as indicated by the single dimensional chromatographic series. Results are shown in Table III.

Table III

Two-dimensional chromatographic examination of fractions displaced by 0·1N *potassium hydroxide*
(Components arranged in decreasing order of spot intensity)

Fraction No. 50	Fraction No. 54	Fraction No. 60	Fraction No. 66
Phosphoethanolamine	Phosphoethanolamine	α-Alanine	α-Alanine
Glutamine	Serine	Serine	Phosphoethanolamine
Tyrosine	α-Alanine	Phosphoethanolamine	Glycine
Glutamic acid	Glutamic acid	Glutamic acid	Serine
Aspartic acid	Threonine	Threonine	Valine + tryptophan
Methionine sulphoxide	Glutamine	Glycine	Leucine + isoleucine
Asparagine	Aspartic acid	Glutamine	Glutamic acid
Serine	Valine + tryptophan	Valine + tryptophan	Glutamine
Threonine	Leucine + isoleucine	Leucine + isoleucine	Tyrosine
Cysteic acid	Tyrosine	Aspartic acid	Cysteic acid
Unknown No. 1	Methionine sulphoxide	Methionine sulphoxide	Aspartic acid
Leucine + isoleucine	Unknown No. 3	Tyrosine	Threonine
Valine + tryptophan	Asparagine	Asparagine	Unknown No. 1
Unknown No. 2	Cysteic acid	Unknown No. 3	Unknown No. 3
	Unknown No. 1	Cysteic acid	α-Amino-n-butyric acid
	Ethanolamine	Unknown No. 1	
	α-Aminoadipic acid		

Fraction No. 72	Fraction No. 78	Fraction No. 84
α-Alanine	Histidine	Arginine[a]
γ-Amino-n-butyric acid	Lysine	Unknown No. 4
Phosphoethanolamine	γ-Amino-n-butyric acid	Unknown No. 6[b]
Leucine + isoleucine	Phosphoethanolamine	Valine + tryptophan
Glycine	Unknown No. 4	Unknown No. 7[c]
Unknown No. 3	α-Alanine	Unknown No. 8[d]
Tryptophan + valine	Methionine sulphone (?)	
Glutamic acid	Arginine	
Unknown No. 1	Leucine + isoleucine	
α-Amino-n-butyric acid	Cystine and/or cysteine	
	Glycine	
	Unknown No. 5	
	Valine + tryptophan	
	Phenylalanine	

[a]Arginine by far the most intense spot. [b]δ-Amino-n-valeric acid? [c]Piperidine-2-carboxylic acid? [d]*N*-Methylhistidine?

The analysis of the fractions for threshold dilution and ninhydrin blue colour is shown in Fig. 1.

Considering, firstly, the ninhydrin blue values, the nitrogenous compounds were recovered quantitatively from the column (99·4%). On the other hand, the total flavour of the recovered fractions greatly exceeded that of the applied solution (154%). Of those fractions, the early ones could not have contained any mushroom components and the flavour intensity determined must have arisen from impurities derived from the column or the regenerating reagents. The average threshold dilution of the first 56 fractions is 19 and, if it is assumed that in all the remaining fractions 19 units of the threshold dilution were due to this background flavour, the total artefact contribution becomes 1653 and the corrected flavour recovery 6037 units or 121%.

Alternatively, if it is assumed that all the mushroom flavour was, in fact, recovered and that the excess unwanted flavour was distributed equally throughout the chromatogram, the contribution to each fraction would have been 31 units. (The quantitative ninhydrin blue value balance lends weight to the validity of this hypothesis.)

The agreement between the two figures (19 and 31) is acceptable when the errors inherent in this type of analysis are considered. In either case, the artefact flavour was insignificant compared to that of the true flavour components contained in the later fractions. It was therefore found possible to draw useful conclusions concerning the nature of the flavour components separated in the chromatogram.

Reference to Fig. 1, which depicts the threshold dilution of the various fractions, indicates a very small flavour peak extending from fraction 40 to fraction 52. These fractions had an acid taste together with the ' chemical ' flavour normally associated with the artefact compounds.

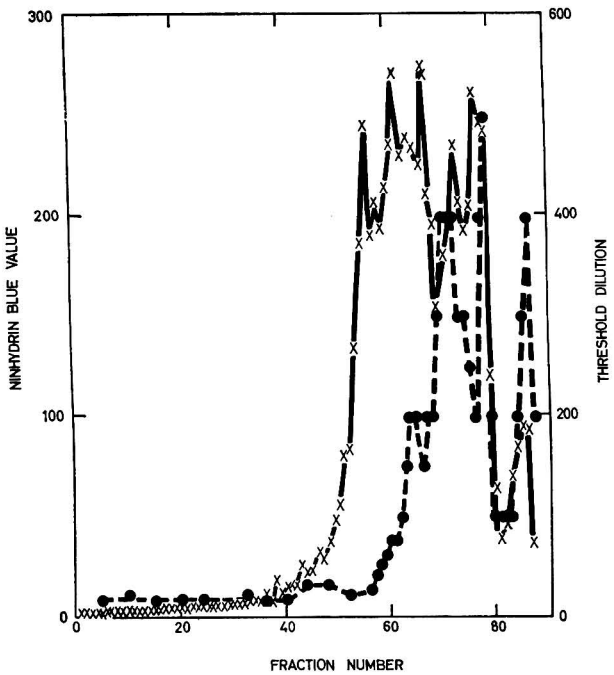
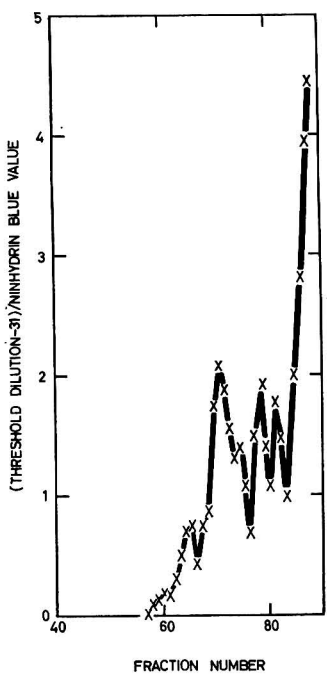


FIG. 1.—*Ninhydrin blue value and threshold dilution of chromatographic fractions*

×——× blue value ●---● threshold distributor



After fraction 56, the flavour intensity rose rapidly and the unwanted contribution from the artefact compounds could no longer be detected qualitatively. As the chromatogram continued through fractions 57 to 78, the flavour character altered progressively from 'acid' through 'sweet' to 'meaty'. At this point the flavour intensity dropped sharply from 500 for fraction 78 to 100 for fraction 80. The intensity remained low to fraction 83 and then gave a final flavour peak before completion elution of the nitrogenous compounds. At the same time, the flavour character altered markedly. The final fractions exhibited a distinctly musty or fungal note which is characteristic of mushrooms.

The contribution to the flavour balance of the compounds contained in these last fractions is emphasised by Fig. 2 in which ninhydrin blue value has been plotted against threshold dilution (corrected for background flavour). It can be readily seen that, in general, the flavour intensity per unit weight of the amino-acids increased with increase in basic strength.

FIG. 2.—*Relationship between threshold dilution, corrected for background, and ninhydrin value*

It was further noted that the determination of threshold dilution of the last fractions was most difficult as the flavour sensation clung tenaciously in the mouth. Only by carrying out the determination several times and by allowing long time intervals (5–10 min.) between successive tastings could a satisfactory threshold dilution figure be achieved. In other words, the flavour contribution of these fractions is of greater significance than simple examination of the threshold dilution figure would indicate.

Conclusions

It seems evident that all the nitrogenous compounds contribute something to the overall mushroom flavour profile. However, the highly basic components are the ones most responsible for the characteristic fungal note.

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THE ORGANIC ACIDS IN TEA PLANTS. A STUDY OF THE NON-VOLATILE ORGANIC ACIDS SEPARATED ON SILICA GEL

By G. W. SANDERSON and R. R. SELVENDRAN*

1. Succinic, oxalic, malic, citric, isocitric, and five unknown acids have been found at appreciable levels (>0·1 μequiv./g. fresh wt.) in tea shoot tips. The major acids quantitatively were oxalic, malic and citric acids. The acids studied were recovered quantitatively from plant extracts, but they accounted for less than one-fourth the total acidity present in tea shoot tips.

2. The acid levels in shoot tips varied quantitatively between clones and between sampling dates, and also both quantitatively and qualitatively between different parts of the tea plant.

3. Changes in the levels of organic acids take place in tea shoot tips during the manufacture of tea which are typical of senescing plant tissues. Notably, the levels of succinic and malic acids are markedly reduced during withering. The level of oxalic acid, however, remains nearly constant throughout manufacture.

4. Studies with $^{14}CO_2$ on intact plants showed that all of the organic acids studied are metabolically active except oxalic acid.

Introduction

The organic acids in tea plants have not been thoroughly investigated, although a number of studies have been reported.[1-11] Most of these investigations have been qualitative studies of the polyphenolic acids, viz., gallic, chlorogenic and *p*-coumarylquinic acids, and theogallin, which occur in appreciable amounts in tea shoot tips.[12]

Interest in organic acids of tea plants stems from their role in respiration and in flavanol biogenesis.[13] Organic acid metabolism during the manufacture of black tea is also of interest because of its possible importance in affecting the quality of the finished product.[14] The paucity of information on this latter subject has been mentioned in a recent review by Forsyth.[15]

This paper reports the results of initial investigations on the organic acids in tea plants. The acids studied here are confined to those which can be separated on silica gel, and their levels in tea plants is reported for the first time. Studies of their metabolism in intact shoot tips and during tea manufacture are also reported. Particular interest was placed in tea shoot tips (flush) comprising the bud, the first two leaves, and the included stem, because it is this part of the plant which is harvested and used in the manufacture of tea.[16,17]

Experimental

Source of plant material

All plant material used in this investigation was collected from a field of clonal blocks of tea near the laboratory (1500 m. elevation). Processing of plant material was begun within 1 h. of harvesting in the field. Unless otherwise stated, all experiments were carried out on material from clone TRI. 2024.

Extraction of acids

Shoot tips (40 g.) were plunged into 150 ml. of boiling 80% (v/v) ethanol for 5 min., cooled, and then macerated for 5 min. in a blendor. The macerate was filtered and the residue was re-extracted 3 times with 100 ml. of 80% (v/v) ethanol for 30 min. on a boiling water bath. It was found that the use of 0·1 N-hydrochloric acid in the extraction media seriously interfered with silica-gel chromatography by causing dehydration of the gel. However, comparative studies showed that organic acids, notably oxalic acid, were quantitatively extracted without the use of 0·1 N-hydrochloric acid in the extraction media.

The combined ethanolic extracts were concentrated to 75 ml. under reduced pressure and clarified by centrifugation at 1600 *g* for 5 min. The clarified extract was passed through a

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cation-exchange column (Amberlite CG–120, H^+ form, 100–200 mesh, 2 × 10 cm.). The column was washed with four bed volumes of distilled water (200 ml.) and the effluent was concentrated to 75 ml. under reduced pressure. This solution was passed through a weak anion-exchange column (Amberlite CG–4B, OH^- form, 100–200 mesh, 2 × 15 cm.). The column was washed with four bed volumes of distilled water and acids were eluted with 125 ml. of 3 N-aqueous ammonia. Treatment with ammonia was important in causing an oxidation of polyphenolic substances which prevented their elution from silica gel. The eluate was evaporated to dryness under reduced pressure, and the residue taken up in 5 ml. of distilled water and stored at − 15° until required for analysis.

Determination of total acidity

Total acidity was determined by titration with 0·1 N-sodium hydroxide to pH 7·0. In the experiment reported in Table III the effluent from the cation-exchange column was divided into two portions, one portion being purified in the usual way and the other passed through a strong anion-exchange column (Dowex 1, formate, 200–400 mesh, 2 × 10 cm.) at the rate of 15 drops/min. The column was washed and the acids eluted with 125 ml. of 7 N-formic acid. The eluate was evaporated to dryness under reduced pressure and taken up in 5 ml. of distilled water for total acid determination. The formic acid which was present as a contaminant was determined by silica-gel chromatography for correction of total acidity values.

Silica gel chromatography

Non-volatile organic acids were separated by chromatography on a silica-gel column and determined by titration as described by Isherwood[18] and Wager & Isherwood[19] with the following modifications.

Sample solutions were made strongly acidic with conc. sulphuric acid before chromatography.[19] Aliquots (1·0 ml.) of sample solution were absorbed onto 1·2 of silica-gel which was transferred to the top of the silica gel column containing 4·4 g. (dry wt.) of silica gel.[20] Stepwise elution was carried out with 75 ml. of 20%, 160 ml. of 35% and 260 ml. of 50% (v/v) n-pentanol in chloroform. Each solvent mixture was equilibrated with 0·5 N–sulphuric acid, filtered, and passed through a column containing 3·0 g. of silica gel mixed with 2·5 ml. of 0·75 M-sodium sulphate in 0·5 N-sulphuric acid before use.

The effluent was collected in 5-ml. fractions; complete separation required about 15 h. Acids were determined by titration with 0·01 N-sodium hydroxide and mixed thymol blue–cresol red indicator.[21]

Recoveries of authentic acids in mixtures are shown in Table I. This method gave good separation, with well-defined peaks, for fumaric, succinic, oxalic, malic, citric and isocitric acids.

Table I

Recovery of authentic acids chromatographed on silica gel

(Results given as μequiv. of acid)

Acid	Acids chromatographed directly[a]		Acids processed alone[b]		Acids processed with shoot tips[c]	
	Added	Recovered	Added	Recovered	Added	Recovered
Fumaric	69·0	67·5 (97·8%)	—	—	—	—
Succinic	84·7	83·9 (99·0%)	50·8	49·3 (97·0%)	84·7	83·0 (98·0%)
Oxalic	158·6	152·3 (96·0%)	111·0	105·5 (95·0%)	111·0	105·5 (95·0%)
Malic	149·5	148·0 (99·0%)	104·7	100·5 (96·0%)	119·6	114·8 (96·0%)
Citric	71·4	69·6 (97·6%)	7·00	6·54 (93·4%)	—	—
Isocitric	71·3	69·9 (98·0%)	—	—	99·9	92·9 (93·0%)

[a] Acids added directly to the silica gel column and chromatographed
[b] Acids in solution were subject to the complete extraction procedure before chromatography
[c] Acids added to one portion of an ethanolic extract of shoot tips. Both portions were then purified and the organic acids were determined in the usual way

Identification of acids

Acids separated by silica gel chromatography were passed through a cation-exchange column, the solution was concentrated, and the acids identified by paper chromatography.[22,23]

$^{14}CO_2$ experiments

Intact shoot tips were treated with $^{14}CO_2$ in the field as described by Hale & Weaver.[24] Shoot tips were exposed to 170 ± 20 μc of $^{14}CO_2$ for 50 min. Two shoot tips were treated in each experiment and they were mixed at the time of sampling with a known weight of untreated shoot tips to make 40 g. in each sample.

$^{14}CO_2$ treatments were begun at 11.00 a.m. and sunlight varied from strong to little due to passing of heavy clouds. Shoot tips were removed for processing 4, 12 and 22 h. after $^{14}CO_2$ treatment in Experiments 1, 2 and 3 respectively. The organic acids were determined as described above.

Radioactivity was determined by drying 0·4 ml. of each fraction from the silica gel column on a planchet and counting as an infinitely thin layer with a thin end-window Geiger counter. The remainder of each fraction was titrated to determine the acid content.

Results

Acids present in tea shoot tips

A typical chromatogram of the non-volatile organic acids in tea shoot tips is shown in Fig. 1. Unknown 1 was identified as being polyphenolic (probably a mixture of acids) from its R_F value on paper, its fluorescence under ultra-violet light which increased after treatment with gaseous ammonia, and its strong absorption at 275 mμ.[12] Unknowns 2, 3, 4 and 5 were not characterised, but chromatography of authentic acid showed that unknown 5 is possibly shikimic acid. Unknown 4 was sometimes not detectable. Succinic, oxalic, malic, citric and

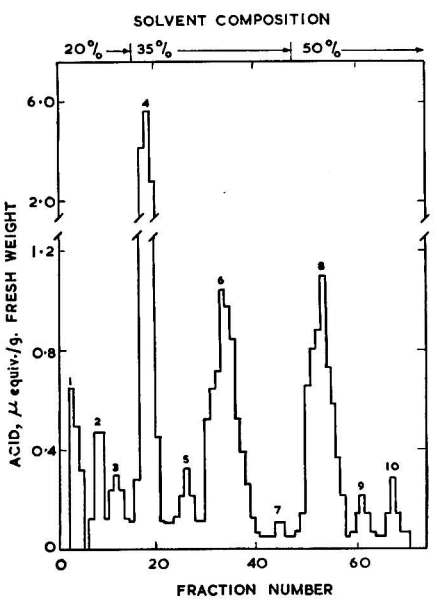

FIG. 1.—*Separation of organic acids in an extract of tea shoot tips*

Solvent 20, 35 or 50 V/v *n*-pentanol in chloroform. Peaks correspond to the following acids: 1 Unknown 1; 2 Succinic; 3 Unknown 2; 4 Oxalic; 5 Unknown 3; 6 Malic; 7 Unknown 4; 8 Citric; 9 Unknown 5; and 10 Isocitric

isocitric acids were characterised by co-chromatography with authentic acids on paper and silica gel. Further evidence that peaks from silica gel chromatography contained only one acid was the finding that acid titration curves and radioactivity curves were coincident.

The levels of the organic acids found are shown in Table II. The large standard deviations shown are due to the large seasonal variations over the period of the investigation; the levels were consistently lower during the latter (monsoon) part of the period covered by this investigation.

Table II

Level of organic acids in tea shoot tips

Acid	Amount of acid*	
	μequiv./g. fresh wt.	mg./100 g. fresh wt.
Unknown 1	1·11 ± 0·46	—
Succinic	1·17 ± 0·81	6·9 ± 4·8
Oxalic	21·58 ± 5·56	97·1 ± 25·1
Malic	5·33 ± 1·44	35·7 ± 9·6
Citric	4·09 ± 2·68	26·2 ± 17·2
Unknown 5	0·44 ± 0·31	—
Isocitric	0·86 ± 0·77	5·5 ± 4·9

* Acid values are based on 9 determinations made on clone TR1.2024 between 20/12/63 and 27/7/64. The mean values and standard deviations are given

Total acidity in tea shoot tips

Typical results are shown in Table III. Passing the crude extract through the cation-exchange column increased the apparent total acidity about sixfold in the first 200 ml. of effluent. All of this acidity could be retained on a strong anion-exchange column, but not on the weak anion-exchange column used in the standard procedure for determining organic acids It was found, however, that additional acidic material could be removed by further washing of the cation-exchange column and that it was not possible to remove all acidic material even with large volumes of wash water (500 ml.). Trials with authentic pectic acid showed that the presence of this substance in tea shoot tips[7, 9] could account for these results. Since the acidic material could not be removed quantitatively from the cation-exchange column, no accurate estimate of total acidity could be made, and the values shown in Table III must be considered to be minimal.

When the weak anion-exchange column eluate was chromatographed on silica gel, the total acidity was again reduced to about one-quarter of the total acidity in the cation-exchange column effluent. The recovery of the organic acids studied from the plant material was, however, quantitative (Table I).

Distribution of organic acids within the tea plant

A survey of the organic acid content of several parts of the tea plant was made with samples of mature bushes collected simultaneously, except for tea seeds which were collected separately. The results (Table IV) showed that different organs were distinctive in their

Table III

Total acidity in extracts of shoot tips at various stages of purification

Fraction examined*	pH	Total acidity (μequiv. acid/g. fresh wt.)
1. Crude extract	5·5	20
2. Effluent from cation-exchange column, first 200 ml.	2·2	117
3. Eluate from strong anion-exchange column (formate)	—	110
4. Eluate from silica gel column	—	30

* Fractionation procedure (described in materials and methods section) was as follows: Crude extract (1) ——→ Cation-exchange column (2) ——→ Strong anion-exchange column (3). Weak anion-exchange column ——→ Silica gel (4)

Table IV

Distribution of organic acids within the tea plant

(Values expressed as μequiv./g. fresh weight of sample)

Acid	Plant part						
	Buds, apical	1st leaf	2nd leaf	Stem from shoot tip[a]	Mature leaves[b]	Feeder roots[c]	Seeds[d]
Unknown 1	2·26	0·82	0·82	1·03	2·33	<0·1	<0·1
Succinic	2·19	1·88	1·73	1·51	2·11	2·03	5·73
Unknown 2	0·30	0·23	<0·1	<0·1	<0·1	<0·1	<0·1
Oxalic	31·92	35·55	32·77	11·97	18·10	3·78	9·12
Unknown 3	0·49	<0·1	<0·1	0·25	0·66	<0·1	<0·1
Malic	4·64	6·13	6·80	3·32	5·80	1·74	11·60
Citric	11·37	6·15	6·52	3·46	15·28	9·41	47·51
Unknown 5	2·61	0·37	0·56	<0·1	4·10	1·77	<0·1
Isocitric	3·73	0·28	0·37	<0·1	0·75	0·65	1·12
Moisture content (% fresh weight)	72	71	72	80	62	71	46

[a] Stem between bud and 3rd leaf of shoot tip
[b] Fully grown leaves from beneath the plucking table
[c] Small fleshy roots growing in top soil, 1–4 mm. diameter
[d] Cotyledons only from mature fruits picked from seed-bearing trees

organic acid contents. Notably, the oxalic acid content of the shoot tips was markedly higher than in other parts of the plant studied, malic and citric acids were most abundant in seeds, and unknown 5 was higher in mature leaves.

Clonal survey

A survey was made of the organic acid content in the shoot tips of six clones of tea. The plants growing in adjacent replicated plots were sampled simultaneously. The organic acid content of the clones examined were qualitatively similar, but quantitative differences were present between clones (Table V). These differences appeared to be unrelated to their quality potential,[25 26] but more results are required for conclusions to be drawn.

Changes occurring during the manufacture of tea

The manufacture of tea is carried out in four distinct stages; namely, withering, rolling, fermenting and firing; [16,17] and it is not until the final stage that the tissues of the shoot tips are killed by heat and desiccation. It is known that the metabolic changes taking place in the plucked shoot tips during manufacture are of importance in determining the quality of the final product,[14 27] and it was therefore of interest to determine the changes taking place in the level of the organic acids.

Table V

Organic acid content of shoot tips from six clones

(Values expressed as μequiv./g. fresh weight. All clones were sampled simultaneously on the same date

Acid	Clone (quality classification)					
	TRI.777 (A_1)	DT.1 (A_1)	TRI.2024 (A_2)	TRI.2025 (B)	TRI.2026 (C)	KEN.16/3*
Unknown 1	0·67	0·88	1·06	1·20	0·80	0·69
Succinic	0·86	1·57	0·60	1·06	1·06	1·07
Unknown 2	<0·1	<0·1	0·27	0·13	<0·1	0·13
Oxalic	14·73	20·82	26·68	27·80	23·32	20·16
Unknown 3	<0·1	0·30	<0·1	<0·1	<0·1	0·15
Malic	10·31	7·33	6·38	9·49	6·63	4·09
Citric	4·88	4·61	6·15	5·88	5·07	4·10
Unknown 5	1·27	0·26	0·36	0·63	0·36	0·34
Isocitric	0·90	0·17	0·18	0·36	<0·1	0·17
Total acids	33·69	35·93	41·68	46·55	37·24	30·89
Moisture content (% fresh weight)	75	79	78	78	77	78

* Not classified

Tea was manufactured under laboratory conditions and samples were taken for analysis as shown in Table VI. It was found that succinic and malic acids decreased to a very low level during 18 h. of withering and remained at a low level thereafter, citric acid varied slightly throughout manufacture, and oxalic acid remained nearly constant throughout this process. Isocitric acid remained at a low constant level.

Changes in acid levels occurring during withering were found to be dependent on time and not on withering itself. This is shown by the similarity of the acid levels in samples 3 and 3x (Table VI) which were stored while being withered (exposed) and not withered (in polythene bags), respectively, for the same period of time.

Table VI

Changes in level of organic acids in shoot tips during manufacture of black tea

(Values given as μequiv./g. fresh weight at the time of plucking the samples)

	Time of sampling (hours from plucking)					
Time sampled, h. after plucking	Sample 1. Freshly plucked flush 0·5	Sample 2. After 8·5 h. of withering 9	Sample 3. After 18·5 h. of withering 19	Sample 3x. After 18·5 h. of storage with no withering 19	Sample 4. After 2·5 h. of fermentation 21	Sample 5. After firing 21·5
Acid						
Unknown 1	0·86	1·29	0·38	0·53	0·45	0·68
Succinic	1·65	1·34	0·58	0·58	0·33	0·58
Oxalic	25·69	25·13	26·86	27·76	26·97	28·14
Malic	6·31	2·68	0·73	0·91	0·73	0·64
Citric	3·40	7·68	1·74	2·04	2·14	4·39
Unknown 5	0·53	0·39	0·82	0·41	0·41	1·23
Isocitric	1·75	1·36	1·43	0·92	1·74	1·84

Metabolism of organic acids in intact shoot tips

The metabolism of the organic acids under study was investigated by use of $^{14}CO_2$. Shoot tips were allowed to grow in the presence of $^{14}CO_2$ and the distribution of radioactivity in the organic acids at various times after exposure to $^{14}CO_2$ was determined. The organic acids contained almost 10% of the total soluble radioactivity 4 h. after $^{14}CO_2$ treatment (Table VII). The amount of radioactivity found in the organic acids decreased with time from treatment, until after 22 h. only half the 4-h. level remained in this fraction. There was some evidence for translocation, and conversion to insoluble cell constituents, of labelled soluble compounds with time, but the design of the experiment did not permit an estimation of the magnitude of these changes.

As shown in Table VIII, malic and citric acids are the most heavily labelled acids 4 h. after exposure to $^{14}CO_2$ (Experiment 1). It was found in other experiments that the shorter the period of time between $^{14}CO_2$ treatment and sampling, the greater was the relative labelling

Table VII

Distribution of radioactivity in soluble fractions obtained during purification of organic acids

(Radioactivity as percentage of total counts in soluble fractions)

Soluble fraction	Expt. 1 4 h. on treated plant	Expt. 2 12 h. on treated plant	Expt. 3 22 h. on treated plant
1. Eluate from cation-exchange column (amino-acids, etc.)	8·3	12·1	10·7
2. Effluent from anion-exchange column (carbohydrates, polyphenols, etc.)	81·9	85·5	84·2
3. Eluate from anion-exchange column (organic acids)	9·8	5·4	5·1
Total soluble counts/min./g. fresh weight	557,000	254,500	170,000

Table VIII

Distribution of radioactivity in organic acids of shoot tips at various times after fixation of $^{14}CO_2$
(The experiments are the same as those in Table VII)

Acid	Acid content (μequiv./g. fresh weight)			Radioactivity (counts as % of counts in acid fraction)			Specific activity (radioactivity/acid content)		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
Unknown 1	1·63	0·71	0·84	3·45	5·02	9·95	2·12	7·05	11·80
Succinic	1·04	0·17	0·58	5·74	1·91	<0·5	5·52	11·57	—
Unknown 2	0·39	<0·1	<0·1	0·68	3·46	4·44	1·74	—	—
Oxalic	13·04	12·18	18·26	3·75	5·37	6·65	0·29	0·44	0·36
Unknown 3	0·33	<0·1	<0·1	2·00	4·68	5·13	6·08	—	—
Malic	5·07	2·43	3·86	32·62	28·74	17·40	6·43	11·85	4·51
Citric	4·15	1·58	2·18	47·52	24·97	26·76	11·44	15·81	12·29
Unknown 5	0·16	0·13	0·13	1·18	2·77	8·90	7·19	20·67	66·42
Isocitric	0·39	0·26	0·13	2·60	11·98	2·74	6·63	46·43	20·45

in malic acid. For example, 1·5 h. after $^{14}CO_2$ treatment malic acid contained 64·0% of counts in the organic acid fraction compared with only 10·5% in citric acid.

The results of these experiments (Table VIII) show that a marked redistribution of radioactivity takes place with time. The level of activity in succinic acid decreases steadily and disappears completely after 22 h. The activity of isocitric acid increased in the night (Experiment 2), whereas in the daytime (Experiments 1 and 3) the activity of this acid was low. Malic acid shows similar but less marked changes. It is noteworthy that the specific activity of unknown acids 1 (polyphenolic acids) and 5 (probably shikimic acid) increases appreciably and steadily up to 22 h. after $^{14}CO_2$ treatment. Oxalic acid appears to be very unreactive, as it was found to change only slightly in amount and in level of labelling with time.

Discussion

The non-volatile organic acids found in tea shoot tips in this investigation agree fairly well with those previously reported. Bhatia[1] reported the presence of fumaric, succinic, malonic, citric, oxalic and malic acids. We found that fumaric acid co-chromatographed, on silica gel and on paper, with unknown 1 which was found to contain a mixture of polyphenolic acids, but the results do not exclude the possibility of low levels of fumaric acid also being present. The presence of malonic acid is uncertain as yet. This acid co-chromatographs with unknown 2, but other carboxylic acids also co-chromatograph with malonic acid[19,28] and a positive identification has not been made. Attempts to find tartaric acid gave negative results contrary to an earlier report by Bhatia & Chanda.[2] The presence of quinic acid in appreciable amounts reported by Bokuchava & Soboleva[4] was not confirmed. In addition to acids reported by other investigators, we found isocitric acid to be present in tea plants at an appreciable level.

The large variation in the level of organic acids in tea shoot tips with time (Table II) is probably related to changes in climatic conditions, but further investigation is required to determine what climatic factors are involved. Variations of similar magnitude have also been reported for other constituents of tea shoot tips.[29–31]

The acids studied in this investigation represent less than one-fourth the total acidity in tea shoot tips (Table III). Pectic acid has been reported to be present at levels varying from about 0·3% to about 0·2% fresh weight[7,9] and it must therefore account for a large part of the undetermined acidity. Volatile acids, which were not determined, and polyphenolic acids, which were probably not determined quantitatively, may also be important.

Oxalic acid is the major acid present in tea shoot tips (Tables III–V), but it has low metabolic activity as shown by its small changes during withering (Table VI) and its slow rate of incorporation of radioactivity from photosynthetically assimilated $^{14}CO_2$ (Table VIII).

Succinic and malic acid levels are markedly reduced during the 18-h. withering stage of tea manufacture (Table VI) and their high metabolic reactivity is indicated by the changes in specific activity of these acids with time in intact shoot tips (Table VIII). Citric acid is heavily labelled 4 h. after exposure to $^{14}CO_2$ and the level of activity remains high over the period studied (22 h.). Some possible relationships between these changes and changes in other constituents are discussed elsewhere.[32]

The manufacture of tea is largely a physiological process involving senescing tea shoot tips[15] which is regulated to some extent by mechanical manipulation[16,17]. Changes in the level of organic acids in tea shoot tips during this process, especially during withering, appear to be typical of senescing tropical and sub-tropical tissues.[33] The importance of these changes, and other physiological changes taking place during withering,[14,32,34-36] in determining the character of the finished product (black tea) has recently been discussed.[14]

The marked increase in labelling of unkown 5 with time in $^{14}CO_2$ experiments (Table VIII) is of particular interest because it is likely that this acid is shikimic acid, a key intermediate in flavanol biogenesis.[13] The slower concomitant increase in labelling of unknown 1, which was identified as being polyphenolic, is also suggestive of a biogenetic relationship between the two acids. The relatively high levels of unknown 5 in mature leaves and buds (Table IV) are of interest in connexion with the site of flavanol synthesis in tea plants.[37] This aspect of the work deserves special consideration in future investigations.

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PROTEIN DENATURATION IN FROZEN FISH. X.*—Changes in cod muscle in the unfrozen state, with some further observations on the principles underlying the cell fragility method

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A study was made of the changes in the muscle proteins that occur when cod are kept in ice, using both protein extractability in salt solution and ' cell fragility ' values as criteria. The results by the two methods did not agree, and the reason for this is discussed with the aid of photomicrographs of homogenates of cod muscle. It appears that changes in extractability are the consequence of a binding together of structural protein molecules and perhaps myofilaments, while a binding together of myofibrils is the agent causing changes in cell fragility readings. Cold storage changes the fragility of the cells (breakdown to fibril level), while bacterial action during stowage in ice changes the fragility of the myofibrils.

Introduction

It has already been shown[1] that the phenomena associated with the onset and resolution of rigor mortis can alter the properties of the proteins of cod muscle. The effects of keeping fish for much longer periods at around the freezing point are not so well established, however, since the published information from other laboratories is scanty and leads to opposing conclusions. Dyer[2] in 1953 reported that the initial freshness of the fish did not affect the extractability of the actomyosin (decrease of extractability is one of the criteria of ' denaturation ') but that, after freezing, the extractability decreased as the pre-frozen storage time progressed. On the other hand, Luijpen,[3] using the same criterion, found that cod frozen after ' rather advanced spoilage ' denatured in the cold store more slowly and to a lesser ultimate extent than cod frozen in the fresh condition. He postulated that the action of bacteria and enzymes had decomposed the protein to such an extent that it was less liable to denaturation. In spite of this, Tokunaga & Nakamura[4] took it ' as a matter of course ' that the rate of freezing denaturation would be greater in stale than in fresh fish.

Measuring the salt extractability of fish muscle protein after 0 and 16 days in ice, Moorjani *et al.*[5] found that some species showed a slight decrease during this time, while others did not: one species still showed a high extractability after 28 days in ice. These authors also found no significant increase in the non-protein nitrogen of the muscle during the 16 days in ice, which indicated no bacterial or autolytic breakdown of the proteins.

The present work was undertaken because of the unsatisfactory state of the published evidence. Since it appeared likely that any changes occurring during stowage in ice would be fairly small, the stowage was prolonged beyond the customary time (usually the limit of edibility of the fish) to enhance any effects that there might be.

Experimental

Material and methods

Immature North Sea cod (*Gadus morhua*, L.)[6] caught by trawl net about 30 miles S.E. of Aberdeen were used throughout the work.

Changes occurring in the muscle protein were assessed either by measuring the decrease of extractability of the homogenised muscle by 5% sodium chloride,[7,8] or by measuring the decrease of fragility of a population of individual muscle cells (the ' cell fragility method ').[9,10] Readings by the latter method are in the form of optical density, a decrease of which signifies a change in the protein from the native state.

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For pre-freezing treatment the whole fish, with guts removed, were packed in an excess of crushed ice in wooden boxes and kept at an ambient temperature of 4°, so that the ice melted continuously but not excessively. The fish were re-iced as necessary.

Results

Studies using protein extractability

The effect of time in ice on the extractability of the protein was studied in the first experiment. Fish caught 14.8.62 were used, and the results (Fig. 1) show a clear decrease with time, in spite of considerable scatter in the experimental values. Each point is the mean of duplicate determinations on the pooled tissue from 6 fish, dissected as described previously.[7]

A few earlier observations (1.10.60) had appeared to show that a different relationship applied after cold storage for a period, so it was decided to carry out a comprehensive experiment in which the protein extractability of fish of different degrees of staleness would be measured before freezing and also after various periods of cold storage.

The pooled tissue from 6 fish (caught 18.10.62) was used for each experimental point, duplicate determinations being carried out and averaged as before. The findings are illustrated in Fig. 2. It can be seen that the extractability of the protein of unfrozen fish declined from 94% to about 70% over 32 days (cf. Fig. 1), although again there was considerable scatter. The slope of the regression line drawn through the points is — 0·712 units per day in ice, and the effect is significant at the 5% level. Freezing and thawing without storage resulted in essentially the same pattern at a lower solubility, but the slope of the regression line had been reduced to — 0·482 units per day. After storage at — 14° for 4 weeks, the slope was reduced still further to — 0·381 units per day, and, most strikingly, the downward slope vanished altogether after 8 weeks at — 14°, so that the stalest fish were probably less denatured than the freshest. The slope of + 0·188 units per day is not significant at the 5% level.

Studies using the cell fragility technique

Stowage of fresh fish in ice.—It was early observed, using fish caught 22.10.58, that the optical density of homogenates obtained in the cell fragility technique[9] decreased from 0·623,

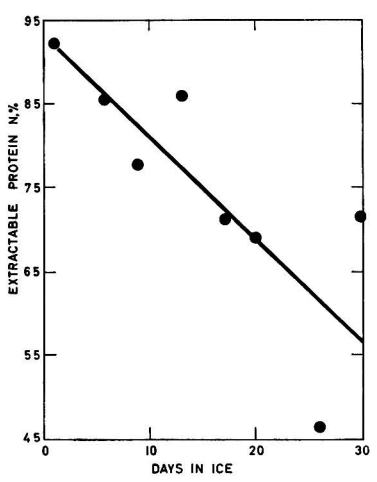


FIG. 1.—*Effect of keeping whole cod in ice on the percentage of total protein nitrogen that will dissolve in 5% NaCl*

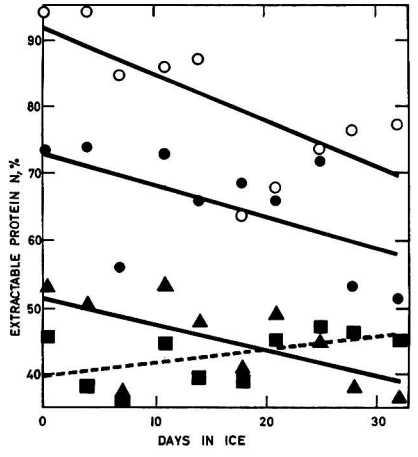


FIG. 2.—*Extractability of cod muscle proteins in 5% NaCl after fish of various degrees of staleness are frozen and cold stored*

○—○ Unfrozen
●—● Frozen and thawed without storage
▲—▲ Stored for 4 weeks at — 14°
■—■ Stored for 8 weeks at — 14°

when the fish had been 14 days in ice, to 0·304 after 23 days. The values remained fairly steady up to 14 days after rising from the low level characteristic of pre-rigor mortis muscle, so the phenomenon was attributed to a fault in the homogeniser. However, an extended repetition of the work (fish caught 9.12.58) led to closely similar results which are illustrated in Fig. 3. Each point is the mean from 10 fish, each determined in duplicate.

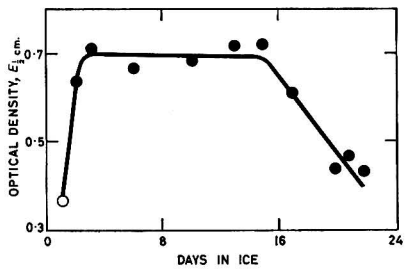


FIG. 3.—*Effect of time in ice on the cell fragility values (read as optical density) of cod muscle*

○ Fish in full rigor
● Post-rigor

The low value 16 h. after death is again the result of rigor mortis phenomena, which have been described in detail in an earlier publication.[1] The lag period was longer than in the first experiment, no clear decrease in optical density occurring until 17 days had elapsed. During this period the values were lower than are customarily found nowadays in fresh fish (about 0·9) because a 5-sec. homogenisation time was used in this and the earlier experiment, instead of 30 sec. which became standard practice later.

Two factors have so far been observed to cause a decrease in the optical density of homogenates of cod muscle obtained under standard conditions. (1) Cold storage for increasing periods causes the cells of the thawed fish to develop an increasing resistance to breakage by the homogeniser, so that homogenates of low optical density, consisting of suspensions of small numbers of unbroken cells in a clear liquid, are obtained. (2) When the fish are starved severely, the protein content of the muscle falls, so that there is less proteinaceous material in a homogenate to scatter the incident light during measurement. The appearance of such a homogenate is characteristic, the protein suspension having a ' misty ' appearance, as though it were less coagulated by the formaldehyde than usual.[9] Few, if any, intact cells can be seen. Photomicrographs of homogenates representing each type of change are shown in Figs. 6–8.

The decrease in optical density shown in Fig. 3 coincided with the appearance of ' pale mistiness ' in the homogenate, although a very few whole cells or large fibrillar aggregates were sometimes seen when the fish had been kept for over 30 days in ice.

The effect of changing the rate of bacterial activity.—For this work, five pieces of muscle, each weighing about 50 g., were taken from the anterior halves of each cod fillet. Ten such pieces, each from a different fish, constituted one ' sample ', which was wrapped in aluminium foil and stored in a cabinet maintained at 0°. Single determinations of cell fragility were carried out on each piece of the sample every two or three days, and averaged.

Ten ' samples ' of fish caught 21.3.62 were stored in this way without further treatment, as controls. A further 10 samples (100 pieces) from the same batch of fish were smeared all over with the slimy muscle of very stale fish, so that they were heavily contaminated with spoilage bacteria.

The results of the cell fragility determinations are shown in the lower two curves of Fig. 4. The decrease in optical density of the controls occurs after only 8 days, which is earlier than in the experiment illustrated in Fig. 3, but the decrease occurs earlier still in the heavily contaminated group—indeed, the lag period has disappeared altogether. This suggests that the decrease is the result of bacterial activity. The decline in optical density in both cases was due to an increasing translucence in the homogenates, and not to an increase in the number of intact cells in suspension.

As a further check, 10 samples, this time from fish caught 14.6.62, were wrapped and stored as before, after the individual pieces had been immersed in 100 p.p.m. chlortetracycline for 30 sec. and drained. The aluminium foil was also treated in the same way.

The results of this treatment, which inactivates most of the spoilage bacteria, are shown in the upper curve of Fig. 4: the onset of the decline in optical density is greatly retarded, and

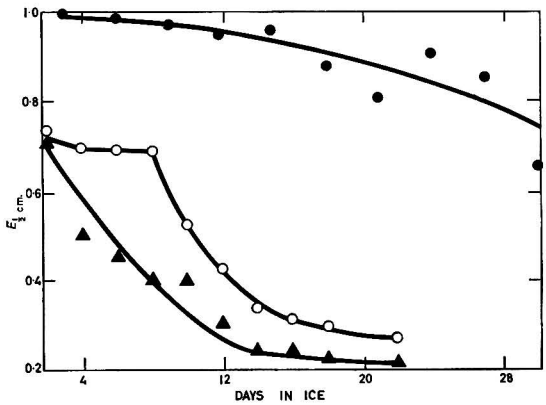


FIG. 4.—*Effect of accelerating and retarding bacterial spoilage on the cell fragility values of unfrozen cod kept at 0°*

○—○ Untreated controls
▲—▲ Heavily contaminated with spoilage bacteria
●—● Treated with antibiotic to retard bacterial activity

it is less marked when it occurs. It will be noticed that the initial values of the uppermost curve are higher than in the other two. This is probably a seasonal effect—the fish for the lower two curves were caught at the time of spawning, when even immature fish suffer emaciation from lack of food.[11] The fish for the upper curve, being caught in June, would be fully recovered and have a higher protein content.

It is clear from these experiments that the effect illustrated in Fig. 3 is, at any rate partly,

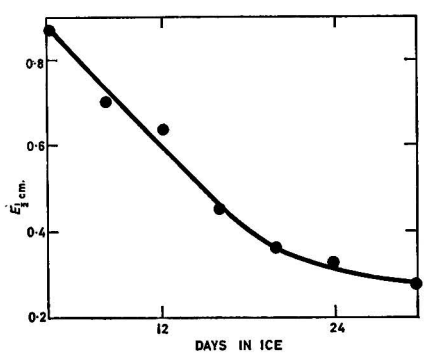


FIG. 5.—*Cell fragility values of cod which were frozen to −14°, thawed, and then stowed in ice*

caused by spoilage bacteria. That it becomes measurable after the fish have been in ice for about 2 weeks accords with general experience: bacterial activity, as measured by various criteria, only becomes marked after such a period.[12]

The shorter lag period in the controls (middle curve, Fig. 4) as compared with Fig. 3 probably reflects the heavier initial load of bacteria carried by the small pieces of fish, which

arises from contamination during cutting up, whereas the whole fish used in Fig. 3 are internally sterile for several days after death.

Stowage of fish in ice after freezing and thawing.—Cod fillets were individually wrapped in aluminium foil and frozen by leaving in a room at − 14° for 15 h. The fish had been filleted 3 days after capture. They were then unwrapped and kept in a room at 20° until soft, after which they were wrapped in two layers of aluminium foil and packed in crushed ice. Cell fragility determinations were carried out on 5 fillets every 4 days, and the results are shown in Fig. 5.

There is no indication of a lag period as compared with Figs. 3 and 4, and an examination of the homogenates by eye showed that the mechanism responsible for toughening during the cold storage of cod fillets had been triggered off by the freezing and thawing; the proportion of myofibrillar aggregates and whole cells steadily increased for the first 2 weeks of storage in ice, causing the optical density to fall. Thereafter, the bacterial activity asserted itself, and the homogenates became less dense (' misty '), though still containing some intact cells.

Visual observations on the homogenates

Earlier discussion of the cell fragility method[9] was vague as to which fraction of the tissue gave the characteristic high optical density when fresh cod muscle was homogenised in formaldehyde. The following experiments were designed to answer this question.

Sarcoplasmic and extracellular proteins.—The fillets from a cod 1 day after death were skinned and passed once through a domestic mincer. The minced material was centrifuged at 2300 *g* at 0° for 45 min., and the supernatant fluid decanted off. It was assumed that this fluid would contain a high concentration of extracellular protein and perhaps sarcoplasmic protein as well, but no fibrillar proteins. The fluid was divided into two portions. To one was added an equal volume of 2·2% formaldehyde at 0°, and to the other an equal volume of chilled distilled water. The concentration of formaldehyde in the first sample was now the same as that of a homogenate from a cell-fragility determination.

No precipitation occurred immediately in either sample. After being kept for 5 min. at room temperature, the sample containing formaldehyde was still clear, but a light precipitate had formed in the sample diluted with water.

This was a most important finding, since it indicated that variations in the apparent coagulation of the ' cell fragility ' homogenate, which corresponded with variations in the optical density (e.g., Fig. 3), were the result of alterations in the behaviour of the structural sub-units of the cell and not of the soluble constituents.

Myofibrils and myofilaments.—Fig. 6 shows the appearance of part of a homogenate, made under the standard conditions of the cell fragility method,[9] of the muscle of cod 2 days after death. The preparation was newly made, unstained, and is shown under phase-contrast. The notable fact about this preparation is its ' cleanness '—it appears to consist of intact myofibrils and very little else, thus confirming that the soluble constituents of the cell play little, if any, part in the optical properties of the homogenate.

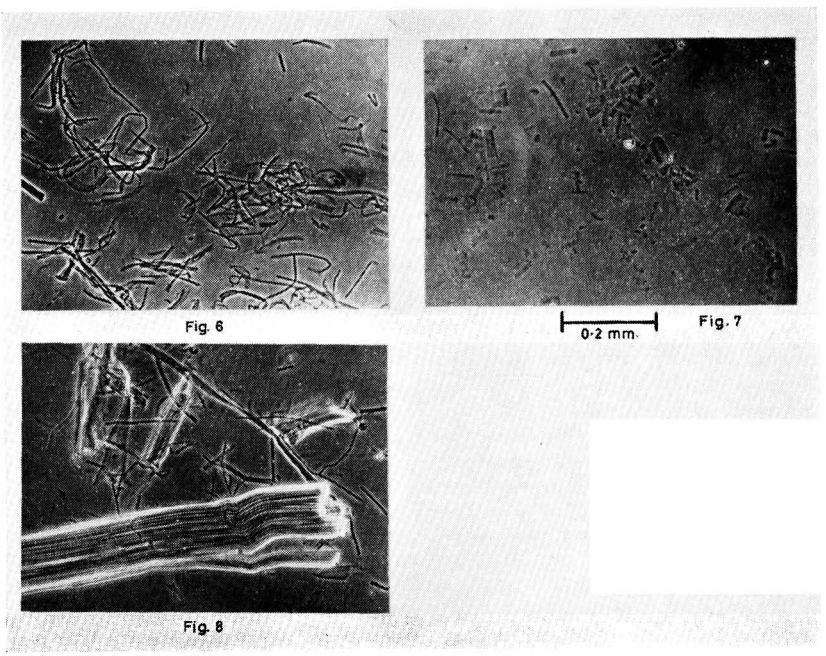
Fig. 7, made and photographed under the same conditions, represents cod muscle after the whole fish had been kept in ice for 30 days. The optical densities of the two homogenates were 0·84 for the fresh fish and 0·33 (cf. Fig. 3) for the stale, each being the average of 3 determinations. The reduced contrast of Fig. 7 as compared with Fig. 6 is real.

The two figures show that the effect of keeping cod in ice is to deplete or degrade the protein of the myofibrils, and thus to weaken them mechanically, so that they are chopped into shorter lengths by the homogeniser. The breakages are almost always clean and at right angles to the long axis of the fibrils. It is possible that they are the result of a weakening of the Z-band material. The revelation by the cell fragility technique of the relative mechanical strength of the fibrils is an unexpected outcome of this experiment.

For comparison, a homogenate is shown in Fig. 8 where the optical density was reduced to 0·35 by cold storage at − 9° for a few days. The magnification is the same as in Figs. 6 and 7, and the linking together of some of the myofibrils can be clearly seen. Some separated myofibrils are also present, since the cold storage changes were not complete in this preparation.

After prolonged storage in the frozen state the homogenate would consist entirely of unbroken cells.[9]

These results may appear confusing. When the cellular material is reduced to the smallest



FIGS. 6-8.—*Homogenates of cod muscle obtained by the cell fragility method (phase-contrast, unstained)*

FIG. 6: Fish 2 days after death (control). FIG. 7: After 30 days in ice, showing transverse breaking of the myofibrils
FIG. 8: After cold storage at −9° showing some aggregation of the myofibrils

fragments of all (Fig. 7) the optical density is low. Larger fragments (Fig. 6) yield a higher optical density, but the optical density of the largest fragments (Fig. 8) is again low.

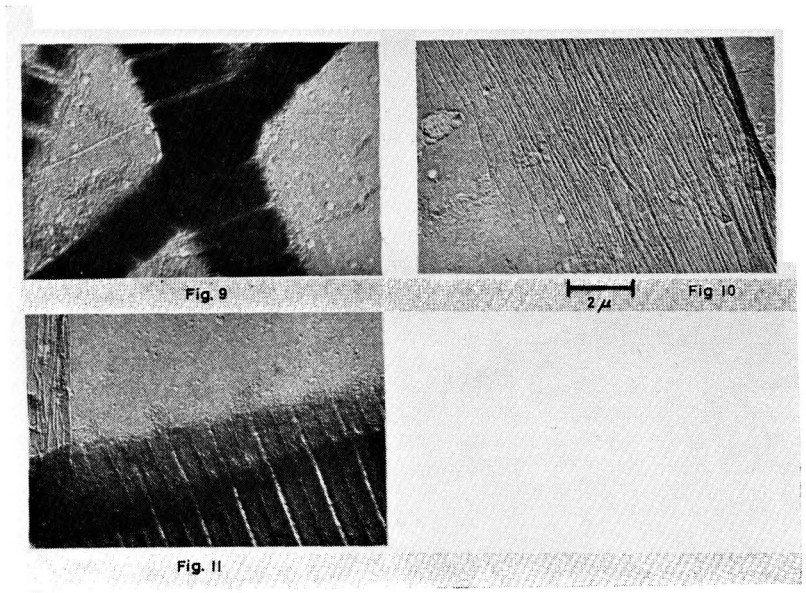
Clearly, it is the increasing preponderance of transparent suspending fluid which causes a drop in optical density during cold storage (i.e., in the largest fragments). The cause of the low optical density of Fig. 7, however, is probably the partial solubilisation of structural proteins—as already stated, the fibrillar bundles show less contrast under the microscope. It is possible in addition that the length of the fibrils plays a part, since in fresh fish the fibrils are long enough to become entangled and matted together, giving rise to floating ' mattresses ' of high opacity, a phenomenon absent from the homogenates of stale fish (Fig. 7).

The electron photomicrograph, Fig. 9, confirms that the homogenates of fresh muscle are essentially intact myofibrils plus some amorphous protein. A few clumps of myofilaments have sometimes been seen, but these are exceptional.

Homogenates of cod muscle prepared with a Waring blendor in potassium chloride–borate buffer (0·06M) was similarly shown by Miss D. P. Young in this Research Station (unpublished) to consist of intact myofibrils surrounded by non-filamentous material.

On the other hand, myofilaments abound in the homogenate after 30 days in ice. Clearly, only a very small part of a homogenate at high magnification can be illustrated here, but after a detailed examination in which many free myofilaments or clumps of myofilaments were seen, it was decided to use two pictures for illustration. Fig. 10 shows a myofibril from muscle 30 days in ice which has been largely degraded. The myofilaments have been spread out, and

on the left-hand side of the picture they are almost free. Fig. 11 shows part of an intact myofibril from the same preparation, bounded by the typical amorphous protein, but with some clumps of filaments lying across it.



FIGS. 9-11.—*Homogenates of cod muscle obtained by the cell fragility method as seen under the electron microscope, shadowed with Au–Pd*

FIG. 9: Fresh fish, showing intact myofibrils and amorphous proteinaceous material
FIG. 10: After 30 days in ice, showing disintegration of a myofibril
FIG. 11: As in Fig. 10, showing filamentous material lying across a myofibril

Discussion

In the foregoing account, for the first time a serious discrepancy has been found between the results as measured by protein extractability (Fig. 1) and by cell fragility (Fig. 3). Previously, in studies on frozen cod[9,10] they had shown close agreement. From the microscopical evidence, the explanation appears to lie in the nature of the structural units involved in the two methods

The changes which gradually occur in the extractability of frozen—and probably unfrozen—fish are thought, from ultracentrifuge and other evidence, to be the result of crosslinkages forming between the myofilaments themselves, although the original configurations of the protein molecules do not seem to alter much during the process.[13] The number of linkages necessary to cause measurable loss of extractability is not at present known. However, Figs. 6 and 8 show that any such changes on a molecular level would probably not affect observations made by the cell fragility method, since the smallest unit involved here is the myofibril. The only conceivable mechanism whereby the myofibrils might be encouraged to stick to each other through changes in the myofilaments is through their becoming more rigid and therefore putting less strain on cementing material while under mechanical stress. Otherwise it seems most likely that the progressive cohesion between adjacent myofibrils, which causes a decrease in optical density, results from an increase in the strength of the sarcoplasmic reticulum. If therefore inextractability in 5% sodium chloride is solely the result of cohesion at the molecular or myofilament level, it is obviously possible for muscle to decrease in solubility while not changing in cell fragility value. This occurs in Figs. 1 and 3, where for the first 14 days in ice a steady decrease in solubility was not matched by any change in cell-fragility reading.

The near-perfect coincidence of reaction kinetics between protein extractability changes and cell-fragility readings during the cold storage of cod at various temperatures[10] appears actually to have been fortuitous, relating as it does to two completely different phenomena. Recent work has shown that the rates of the two reactions do *not* coincide in other species of frozen fish,[14] nor in fish frozen after treatment with sodium tripolyphosphate.[15] These discrepancies do not, of course, invalidate the cell-fragility method as a practical means of measuring changes during the cold storage of other species, since smooth curves of optical density against storage time can be obtained from many species of fish.

In the cell-fragility technique the muscle is homogenised in very dilute formaldehyde solution rather than water, because in fresh fish a higher optical density results, leading to greater sensitivity for the method. In the early work[9] this action of formaldehyde was visualised as a coagulation of the ' proteins ' liberated from the cell by homogenisation, but, since homogenates prepared in water resemble Fig. 7, it is now clear that the formaldehyde acts by preserving the integrity of the myofibrils through its slight hardening influence.

Let us now examine the development of insolubility in iced fish (Fig. 1) and see how it differs from that in frozen fish. Freezing causes profound changes to occur in tissue. Water molecules, which were distributed between the muscle cells, the myofibrils and the myofilaments, associate into relatively large masses as ice, so that the various structural units become displaced and often pushed into close proximity with each other. The increased concentration of protein probably resulting from dehydration of this kind would clearly favour the side-to-side aggregation of structural protein molecules, leading to insolubility as already discussed. It is therefore not surprising to find that at 0°, where the tissue elements are not so crowded together, the rate of development of insolubility is considerably slower than at, say, — 1° in the frozen state,[16] although there is only 1°C of difference between them.

Close proximity, however, is not the only factor involved. Olley & Lovern[17] showed that the release of free fatty acid (FFA) from phospholipid in frozen cod muscle stored at three different temperatures closely resembled in form the development of insolubility of actomyosin.[10] and recent research by Steinberg's group[18–20] has provided more evidence of the important role of FFA in actomyosin insolubilisation.

A further factor intimately involved is the concentration of the tissue salts, which increases through the freezing out of tissue water. The importance of salt concentration in this connexion was recognised many years ago,[21 22] but recent findings suggest[23] that it in fact works in conjunction with FFA, its role being to dissolve the structural proteins and so bring them and the fatty acids into more intimate contact.

Now the changes in the solubility of fish kept in ice do not fit into this scheme. According to Figs. 1 and 2, the extractability of the proteins decreases in a steady, progressive manner right from the time of death, as far as can be judged. In contrast to this, very little FFA is released in unfrozen cod stowed in ice for the first 10 days,[24] but then it increases rapidly, reaching its maximum after about 35 days. By analogy with the frozen tissue, one would have expected the structural proteins to have become completely insoluble after this time, but extrapolation of Fig. 1 shows that about 50% of the protein nitrogen is still soluble. These two discrepancies suggest that, if indeed FFA does make proteins inextractable in frozen muscle, it does not exert the same influence in the unfrozen state, but requires the presence of concentrated salts, as already suggested,[23] and maybe mutual juxtaposition of the protein elements as well.

Possibly the slow insolubilisation of cod muscle protein at 0° is spontaneous and independent of FFA concentration, much as protein extracts from cod muscle will slowly aggregate at 0° in dilute solution.[25] The appearance of Fig. 7 suggests, however, that some kind of degradation is going on at the same time (as postulated by Luijpen[3]), and it is therefore improbable that the protein extractability, reduced to around 50% after 35 days in ice, would ever drop to the usual lower limit of 28% observed after prolonged frozen storage[8] of fresh cod.

The breakdown of proteins during spoilage is reproducible enough to have been adapted as a quality test. Kurtzmann & Snyder discovered that an ethanol extract of shrimp[26] or crab[26] muscle during the development of spoilage gave more and more turbidity (which could

be measured) after picric acid was added. Picric acid is a more powerful protein precipitant than is ethanol, so it could precipitate the increasing proportions of protein fragments broken off larger molecules by the bacteria.

What, then, of the controversy in the literature, stated in the introductory section?

It seems likely that the considerable scatter inherent in the protein solubility method may have concealed the phenomena described in this paper from some workers, but in addition to this is the fact that the rate of bacterial penetration into fish muscle after death is a notoriously variable quantity, which may vary in different parts of the world and in different seasons.

Conclusions

Protein extractability slowly falls when fish are kept in ice. This is due to an aggregation of the structural proteins at the molecular level which appears to be uninfluenced by free fatty acid formation, and which shows no sudden break with the advent of bacteria after the second week. Some of the structural proteins are, however, altered in some way, so that in stale fish which is subsequently frozen they appear to aggregate less readily than in fresh fish.

An increase in the ability of the muscle cells to withstand disruptive forces, which is a known characteristic of cold storage in fish, is now envisaged as an increase in the strength of the sarcoplasmic reticulum or other cementing agent. Cell fragility can change independently of protein extractability, as can the fragility of the myofibrils, which, in contrast with protein extractability, is influenced by bacterial action. During the early stages of spoilage, when bacterial penetration is slight, myofibrillar fragility does not change, but increased bacterial activity rapidly weakens the myofibrils so that they are easily broken under the standard conditions. Extracellular and sarcoplasmic proteins appear to play no part in the 'cell fragility method', and in studies by this method on cold-stored fish the structural units involved are whole cells and myofibrils: very little further degradation to myofilaments takes place.

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STUDIES ON RUMEN METABOLISM. IV.*—Effect of carbohydrate on ammonia levels in the rumen of pasture-fed cows and in rumen liquors incubated with ryegrass extracts

By J. A. ROBERTSON and J. C. HAWKE

Paired feeding of rumen-fistulated cows showed that 7·4–14·8% increases in nitrogenous constituents in pasture led to higher maximum concentrations of ammonia in the rumen 2–4 h. and higher minimum values 6 h. after feeding. Infusion of 500 g. of starch into the rumen was not always effective in lowering ammonia content to the control levels when additional nitrogen, equivalent to about 130–160 g. of protein each feeding period, was included as a pasture constituent. In general, 900 g. of starch in similar conditions led to ammonia levels of the same order as recorded in the controls. Infusion of starch did not increase VFA concentrations.

Incubation of rumen liquor with ryegrass extract gave rapid production of ammonia. This could be largely prevented by the addition of galactose, sucrose, lactose and glucose. The results suggest that one of the factors that can limit the utilisation of pasture protein is likely to be the amount of soluble sugars and other readily hydrolysed carbohydrate available in the pasture.

Introduction

Because the type and the relative amounts of dietary protein and carbohydrate regulate the ammonia concentration and presumably the extent of microbial synthesis in the rumen,[1] the high concentrations of ammonia frequently found in the rumen of animals grazing on pasture has led to speculation on the efficiency of utilisation of nitrogenous constituents of pasture species.[2] The concentrations of sugars in grasses and clovers are influenced by factors such as stage of growth and climate,[3–5] and accompanying fluctuations in the levels of protein and other nitrogenous constituents result in considerable variation in the soluble carbohydrate/protein ratio throughout a growing season, with low values in early spring and in late autumn when growth is rapid.[6]

The significance of the variation in the protein level during a growing season is difficult to ascertain in a seasonal study because of the introduction of uncontrollable factors. Furthermore, the influence of the physical and chemical properties of a protein on its rate of utilisation makes supplementation of pasture protein with protein from another source an unreliable guide to the effects of variations in the level of protein in pasture on rumen digestion. Paired feeding experiments in which the protein intake was varied by growing fresh fodder with different nitrogen levels, provided a method for simulating natural variations in pasture protein and examining the effects of carbohydrate supplementation. Starch was infused into the rumen to examine carbohydrate supplementation, because it had been found greatly to increase the rate of disappearance of ammonia in the rumen of cows on hay and concentrate diets and to improve the conversion of urea to protein.[7]

The role of soluble sugars in the conversion of ryegrass protein to bacterial protein was followed *in vitro*. Ryegrass extract served as an appropriate protein substrate.

Experimental

Treatment of animals for in vivo *experiments*

Lactating twin Jersey cows (pair *a*, A) with rumen fistulas, which were being stall-fed twice daily (9.30 a.m. and 4.00 p.m.) on freshly cut clover–ryegrass pasture, were used to investigate the influence of readily fermentable carbohydrate on the utilisation of dietary protein as measured by ammonia levels in the rumen. One-half of the available pasture had previously been top-dressed with ammonium sulphate at the rate of 200 lb./acre. After a preliminary period of nine days, during which cow *a* was fed top-dressed pasture and cow A normal pasture, 500 g. of commercial wheat starch was infused, as a 50 : 50 aqueous suspension,

* Part III: *J. Sci. Fd Agric.*, 1964, **15**, 890

into the rumens of both animals during the morning (9.30–11.30 a.m.) and afternoon (4.00–6.00 p.m.) periods for two consecutive days (Experiment i). Rumen samples were collected immediately before the morning feeding period and 2, 4, 6, 8·5, 10·5, and 12·5 h. after feeding on the day before infusion of the starch and on the 2 days of infusion.

After the second day of infusion, cow *a* was fed normal pasture and cow A fed top-dressed pasture for a period of 14 days. Both animals then received 900 g. of starch twice daily for 2 consecutive days, with infusion and sampling times as described above (Experiment ii).

Treatment of animals for the in vitro *measurement of fermentation rates*

A non-lactating Jersey cow with a rumen fistula, which was being stall-fed on freshly cut red clover and fasted overnight before sampling, provided the source of rumen liquor. The collection of rumen fluids, preparation of ryegrass juice extract, and the measurement of fermentation rates at 39° were as described previously.[8]

Incubation of rumen liquor with carbohydrate

In the first series of experiments, the eight bottles of 1-pint capacity used as experimental vessels were arranged as follows: two blanks containing rumen liquor (100 ml.) and water (20 ml); two or three controls containing rumen liquor (100 ml.), perennial ryegrass juice (10 ml.) and water (10 ml.); three or four bottles containing rumen liquor (100 ml.), perennial ryegrass juice (10 ml.) and carbohydrate solution (10 ml.) which contained either 0·1 or 0·2 g. of carbohydrate. In order to determine gas formation, one of the blanks was acidified at zero time and the remaining bottles after incubation for 3 h.

In the second series of experiments, four bottles were used as controls (rumen liquor, 100 ml.; ryegrass juice, 10 ml.; water, 10 ml.) and three with 10 ml. water replaced by 10 ml. of a 10% solution of sugar. As the supply of α-D-galacturonic acid was limited, 10 ml. of a 5% solution were used. One of the controls was acidified at zero time and one control and one containing sugar after incubation for 0·5, 1·5 and 3 h.

Ammonia and VFA* concentrations were measured after acidification in all experiments.

Analytical methods

Moisture.—100 g. freshly cut herbage were chopped into 1–2 in. lengths and dried in a forced-draught oven on aluminium trays at 100° for 16 h.

Crude protein.—Crude protein was determined as described previously.[8]

Non-protein nitrogen.—5 g. of dried material were boiled with 250 ml. of 80% ethanol for 3 min. and filtered. The filtrate was evaporated to dryness on a water-bath and re-dissolved in 10 ml. of water.[9] Total nitrogen was determined on a 5-ml. aliquot by the Kjeldahl method.[10]

Ammonia and total VFA in the rumen liquors and total nitrogen and soluble sugars in the grass juice extracts were determined as described previously.[8]

Separation and identification of soluble sugars in grass juice extract

An aliquot (approx. 100 ml.) of the grass juice extract was centrifuged at 40,000 *g* for 30 min. to remove suspended particulate material.[4] Soluble sugars in the supernatant were then separated by chromatography on either Whatman No. 1 or No. 3 MM paper. The most useful eluting solvent for the separation of glucose, galactose, mannose, arabinose, ribose and fructose was ethyl acetate–acetic acid–water (3 : 1 : 3, by vol.). *p*-Anisidine hydrochloride was used as the spray reagent for pentoses and hexoses[11] and urea phosphate for ketoses.[12]

Quantitative estimation of sugars

The concentrations of the various sugars in ryegrass juice extract were estimated after purification by chromatography[13] on Whatman No. 3 MM paper. A volume of 0·003–0·015 ml. of supernatant prepared from ryegrass juice was applied to the paper as spots by an 'Agla'

* Acetic, propionic and butyric acids

micrometer syringe, and the papers (46 in. × 18 in.) eluted for 32–70 h. with ethyl acetate–acetic acid–water (3 : 1 : 3, by vol.). The papers were dried and, except for fructose determinations, dipped in aniline hydrogen phosphate[14] and heated at 105° for 10 min., 50–100 μg. of the sugars being estimated were used as standards throughout the procedure. Elution and the determination of the sugars was by the method described by Richards.[15]

For the determination of fructose, an additional marker strip containing the standard was chromatographed. This strip was cut off, sprayed with urea phosphate solution and heated. The areas on the main chromatogram corresponding to the position of fructose on the marker strip were cut out and eluted in 6 ml. of water. Fructose was estimated in 2 ml. of the eluted solution by the method of Bell,[16] and total soluble sugar by the method of Bath.[17]

Results

(1) In vivo *experiments*

Nitrogen levels in the feed and the level of intake

Total nitrogen in pasture top-dressed with ammonium sulphate at the rate of 200 lb./acre ranged from 3·18 to 3·72% of the dry matter and was 7·4–14·8% higher than the levels in untreated pasture obtained on corresponding days (Table I). Levels of non-protein N (NPN) were also higher in top-dressed pasture, and consequently the relative proportions of the NPN and the protein components in the two pastures were similar.

With the availability of pastures of different nitrogen levels, the two cows in paired feeding experiments were maintained on appreciably different nitrogen intakes except on the fourth day when cow *a*, feeding on pasture of lower nitrogen content, had an unusually high intake (Table I).

Table I

Nitrogen intake of twin cows fed on pasture containing different levels of nitrogenous constituents

		Pasture top-dressed with 200 lb. of $(NH_4)_2SO_4$/acre					Pasture without $(NH_4)_2SO_4$					
		N content of pasture, % of dry matter		Total N intake, lb.				N content of pasture, % of dry matter		Total N intake, lb.		
Date	Animal	Total-N	NPN	Morning	Afternoon	Total	Animal	Total-N	NPN	Morning	Afternoon	Total
31.10.62	Cow *a*	3·56	0·76	0·345	0·328	0·673	Cow A	3·28	0·71	0·266	0·289	0·554
1.11.62*	Cow *a*	3·41	0·74	0·351	0·368	0·719	Cow A	3·16	0·69	0·288	0·300	0·588
2.11.62*	Cow *a*	3·72	0·84	0·379	0·372	0·751	Cow A	3·16	0·72	0·237	0·303	0·540
15.11.62	Cow A	3·18	0·74	0·308	0·347	0·655	Cow *a*	2·96	0·64	0·355	0·355	0·710
16.11.62†	Cow A	3·25	0·79	0·361	0·397	0·758	Cow *a*	2·83	0·70	0·286	0·314	0·600
17.11.62†	Cow A	3·33	0·75	0·326	0·290	0·616	Cow *a*	2·94	0·66	0·303	0·285	0·588

* 500 g. of an aqueous suspension of starch infused into rumens of both animals during morning and afternoon feeding periods
† 900 g. of an aqueous suspension of starch infused into rumens of both animals during morning and afternoon feeding periods

Ammonia concentrations in the rumen and the effect of addition of starch

Changes in the ammonia concentration in the rumen followed much the same pattern throughout the 6 days of the experiment, with peak concentrations occurring 2–4 h. after the commencement of each feeding period (Fig. 1). Concentrations of ammonia were appreciably higher in the rumen when the cows were fed pasture with the higher nitrogen content. Without starch, the average ammonia concentration 6 h. after the start of feeding on high-N pasture was more than twice that for normal pasture (15·8 mg./100 ml. of rumen liquor compared with 6·9 mg./100 ml.).

The addition of 500 g. of starch twice daily had a variable effect on ammonia levels. On the second day of addition, together with a diet of high-N pasture, the recorded maxima and minima were similar to those for untreated pasture without starch. However, on the first day, when additional nitrogen equivalent to about 130–160 g. of protein was present in the high-N pasture given at each feeding period, this level of supplementation was insufficient to bring about a lowering of ammonia content. Increasing the dose to 900 g. of starch twice daily brought about appreciable and consistent reductions in ammonia level; in general, concentrations in the rumen of cows feeding on high-N pasture were of the same order as those

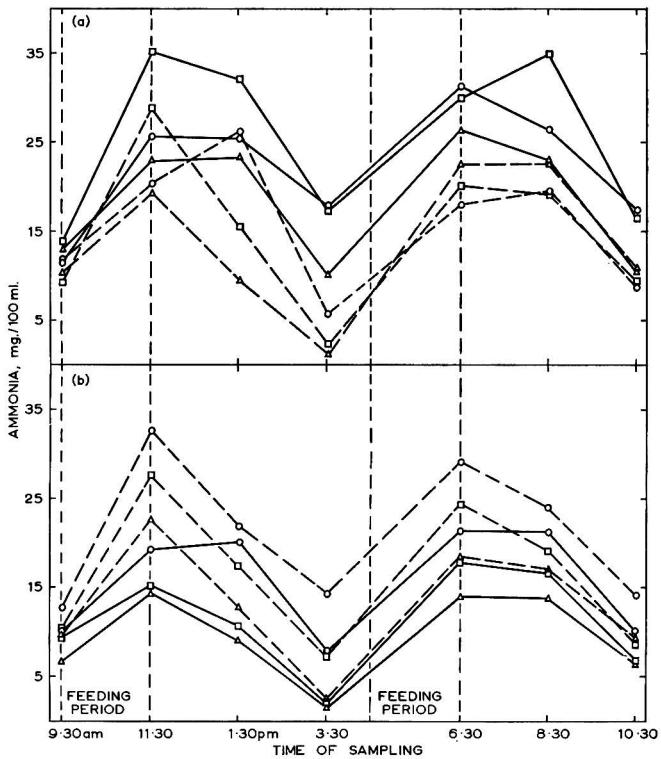

FIG. 1.—*Variations in rumen ammonia concentrations in twin cows fed on freshly cut mixed pasture with and without the addition of starch*

(a) —— Cow *a*—fed top-dressed pasture
--- Cow A—fed untreated pasture
○ 31/10/62—no starch
□ 1/11/62—500 g. starch added to rumen during each feeding period
△ 2/11/62—500 g. starch added to rumen during each feeding period

(b) —— Cow *a*—fed untreated pasture
--- Cow A—fed top-dressed pasture
○ 14/11/62—no starch
□ 15/11/62—900 g. of starch added to rumen during each feeding period
△ 16/11/62—900 g. of starch added to rumen during each feeding period

for normal pasture feeding without starch. The very low minima recorded 6 h. after feeding (< 5 mg./100 ml. on many occasions) was a further feature when starch was added.

VFA concentrations in the rumen

The concentrations of VFA generally rose steadily throughout the day, reaching maximum levels after the afternoon feeding period (Fig. 2). Values ranged from minima of 50–94 mmoles/l. to maxima of 124–162 mmoles/l. and, in general, the cow with the higher intake had slightly higher VFA levels. There was no apparent effect of either the nitrogen level in the feed or of added starch.

(2) In vitro *experiments*

Soluble sugars and crude protein in ryegrass juice extract

The concentrations (mg./ml.) of six sugars found in the grass juice extract as measured by quantitative paper chromatography were: galactose, 1·38; glucose, 4·00; mannose, 1·55;

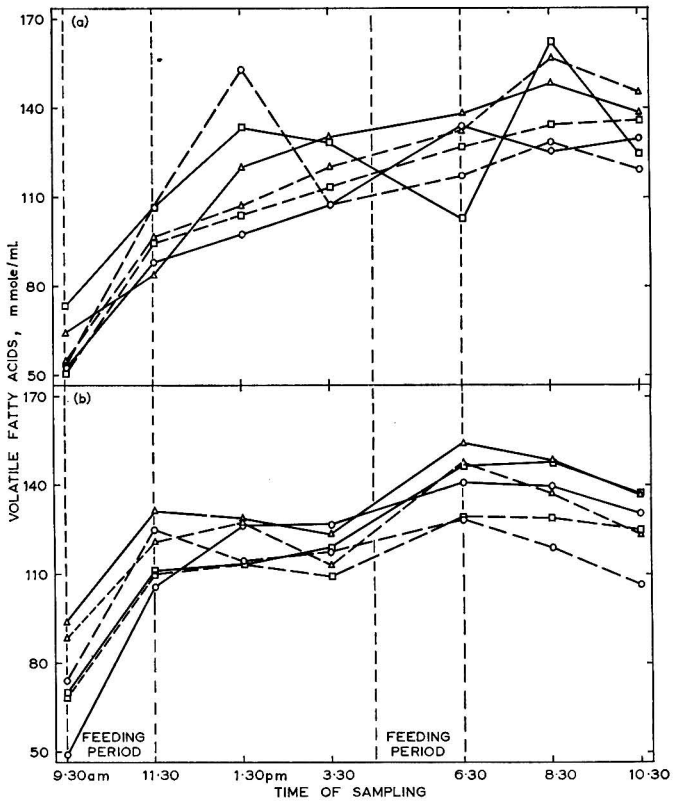

FIG. 2.—*Variations in VFA concentrations in the rumen of twin cows fed on freshly cut mixed pasture with and without the addition of starch*

(a) —— Cow *a*—fed top-dressed pasture
--- Cow A—fed untreated pasture
○ 31/10/62—no starch
□ 1/11/62—500 g. of starch added to rumen during each feeding period
△ 2/11/62—500 g. of starch added to rumen during each feeding period

(b) —— Cow *a*—fed untreated pasture
--- Cow A—fed top-dressed pasture
○ 14/11/62—no starch
□ 15/11/62—900 g. of starch added to rumen during each feeding period
△ 16/11/62—900 g. of starch added to rumen during each feeding period

fructose, 7·88; arabinose, 0·85; ribose, 0·29. Galacturonic acid was also identified. The total concentration of the individual sugars determined by this method (15·95 mg./ml. extract) was 0·7 mg./ml. higher than the value obtained for total soluble sugars by the method of Bath.[17] The ryegrass extract contained 4·4 mg. of crude protein/ml.

Effect of addition of 0·1 *and* 0·2 *g. soluble carbohydrate on metabolic activity* in vitro

In the absence of added substrate, small changes occurred in gas production, ammonia and VFA concentration after incubation of rumen liquors obtained from a cow fasted overnight for 3 h. (Table II). The considerable variations (55–155 ml.) in the gas released on acidification of collected rumen liquor was related to the initial level of VFA, and therefore to the pH—high VFA concentrations giving low pH values and hence low bicarbonate concentration in rumen liquor. Compared with values for the incubation of rumen liquor alone, the increases brought about by the addition of 10 ml. of ryegrass juice extract to 100 ml. of rumen liquor were as follows: gas formation, 33–76 ml.; ammonia concentration, 12·1–15·7 mg./100 ml.; VFA concentration, 18·1–32·9 m.moles/l.

Table II

Gas, ammonia and VFA formation in rumen liquor (100 ml.) incubated with ryegrass juice alone (10 ml.) or with ryegrass juice (10 ml.) and soluble carbohydrates for 3 h. at 39°

Exp. no.	Blank		Ryegrass juice alone	Ryegrass Juice and Glucose		Sucrose		L-Arabinose		Fructose	Galactose	Xylose
	0 h.	3 h.		0·1 g.	0·2 g.	0·1 g.	0·2 g.	0·1 g.	0·2 g.	0·2 g.	0·2 g.	0·2 g
Gas production, ml.												
1	155	176	209	222	—	220	—	—	—	—	—	—
2	122	138	180	192	—	193	—	188	—	—	—	—
3	67	82	151	—	170	—	170	—	158	—	—	—
4	126	142	205	—	226	—	225	—	—	225	—	—
5	55	77	153	—	175	—	168	—	—	—	173	167
NH_3 concentration, mg./100 ml.												
1	15·2	22·8	34·9	33·7	—	34·0	—	—	—	—	—	—
2	21·0	27·5	40·1	38·0	—	38·4	—	40·3	—	—	—	—
3	18·6	22·5	38·2	—	34·6	—	34·6	—	37·8	—	—	—
4	16·3	21·7	36·4	—	31·7	—	32·9	—	—	32·2	—	—
5	18·7	26·2	41·4	—	37·7	—	37·3	—	—	—	36·1	35·8
VFA, mmoles/l.												
1	—	—	—	—	—	—	—	—	—	—	—	—
2	56·5	53·5	76·4	85·6	—	84·3	—	84·4	—	—	—	—
3	76·1	76·0	94·1	—	110·2	—	110·7	—	109·5	—	—	—
4	52·7	53·4	79·5	—	85·8	—	85·7	—	—	85·6	—	—
5	81·1	79·2	112·1	—	117·5	—	117·8	—	—	—	118·5	117·8

It is apparent from Table II that at the levels investigated, glucose, sucrose, fructose, galactose and xylose produced very similar increases in the fermentation activity of the rumen micro-organisms in terms of gas and VFA formation and ammonia utilisation. Increasing the glucose or sucrose from 0·1 to 0·2 g. produced an almost proportional increase in this activity. Of the carbohydrates tested, L-arabinose was the least effective in increasing the utilisation of ammonia-N but increased gas formation and VFA levels by about the same as the other sugars.

Comparison of rates of fermentation of soluble carbohydrates

Preliminary experiments showed that the effects of glucose on gas production, ammonia and VFA concentrations increased as the levels of glucose were raised from 0·1 to 1·0 g. (Table III). Excepting for ammonia, these changes were approximately proportional to the increases in the amount of glucose added. Consequently, it was decided to test a series of carbohydrates at the 1·0 g. level since differences could then be expected to be more apparent than at the lower levels used above. Practical difficulties permitted rate measurements to be made using only one carbohydrate at a time: therefore direct comparisons of gas formation and VFA and ammonia concentrations are not valid because of the variations in the initial values for the different samples of rumen liquor.

A consistent feature of the incubation experiments was the high rate of gas, ammonia and VFA formation in the first half hour of incubation (Table IV). The increase in ammonia levels in the controls during this period was usually between one-half and three-fourths of the total increase over the 3-h. period, whereas increases in gas and VFA formation represented between one-third and one-half and between one-quarter and one-half of the 3-h. increases respectively. Although gas production and VFA formation in the controls increased at a slower rate between 30 min. and 3 h., the presence of certain sugars had the effect of sustaining the initial high rates.

Table III

Gas, ammonia and VFA formation in rumen liquor (100 ml.) incubated with ryegrass juice alone (10 ml.) or with ryegrass juice (10 ml.) and glucose (0·1–1·0 g.) for 3 h. at 39°

	Blank		Ryegrass juice alone	Ryegrass juice and glucose			
	0 h.	3 h.		0·1 g.	0·2 g.	0·5 g.	1·0 g.
Gas production, ml.	91	124	196	208	211	237	278
Ammonia, mg./100 ml.	28·1	33·9	50·6	48·6	46·9	44·7	43·6
VFA, m.moles/l.	66·0	73·8	85·0	87·5	92·7	103·6	111·5

Table IV

Gas, ammonia and VFA formation in vitro *with and without* 1 *g. of additional carbohydrate*

Carbohydrate		Gas production, ml.				Ammonia, mg./100 ml. Incubation time (h.)				VFA, mmoles/l.			
		0	0·5	1·5	3·0	0	0·5	1·5	3·0	0	0·5	1·5	3·0
Glucose	C*	150	183	201	218	23·8	34·8	36·7	37·9	54·2	61·3	70·0	81·0
	E†	150	200	249	296	23·8	32·1	31·6	26·9	54·2	75·0	92·5	103·0
Galactose	C	127	168	186	199	27·3	38·5	38·8	42·2	59·9	66·1	78·0	83·1
	E	127	174	238	285	27·3	37·1	32·4	26·1	59·9	73·4	96·6	113·8
Lactose	C	107	132	152	164	22·5	29·8	32·9	33·2	71·8	80·4	86·1	91·6
	E	107	144	179	239	22·5	30·5	26·1	24·2	71·8	87·0	96·9	113·8
Sucrose	C	129	164	185	195	21·0	30·0	32·4	33·2	62·0	72·4	80·3	85·7
	E	129	174	240	270	21·0	27·9	24·4	24·5	62·0	82·4	98·4	109·0
Xylose	C	175	200	218	233	17·9	24·6	38·8	30·9	45·3	52·9	61·9	63·8
	E	175	202	224	256	17·9	25·4	27·1	24·9	45·3	53·8	63·5	75·4
D-Arabinose	C	137	173	186	215	26·2	35·6	37·3	39·7	69·0	79·0	85·4	94·8
	E	137	176	187	208	26·2	34·1	37·7	40·5	69·0	76·5	87·2	99·2
α-D-galacturonic acid (0·5 g.)	C	170	194	219	240	18·6	28·3	31·9	33·7	49·7	56·4	67·7	69·9
	E	170	195	226	270	18·6	27·1	28·9	27·5	49·7	62·1	73·4	86·7

* C—control = rumen liquor (100 ml.) and ryegrass juice extract (10 ml.) and water (10 ml.)
† E—experimental = rumen liquor (100 ml.), ryegrass juice extract (10 ml.) and carbohydrate solution (10 ml.)

These same sugars had an even more marked effect on the levels of ammonia during the later stages of fermentation as shown by the subsequent decrease in ammonia concentration, whereas the levels continued to increase in the controls.

Comparison of the effect of sugars on gas production and ammonia formation shows that there is a close relationship between the fermentation of readily available substrate and the utilisation of nitrogen for protein synthesis (Fig. 3). Galactose, glucose, sucrose and lactose, which were fermented rapidly, brought about the greatest reduction in ammonia concentration. Galacturonic acid appears to behave in a similar way allowing for the lower concentration used. On the other hand, when D-arabinose was present, a negligible effect on fermentation rate was accompanied by practically no change in ammonia concentration.

Similarly, galactose, sucrose, lactose, glucose and galacturonic acid caused the greatest increases in VFA formation (16·8–30·7 mmoles/l. above the controls after incubation for 3 h.). Xylose was less effective (11·6 mmoles/l.), and D-arabinose had a very small effect (4·4 mmoles/l.).

Discussion

The higher ammonia levels present in rumen samples from animals fed top-dressed pasture illustrate a relationship between the nitrogen content of the pasture nitrogen intake and the concentration of ammonia in the rumen. Other workers[18-20] have shown that the time required for ammonia levels to reach a maximum after feeding, and the magnitude of the increases, depend largely on the solubility of the dietary protein. For example, a dose of 100 g. of casein gave a maximum ammonia concentration of 63·5 mmoles/l. 3 h. after administration, whereas zein, which is much less soluble, gave a peak of 11·0 mmoles/l. 10 h. after administration.[18] In the present work, maximum concentrations occurred 2–4 h. after feeding, which indicates that the plant protein was readily metabolised. McDonald[18] also observed lower ammonia concentrations a few hours after feeding than before feeding. It was suggested that, as a result of large amounts of energy becoming available for protein synthesis after feeding, ammonia utilisation would exceed formation. Later, when the rate of bacterial growth dropped, production could again exceed utilisation.

Increases in the level of nitrogen in the pasture as a result of top-dressing were similar to those found in hay which had received the same rate of application 2 weeks before cutting.[21] Ferguson[21] also noted that top-dressing had no effect on the protein-N/NPN ratio. In spite of a lower intake of nitrogen on the first day of Experiment (ii), cow *a*, fed on high-N pasture, had higher concentrations of ammonia in the rumen. This suggests that, either the protein in the top-dressed pasture was more readily hydrolysed, or the higher level of soluble carbohydrate

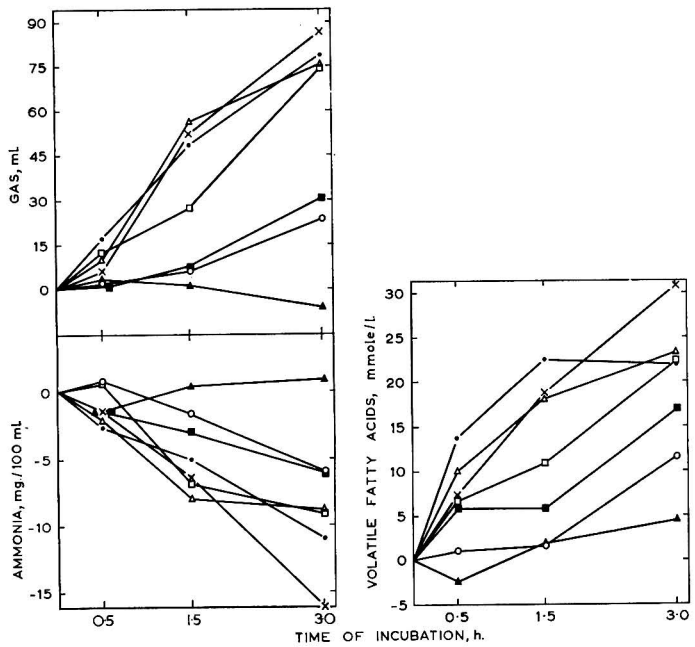

FIG. 3.—*Effect of soluble carbohydrates on gas production and VFA concentrations in rumen liquor* (100 *ml.*) *and ryegrass juice extract* (10 *ml.*) *incubated at* 39°

(Rates with rumen liquor and ryegrass extract = 0)
× galactose; △ sucrose; □ lactose; ● glucose; ■ galacturonic acid; ○ xylose; ▲ D-arabinose (1·0 g. of sugars; 0·5 g. of galacturonic acid)

in the untreated pasture (approximately 2·2% of dry matter) increased protein synthesis sufficiently to utilise the additional nitrogen ingested.

The effect of starch in lowering ammonia concentrations in the rumen of pasture-fed animals is in agreement with observations of Annison[20] and of Lewis & McDonald[1] after the dosage of animals on hay and concentrate diets with starch. In so far as ammonia levels in the portal blood are related to ammonia levels in the rumen,[22] starch supplementation would seem to improve the utilisation of dietary nitrogen in ruminants grazing on pasture high in protein.

The lack of any consistent difference in the levels of total VFA as a result of either the difference in the level of nitrogen in the feed or the addition of starch was unexpected in view of the findings of Lewis & McDonald[1] that casein added to the rumen of sheep at the same time as starch resulted in higher concentrations of total VFA. In addition, the present *in vitro* experiments show that there is a direct relationship between the effect of added sugar on ammonia utilisation and the formation of VFA. Reduction in ammonia concentration, without an accompanying increase in VFA concentration when starch is added, may well indicate that starch was being utilised as an energy source in protein synthesis by rumen bacteria.

With the exception of ribose, the sugars present in ryegrass juice extract are among those commonly found in grasses.[3] The absence of sucrose in the extract was likely to be due to enzymic hydrolysis during extraction and subsequent storage. Similarly, arabinose and galactose are usually present in polymeric form in the hemicellulose and pectic fractions respectively[23] and their occurrence as free sugars was probably due to enzymic degradation.[24]

The reduction in ammonia concentration in the rumen liquor when certain soluble sugars were added to the incubation mixture agrees with observations made previously by Pearson

& Smith[25] and McNaught[26] when studying the *in vitro* conversion of non-protein to protein. The rapid fermentation of galacturonic acid in the present work indicates that the presence of a primary alcohol group is not essential to the utilisation of a carbohydrate as was suggested by McNaught. Lewis & McDonald[1] found that the greatest increases in protein utilisation occurred when the food material acting as an energy source was attacked at a rate comparable to the rate at which NPN becomes available in the rumen. In the present work, the very high rate of ammonia formation during the first half-hour of incubation indicated that the nitrogen in the grass juice extract was readily metabolised, and in these circumstances readily metabolised sugars should be effective in facilitating the incorporation of ammonia into bacterial protein.

The considerable reduction in ammonia formation brought about by supplementation of soluble sugars in ryegrass extract with galactose, glucose, sucrose and lactose stresses the importance of the concentrations of readily fermented carbohydrate in pasture grasses and clovers. This will apply to sucrose and mannose in particular and to a lesser extent ribose. Hemicelluloses and pectic substances probably would also be broken down sufficiently quickly to enhance ammonia utilisation. Thus, provided the amount of protein being ingested by an animal is not in excess of the protein requirements of that animal, the availability of soluble sugars and other readily hydrolysed carbohydrates is likely to be the main factor limiting the utilisation of pasture protein when carbohydrate/protein ratios are low, as occurs in grass and clover pastures in early spring and late autumn. The chemical composition of pasture as affected by season requires further investigation before the most efficient utilisation of grassland by ruminants is likely to be achieved.

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DETERMINATION OF TOTAL CARBON IN SOILS BY WET COMBUSTION

By W. O. ENWEZOR and A. H. CORNFIELD

A digestion–purification train using sulphuric–phosphoric acid and potassium dichromate and employing a gravimetric finish is described for the determination of total organic carbon in soils. Interference of volatile mineral acids, chlorine and chromyl chloride (derived from chloride present in the soil) is prevented by using a bubbler containing a solution of sodium acetate, acetic acid and potassium iodide. The results obtained for total carbon in a number of mineral soils were very similar to those obtained by the Pregl dry combustion method.

Introduction

The main advantages of wet combustion over dry combustion methods for the determination of total carbon in soils are the simpler and cheaper apparatus and the shorter time of determination required in the case of the former group. Bremner[1] used the Van Slyke–Folch reagent (a mixture of sulphuric, phosphoric, chromic and iodic acids) in the Van Slyke–Neil manometric apparatus[2] and found that the method gave complete recovery of carbon from soils. Cornfield[3] replaced the expensive manometric apparatus with a digestion–purification train, using a column of heated silver to eliminate chloride interference and employing a gravimetric finish. Earlier, Clark & Ogg[4] showed that a simpler oxidising reagent (a mixture of sulphuric and phosphoric acids and potassium dichromate) gave complete recovery of carbon from soils. Shaw[5] also used this reagent, but considered that the Clark & Ogg technique for absorbing carbon dioxide was inconvenient and so employed a gravimetric finish. Neither Clark & Ogg nor Shaw took precautions to eliminate chloride interference, but Allison[6] used successive traps of potassium iodide solution and saturated silver sulphate to achieve this.

All methods employing a purification train have included a column of metallic zinc to absorb volatile mineral acid vapours. It was considered that the apparatus could be simplified by eliminating the zinc column and employing a single trap, containing a solution of sodium acetate, acetic acid and potassium iodide, which would fix volatile mineral acids as well as the chlorine and chromyl chloride derived from chloride present in the soils. With this in view, experiments were carried out and the method finally adopted is described below. ' Quickfit ' interchangeable glassware is used for the combustion portion of the apparatus so that no glassblowing is required.

Apparatus (see Fig. 1)

Experimental

The tube A (25 cm. long by 2 cm. bore, filled with 14–20 mesh ' Carbosorb ' soda-lime) removes carbon dioxide from the incoming air. Stopcock B is used to regulate the air flow. The ' Quickfit ' inlet tube C connects the stopcock with the 100-ml. ' Quickfit ' digestion flask D. The ' Quickfit ' condenser E (20 cm. effective length, 1·5 cm. bore) is connected through the ' Quickfit ' adaptor F to the ' Quickfit ' 100-ml. funnel G, with tap H, and also to the bubble counter I containing syrupy phosphoric acid. The ' acid fume and chlorine ' trap J (a 250-ml. Dreschel bottle with sintered glass inlet) contains the absorbing solution described under Reagents. The tube K contains a 5-cm. layer of 4–10 mesh potassium iodide at the bottom, next an 18-cm. layer of 14–20 ' Anhydrone ' (anhydrous magnesium perchlorate), and at the top another 2-cm. layer of 4–10 mesh potassium iodide. L is a stopcock in the tube used for by-passing the U-tube M, with stopcocks N and O, which absorbs carbon dioxide. The U-tube M (1·5 cm. bore by 25 cm. effective length) is filled with 14–20 mesh ' Carbosorb ' except for a 1-cm. deep layer of Anhydrone (14–20 mesh) at the inlet and an 8-cm. deep layer at the outlet end. The guard tube P (25 cm. long by 2 cm. bore) is filled with 3–8 mesh ' Carbosorb ' and is connected to a water pump and manometer.

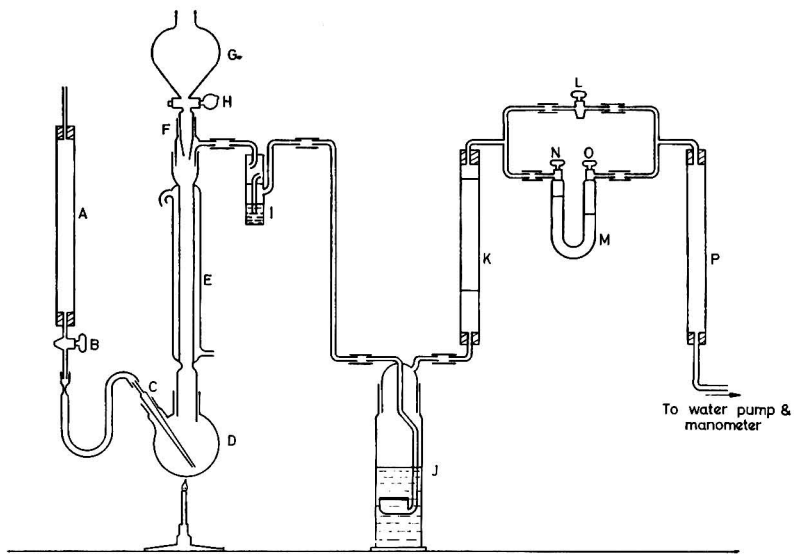

FIG. 1.—*Diagram of apparatus and absorption train*
(For A-P see text)

All absorption tubes have a glass wool plug at each end. Where more than one solid reagent is present in a tube, these are separated by a thin layer of glass wool. All ground joints and tap H are lubricated with phosphoric acid and all other taps with stopcock grease.

Reagents

Digestion acid (as used by Clark & Ogg[4]).—Three parts by volume of conc. sulphuric acid are mixed with two parts by volume of 85% phosphoric acid. This mixture may be prepared in bulk. *Potassium dichromate.*—Powdered 'Analar' potassium dichromate. *'Acid fume and chlorine' trap absorption mixture.*—100g. of potassium iodide, 30 g. of sodium acetate trihydrate, and 2 ml. of glacial acetic acid are dissolved in 100 ml. of water. The quantities are not critical.

Procedure

The U-tube M, with taps N and O closed, is weighed and placed in position in the train. Tap L is opened. A sample of the dry soil (ground to pass an 0·25 mm. sieve) containing not more than 0·1 g. of carbon is weighed into the dry digestion flask D and mixed with 3–4 g. of potassium dichromate and about 3 ml of water. After lubrication of both joints with phosphoric acid, the flask is placed in position in the train. Air is sucked through the apparatus using a suction head of about 5 cm. Hg at 3–4 bubbles per sec. (by adjusting tap B) for about 5 min. to clear it of carbon dioxide. Taps N and O are then opened and L closed and the air flow reduced to 1–2 bubbles per second. Twenty-five ml. of digestion acid are added to the funnel G and allowed to run into the digestion flask by opening tap H, which is closed immediately the acid has run through. The flask is heated with a small flame of a micro-burner, which is removed when the initial vigorous reaction begins. When this has subsided the flask is again heated so that the contents are brought to boiling in 3–5 min. Boiling is continued for 10 min. and the flame is then removed. The air flow is increased to about 8 bubbles per sec. and aeration continued at this rate for at least 10 min. Aeration is then stopped, the taps N and O of the U-tube closed and the U-tube removed and weighed.

The initial sweeping-out of carbon dioxide from the apparatus need only be done at the beginning of each set of determinations, providing another digestion flask containing the next

sample mixed with potassium dichromate and water is placed in position immediately after the previous digestion flask has been removed. Time may also be saved by having a second weighed U-tube ready to be placed in position. A blank should be run for each series of determinations to allow for the presence of carbon in the reagents.

The heating of the digestion flask must be controlled to prevent excessive frothing, which tends to carry organic material to the sides of the flask and out of contact with the digestion mixture. The contents of the U-tube must be examined regularly and replaced well before they are exhausted. This is shown clearly by the 'Carbosorb' self-indicating granules and also fairly clearly by the Anhydrone, the exhausted portion of which assumes a 'wet' appearance.

Providing the sample contains between 0·05 and 0·1 g. of carbon accurate results will be obtained. If much more than 0·1 g. of carbon is present, then the potassium dichromate will be used up and digestion will be incomplete. If even an approximate knowledge of the carbon content of the soil is not known, this can be determined quickly by the Walkley–Black titration method[7] and multiplying by a factor of 1·3.

Results

Comparison of the proposed method with the Pregl dry combustion method

The results obtained by these two methods on a number of mineral soils are shown in Table I. It is seen that reproducibility of the proposed method is good and the mean results agreed well with those given by the dry combustion method.

Chloride interference

The effects of increasing amounts of chloride (up to 0·08 g.) added as sodium chloride to 2-g. samples of a chloride-free soil are shown in Table II. It is seen that when the 'acid fume and chlorine' trap was excluded from the train chloride in excess of 0·004 g. (equivalent to the presence of more than 0·2% chloride in the soil) gave high results. When the trap was included in the train even 0·08 g. of chloride (equivalent to 4% of chloride in the soil) did not interfere. Similar results were obtained with another soil, and with this it was shown that even 0·6 g. chloride (equivalent to 30% chloride in the soil) did not interfere.

When the contents of the digestion flask are heated chloride is converted to chromyl chloride, which appears as reddish fumes which deposit on the cooler parts of the flask and condenser. However, when the digestion mixture is brought to boiling the red deposit disappears except for a small amount at the top of the condenser. Chromyl chloride decomposes at 180–190°,[6] which is lower than the boiling point (210°) of the digestion mixture, releasing chlorine. This chlorine and any traces of chromyl chloride react with the contents of the

Table I

Comparison of the proposed wet combustion method with the Pregl dry combustion method

Soil	Carbon, %			Soil	Carbon, %		
	Wet combustion	Mean	Dry combustion		Wet combustion	Mean	Dry combustion
1	1·42	1·41	1·39	5	4·04	4·02	4·02
	1·41				4·00		
2	1·41	1·41	1·43	6	4·19	4·18	4·21
	1·41				4·17		
3	2·54	2·52	2·54	7	5·66	5·66	5·60
	2·50				5·66		
4	3·95	3·98	3·98	8	8·07	8·06	8·07
	4·01				8·05		

Table II

Effect of chloride additions on the proposed method

Chloride added[a]		Carbon, %	
mg.	% in soil	Trap absent[b]	Trap present[c]
0	0·0	2·08	2·08
4	0·2	2·09	2·08
8	0·4	2·12	2·07
12	0·6	2·14	2·09
16	0·8	2·20	2·07
20	1·0	2·34	2·09
40	2·0	2·59	2·07
80	4·0	3·09	2·08

[a] Added as sodium chloride to 2 g. of soil
[b] ' Acid fume and chlorine ' trap excluded from the train
[c] ' Acid fume and chlorine ' trap present in the train

' acid fume and chlorine ' trap, releasing iodine. Most of the iodine is retained in this trap, but any passing through is retained by the layer of solid potassium iodide at the bottom of tube K. The layer of potassium iodide at the top of tube K is useful for indicating when potassium iodide in the ' acid fume and chlorine ' trap as well as that at the bottom of tube K are exhausted.

Possible interfering factors

The main volatile mineral acid formed during digestion is nitric acid, derived from oxidation of soil organic nitrogenous materials. With soils free from chloride, satisfactory results were obtained even when the ' acid fume and chlorine ' trap was excluded from the train and the column K contained Anhydrone only. This testified to the high efficiency of the condenser E in removing volatile mineral acids.

Soils sometimes contain carbon in forms such as coal, coke, charcoal and peat, which are relatively resistant to oxidation. The recovery of carbon, as carbon dioxide, by the proposed method from these materials was studied by adding known weights of the materials (ground to pass an 0·25 mm. sieve) to a soil of known carbon content. It was found that the normal digestion period (boiling gently for 10 min. after the contents of the digestion flask had been brought to boiling) resulted in complete recovery of carbon from peat, but not more than 89–95% recovery of the carbon from coal, coke and charcoal. There was, however, complete recovery of carbon from these materials when the digestion mixture was boiled for 30 min. instead of 10 min. Thus, if significant amounts of these materials are suspected of being present, the longer digestion period should be used.

Carbonates, if present, may be removed by treatment of the weighed sample in the digestion flask with sulphurous acid followed by drying in a vacuum desiccator over solid sodium hydroxide.[1, 5]

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EFFECTS OF FAT CONTENT ON DIFFUSION OF WATER IN FISH MUSCLE*

By A. C. JASON

The coefficients of diffusion of water, D_i and D_{ii}, associated with states of ' high ' and ' low ' hydration respectively in fish muscle are sensitively dependent on the amount of fat present. The diffusion resistivity D^{-1} for various species may be represented in both cases by straight lines of the form

$$D^{-1} = \alpha + \beta F$$

where F is fat content and α and β are constants. Although the results for various species appear to fall about common lines for D_i^{-1} and D_{ii}^{-1}, the diffusion of water in the muscle of dogfish (*Squalus acanthias* L.) is anomalous in certan respects.

The experimental evidence is generally incompatible with a muscle microstructure consisting of a dispersion of fat in a non-fatty medium, as is commonly supposed, but is more in accord with one consisting of relatively non-fatty tissue entirely surrounded by fat, the thickness of which increases linearly with fat content.

Introduction

The coefficient of diffusion of water in the muscle tissue of lean fish has been shown[1] to be independent of species. The mechanism is one of thermal activation in which water molecules hop from one site of strong bonding to an adjacent site over a saddle point on a potential surface. The coefficient of diffusion D thus varies with temperature T in accordance with Boltzmann's energy distribution so that

$$D = D_0 \exp(-E/\boldsymbol{R}T) \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where E is the height of the potential barrier, $\boldsymbol{R}$ is the gas constant and $D_0 \approx d^2kT/3h$, d being the separation between adjacent sites, k Boltzmann's constant and h Planck's constant.

The coefficient of diffusion is independent of concentration over a wide range of water content. When the water concentration falls below a value corresponding to the unimolecular layer on the surface of the protein molecules, however, the diffusion coefficient at a given temperature is considerably reduced and there is a corresponding increase in the energy of activation, while D_0 remains practically unchanged. It may be supposed that the higher activation energy in the drier condition results from strong double hydrogen-bonding between water molecules and reactive groups on the protein surfaces and that this is slightly reduced, perhaps by electrostatic screening, when successive layers of water molecules surround the unimolecular layer in the more hydrated state.

Under practical conditions, the weight W_t of a sample of fish at time t, drying under conditions in which the constant-rate period may be neglected, is given by the expression

$$W_t - W_e = (W_0 - W_e)(A_i e^{-t/\tau_i} + A_{ii} e^{-t/\tau_{ii}}) \quad . \quad . \quad . \quad . \quad (2)$$

where W_e is the equilibrium weight of the sample when $t \to \infty$, W_0 is the initial weight and A_i, A_{ii}, τ_i, τ_{ii} are constants. $A_i \gg A_{ii}$ and $\tau_i < \tau_{ii}$, τ_i and τ_{ii} are termed 'drying time-constants', the suffixes denoting values corresponding to states of ' high ' and ' low ' hydration respectively. For convenience, drying, as characterised by these parameters, in this, the falling-rate period, may be said to take place in two phases, phase i and phase ii respectively.

Fig. 1 illustrating the typical drying behaviour of herring muscle, shows the unaccomplished weight loss $W_t - W_e$ of a small slab plotted on a logarithmic scale as a function of t on a linear scale. The two straight portions of the curve, corresponding to each of the drying phases, here clearly observed, are in this respect characteristic of most species of fish, both lean and fatty. It has been found, however, that the drying behaviour of dogfish muscle has an additional feature in that drying curves, of which Fig. 2 is a typical example, exhibit a third distinct phase, so that such behaviour may be represented by an equation of the type

$$W_t - W_e = (W_0 - W_e)(A_i e^{-t/\tau_i} + A_{ii} e^{-t/\tau_{ii}} + A_{iii} e^{-t/\tau_{iii}}) \quad . \quad . \quad . \quad (3)$$

where A_{iii} and τ_{iii} are constants relating to the third phase.

* Read at 1st International Congress of Food Science and Technology held in London, 1962

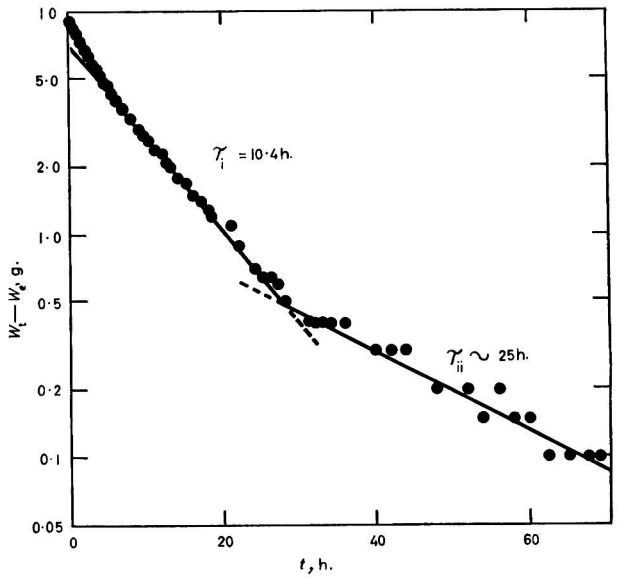

FIG. 1.—*Unaccomplished weight loss* $W_t - W_e$ *at* 30° *as function of time* t *of sample of herring muscle* 10 × 5 × 0·5 cm.

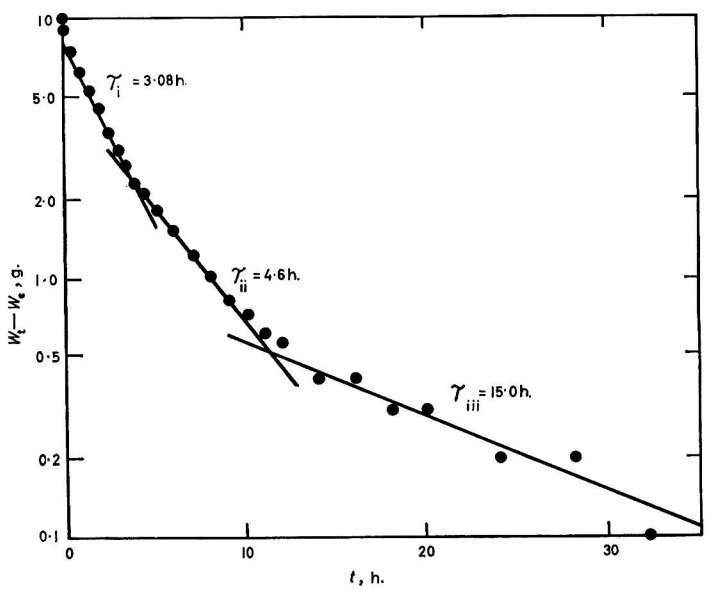

FIG. 2.—*As Fig.* 1 *for dogfish muscle*

It has been shown[1] that Fick's law

$$f = -D \operatorname{grad} C \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

holds for fish muscle provided that concentration C is related to initial volume of the sample (f is the flux). By analogy with Ohm's law for the flow of current in a conductor, it is convenient to consider D^{-1} as ' diffusion resistivity '.

Diffusion theory shows that

$$\tau_i = 4/\pi^2 D_i f(\chi);\ \tau_{ii} = 4/\pi^2 D_{ii} f(\chi);\ \tau_{iii} = 4/\pi^2 D_{iii} f(\chi) \quad . \quad . \quad . \quad (5)$$

where $f(\chi)$ is a factor depending on the geometrical configuration of the sample. For example, in the case of a rectangular parallelepiped of dimensions $2a$, $2b$, $2c$, $f(\chi) = a^{-2} + b^{-2} + c^{-2}$. D_i, D_{ii} and D_{iii} are the coefficients of diffusion in phases i, ii and iii respectively.

Coefficients of diffusion in the muscle of a number of species of fatty fish have been measured to determine the way in which the movement of water is influenced by the presence of fat. The method has been to measure the slopes of the straight portions of the drying curves plotted as in Figs. 1 and 2 for samples of regular shape dried in a wind tunnel under controlled conditions of temperature, humidity and air-flow, and thus to derive the various coefficients of diffusion.

Experimental

In the present experiments attention was confined to samples of only one shape, a slab measuring 10 × 5 × 0·5 cm., dried at 30° in a stream of air at 30% R.H. moving at 152 cm./sec. Fat content was determined by ether extraction with a Soxhlet apparatus. Owing to the relatively small weight change of the sample (~1 g.) during the period in which D_{ii} dominates and to the inherent accuracy of the balances used (±0·05 g.), D_{ii} could not be determined with as great an accuracy as D_i. In the case of dogfish the accuracy decreased in the order D_i, D_{ii}, D_{iii}.

Results

Table I shows values of D_i determined on samples of various fat contents for species of fatty fish, including herring, for which most results were obtained, and D_{ii} for herring only. Values of D_i, D_{ii} and D_{iii} for various samples of dogfish are shown in Table II. All values of these coefficients are seen to decrease with increasing fat content.

In Fig. 3, D_i^{-1} is plotted as a function of fat content F for each of the six species listed in Table I and D_{ii}^{-1} for herring only. Fig. 4 shows D_i^{-1}, D_{ii}^{-1} and D_{iii}^{-1} plotted against F for dogfish. Straight lines of the form

$$D^{-1} = \alpha + \beta F \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

have been fitted to each set of results and estimates of the slope β and the intercept α are given in Table III. From these results the following observations are made:

(1) The different sets of data for dogfish and herring can be grouped in order of increasing slope as follows:

D_i^{-1}	Dogfish	
D_{ii}^{-1}	Dogfish	indistinguishable
D_i^{-1}	Herring	
D_{iii}^{-1}	Dogfish	indistinguishable
D_{ii}^{-1}	Herring	

(2) No real differences can be detected between the intercepts for the five sets of results.

(3) Values of D_i^{-1} for white fish, obtained from previous results for cod[1] as well as from present values for dab, megrim, plaice and witch, are indistinguishable from the extrapolated values for herring at the same fat content, as shown in Table IV.

Discussion

The lipid extracted with ether from the white flesh (the part used in this work) of fish, whether lean or fatty species, consists except in the case of dogfish, almost entirely of normal fat (triglycerides), as opposed to phospholipids, and is not chemically bound to the protein.* Unfortunately, it is not at present known in what manner the fat is distributed on the microscopic scale, for example, whether fat pervades the muscle cells, or surrounds them completely, or both; or whether the fat is a suspension of globules in a medium of non-fatty tissue. To

* In the dogfish the lipid is a mixture of triglycerides and alkoxydiglycerides, but the physical properties of the latter are so close to those of triglycerides that they are unlikely to account for any differences of behaviour.

Table I

D_i *and* D_{ii} *for various species of fish muscle of various fat contents at* 30°

Species*	Run No.	Fat content, %	D_i (10^{-6} cm.^{2} sec.$^{-1}$)	D_{ii}
Herring (*Clupea harengus* L.)	174B	12·8	0·604	—
	176B	6·4	0·948	—
	177B	11·5	0·686	—
	178B	9·2	0·780	—
	179B	16·2	0·453	0·130
	180B	12·5	0·388	0·159
	181B	1·5	1·71	0·571
	182B	2·0	1·85	—
	183B	8·5	1·01	—
	184B	6·9	1·01	0·250
	185B	10·0	0·716	0·298
	186B	7·5	1·03	0·375
	187B	6·5	1·18	—
	188B	11·5	0·845	0·490
	189B	4·1	1·28	—
	191B	4·4	1·55	—
	192B	2·9	1·39	0·785
	194B	7·9	0·858	0·358
	195B	2·0	1·44	0·751
	196B	10·1	0·564	0·396
Mackerel (*Scomber scombrus* L.)	252A	0·7	2·19	—
	252B	0·7	2·49	—
	328A	4·3	1·22	—
	328B	6·3	1·08	—
	336A	3·4	1·17	—
	336B	2·6	1·43	—
Dab (*Pleuronectes limanda* L.)	242B	0·5	2·66	—
Megrim [*Arnoglossus megastoma* (Day)]	333A	0·5	3·14	—
	333B	0·5	2·65	—
Plaice (*Pleuronectes platessa* L.)	241B	0·3	2·99	—
Witch (*Pleuronectes cynoglossus* L.)	330A	0·2	2·58	—
	330B	0·2	2·50	—

* The scientific classification given in this paper is that of Jenkins[7]

account for the observed variation of diffusion resistivity with fat content it is therefore necessary to assume various types of fat distribution for which theoretical treatment is possible and to compare the computed results with those of observation.

Various treatments for diffusive flow in heterogeneous media have been given (for references see Barrer & Petropoulos[2]) in which the media usually consist of a disperse phase of particles of

Table II

D_i, D_{ii} *and* D_{iii} *for dogfish* (Squalus acanthias *L.*) *muscle of various fat contents at* 30°

Run No.	Fat content, %	D_i	D_{ii} (10^{-6} cm.^{2} sec.$^{-1}$)	D_{iii}
326A	9·5	1·55	1·14	0·503
326B	9·0	1·73	1·01	0·291
327A	5·3	2·26	1·43	0·465
327B	6·6	2·42	1·45	0·505
331B	7·2	1·61	1·08	0·470
332A	14·6	1·31	0·596	0·268
332B	16·3	0·80	0·525	—
337A	4·0	2·19	1·60	0·764
337B	6·0	2·37	1·51	0·395
338A	7·4	1·98	1·03	0·386
338B	8·8	1·71	1·01	0·362
340A	10·0	1·14	0·763	0·214
340B	8·0	1·49	0·701	0·320

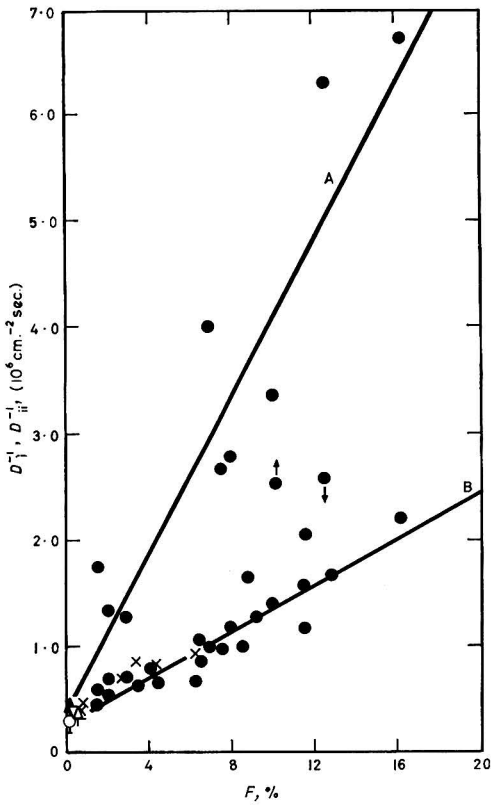

Fig. 3.—*Relationship between* D_i^{-1} *and fat content* F *at* 30° *for 6 species of fish and* D_{ii}^{-1} *for herring only*
● herring × mackerel △ dab + megrim
▽ plaice ▲ witch ○ cod (Jason[1])
Curve A D_{ii}^{-1} for herring only
Curve B D_i^{-1} for 6 species of fish

low permeability in a continuous medium of high permeability. Consideration is given here to three widely different model systems in which the volume fraction of fat is

$$v_F = F/92 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

since the density of fish fat is 0·92 g. cm.$^{-3}$

Maxwell[3] derived the following equation for the effective resistivity R of a medium of resistivity r_2 containing spheres consisting of a material of resistivity r_1

$$R = \frac{2r_1 + r_2 + v_1(r_1 - r_2)}{2r_1 + r_2 - 2v_1(r_1 - r_2)} \cdot r_2 \quad . \quad . \quad . \quad . \quad . \quad (8)$$

where v_2 is the volume fraction of the disperse phase. Writing

$$R = D^{-1};\ r_1 = D_F{}^{-1};\ r_2 = D_N{}^{-1};\ v_1 = v_F$$

(where D_F = coefficient of diffusion of water in fat and D_N = coefficient of diffusion of water in non-fatty muscle), Equation (8) becomes

$$\frac{D^{-1}}{D_N{}^{-1}} = \frac{2D_F{}^{-1} + D_N{}^{-1} + v_F(D_F{}^{-1} - D_N{}^{-1})}{2D_F{}^{-1} + D_N{}^{-1} - 2v_F(D_F{}^{-1} - D_N{}^{-1})} \quad . \quad . \quad . \quad (9a)$$

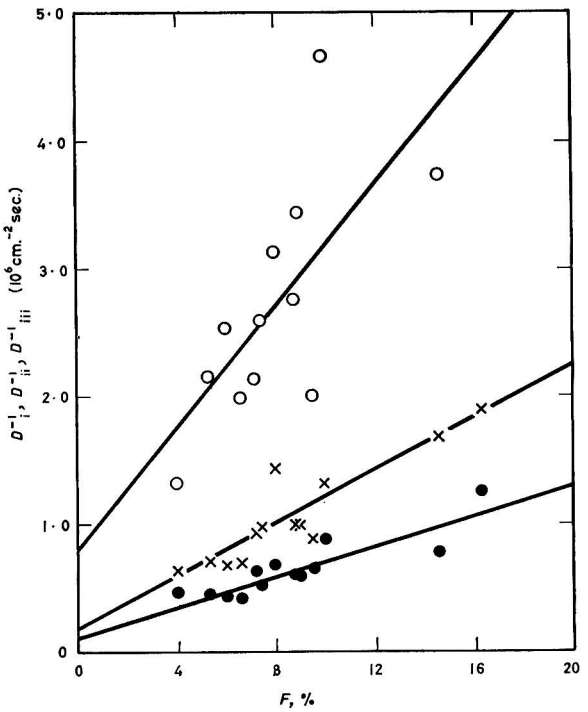

FIG. 4.—D_i^{-1}(○), D_{ii}^{-1}(×) *and* D_{iii}^{-1}(●) *at* 30° *plotted against* F *for dogfish*

Table III

Values of α *and* β

Data	Slope, β (10^5 cm.$^{-2}$ sec./% fat)		Intercept, α (10^5 cm.$^{-2}$ sec.)	
	Estimate	Standard error	Estimate	Standard error
Herring, Mackerel, White fish* } D_i^{-1}	0·88	0·11	3·8	0·8
Herring, D_i^{-1} (from same data)	1·08	0·17	2·8	1·4
Herring, D_{ii}^{-1}	3·67	0·88	4·3	8·0
Dogfish, D_i^{-1}	0·58	0·10	1·3	0·9
Dogfish, D_{ii}^{-1}	1·06	0·15	1·4	1·4
Dogfish, D_{iii}^{-1}	2·37	0·76	8·0	6·4

* Dab, megrim, plaice, witch

Table IV

Comparison of experimental values of D_i^{-1} *for white fish and values from extrapolation of herring data*

Species	Fat content, %	D_i^{-1}(10^5 cm.$^{-2}$ sec.)	
		Estimate	Standard error
Cod (previous results) }	0·1	2·98	0·59
Herring (extrapolated) }	0·1	2·9	1·4
White fish (present results) }	0·4	3·7	0·13
Herring (extrapolated) }	0·4	3·2	1·4

Results for herring are not sufficiently accurate to make comparison of the extrapolated values of D_{ii}^{-1} with the corresponding value for cod.

and for the extreme case giving the highest value for D^{-1}, i.e. when $D_F = 0$

$$\frac{D^{-1}}{D_N^{-1}} = \frac{2 + v_F}{2 - 2v_F} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9b)$$

Barrer & Petropoulos[2] considered the permeability of a regular lattice of rectangular parallelepipeds in a continuum. The expressions derived are very complicated except when $D_F = 0$, in which case

$$\frac{D^{-1}}{D_N^{-1}} = \frac{1 - v_F}{1 - v_F^{2/3}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

For a system in which parallel tubes of fat are embedded in non-fatty muscle and are parallel to the direction of flow of water

$$D = (1 - v_F)D_N + v_F D_F \quad . \quad . \quad . \quad . \quad . \quad . \quad (11a)$$

so that for $D_F = 0$

$$D^{-1}/D_N^{-1} = 1/(1 - v_F) \quad . \quad . \quad . \quad . \quad . \quad . \quad (11b)$$

Calculation shows that the values obtained for the diffusion resistivity D_i^{-1} of fatty muscle in the more hydrated condition, based on the models represented by Equations (9), (10) and (11), are considerably less than observed values. Even when it is assumed that fat is completely impermeable to water, the theoretical values of D_i^{-1} are not sufficiently sensitive to fat content (Table V). Similar results follow when diffusion resistivity D_{ii}^{-1} in the near-dry state is considered.

It is clear that the sensitive dependence of diffusion resistivity on fat content in fish muscle cannot be accounted for on the basis of a dispersion of fat in a non-fatty medium. It is therefore of interest to consider the possibility that the fat imposes a finite series resistance to the flow of water. This is feasible if it is postulated that on a microscopic scale relatively non-fatty tissue is entirely surrounded by fat, the thickness of which increases linearly with fat content; and if, in accord with Brandes & Dietrich,[4] the fat displaces water but not non-aqueous material.

Crank[5] has shown that diffusion resistivity of a composite membrane is equal to the sum of the diffusion resistivities of the component layers. If then a tortuosity factor γ is introduced to account for the detailed configurational arrangement of the muscle components, the effective diffusion resistivity may be written

$$D^{-1} = D_N^{-1} + v_F D_F^{-1}/\gamma \quad . \quad . \quad . \quad . \quad . \quad (12a)$$

or, arranged in accord with the above treatments

$$D^{-1}/D_N^{-1} = 1 + v_F D_F^{-1}/\gamma D_N^{-1} \quad . \quad . \quad . \quad . \quad (12b)$$

Substituting $F/92$ for v_F in Equation 12(a) and comparing with the empirical expression (6), it is seen that

$$\alpha = D_N^{-1} \text{ and } \beta = D_F^{-1}/92\gamma \quad . \quad . \quad . \quad . \quad . \quad (13)$$

Tables III and IV clearly suggest that the diffusion resistivity in the non-aqueous, non-fatty

Table V

Ratio of diffusion resistivity of fatty muscle to that of lean muscle ($F = 0$)

Fat content, %	Theoretical value of D_i^{-1}/D_N^{-1}			Experimental value of D_i^{-1}/D_N^{-1} for herring muscle
	Equation (9b)	Equation (10)	Equation (11b)	Equation (6)
0	1·0	1·0	1·0	1·0
5	1·09	1·10	1·06	2·93
10	1·18	1·15	1·12	4·86
15	1·29	1·19	1·20	6·80
20	1·42	1·22	1·28	8·72

component (i.e., in cod and lean white fish) is indeed numerically equal to α, and that fat imposes a resistivity in addition to this value and in proportion to the fat content.

It is difficult to estimate γ, but if a value of $\sqrt{2}$ is adopted[6] it is possible to estimate D_F from values for β given in Table III. These values of D_F obtained with various combinations of fish are given in Table VI. This shows that the coefficient of diffusion of water in fish fat is of the order of 10^{-7} cm.^{2} sec.$^{-1}$ at 30° a value that would be interesting to confirm directly, but the determination of which presents rather special experimental difficulties.

Table VI

Estimated values of coefficient of diffusion of water in fish fat

	$D_F(10^{-8}$ cm.^{2} sec.$^{-1})$	
	Estimate	Standard error
Herring, Mackerel, Whitefish } D_i^{-1}	9·6	1·2
Herring, D_i [1]	6·9	1·1
Dogfish, D_{ii}^{-1}	7·2	1·0

The mechanism of diffusion in the second phase of drying in most fatty fish and in phases i and iii in dogfish is somewhat more complicated and its elucidation requires additional investigation.

Acknowledgments

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

MAY, 1965

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Slope soils in Wales and the Welsh borderland. D. Mackney and C. P. Burnham (*J. Soil Sci.*, 1964, **15**, 318—330).—The characteristics of four profiles developed in materials of loam or clay loam texture on moderate or steep slopes are represented. A. H. CORNFIELD.

Classification of humus forms and micro-fabrics of temperate grasslands. B. C. Barratt (*J. Soil Sci.*, 1964, **15**, 342—356).—Grassland humus forms are subdivided on the basis of field morphology into mull and mor groups, although a mor-like mull intergrade is also recognised. According to variation in horizon thickness, structure, and consistence, 16 humus forms are recognised and described. Each horizon of a humus form may have one or more distinct micro-structures of fabrics. Twelve fabrics so far recognised under grassland are described. A. H. CORNFIELD.

Determination of dissolved oxygen concentrations in soils with bare, stationary platinum electrodes. G. H. Brandt (*Dissert. Abstr.*, 1964, **25**, 11—12).—A preliminary investigation of appropriate apparatus and techniques. A. G. POLLARD.

Effects of low-temperature storage on the oxygen uptake of soil. D. J. Ross (*Nature, Lond.*, 1964, **204**, 503—504).—The storage of four different soils at —20° for several weeks did not appear to affect consistently their O_2 uptake. S. A. BROOKS.

Gas-tight growth chamber for investigating gaseous nitrogen changes in the soil/plant/atmosphere system. P. J. Ross, A. E. Martin and E. F. Henzell (*Nature, Lond.*, 1964, **204**, 444—447).—Full details are given of a small gas-tight chamber which allows prolonged growth of enclosed plants. Experiments are described using the chamber, with ^{15}N in the atm. or $(^{15}NH_4)_2SO_4$ fertiliser, in which a N balance has been obtained. (16 references.) S. A. BROOKS.

Soil conservation and improvement in Spain. C. Roquero de Laburu (*An. Inst. nac. Invest. agron., Madrid*, 1964, **13**, pp. 477+xxvii).—An extensive review, intended as a reference guide to the application of modern principles of soil conservation to Spanish conditions, covering (i) the causes and effects of impoverishment and loss of fertility of the soil (9 chapters, 134 references), (ii) the defence, conservation and improvement of soil fertility (10 chapters, 108 references) and (iii) practical applications (6 chapters, 48 references), together with a brief appendix on relevant Spanish legislation. Available information on Spanish conditions is fully documented and discussed, with many maps, illustrations, tables and diagrams. E. C. APLING.

Cobalt status of Tasmanian soils. II. Recovery of applied cobalt in pot experiments. K. D. Nicolls and J. L. Honeysett (*Aust. J. agric. Res.*, 1964, **15**, 609—624).—Adding Co to the soil before sowing, and covering the soil in the pots with gravel, successfully avoided contamination of the plant tops with applied Co or with soil. Tests were run with subterranean clover, ryegrass and white clover. pH appeared to govern the ability of plants to absorb applied Co, more being recovered at the lower pH levels (4·9—6·2). (16 references.) E. G. BRICKELL.

Total and exchangeable iron in Egyptian soils. A. M. Abdel Ghani (*Z. PflErnähr. Düng.*, 1964, **107**, 136—145) —The total Fe content of Nile alluvial soils ranges from 1 to 7%, depending on the clay plus silt content % rather than on the clay. Soils with > 7% of $CaCO_3$ are generally poor in Fe and typical calcareous soils with > 70% of $CaCO_3$ contain less than 0·64% of Fe. The clay and next-finer fractions of typical Nile alluvial soils contain most of the Fe content of the soil, indicating that Fe exists as a partial substitute for Al of the octahedral layer, if it does not occur as free oxides. N-NH_4OAc at pH 3·0 (**I**) is the most suitable extractant for Fe in the alkaline Egyptian soils. Neutral aq. NH_4OAc without a reducing agent fails to extract Fe from these soils. Fe extracted by **I** ranges from 1 to 15 μg./g. of soil. Soils with more than 7 μg./g. are suffering from anaerobic conditions. There is no correlation between total Fe or soil pH and Fe extracted by **I**. M. LONG.

Sorption and leaching of nutrients in horticultural soils. III. $H_2PO_4^-$ ions. J. Soukup (*Z. PflErnähr. Düng.*, 1964, **107**, 113—118).—Of phosphate applied to a pure peat column 80% is elutriated by fifteen 25-ml. distilled water elutriations; 69% is elutriated from a 6 : 1 peat/bentonite mixture, 39% from a 3 : 1, 6% from a 1 : 1 and only about 1% from pure bentonite under the same conditions. Phosphate, retained on the column, can be separated into adsorbed, acid-sol. and acid-insol. fractions. The first fraction is removed from the column by elutriation with 0·5N-NH_4F at pH 7·0 and the second by 0·1N-HCl. Fixed phosphate is also vertically distributed down the column. M. LONG.

Biologically controlled decomposition process in soil. III. G. Müller, D. Kleinhempel and I. Förster (*Zbl. Bakt.*, 1964, II, **118**, 134—147).—Incubation of finely-divided org. matter with soil resulted in a marked increase in base-exchange capacity, which persisted for a year although 70% of the org. matter had been mineralised in the interval. Comparison is made of the action of colour-forming and non-colour-forming soil microfungi on org. matter (ground lucerne roots) added to soil and on concomitant changes in degree of saturation with bases. Humic acids produced by the colour-forming fungi occurred exclusively in the spores. The i.r. absorption spectra of extracts of the colour-forming fungi closely resembled those of corresponding extracts of a chernozem soil. A. G. POLLARD.

Effect of blue-green algae on growth of micro-organisms in the soil. G. N. Perminova (*Mikrobiologiya*, 1964, **33**, 472—476).—Algologically pure cultures of blue-green algae, isolated from the soil of Kirov region, contained considerable amounts of micro-organisms, including *Azotobacter*, *Clostridium pasteurianum*, and oligonitrophils. Laboratory tests showed that the introduction of specific organisms isolated from blue-green algae into the soil, with and without mineral N, increased the overall no. of micro-organisms in the soil. Vegetative tests on barley showed that the introduction into the topsoil of algae, and also of specific organisms prepared from it, resulted in an increase of N-fixing organisms in the soil, and in an increased yield of barley. In field tests seed of barley was inoculated before sowing with *Stratonostoc linckia* from blue-green algae. The azotobacter content of the soil was increased on average 89%, the content of oligonitrophils by 24%, and of *Clostridium* by 21%. A. S. LEVESLEY.

Influence of chlorocholine chloride (CCC) on soil biological processes. J. Jung (*Z. PflErnähr. Düng.*, 1964, **107**, 153—157).—On the basis of CO_2 production and nitrification chlorocholine chloride has no effect on soil microbiological activity when applied at the rates of 3, 30 or 300 kg./ha. M. LONG.

Lateral drag of chemicals in soil by a plough and a rotavator. L. N. Staniland (*Nature, Lond.*, 1964, **204**, 503).—A green fluorescent insol. powder mixed with powdered talc (to simulate aldrin dust) was spread over strips of soil which were then ploughed or rotavated. Analysis of samples of soil showed that the plough dragged ~50% of the tracer forward for 1 ft. and up to 10% for 4 ft., the rotavator threw 75% backwards 1 ft. and pushed ~25% forwards 3 ft. S. A. BROOKS.

Theoretical aspects of the use of fertilisers. L. Tombesi and M. T. Calè (*Ann. Staz. chim.-agr., Roma*, 1963 [1964], Ser. iii, No. 212, 8 pp.).—Some questions of the mode of action and the optimal relationship between nutrient additions, the consideration of nutrient availability in the soil in the light of thermodynamic principles, and the relationship between availability of mineral nutrients and enzymic activity in the plants are briefly discussed in relation to recent research. E. C. APLING.

Delayed effect of mineral nitrogenous fertilisers. R. Morel, A. Récamier and G. Pasqualini (*C.R. Acad. Agric. Fr.*, 1964, **50**, 954—963).—Plots under barley treated annually during 8 years with 120, 65, and 0 kg. of NH_4NO_3 per ha. showed but small differences as regards inorg. available soil-N and crop yields. Evidence was obtained of the passage of an appreciable part of the excessive inorg. N into org. soil-N. P. S. ARUP.

Addition of nitrogen to lupin green manure. G. Gattorta (*Ann. Staz. chim.-agr., Roma*, 1963 [1964], Ser. iii, No. 210, 247—258).—The effects of additions of NH_4NO_3 with lupin green manure to

alluvial loam low in humus and maintained at 50% of its water-holding capacity during the six hot months are reported. Additions of N promoted better humification of the green matter and led to an increase in org. C and total N and to an improvement in structural stability of the soil. (15 references.) E. C. APLING.

Effects [on crops] of increasing annual applications of potassium salts. J. Hébert and J.-C. Rémy (*C. R. Acad. Agric. Fr.*, 1964, **50**, 946—953).—Experiments were carried out over 6 years with sugar beet alternating with wheat on soil containing 0·019% of K. Moderate annual applications of K sufficed to maintain yields even when small decreases in total soil-K occurred. Large applications offered no advantage. In plots not receiving K there was evidence of partial replenishment of available K at the expense of the fixed K. The aim should be to compensate for the K removed by the crops. The remains of previous harvests should be left on the field. P. S. ARUP.

Granulation characteristics of a 5-4-12 (5-10-15) fertiliser containing potassium nitrate. D. R. Boylan and D. V. Kamat (*J. agric. Fd Chem.*, 1964, **12**, 423—428).—An economically attractive fertiliser grade of KNO_3 is now available in the U.S.A. Factors influencing the granulation of a mixed fertiliser containing KNO_3 are reported and an attempt is made to relate mathematically the yield and deviation in nutrient analysis between product and feed with moisture in the feed and temp. of granulation. W. ELSTOW.

Determination of utilisation coefficients of potassium fertilisers by isotopic dilution, by the differential method and by the relationship between the amounts assimilated. S. Fortini and S. Panella (*Ann. Staz. chim.-agr., Roma*, 1963 [1964], Ser. iii, No. 214, 15 pp.).—Assimilation of K from fertilisers in pot experiments with lucerne grown over 36 and 163 days was estimated by three methods: (i) the differential method (cf. Demolin A., ' Croissance des Végétaux cultivés ', 1956), (ii) with ^{86}Rb as tracer, and calculation using empirically determined correction factors, and (iii) on the assumption that the ratio of assimilable K in the fertiliser to total assimilable K in the soil is equal to the ratio of K absorbed by the plant from the fertiliser to the total K absorbed. The calculated utilisation coeff. given by the three methods were in good agreement for 36-day trials, but were non-concordant in a 163-day trial. E. C. APLING.

X-Ray analysis of the crystalline structure of some phosphatic fertilisers. H. Beckmann (*Z. PflErnähr. Düng.*, 1964, **107**, 97—104).—*X*-Ray analysis indicates that carbonate-apatite occurs in ' Hyperphosphate ', $NaCaPO_4$ in ' Rhenaniaphosphate ', $Ca(H_2PO_4)_2.H_2O$ in ' Superphosphate ', and $CaHPO_4$ and its hydrate in the mixed fertilisers ' Complesal ' (blue), ' Kampka ' (green), ' Nitrophoska ' (red) and ' Rustika ' (red). In aq. solution all these phosphates change into an apatite-like compound. M. LONG.

One-step continuous quick-curing triple superphospate process employing rod mill grinding. D. R. Boylan and A. B. Admin (*J. agric. Fd Chem.*, 1964, **12**, 428—432).—Experiments on the pilot plant scale indicate that a continuous one-step quick curing process for triple superphosphate of high conversion is possible. (15 references.) W. ELSTOW.

Chemical and physical characteristics of some Italian lignites from the point of view of their use as fertilisers. C. Nigro (*Ann. Staz. chim.-agr., Roma*, 1963 [1964], Ser. iii, No. 215, 19 pp.).—Determinations of NaOH-sol. humus, ash, N, water holding capacity, cation-exchange capacity and the variation of P absorption with pH are reported and discussed for five varieties of Italian lignite. *In vitro* studies indicated that mineralisation of pulverised lignites was not improved by N, P treatments. (31 references.) E. C. APLING.

Fertilisers. Imperial Chemical Industries Ltd. (Inventors: O. T. W. Price and J. S. Gow) (B.P. 942,090, 13.6.61).—There is claimed a granular mixed fertiliser comprising NH_4NO_3 and finely divided rock phosphate, especially Christmas Island rock phosphate, such that the N/P_2O_5 wt.-ratio is in the range of 90 : 29 to 1 : 2 and the total no. of plant food units per 100 pt. of fertiliser is $\leqslant 20$. F. R. BASFORD.

Plant Physiology, Nutrition and Biochemistry

Effects of ageing and temperature on respiratory metabolism of green leaves. J. Geronimo and H. Beevers (*Plant Physiol.*, 1964, **39**, 786—793).—Declining respiratory rates during ageing were correlated with striking decreases in mitochondrial activity. These in turn appeared to be related to increased disorganisation of the internal structure of the mitochondria. E. G. BRICKELL.

Photosynthesis in climatic races of *Mimulus*. II. Effect of time and carbon dioxide concentration on rate. H. W. Milner and W. M. Hiesey (*Plant Physiol.*, 1964, **39**, 746—750).—Climatic races of *M, cardinalis* show different changes in photosynthetic rate at 0·0425% CO_2 during 12 h. under conditions producing max. rate, the loss of rate with time increasing with the elevation of their native habitats. Continuous photosynthesis with 0·150% CO_2 showed a high initial rate falling rapidly with time. A relation is noted between these findings and the work of others on enhancement of crop yields by CO_2 fertilisation. E. G. BRICKELL.

Photosynthesis in healthy and rust-infected plants. A. Livne (*Plant Physiol.*, 1964, **39**, 614—621).—Bean and wheat plants were inoculated with spores of *Uromyces phaseoli* var. *typica*, and *Puccinia graminis tritici*, respectively, and rates of photosynthesis were examined by use of $^{14}CO_2$. During the first 4-5 days following inoculation photosynthesis occurred at substantially the same rate in healthy and infected tissues, but later declined in infected tissues to ⅓-½ that of normal leaves. Under some conditions photosynthesis was stimulated to extents related to the intensity of the infection. The distribution of ^{14}C among neutral, basic and acidic components of leaves was modified by infection. Inhibition of photosynthesis in infected tissue may be compensated, in part, by its stimulation in organs at a distance from infected tissue. A. G. POLLARD.

Effect of light on the isotopic composition of oxygen secreted by plants. V. M. Kutyurin, I. V. Matveeva, N. M. Nazarov and K. N. Semenyuk (*Dokl. Akad. Nauk SSSR*, 1964, **157**, 1474—1476).—A special apparatus was assembled for the study of the composition of O_2 released by *Chlorella pyrenoidosa* and *Elodea canadensis* when irradiated with light up to an intensity of 75,000 lux. The $^{18}O_2$ content of the O_2 released was the same as that of the absorbed water, 0·1981%. Further tests with CO_2 and O_2 mixtures indicated that the evolved O_2 originated from the water imbibed by the plants. (10 references.) R. A. KEEN.

Control by iron of chlorophyll formation and growth in *Euglena gracilis*. C. A. Price and E. F. Carell (*Plant Physiol.*, 1964, **39**, 862—868).—*Euglena gracilis* grown first under low light intensity and then shaken under high light intensity in a non-nutrient buffer, synthesises chlorophyll rapidly and in absolute dependence on the Fe content of the cells. The synthesis is linear from zero to a saturating value of Fe beyond which the rate remains constant over a 20-fold range of Fe contents. Chlorophyll synthesis may occur without net synthesis of protein and is not inhibited by 2, 4-dinitrophenol. E. G. BRICKELL.

Interrelationship of iron and manganese supply in growth, chlorophyll and iron porphyrin enzymes in barley plants. S. C. Agarwala, C. P. Sharma and A. Kumar (*Plant Physiol.*, 1964, **39**, 603—609).—Nutrient solutions for sand-cultured barley plants contained varied concn. and ratios of Fe and Mn. Mn toxicity symptoms, produced when [Fe] were low (0·28 and 0·56 p.p.m.) and Mn concn. were excessive, were distinct from the chlorosis produced by Fe deficiency. Plant yields (dry matter) were depressed by both deficiency and excess of Mn and also by deficiency but not by excess of Fe; the latter had no characteristic effects. The optimum Fe/Mn ratio varied widely with the actual concn. of the two elements supplied. Deficiency of Fe or Mn and excessive [Mn] restricted the production of chlorophyll, for which both elements are probably essential. Deficiency of Fe depressed catalase activity, as also did deficiency and excess of Mn over practically all the range of [Fe] examined. (42 references.) A. G. POLLARD.

Ozone effects on cell wall metabolism of *Avena* coleoptile sections. L. Ordin and B. P. Skoe (*Plant Physiol.*, 1946, **39**, 751—755).—Pretreatment with 90 to 140 p.p.m. (vol.) of O_3 resulted in a subsequent stimulation of glucose uptake without destroying the final growth capacity of the tissue. Ozone inhibition of glucose and cellulose metabolism may be a factor in the inhibition of plant growth. E. G. BRICKELL.

Response of plants to air pollutants. III. Relation between ascorbic acid levels and ozone susceptibility of light-preconditioned tobacco plants. H. A. Menser (*Plant Physiol.*, 1964, **39**, 564—567).—Two varieties (susceptible and resistant to O_3) of tobacco plants were light-conditioned by exposure to incandescent light (900 ft. candles) between 4.0 p.m. and 4.0 a.m., control plants being in darkness during this period. Treated plants showed increased (2·5—3·3-fold) ascorbic acid (**I**) contents and injury by O_3 was halved. The protective effect of light-preconditioning probably resulted from preferential oxidation of **I** by O_3 although no relation was apparent between **I** content and varietal differences in susceptibility or resistance to O_3 injury. A. G. POLLARD.

Ion uptake in relation to cell structure of barley roots as affected by calcium supply. H. Marschner and I. Günther (*Z. PflErnähr. Düng.*, 1964, **107**, 118—136).—Roots grown in a moisture-saturated atm. without an external supply of Ca grow well for a few days and then start to die, showing the typical brown coloration of Ca deficiency. Lack of Ca in the roots reduces Na and K uptake and

the addition of Ca to the nutrient solution has the same effect. Uptake of Na by roots is coupled with a high simultaneous release of K, the latter being unaffected by Ca concn. in the nutrient solution. Electron microscope studies indicate that cells 2 mm. from the tip of Ca-deficient roots show breakdown of tonoplast with resulting loss of vacuoles already formed. whilst cells 3 mm. away from the tip are structureless. Treatment of Ca-deficient roots with aq. $CaSO_4$ reverses the damage and roots regain their ability to accumulate ions after a 6—24 h. period. M. LONG.

Phosphorus tolerance and sensitivity of soya-beans as related to uptake and translocation. B. D. Foote and R. W. Howell (*Plant Physiol.*, 1964, **39**, 610—613).—Soya-bean varieties tolerant (*T*) and sensitive (*S*) to high [P] were grown in water-culture. With increase in P supply, the uptake of P by the *S* variety increased more rapidly than did that of the *T* variety. Symptoms of P toxicity occurred when the P content of the cotyledons reached 0·45 mg./plant or 1·2% of the dry wt. This level was attained in the *S* plants with the [P] in the nutrient at 1·6mM; in the *T* plants [P] at 16mM was needed. Accumulation of P in the tops of the *T* variety grafted on *S* roots reached toxic limits when the [P] of the nutrient was 1·6mM. *S* plants did not develop toxicity symptoms when grafted on *T* roots. A. G. POLLARD.

Technique for studying absorption and translocation in aquatic plants. P. A. Frank and R. H. Hodgson (*Weeds*, 1964, **12**, 80—82).—The technique, which allows for application of labelled herbicides to selected portions of the plant, is described. A. H. CORNFIELD.

Influence of foliar leaching on root uptake and translocation of calcium-45 to the stems and foliage of *Phaseolus vulgaris*. R. A. Mecklenburg and H. B. Tukey, jun. (*Plant Physiol.*, 1964, **39**, 533—536).—Young bean plants were grown in nutrient solutions containing ^{45}Ca, in apparatus permitting leaching of leaves by a water mist, collection of leachates, and variations in environmental conditions. Ca was leached from the leaves in considerable quantities and subsequent re-absorption from the leachates via roots increased the intake and translocation of Ca to stems and leaves. Meanwhile, the loss of Ca from leaves lowered the accumulation of dry matter by the plants and temporarily slowed growth. A. G. POLLARD.

Characterisation of leachate from plant foliage. J. V. Morgan and H. B. Tukey, jun. (*Plant Physiol.*, 1964, **39**, 590—593).—Foliage of seven species of plants was subjected to leaching by rain or mist and org. matter present in the leachates was fractionated by exchange resins and determined chromatographically. All leachates contain a similar series of amino-acids (21 of which were identified) and only minor differences between species in this respect were apparent. Fourteen org. acids, including Krebs-cycle acids, were detected in leachates, species differences being more marked than in the case of amino-acids. Four free sugars, some polysaccharides and other carbohydrate material were also present. A. G. POLLARD.

The calcicole-calcifuge habit. II. Influence of calcium on the growth and establishment of four species in soil and sand cultures. R. L. Jefferies and A. J. Willis (*J. Ecol.*, 1964, **52**, 691—707).—The four species examined were *Juncus squarrosus* and *Nardus stricta* (calcifuges with narrow distribution range in respect of Ca), *Sieglingia decumbens* (with a width tolerance range in respect of exchangeable bases, especially Ca) and *Origanum vulgare* (occurring only in soils rich in Ca). The plants were grown in water or sand cultures in which nutrient conditions paralleled those in soils of their natural habitats when the concn. of all nutrients in the culture solutions were low. *N. stricta* could be grown with high ratio of Ca to other cations but with relatively high concn. of nutrients even *O. vulgare* failed to grow. Leaves of plants making poor growth in culture media differing widely from conditions in their natural habitats showed nutrient levels very different from those in naturally grown plants. A. G. POLLARD.

Effect of calcium on intracellular sodium and potassium concentrations in plant and animal cells. G. A. Morrill, H. R. Kaback and E. Robbins (*Nature, Lond.*, 1964, **204**, 641—642).—Evidence is presented to show that Ca is essential in maintaining both high intracellular K and low intracellular Na concn. in several different plant and animal systems. This Ca requirement has not been demonstrated in all cell systems previously investigated; an explanation for this is offered. (15 references.) S. A. BROOKS.

Reduction of strontium-90 uptake by maize and soya-beans with deep placement, irrigation, and soil amendments. D. L. Myhree, R. G. Menzel, H. Roberts, jun., M. H. Frere, M. Amemiya, O. W. Beale, D. R. Timmons and E. H. Wood (*Agron. J.*, 1964, **56**, 463—467).—The ^{90}Sr content of maize and soya-bean grain was less when the contaminated upper 4-in. layer of soil was placed at a depth of 20—24 in. than when the soil was ploughed to a 6-in. depth. Irrigation had no effect. Application of lime or gypsum reduced grain-^{90}Sr content of both species whilst K application reduced ^{90}Sr in maize grain. A. H. CORNFIELD.

Molybdenum deficiency for tropical crops. M. Riandey (*C. R. Acad. Agric. Fr.*, 1964, **50**, 967—969).—Comments on a report of investigations of African soils carried out at the Centre Scientifique et Technique de l'O.R.S. T.O.M., Bondy. P. S. ARUP.

Preliminary investigations of the influence of molybdenum on the absorption capacity of the roots of some cultivated plants. A. Baroccio and G. Celi (*Ann. Staz. chim.-agr., Roma*, 1963 [1964], Ser. iii, No. 217, 13 pp.).—In pot experiments, lucerne, pea, oat, sunflower and pepper plants were raised on gravel and treated with increasing doses of NH_4 molybdate. Moderate doses of Mo increased K absorption and lowered that of Ca. With > 1 p.p.m. of Mo both absorption and K and Ca and protein formation in the roots were reduced. (33 references.) E. C. APLING.

Influence of cobalt on leaf expansion and oxidative phosphorylation. L. Loercher and J. L. Liverman (*Plant Physiol.*, 1964, **39**, 720—725).—Co^{2+}-induced leaf expansion is not susceptible to inhibition by 2,4-dinitrophenol nor does Co affect the content of adenosine triphosphate in bean leaf tissue although it prevents a decrease in the presence of DNP. Activity of ATP in sweet potato mitochondria is lower in the presence of Co ions. E. G. BRICKELL.

Metabolic changes associated with the germination of maize. I. Changes in weight and metabolites and their redistribution in the embryo axis, scutellum and endosperm. J. Ingle, L. Beevers and R. H. Hageman (*Plant Physiol.*, 1964, **39**, 735—740).—Over a 5-day period growth of the axis was largely at the expense of the reserves of the endosperm which lost extensively in protein and insol. carbohydrates. The fat content of the scutellum decreased after the second day with a concurrent increase in sol. carbohydrates and sol. nitrogenous components, although the scutellum dry wt. remained relatively constant throughout the experimental period. E. G. BRICKELL.

Relationship between ribonucleic acid content and the rate of growth of maize roots. J. Ingle and R. H. Hageman (*Plant Physiol.*, 1964, **39**, 730—734).—The direct proportionality between growth rate and RNA content of the root tip observed for the inbred lines did not hold true for the hybrid maize, although a relationship was indicated between growth rate and cellular RNA content for both inbred and hybrid lines. Results suggest that RNA content is not the limiting factor in the control of growth rate during the early stages of root growth. E. G. BRICKELL.

^{14}C-Amino-acid incorporation by spinach chloroplast preparations. A. App and A. T. Jagendorf (*Plant Physiol.*, 1964, **39**, 772—776).—Apparent incorporation of amino-acids into protein by isolated *Spinacis oleracea* chloroplasts is due to contaminating bacteria which are sedimented together with the chloroplasts during their initial isolation. Previous reports of stimulation of incorporation by light are confirmed but enhancement may be due to evolution of a small amount of O_2 by the chloroplasts. E. G. BRICKELL.

Influence of elevated shoot and root temperature on nitrogen fixation. J. V. Possingham, D. V. Moye and A. J. Anderson (*Plant Physiol.*, 1964, **39**, 561—563).—In subterranean clover plants high (30°) root temp. increased the no. of nodules per plant, but lowered the total N and total protein contents of the plants unless fertiliser N was applied. High temp. of shoots did not restrict N fixation but diminished the total dry matter per plant. A. G. POLLARD.

Factors influencing dormancy of groundnut seeds. V. K. Toole, W. K. Bailey and E. H. Toole (*Plant Physiol.*, 1964, **39**, 822—832).—Germination was improved by leaching, daily changing of towel substratum, and by increased vol. of available water. Germination was also improved by removal of the outer layer or, better still, removal of the entire seed coat, by damage to the coat, by enclosing the seeds on wet substratum within a sealed box, and sometimes by passing CO_2 through boxes containing imbibed seeds. Effects of light on breaking dormancy were inconclusive. E. G. BRICKELL.

Loss of adenosine triphosphate synthesis caused by freezing and its relationship to frost hardiness problems. U. W. Heber and K. A. Santarius (*Plant Physiol.*, 1964, **39**, 712—719).—ATP synthesis is primarily damaged by freezing due to inactivation of the phosphorylating systems of isolated chloroplasts and mitochondria. Uncoupling can be prevented by the addition of small amounts of glucose, sucrose and raffinose to the chloroplast system. E. G. BRICKELL.

Evaluation of cold-hardness by controlled freezing of field-hardened forage crops. R. W. Peake (*Canad. J. Plant Sci.*, 1964, **44**, 538—543).—Highly significant correlations were obtained between

survival after controlled freezing and survival after natural winter exposure in studies with 10 orchardgrass-, 15 lucerne varieties, 16 grass species, and 43 lines of white clover. E. G. BRICKELL.

Conditions determining effects of far-red and red irradiations on flowering response of *Pharbitis nil*. H. Fredericq (*Plant Physiol.*, 1964, **39**, 812—816).—Far-red light at the end of 2- or 4-h. photoperiods and, if of low intensity, after 8-h. periods, inhibits flowering. Inhibition at the beginning of the dark period is completely and repeatedly reversible. Far-red reversibility of flowering in the middle of the dark period is successful if the red and far-red irradiances are no more than 30 sec. each and are not separated by darkness. E. G. BRICKELL.

Preparation of indole extracts from plants for gas chromatography and spectrophotofluorometry. L. E. Powell (*Plant Physiol.*, 1964, **39**, 836—842).—A procedure is described encompassing (*a*) fractionation of the indoles in a plant into four major indole groups in acidic, basic, neutral and water-sol. compounds, (*b*) preliminary purification by SiO_2-gel column chromatography, (*c*) further purification of the neutral and acidic indoles by gas chromatography and (*d*) measurement of the fluorescence intensity of individual indoles spectrofluorometrically. E. G. BRICKELL.

Persistence and translocation of exogenous regulating compounds that exude from roots. P. J. Linder, J. W. Mitchell and G. D. Freeman (*J. agric. Fd Chem.*, 1964, **12**, 437—438).—When applied to the leaves of bean plants the growth regulators 2,3,6-trichlorobenzoic acid, α-methoxyphenylacetic and 2-methoxy-3,6-dichlorobenzoic acids are translocated to the roots and exuded as such. 2,5-Dichlorobenzoic, 3,4-dichloro-α-methoxyphenylacetic and naphthylacetic acids are apparently degraded and bound by the plant and not exuded. The ability of a plant to exude a growth regulator does not affect its susceptibility to the regulator. (10 references.) W. ELSTOW.

Synthesis, properties and biological activity of some amino-acid derivatives of halogen-substituted phenoxy acids. J. F. Carmichael, E. J. Saggese, J. S. Ard, C. F. Krewson and E. M. Shantz (*J. agric. Fd Chem.*, 1964, **12**, 434—436).—The synthesis of 39 new leucine deriv. of chlorinated phenoxyacetic acids is reported with a progress report on the growth-regulating properties of many of them. Deriv. of D-leucine and D-isoleucine were without effect. The L-leucine and L-isoleucine derivatives of 2,4-dichloro- and 2-methyl-4-chlorophenoxyacetic acids exhibited growth accelerating effects. (14 references.) W. ELSTOW.

Interaction of growth substances in growth and organ initiation in the embryos of *Capsella*. V. Raghavan (*Plant Physiol.*, 1964, **39**, 816—821).—In media containing low concn. of IAA and an optimum concn. of gibberellic acid (**I**), the root, hypocotyl and cotyledons of the embryos were longer than those in corresponding treatments with IAA alone. Low concn. of IAA overcame the inhibition by kinetin (**II**), of the growth of the hypocotyl and cotyledons but embryos grown in media containing (**II**) + IAA lacked organised root and shoot systems. In media containing both **I** and **II**, **II** did not appreciably affect the growth of the root, hypocotyl or cotyledons of the embryos. E. G. BRICKELL.

Interaction of nucleotides with auxins in growth of pea stem segments. R. T. Wedding and M. K. Black (*Plant Physiol.*, 1964, **39**, 799—803).—The growth of pea epicotyl segments is synergically stimulated by several nucleotides in combination with 2,4-D or IAA. The greatest effect is produced by NADP with somewhat less response to NAD and ADP. E. G. BRICKELL.

Occurrence and biosynthesis of indolepyruvic acid in plant tissues and bacteria. B. I. Sahai Srivastava (*Plant Physiol.*, 1963, **39**, 781—785).—Maize kernels and tomato seedlings were examined for the natural occurrence of the acid (IPyA), and maize kernels, and coleoptiles, tomato seedlings and crown gall bacteria tested for their capacity to synthesise this auxin from tryptophan. No evidence for the natural occurrence of IPyA or of its formation from tryptophan was obtained. E. G. BRICKELL.

Distribution of indoleacetic acid oxidase and inhibitors in light-grown cotton. P. W. Morgan (*Plant Physiol.*, 1964, **39**, 741—746).—Evidence for an increase, with age, of IAA-oxidase activity in light grown cotton tissues is presented. *In vitro* activity of the enzyme increased in leaves and stems from apex to base of plants; inhibitor activity was highest in the apex and decreased basipetally. This finding is compatible with a hypothesis that IAA-oxidase functions *in vivo* in cotton to help regulate auxin levels and that the activity of the enzyme is controlled by an inhibitor system. E. G. BRICKELL.

Effects of indoleacetic acid and kinetin on activities of enzymes of the hexose monophosphate shunt in tissue cultures of Nicotiana. K. J. Scott, J. Daly and H. H. Smith (*Plant Physiol.*, 1964, **39**, 709—711).—With the enhancement of normal tissue growth in the presence of IAA and kinetin there was a decrease in the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and transketolase, but enolase, isocitrate dehydrogenase and malate dehydrogenase activities were unaffected. These results indicate that activities of the hexose monophosphate shunt of normal tissue are decreased by increasing the growth rate of the plant material. E. G. BRICKELL.

Ribonuclease levels in the mesocotyl tissue of *Zea mays* as a function of 2,4-dichlorophenoxyacetic acid application. J. C. Shannon, J. B. Hanson and C. M. Wilson (*Plant Physiol.*, 1964, **39**, 804—809).—Application of 2,4-D to intact tissue promotes or inhibits growth depending on concn. but ribonuclease, protein and nucleic acids increase with the concn. of 2,4-D applied even into the herbicidal range. Herbicidal action correlates with excessive nucleic acid and protein synthesis. E. G. BRICKELL.

Chemical inhibition and promotion of citrus flower bud induction. S. P. Monselise and A. H. Halevy (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 141—146).—Application of gibberellin (200 p.p.m.) at 2-weekly intervals, beginning in Nov. and repeated a different no. of times to the end of Jan., inhibited flower induction of orange trees. Providing the treatment was not given after mid-Dec. some delayed blossoming occurred the following spring. Application of benzothiazolyloxyacetate (50 p.p.m.) in summer induced flowering in lemon trees even on young non-bearing shoots. 0·2% *N*-dimethylaminosuccinamic acid induced flower buds to a limited extent. A. H. CORNFIELD.

Straw length reducing action of chlorocholine chloride (CCC) on wheat and its dependence on soil type. J. Jung (*Z. PflErnähr. Düng.*, 1964, **107**, 146—153).—CCC generally reduces straw length whether applied to the soil or as a foliar spray, although the latter is more effective. The differences are especially related to sorption and exchange processes in the soil. Applied to peaty, mineral and neutral loam soils, the effect of CCC is much less marked; with a clay, only spraying has any effect. CCC is markedly inactivated after 2 weeks at 20° in soil. The lowest node is the most shortened. M. LONG.

Movement and fate of labelled *N*-dimethylaminosuccinamic acid, a size controlling compound, in apple seedlings. G. C. Martin, M. W. Williams, and L. P. Batjer (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 7—13).—Application of ^{14}C-labelled *N*-dimethylaminosuccinamic acid (B9) to the petiole, severed stem or single root of apple seedlings showed that the compound was quite mobile in the plant, its speed of movement being comparable with that of inorg. ions. The compound was resistant to breakdown in the plant. A. H. CORNFIELD.

Effect of nitrogenous materials in soya flour on quality of gibberellins produced in deep culturing of *Gibberella fujikuroi*, (SAW), Wr. Ĭ. Fuska, U. Kugr and Ĭ. Zaĭichek (*Mikrobiologiya*, 1964, **33**, 783—786).—N-containing substances in defatted soya flour (Total N 7·25, fat 0·25%) were separated by extraction with water, H_2SO_4 and alkali at various pH levels into nine sol. and insol. fractions with N content between 1·96 and 11·35%. Fractions were used as N sources in fermentation of Roulin–Thom maize extract medium by a strain of *G. fujikuroi*, V_1. N content was constant in each fermentation. A table shows pH of medium, total production (65—600 μg./ml. of medium) and composition of gibberellins produced. Except with one fraction (solid residue after extraction at pH 12) which yielded only gibberellic acid (GA_3), a mixture of gibberellin A_1 (GA_1) and gibberellic acid (GA_3) was always produced. Production of GA_1, when soya flour is source of N, is attributed to a constituent containing N which is stable in alkaline medium and thermally stable at pH 3. Whether or not a N-containing component of soya bean flour has significant effect on total production of gibberellins depends on its solubility. P. W. B. HARRISON.

Evaluation of flame photometric determination of magnesium in plant material. A. A. MacLean (*Canad. J. Plant Sci.*, 1964, **44**, 520—534).—Flame methods were compared with EDTA titration and a thiazole yellow procedure. Precision was highest for the oxyhydrogen flame method and lowest when the oxyacetylene flame was used. SiO_2 repressed Mg emission with either flame source but interference was greater with the oxyhydrogen flame. E. G. BRICKELL.

Crops and Cropping

Effect of artificial lodging on winter wheat grain yield and quality. R. O. Weibel and J. W. Pendleton (*Agron. J.*, 1965, **56**, 487—488).—When winter wheat was artificially lodged, by being pushed over

and held in position with wire mesh, grain yield, test wt., and kernel wt. were reduced compared with these values in erect plants. The yield reduction due to lodging decreased as lodging was done from the head to the hard dough stage. Grain protein % was higher in lodged than in erect plants. A. H. CORNFIELD.

Response of four winter wheat varieties to nitrogen fertilisation. F. C. Stickler and A. W. Pauli (*Agron. J.*, 1964, **56**, 470—472).—There were only slight differences due to variety in the response of four varieties of winter wheat to N applications (25—100 lb. per acre) in seven trials. Ottawa gave a significantly higher response to N than did the other varieties in two of the trials. This was considered to be due to the higher resistance of Ottawa to rusts. A. H. CORNFIELD.

Effects of soil moisture stress on growth of barley. I. Vegetative development and grain yield. D. Aspinall, P. B. Nicholls and L. H. May (*Aust. J. agric. Res.*, 1964, **15**, 729—745).—The effects of soil moisture stress on tillering, stem elongation and grain yield of barley were studied by subjecting the plants to periods of stress at different stages of development. The results support the contention that the organ which is growing most at the time of a stress is the one most affected. (40 references.) S. A. BROOKS.

Effect of irrigation on the agronomic and malting characteristics of barley. F. W. Sosulski and V. M. Bendelow (*Canad. J. Plant Sci.*, 1964, **44**, 509—514).—Light irrigation increased grain yields, plant height, lodging, and days to maturity. Heavy irrigation increased kernel size and malt extract but decreased saccharifying activity. Grain N content decreased with increasing soil moisture level. Results suggest that two-rowed malting varieties might be useful alternatives to feed barleys for the irrigated districts of W. Canada, the variety Betzes appearing more promising than Hannchen for this purpose. E. G. BRICKELL.

Effect of N, P, Ca and Mg treatments on yield of barley varieties grown on acid soils. T. C. Chiasson (*Canad. J. Plant Sci.*, 1964, **44**, 525—530).—Twelve varieties were grown on soils of pH 5·0 and 5·4. Those which performed best at the higher pH developed yellowing symptoms, had stunted growth, were late tillering, and gave very low yields when grown at the lower pH. Applications of N and P at 40 and 44 lb./acre respectively, in direct contact with the seed eliminated all symptoms and generally doubled yields on soils of pH 5·0. Lime and/or Mg in direct contact with the seed had no influence on symptoms or yield. E. G. BRICKELL.

Maize growth as affected by aggregate stability, soil temperature and soil moisture. W. B. Anderson and W. D. Kemper (*Agron. J.*, 1964, **56**, 453—456).—Maize yields in a sandy clay loam were higher when the soil contained initially 53% than when it contained 32% or 73% of water-stable aggregates. Maize roots continued to use soil O_2 even when the O_2 content of the soil air was reduced to $<5\%$, whilst micro-organisms appeared to stop using O_2 below this level. Rate of use of O_2 by roots was higher at 30° than at 17° when the soil air was high in O_2, but the reverse was true when the soil air was low in O_2. A. H. CORNFIELD.

Water requirements of irrigated maize in Nyasaland. J. M. Munro and R. A. Wood (*Emp. J. exp. Agric.*, 1964, **32**, 141—152).—The ratio of the water requirement of maize to open-pan evaporation was not consistent throughout the growing season, but rose sharply about the time of tassel emergence. Over 2 years the same irrigation factors gave similar patterns of soil moisture use, although evaporation, and hence the amount of irrigation water applied, was appreciably different. A. H. CORNFIELD.

Efficiency of *Azotobacter* used together with mineral fertilisers on rice crops. Sh. T. Shende and L. M. Kokorina (*Mikrobiologiya*, 1964, **33**, 467—471).—In field trials on 70·6 sq./m. plots in the Kuban, fertiliser was added to the soil at sowing at the rates, NPK 60, or N 120, or PK 90 kg./ha. Seed was sown both with and without preliminary treatment with *Azotobacter* (400 milliard cells per ha.). Treatment of the seed, particularly with fertiliser NP and NPK, increased the content of *Azotobacter* in the soil. It stimulated the microbiological processes in the soil under rice crops, and improved the accumulation of readily accessible forms of N e.g. NO_3^- and NH_3. *Azotobacter* alone was not a substitute for fertilisers, but appreciably increased their efficiency. NPK with *Azotobacter* increased the yield of rice by 24—42%. (21 references.) A. S. LEVESLEY.

Soil water and the growth of grasses. I. Interaction of water-table depth and irrigation amount on the growth of *Agrostis tenuis* and *Alopecurus pratensis*. J. R. Etherington and A. J. Rutter (*J. Ecol.*, 1964, **52**, 677—689).—The two grasses were grown in concrete tanks in which the water-table was maintained at 20 or 60 cm. below the surface or was uncontrolled. Three levels of irrigation were applied, calculated to maintain the water content at high (*H*) level, i.e., field capacity; medium (*M*) level, 50% full capacity increasing to 66% in June or low (*L*) level 25% of field capacity increasing to 33% in June. With the water table held at 30 cm. reducing conditions were set up in the soil and growth of grasses was restricted. With irrigation, *L*, without a maintained water-table, high soil-moisture tensions reduced the growth of the grasses by 50%. Irrigations *M* with no maintained water-table and *L* with 60 cm., water-table caused less severe moisture tensions and smaller reductions in yield. No interaction treatment × species was apparent. A. G. POLLARD.

Influence of nitrogen and potassium on the yield and chemical composition of orchardgrass. W. K. Griffith, M. R. Teel and H. E. Parker (*Agron. J.*, 1964, **56**, 473—475).—Orchardgrass was treated factorially with three levels of N (25, 15 and 300 lb. of N, as NH_4NO_3) and K (0, 166 and 322 lb. of K, as KCl, per acre) and cut every 5·5 weeks (4 cuttings) during the growing season. Yields at all cuttings increased with level of applied N but where K was applied only at the third cutting and, at the first cutting, only where the lowest level of N was applied. N reduced total fructose % in the plant at the first two, and K at the first three, cuttings. N accentuated K deficiency, particularly where high levels of the former were applied. The deficiency was associated with an abnormal accumulation of asparagine in the plant tissue When N was not limiting there was a high negative correlation between K% and asparagine % in the tissue. A. H. CORNFIELD.

Effect of potassium rate and source on yield and composition of bromegrass in Alaska. W. M. Laughlin and S. H. Restad (*Agron. J.*, 1964, **56**, 484—487).—Increasing rates of application of K (33—133 lb. per acre) increased dry matter yields of bromegrass, K% and % total cations (mequiv.) in the forage and reduced forage-Ca% and Ca/Mg ratio. P% and Mg% in the forage were unaffected by K applications. K_2SO_4 and KCl were equally effective as sources of K. A. H. CORNFIELD.

Distribution of potassium, calcium, magnesium and sodium in grasses at progressive stages of maturity. G. I. Pritchard, W. J. Pigden and L. P. Folkins (*Canad. J. Plant Sci.*, 1964, **44**, 318—324).—Bromegrass and timothy were sampled eight times during the season. Concn. of the three cations were highest in leaves and lowest in stem bases. With advancing growth concn. of K, Ca and Mg in stems diminished whereas, in leaves, K diminished, Ca increased and Mg showed no appreciable change. Na concn. were small throughout and changed very little in successive samplings. In both grasses the ratios, K/Ca, K/Mg and K/(Ca+Mg) were high and did not alter greatly in leaves but decreased in stems and heads during the season. A. G. POLLARD.

Soil moisture extraction trends of legume–grass mixtures as affected by cutting frequency and nitrogen fertilisation. D. D. Wolf (*Agron. J.*, 1964, **56**, 467—469).—Soil moisture extracted under grass–legume mixtures cut three times was less at the 12-in. depth, but greater at the 48-in. depth, than when five cuttings were made during the season. Extraction under lucerne–grass mixtures receiving N (200—400 lb. NH_4NO_3-N per acre) was greater at 12-in., but usually less at 48 in., than under mixtures receiving no N. Moistures containing lucerne extracted less soil moisture at 12 in., but more at 48 in., than did mixtures containing birdsfoot trefoil or ladino clover. A. H. CORNFIELD.

Tolerance of five turfgrass species to soil alkali. O. R. Lunt, C. Kaempffe and V. B. Younger (*Agron. J.*, 1964, **56**, 481—483).—*Puccinellia distans* and Seaside bentgrass showed good tolerance to Na up to 26—28 ESP (exchangeable Na as % of total exchangeable bases, in mequiv.). Alta fescue, Kentucky bluegrass and common Bermudagrass showed reduced growth when ESP exceeded 11—12. The two tolerant grasses showed either an ability to accumulate Na or a low uptake of Ca and Mg. The growth of *P. distans* and Alta fescue was not affected by addition of 0·1% vinyl acetate–maleic acid copolymer to the soil, whilst growth of the other grasses was improved. A. H. CORNFIELD.

Yield and chemical composition of Sudangrass and forage sorghum under three systems of summer management for late autumn *in situ* utilisation. J. C. Burns and W. F. Wedin (*Agron. J.*, 1964, **56**, 457—460).—Dry matter yields and crude protein% of the aftermaths of forage sorghum and Sudangrass were no different whether 3, 1 or no summer cuts were made. The HCN% in forage sorghum aftermath decreased with delay in harvesting, whilst that in Sudangrass was not affected. A. H. CORNFIELD.

Effects of row spacing and time of fertilisation on grass seed production. H. M. Austenson and D. V. Peabody, jun. (*Agron. J.*, 1964, **56**, 461—463).—Seed yields of orchardgrass and timothy on a silt loam were higher when grown in 42- than in 7-in. rows, but the reverse was true for colonial bentgrass. Red fescue yields were unaffected by row spacing. Split fertiliser applications gave highest yields of orchardgrass in 42-in. and autumn applications

gave highest yields in 7-in. rows. Red fescue and colonial bentgrass gave highest seed yields when all or part of the fertiliser was applied in spring. Seed yields of timothy were not affected by time of fertilisation. Seed quality was not affected by row width or time of fertiliser application in any of the four species.
A. H. CORNFIELD.

Silica in medusahead, *Elymus caput-medusae*, L. C. F. Swenson, D. le Tourneau and L. C. Erickson (*Weeds*, 1964, **12**, 16—18).—Silica accounted for 72—89% of the ash of medusahead, a winter annual grass which is a major weed problem in many states. Ash and SiO_2 content decreased with advancing maturity. The SiO_2 was present as opal and was particularly high in the barbs of awns and in the epidermis of leaves, culms, glumes and seeds. The high SiO_2 content may account for its poor palatability to livestock and consequent persistence as a weed. A. H. CORNFIELD.

Interactions in forage yield trials. D. B. Wilson (*Canad. J. Plant Sci.*, 1964, **44**, 344—350).—The effects of interactions of common management treatments on yields of forage are examined. Fertiliser treatment, stage of growth just prior to harvest, height of stubble when cut and method of sowing are compared. Response to N fertiliser varied with the height of stubble left after cutting and with the method of sowing. Methods of avoiding the effects of the interactions are indicated. A. G. POLLARD.

Differential yields in lucerne (*Medicago sativa*, L.) with special reference to factors affecting net production and photosynthetic activity. F. W. Fuess (*Dissert. Abstr.*, 1964, **25**, 13).—Lucerne, cut three times per season for 2 years, yielded more than when cut twice per season, leaf loss being greater in the latter case and photosynthesis being more active in the relatively younger leaves of the thrice-cut crop. Leaf area did not provide a sound basis for prediction of the seasonal production, but was positively related to the yield from an individual cutting. A. G. POLLARD.

Non-fermentable free sugars in the leaf-petiole fraction of lucerne (*Medicago sativa*). V. V. Rendig, E. A. McComb and C. L. Hu (*J. agric. Fd Chem.*, 1964, **12**, 421—423).—The following non-fermentable free sugars were isolated from the leaf petioles of lucerne, D-glycero-D-manno-octulose, galactose, manno-heptulose, arabinose, altro-heptulose, xylose and ribose. An additional unknown ketose was separated but not identified. (30 references.)
W. ELSTOW.

Influence of levels of irrigation on the nutritive value of lucerne. L. M. Bezeau and L. G. Sonmor (*Canad. J. Plant Sci.*, 1964, **44**, 505—508).—Fertilised plots of Ladak lucerne were irrigated at 75, 50 and 25% levels of available water within the root zone. The 50% level was superior to both the 76% and the 25% levels in all criteria studied. It produced more protein, more digestible cellulose per acre and the ' nutritive value index ' was higher.
E. G. BRICKELL.

Water-soluble phytotoxic substances in lucerne forage; variation with variety, cutting year and stage growth. W. D. Guenzi, W. R. Kehr and T. M. McCalla (*Agron. J.*, 1964, **56**, 499—500).—There were no differences between two varieties of lucerne in respect of reduction of root and shoot growth of maize seedlings treated with water extracts of the dried lucerne leaf. Extracts from various stages of growth and cuttings of lucerne differed significantly in their effect on root growth of maize; forage cut when 10 in. high had the greatest phytotoxic effect. The extent of phytotoxicity of water extracts of lucerne also varied from year to year.
A. H. CORNFIELD.

Effect of naphthylacetic acid-type materials and 1-naphthyl *N*-methylcarbamate on fruiting, flowering and keeping quality of apples. F. W. Southwick, W. D. Weeks and G. W. Olanyk (*Proc. Amer. Soc. Hort. Sci.*, 1964, **84**, 14—24).—Application of 1-naphthyl *N*-methylcarbamate (NMC, 1·5 lb. per gal.) or of naphthylacetamide (NAD) 25—37·5 p.p.m. to heavy-flowering apple trees 12—24 days after petal fall reduced fruit set to the same degree and also had similar effects on fruit size and yield. Neither material affected pre-harvest crop. NAD and naphthylacetic acid applied 10—18 days after petal fall reduced the rate of fruit abscission for 8—10 days after treatment, but this was then followed by a sharp increase in fruit drop. NMC on the other hand had no temporary inhibiting action on abscission, but stimulated a gradual increase in the rate of June drop. Cold and controlled atm. storage studies with fruit from plots where fruit size was not greatly increased by NMC and NAD thinning treatments, indicated that neither material had any direct influence on fruit firmness, brown core, or scald of McIntosh apples. A. H. CORNFIELD.

Effect of exchangeable magnesium, potassium and calcium in the soil cation content of apple seedlings. J. L. Mason (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 32—38).—The effect of various ratios of exchangeable K, Ca and Mg in a gravelly sandy loam on the leaf cations of apple seedlings was studied. When the soil Ca/Mg ratio was reduced from 16 to 1, with 5% K saturation leaf-Mg increased and leaf-Ca and -K decreased. When the soil Mg/K ratio was reduced with Ca saturation 60%, leaf-Mg and -Ca were reduced and leaf-K increased. Leaf-N decreased slightly with increasing leaf-Mg. A. H. CORNFIELD.

Response of apple (*Malus sylvestris*) to various levels of soil moisture. S. J. Gamble (*Dissert. Abstr.*, 1964, **25**, 1).—Apple trees (18 years old) were grown in a sandy loam, the moisture content of which was allowed to fall to pre-arranged levels or was increased by sprinkler irrigation. The available water content of the soil was measured at 18 in. depth. Yields of Northern Spy were increased by irrigation when the soil moisture level had fallen to 70% of the field capacity. McIntosh and Red Delicious showed no significant yield increase attributable to irrigation. Fruit size was related more closely to total yield than to irrigation treatment. Terminal shoot growth was unrelated to soil moisture content but the trunk dia. of the trees was correlated significantly with soil moisture level. Leaf-N, -K, -P, -Ca, -Mn, -Mg, -Fe, -Cu, -B, -Zn, -Mo and -Al showed no consistent relation to soil moisture content. A. G. POLLARD.

Environmental studies of maturation in the McIntosh apple. A. M. Badran (*Dissert. Abstr.*, 1964, **25**, 11).—Effects of night temp., light intensity and day length on rates of vegetative growth, fruit development, starch accumulation, fruit maturity and storage quality are examined. High night temp. during the first month after flowering increased the rate of vegetative and fruit growth and hastened maturity. Low light intensities increased vegetative growth and depressed that of fruit growth; maturity was delayed. High night temp. counteracted the influence of low light intensity on fruit growth. Short-day conditions had little effect on fruit growth; short days with low light intensities caused a secondary flush of terminal growth. The interval between flowering and starch accumulation was unrelated to that between flowering and the physiological maturity of the fruit. Prediction of fruit maturity by heat-summation methods developed in other localities could not be applied satisfactorily to the site under observation.
A. G. POLLARD.

Effect of growth regulators on flowering and fruit set of apple trees. L. J. Edgerton, M. B. Hoffman and C. G. Forshey (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 1—6).—A no. of benzoic acids, including 2,3,5-tri-iodobenzoic acid (TIBA), did not promote flower formation consistently on mature apple trees bearing a crop of fruit. Treatment with TIBA did not increase flowering the year following treatment. TIBA applied 2—4 weeks following bloom increased flower bud formation in Baldwin trees.
A. H. CORNFIELD.

Volatile constituents of apple fruits as influenced by fertiliser treatments. L. P. Somogyl, N. F. Childers and S. S. Chang (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 51—58).—Volatiles were determined in the juice of apples by gas–liquid chromatography. With a no. of varieties and locations, application of N to the soil resulted in increased volatiles in the fruit. Application of P, K and trace elements increased volatiles further. Foliar applications of urea and $MgSO_4$ also increased volatiles. In many cases the individual volatile components were also affected by the nutrient treatments.
A. H. CORNFIELD.

Response of Yellow Newton apple leaves to foliar applications of manganese and zinc. K. Uriu and E. C. Koch (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 25—31).—An intervenal chlorosis of Yellow Newton apple leaves was cured by application of Mn-EDTA and Zn-EDTA, each at 1 lb. per 100 gal. Zn alone gave no correction. Mn alone cured the intervenal chlorosis but left a mottled pattern suggestive of Zn deficiency, which was cured by application of Zn. Early spring sprays of Mn + Zn applied at least twice corrected the symptoms for the entire year, but symptoms reappeared the following season. A. S. CORNFIELD.

Influence of calcium and boron tree sprays on York spot and bitter pit of York Imperial apples. W. C. Stiles (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 39—43).—Reductions in the % of fruit affected with York spot after spraying with 0·05M-$CaCl_2$ were associated with reductions in the size of fruit at harvest. The average no. of York spots per fruit were decreased by $CaCl_2$ and B (1 lb. of borax per 100 gal.) sprays without significant reduction in final fruit size. The severity of bitter pit was reduced by summer sprays of $CaCl_2$. Fruit wt. was reduced at one of two locations. A. H. CORNFIELD.

Effect of varying concentrations of oxygen, with and without carbon dioxide, on senescent changes in stored McIntosh apples grown under two levels of nitrogen fertilisation. C. A. Eaves, F. R. Forsyth, J. S. Leefe and C. L. Lockhart (*Canad. J. Plant Sci.*, 1964, **44**, 458—465).—The apples were stored in atm. containing 0, 5 or 7% of CO_2 and from 2·5 to 20% of O_2. Fruit from trees of

low N status were susceptible to core-browning in presence of CO_2 with all $[O_2] > 2{\cdot}5\%$. With fruit from high-N trees the presence of CO_2 in the storage atm. tended to decrease the incidence of the browning. In presence of CO_2, the firmness, sol. solids and acid contents of the fruit were greater than when the atm. contained no CO_2. With $[CO_2]$ 5% the no. of fungal rots occurring in the stored fruit diminished. Reduction in $[O_2]$ in absence of CO_2 improved the control of fungal rotting. A. G. POLLARD.

Aerobic and anaerobic carbon dioxide production by apple fruits following air and controlled atmosphere storage. D. R. Dilley, D. C. MacLean and R. R. Dedolph (*Proc. Amer. Soc. hort. Sci.*, 1964, **84,** 59—64).—The rate of CO_2 evolution under both aerobic and anaerobic conditions from apple fruits was markedly lower following 5 months in an atm. of 3% O_2–3% CO_2–94% N_2 at 0° as compared with the same duration in air at 0°. The ratio of anaerobic to aerobic CO_2 evolution of fruits following controlled atm. storage was lower than that of fruits following air storage, indicating a conserving effect of the modified atm. on the mechanism for aerobic CO_2 production. The ratio of anaerobic to aerobic CO_2 evolution by apple fruits increased during ripening at 20° immediately following harvest. The ratio may be useful as a post-harvest measure of physiological age of apple fruits. A. H. CORNFIELD.

Seasonal changes in electrical resistance of apple shoots as a criterion of their maturity. J. Wilner (*Canad. J. Plant Sci.*, 1964 **44,** 329—331).—In three varieties of apple trees the electrical conductivity of twigs declined with the advancing season summer→autumn to extents comparable with the rate of advance of autumn maturity. The use of such measurements in the selection of early- and late-maturing varieties is indicated. The relatively high autumn resistance of the Antonovka was transmitted to progenies of crosses. A. G. POLLARD.

Mineral composition of the grape petiole at bloomtime in relation to rootstock and scion variety behaviour. J. A. Cook and L. A. Lider (*Proc. Amer. Soc. hort. Sci.*, 1964, **84,** 243—254).—Petioles at bloomtime were analysed for NO_3^-, K, P, Mg and Ca and the results were compared with fruit yields and pruning wt. in trials involving 22 scion varieties and 3 rootstocks. The stock producing the highest fruit yields showed intermediate petiole-NO_3^- levels. The weakest stock was the most efficient in terms of fruit yield per unit of growth. The stock producing the greatest vegetative growth generally produced the highest petiole nutrient levels. With this stock petiole-NO_3^- levels were inversely related to fruit production. A. H. CORNFIELD.

Effect of gibberellic acid on Anab-E-Shahi grape, *Vitis vinifera*. L. Venkataratnam (*Proc. Amer. Soc. hort. Sci.*, 1964, **84,** 255—258).—Dipping the grape inflorescence in aq. gibberellic acid 40 p.p.m., 24—48 h. before anthesis, increased the size of the bunch and berry. Higher concn. of the acid (60—100 p.p.m.) reduced the % set and did not increase berry size very much. Although maturity and sugar production were delayed, this effect was overcome by delaying harvest for 3 weeks. A. H. CORNFIELD

Effects of 4-thianaphthenacetic acid on ripening of Concord grapes. R. K. N. Singh and R. W. Campbell (*Proc. Amer. Soc. hort. Sci.*, 1964, **84,** 259—262).—Application of thianaphthen-4-yl-acetic acid (4-TNA), 25—100 p.p.m. at weekly intervals 1—6 weeks before the date of harvest, failed to increase the uniformity of ripening and resulted in delayed maturity. The treatments caused no external injury to leaves or berries, but the higher levels increased the colloidal N% in the leaves. A. H. CORNFIELD.

Changes in methoxyl content in the peach endocarp and some of its soluble phenolic constituents during lignification. K. Ryugo (*Proc. Amer. Soc. hort. Sci.*, 1964, **84,** 110—116).—Lignin in the peach endocarp increased in methoxyl content to 16·6% in about 50 days after initiation of lignification. The ratio of di- to mono-methoxylated alcohol-sol. phenolic aldehydes decreased whilst lignin was still increasing, but became constant as the rate of lignin synthesis diminished in July. A. H. CORNFIELD.

Effect of post-harvest soil moisture depletion on subsequent yield of apricots. K. Uriu (*Proc. Amer. Soc. hort. Sci.*, 1964, **84,** 93—97).—Low yields of apricots in some years occurring in unirrigated treatments were associated with early depletion of soil moisture during the previous summer. When soil moisture did not reach the permanent wilting point (PWP) until mid-Aug., yields in the following year were not appreciably affected, but when PWP was reached in early July and remained at that level, yields in the following year were reduced. Irrigation in July prevented crop reduction. Adequate soil moisture at the time of floral bud differentiation in the immediate post-harvest period (late July to early Aug.) is essential for satisfactory yields of apricots in the following year. A. H. CORNFIELD.

Effects of soil pH on the mineral composition and growth of the low-bush blueberry. I. V. Hall, L. E. Aalders and L. R. Townsend (*Canad. J. Plant Sci.*, 1964, **44,** 433—438).—Growth of the blueberry was optimal in media of low pH (4·2—5·5). Addition of dolomitic limestone to the medium, reduced growth, decreased leaf-Mn, -P and -K and increased leaf-Fe in most cases. The influence of changes in pH on growth and uptake of nutrients differed considerably with the nature of the growth medium (soil, soil and sawdust, potting compost). A. G. POLLARD.

Deficiency states in citrus fruit trees. J. Maria del Rivero (Inst. nac. Invest. Agronom., Madrid, 1964, 383 pp.).—An extensively documented and illustrated treatise on the biochemistry, physiology, diagnosis and control of trace element deficiencies in citrus fruit trees. E. C. APLING.

Influence of nitrogen source and soil organic matter on the cranberry. L. P. Somogyi, N. F. Childers and P. Eck (*Proc. Amer. Soc. hort. Sci.*, 1964, **84,** 280—288).—In a sand substrate $(NH_4)_2SO_4$ (**I**) was a more effective source of N (50 lb. per acre) in increasing vegetative growth of cranberry over one season than were urea or urea + urea–formaldehyde (**II**), whilst the latter was the least effective. In a cranberry bog soil urea + **II** was the most effective source. At the end of the second growing season leaf-N was higher where the org. sources than where **I** had been applied. A. H. CORNFIELD.

Seasonal changes in Florida avocados. T. T. Hatton, jun., P. L. Harding and W. F. Reeder (*U.S. Dep. Agric. tech. Bull.*, 1964, No. 1310, 47 pp.).—Results of the research on 7 crop years covering more than 40 commercial varieties picked from more than 30 groves, are reported. Picking date, fruit wt. and dia. correlation, oil content gave a good index of maturity. (26 references.) E. G. BRICKELL.

Identification of citrus species and varieties by instrumental analysis of citrus leaf oils. A. P. Pieringer, G. J. Edwards and R. W. Wolford (*Proc. Amer. Soc. hort. Sci.* 1964, **84,** 204—212).—Leaf oils of 8 different citrus varieties and 2 specimens of sour orange were analysed by gas chromatography, i.r. and u.v. spectrophotometry and n measurements. The varieties were differentiated by gas–liquid chromatograms, whilst i.r. and u.v. spectrophotometry permitted only the differentiation between citrus species; n values were of limited use. A. H. CORNFIELD.

Application of gas–liquid chromatography to the citrus leaf oils for the identification of kinds of citrus. J. W. Kesterson, A. P. Pieringer, G. J. Edwards and R. Hendrickson (*Proc. Amer. Soc. hort. Sci.*, 1964, **84,** 199—203).—Gas–liquid chromatography was used to identify the oils, obtained by steam distillation, of leaves of 11 kinds of citrus. The composition of the leaf oils differed among species of the same genus and may be used for identifying the source of the oil. A. H. CORNFIELD.

Amendment of sandy soil by soft rock phosphate or Fullers' earth and response of young citrus trees. R. B. Diamond (*Dissert. Abstr.*, 1964, **25,** 12—13).—In lysimeter trials a fine sandy soil treated with soft rock phosphate, (**I**), (P, 8%, largely as apatite) and lime (1·5 ton/acre) mixed with the surface 4 in., produced better top- and root-growth than when lime alone was applied. In amounts > 1% of the surface soil, **I** did not improve growth further, but 5% of **I** mixed with the whole depth of soil diminished the leaching losses of K, increased the pH and Ca content and the available P and K of the soil under greenhouse conditions. **I**, at 20 tons/acre increased the wt. of roots (doubled) and of tops (trebled), the P, K and Mn concn. in leaves and the pH and extractable-Mg and -P in the soil. In the field, **I** or Fullers' earth (**II**), placed in the planting hole of young orange trees, doubled the increase in dia. in 1 year. Lime, applied broadcast, increased the production of foliage by 100% and, when placed in the planting hole, by 50% of that in controls. Leaf-Ca levels were increased more by placement of the amendments in the planting hole than when broadcast. In a soil carrying mature trees the pH was increased by lime but not by **I** or **II**. Extractable-Ca in soil was increased by lime or **I**, extractable-Mg by **II** and extractable-P by **I**. A. G. POLLARD.

Uptake and distribution of chloride in citrus cuttings during a short-term salt test. A. A. Hewitt, J. R. Furr and J. B. Carpenter (*Proc. Amer. Soc. hort. Sci.*, 1964, **84,** 165—169).—Over 8 weeks the rate of accumulation of Cl^- in the leaves of 5 selections of citrus cuttings receiving NaCl (6000 p.p.m.) in the nutrient was proportional to the relative salt tolerance of the selections as judged by visual symptoms. Accumulation of Cl^- in the roots was similar for all selections. A. H. CORNFIELD.

Seasonal changes in the organic acid content of Valencia orange fruit. G. K. Rasmussen (*Proc Amer. Soc. hort. Sci.*, 1964, **84,** 181—187).—Citric acid was the major acid present in orange pulp from 8 weeks after fruit set until after maturity and reached its max. concn. in early autumn. Very little citric acid was found in

the peel at any time. Malic acid was the major acid present in the peel and reached its max. concn. in early autumn. The concn. of malic acid was slightly higher in the pulp than in the peel. Very little oxalic acid occurred in the pulp, but relatively large amounts (present as an insol. salt) occurred in the peel, especially in the summer. Malic and citric acids constituted 95% of the total water-sol. acid of the pulp throughout the life-cycle of the orange.
A. H. CORNFIELD.

Effects of foliar applications of manganese, zinc and urea on Valencia orange yield and foliage composition. C. K. Labanauskas and R. E. Puffer (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 158—164).—Foliar application of $MnSO_4$ (Mn 1 lb. per 100 gal.) to Mn-deficient orange trees increased dry wt. of and Mn% in the leaves and the yield of oranges. Foliar sprays of $ZnSO_4$ (Zn 1 lb. per 100 gal.) on moderately Zn-deficient trees increased Zn% in the leaves, but had no effect on dry wt. of leaves or yield of oranges. Sprays of urea (3·5 lb. N per 100 gal.) increased leaf N% and Mn%, but had no effect on leaf dry wt. or fruit yields. Single sprays of Mn and Zn, separately or in combination, corrected deficiencies in existing leaves, but not in leaves which developed after spraying.
A. H. CORNFIELD.

Chlorophyll concentration in the navel orange rind as related to application of potassium gibberellate and light intensity. L. N. Lewis, C. W. Coggins, jun., and M. J. Garber (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 177—180).—The normal rate of reduction of chlorophyll a and b during colour change of the rind of oranges was reduced by treating the fruit with K gibberellate (500 p.p.m. acid equiv.) and increased by reducing the light intensity. There was no interaction between light and gibberellate treatments.
A. H. CORNFIELD.

Cause and control of oleocellosis on lemons. G. A. Cahoon, B. L. Grover and I. L. Eaks (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 188—198).—Oleocellosis (rind oil spotting) on desert-grown lemons was associated with conditions contributing to high turgidity of the rind, i.e., high soil moisture and atm. R.H. and low temp. The condition developed when high-turgid fruit was picked and handled. The trouble may be avoided by picking the fruit only when soil moisture and atm. R.H. are both low. A. H. CORNFIELD.

Effect of stage of maturity and ethylene treatment on respiration and ripening of tomato fruit. J. M. Lyons and H. K. Pratt (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 491—500).—The internal concn. of O_2, CO_2 and C_2H_4 in tomato fruit followed a pattern similar to that shown by most fleshy fruits. Fruit harvested as early as 17 days after pollination (approx. 38% of the total growth period) were induced to colour at 20° in an atm. containing C_2H_4 (1000 p.p.m.), although they required 12—15 days of continuous treatment and were not of edible quality. A. H. CORNFIELD.

Effect of calcium on cracking in tomato fruits. D. B. Dickinson and J. P. McCollum (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 485—590).—Cracks were induced in detached tomato fruits by infiltration with water. Infiltration with 0·1M-$CaCl_2$ prevented cracking in a crack-resistant variety and reduced the severity of cracking in a susceptible variety. Infiltration with 0·1M-NaCl or -KCl was ineffective in reducing cracking. Spray application of 0·04M-$CaCl_2$ to tomato plants reduced the incidence of cracking in fruit after picking. A. H. CORNFIELD.

Effects of soil temperature and phosphorus fertilisation on snap beans and peas. H. J. Mack, S. C. Fang and S. B. Apple, jun. (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 332—338).—Increasing soil temp. from 12·2—25·5° increased dry matter yields of snap beans 15-fold, but had little effect on peas. P% in both species increased slightly with temp. Increases in dry wt. and P% from application of P were in general similar at all temp. Application of high rates of P did not compensate for the inhibitory effect of low soil temp. on the growth of snap beans. Recovery of applied P was better by snap beans than by peas. A. H. CORNFIELD.

Relation of calcium content and pectic substances in bean hypocotyls of different ages to susceptibility to *Rhizoctonia solani*. D. F. Bateman and R. D. Lumsden (*Phytopathology*, 1964, **54**, 887).—Susceptibility of hypocotyl tissue of kidney bean to *Rh. solani* was high during the first 2 weeks of growth and subsequently declined; it was resistant in the fourth week. This effect was associated with increase in Ca content and with conversion of pectin into pectate in the maturing hypocotyl, thus rendering pectic materials resistant to polygalacturonase. A. G. POLLARD.

Some nutritional and metabolic factors affecting the formation of oxalates in spinach *(Spinacia oleracea)*. J. W. Kitchen (*Dissert. Abstr.*, 1964, **25**, 13—14).—The oxalate content of spinach is a biological characteristic of the plant and is related to the morphological leaf type; it can be lowered by suitable cross-breeding and selection. The contents of total and sol. oxalate in leaves, petioles and roots are in the (decreasing) order named; they diminish with advancing vegetative growth of the plants and are predominantly sol. In sand-cultured plants, P affected the vegetative growth of the plants but not necessarily their oxalate content. Mg produced a similar effect. Micro-nutrients did not affect growth or oxalate content; the latter was influenced by a soil factor other than K, Ca or P. Oxalate might serve as a substrate for respiration; it was slowly metabolised to CO_2 and was formed in absence of photosynthetic activity. A. G. POLLARD.

Selection of rape plants *(Brassica napus)* with seed oil practically free from erucic acid. B. R. Stefanson and F. W. Hougen (*Canad. J. Plant Sci.*, 1964, **44**, 359—364).—The erucic acid (**I**) and other fatty acid contents of seed oils of numerous strains of *B. napus*, *B. campestris* and *B. juncea* showed considerable variability. By repeated selection strains of *B. napus* yielding oils with only traces of **I** were isolated. In these oils oleic acid had replaced **I** as the major fatty acid. Genetic considerations are discussed.
A. G. POLLARD.

Effects of planting density and manuring on the yields of bunch-type groundnuts. R. M. Meredith (*Emp. J. exp. Agric.*, 1964, **32**, 136—140).—Yields of kernels (wt.) were not significantly different with plant population ranging from 14,000 to 70,000 per acre, although no. of nuts per plant increased with decreasing population. Superphosphate (56—112 lb. per acre) increased no. of nuts per plant and kernel yields at all plant population levels.
A. H. CORNFIELD.

Soil fertility and response of groundnuts to fertilisers in the Gambia. S. H. Evelyn and I. Thornton (*Emp. J. exp. Agric.*, 1964, **32**, 153—160).—Characteristics of soils representing the four major soil types of the Gambia are described. Significant responses in yields of groundnuts were obtained by P application in three of the four soils. Responses to P were better in the presence than in the absence of applied K. There were no responses to N applications.
A. H. CORNFIELD.

Sodium injury in chrysanthemum cuttings. J. Vlamis and R. D. Raabe (*Phytopathology*, 1964, **54**, 911).—Poor rooting of chrysanthemum cuttings and poor growth with red vascular coloration in survivors is examined. No pathogenic organisms were traced but the effect was associated with a change in water supply from one of high salt content (mostly Ca) to one of lower salt content (largely Na). It occurred only when peat was incorporated with the rooting medium. The disorder resulted from accumulation of Na by the peat in amounts equivalent to 1 ton/acre: it was severe with the equivalent of 3 tons/acre. Gypsum at the rate of 2 tons/acre counteracted the disorder. A. G. POLLARD.

Influence of light on the germination of seeds of forest trees. Z. Jacopi (*Ric. sci., R.C.*, [B], 1964, **4**, 481—484).—The influence of i.r. or red irradiation, and the interaction of red and white light in the germination of various seeds has been examined. The germination of *Larix decidua* and *Pinus pinaster* was promoted by red radiation.
L. A. O'NEILL.

Response of rubber trees to sulphur dioxide in the atmosphere. E. Brennan, I. A. Leone and R. H. Daines (*J. Rubb. Res. Inst. Malaya*, 1964, **18**, 175—184).—Chamber tests showed that the foliage of *Hevea brasiliensis* was unaffected by 15 min. exposure to SO_2 concn. below 75—100 p.p.m. whereas 4 h. exposure to 0·78 p.p.m. resulted in damage. Exposure for 3 weeks to 0·30 p.p.m. (the max. likely to be encountered in practice) was without effect. Wet leaves are more susceptible than dry ones and young leaves more than old. During longer fumigations (>4 h.), the leaf S content gradually, but not proportionally, builds up but injury occurs only when the S accumulates rapidly. (15 references.)
J. W. WALPOLE.

Influence of formulation on yield response and bark damage following the application of yield stimulants above the tapping cut. P. D. Abraham and S. G. Boatman. **Appendix: anatomical observation on *Hevea* bark treated with 2,4-D.** J. B. Gomez (*J. Rubb. Res. Inst. Malaya*, 1964, **18**, 211—225, 226—230).—Yield stimulant compositions which are applied at intervals to *H. brasiliensis* above the tapping cut, consists of 2,4-D or 2,4,5T in vegetable and/or mineral oil carriers. The nature and η of the carrier influence the degree of bark renewal but have relatively little effect on the latex yield. In general it seems that stimulants are better applied below the tapping cut. The appendix examines the effects of 2,4-D stimulants on the bark tissues. J. L. WALPOLE.

Effects of cover plants on soil nutrient status and on growth of *Hevea*. V. Loss of nitrate-nitrogen and cations under bare soil conditions. G. A. Watson, Wong Phui Weng and R. Narayanan (*J. Rubb. Res. Inst. Malaya*, 1964, **18**, 161—174).—Jungle cleared for rubber cultivation is also planted with cover plants (leguminous creepers, grasses or natural vegetation) as a soil conservation measure. Soil analyses at yearly intervals show higher levels of

NO_3^--N and lower levels of C and soil cations (Ca, Mg and K) in bare soil compared with the under-planted areas. Under bare soil conditions the NO_3^- concn. increases with depth—presumably as the result of leaching. Higher levels of NH_4+-, NO_3^-- and total N were found under leguminous creepers than under the other two covers. (16 references.) J. L. WALPOLE.

Chemical analysis of plant material. K. R. Middleton, P. R. Gyss, J. C. Fallows and J. A. Varley (*J. Rubb. Res. Inst. Malaya*, 1964, **18**, 194—210).—The methods used in a collaborative study by four laboratories for the analysis of *Hevea* and oil palm leaves are compared and discussed. The amounts of N, P, K, Mg, Ca and Mn found were in good agreement but are dependent on the position of the leaves on the tree and on the presence or absence of midribs in the samples. Precise analytical details are given.
J. L. WALPOLE.

Comparison of fertility treatments in a crop rotation experiment. F. B. Cady and D. D. Mason (*Agron. J.*, 1964, **56**, 476—479).—The statistical treatment of an experiment involving a rotation of maize and soya-beans over 6 years is described.
A. H. CORNFIELD.

Pest Control

Rôle of chemistry in pest control. H. Martin (*Chem. & Ind.*, 1965, 157—160).—A general review. C. V.

Surfactants as fungicides. F. R. Forsyth (*Canad. J. Bot.*, 1964, **42**, 1335—1347).—The activities of some cationic, anionic and non-ionic surfactants as inhibitors of respiration, as agents promoting exudation of amino-acids, and as inhibitors of germination of the spores of one or more of the test organisms *Monilinia fructicola*, *Alternaria solani*, *Puccinia recondita* and *P. coronata*, are reported. Although causing reduction in respiration rate paralleling their fungicidal activity, this effect is considered to be secondary to the irreparable damage caused to the semipermeable outer membrane of the cytoplasm. (23 references.) E. G. BRICKELL.

Effects of surfactants on fungi. G. W. Steiner (*Phytopathology*, 1964, **54**, 909).—Growth of species of *Penicillium*, *Fusarium*, *Gliocladium* and *Aspergillus* was not greatly affected by the Na salts of sulphated alkyl (anionic) or quaternary NH_4 (cationic) surfactants whereas others, including *Lenzites trabea*, *Fomes pinicola* and *Polyporus cinnabarinus* were inhibited by these substances in concn. ~50 p.p.m. All the fungi tested were inhibited by three non-ionic surfactants containing a hydrophobic portion, nonylphenol, to extents increasing with concn. up to 100 p.p.m.
A. G. POLLARD.

Actinomyces: antagonists to cotton wilt agent. S. M. Khodzhibaeva and E. Tokhtamuratov (*Mikrobiologiya*, 1964, **33**, 477—482).—Of 1563 actinomyces strains isolated from the soil of the West Pamirs 428 showed antifungicidal activity. Cultivated on a peptone, maize extract, agar medium, their activity against *Verticillium dahliae* fungus was determined, and also their u.v. absorption spectra. Of the 428 strains, 67 produced polyene antibiotics; the actinomyces producing tetraene antibiotics were most active against *V. dahliae* fungus, and those producing pentaene and hexaene antibiotics were least active. The polyene antibiotics were produced mainly by unpigmented actinomyces, belonging to the white, grey and globosporinic groups. Almost all the globosporinic actinomyces produced hexa and heptaene antibiotics.
A. S. LEVESLEY.

Antibiotics against plant disease. VIII. Screening for nonpolyenic antifungal antibiotics produced by streptomycetes. L. A. Lindenfelsen, O. L. Shotwell, M. J. Bachler, G. M. Shannon and T. G. Pridham (*Appl. Microbiol.*, 1964, **12**, No. 6, 508—512).—Of 500 strains grown in shaken flasks, 240 of the culture liquors contained active factors as shown by paper disk assay against *Mucor ramannianus*. Filtrates and extracts were examined by i.r. spectrophotometry; 196 were nonpolyenic as determined by absorption spectra and heat-stability. Tests of the nonpolyenic antibiotics over a broad pH range showed 15 to be stable under all test conditions, 70 were moderately stable and 81 unstable. Two of the most interesting antibiotics were identified as cycloheximide and musarin. (14 references.) C. V.

New foliage protectant fungicide, tetrachloroisophthalonitrile. N. J. Turner, L. E. Limpel, R. D. Battershell, H. Bluestone and D. Lamont (*Contr. Boyce Thompson Inst.*, 1964, **22**, 303—310).—The results of laboratory, greenhouse and field tests are given and tetrachloroisophthalonitrile (**I**) is shown to control tomato blight and grey leaf spot, bean rust, apple scab, black spot, etc. **I** has good crop tolerance, residual activity and rain resistance and a low mammalian toxicity. E. C. DOLTON.

Decomposition of ferbam. G. D. Thorn and L. T. Richardson (*Phytopathology*, 1964, **54**, 910).—The formation of thiram by decomposition of ferbam (**I**) at low pH levels is confirmed. At pH 6≮, **I** was hydrolysed to give free dithiocarbamate (**II**) ions. When shaken with water **I** was converted into thiram, which in presence of fungus spores yielded **II** ions. At pH 7 there was no uptake of **II** by spores. A. G. POLLARD.

Effect of sodium dimethyldithiocarbamate on water uptake, transpiration and stomatal opening. G. D. Thorn and W. H. Minshall (*Canad. J. Bot.*, 1964, **42**, 1405—1410).—Applied to the roots of tomato and bean plants Na dimethyldithiocarbamate markedly decreased the rate of transpiration and prevented stomatal opening. It also caused a large reduction in water movement associated with metabolic root pressure. (17 references.) E. G. BRICKELL.

Compatibility of certain soil fungicides with the nodule bacterium, *Rhizobium leguminosarum*. J. A. Bauling and M. B. Linn (*Phytopathology*, 1964, **54**, 887—888).—Limiting concn. of a no. of soil fungicides which just failed to suppress *Rh. leguminosarum* in liquid and solid media and in soil were determined. Botran (50% 2,6-dichloro-4-nitroaniline, Lanstan (45% 1-chloro-2-nitropropane) and PCNB at <400 p.p.m. did not inhibit the bacterium. Other critical concn. determined included H 3944 (50% 4-phenyl-5-chloro-1,2-dithiol-3-one), 200 p.p.m.; captan, 100 p.p.m. and DAC 649 (75% 3,3,4,4-tetrachlorotetrahydrothiophen-1,1-dioxide), 50 p.p.m. Nabam, thiram and maneb showed limiting concn. ≯25 p.p.m. and much more persistent action. A. G. POLLARD.

Release of methyl isothiocyanate from soils treated with Mylone(3,5-dimethyltetrahydro-1,3,5-2H-thiadiazine-2-thione). D. E. Munnecke and J. P. Martin (*Phytopathology*, 1964, **54**, 941—945).—Breakdown of Mylone in columns of non-sterile soil was measured by the concn. of the methyl isothiocyanate (**I**) in the effluent air. The rate of release of **I** increased with temp. (1—23°) with soil moisture content up to 80% saturation and with pH (2·3—6·5) declining somewhat at higher pH (up to 7·7) and also with increase in clay or peat content of the soil. Formation of **I** occurred independently of soil micro-organisms. A. G. POLLARD.

Effect of spray gallonage and leaf character on deposition and retention of copper-containing fungicides. J. D. Wilson and O. K. Hedden (*Phytopathalogy*, 1964, **54**, 912—913).—Initial deposits of Cu from 'tribasic Cu sulphate' on a particular plant species were not greatly different when 25 than when 200 gal./acre were used to make the spray. The initial deposit on the pubescent leaf of egg-plant was much greater than on a smooth-leaved pepper. After 10 days' weathering the egg-plant retained much more Cu deposit than did the pepper with the low-gallonage spray and the difference was still greater when the high-gallonage formulation was used. Initial deposits were greater after spraying on dry leaves than on leaves moistened with dew but retention after weathering was greater on leaves which were wet when sprayed.
A. G. POLLARD.

Action of propionate on wheat stem rust and sorghum head smut. M. C. Futrell and J. W. Berry (*Phytopathology*, 1964, **54**, 892).—Sand-cultured wheat plants were treated with various concn. of aq. Na propionate applied to the sand 24 h. before inoculation with *Puccinia graminis tritici*. The no. of stem rust pustules developing was lowered by 10^{-1} and 10^{-2} M-propionate. In corresponding tests with sorghum inoculated with the head smut fungus (*Sphacelotheca reiliana*) growth of the pathogen was stimulated by the propionate in concn. 10^{-4} and 10^{-3} M, diminished by 10^{-2} M and totally inhibited by 10^{-1} M concn. A. G. POLLARD.

Factors affecting field performance of nickel salts plus dithiocarbamate fungicide mixtures for control of wheat rust. J. B. Rowell (*Phytopathology*, 1964, **54**, 999—1008).—Control of damage to Marquis wheat caused by *Puccinia graminis*, f.s.p. *tritici* and *P recondita* by combinations of Ni salts (Cl^- or SO_4^{2-}) and dithiocarbamate fungicides depended primarily on timing the applications to delay the rate of development of the epidemic. Outbreaks of leaf rust reached 50% severity on average 13 days and that of stem rust about 20 days after heading (Minnesota, U.S.A.). The rust incidence increased approx. 10-fold in 4—5 days. The optimum time of application was when the no. of uredia formed reached 10—100 per culm. with a second application 10 days later. 66% control of rust was thus obtained. The effects of the Ni salts and the dithiocarbamate appear to be additive. Surfactants improved the degree of control in some cases. A. G. POLLARD.

Effects of pantothenic acid and related compounds on infection of wheat by uredospores of *Puccinia graminis tritici*. C. D. Hobbs (*Dissert. Abstr.*, 1964, **25**, 16—17).—Suspensions of the uredospores in aq. Ca pantothenate (**I**), or other vitamins and sources of Ca and N were injected hypodermically into the plants. The no. of pustules formed was increased by **I**, optimal concn. of which differed among varieties. In some cases pustule formation was increased by

$CaCl_2$ and also by KNO_3. Among other substances tested, those having no effect on pustule formation included, choline chloride, thiamine hydrochloride, nicotinic acid, riboflavin, ascorbic acid, pyridoxal and β-alanine. Pantoyl-taurine in concn. 10^{-2} and 10^{-5} M, and also β-alanine (10^{-2} M) diminished pustule formation. Treatment of seedlings with **I**, 4 days after inoculation, had no effect on no. of pustules. Pantoyl taurine, under these conditions, reduced pustule formation. A. G. POLLARD.

Root and stalk rot of maize in south-western Ontario. III. Sugar levels as measure of plant vigour and resistance. C. G. Mortimer and G. M. Ward (*Canad. J. Plant Sci.*, 1964, **44**, 451—457).—In physiologically mature maize plants, high levels of sol. sugars in the pith are associated with resistance to root and stalk rot. In the field, treatments which predispose the plants to stalk rot; e.g., high population density or late defoliation, lowered the sugar content. Conversely, prevention of kernel development and low population density causing high sugar contents in the pith, tended to increase resistance to the disease. A. G. POLLARD.

Root rot of beans caused by *Fusarium solani* f. *phaseoli*. D. M. Huber (*Dissert. Abstr.*, 1964, **25**, 17).—Growth of barley previous to the bean crop increased the incidence of root rot, whereas maize had the opposite effect. The presence of tyrosinase, peroxidase and β-glucosidase in the endodermis, phloem, cambium and xylem and their activation round a wound were associated with resistance to root rot. This resistance was characterised by a non-specific wound response which consisted of a rapid deposition of inhibitory compounds at the site of infection and by the formation of a wound periderm which prevented deep penetration into the tissue.
A. G. POLLARD.

Influence of soil moisture on infection of peas by *Pythium ultimum*. A. Kerr (*Aust. J. biol. Sci.*, 1964, **17**, 676—685).—Laboratory experiments show that increases in soil moisture have only slight effects on the proliferation of *Pythium*, and that the actual cause of the rapidly increasing seed-infection with increasing soil moisture is attributable to the effect of the moisture on the amount of sugar (mostly sucrose) exuded from the seeds (cf. following abstract). Considerably more sugar is exuded from seeds sown in sand than from seeds sown in aggregating sandy loam or loam. (14 references.)
P. S. ARUP.

Pre-emergence rotting of peas in South Australia. I. Factors associated with the seed. II. Factors associated with the soil. III. Hostenpathogen interaction. N. T. Flentje and H. K. Saksena (*Aust. J. biol. Sci.*, 1964, **17**, 643—650, 651—664, 665—675).—I. Stunted seedling growth occurs sometimes and in varying degree with wrinkled-seeded, but not with smooth-seeded peas. The occurrence is connected with heavy bacterial and fungal infections of the seed.

II. The rotting is due to attacks by *Pythium ultimum* and (secondarily) by *Fusarium* spp. The % of seeds attacked is proportional to the soil moisture content.

III. Laboratory experiments show that wrinkle-seeded peas exude sugar during the first 24 h. of germination, but that smooth-seeded peas do not do so. These and further observations support the theory that the exudate stimulates the growth of *Pythium* in the soil near the seeds, and thus renders the embryo liable to attack during an early stage of emergence. The effect is probably enhanced by soil moisture. Both types of seed can be attacked during the subsequent lengthening of the epicotyl, but the conditions for attack are much less favourable in the case of the smooth seeds. (11 references.) P. S. ARUP.

Control of *Verticillium dahliae* and *Meloidogyne incognita* in greenhouse tomatoes by soil fumigation. C. D. McKeen and R. M. Sayre (*Canad. J. Plant Sci.*, 1964, **44**, 466—470).—Fumigation of greenhouse tomato soils with prep. containing methyl isothiocyanate as active agent, controlled the root-knot nematode and simultaneously lowered the incidence of verticillium wilt to a very great extent; fruit yields were increased. Plate counts from a soil suspension showed that the survival of *Fusarium* spp. was an effective index of the destruction of *V. dahliae* by the fumigation.
A. G. POLLARD.

2-Aminobutane salts for control of post-harvest decay of citrus apple, pear, peach and banana fruits. J. W. Eckert and M. J. Kolbezen (*Phytopathology*, 1964, **54**, 978—986).—Organisms causing loss of harvested fruits, viz. *Penicillium digitatum* and *P. italicum* on citrus, *P. expansum* on apples and pears, *Monilinia fructicola* on peaches and *Gleosporium musarum* on bananas, were controlled by salts of 2-aminobutane (**I**). Twenty other aliphatic amine salts failed to destroy *P. digitatum* on lemons. The efficiency of **I** increased with the concn. applied (0·25—1·0%), with the time of contact with the fruit (2 sec.—4 min.) with increasing pH (9—11) and temp. (20—40°). Optimum results were obtained by immersing the fruit for ≮1 min. in 0·5% aq. **I** at pH <9 and temp. ⋗45°. The fruit is preferably not rinsed after treatment. A. G. POLLARD.

Root disease inspection and treatment. A. Newsam (*R.R.I. Plant Bull.*, 1964, No. 75, 238—243).—Regular quarterly inspections, and application of protectants to trees that are opened for white root disease, will reduce the need for expensive and injurious excisions. J. L. WALPOLE.

Minor root diseases: a reappraisal. K. P. John (*R.R.I. Plant. Bull.*, 1964, No. 75, 244—248).—Three fungi, *Ustulina zonata*, *Sphaerostilbe repens* and *Poria hypobrunnea* and the root diseases they cause in rubber trees are described in detail; differences of habit and mode of infection are noted. J. L. WALPOLE.

Spore dissemination of root disease. K. P. John (*R.R.I. Plant Bull.*, 1964, No. 75, 233—237).—Root diseases of rubber trees commonly become established through air- or insect-borne spore infection of stumps. This is now generally minimised by tree or stump poisoning with Na arsenite or 2,4,5-T and treatment of cut surfaces of felled timber with preservatives such as creosote.
J. L. WALPOLE.

Principles of root disease control. R. A. Fox (*R.R.I. Plant. Bull.*, 1964, No. 75, 210—217).—Modern procedures for root disease control in rubber plantations which afford considerable potential savings, consist of pre-planting collar and root inspections, creosoting of the cut surfaces of felled stumps and eradication of infected roots only within the limits of the planting row. The use of creeping cover plants is also recommended. J. L. WALPOLE.

Mechanical cultivation and root disease. Boon Weng Siew (*R.R.I. Plant. Bull.*, 1964, No. 75, 218—224).—Mechanical clearance methods used for replantings and new plantings on different terrains are described and shown to be effective in reducing root disease. Typical operational costs and means of minimising any resulting soil erosions are given. J. L. WALPOLE.

Effects of clearing methods on root disease incidence. A. Newsam (*R.R.I. Plant. Bull.*, 1964, No. 75, 225—232).—The results of *Hevea*-replanting trials, presented and discussed, show that root disease can be controlled by felling diseased trees followed or preceded by poisoning with Na arsenite or butyl 2,4,5-T.
J. L. WALPOLE.

Biological methods of combating cotton root rot caused by *Rhizoctonia solani*. S. Achilova and A. G. Kuchaeva (*Mikrobiologiya*, 1964, **33**, 900—903.—Experimental results are presented on the action of *Actinomycetes* (**I**) antagonistic to *R. solani*, the agent of cotton root rot. Three strains active in laboratory experiments were used: (*a*) from *A. fradiae*, (*b*) from *A. glaucus* and (*c*) from group of *A. rimosus*, and were cultivated in fish media in a shaker. In field experiments sterilised seeds were treated with aq. suspension of 7-day culture of *R. solani* mould, dried and immersed for 18 h. in antibiotic liquor from *Actinomyces* cultures. In further experiments uninfected seeds were grown in mixture of 90% soil + 10% infected cotton seed oil cake to which the test cultures were added. Treatment with *Actinomyces* cultures increased field germination up to 96%, reduced the no. of infected plants from extremes of 72 to 3% and increased yields of cotton from 4 to 6·9 kg./10 m². P. W. B. HARRISON.

Use of flowering crab apples for detecting latent viruses in apples. J. E. Reynolds (*Dissert. Abstr.*, 1964, **25**, 18—19).—Among numerous varieties and species of crab apple examined, *Malus huphensis* was the most effective indicator of the presence of latent virus in apple varieties. Experimental data obtained with several viruses are recorded. Movement of virus in inoculated crab trees was not at uniform rates. A. G. POLLARD.

Determination of traces of phosphoric esters by oscillopolarography [cathode-ray polarography]. P. Nangniot (*Anal. chim. Acta*, 1964 **31**, 166—174).—Esters that contain the groups ≡P : S or ≡P·S give rise to adsorption peaks in cathode-ray polarography. Measurement of such peaks provides a means of determining the esters in concn. down to <0·5μg. per ml. Relevant characteristics are given for 26 phosphoric esters commonly used as pesticides. H. N. S.

Review of herbicide penetration through plant surfaces. C. L Foy (*J. agric. Fd Chem.*, 1964, **12**, 473—476).—The known factors affecting the penetration of herbicides into leaves are discussed with particular reference to the four main components of leaf surface structure, i.e., cutin, waxes, pectin and cellulose. (29 references.) W. ELSTOW.

Modification of plant transpiration rate with chemicals. Don Smith and K. P. Buchholtz (*Plant Physiol.*, 1964, **39**, 572—578).—In potometer tests various plant species were tested via roots or by foliar spray, with a no. of herbicides and effects on transpiration rates were determined. Among herbicides examined six triazines and three substituted urea compounds, at 20 m./l., via roots,

lowered the transpiration rates by 50% within 6 h. Smaller effects were produced by dinitrophenol, 2,4-D and amitrole. Hydroxy-atrazine had no effect. Foliar sprays (300 mg./l. + wetting agent) producing similar effects included triazines, HgPh acetate, isocil and F.W.-734 (3,4-dichloropropionanilide). Atrazine (5—1000 mg./l.) strongly inhibited translocation (max. at 100 mg./l.).
A. G. POLLARD.

Placement of Di-allate and Tri-allate for control of wild oats in wheat. E. S. Molberg, H. A. Friesen, E. V. McCurdy and R. D. Dryden (*Canad. J. Plant Sci.*, 1964, **44,** 351—358).—Di-allate (2,3-dichloroallyl di-isopropylthiolcarbamate) or Tri-allate (the corresponding 2,3,3-trichloro compound) at the rate of 1·0—1·5 lb./acre effectively controlled wild oats provided the wheat was sown 3 in. deep and herbicide was incorporated with a very shallow surface layer of soil (e.g., by light harrowing). Wheat tended to be more tolerant of the Tri- than of the Di-allate. A. G. POLLARD.

Structural requirements of 3-amino-1,2,4-triazole for physiological activity. E. E. Schweizer and B. J. Rogers (*Weeds*, 1964, **12,** 7—10).—Of 12 heterocyclic compounds tested only 3-amino-1,2,4-triazole (amitrole) and 5-chlorobenzo-1,2,3-triazole were effective in inhibiting wheat root elongation and growth of carrot explants, in producing chlorosis, and in depressing catalase activity. The other 10 compounds were effective only in reducing growth of wheat roots and carrot explants. A. H. CORNFIELD.

Photo-decomposition of substituted phenylureas. L. S. Jordan, C. W. Coggins, jun., B. E. Day and W. A. Clerx (*Weeds*, 1964, **12.** 1—4).—The effect of u.v. light and sunlight on the degradation of the substituted phenylurea herbicides monuron, diuron, neburon and fenuron was studied. All four herbicides were degraded by u.v. light in particular and also by sunlight. The degradation began within 2 h. of exposure to sunlight and the rate of degradation decreased with time, indicating that the decomposition products formed a partially protective barrier against further degradation. A. H. CORNFIELD.

Decarboxylation of phenoxyacetic acid herbicides by excised leaves of woody plants. E. Basler (*Weeds*, 1964, **12,** 14—16).—Only traces of 2,4,5-trichlorophenoxyacetic acid absorbed by the petioles of excised blackjack oak leaves were decarboxylated. About 1% of the 2,4-D and ~7% of 2-chloro-4-fluorophenoxyacetic acid absorbed were decarboxylated. The decarboxylation rate of 2,4-D in June varied between the leaves of five different woody species and there was no correlation between decarboxylation rate and susceptibility to 2,4-D. A. H. CORNFIELD.

Effect of *s*-triazine and phenylurea herbicides on soil fungi in maize- and soya-bean cropped soil. D. D. Kaufman (*Phytopathology*, 1964, **54,** 897).—Comparative effects of simazine (**I**), atrazine (**II**) on fungi in maize-cropped soil and linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] (**III**) and diuron (**IV**) in a soya-bean soil were examined. In all cases significant qual. and quant. effects were produced; these varied with the herbicide and the crop. **II** was more effective than **I** in lowering the no. of *Fusarium* spp. **III** and **IV** suppressed *Fusarium* spp. in soya-bean soil but not in maize soil. All four herbicides stimulated growth of one or more genera of soil fungi antagonistic to *Fusarium* spp.
A. G. POLLARD.

Herbicides and mixtures for annual weed control in grain sorghum. R. G. Robinson, W. W. Nelson, R. L. Thompson and J. R. Thompson (*Weeds*, 1964, **12,** 77—79).—Pre-emergence applications of CDAA (2-chloro-*NN*-diallylacetamide, 4 lb.) plus atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 2 lb.) or Propazine [2-chloro-4,6-bis(isopropylamino)-*s*-triazine, 2 lb. per acre] gave excellent control of grasses and weeds without injuring sorghum. The mixtures gave better weed control than did either component alone, even at higher rates. Pre-emergence application of CDAA followed by post-emergence application of atrazine or propazine also gave excellent results. In general atrazine was more effective than propazine. A. H. CORNFIELD.

Volatility of seven *s*-triazines. P. C. Kearney, T. J. Sheets and J. W. Smith (*Weeds*, 1964, **12,** 83—87).—The volatility at 25° of 7 *s*-triazines decreased in the order prometone = Tiretazine > atrazine = Ametryne = Prometryne > Propazine > simazine. Loss of Prometone from soils was directly related to sand % and inversely related to clay % and org. matter % in the soil, whilst losses of atrazine and simazine were less affected by soil properties.
A. H. CORNFIELD.

Photodecomposition of triazines. L. S. Jordan, B. E. Day and W. A. Clerx (*Weeds*, 1964, **12,** 5—6).—The herbicides atrazine, simazine and Ametryne were degraded when exposed to u.v. light or sunlight. The rate of degradation decreased with time, probably due to the protective effects of the decomposition products.
A. H. CORNFIELD.

Use of simazine for control of weeds in strawberries in coastal British Columbia. J. A. Freeman (*Canad. J. Plant Sci.*, 1964, **44,** 555—560).—A complete simazine schedule (4 applications) at 2 lb./acre per application over a 3-year period provided excellent weed control without adversely affecting crop yield and a 1½ lb./acre application rate is suggested as being adequate. Fruit quality was not affected. E. G. BRICKELL.

Influence of some representative herbicidal chemicals on the growth of some soil fungi. D. F. Millikan and M. L. Fields (*Phytopathology*, 1964, **54,** 901).—The action of herbicides was examined on *in vitro* cultures of soil fungi, including pathogens. Addition of 2,4-D (100 p.p.m.) to the nutrient medium reduced the growth of colonies of *Fusarium culmorum, Trichoderma viride, Pythium ultimum* and *Rhizoctonia solani* by 70—93%. Simazine (**I**) and amitrole (**II**) (10 p.p.m.) suppressed *F. culmorum* by 71 and 94% respectively or, when used together, by 87%. **I** suppressed *T. viride* and *Rh. solani* by 92—93% but **II**, alone or in combination with **I**, had no effect. *P. ultimum* was unaffected or stimulated by **I, II** or both together. A. G. POLLARD.

Effects of five herbicides on numbers of certain invertebrate animals in grassland soil. C. J. S. Fox (*Canad. J. Plant Sci.*, 1964, **44,** 405—409).—On grassland plots application of dalapon increased the no. of millipedes (**I**), springtails (**II**) and mites (**III**). Monuron reduced the no. of wireworms (**IV**), **I**, earthworms (**V**), **II** and **III**. TCA increased the **I, II** and **III** and diminished those of **V.** 2,4-D had no effect on **II, III** or **IV.** Changes in the fauna due to the herbicides probably result from their action on the botanical composition of the herbage. The possibility of integrated control programmes involving both herbicides and fungicides are noted.
A. G. POLLARD.

Control of root disease in peas by seed treatment in S. Alberta. F. R. Harper (*Canad. J. Plant Sci.*, 1964, **44,** 531—537).—Emergence and yield were higher from treated than from untreated seed, Captan, Semesan and Bayer 47531 being the most effective fungicides. Captan protected both sound and damaged seed from attack by pathogens. E. G. BRICKELL.

Pre-emergence control of annual bluegrass, *Poa annua* L. R. L. Goss (*Agron. J.*, 1964, **56,** 479—483).—Application of Dacthal (10 lb.), Zytron (15 lb.), Dipropalin (6 lb.), Trifluralin (2 lb.), Betasan (15 lb.) and Enide (4 lb. per acre) to the soil surface before sowing controlled annual bluegrass for 9—12 weeks. The action of the materials was largely associated with inhibition of root development. A. H. CORNFIELD.

Control of bristly thistle, *Cirsium horridulum*, Michx. D. E. Davis, H. H. Funderburk, jun., and D. R. Roberts (*Weeds*, 1964, **12,** 22—25).—From 2 to 6 successive cuttings below the soil surface were necessary to kill bristly thistle plants. The thistle was controlled by application of 2,4-D propylene glycol butyl ether esters and 2-methyl-4-chlorophenoxyacetic acid (3 lb. per acre).
A. H. CORNFIELD.

Subaqueous release of herbicides from granules. R. E. Wilkinson (*Weeds*, 1964, **12,** 69—76).—Factors affecting the rate of release of hormone-type weedkillers from granules used for controlling aquatic weeds were studied. In general the rate of release of 2,4-D from attapulgite granules increased with temp. and pH and decreased with increasing granule size. Of 11 granular herbicides tested the most rapid rate of release of active ingredient occurred from the K salt of 2-(2,4,5-trichlorophenoxy)propionic acid.
A. H. CORNFIELD.

Determination of polychlorinated benzoic acid herbicide residues by gas chromatography. J. J. Kirkland and H. L. Pease (*J. agric. Fd Chem.*, 1964, **12,** 468—472).—The chlorobenzoic acids are extracted with methyl ethyl ketone and methylated, and the methyl esters determined by programmed temp. mincrocoulometric gas chromatography. Recoveries of about 80% are obtained and no interference in the method has yet been encountered. (13 references.)
W. ELSTOW.

Fungicidal compositions containing cycloheximide. Upjohn Co. (B.P. 942,066, 18.3.60. U.S., 22.4.59).—A fungicidal composition for use in the control of blister rust and fungal infection in trees comprises cycloheximide (1—10%) in association with a polar solvent, especially cyclohexanone (0·2—15) and a non-volatile hydrocarbon oil (100 pt.). The composition may be mixed with water for use, to form an oil-in-water emulsion containing 25—1000 p.p.m. of active agent. F. R. BASFORD.

Organotin derivatives. Stauffer Chemical Co. (B.P. 942,115, 25.6.62, U.S., 27.6.61).—Compounds claimed have the general formula $SnR_n(Z{\cdot}SO_2Me)_{4-n}$ (R is 1-12-C-alkyl, Ph or naphthyl; Z is O or S; *n* is 2—3). They are especially useful as pesticides and are prepared by interaction of SnR_4, SnR_2O, SnR_2Cl, or SnR_2Cl_2

with M.Z.SO_2Me (M is H; M is H, NH_4, or alkali metal). The prep. is described of *dibutyltin-di-methanesulphonate* m.p. 306—309°. F. R. BASFORD.

New derivatives of carbamic acid and their manufacture and use. CIBA Ltd. (B.P. 943,161, 22.11.61. Switz. 22.11.60).—The compounds claimed comprise selective herbicides of the general formula *m*-$CF_3 \cdot C_6H_4 \cdot NH \cdot CO \cdot NRR'$ (R is hydrocarbon radical of 1—2 C; R' is hydrocarbon radical of 2—4 C; or NRR' is heterocyclic residue of -6 ring atoms). One example is NN-*diethyl*—N'-m-*trifluoro-phenyl urea*, m.p. 83—84° (prep. described). F. R. BASFORD.

Treatment of plant growth media and especially of soil. Dow Chemical Co. (B.P. 943,201, 29.1.60. U.S., 4.2.59).—Nematodes in plant-growth medium or soil are destroyed or controlled by incorporation of 0·005—50 p.p.m. of a compound of the general formula $RR^I \cdot PY \cdot O \cdot C_6H_{5-n}X_n$ (R and R^I are $NR^{II}R^{III}$ or R is alkoxy or alkoxyalkoxy of 1—6 C; R^{II} and R^{III} are H or alkyl of 1—6 C; X is H, Cl, OMe, and/or Me; *n* is 1 or 2; Y is O or S), e.g., *O*-phenyl-*NN'*-dimethylphosphorodiamidate. F. R. BASFORD.

Phosphoramidates. Dow Chemical Co. (Inventors: C. L. Moyle and L. L. Wade) (B.P. 943,919, 19.4.60).—Compounds claimed have the general formula $OR(NHR^I) \cdot PO \cdot (O \cdot C_6H_{5-n}Cl_n)$, wherein R and R^I are alkyl of 1—4 C, or R is H; *n* is 1—5. They are useful as insecticides, microbicides and herbicides. They are prepared by reacting $POCl_2(O \cdot C_6H_{5-n}Cl_n)$ with ROH (or a functional deriv. thereof) and then with NH_2R^I. Or, $NHR^I \cdot POCl_2$ may be reacted successively with the phenol (or a functional deriv. thereof) and ROH. The process is exemplified by the prep. of *Et* 2,4,5-*trichlorophenylethylphosphoroamidate*, a yellow oil. F. R. BASFORD.

Insecticidal formulation. Montecatini Società Generale per l'Industria Mineraria e Chimica (B.P. 943,120, 24.4.61. It., 28.4.60).—The period of activity of the insecticide Me_2 dithiophosphorylacetamide is increased by compounding with a glycol ether acetate. Thus, a mixture of the insecticide (20), cellusolve acetate (78), and non-ionic emulsifier (2 g.), of initial activity 95·2% has 95·1% activity after subjecting to accelerated stability test during 60 days at 50°. F. R. BASFORD.

Dithiophosphoric acid esters and compositions containing them. S.A.R.I.A.F. Società Azionaria Romagnola Industrie Agricolo Farmaceutiche s.p.A. (Inventors: F. Cano and R. Martelli) (B.P. 941,810, 12.7.61).—Insecticidal esters of the general formula $OR^I(OR^{II}) \cdot PS_2 \cdot SO_2 \cdot R$ are claimed and are obtained by interaction of $OR(OR') \cdot PS_2H$ with $R \cdot SO_2X$ in an org. solvent at 20—90° in presence of acid-binding agent (R^I and R^{II} are alkyl or cycloalkyl optionally substituted by halogen, or are alkenyl, aryl, or aralkyl optionally substituted by halogen, NO_2 or OH; R is alkyl, aryl or aralkyl, the aryl portion optionally containing halogen, NO_2 or OH; X is halogen). One example is OO-*dimethyl* S-*methanesulphonyl phosphorodithioate*, prepared in 90—92% yield. F. R. BASFORD.

Pyrophosphoric acid esters. F. D. Cramer (B.P. 942,943, 19.4.60. Ger., 21.4.59).—*Sym.* and *asym.* pyrophosphate esters (insecticides, bactericides and fungicides) are obtained in good yield by interaction of $OH \cdot PO(OR^I) \cdot OR^{II}$ with $OR^{III}(OR^V) \cdot PO \cdot O \cdot CO \cdot NHR^{IV}$ at <50° under anhyd. conditions, preferably in pyridine (R^I is esterforming group; R^{II} is H or as R^I; R^{III} is H or cation derived from a base or an ester-forming org. group; R^{IV} is org. radical; R^V is H or cation). Some of the esters may be isolated as Li salts. The prep. is detailed of the Li_2 *salt* of sym. *diphenyl pyrophosphate* in 80% yield. By the same process P^I-β-D-1,2,3,4-*tetra-acetylglucosyl* P^{II}-*phenyl pyrophosphate* is obtained. F. R. BASFORD.

Physiologically-active heterocyclic phosphorus-containing compounds. N. V. Philips' Gloeilampenfabrieken (B.P. 943,633, 2.3.60. Netherl., 3.3.59).—There are claimed compounds of the general formula $Q \cdot PX(Y^I \cdot R^I) \cdot Y^{II} \cdot R^{II}$, also compositions comprising them for use in the combating of noxious organisms (Q is residue of a *N*-heterocyclic, aromatic-type compound of >3N connected immediately adjacently to each other and containing at least 1 NH group of which the H is replaced by the phospho group; X and Y^I are O or S; R^I and R^{II} are aliphatic hydrocarbon radical; Y^{II} is O, S, NH, or NR^{III}; R^{III} is alkyl of 1—5). One example (prep. described) is N-(O-*ethyl*-N-*dimethylaminophosphoryl*)-3-*amino*-1,2,4-*triazole*, m.p. 57—64°. It is highly active against spider mites and moulds. F. R. BASFORD.

Heterocyclic o-dithio compounds. Farbenfabriken Bayer A.-G. (B.P. 943,567, 22.11.61. Ger., 22.11.60).—Compounds claimed, useful as insecticides, acaricides, and in some cases fungicides, are obtained by interaction of a 2,3-di-mercapto-pyrazine with phosgene, thiophosgene or an alkyl halogenocarboxylate in presence of an acid-binding agent. Thus, thiophosgene is added dropwise at 0—5° to an aq. solution of 2,3-dimercaptopyrazine containing NaOH, then after stirring during a further 1 h. at room temp. the product, viz. the *pyridazine* 2,3-*trithio carbonate* (2'-*thio*-1', 3'-*dithiolopyriadazine*), m.p. 169—171° is isolated. F. R. BASFORD.

Halogenated aromatic carboxylic halides and esters. Diamond Alkali Co. (B.P. 942,911, 9.5.62. U.S., 11.5.61).—Herbicidal compounds $C_6X_{6-n}(CO_2R)_n$ (R is alkyl or aralkyl; *n* is 2—4; X is halogen), especially Me_2 2,3,5,6-tetrachloroterephthalate (**I**) are obtained in good yield free from C_6X_6 by halogenating $C_6X_{6-n}(CH_2 \cdot OH)_n$, then treating the resulting carbonyl halide with ROH (M is H or alkali metal). Residue is washed with CCl_4, to give 2,3,5,6-tetrachloroterephthaloyl dichloride (**I**) (10·3) containing 1—2% of $C_6Cl_5 \cdot COCl$ and no C_6Cl_6. A further amount (3·5 g.) of impure material is present in the mother liquor. The pure **I**, m.p. 145—147°, is heated (50) with MeOH 13 gal. in presence of 26·7 litres of a 25% solution of NaOMe in MeOH during 8·5 h. at 65° with formation of **I** (41·5 lb.) in 95·2% yield and of 87·5% purity. F. R. BASFORD.

Compounds having herbicidal and fungicidal properties. Fabriek van Chemische Producten Vonderlingenplaat N.V. (Inventor: K. van den Boogaart) (B.P. 941,709, 30.4.60).—Compounds claimed have the general formula 2,4,6,1-$C_6H_2YX^IX^{II} \cdot O \cdot CO_2R$, wherein X' and X'' are H or NO_2 but at least one of them is NO_2; Y is H, halogen or alkyl of 1—4 C; and R. is alkyl of 3—8 C, alkenyl of 3—6 C, Cl-substituted Me or Et, aralkyl or cycloalkyl. They are obtained by reacting the corresponding phenol with $ClCO_2R$, or with phosgene and then with ROH. The prep. is described of *hexyl* 6-*methyl*-2,4-*dinitrophenyl carbonate*. Its fungicidal and herbicidal activities are favourably compared with those of DNOC. F. R. BASFORD.

***s*-Triazine derivatives and herbicidal compositions containing them.** J. R. Geigy A.-G. (B.P. 944,063, 1.11.61. Switz., 2.11.60).—The herbicidal compounds comprise 2-cyano-*s*-triazines substituted in the 4- and 6-positions by NR^IR^{II} and $NR^{III}R^{IV}$ respectively (R^I is alkyl or alkenyl of 1—6 C, alkoxyalkyl, alkylthioalkyl, alkenoxyalkyl, or alkenylthioalkyl, in which total C is >6; R^{II} and R^{IV} are H or alkyl of 1—6 C; R^{III} is H, or as R^I), and obtained by heating a 2-halogeno analogue with metal or NH_4 cyanide. The prep. of 4,6-*bis*(*diethylamine*)-2-*cyano*-*s*-*triazine*, m.p. 212—214, is detailed. F. R. BASFORD.

Thallophyticidal compositions. Minnesota Mining & Mfg. Co. (B.P. 944,219, 23.12.59. U.S., 29.12.58).—Products active against, e.g., Schizomycetes, Ascomycetes, etc., contain as active ingredient a complex of a triarylborane (tritolylborane) and an amine (benzylamine, morpholine). F. R. BASFORD.

Animal Husbandry

Symposium on growth; environment and growth. C. F. Winchester (*J. Anim. Sci.*, 1964, **23**, 254—264).—A critical review with 45 references. A. G. POLLARD.

Protein quality of feeding-stuffs. II. Comparative assessment in three fish meals by microbiological and other laboratory tests and by biological evaluation with chicks and rats. J. Bunyan and A. A. Woodham. **III. Comparative assessment of protein quality of three fish meals given to growing pigs.** R. S. Barber, R. Braude, A. C. Chamberlain, Z. D. Hosking and R. G. Mitchell. (*Brit. J. Nutr.*, 1964, **18**, 537—544; 545—554).—**II.** A British white-fish meal and two Peruvian anchovy meals were examined for total amino-acids, etc. One of the anchovy meals was markedly inferior but the differentiation between the two good meals was not clear cut. (20 references.)

III. All three meals were of similar crude protein content and were used as supplements in the rations of growing pigs. The findings were similar to those obtained in the laboratory examination (Part II). C. V.

Browse plants in Ghana. I. Monthly chemical composition of seven species of trees, shrubs and vines browsed by free-ranging cattle on the Accra plains. R. Rose Innes and G. L. Mabey. **II. Digestibility of *Griffonia simplicifolia* from the Accra plains using local cattle as experimental animals.** G. L. Mabey and R. Rose Innes (*Emp. J. exp. Agric.*, 1964, **32**, 114—124, 125—130).—**I.** Feeding value, habit, palatability, frequency and habitat of the seven species are presented.

II. Digestibility of the highly palatable indigenous shrubby evergreen leguminous climber *G. simplicifolia* was investigated. Using West African cattle as the experimental animal digestion coeff. were, for dry matter 69%, crude protein 81%, crude fibre 59%, N-free extract 75%, ether extract 63%, ash 55%, and org. matter 70%. A. H. CORNFIELD.

Intake of soil by the grazing sheep. A. C. Field (*Proc. Nutr. Soc.*, 1964, **23,** xxiv—xxv).—Ingested soil is a source of minerals to a grazing ruminant but some constituents, e.g., clay, may interfere with the absorption of some minerals by the animal from the alimentary tract. Observations were carried out on four adult wethers over nine periods of six days. The amount of soil ingested was calculated from the ratio of the concn. of Ti in the faeces to that in the soil, assuming that the soil was indigestible. Of the nutritionally important elements with a high concn. in the soil relatively to herbage, e.g. Co, soil is an important source throughout the year and in winter it may be the main source. On the other hand, those mineral elements with relatively low soil to herbage ratio, e.g. Mg, could be an important source only during periods of high soil intake and low herbage availability. The degree of utilisation of these elements still requires elucidation. C. V.

Course of fermentation of lucerne silage under different conditions. J. Rojahn, J. Wagner and W. Harnisch (*Zbl. Bakt.*, 1964, II, **118,** 148—170).—Comparison is made of the effects of various additives, on fermentation in pressurised vessels and in an atm. of N_2 under normal pressure, on the quality of silage produced. On the basis of changes in bacterial population, of the composition of the mixed org. acids produced and of the pH of the product, addition of cultures of lactic acid organisms to high-protein material under pressurised conditions had very favourable effects on the silage obtained. A. G. POLLARD.

Estimation of the digestibility and nutritive value of forages by cellulose and dry-matter solubility methods. B. A. Dehority and R. R. Johnson (*J. Anim. Sci.*, 1964, **23,** 203—207; cf. *ibid.*, 1963, **22,** 222).—The method developed earlier, and based on the extraction of cellulose by cupriethylenediamine, gave low results for lucerne forage. The dissolution of cellulose was increased by preliminary autoclaving and still further increased by subsequent shaking with 2N-H_2SO_4. Data obtained by thus modifying the method were correlated with *in vivo* determinations of dry matter digestibility, (**I**), energy digestibility, (**II**), and nutritive value index (*NVI*) for grasses and mixed forage but not with those for legumes. Dry-matter solubility in N-H_2SO_4 determined after preliminary autoclaving, by weighing the residue after 30 min. shaking with the acid, yielded values correlated with relative intake, as well as with **I, II** and *NVI* for grasses, mixed forage and legumes. A. G. POLLARD.

Irrigated grass–legume mixtures as summer pasture for yearling steers. W. A. Hubbard and H. H. Nicholson (*Canad. J. Plant Sci.*, 1964, **44,** 332—336).—Three irrigated mixed pastures, viz., bromegrass–orchardgrass–ladino clover (**I**), bromegrass–orchardgrass–lucerne and Reed canary grass–orchardgrass–ladino clover were compared by the gain in wt. of long-yearling steers. On the basis of feed consumption per unit gain in wt., **I** was the most efficient although the daily gain in wt. was the same with all three forages. Probably beef production is limited by forage potential and the value of pasture is, in general, more reliable when based on animal production than on the measured total digestible nutrients of the forage. A. G. POLLARD.

Forage utilisation: nutritive value of forage as affected by physical form. I. General principles involved with ruminants and effect of feeding pelleted or wafered forage to dairy cattle. L. A. Moore. **II. Beef cattle and sheep studies.** D. W. Beardsley. **III. Effects of fertility levels and stage of maturity on forage nutrient value.** R. E. Blaser (*J. Anim. Sci.*, 1964, **23,** 230—238, 239—245, 246—253).—A symposium. A. G. POLLARD.

***In vitro* destruction of vitamin A by abomasal and ruminal contents.** F. J. Klatte, G. E. Mitchell, jun., and C. O. Little (*J. agric. Fd Chem.*, 1964, **12,** 420—421).—Incubation of vitamin A with ruminal fluid, abomasal fluid, autoclaved ruminal fluid and distilled water for 4 h. at 37° resulted in losses of 36·1%, 33·6%, 13% and 16%, respectively, of the vitamin. W. ELSTOW.

***In vitro* and *in vivo* comparisons on the utilisation of urea, biuret and diammonium phosphate by sheep.** R. R. Johnson and K. E. McClure (*J. Anim. Sci.*, 1964, **23,** 208—213).—Sheep were fed rations containing equal proportions of roughage (maize cobs, soya-bean flakes) and concentrates (starch, shelled maize, supplemented with urea (**I**), biuret (**II**), or $(NH_4)_2HPO_4$ (**III**). Utilisation of **II** and **III** was less efficient than that of **I**. Blood-**I** levels were higher when **I** than when **II** was used. The sheep showed a measure of ' adaptation ' to **II** in respect of apparent digestibility but not in those of N retention or biological value. No comparable effects with **I** were apparent. Feeding **II** had no effect on rumen-NH_3 or blood-**I**; both values were increased considerably when **I** was given. Theories of the ' adaptation ' of animals to **II** are discussed. A. G. POLLARD.

Effect of different systems of cattle grazing on botanical composition of permanent downland pasture. D. D. Kydd (*J. Ecol.*, 1964, **52,** 139—149).—Changes in species distribution due to grazing were slow and limited over the five-year period of the experiment although finally becoming definite. Comparison is made of continuous grazing (always over-grazed), frequent grazing (always under-grazed or with stocking adjusted to the growth of herbage), rotational grazing with stocking adjusted and grazing aftermath after cutting hay. Fouling with dung and urine resulted in patches of uneaten herbage which later became long, coarse and unattractive to cattle, but was followed by gradual return to the initial state in about a year in most cases. Rotational grazing tended to produce swards of improved botanical composition. A. G. POLLARD.

Variations in initial composition of orchardgrass as related to silage composition and feeding value. C. H. Gordon, J. C. Derbyshire, H. G. Wiseman and W. C. Jacobson (*J. Dairy Sci.*, 1964, **47,** 987—992).—Orchardgrass grown on heavily fertilised land contained higher levels of crude protein but lower levels of dry matter and N-free extract sugar than had grasses grown without fertiliser. Silage made from fertilised grasses had a higher pH value and contained more butyric acid and NH_3 than had that from unfertilised grasses. In three out of four trials silage made from fertilised grasses had a lower feeding value than that from unfertilised grasses. The liberal use of N fertilisers on grass crops should continue, but before ensiling such grasses the sugar content should be determined and if necessary raised to $\not<2\%$ by either wilting or the use of additives. (16 references.) M. O'LEARY.

Nutrition of forages and pastures. [A]. Collecting forage samples representative of ingested material of grazing animals for nutritional studies. C. W. Cook. **[B]. Selenium in forages as related to the geographic distribution of muscular dystrophy in livestock.** W. H. Allaway and J. F. Hodgson (*J. Anim. Sci.*, 1964, **23,** 265—270, 271—277).—A symposium. A. G. POLLARD.

Response of dairy heifers to diethylstilboestrol. E. W. Wickersham and L. H. Schultz (*J. Anim. Sci.*, 1964, **23,** 177—182).—Diethylstilboestrol (**I**), was added to a standard ration to provide 10 mg./head/day for heifers from 6 months until the expected date of first calving. The animals were fed individually, feed intakes being recorded. Over the period from 6 to 15 months of age, **I** increased, significantly, the average daily intake of roughage (dry basis) although the average increase in daily gain was not significant. From 15 to 24 months the increase in roughage intake due to **I** was consistent but not significant. **I** had no effect on skeletal growth but from 6 to 12 months of age increased the length of the mammary gland, although subsequent milk yields were unaffected. The reproductive organs and performance were unaffected by **I** except that first calves had lower birth wt. Cessation of **I** feeding after the first calving did not influence the subsequent reproductive performance. A. G. POLLARD.

Body composition *in vivo*. VI. Composition of ewes during prolonged undernutrition. B. A. Panaretto (*Aust. J. agric. Res.*, 1964, **15,** 771—787).—Ten ewes were undernourished by feeding progressively diminishing quantities of lucerne chaff and oats (1 : 1) until they had lost 32—39% of their initial wt. in 150—200 days. The fat and protein reserves were gradually depleted, thiocyanate spaces expanded relative to body wt. and red cell vol. decreased while the plasma vol. was maintained. Three very fat ewes gradually passed into a phase of inappetence and died while still very fat. (22 references.) S. A. BROOKS.

Analysis of fat deposition in swine by gas–liquid chromatography. J. D. Sink, J. L. Watkins, J. H. Ziegler and R. C. Miller (*J. Anim. Sci.*, 1964, **23,** 121—125).—Ether extracts of homogenised fat samples were dried and after removal of the solvent were examined by gas–liquid chromatography. Changes in the qual. and quant. deposition of fatty acids followed a definite pattern beginning in animals of approx. 130 lb. live-wt. Deposition of saturated fatty acids increased with live-wt. and occurred preferentially in perirenal rather than in subcutaneous zones. The pattern of deposition of unsaturated fatty acids was the reverse of this. A. G. POLLARD.

Effect of chlortetracycline, sulphamethazine and procaine penicillin on the performance of starting pigs. R. F. Elliott, D. D. Johnson and A. L. Shor (*J. Anim. Sci.*, 1964, **23,** 154—159).—Starter rations for pigs weaned at 3 weeks were supplemented with chlortetracycline (100) alone or with sulphamethazine (100) and/or procaine penicillin (50 g./ton of feed). Significant increases in average daily gain in wt. and in average daily feed consumption resulted with diminution in feed consumed per unit gain. Mild infection of the pigs by *Salmonella choleraesuis* lowered the rate of gain in wt. and feed efficiency; this condition was improved by the three

supplementary antibiotics. Severe infections causing death or slow recovery were rapidly eliminated by the combined antibiotic supplement, the animals recovering their normal rate of growth after 7 days. A. G. POLLARD.

Utilisation of dietary energy and protein by poultry. J. Davidson (*Annu. Rep. Anim. Nutr., Rowett Res. Inst.*, 1964, **20,** 61—67).—The report describes work concerning energy and protein utilisation in growing chickens and protein utilisation in laying hens. The following are discussed : choice of a criterion of dietary energy value ; metabolisable energy preferred ; factors affecting utilisation of metabolisable energy ; indigestible org. matter ; crude protein ; metabolisable energy ratio ; dietary fat ; breed effects ; amino-acid requirements ; proportion of tissue energy available for man ; relative values of cereal proteins for chick growth ; protein utilisation for egg production. (22 references.) E. M. J.

Nutritional evaluation of meat meals for poultry. II. Effect of increasing protein concentration, removal of bone, and folic acid, pyridoxine and pantothenic acid supplementation of diets based on high- and low-quality meals. B. S. Sathe, R. B. Cumming and G. L. McClymont (*Aust. J. agric. Res.*, 1964, **15,** 698—718).—The growth-promoting ability of meat meals in chick diets depends tentatively on (i) any effect of excess ash (particularly Ca), (ii) quality (digestibility, biological value) of their protein which is not related to the proportion of collagen and the proportion of meat protein in the diet and (iii) the folic acid and/or pyridoxine contents of the diets, and/or the requirements of the chicks.
E. G. BRICKELL.

Methionine supplementation of breeder diets. N. J. Daghir, S. S. Akrabawi and K. Rottensten (*Poultry Sci.*, 1964, **43,** 1106—1109).—Addition of 0·05—0·15% Ca methionine hydroxy-analogue (90%) to an all-plant maize–soya-bean diet for 224 days had no effect on feed consumption, egg production, body wt., egg wt. or mortality. 0·10—0·15% of the supplement increased hatchability of fertile eggs. A. H. CORNFIELD.

Effects of different isomers of methionine on growth of chicks fed amino-acid diets. L. Marrett, H. R. Bird and M. L. Sunde (*Poultry Sci.*, 1964, **43,** 1113—1118).—When a DL-amino-acid mixture, containing 15% D-amino-acids (3·8% of the total ration), was used and different levels of D- and L-methionine were added, the L-form was utilised better by chicks (as shown by wt. gains) than was the D-form. With 0·5% methionine the L-form was almost twice as active as the D-form, whilst with 0·8% methionine there was little difference between the two forms. With the L-amino-acid basal mixture, the two forms of methionine were utilised equally well.
A. H. CORNFIELD.

Enzyme supplements in rations containing legume seed meals or gums. J. O. Anderson and R. E. Warnick (*Poultry Sci.*, 1964, **43,** 1091—1097).—The reduction in growth rate and feed efficiency and the occurrence of sticky droppings when guar meal or gum or locust gum replaced cottonseed meal in chick rations was prevented when certain enzyme prep. were added. An enzyme which improved the performance of chicks receiving barley was ineffective in improving the performance of chicks receiving guar meal. An enzyme mix which improved the value of a guar meal diet only slightly improved the value of a soya-bean meal diet.
A. H. CORNFIELD.

Physiological effects of feeding high levels of vitamin A acetate to chicks. W. J. Pudelkiewicz, L. Webster, G. Olson and L. D. Matterson (*Poultry Sci.*, 1964, **43,** 1157—1164).—Temporary toxic effects occurred in chicks receiving 5 g. of vitamin A acetate per kg. of diet, but not in chicks receiving 0·5 g. or lower levels. Liver wt. and vitamin A level and plasma vitamin A were at a max. when 0·5 g. of the vitamin was supplied. An intake of 220 i.u. per g. body wt. had no toxic effects, whilst 1120 i.u. reduced feed consumption. A. H. CORNFIELD.

Growth inhibitory effect of polysaccharides for chickens. P. Vohra and F. H. Kratzer (*Poultry Sci.*, 1964, **43,** 1164—1170).—Addition of 2% guar gum, locust gum, gum tragacanth, gum karaya, carrageenin, dried okra or psyllium husk or of 4% pectin depressed wt. gains of chickens, whilst cellulose, methylcellulose, carboxymethylcellulose, dextrin, dextran, linseed mucilage, caramel, gum ghatti, agar-agar and kelp had no effect. The growth-depressing effects of pectin and guar gum were overcome by treatment with pectinase and cellulase respectively. A. H. CORNFIELD.

Availability of calcium in feed grade phosphates to the chick. B. C. Dilworth, E. J. Day and J. E. Hill (*Poultry Sci.*, 1964, **43,** 1132—1134).—When the availability of Ca, as measured by wt. gains and tibia ash%, from $CaCO_3$ was taken as 100, that from two defluorinated phosphates was 92 and 95, from low-fluorine rock phosphate 90, and from soft phosphate was 68.
A. H. CORNFIELD.

Use of chick plasma non-protein nitrogen in evaluating fish meals. G. R. Childs and G. F. Combs (*Poultry Sci.*, 1964, **43,** 1220—1222).—The quality of fish meals, as determined by chick assay, was correlated with the levels of non-protein-N and methionine in the plasma of chicks fed with the materials for 1 h. following a 22-h. period of fast. A. H. CORNFIELD.

Calcium and phosphorus requirements of broilers as influenced by energy, sex and strain. R. J. Lillie, P. F. Twining and C. A. Denton (*Poultry Sci.*, 1964, **43,** 1126—1131).—Gains in wt. of broilers to 8 weeks of age on maize–soya-bean meal diets were greater for males than for females, were greater on the high- (1550) than on the low-energy diet (1400 kcal. metabolisable energy per lb.), and were also different between strains. With varying P (0·5—0·9%) in the diet, wt. gains were highest with 0·7% dietary P. Wt. gains were not affected by dietary Ca level (0·9—1·2%) at any level of P or energy. Feed efficiency was higher on high- than on low-energy diets. There were no differences in % bone ash of the tibia due to treatments. A. H. CORNFIELD.

Effect of iron and magnesium on manganese metabolism in turkey poults. H. R. Woerpel and S. L. Balloun (*Poultry Sci.*, 1964, **43,** 1134—1142).—Turkey poults required approx. 22 p.p.m. of Mn in the diet for optimum growth. Liver-Mn% reflected the intake of Mn. Addition of Mg (4400 p.p.m.) to a maize–soya-bean diet depressed feed efficiency, reduced bone-ash% and increased bone-Mg%. Mn requirement for normal bone structure development was increased when high levels of Mg and Fe (440 p.p.m.) were added to the diet. A. H. CORNFIELD.

Comparisons of sorghum grain and maize proteins in various combinations with soya-bean meal protein in laying diets. D. J. Bray (*Poultry Sci.*, 1964, **43,** 1101—1106).—Egg production from pullets receiving 8·5%-protein diets was higher when intermediate combinations of sorghum grain and soya-bean protein were used than when either material provided the bulk of the protein. When no supplemental methionine was supplied egg production was higher with a 55 : 45 combination of soya-bean/maize-protein than a similar combination of soya-bean/grain-sorghum protein ; there were no differences when 0·2% methionine was supplied. Responses to methionine increased as the proportion of protein supplied by soya-bean increased from 25% to 85% of the total protein in both sorghum and maize diets. A. H. CORNFIELD.

Changes in egg yolk-cholesterol, serum-cholesterol and serum-glutamic oxaloacetic transaminase due to feeding cholesterol and vegetable oil to mature hens. B. J. Hulett, R. E. Davies and J. R. Couch (*Poultry Sci.*, 1964, **43,** 1075—1078).—Addition of 1—5% of cholesterol to hen diets increased the cholesterol level in both serum and egg yolk. These increases together with that in the serum-glutamic oxaloacetic transaminase were less when no rice oil than when 10% of rice oil was added to the diet. Egg production was reduced by addition of cholesterol to the diets and further reduced when rice oil was added also. A. H. CORNFIELD.

Effect of protein level and source and grain source on performance of egg production stock. J. W. Daeton and J. H. Quisenberry (*Poultry Sci.*, 1964, **43,** 1214—1219).—Birds receiving a 16%-protein diet had heavier body and egg wt. and higher egg production and feed efficiency than had those receiving a 14%-protein diet. Maize was a more effective grain source than sorghum grain in three of the four diets. Egg wt. was greater where sorghum grain than where maize was supplied in the 16%-protein diet, but the reverse was true in 14%-protein diet. A. H. CORNFIELD.

Effect of high levels of lucerne meal on egg production, yolk colour, fertility and hatchability. J. R. Kingan and T. W. Sullivan (*Poultry Sci.*, 1964, **43,** 1205—1209).—Egg production, feed efficiency with respect to egg production and fertility were not affected by addition of 8—20% of dehydrated lucerne meal, in comparison with a 4% addition, to the hens' diet. Hatchability was significantly greater with 12—20% than with 6% of lucerne meal in the diet. Egg yolk colour and β-carotene equiv. per g. of fresh yolk increased with level of lucerne meal supplied. Egg yolk colour with all treatments tended to decline with the onset of warm weather. A. H. CORNFIELD.

Relation between egg-weight before incubation and the weight of hatched chicks. Alberto M. Gamero (*Rev. Fac. Agron., La Plata*, 1963, **39,** (No. 1a), 11—21).—Relationships between wt. of eggs and hatched chicks were studied from July to Nov. in four years, 1958—1961, with five different breeds. Mean chick wt. as % of egg wt. at commencement of incubation and overall correlation coeff. calculated for each breed were : S.C. White Leghorn, 64·6%, 0·88 ; White Plymouth Rock, 60·8%, 0·84 ; Barred Plymouth Rock, 59·4%, 0·75 ; New Hampshire, 56·6%, 0·81 ; and Rhode Island Red, 58·3%, 0·54. Variations of the correlation coeff. with colour of egg, date of laying and age of hen are reported and analysed.
E. C. APLING.

Degradation of manure collected in water under chickens. C. E. Ostrander and S. A. Hart (*Poultry Sci.*, 1964, **43**, 1144—1151).—Manure from chicken cages collected in water was usually less odorous than solid manure. Strength of odours increased with temp., as did breakdown and loss of org. matter. Altering the pH (7·2) of the water-collected manure did not control scours and often produced more offensive odours. A. H. CORNFIELD.

Emerging diseases of animals. *F.A.O. agric. Stud.*, 1963 [1965], No. 61, 241 pp.—The term ' emerging diseases ' is applied to some which have been confined entirely, or almost entirely, to certain regions, but are now appearing in areas far removed from those where they were originally observed. Some are associated with improved nutrition. Six of the important emerging diseases are fully dealt with : (i) **African swine fever.** W. O. Neitz, pp. 1—70 ; (ii) **African horsesickness.** P. G. Howell, pp. 71—108 ; (iii) **Bluetongue.** P. G. Howell, pp. 109—153 ; (iv) **Johne's disease (paratuberculosis).** R. Worthington, pp. 155—176 ; (v) **Lumpy skin disease.** K. E. Weiss, pp. 177—201 ; and (vi) **Enterotoxaemias of sheep caused by organisms of the Welch group.** P. W. Thorold, pp. 203—220. Bibliography (i) 130 references, pp. 221—226 ; (ii) 83 references, pp. 226—229 ; (iii) 76 references, pp. 229—232 ; (iv) 86 references. pp. 232—235 ; (v) 38 references, pp. 235—237 ; (vi) 109 references, pp. 237—241. E. M. J.

Reviews of the progress of dairy science. E. Diseases of dairy cattle. Brucellosis. W. J. B. Morgan (*J. Dairy Res.*, 1964, **31**, 315—359).—The incidence of brucellosis in cattle, sheep, goats and pigs during 1960—63 together with methods developed during that period for its diagnosis and treatment, are reviewed. (12 pp. of references.) M. O'LEARY.

Effectiveness of a combination of antibiotics in a bolus and of potassium laevopropylcillin in the prevention of pasture bloat. P. R. Shellenberger, N. L. Jacobson, P. A. Hartman and A. D. McGilliard (*J. Anim. Sci.*, 1964, **23**, 196–202.—Mixtures of streptomycin sulphate, tylosin phosphate, erythromycin thiocyanate and procaine penicillin in the form of boluses were administered to dairy cattle and sheep grazing lucerne pasture. Bloat was reduced in cattle for several weeks. After administration of three boluses, further dosages at intervals of several weeks prolonged the protective effect; average daily gains in wt. were increased. Initial administration of a half-bolus to sheep gave protection for 3—4 weeks but a second dosage at 4 weeks had no further effect. In preliminary tests K laevopropylcillin gave promising results in which the period of protection was increased considerably. A. G. POLLARD.

Toxicology of cadmium. H. D. Fayle (*Dissert. Abstr.*, 1964, **25**, 2201).—Exhaustive physiological and chemical tests were carried out on the effects of Cd on 39 mongrel dogs, an acutely affected group of 11 dogs, a chronically affected group of 18 and a control group of 10 dogs. Blood was obtained from the femoral or jugular veins. In dogs, for intravenously administered Cd, the approx. LD_{50} was 2 mg. Cd/kg., the M.L.D. was $<2{\cdot}0$ mg. Cd/kg. and the LD_{100} >5 mg. Cd/kg. It was assumed that the toxicity of the intravenously injected $CdSO_4$ is due to the concn. of the Cd^{2+} ion available for combination with enzymes containing the SH^- groups. F. C. SUTTON.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Dehydration of whole rice. I. Determination of equilibrium kinetics. A. Escardino and F. Ruiz (*Rev. Agroquim. Tecnol. Aliment.*, 1964, **4**, 375—380).—Equilibrium humidities were determined at 25° by the method previously described (*ibid.*, 1963, **3**, 21) for three rice varieties (Balilla, Stirpe and Americano 1600), and equations correlating the results obtained are reported. The influence of initial moisture content of the rice on the equilibrium curve was studied and a hysteresis effect was found. It is concluded that rice should be stored after drying at R.H. from 40—70% in order to maintain the moisture content within the optimum range (11—14%). (18 references.) E. C. APLING.

Radionuclides in wheat and other agricultural products: a review. V. F. Pfeifer (*Cereal Sci. Today*, 1964, **9**, 354—357).—The radionuclides produced in nuclear explosions and present in foods are described and the concn. in wheat and milling products over the last few years given. Recommended max. intakes of these radionuclides and methods of reducing the concn. in wheat are discussed. (26 references.) S. A. BROOKS.

α-Amylase activity of varieties of English wheat. B. A. Stewart (*Nature, Lond.*, 1964, **204**, 1088).—It is suggested that a detailed examination of present and future varieties of wheat is undertaken to ascertain which varieties are likely to be unsuitable for flour due to high and variable α-amylase activity. S. A. BROOKS.

Moisture adsorption of wheat flour as affected by physical and chemical characteristics. Chaim Gur-Arieh (*Dissert. Abstr.*, 1964, **25**, 399—400).—Apparatus described provides for circulation of air at pre-determined R.H. through tubes loosely filled with particles which can thus reach a water content in equilibrium with the R.H. Twelve circuits, each at different R.H. can be operated simultaneously, and a complete adsorption isotherm (*AI*) can be produced in 24—36 h. The *AI* observed were independent of the particle size-distribution of the flour ; sp. surface areas calculated from the *AI* were >1000-fold those determined by direct methods. The sorptive capacity of flour diminished with rise in protein content ; that of starch separated from flour exceeded that of the gluten fraction. Sorption capacities for alkaline water and the apparant η of acidulated-flours increased with diminution in particle size. Cakes baked from flours of decreasing particle size showed increasing vol. and lowered adsorption capacity. The *d* of flour at different moisture contents was independent of particle size-distribution. A. G. POLLARD.

Investigation of the relationship between maltose figure and the extent of starch damage in wheat flour. E. F. Wolf (*Getreide u. Mehl*, 1964, **14**, 115—117).—The increase in maltose figure (Δ-maltose fig.) resulting from the addition of a constant amount (e.g., 1%) of malt flour is shown to be directly related to the amount of damaged starch in the flour, and is proposed as a practical index of starch damage. E. C. APLING.

Short term effects of chlorine dioxide on flour. W. Lewis Jones (*Cereal Sci. Today*, 1964, **9**, 358, 360, 381).—The extensigram parameters of commercially milled 72%-extraction bakers' flour treated with 35 p.p.m. ClO_2 were examined. A rapid rate of decay in apparent oxidation effects during the first 48 h. was noted, declining to a more stable state after 8 days. Possible explanations of this are discussed. S. A. BROOKS.

Analysis of bread flour constituents. J. A. Johnson and Yu-Yen Linko (*Qualitas Plant.*, 1964, **11**, 256—268).—Org. acids, esters and carbonyl compounds (*CC*) were studied. *CC* are present in greater concn. in the crust than in the crumb and have an influence in the browning. The composition of *CC* can be changed according to the amino-acids present. When isoleucine was added to a bread with a sufficiency of sugar, isovaleraldehyde increased in the crust from 3·1 to 17·4 mg./100 g., when valine was added, isobutyraldehyde rose from 0·1 to 0·7 mg./100 g. in the same site. The influence on flavour as the result of these additions is discussed. Hydroxymethylfurfural was produced in relatively high concn. in the crust as the result of the browning involving glucose while furfural production was minimal because of the relatively low concn. of pentose sugars in the dough. Analytical details, separation into classes, concn. developing solvents and the appropriate techniques for each group of compounds are provided. (35 references.) C. V.

Rôle of wheat flour pentosans in baking. III. Enzymic degradation of pentosan fractions. P. M. Wrench (*J. Sci. Fd Agric.*, 1965, **16**, 51—57, cf. J.S.F.A. Abstr. 1965, i, 150.).—Chromatography of the water-sol. pentosans of an Australian flour on DEAE cellulose yielded five fractions ; B and C are respectively a galactose–arabinose–xylose polymer and an arabogalactan, and each contains some protein. A partially resolved second component of peak A also appears to be degraded by snail juice enzymes, but fractions D and E are not attacked. If doughs are made containing pentosans degraded by snail juice enzymes, the vol. of the loaf is smaller. This effect is not observed in presence of oxidising agents; the action is discussed. (10 references.) E. M. J.

Utilisation of ammonia gas liquor for the production of yeast proteins. J. Bárta, F. Štros and R. Zábojník (*Kvasný průmysl*, 1964, **10**, 256—257).—Trials were made by replacing the conventional nutritive salts with the waste water from pressure gas works. From the favourable results obtained on laboratory scale, the method was introduced in an industrial plant. After 1-year's experience no impairment of the final product quality was observed, the fermentation was regular, and the method could be applied in a baker's yeast production plant. J. S. B.

Use of amyloglucosidase in bread making. Y. Pomeranz, G. L. Rubenthaler and K. F. Finney (*Food Technol.*, 1964, **18**, No. 10, 138—140).—The use of enzyme prep. to supply sugars for panary fermentations was examined. Adding amyloglucosidase (**I**) from *Aspergillus niger* or a mixture of **I** and α-amylase (**II**) to a bread formula containing 2 g. of sugar/100 g. of flour produced a loaf comparable to that from using 6 g. of sugar. The effects of **II**

alone were smaller than those of mixtures of **I** and **II**. The improving effects of enzyme supplementation was most pronounced in doughs of low sugar-content. **I** derived from *A. niger* gave better results than did **I** from *Rhizopus delemar*; the enzyme from *A. niger* remains relatively stable up to 70°. E. M. J.

Flour protein solubility and baking quality. X. Influence of salts and acids. E. Maes (*Getreide u. Mehl*, 1964, **14**, 109—111).—The effects of NaCl, citric acid, KCN, Na_2SO_4, $CuSO_4$ and $KMnO_4$ on protein solubility in water, isopropanal, lactic acid and 0·5% KOH (cf. *ibid*, 1963, **12**, 70) and on baking quality are reported and discussed. The effects appear in some cases to be contradictory, e.g., small quantities of citric acid and KCN both increased protein solubility in water and reduced solubility in the other solvents, but effects on baking quality are opposite (improvement and deterioration, respectively). E. C. APLING.

Fluid shortenings for white bread. E. G. Bayfield and W. E. Young (*Cereal Sci. Today*, 1964, **9**, 363—364, 366—368, 370—371, 381).—Fluid or liquid shortenings for white bread were examined and compared with powdered hard fat and fatty acid mixtures. Liquid shortening improved the crumb softness and shelf life of the bread. Fatty acids were not as effective as hard triglycerides. Increasing the amount of oxidant did not change the shortening effect very much. (15 references.) S. A. BROOKS.

Effect of additives on the elastic and plastic properties of breadcrumb. V. Effect of gluten. Summary and evaluation of publications I—V. L. Telegdy Kováts and R. Lásztity (*Period. Polyt., Budapest*, 1964, **8**, 95—103).—The rheological properties of crumbs of bread prepared with different quantities of dried native gluten were investigated. Total, plastic and elastic deformation increased with increase in gluten content. The measured relative elasticity decreased due to increase in vol. on addition of gluten; when a vol. correction was made relative elasticity was found to increase. (11 references.) S. A. BROOKS.

Volatile aromatics in bakery products. H-D. Ocker (*Qualitas Plant.*, 1964, **11**, 269—280).—Samples are divided immediately after removal from the oven ; one half is examined at once, the other after 24 h. They are heated in a flask at 60°, in a flow of N_2 the volatiles being condensed in a liquid-N trap ; they are dissolved in acetic ester and examined by gas chromatography. EtOH (**I**), $CH_3CO{\cdot}CH_3$ and CH_3CHO (**II**) are the main components of the aroma from rolls for diabetics together with four other compounds, one of which is methyl mercaptan. Products examined on removal from the oven chiefly contained **I** together with **II** the conc. of this latter being 0·001 of **I** ; another volatile compound is present in a 0·0001 concn. of **I**, but this has not been identified. Comparison is made between bread leavened with yeast and with chemicals ; the observations suggest that the volatile aromatic compounds are formed by the yeast during the fermentation. C. V.

Potential vitaminisation of bakery products with vitamin B_1 using fermented grain bran steep liquor and brewer's yeast. P. Němec and L. Brezák (*Prům. potravin*, 1964, **15**, 565—567).—Experiments aiming at enriching bakery products by addition to the dough of components containing vitamin B_1 are reported. The vitamin sources were fermented water extract of grain bran, and brewer's yeast, added to the dough as a portion of the necessary water quantity. Best results, both as to the vitamin content and as to organoleptic qualities were obtained, if the steep liquor was prepared by extracting bran (2000 g.) with water (10 l.) at 52°, fermenting the extract under addition of a nutrient and lactic acid for 48 h., during which time non-debittered brewer's yeast (1000 g. with 14% dry substance) was introduced. Within 24h. in the fermenting medium the objectionable taste of brewer's yeast is removed. J. S. B.

Protein fortification of doughnuts. I. I. Rusoff, A. H. Goodman, J. Sommer and S. M. Cantor (*Food Technol.*, 1964, **18**, No. 11, 131—134).—As a relatively easily mass-produced food product, the doughnut represents an acceptable means for incorporating quality protein (20%) into the diet. This was studied in relation to the proteins available in the country in which the product is to be consumed. Toasted soya flour, defatted fish fillets and defatted whole fish protein were used to enrich the nutritive value of doughnuts when added at an equal level. Such doughnuts contain an adequate content and balance of essential amino-acids as compared with values for standard casein by rat growth. (15 references.) E. M. J.

Defrosting of frozen bread. B. Belderok and W. H. G. Wiebols (*Food Technol.*, 1964, **18**, No. 11, 155—160).—Defrosting of wrapped and unwrapped white, brown and currant bread at air temp. of 50, 60, 70 and 80°, R.H. of 40, 50, 60, 70 and 80%, and air velocities of 0·75 and 2·50 m./sec. showed that defrosting time was reduced with each 10° increase in air temp. when R.H. was constant. When air temp. was constant, defrosting times were shorter at higher than at lower R.H. Under all these conditions, crumb was of excellent quality, but crust varied considerably. At intermediate R.H. a good, crisp crust was obtained. E. M. J.

Modification of the gluten of flour. Research Ass. of British Flour-Millers, J. Pace and E. E. McDermott (B.P. 943,848, 17.5.61). —Gluten properties of flour are modified (so that dough made therefrom has reduced elasticity and increased flexibility) by addition of supplementary protein containing sulphydryl groups, e.g., thiolated gelatin. R. R. BASFORD.

Oxidation of starch. Miles Laboratories Inc. (B.P. 943,664, 15.1.61. U.S., 27.6.60).—Dialdehyde starch is obtained in a state of higher purity and in improved yield by oxidising starch at 30—35° during 3—5 h. with HIO_4 in solution at pH 1·5 (maintained with strong acid, e.g., H_2SO_4, H_3PO_4, or HCO_2H). F. R. BASFORD.

Compacted starch. Corn Products Co. (Inventors : A. Kott and R. M. Olson) (B.P. 944,224, 29.11.60. U.S., 2.12.59).—There is claimed a method of continuously compacting starch into a strip of indefinite length by force-feeding a stream of highly mobile powdered starch with entrained air in a continuous path into a compaction region ; compressing the starch and entrained air as the stream advances ; discharging the compressed air adjacent to and in advance of the compaction region whilst maintaining the starch under pressure in the compaction region ; and continuously compressing the deaerated starch. The product, which is dustless, is a hard, grain-like mobile form of starch, is readily dispersible in water and shows no change of physical and chemical properties over the uncompacted starch. It is suitable for household and other uses. Apparatus is figured. F. R. BASFORD.

Sugars and confectionery

Chemical mechanism of white sugar moistening. II. Reaction of sucrose and water vapour from the viewpoint of chemical kinetics. K. Čiž (*Listy cukrovar.*, 1964, **80**, 299—301).—The initial phase of sugar moistening may be described by an equation for a first-order reaction, the storage property being characterised by the rate constant. J. S. B.

Influence of natural and artificial aeration of stored sugar beets. L. Schmidt and J. Zahradníček (*Listy cukrovar.*, 1964, **80**, 313—315).—Storage trials of beets in piles under pilot plant conditions, naturally and artificially aerated, were made to compare the daily losses of digestion sugar. In naturally aerated beets the losses were lower by 4·2%, in the artificially aerated by 13·3% lower, compared with non-aerated piles. The favourable influence of both natural and artificial aeration on the technological quality of beets was verified also by classification of the condition and microbiological analysis of affected beets. J. S. B.

Validity of Lambert–Beer law in sugar colorimetry [and the correlation with the colour of solutions]. V. Valter (*Listry cukrovar.*, 1964, **80**, 319—327).—In an extensive study of the problem, reported in detail and accompanied with numerous tables and graphs, the validity of the Lambert–Beer law for sugar solutions was verified. The most suitable wavelength for measuring the colour of sugar solutions differs only very slightly from the value of 560 mμ, recommended by ICUMSA. J. S. B.

Sugar crystallisation: Aspects affecting conglomeration. H. E. C. Powers (*Socker*, 1964, **19**, 51—65).—A review, and general discussion. C. V.

Ethers of sugars. Monsanto Chemical Co. (B.P. 941,950, 27.6.60. U.S., 26.6.59).—The prep. is described of the Na salt of carbonyvinyl glucose (and other sugar) ether, which has soil fungicidal properties against, e.g., *Rhizoctonia solani* and other fungi, at concn. of 100 p.p.m H. S. R.

Fermentation and Alcoholic Beverages

Higher alcohol formation by yeasts. J. F. Guymon (*Qualitas Plant.*, 1964, 11, 194—201).—Gas liquid chromatography was used to determine the % of individual alcohol components as well as for the separation and collection of specific alcohols. Recent work indicates that these alcohols are formed by reactions closely related to the synthesis of valine, leucine and isoleucine but the amounts formed by the Ehrlich pathway of direct breakdown of endogenous amino-acids are often negligible or non-existent. There is evidence to support the hypothesis that 2-ketobutyric acid is the intermediate from which both n-propanol and n-butanol are formed in fermentations by mutant strains of yeast. (22 references.) C. V.

Influence of sulphurous acid on formation of acetaldehyde by yeasts in fermentation of must and sparkling wines. H. Schanderl and T. Staudenmayer (*Mitt. Wein u. Obstbau, Wien*, 1964, **14A**, 267—281).—This influence is strong as regards ordinary wine yeasts, but slight as regards acetogenous yeasts. After the second fermentation in the prep. of sparkling wines, large amounts of acetaldehyde (which gives an oxidation flavour) are formed unless the wine is kept out of atm. contact. Clarification with yeast decreases the rH value and the acetaldehyde content, and removes metallic impurities. (18 references.) P. S. ARUP.

Stabilisation of wines with metatartaric acid (I), and the preparation thereof. V. Beneš and V. Krumphanzl (*Kvasný průmysl*, 1964, **10**, 258—261).—Experimental results indicate that **I** is an outstanding stabilising agent preventing pptn. of wine stone in natural wine. **I** was prepared by heating tartaric acid at 160—170° for ~1·5 h., and meets the requirements in every respect. It secures the necessary stabilisation, and is harmless to the organoleptic qualities of the wine. The quality of the **I** is equal to that of the imported fine chemical. J. S. B.

Diethyl pyrocarbonate. B. C. Rankine (*Bottler and Packer*, 1964, **38**, No. 12, 68, 70, 72, 74).—History, physical and chemical properties, germicidal effect, mixing difficulties (with wine) influence of SO_2, taste threshold, legality, advantages and disadvantages are discussed. E. M. J.

Aromatic qualities of wine. A. D. Webb (*Qualitas Plant.*, 1964, **11**, 234—243).—A vapour-sampling chromatograph is discussed; the extreme sensitivity enables the changes with age to be followed in bottled wines. The technique is discussed and some examples are considered. (15 references.) C. V.

Ethyl acetate in wines. P. Ribéreau-Gayon (*Qualitas Plant.*, 1964, **11**, 249—255).—A very general, brief discussion. It is claimed that it is this ester, not acetic acid, that results in off-odours. C. V.

Gas chromatography of the aroma compounds of alcoholic beverages. E. Sihto, L. Nykänen and H. Suomolainen (*Qualitas Plant.*, 1964, **11**, 211—228).—A triethanolamine column was used to separate isoamyl and optically active amyl alcohol. When the heads fraction obtained in the production of spirit was analysed a considerable amount of acetal was present. The fusel oil fraction of beer was studied together with the ester-odours; the aromatic substances were concentrated by extraction in an ether–pentane mixture. The most important substances separated and identified were phenylethylalcohol, ethyl caprylate, isoamyl acetate and phenylethyl acetate. An interesting comparison is afforded between the gas chromatograms for Scotch whisky and an imitation; the latter contains peaks which are not present in the former while the proportions of the components differ markedly. C. V.

Manganese content of Hungarian wines. I. Tuzson (*Mitt. Wein u. Obstbau, Wien*, 1964, **14A**, 299—305).—The limits of variation in 842 samples were 0·44—6·73 mg./l.; about 40% of the samples contained 1·0—1·5 mg./l. of Mn. The content of wines from hybrid vines was greater than that of wines from European vines. Seasonal and cultural variations were noted. (10 references.) P. S. ARUP.

Sensitivity of barley to water. R. Scriban (*Brasserie*, 1964, **19**, 310—315).—Comparative germination experiments with different varieties of barley confirm the observation of Isebaert that the grains should be placed resting on the dorsal side in order to obtain max. reproducibility of results. Furthermore, the grains should be disposed out of contact with one another in all the conventional tests. P. S. ARUP.

New conception of the processes taking place in barley during germination. V. Karel (*Kvasný průmysl.*, 1964, **10**, 245—246).—Some new views on the rôle of the embryonal and aleurone layers in the germination processes of malting barley are discussed. The embryo produces substances, belonging to the gibberellin group, which diffuse into the endosperm, and influence the formation of enzymes in the aleurone layer. Therefore the latter is the main source of enzymes, being a live tissue, and not the embryo. J. S. B.

Determination of 2-phenylethanol in cider. M. E. Kieser, A. Pollard, P. M. Stevens and O. G. Tucknott (*Nature, Lond.*, 1964, **204**, 887).—The 2-phenylethanol content of cider has been extracted from distillates with EtCl. Ethanol was added to the extracts, EtCl removed and the 2-phenylethanol content determined by gas chromatography by comparing the retention time with that of a standard. (13 references.) S. A. BROOKS.

Method and apparatus for preparing brewer's wort. A. Guinness Son & Co. (Park Royal) Ltd. (Inventors: P. H. Watts, M. E. Ash and G. C. Phillpotts) B.P. 943,811, 14.1 and 20.10.61).—A continuous mashing process for the production of brewer's wort is described. Apparatus is figured and claimed. F. R. BASFORD.

Continuous brewing of beer. R. Ramsden & Son Ltd. and R. P. Williams (B.P. 943,091, 4.5.60).—The process comprises feeding ground malt and water continuously to a mashing unit; withdrawing wort after a predetermined time and passing it to storage; drawing off successive batches therefrom into a boiling copper (or coppers); adding hops and boiling; transferring the batch(es) to one or more hopbacks wherein spent hops are removed; cooling; introducing yeast; passing the fermenting liquor through the fermentation plant; and continuously drawing off the resulting beer. Apparatus is figured and claimed. F. R. BASFORD.

Fruits, Vegetables, etc.

Effect of temperature on postharvest physiology and storage life of pears. S. W. Porritt (*Canad. J. Plant Sci.*, 1964, **44**, 568—579).—Anjou and Bartlett pears were studied over nine storage temp. ranging from 20 to 70°F. After harvest, low metabolic activity persisted about 4 days in Bartlett and over 50 days in Anjou at 50—70°F and the latter ripened only after a period of cold storage. Storage life of both varieties was 35 to 40% greater at 30° than at 32°F. E. G. BRICKELL.

Quality of orange varieties. V. Standardisation of methods. Standardised method for determination of juice content. E. Primo, J. M. Sala and P. Asensi (*Rev. Agroquím. Tecnol. Aliment.*, 1964, **4**, 361—370).—The method is based on weighing the juice obtained by mechanical expression using rotary cones and further mechanical separation of juice and pulp in specially designed apparatus. The method is simple and reliable, and is suitable for comparing different varieties of oranges. Results for juice content obtained by conventional hand-operated procedures are invariably much too low. (10 references.) E. C. APLING.

Pectic substances and pectic enzymes of fresh and processed Montmorency cherries. K. A. Al-Delaimy (*Dissert. Abstr.*, 1964, **25**, 2198—2199).—The carbazole-colorimetric method was used for determining the pectic constituents in *Prunus cerasus*, L. var. Montmorency. The titrimetric method was used for measuring pectinesterase (PE) activity of fresh, frozen and irradiated cherries. The polygalacturonase (PG) activity was measured by titrating reducing groups or by colorimetry by Willaman and Davison's method. The chromotropic acid-colorimetric method was used for methanol content determination. The findings at different stages of maturity are recorded. Total pectin, water-sol. and water-insol. pectin contents of premature cherries were considerably higher than those of the mature and over-mature cherries. PE activity increased with maturity. No PG activity was detected in fresh or frozen cherries picked at various stages of maturity. Methanol content increased on storage reaching max. at the end of 12 months. F. C. SUTTON.

Effect of γ-irradiation on table grapes. E. C. Maxie, K. E. Nelson and C. F. Johnson (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 263—268).—γ-Irradiation (300 krads. per h. for 20—60 min.) of grapes seriously impaired their eating quality after storage and was relatively ineffective, compared with SO_2 treatment, in preventing decay during 3 months' cold storage. A. H. CORNFIELD.

Methods of moisture determination in dried grapes. F. Radler and M. Grncarevic (*Food Technol. Aust.*, 1964, **16**, 732—733, 735—737).—Methods available for determination of moisture in solids, viz., by vac. oven, different types of resistance meters, a capacitance meter and hygrometers, are discussed in respect of their application to dried fruits. Standard deviations and coeff. of variance were determined for moisture in sultanas. The indirect method by use of resistance meters gives results that are comparable to the official vac. oven method. (18 references.) E. M. J.

Refractometric dry solids as indicator of sugar content of papaya fruit. T. Murashige and Y. Abuzeid (*J. agric. Fd. Chem.*, 1964, **12**, 520—522).—For comparatively wide ranges (of ~ 7%) of difference in the refractometric dry solids (RDS) of the juice of *Carica papaya* L. the correlation between the RDS and the actual sugars (as glucose) was nearly perfect. Deductions of 2% from the RDS gave close approximations to the actual sugars %. For small ranges of RDS (as between fruits of different harvests) the coeff. was 0·61 only ($P = 0·95$) which, however, was considered satisfactory for control purposes. P. S. ARUP.

Changes in phenolic content in persimmons during ripening and processing. M. A. Joslyn and J. L. Goldstein (*J. agric. Fd. Chem.*, 1964, **12**, 511—520).—The phenolic substances extracted with MeOH followed by 50% MeOH were determined colorimetrically

by the Folin-Denis and the vanillin reagents. Decreases in the phenolic content were accompanied by decreases in organoleptic astringency and a shrinking and loss of fluidity of the tannin cells ; such decreases occurred through oxidation during air-drying or high-speed blending of the tissue, and also, despite additions of ascorbic acid or SO_2, when purees of the pulp were frozen. P. S. ARUP.

Quality of tomato varieties. III. Quality of some varieties for canning in the form of whole peeled tomatoes, juices and concentrates. L. Durán, P. Cuñat, J. Flores, J. Morell and S. Plaja (*Rev. Agroquím. Tecnol. Aliment.*, 1964, **4**, 351—360).—Quality evaluations of seven varieties in relation to both yield and quality of material are reported. The variety Roma was the most suitable for canning of whole tomatoes and the varieties Lampedusa, Roma and Pearson Improved were all of good quality for juice and concentrate manufacture. (21 references.) E. C. APLING.

Colour measurements of tomato products. R. A. Edwards and F. H. Reuter (*Food Technol. Aust.*, 1964, **16**, 694—695, 697, 699, 701).—Efforts to establish colour grades and quality standards of tomato products by objective techniques over the last 30 years are reviewed, including the work of Mackinney and Little (1962) and that of Kefford (1963) in which samples of canned tomato pulps representing different fruit samples and maturities were judged by visual ranking and spectral reflectance measurement. (35 references.) E. M. J.

Effect of pH adjustment and high-temperature short-time [HTST] processing on colour and pigment retention in spinach puree. S. M. Gupte and F. J. Francis (*Food Technol.*, 1964, **18**, No. 10, 141—144).—A combination of a HTST process, with a pH adjustment was found to maintain the green colour of spinach puree better than either method alone. Spinach puree adjusted to pH 8·5 with $MgCO_3$ and processed at 300° F to F_0=4·9 in glass thermal-death-time tubes, retained approx. 24% of the chlorophyll after 6 months at room temp. whereas a similar sample at a normal pH processed at 250° F retained only 6% after 6 months. Improvement in colour was caused primarily by decrease in degradation of chlorophyll *a*. E. M. J.

Preservation of the natural colour in processed sweetpotato products. II. Precooked frozen. M. W. Hoover (*Food Technol.*, 1964, **18**, No. 11, 135—138).—Of Na acid pyrophosphate (**I**), Na_4 pyrophosphate (**II**) and a 3 : 1 mixture of **I** and **II**, **I** was very effective in preventing the discoloration of frozen sweetpotatoes. When pieces were cooked in 25% sucrose solution prior to freezing and concn. of >0·5% of **I** were added to the sugar solution, the pieces acquired a slightly acid flavour. **II** was effective in preventing discoloration ; it produced off-flavour and sloughing of the outer tissue at levels of >0·02%. The 3 : 1 mixture caused some sloughing at concn. >0·6%. (13 references.) E. M. J.

Stability studies with cooked legume powders. I. Flavour-judging procedure. M. M. Boggs, H. J. Morris and D. W. Venstrom (*Food Technol.*, 1964, **18**, No. 10, 114—117).—In addition to data concerned with performance of individual judges, detailed procedures are described for conducting the flavour-judging tests (duo-trio tests comparing bean powder air-packed and stored at 32° with control, N_2-packed and stored at − 34°) and for analysing the panel results. Of a 56-member panel, the most sensitive (~19) found flavour change in the air-packed sample stored at 32° after 26 days ; the medium sensitive after 44 days ; and the least sensitive after 66 days. E. M. J.

Gas chromatography applied to the determination of squalene in vegetables. J. Brossard, S. Q. Alam and G. Mackinney (*Qualitas Plant.*, 1964, **11**, 403—406).—The method used is described and the concn. present in olive oil (150 mg./100 g.), olive tree leaves (4 mg./100 g.), lucerne (2 mg./100 mg.) and the leaves of elder, acanthus, lettuce and carrots (0·1/100 mg.). C. V.

Free amino-acids in English walnut (*Juglans regia*) kernels. L. B. Rockland and B. Nobe (*J. agric. Fd. Chem.*, 1964, **12**, 528—535).—An aq. extract of defatted walnuts was fractionated on a column of Dowex-50 by gradient elution with an NH_4 formate buffer. Paper chromatography of the eluates revealed the presence of 17 identifiable and six tentatively identifiable amino-acids as major constituents. Minor ninhydrin constituents (39) were chromatographically characterised. (29 references.) P. S. ARUP.

Non-alcoholic beverages

Detection of emulsifying agents in ingredients for alcohol-free beverages. H. Rother (*Riechstoffe u. aromen.*, 1964, **14**, 359—367).—Polyhydroxyethylene compounds can be detected in the oily matter extracted with $CHCl_3$ from the sample by a method depending on the evolution of acetaldehyde when the (prepared) matter is heated with H_3PO_4, or alternatively by a paper chromatographic procedure. A thin-layer chromatographic method is described for the detection of sucrose fatty acid esters in the same material. In prep. for the detection of mono- and diethanolamine or of fatty acid monoglycerides the oily material is saponified, and the fatty acids are removed by acidification and extraction with C_6H_6. The ethanolamines (<0·05%) can be detected in the (conc.) aq. phase by thin-layer chromatography, and glycerides by oxidation with HIO_4 and testing for formaldehyde with chromotrophic acid ; a paper chromatographic method is described for the detection of glycerol and other polyhydric alcohols. Monoglyceride (<0·01%) can be detected by these methods. (18 references.) P. S. ARUP.

Influence of different methods of preparation and storage conditions on changes in vitamin content of stabilised grape juice. J. Schneyder (*Mitt. Wein u. Obstbau, Wien*, 1964, **14A**, 282—298).—A lecture covering published data concerning vitamins in general and the vitamins of grape juice in particular. (61 references.) P. S. ARUP.

Apple juice volatiles. D. R. MacGregor, H. Sugisawa and J. S. Matthews (*J. Fd Sci.*, 1964, **29**, 448—455).—Material for gas chromatography was prepared by concentrating volatiles 10-fold from McIntosh apple juice. Volatile components tentatively, identified by i.r. spectra and retention results were : four aldehydes, one ketone, 11 alcohols, 10 esters and four fatty acids. Seven peaks were found but not identified. (16 references.) E. M. J.

Viscosity of orange juice concentrates. Effect of ultrasonic treatment and concentration. Z. Berk (*Food Technol.*, 1964, **18**, No. 11, 153—154).—Ultrasonic wave treatment (20,000 c.p.s.) of conc. orange juice causes marked reduction in η. The change is irreversible. Some disintegration of the suspended pulp particles occurs. When the treatment takes place in air, off-flavours are formed, but not in an atm. of N_2 or in vac. E. M. J.

Detection of adulterations in citrus juices. II. Identification of acids in orange varieties. J. Sánchez, J. Alberola and I. García (*Rev. Agroquím. Tecnol. Aliment.*, 1964, **4**, 371—374).—The non-volatile acids of fruits of the varieties Cadanera, Sanguina, Comuna and Valencia Late picked at two different stages of maturity were examined by the methods previously described (*ibid.*, 1963, **3**, 349). Free galacturonic acid was found in higher concn. in ripe fruits. The presence of lactic, adipic, isocitric and aconitic acids in orange juice is reported for the first time. E. C. APLING.

Citrus fruit solid concentrates. Salada Foods Ltd. (Inventor : S. A. Spross) (B.P. 942,004, 14.3.60).—The method of prep. of the concentrate comprises extracting the juice ; removing excess of pulp ; extracting from remaining juice both sol. and insol. fruit solids ; removing the larger insol. solids ; heat stabilising the juice, to sterilise it and inactivate naturally occurring pectin enzymes in the juice solids ; treating the latter juice with enzymes, to effect flocculation of remaining insol. solids, hydrolysis of the pectin and debittering of the juice ; then separating out flocculated solids ; and concentrating the filtered juice. F. R. BASFORD.

Marmalade base. Salada Foods Ltd. (Inventor: S. A. Spross) (B.P. 942,204, 23.3.60).—There is claimed a marmalade base product comprising clarified, stabilised, conc. citrus fruit solids from which the natural pectin has been eliminated and into which has been incorporated citrus pectin in an amount equal to that of the eliminated pectin, citric acid and $NaHCO_3$. F. R. BASFORD.

Tea, coffee, cocoa

Quality and flavour of Ceylon tea. R. L. Wickremasinghe and T. Swain (*J. Sci. Fd. Agric.*, 1965, **16**, 57—64).—Results of comparative qual. and quant. studies of the polyphenols, amino-acids and low-boiling volatile compounds in two specially prepared samples of Ceylon tea and those in 20 commercial samples of black tea, eight of which were manufactured from Ceylon-grown tea are presented. The correlation between polyphenol content and commercial valuation (quality), and the contribution of low-boiling volatiles to the flavour is discussed. *N*-Ethylasparagine was tentatively identified in tea leaf. (23 references.) E. M. J.

Precursors of chocolate aroma: [A] comparison of fermented and unfermented beans. T. A. Rohan. **[B] Flavonoids and phenolic acids.** T. A. Rohan and M. Connell (*J. Fd Sci.*, 1964, **29**, 456—459, 460—463).—[A] Aq. methanolic-sol. components of fermented and unfermented cocoa beans comprise amino-acids, sugars and flavonoids. The extract from fermented but not from unfermented cocoa produced chocolate aroma when heated. Probably the carbohydrate fraction (sucrose) in the unfermented cocoa is the precursor of glucose and fructose in the fermented material, flavonoid compounds the precursors of more complex substances and the insol.-protein fraction of the free amino-acids respectively. (10 references.)

[B] Flavonoids and phenolic acids were identified in the ethyl acetate-sol. fraction of the precursors of chocolate aroma. In addition to compounds identified two flavonols (quercetin and quercetrin) and three phenolic acids (*p*-coumaric, caffeic and chlorogenic) were found. The aq. methanolic sol. fraction of fermented cocoa beans gives chocolate aroma when heated. The quality of the aroma was not affected by ethyl acetate extraction of the concentrate prior to heating. (16 references.) E. M. J.

Determination of theobromine and calculation of fat-free cacao paste in chocolates. Examination of Pritzker and Jungkunz method; theobromine content of cacao beans and cacao pastes. H. Hadorn (*Mitt. Lebensm. Hyg. Bern*, 1964, **55**, 217—242).—Some details of the Pritzker and Jungkunz method (cf. *Swiss Food Book*, 5th. Edn.) have been modified in order to improve the accuracy and to avoid operating difficulties. Although MgO (fresh) was found in model experiments to absorb theobromine (**I**), this did not occur during analyses of cacao beans, pastes or chocolates. The alkaloids (**I** + caffeine) as finally extracted with $CHCl_3$ were often too impure to be determined by wt.; determination (as **I**) by means of the Kjeldahl method is recommended. The alkaloid content of the solids-not-fat of cacao beans and pastes (25 samples) was 2·45—3·51, the average (for the pastes) being 3·21%; the content of caffeine was 0·18—1·39%, and that of **I** 1·79—3·34%. The standard deviation of results for the total alkaloids (as **I**) calculated on 64 analyses of 25 samples, was ± 0·043. (13 references.) P. S. ARUP.

Milk, Dairy Products, Eggs

Influence of a change in farm dairy practice on the bacterial flora of fresh and stored raw milk. C. Higginbottom, S. M. Jones and M. M. Taylor (*J. appl. Bact.*, 1964, **27**, 385—391).—Steam sterilisation of dairy equipment was formerly used in Scotland; with the introduction of refrigerated bulk milk tanks, chemical sterilisation was permitted. This resulted in an increase in the % of pseudomonads in milks stored at 22, 15 and 5° and in the no. of Gram-negative rod cultures which were resistant to hypochlorite. (12 references.) C. V.

Analysis of milk by infra-red absorption. J. D. S. Goulden (*J. Dairy Res.*, 1964, **31**, 273—284).—The construction of an automatic i.r. milk analyser capable of determining the fat, protein, and lactose contents of milk from measurements of the intensities of their respective absorption peaks at 5·73, 6·46 and 9·6 μm. is described. (10 references.) M. O'LEARY.

Separation of milk protein on dextran gel. R. D. Hill and R. R. Hansen (*J. Dairy Res.*, 1964, **31**, 291—295).—Four fractions were separated from skim-milk on Sephadex G100 dextran gel and were further examined by starch gel electrophoresis. Three of the fractions corresponded to the casein micelle, lactoglobulin and lactalbumin and the fourth was a non-protein fraction. The usefulness of the gel filtration method for the systematic study of variations in the composition of milk protein is discussed. M. O'LEARY.

Isolation and physical-chemical characterisation of kappa-casein from cow's milk. H. E. Swaisgood (*Dissert. Abstr.*, 1964, **25**, 82).—For the isolation of κ-casein sedimentation-velocity and equilibrium ultracentrifugation, electrophoresis, η, and various chemical techniques were used and the physical properties of κ-casein were determined in strong dissociating solvents. Reduction of the disulphide bonds lowered mol. wt. to 28,000 in 5·0 M-guanidine hydrochloride and 67% AcOH–0·15 M-NaCl. Determination of the sulphydryl groups indicated 2—3 -SH groups per 28,000 g. It was concluded that the basic unit of κ-casein was composed of two 28,000 mol. wt. sub-units joined by disulphide bond(s). F. C. SUTTON.

Attempts to relate the selenium content of heat-treated milks with their nutritive properties. K. M. Henry (*J. Dairy Res.*, 1964, **31**, 239—245).—Analysis of the Se content of spray- and roller-dried skim-milks, evaporated milk, milk sterilised by the ultra high temp. process, and liquid skim-milk suggested that the Se content of heated milks is related to the severity of the heat treatment. Feeding trials with rats indicated that considerable differences exist within and between different strains in their susceptibility to liver necrosis and in their ability to utilise Se. (24 references.) M. O'LEARY.

Storage of chilled milk in relation to butter quality. A. K. R. McDowell (*J. Dairy Res.*, 1964, **31**, 247—251).—Storage tests showed that butter made from milk accumulated for 3 days in a refrigerated tank was of equal quality with butter from unchilled milk pasteurised and separated daily, when both milks were of good quality. When both milks were of inferior quality the butter from the chilled stored milk was superior to that from the unchilled milk used daily. (11 references.) M. O'LEARY.

Effect of acidity, salt and copper and iron contamination on the keeping quality of butter. A. K. R. McDowell (*J. Dairy Res.*, 1964, **31**, 221—232).—The addition of 0·02—0·03 p.p.m. of Cu to sour cream butters with pH values <6·0 and containing up to 1·5% salt resulted in the development of oily and fishy flavours during storage at 14°F. The addition of similar amounts to unsalted sour cream butter or to sour cream butter with pH values >6·0 had no effect on flavour. A Cu content >0·08 p.p.m. in sweet cream salted butter and >0·12 p.p.m. in sweet cream unsalted butter caused the development of oxidative defects during storage. The addition of 0·5 p.p.m. Fe resulted in the development of a metallic flavour in fresh butters. (25 references.) M. O'LEARY.

Fatty acid content of butters. C. Antoniani and A. Daghetta (*Qualitas Plant.*, 1964, **11**, 299—308).—Gas chromatography is used to examine a large number of Italian and imported butters. The max. and min. values obtained are: butyric acid, 2·7—6·11; capric acid, 1·26—3·86; caprylic acid, 0·85—1·93; caproic 1·46—3·97; lauric, 2·0—4·44; myristic, 8·05—13·97; myristoleic, 1·3—4·08; palmitic, 20·47—45·41; palmitoleic, 1·44—5·67; stearic, 4·83—12·91; oleic, 16·53—32·22; linoleic, 0·15—3·73 and linolenic acid trace to 4·64 %. (10 references.) C. V.

Manufacture of unwashed butter. M. Vedlich (*Prům. potravin*, 1964, **15**, 492—496).—Favourable results of pilot experiments aiming at obtaining high quality in butter prepared without washing are reported. A manufacturing technology directed to most uniform scattering of fine water particles is suggested, which on account of the low consumption of processing water is particularly suitable for dairies short in water supply. The product has full organoleptic quality with the characteristic taste due to pure lactic fermentation, which cannot be obtained in butter made of sweet cream. Although unwashed butter is designed in the first place for consumption while fresh, its storage ability is the same as that of the product prepared with washing. J. S. B.

Production of natural flavour in chemically acidified cream and skim milk. W. L. Hempenius and B. J. Liska (*J. Dairy Sci.*, 1964, **47**, 1099—1101).—A laboratory trial is described in which acceptable flavours were produced in cream and skim milk, acidified to pH 4·4 with glucono delta-lactone, by the citric-acid fermenting organism *Streptococcus citrovorus* after 12 to 14 days' incubation at 20°. M. O'LEARY.

Compound responsible for mushroom flavour in dairy products. W. Stark and D. A. Forss (*J. Dairy Res.*, 1964, **31**, 253—259).—The mushroom-like flavour of oxidised butter was shown to be due to the formation of oct-1-en-3-ol. This compound has a flavour threshold value of 1 part in 10^9 in water, 1 part in 10^8 in skim-milk, and 1 part in 10^8 in butterfat. (16 references.) M. O'LEARY.

Processing methods and the use of additives in sterilised milk concentrate. M. E. Seehafer (*Dissert. Abstr.*, 1964, **25**, 2436—2437).—Trials were made to develop a whole milk concentrate containing 36·5% total solids worthy of commercial evaluation. Emphasis was placed on initial product properties and the behaviour of product during storage. These evaluations consisted of tests for solubility index, sedimentation, apparent η and flavour. By combining the correct treatment combinations (e.g., to control η changes and sedimentation, etc., on storage) a sterilised milk concentrate was produced having commercial possibilities. F. C. SUTTON.

Measurement of protein stability in sterile concentrated milk. S. Nakai, H. K. Wilson and E. O. Herreid (*J. Dairy Sci.*, 1964, **47**, 1056—1061).—A method for determining the stability of milk proteins by measuring the optical densities of gold sol—protein solutions at 535 mμ is described. Using this technique, a loss of 60% in the stability to Ca^{2+} of conc. milk after treatment with rennet was detected. Conc. milk lost 30% of its stability on gelation during storage at 35°. (14 references.) M. O'LEARY.

Influence of κ-casein and β-lactoglobulin on the heat stability of skim-milk. H. Tessier and D. Rose (*J. Dairy Sci.*, 1964, **47**, 1047—1051).—A study of the heat stability of milks from individual cows at various pH values in the range 6·4—7.0 showed that one group (type A) had max. and min. stabilities, whereas a second group (type B) had a gradually increasing stability over the pH range. The heat stability responses of the milks could be reversed by the addition of κ-casein and β-lactoglobulin to types A and B respectively. The addition of κ-casein to type A milk which had been heated to 90° for 10 min. had no effect on its heat stability. The addition of either α_s- or β-casein did not affect the heat stability of either group of milks. (20 references.) M. O'LEARY.

Application of *Propionibacterium shermanii* for increasing the content of vitamin B_{12} in fermented milk. R. Karlinová (*Prům. potravin*, 1964, **15**, 529—531).—Fermented milk has bacteriostatic effect, due chiefly to lactic acid, and to a certain degree to the presence of antibiotics in low concn., regulating the intestinal bacterial flora. Kefir, especially owing to the presence of yeasts, can supply the vitamins of B-group. It can be enriched with vitamin B_{12} by introducing into milk 0·5% of peptone and *P. shermanii*, without affecting the taste and colour of the product. After an incubation period of 3 days the content of vitamin B_{12} is 2—3 times higher than the initial. In the tropics kefir can be prepared from dehydrated milk. (24 references.) J. S. B.

Bacteriological studies on milk and milk products. I. Resazurin test of raw milk. II. Abortus Bang Ringprobe of raw milk. S. Hamada, M. H. Jousif, I. H. Sherif, M. El-Hedik, A. Shebeita and H. M. El-Sawah (*J. Arab Vet. Med. Ass.*, 1964, **24**, 6—11, 12—16).—**I.** Samples of raw milk (buffalo 288, cow 212) were examined by the resazurin test. Results were 43·2% Grade A, 14·8% Grade B and 42% Grade C. From a collecting and cooling centre Grade C milk was 89·2%.

II. Five hundred samples of raw milk (buffalo 288, cow 212) were tested for brucellosis with antigen prepared from *Brucella abortus* strain 19. Suspected cases were six buffalo samples and six samples of the cows' milk. The tests with buffalo milk need further study. E. M. J.

Factors affecting the multiplication and survival of coagulase-positive staphylococci in Cheddar cheese. B. Reiter, B. G. Fewins, T. F. Fryer and M. E. Sharpe (*J. Dairy Res.*, 1964, **31**, 261—272).—Cheesemaking trials showed that growth of coagulase-positive staphylococci was more rapid in cheese made with phage-infected starter than with cheese made with normal starter. There was little decrease in the staphylococcal count of cheese of the former type even after 18 months' storage, whereas the count of normal cheese declined rapidly. The results of laboratory experiments suggested that the low survival rate of staphylococci in cheese made from milks heated at sublethal temperatures is due to a lag in the recovery of heat-shocked cells and their inability to multiply at the pH level of the cheese curd. (33 references.) M. O'Leary.

Comparison of media for counting and isolating the bacteria from Cheddar cheese. P. S. Robertson (*J. Dairy Res.*, 1964, **31**, 297—302).—Ten media were compared for their ability to yield max. counts with each of 23 types of bacteria common in Cheddar cheese. As none of the media tested gave max. counts with all strains it is suggested that the bacteriological examination of cheese be carried out using at least two complementary media of widely different composition. (13 references.) M. O'Leary.

To improve the quality of reconstituted milk cheese. II. Variables: acid, salt and ripening. I. I. Peters and J. D. Williams (*Food Technol.*, 1964, **18**, No. 10, 111—113).—The reconstituted milk before setting was treated with $CaCl_2$ (0·02%) (control) or lactic, citric acid or HCl to bring the milk to 0·225% titratable acidity (pH 6·4). This varied treatment gave only minor differences in curd character at cutting and milling time and no statistically significant differences in flavour and body scores of the ripened cheeses. Modified salting plays only a minor rôle in improving the quality. Cheeses that were ripened 6 months at 10° were superior in flavour and body to cheeses ripened 6 months at 16° or 3 months at 16° + 3 months at 10°. E. M. J.

Nitrogenous substances in brines used for ripening and storage of some Bulgarian cheeses. P. Prodanski (*Prům. potravin*, 1964, **15**, 497—502).—A comparative study of the interchange of N substances taking place between the cheese and the brines used in ripening and storage is presented. It relates to Bulgarian white sheep-milk, cow-milk, type 83-, and Chemus cheeses. During ripening of cheese in brine much of the N substance, mostly free amino-acids, is leached, whereby a considerable portion of valuable and digestive components gets lost, and the flavour is affected. In the composition of the amino-acids leached from the individual cheese samples by various brines no significant difference has been found. J. S. B.

Changes in concentrated milk during frozen storage. T. A. Nickerson (*J. Fd. Sci.*, 1964, **29**, 443—447).—One of the principal defects is a deterioration in physical stability. Agitation or cooling procedures that induce nucleation accelerate destabilisation, as does the presence of foreign nuclei. Whey protein components change little, but changes in caseins and whey fractions occur during frozen storage as shown by starch-gel electrophoresis. Results indicate that the mechanism of protein insolubilisation during frozen storage is more complex than previously suggested. (19 references.) E. M. J.

Indices of protein quality in dried soya-bean milks. J. P. van Buren, K. H. Stein-Kraus, L. R. Hackler, I. El Rawi, and D. B. Hand (*J. agric. Fd. Chem.*, 1964, **12**, 524—528).—The Learmonth test for the extent of destruction of the trypsin inhibitor affords a useful index of the adequacy of the heating of the beans or of water-extracted milks ; tests for urease activity or sol. N are of little value. The extent of overheating is best estimated by the Carpenter method for determining the available lysine (cf. *Anal. Abstr.*, 1961, **8**, 1717) : useful indications are also afforded by the Hunter L values and the degree of browning. (19 references.) P. S. Arup.

Influence of rapid cooling and storage conditions on shell egg quality. F. R. Tarver, jun., and R. E. Choate (*Food Technol.*, 1964, **18**, No. 10, 100—102).—Haugh units (HU) of large eggs immediately after rapid cooling to 27 ± 2°F were significantly higher than the HU of eggs before cooling. After storage for 7 days at 45°F the HU of rapidly and non-rapidly cooled eggs were similar to the HU of eggs before rapid cooling. Eggs once cooled should be maintained at a reduced temp. during storage. Storage temp. and days of storage had a greater influence on the decline of HU than method of cooling. (12 references.) E. M. J.

Relationships between selected physical characteristics and the resistance to shell failure of *Gallus domesticus* eggs. F. R. Frank, M. H. Swanson and R. E. Burger (*Poultry Sci.*, 1964, **43**, 1228—1235).—The resistance to breakage of the shell of the hen's egg, as determined by either a crushing or impact device, was highly correlated with shell thickness, sp. gr. of the intact egg, % egg as shell, and shell wt. Multiple regressions showed that no one variable was consistently superior as an indicator of shell strength. Only 60% of the total variation in shell strength was accounted for by all the factors measured. A. H. Cornfield.

Experimental pasteurisation of egg paste by γ-radiation. V. Jedlička, K. Otta, S. Matějová and A. Linhart (*Prům. potravin*, 1964, **15**, 528—529).—Several sorts of egg paste in consumer's paper and carton packing were irradiated with a ^{60}Co-source, each with 100, 250 and 500 krad, and then microbiologically tested for the micro-organisms (expressed in % of the initial count), and moisture content and acidity thereof determined. The physico-chemical examination did not show any difference from the non-irradiated controls, while microbiological tests showed a decrease of surviving micro-organisms, proportional to the irradiation dose, the decrease being substantial already at 100 krad, and at 500 krad practically all organisms were destroyed. In organoleptic tests the samples irradiated with 100 krad were undistinguishable from the non-irradiated controls, whereas higher irradiation doses proved unjustifiable in view of the proportional deterioration. J. S. B.

Effects of ultra-violet irradiation of egg liquids on *Salmonella* destruction and performance quality with emphasis on egg white. K. Ijichi, O. A. Hammerle, H. Lineweaver and L. Kline (*Food Technol.*, 1964, **18**, No. 10, 124—128).—Destruction of bacteria, e.g. *Salmonella*, by application of u.v. irradiation to thin films of egg liquids in a Centrifilmer is described. Bacterial counts decreased with increasing irradiation and increased with increasing rate of feed of the egg liquid. When the feed rate was 100 ml./min. and u.v. energy $7·22 \times 10^4$ ergs/cm²/sec., counts of *S. typhimurium* were reduced by a factor of 10^6 to 10^7. Heat-resistant *S. senftenberg* was killed at about the same rate. The degree of off flavour detected in unflavoured cakes made from egg whites exposed to intermediate intensities of irradiation was so slight that it might not preclude their use in flavoured angel food cakes. With irradiated whole liquid egg the degree of off flavour in scrambled eggs or layer cake was much more severe. (13 references.) E. M. J.

Milk-based foods. R. A. E. C. Paul (B.P. 942,109, 30.3.62. Fr., 4.4. 31.10, and 7.12.61, and 16.3.62).—A mixture of milk solids, sugar and water is heated without local heating (e.g., at 90—110°) to effect gelling of the milk proteins and form a food composition containing > 72% of water, with non-milk sugar (as sucrose), non-fat milk solids wt. ratio in the range of 0·24—1 : 1 (e.g., sugar + milk solids 32—48% of the composition). F. R. Basford.

Edible Oils and Fats

Value of the thiobarbituric number in the investigation of edible vegetable oils and of lard. F. Mihelić (*Kem. u Industr., Zagreb*, 1964, **13**, 682—684).—Determinations of thiobarbituric acid no. (TBA) (cf. J. Sedlaček, *Nahrung*, 1958, **2**, 658 ; R. Sinnhuber, *Food Technol.*, 1957, No. 12, 9 ; H. Schmidt, *Fette und Seifen*, 1959, **61**, 127 ; and K. Taufel, *Naturwissenschaften*, 1960, **47**, 135) in refined vegetable oils and in lard were carried out in parallel with determinations of the peroxide no., the acidity, and the epihydrin-

aldehyde no. The relation between the TBA no. and the peroxide no. was observed in refined soya-bean oil and in lard at 70° over 216 h. The effect of TETD (tetraethylthiuram disulphide) and PG (propyl gallate) additives on the quality of lard stored at room temp., its TBA and peroxide no. and other related factors was studied over 6 months. An increase in the TBA no. and peroxide value was parallel to the decrease in organoleptic qualities of edible oils. After storage for 6 months the TBA and peroxide no. of lard free of additives equalled 9·1 and 0·355 respectively, and of lard containing TETD 2·0 and 0·062, respectively. The TBA curves gave better picture of changes taking place in oils and fats, and the use of TBA no. determinations in the control of the freshness of fats was recommended. A. L. GROCHOWSKI.

Application of gas chromatography in the determination of purity of fats. E. P. Magré and F. D. Tollenaar (*Qualitas Plant.*, 1964, **11**, 286—297).—Twelve pure cocoa butters have been analysed together with several commercial samples and a substitute. The purity of lard is related to the ratio of pentadecanoic acid (C_{15}) to eicosanoic acid (C_{20}) in Bömer's glyceride. C. V.

Detection of aldulterant castor oil in other vegetable oils by thin-layer chromatography. G. Lakshminarnayana and V. V. S. Mani (*Indian J. Technol.*, 1964, **2**, 320).—A 1% solution of oil in $CHCl_3$ is spotted on to silica-gel plates, developed with 60 : 40 : 20 light petroleum : ether : AcOH and indicated with I_2 vapour or phosphomolybdic acid. Adulteration with 1% castor oil can be detected. E. C. DOLTON.

Use of gas chromatography in the examination of Italian olive oils. A. Montfredine and L. Laporta (*Qualitas Plant.*, 1964, **11**, 309—315).—Raw oil, 245 samples from 35 provinces, was examined. The findings are tabulated showing the individual fatty acid content, solidifying point, etc., for the oils from the different areas. C. V.

Infra-red examination of the fractions obtained by gas chromatographic separation of the fatty acid methyl esters. F. de Francesco, M. Ghirardoni and D. Avancini (*Qualitas Plant.*, 1964, **11**, 316—328).—The difficulties associated with identifying the components of a mixture which have been separated by gas chromatography have been overcome by i.r. spectrographic techniques. The identification of oleic acid (**I**) and its isomer elaidic acid is specially studied and the preliminary results obtained are discussed. A *trans*-isomer of **I** (2%), is reported. C. V.

Glyceride structure and fat digestibility. R. H. Davis and D. Lewis (*Proc. Nutr. Soc.*, 1964, **23**, xxx—xxxi).—The position of the fatty acid within the glyceride mol. may influence the extent to which it is absorbed. Palmitic and (**I**) stearic acids forming the major portion of the available fats, the optimum conditions for their best utilisation are of special importance. The fats used were natural lard in which **I** was predominantly β-esterified, interesterified lard with a completely random distribution of fatty acids and a beef tallow where the saturated acids were predominantly α-esterified. Glyceride structure influenced the digestibility of the fat, the bulk of the undigested food fat being voided as free acids. Lard was better digested than interesterified lard and both were better digested than tallow, the actual values recorded being 83, 80 and 71% respectively C. V.

Interpretation of fluorescence spectra of vegetable oils. L. Jung and P. Morand (*Ann. Falsif., Paris* 1964, **57**, 17—25).—Investigations were made into the nature of the peaks in the fluorescence spectra (Woods light) of various crude and refined oils. The chief substances contributing to the peaks are : carotenoids, chlorophyll, pyrene, benzo-3,4,pyrene and benzo-1,2,pyrene. Certain carotenoids and chlorophyll are removed during the refining of olive oil. J. V. RUSSO.

Meat and Poultry

[A] Release of aqueous extracts by beef homogenates, and factors affecting release volume. [B] Beef microbial quality determined by extract-release volume (ERV). J. M. Jay (*Food Technol.*, 1964, **18**, No. 10, 129—132, 133—137).—[A] Homogenates of fresh beef allowed to filter through filter paper released relatively large vol. of extract in a given period ; spoiled and protease-treated beef homogenates released less or none. The max. amount of extract released in the extract release vol. prodecure is ~80 ml. (after 1—2 h.) when 100 ml. of distilled water are added to 25 g. of meat. The phenomenon appears to resemble that of water-holding capacity in beef.

[B] The ERV of beef decreases in a straight line manner as beef undergoes spoilage and values become 0 when putrefaction has set in. Decrease in ERV is accompanied by an increase in bacterial no. Ninhydrin-positive substances increased with bacterial no. up to a point then fell. Lowered ERV values were not the direct result of large no. of bacterial cells *per se*. Chlortetracycline (10 p.p.m.) slowed the reduction of ERV below that in controls, in which ERV fell sharply. Phosphate buffer at pH 5·8 and 30° are suggested as the extractive and test temp. Beef with ERV values <30 was considered spoiled. Only three of 40 samples, when fresh, failed to produce ERV of 30 and above. The average no. was 40 with an approx. bacterial no. of log 6·7. The degree of correlation between ERV and bacterial no. was very high. E. M. J.

Effects of soaking in water, thermal enzyme inactivation and irradiation on the textural factors of beef. A. A. El-Badawi, A. F. Anglemier and R. F. Cain (*Food Technol.*, 1964, **18**, No. 11, 149—152).—Beef soaked in distilled water for 72 h. prior to heat inactivation of the enzymes and irradiation (**I**) 4·5 Mrads was much firmer in texture than unsoaked samples ; beef heated to 160°F prior to **I**, was firmer also. **I** tends to reverse the effects of soaking and heating. Effects on the proteins and the possibility of using the Zn content of the meat juice as a measure of the solubility of meat proteins are discussed. (13 references.) E. M. J.

Factors that influence tenderness of beef and the development of suitable methods for appraising tenderness. C. H. Adams (*Dissert. Abstr.*, 1964, **25**, 2435—2436).—The Warner-Bratzler shear force, grinder energy and the Carver tenderness press were the three objective methods used for measuring tenderness of beef. Secondly, maturity measurements were devised to determine whether they could be used to estimate tenderness, and thirdly, different live animal and carcass measurements which might indicate tenderness were obtained. From results collected a paternal half-sib heritability estimate of 0·51 for shear force was determined, indicating that tenderness of beef must be a fairly heritable characteristic. F. C. SUTTON.

Correlation of meat and milk traits in dairy cattle. C. J. R. Nichols and J. M. White (*J. Dairy Sci.*, 1964, **47**, 1149—1155).—The literature on the genetic relationship between milk production and beef production in dairy cattle is reviewed. (43 references.) M. O'LEARY.

Dairy beef in the packing industry. W. R. Marquart (*J. Dairy Sci.*, 1964, **47**, 1145—1149).—The types of dairy cows and steers suitable for use in the United States manufacturing industry are discussed. M. O'LEARY.

Eating quality of lamb. I. Effect of age. II. Effect of pre-slaughter nutrition. III. Overall comparisons and interrelationships. P. C. Paul, J. Torten and G. M. Spurlock (*Food Technol.*, 1964, **18**, No. 11, 121—124, 125—127, 127—130).—**I.** New-crop lambs (**I**), 5½ months old, and old-crop lambs (**II**), 11—12 months old, were used. The cuts from **II** generally weighed more, contained a higher % of lean, a lower % of bone and had higher cooking losses than had cuts from **I**. There was considerable difference among the cuts in yield of lean, fat, bone and waste, in cooking losses, and in amount of moisture and ether extract in the muscles. In general, there was much less difference between the cuts from the choice and good-grade carcasses than between choice or good and utility. Choice-grade cuts were generally highest in separable fat. Detailed analyses are given. (14 references.)

II. Differences attributable to feedlot vs. pasture feeding were not very marked and did not present clear-cut suggestion of superiority for one feeding method over the other. Differences were somewhat more definite between the two grades (choice or prime) than between the two types of feeding. The choice-grade cuts contained more fat and less bone, had higher scores for certain palatability factors and had less moisture and more ether-extractable material in the lean.

III. When evaluating the above results, certain types of information were considered as a total according to three grading systems : live-, federal carcass-, and laboratory grades based on firmness of cuts and amount and distribution of external and internal fat. The federal carcass grades were better than the laboratory grades for ordering the raw marbling scores. The live wt. of the animals, yield of chilled carcass, and yield of edible cuts decreased with grade. The thawing drip from the frozen cuts was quite small and showed no relation to % moisture or ether extract of the lean tissue. Tenderness scores and the force required to shear had the highest correlation coeff. in the chops and lowest in the legs. E. M. J.

Transparent hot-melt coating material for prolonging storage life of frozen pork chops. J. C. Ayres (*Food Technol.*, 1964, **18**, No. 10, 117—120).—The coating material is heat-fused cellulose acetate butyrate (35%) with acetylated monoglycerides (65%) (cf. U.S. Pat. 3,000,748, Lepak). Each chop was weighed then quickly immersed in the hot solution and instantly withdrawn. Temp. tried ranged from 180 to 220°. The chops were returned immediately to the freezer and stored up to 150 days. Taste panel analysis of the fried meat for flavour, appearance and texture

showed no differences between dip-coated or Al foil-wrapped chops frozen for 5 months and fresh frozen chops. E. M. J.

Effect of internal temperature, pumping and antibiotics on spoilage of hams given a dry salt cure. R. F. Kelly (*Food Technol.*, 1964, **18,** No. 10, 103—107).—Hams chilled to an internal temp. of 1° had less spoilage than those chilled to 8 or 16° or not chilled (38—40°) prior to curing. Hams soaked in an antibiotic solution for 1 h. before being placed in cure had significantly less spoilage than had the controls, antibiotic infused or pickle-pumped lots. The hams cooled to 1° before curing contained the highest levels of salt. No residual antibiotic was found in hams stored for 125 days. (23 references.) E. M. J.

Assessing gain in the lean of hams. L. Kamm, J. C. Bartlet and D. Morison Smith (*Food Technol.*, 1964, **18,** No. 10, 144—148).—The unknown wt. of the uncured leg of pork may be estimated from analysis of the finished product, protein on a fat-free basis being the most suitable criterion. An equation was derived relating % protein to change in wt. of lean on curing. The equation gave a better estimate of the change in wt. of the lean than estimates determined by the moisture–protein ratio. (16 references.) E. M. J.

Structure of collagen. J. Rosmus and Z. Deyl (*Prům. potravin*, 1964, **15,** 598—601).—The existing state of knowledge on the structure of collagen, involving the problems of the primary structure, amino-acid sequence, the secondary, tertiary, and fourth-order structure, and the stability of collagen, is reviewed. (60 references.) J. S. B.

Effect of emulsifiers on the stability of sausage emulsions. J. A. Meyer, W. L. Brown, N. E. Giltner and J. R. Guinn (*Food Technol.*, 1964, **18,** No. 11, 138—140).—Eight commercial food emulsifiers (additions of 0·1, 1·0 or 3·0%), lecithin or oleic acid, were examined with regard to stability of sausage emulsion. The type of fat (beef or pork) of the raw material markedly affected the response of a specific emulsifier. Lecithin did not improve emulsion stability and imparted an off flavour to the finished product. None of the emulsifiers used were effective except oleic acid. E. M. J.

Presence of *Staphylococcus aureus* in meat processing plants. J. Zlámalová (*Prům. potravin*, 1964, **15,** 522—525).—Results of microbiological tests for the presence of *S. aureus* in meat and meat products during the various stages of the processing technology at several industrial plants are reported. Fresh and cured meat, sausage filling, and ready sausages were checked, and samples of the surface films from the equipment. *S. aureus* occured on average in 10% of all the tests, in cured meat in 30% and in filling in 22% of cases. By means of the plasma coagulase test no active strain could be detected. Existing methods do not provide sufficient information for deciding whether the source of contamination is in the plant equipment, or in the meat material. J. S. B.

Flavour studies of irradiation-sterilised chicken. H. L. Hanson, M. J. Brushway and H. Lineweaver (*Food Technol.*, 1964, **18,** No. 11, 141—146).—The tests were made on vac.- or N_2-packed chicken irradiated with 4·5—4·6 Mrads and stored at 21 or 38°. Odour and flavour induced in chicken by 0·1 Mrad of irradiation at ambient temp. were readily detected by the panel. Deep fat frying usually reduced unpleasant odour and flavour more than cooking methods without fat. When samples were irradiated at —20° or lower, very slight unpleasant odour and flavour occurred. A combination process, viz., inactivation of enzymes prior to irradiation, irradiation at sub-freezing temp., packing with C, and deep-fat frying should give irradiation-sterilised chicken with relatively few flavour disadvantages. (26 references.) E. M. J.

Effect of polyphosphates on oxidative deterioration of commercially cooked fryer chickens. J. E. Thomson (*Food Technol.*, 1964, **18,** No. 11, 147—148).—Commercially produced frozen cooked chicken, which after evisceration and chilling, had been treated with polyphosphates (Na tripolyphosphate + Na pyrophosphates, 50 lb. in 500 gal.) and held for 1 week at 40°F had very slight off-odour and a 2-thiobarbituric acid (TBA) value of ~1. Untreated control chicken had a slightly-strong to medium-strong off-odour and a TBA value of ~6. (10 references.) E. M. J.

Food products. Unilever Ltd. (Inventor: S. Zwart) (B.P. 944,278, 7.3.60).—There is claimed a method for rapidly preparing packed, cured and matured meat slices, wherein meat is sliced, curing salt in solid particulate form is applied to the surface of each slice, the slices are then packed in air-impermeable moisture-proof containers and sealed therein in absence of free O_2 and are then allowed to mature *in situ*. F. R. Basford.

Fish

Better-quality salt-cured and sun-dried mackerel ***(Rastrelliger canagurta).*** D. P. Sen and N. L. Lahiry (*Food Technol.*, 1964, **18,** No. 10, 107—110).—A mixture comprising NaCl, Na benzoate, Na acid phosphate, $NaHCO_3$, Na hexametaphosphate and ascorbic acid was formulated for dry-curing and sun-drying of mackerel. The fish were afterwards given a dip-treatment in K sorbate solution and dried. Keeping quality was improved from ~2 months to ~7 months at room temp. in South-East Asia. (22 references.) E. M. J.

Thermal conductivity of some freeze-dried fish. G. Lusk, M. Karel and S. A. Goldblith (*Food Technol.*, 1964, **18,** No. 10, 121—124).—Thermal conductivities of freeze-dried salmon steaks, slabs of haddock and ocean perch were determined under quasi-steady-state conditions. For the last two named, results were in the range 0·011 to 0·020 BTU/ft./h./°F; for salmon they were less consistent. The conductivities observed under actual drying conditions were in good agreement with those reported for freeze-dried tissues. E. M. J.

Spices, Flavours, etc.

Colouring matters

Presence of several phenolic components in fruit proanthocyanidins. S. Ito and M. A. Joslyn (*Nature, Lond.*, 1964, **204,** 475—476).—Apple, carob, grape and persimmon proanthocyanidins were hydrolysed with 0·1N-HCl at 100° and the hydrolysates analysed by two-dimensional paper chromatography. The no. of phenolic compounds identified varied from two for apple to eight or more for carob and grape. (11 references.) S. A. Brooks.

Preservatives

Fractionation and identification of some compounds in wood smoke. R. W. Porter (*Dissert. Abstr.*, 1964, **25,** 401).—The steam-volatile and non-volatile phenols, acids and carbonyls are determined in whole smoke and condensates from wood heated at temp. ranges 350—550°. Smoke flavour was assessed by a taste panel using bacon and cheese. The total production of phenols was greatest in smokes generated at 500°, that of total acids at 450° and of carbonyls at 550°. Aliphatic monocarboxylic acids of C_1-C_{10} (largely C_1—C_4) occurred in the steam-volatile portion of whole smoke. The principal monocarbonyls included 2-pentanone, valeraldehyde, 2-butanone, butanal, acetone, propanal and croton- and acet-aldehydes. No 3,4-benzopyrene was detected. A. G. Pollard.

Pesticides in Food

[A] Malathion residues on fruit treated by dipping. P. Koivistoinen, A. Karinpää, M. Könönen and P. Roine. **[B] Disappearance rates of malathion residues as affected by previous treatments with Paraoxon, parathion and malathion.** P. Koivistoinen, A. Karinpää, and M. Könönen. **[C] Stability of malathion residues in food processing and storage.** P. Koivistoinen, M. Könönen, A. Karinpää and P. Roine (*J. agric. Fd. Chem.*, 1964, **12,** 155—555, 555—557, 557—560.—[A] Residues on intact fruits that had been dipped after harvesting in a water emulsion or suspension of malathion disappeared rapidly during storage; thus ~80% disappearance was observed on strawberries after 2 days and on gooseberries after 1 week at 10 or 20°; the rates were somewhat slower at 4°. The observed disappearance rates were practically the same as those previously observed on the growing fruits.

[B] Paraoxon applied by dipping harvested intact strawberries or tomatoes in suspensions or emulsions appreciably retarded the disappearance of malathion similarly applied ~6 h. later. The retardation effects were much less pronounced than those previously observed in experiments with macerated fruits. Applications of parathion or malathion to beans or spinach in the field did not retard the disappearance of the malathion from the spinach leaves on the plants or from the harvested beans during storage. The effect of paraoxon is probably due to the inhibition of carboxy-esterases.

[C] Malathion was applied to fruits and string beans by the dipping method shortly before processing. The approx. losses from fruits during processing were as follows: canning and jam-making 50—60%; cooking for 15—20 min. 30—50%; juice-making by pressing or steaming 70—90%; drying at 75° for 1—2 days 90—100%; freezing 40—50%. Appreciable losses occurred from fruits kept overnight at 4° before jam-making. Losses from string beans during salting, canning and storage were 90—99%. The amounts of malathion removed by washing varied widely according to the kind of fruit; emulsion applications were more resistant to washing than suspension applications. P. S. Arup.

Detection and determination of some chlorinated pesticide residues in crops, soils and animal tissues by gas-liquid chromatography. R. Goulden (*Qualitas Plant.*, 1964, **11**, 381—402).—This rapid method using electron capture ionisation detection is outlined. Only conventional G.L.C. equipment is required and neither 'clean-up' nor concn. of the extract solution of the pesticide is, in general, necessary. In most cases the procedure is applicable to chlorinated pesticide residues in concn. down to 0·1—0·25 p.p.m. and the sensitivity can be further increased by adjustment of sample vol., the operating conditions and/or the introduction of an extract 'clean-up' stage. Interference present in four extracts can be resolved by the use of a polar G.L.C. column or removed by liquid-solid chromatography. The standard procedure requires 50 min. for a single analysis but only 30 min. when a series is being carried out. (13 references.) C. V.

Adventitious toxic factors in protein concentrates. L. Friedman (*Food Technol.*, 1964, **18**, No. 10, 49—50, 55—59).—These factors comprise any substance in plant or animal raw material that is not a genetically determined material or any substance that gains entrance into the final product from source to consumer. In the review the following are covered: source of toxins, toxins from processing, chlorinated naphthalenes, natural toxins, weed contaminants, fungus toxins, trout hepatoma, sanitation, assessing toxicity and problems. (54 references.) E. M. J.

Insecticides applied to pests of olives and their residual toxicity. G. Baluja Marcos (*Rev. Agroquím. Tecnol. Aliment.*, 1964, **4**, 330—335).—Available data on toxicity and residues persisting in the oil from treated olives are discussed. Far lower residual toxicity in the oil results from treatments with P insecticides, particularly Rogor, than with chlorinated insecticides. Field results from a no. of countries (reported to FAO in 1960) also showed best control of the *Dacus* fly by treatment with Rogor. E. C. APLING.

Food Processing, Refrigeration

Dehydration of food. G. Evans (*Chem. & Ind.*, 1965, 173—179).—A general review in which the selection of the appropriate method is specially stressed. The initial cost (is the product seasonal?), versatility, accessibility for cleaning, uniformity in drying, a reasonably high degree of efficiency and the possibility of continuous operation, are all considered. (22 references.) C. V.

Development of formulas for calculating the theoretical temperature history and sterilising value in a cylindrical can of thermally conductive food during heat processing. K. Hayakawa (*Dissert. Abstr.*, 1964, **25**, 2436).—A mathematical expression for the sterilising value of the cooling phase based on the theoretical cooling curve was obtained. The theoretical curve gave satisfactorily close agreement to the experimental curve, the determination of which is described. F. C. SUTTON.

Novel uses of fluidised beds in chemical processing. W. M. Goldberger and H. Nack (*Battelle Tech. Rev.*, 1964, **13**, No. 11, 3—9).—The versatility of this process enables it to be applied to virtually all chemical industries. The general principles are outlined in relation to freezing, high and ultra-high temp. Technology and the operating conditions are outlined over a wide range (—70 to 350°F, gas velocity, coating time, thickness, etc.). The application of this technique to the cooking of food is specially examined in relation to fish, nuts, meat and vegetables. Bed materials are discussed, air being the ideal fluidising gas; smoking can also be carried out at this point. (11 references.) C. V.

Quality changes of vegetable and fruit during freeze-drying. W. Spiess (*Kältetechnik*, 1964, **16**, 349—358).—After a discussion of the thermodynamic principles, freeze-drying experiments on a no. of vegetables and fruits are described and the factors influencing quality are studied. Type and ripeness, degree of comminution, blanching and freezing rates are considered and optimal conditions are given. Most suitable for freeze-drying are beans, cauliflower, peas, peppers, asparagus and spinach. Soft fruit and berries are less suitable. M. SULZBACHER.

Electron microscopical changes in the meat of fish and warm-blooded animals during chilling and freezing. W. Partmann (*Kältetechnik*, 1964, **16**, 341—345).—After a description of muscle structure and cross-striated muscle fibres, the post-mortem changes of the fibres and the alterations of structure during chilling and freezing of meat and during cold storage are illustrated by a number of photographs obtained with electron microscopy. (12 references.) M. SULZBACHER.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Evaluation of popular nutritional knowledge. J. C. McKenzie (*Chem. Ind.*, 1965, Jan. 23, 152—156).—A general discussion. (10 references.) C. V.

Microbiological production of proteins using the residues from the production of industrial alcohol from cane molasses as raw material. (cf. *ibid.*, 1963, May, June, Oct. and Nov.; 1964, Mar.). O. Gonçalves de Lima (*Rev. Quím., industr., Rio de J.*, 1964, **33**, 17—19).—In continuation of earlier work observations are made on the composition of available raw material. A summary of results of laboratory (6 l.) scale production of *Candida utilis* H-141, and discussion of possible utilisation of more thermotolerant organisms (e.g., thermotolerant strains of *C. tropicalis*, *C. utilis* or *Torulopsis inconspicua*) are presented. E. C. APLING.

Urinary sulphur as a measure of the protein value of diets. D. S. Miller and P. Mumford (*Proc. Nutr. Soc.*, 1964, **23**, xliv).—Further work on that of Miller and Donoso (cf. *J.S.F.A. Abstr.* 1963, ii, 107) is reported. Urine collections over 24 h., rather than casual samples, are necessary since post prandial excretion of N and S rises to a max.; creatinine is independent of food intake. Urinary N minus endogenous N is taken as the index of the protein consumed. Only adults were used and it was assumed that there was an adequate intake of calories for protein synthesis but if the method is applicable to all physiological groups it could be advantageously employed for dietary surveys. The adequacy of the diet with respect to calories can possibly be judged by the level of ketone bodies in the urine. C. V.

All-vegetable protein mixtures for human feeding. XIII. Effect of cooking mixtures containing cottonseed flour on free gossypol content. R. Bressani, L. G. Elias, R. Jarquín and J. E. Braham (*Food Technol.*, 1964, **18**, No. 10, 95—99).—The effects of moist heat on INCAP Vegetable Mixture 9 were tested; changes in gossypol, lysine, thiamine and riboflavin were measured. Biological tests of protein quality were made in dogs and pigs. Protein value was not decreased. Free gossypol levels were decreased by moist heat; $CaCO_3$ and more so by $Ca(OH)_2$; also by $FeSO_4$ + $Ca(OH)_2$; and sugar had an effect in the inactivation of free gossypol (24 references.) E. M. J.

Effect of vitamin A degradation products on the determination of α-tocopherol [vitamin E] by the Furter-Meyer method. B. Uhlik (*Kem. u Industr., Zagreb*, 1964, **13**, 685—687).—An earlier report (*ibid*, **13**, 336—339) on the interference of vitamin A in colorimetric determinations of vitamin E in veterinary prep. containing vitamins A, D_2, and E, is confirmed and it is now stated that amounts of vitamin E below 700 i.u. per 25-ml. sample solution yield enough degradation products absorbing the 470 mμ wavelength to affect the result. The sum extinctions of the vitamin A and vitamin E samples oxidised separately was greater than the extinction of a combined vitamin A + E sample, indicating a reaction between the two oxidation products. Determinations by the extinction difference are therefore useless, but the proposed modification (*loc. cit.*) of adding to the standard vitamin E solution before its saponification an amount of vitamin A approx. equal to that in the samples gives results representing 97—99% of actual vitamin E content. A. L. GROCHOWSKI.

Riboflavin tetrabutyrate. K. Yagi (B.P. 943,078, 18.9.62. Japan, 18.10.61).—Aq. $HClO_4$ (60%) is added at room temp. to a mixture of riboflavin and butyric anhydride whilst stirring vigorously, then the product is poured into water. The orange layer is concentrated *in vacuo*, and the residue is recrystallised from ether, to give *riboflavin tetrabutyrate* m.p. 145—147°. The ester is fat-sol., tasteless and odourless, and has vitamin B_2 activity, making it useful for food enrichment and pharmaceutical prep. F. R. BASFORD.

Unclassified

Four principal problems of food technology. D. J. Tilgner (*Chim. et Industr.*, 1964, **92**, 395—404).—The survey covers requirements for adequate nutrition, methods of food preservation such as curing, smoking, dehydration, canning, irradiation and use of antibiotics, the nutritive values of food, factors influencing them, storage losses and additives, the organoleptic qualities and flavouring substances. (15 references.) M. SULZBACHER.

Gas chromatography applied to the analysis of foodstuffs. R. Kohn (*Qualitas Plant.*, 1964, **11**, 150—168).—A literature review. (264 references.) C. V.

Colorimetric determination of copper in some food products by tetraethylthiuram disulphide (Dikupral). H. Nozozámská and J. Zyka (*Prům. potravin*, 1964, **15**, 520—521).—The colorimetric method for determining traces of Cu by the Dikupral reagent,

developed by Michal and Zýka (*Chem. listy*, 1954, **49**, 915, 1043, 1338) was improved and sensitivity increased by introducing a shaking operation, in which the resulting complex compound is treated with ether. The modified method is suitable for determining Cu in various liquid food products, such as wine, cider, syrup, jam, etc. The determination is not affected by the possible presence of other metal ions in the sample. J. S. B.

3.—SANITATION, WATER, etc.

Synthesis and toxicity to houseflies of *ortho*-substituted analogues of DDT. J. J. Hartigan (*Dissert. Abstr.*, 1964, **24**, 3981—3982).—The introduction of an *o*-halogen into the DDT mol. has less effect on lethality to the housefly *Musca domestica* than the removal of a *p*-halogen. In the prep. of various DDT analogues various α-trichloromethylbenzyl alcohols were used. Aldehydes with $CHCl_3$ and KOH, in which the base was dissolved in ethylene glycol ether, gave high yields of these alcohols, which were condensed with H_2SO_4 with suitably chlorinated and/or methylated benzenes to obtain the desired products. F. C. SUTTON.

Action of heat on pyrethrum extract: isomerisation of pyrethrins to isopyrethrins. A. A. Goldberg, S. Head and P. Johnston (*J. Sci. Fd Agric.*, 1965, **16**, 43—51).—The biological activity of pyrethrum extract, after heat treatment, is a function of the ratio of the optical density of the extract at 2700Å to that at 2300Å. This ratio is a measure of the isopyrethrin content of the extract, and if the ratio is $\geq 0{\cdot}095$, the heat treatment has caused no damage. This information is of significance in the problems encountered in the distillation of pyrethrum extract and in the design of thermal fogging machines for dispensing pyrethrum mists. In normal unheated extracts the ratio of the optical densities at 2700Å and 2300Å is 0·08 ; in an extract completely isomerised to isopyrethrins the ratio is 0·62 and this extract has 0·5 of the lethal but only 0·25 of the knockdown activity of normal extract on houseflies. (18 references.) E. M. J.

I. Determination of radioactive niobium in water and foods. II. Determination of radioactive ruthenium in water and sewage. R. F. Skoniecczny (*Dissert. Abstr.*, 1964, **25**, 61).—Radioactive fallout in drinking water, sewage and in animal and plant tissues and the need for special analytical techniques to suit various types of samples are discussed. Nb can be precipitated as Nb_2O_5 from 4 l. samples of both tap and hard water. For estimation of Ru oxidation to its max. state with $KMnO_4$ and then reduction with hydroxylamine hydrochloride was adopted. The pptd. Ru is redissolved in acid and the conventional still for radioelements was then usable. F. C. SUTTON.

Fate of 2,4-D and ester derivatives in natural surface waters. O. M. Aly and S. D. Faust (*J. agric. Fd. Chem.*, 1964, **12**, 541—546).—In laboratory experiments the amounts of 2,4-D (Na salt or esters) adsorbed on clays were insignificant. The solubilities of the Ca and Mg salts of 2,4-D are too high for removal by pptn. Decomposition in aq. solutions by u.v. irradiation was more rapid at pH 9 than at pH 4. In aerobically incubated lake waters 2,4-D persisted up to 120 days. In lake mud suspensions 81—85% decomposition (via 2,4-dichlorophenol) occurred within 24 h. provided that the bacterial flora had previously been acclimatised to 2,4-D. (19 references.) P. S. ARUP.

Unsaturated alcohols. F. Hoffmann—La Roche & Co. A.-G. (B.P. 960,756, 20.12.62. Switz., 12.12.61).—The products claimed have pleasant fragrance (perfumes) and are of value in arresting the development of insects (*Tenebrio molitor, Calliphora erythrocephala, Galleria mellonella*). Specifically mentioned are **3,7,11-*trimethyl*-*trideca*-2,6,10-*trien*-1-*ol*** and **-*dodeca*-2,6-*dien*-1-*ol*** and **3,7,10,11-*tetramethyldodeca*-2,6,10-*trien*-1-*ol***. H. S. R.

Water wastes and sewage

Cultivation of green plankton algae on sewage. G. G. Vinberg (*Mikrobiologiya*, 1964, **33**, 508—515).—Three cultures, *Chlorella vulgaris, Scenedesmus quadricauda* and *Sc. obliquus*, cultivated initially on sewage, were introduced into town sewage filtered through cotton wool, and incubated in sealed vessels at 18—24°, with illumination for 12 h. a day at 3500—4000 lux. A similar series was carried out with air passed through the liquid for 12 h. a day. On the samples at intervals were determined bichromate loss of O_2, ammoniacal N, pH, content of sol. O_2, amount of saprophytic bacteria and titre of intestinal bacilli. Rapid growth of algae began in the initial anaerobic self-purification stage. The duration of this stage was considerably reduced by photosynthetic aeration due to bacterial oxidative mineralisation of dissolved and suspended org. matter. Growth of algae was accompanied by decrease of alkalinity, ammoniacal N and mineral P. The initial mineral forms of N, P and C were soon exhausted, and growth was supported by these elements previously forming part of the sewage org. matter, and released by bacterial mineralisation. Growth of algae was limited mainly by exhaustion of C, and aeration resulted in an increased yield. In aerated cultures, the yield of ash-free material of cells averaged 640 mg./l., whilst in closed vessels the yield was 268 mg./l., other conditions being equal.

A. S. LEVESLEY.

4.—APPARATUS AND UNCLASSIFIED

Description and mode of use of a photoelectric apparatus to measure vegetable surfaces. B. Bonzon (*Fruits, Paris*, 1964, **19**, 577—581).—The apparatus described and illustrated is based on earlier types by Rabechault at Bondy, and Voicey and Mason (*Canad. J. Plant Sci.*, 1963, **43**, 247). If converging light rays are such that the intensity at all points of a transverse section is constant, the quantity of light intercepted by a flat object (in regard to the angle of incidence) in the transverse section is proportional to the projected surface of the flat object on the transverse section. The method has been used to measure foliar and radicle surfaces in the pineapple. Precision is always >2% of the measured surfaces. (15 references.) E. M. J.

Faster analysis for lead-210 in biological specimens. A. L. Aronson and T. B. Hammond (*Nucleonics*, 1964, **22**, No. 2, 90—92).—In the development of a more rapid method for detecting base quantities of Pb it has been established that ^{210}Pb activity in biological specimens can be evaluated prior to secular equilibrium with ^{210}Bi by separating the two using a dithizone extraction at pH 3·8—3·9 followed by holding the specimens at least 11 days before counting. ^{210}Bi arising from the decay of ^{210}Pb can thus be distinguished from ^{210}Bi present in tissue at the time of procurement from the animal. In this way approximately three weeks is saved, recovery of ^{210}Pb from biological material is quant. regardless of the tissue involved when greater than 4 μg. of stable Pb are present in the aliquotes analysed. The technique is detailed and includes the digestion of tissues with a mixture of HNO_3, H_2SO_4 and perchloric acids. J. W. TAYLOR.

Determination of zinc in biological materials by atomic absorption spectrophotometry. Keiichiro Fuwa, P. Pulido, R. McKay and B. L. Vallee (*Analyt. Chem.*, 1964, **36**, 2407—2411).—Sample is diluted with water or metal-free buffer (10^{-3}M-EDTA or *o*-phenanthroline) for direct aspiration into the H_2-air flame of the atomic absorption spectrometer. Results agree with those obtained by colorimetry or ^{65}Zn measurement ; sensitivity (0·002 μg Zn per ml.) is 10 to 100-fold greater than for the dithizone method and a result is obtained in about a tenth of the time, mainly because no preliminary ashing, pptn. or extraction is required. Results for Zn alloys (used to check accuracy), human serum, urine, and bovine carboxypeptidase are reported. (19 references.)

W. J. BAKER.

Hepatoxicity of α-naphthyl isothiocyanate and related compounds. B. A. Becker (*Dissert. Abstr.*, 1964, **25**, 2558).—In a study, the structure-action has shown that α-naphthylisothiocyanate (**I**) and phenylisothiocyanate (**II**) affects liver function of mice by action on the hepatocytes. The action is dose related ; liver parenchyma is damaged but is still capable of metabolising bilirubin. **I** or **II** produce cholestasis in ~16h. after one oral dose. This cholestasis is different from that due to bile obstruction, i.e., extrahepatic cholestasis. The cholestatic action of **I** was demonstrated by two new bile flow techniques. F. C. SUTTON.

Tracer technique for determining dispersion of micro-ingredients in dry mixes [mixed animal feeds, pharmaceutical preparations, flour, maturing agents, vitamins, etc.]. S. Eisenberg (*Cereal Sci. Today*, 1964, **9**, 361—362, 381).—The use of tracers for the determination of micro-ingredients is discussed with particular respect to obtaining the best compromise between cost and accuracy of estimation.

S. A. BROOKS.

Determination of fluoride ions in microgram range. J. Bäumler and E. Glinz (*Mitt. Lebensm. Hyg., Bern*, 1964, **55**, 250—264).—The isolation of F as HF is carried out by micro-diffusion in a small beaker-like vessel made by hollowing out a plastic stopper ; an intact similar stopper is used to close the vessel. The sample (water, milk, or urine solids, tooth paste, pulverised teeth or bones, or salt) is treated (without previous ashing) in the closed vessel with 70% $HClO_4$ at 50—55° for 48h., the HF being absorbed on a strip of filter-paper, moistened with N-NaOH, suspended or supported above the reaction mixture. The F is determined by the colorimetric method of Belcher as modified by Buck (cf. *Anal. Abstr.*, 1963, **10**, 5288). Active C, $Ca_3(PO_4)_2$ or Na_2SO_4 do not interfere, but $AgClO_4$ must be added if the sample is rich in Cl. The range of determination is 0·2—2 mμ of F. (16 references.)

P. S. ARUP.

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ABSTRACTS

MAY, 1965

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

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