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

Published by the Society of Chemical Industry

Volume 21 | No. 6 | June, 1970

SOCIETY OF CHEMICAL INDUSTRY

Offices of the Society: 14 Belgrave Square, London, S.W.1
Telephone: 01-235 3681/5

Annual Subscription to the *Journal of the Science of Food and Agriculture*
£18 post free, single copies £1 17s. 6d. post free

Subscriptions (Non-members) to
S.C.I. Publications, The Chemical Society Publications Sales Office,
Blackhorse Road, Letchworth, Hertfordshire
Telephone: 04626 5817

DETERMINATION OF WATER-SOLUBLE P_2O_5 IN FERTILISER SOLUTIONS CONTAINING SHORT-CHAIN LINEAR POLYPHOSPHATES

I.—A preliminary study

By D. A. PALGRAVE, T. J. PATON and R. ELSON

A study of the official vanadium phosphomolybdate spectrophotometric method, to determine water-soluble P_2O_5 in fertilisers, has shown that incomplete recovery may occur if a substantial proportion of the P_2O_5 is present as short-chain linear polyanions. It is essential that the preparative stage allows for complete conversion of all water-soluble P_2O_5 into orthophosphate, but unless the regulations are very broadly interpreted, degradation may be only partial. Consequently there is an element of uncertainty about the validity of the results. This investigation has shown the need for minor modifications of the method, including the use of a greater volume of acidulant and a more precise definition of the term 'incipient ebullition'.

Introduction

With the overall trend towards power farming techniques, fertilisers are being bulk-handled to an increasing extent. Among the alternative systems available, fertilisers in the convenient form of aqueous solutions have made considerable headway in Britain.[1] By virtue of the raw materials employed, phosphate components of these fertiliser solutions are frequently in the form of short-chain linear polymers, largely pyrophosphates, triphosphates or higher analogues. Fertiliser compositions are evaluated using procedures defined by Act of Parliament. The present investigation was undertaken to assess whether or not the spectrophotometric vanadium phosphomolybdate technique, described in paragraph 4.23 of the Seventh Schedule of the appropriate Statutory Instrument,[2] was valid when a substantial proportion of the water-soluble P_2O_5 was in a polyphosphatic state.

Experimental

Materials

Typical fertiliser solutions were prepared by neutralising 76% P_2O_5 polyphosphoric acid with aqueous ammonia, then adding urea and/or potassium chloride to vary the nutrient ratios. A sample of commercially available sodium pyrophosphate was also examined.

Method

Total P_2O_5 values were determined using the procedure as described in paragraph 4.22 of the Statutory Instrument.[2] However, as the water-soluble P_2O_5 determination set out in paragraph 4.23 seemed to involve some latitude of interpretation, the effects of varying some of the manipulative details were explored.

Since the method was formulated with solid fertilisers in mind, the initial stage refers to extraction. This was appropriate in the case of the sample of sodium pyrophosphate but as far as the fertiliser solutions were concerned this step merely amounted to dilution. Nevertheless each liquid sample was treated as if it required to be extracted and shaken for the 30-min period in the approved manner.

The statutory procedure next called for the aliquot to be acidified by 1 ml of nitric acid. The corresponding A.O.A.C. procedure[3] specified 5 ml on a *pro rata* basis. As rates of polyphosphate hydrolysis[4,5] are pH sensitive, variations in the volume of acid introduced could affect the final results. To study this factor, the procedure was undertaken with 0, 1, 2 and 5 ml additions of nitric acid.

Perhaps the most controversial feature of the method is the practical interpretation of the term 'incipient ebullition'. It cannot mean 'boil', but unless the temperature is specified it is difficult to envisage a satisfactory definition of the meaning of this phrase. Furthermore, the time taken to reach 'incipient ebullition' can vary. The extent of polyphosphate hydrolysis is greatly influenced by the interacting factors of time and temperature. To study this aspect of the problem, an electrothermal Kjeldahl digestor unit was calibrated so that temperatures in the range 94–98° could be readily maintained. Samples, with and without nitric acid, were heated for varying lengths of time up to 1 h; the final temperatures were recorded. In one series of experiments the samples were heated to boiling, prior to the acidification step; this was to ensure that any hydrolysis of polyphosphates was not brought about by the presence of acid during the heating-up period. A further variation was to discover the effect of rapidly cooling the sample after the 10-min treatment, as opposed to allowing it to cool slowly.

The last operation in the official procedure before preparing the sample for colour development, is the neutralisation of the sample by sodium hydroxide. The usefulness of including this step appeared doubtful, particularly when it was not part of the statutory determination of total P_2O_5. To resolve this, a number of determinations were carried out omitting the neutralisation step.

Results

Total P_2O_5 contents are given in Table I.

For comparative purposes, most water-soluble P_2O_5 results have been expressed in terms of percentage recovery of total P_2O_5.

The variation in temperature with time of heating is summarised in Table II.

The implications of these temperature variations on the P_2O_5 recoveries are summarised in Table III.

Data from the experiments where the aliquot was heated to boiling prior to the addition of 1 ml of nitric acid, are presented in Table IV.

The sensitivity of P_2O_5 recovery values to the volume of nitric acid used is demonstrated in Fig. 1.

The omission of the neutralisation step depressed the water-soluble P_2O_5 results by rather less than 0·1% absolute.

Discussion and Conclusions

The typical temperature profile delineated in Table II shows that heating-up occupied 30–40 min, after which the temperature slowly began to level off, presumably at a value identified with what is implied by 'incipient ebullition'. It is inevitable that the instant marking the beginning of the 10-min period, during which the aliquot should be maintained just short of boiling, is almost impossible to define. Unfortunately, this inherent uncertainty affects water-soluble P_2O_5 recovery. If by use of the data in Table III it is assumed that the period of 'incipient ebullition' began 30 min after the aliquot had been placed on the hotplate, recovery could be only 96·8%. On the other hand, 'incipient ebullition' might not be considered to have begun until 20 min later in which case recovery would be 99% or better. When there was no doubt that the temperature of 'incipient ebullition' had been attained, as indicated by the data in Table IV, virtually 100% recovery was assured. However the introduction of a cooling step after 'incipient ebullition' tended to depress the recovery of water-soluble P_2O_5, suggesting that some hydrolysis was still taking place in the hot mixture subsequent to removal from the source of heat. This leads to the conclusion that a more explicit instruction including the phrase 'boil for 10 min' would be less open to misinterpretation.

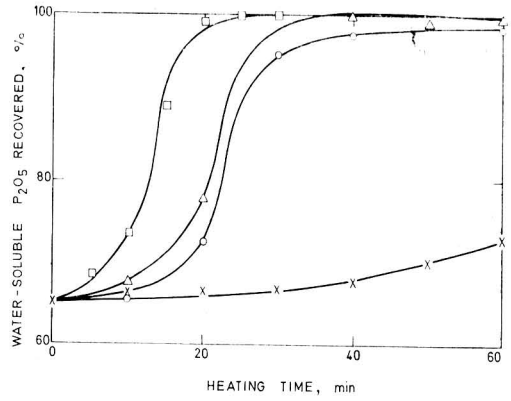


FIG. 1. *Water-soluble P_2O_5 recovered from sample B*
× 0; ○ 1 ml; △ 2 ml; □ 5 ml HNO_3

Fig. 1 clearly shows the effect of changing the volume of nitric acid. In fact by merely doubling the quantity of acid, almost complete hydrolysis of the polyphosphates in sample B could be achieved before reaching the point of 'incipient ebullition'. There would seem to be considerable merit in increasing the volume of nitric acid to bring it in line with the A.O.A.C. procedure.

Neutralisation prior to colour development had no significant effect on the results. However, it is possible that a fertiliser solution containing a fairly high concentration of sequestered cations might give rise to an orthophosphate precipitate at this point. This might introduce difficulties although the excess of acidity in the colorimetric reagent would probably redissolve any such insoluble matter. On balance, the neutralisation step appears somewhat superfluous and could justifiably be omitted.

TABLE I
Total P_2O_5 contents of experimental samples

Identity	Nominal grade	P_2O_5 found, %
A	2–6–12	6·02
B	7–7–10	7·12
C	9–9–9	8·52
D	Sodium pyrophosphate	31·81

TABLE II
Typical temperature profile

Time, min	0	10	20	30	40	60
Temperature, °c	23	63	77	92	94	95

TABLE III
Water-soluble P_2O_5 recovery from aliquots acidified by 1·0 ml nitric acid, %

Identity of sample	Time of heating, min						
	0	10	20	30	40	50	60
A	65·1	66·8	71·1	98·6	99·5	99·7	99·7
B	65·0	65·6	71·5	95·4	98·0	94·0	99·0
C	59·3	67·8	71·6	96·0	98·5	99·8	99·9
D	0·50	8·70	47·0	88·4	96·8	98·6	99·8

TABLE IV
Water-soluble P_2O_5 recovery from samples C and D with varying thermal history, %

	Heated to boiling, acidified and maintained at 94–98°C for 10 min. Then cooled slowly by being left on hotplate	Heated to boiling, acidified and maintained at 94–98°C for 10 min. Then cooled rapidly in cold water
Sample C	100·1	99·6
Sample D	99·7	98·0

Acknowledgments

The authors wish to express their thanks to Mr. J. B. E. Patterson and his colleagues at the Ministry of Agriculture, Fisheries & Food, N.A.A.S. Eastern Region Laboratories, Cambridge, for their helpful observations and also for the

opportunity of discussing certain aspects of this work in detail.

J. W. Chafer Limited,
Milethorn Lane,
Doncaster,
Yorkshire

Received 31 July, 1969;
amended manuscript 5 January, 1970

References

1. Palgrave, D. A., *Agric. Progr.*, 1968, **43**, 15
2. Fertiliser and Feeding Stuffs Regulations, 'Statutory Instrument No. 218—Agriculture', 1968 (London: H.M.S.O.)
3. 'Official Methods of Analysis', (10th Edn) 1965 (Washington, D.C.: Association of Official Agricultural Chemists)
4. Friess, S. L., *J. Am. chem. Soc.*, 1952, **74**, 4027
5. van Wazer, J. R., Griffith, E. J., & McCullough, J. F., *ibid.*, 1955, **77**, 287

AUTOMATED DETERMINATIONS OF PHOSPHATE CONTENT OF SOILS UNDER RUBBER CULTIVATION

By S. K. NG

The phosphate status of rubber-growing soils in West Malaysia is assessed by measuring the phosphate extracted with acid fluoride and with a mixture of perchloric and sulphuric acids. The extracted phosphate can in each case, be determined automatically on a Technicon Auto-Analyzer, using the molybdenum blue colorimetric method with ascorbic acid as reductant. For P soluble in acid fluoride, the automated procedure is slightly more reproducible but gives somewhat lower results than the manual stannous chloride procedure, except for very low concentrations where the position is reversed.

Ascorbic acid prevents interference by Fe in the determination of perchloric acid-soluble P; recovery and replicate tests show that the automated procedure is more accurate than the manual phosphovanadate method. Digestion with $HClO_4/H_2SO_4$ gives as good a measure of total soil P as HF/HNO_3, and although values are slightly lower than with sodium carbonate fusion, the method is more convenient and rapid for routine analysis.

Introduction

Fertiliser experiments on rubber (*Hevea brasiliensis*) have shown that for inland soils of West Malaysia, phosphate increases growth during immaturity[1] and raises girth increment and yield in mature trees.[2] Thus, the use of rock phosphate for rubber establishment and yield maintenance on the strongly acid soils of West Malaysia has become widespread practice in rubber upkeep. To assess the phosphate status of rubber-growing soils Owen[3] introduced the acid fluoride extraction in routine testing.[4] In more recent years, a combination of soil and leaf analysis for manurial recommendations has been advocated by the Rubber Research Institute of Malaya,[5] and Guha & Yeow[6] broadly related 'total' phosphorus as measured by perchloric–sulphuric acid soluble phosphorus of soils to phosphorus levels in rubber leaves.

At present, the Rubber Research Institute of Malaya provides a soil and leaf analysis service to rubber growers; for assessing soil phosphate status, both acid fluoride-soluble and total phosphates are determined. As the area of cultivated rubber land in West Malaysia is in the region of 4·5 million acres, and the period for concurrent soil and leaf sampling limited to about 6–8 months of the year owing to 'wintering' and the process of refoliation, large numbers of soil samples may accumulate during this functional period. In order to avoid any significant backlog of samples and to expedite rapid advice to growers, automation of a number of analytical determinations has been instituted and this paper deals with the automated analysis of these two forms of soil phosphates.

Experimental

Materials

Surface and subsoil layers of rubber-growing soils were sampled. After air-drying they were ground and passed through a 2 mm sieve. Unless otherwise stated, soils of this particle size were used.

Preparation of extracts

Acid fluoride-soluble P

A 2N ammonium fluoride solution was prepared by dissolving 37 g NH_4F in water, diluting to 500 ml and keeping it in a polyethylene bottle.

0·5 N hydrochloric acid was made by diluting 20·2 ml of concentrated HCl to 500 ml with water.

To prepare the extracting solution 200 ml of 0·5 N-HCl and 15 ml of 2 N-NH_4F solution were added to a litre flask and diluted to the mark.

The extraction was carried out as described by Arnold[4] and a portion of the filtrate was taken for automatic determination.

Total P

For the $HClO_4/H_2SO_4$ digestion 2 g of soil were digested in a Kjeldahl flask at moderate heat for 2 h with 6 ml of a mixture of equal volumes of 60% $HClO_4$ and 98% H_2SO_4. Where necessary, a further 1 ml of acid mixture was used to wash down undigested material at the neck of the flask. After cooling, the digest was taken up in water, transferred to a 100 ml flask, made up to the mark and allowed to stand overnight. The supernatant was filtered and the filtrate was taken for analysis. The HF/HNO_3 digestion was carried out by heating 0·5 g of 100-mesh soil in a platinum dish over a sand bath with 10 ml concentrated HNO_3 and evaporating to dryness. The residue was heated to 550° in a muffle furnace and after cooling, was further treated with 2 ml of HNO_3 and 10 ml of 40% HF over the sand bath. The residue was treated twice more with 10 ml of HNO_3 each time to remove fluoride. Finally, the residue was taken up with 5 ml conc. HNO_3 and 20 ml water, filtered, and made up to 100 ml.

A Na_2CO_3 fusion was done by fusing 0·5 g of 100-mesh soil with 5·0 g of sodium carbonate in a platinum crucible which was then transferred to a 250 ml beaker containing about 100 ml of water. 15 ml of concentrated HCl was added and the contents were heated on a water bath until the volume was 50–70 ml. The contents were then transferred into a 100 ml flask, which was made up to the mark when cool. About 20 ml of the extract was centrifuged and the clear supernatant was taken for determination.

Automated determination of P in the extracts

Apparatus

A Technicon Auto-Analyzer Unit comprising Sampler II, Proportioning Pump II, Heating Bath, Colorimeter with 15 mm tubular flowcell and a Double Pen Recorder was used.

Reagents

For the acid fluoride-soluble P determination the following reagents were prepared: 2·5% ammonium molybdate (25 g of ammonium molybdate in 1 l. 10N-H_2SO_4); boric acid solution (49·4 g boric acid in 1 l. H_2O); molybdate–boric acid mixture (150 ml of boric acid solution and 40 ml of 2·5% molybdate solution with 590 ml of water); and ascorbic acid (0·5 g ascorbic acid in 100 ml of water).

For the total P determinations a molybdate solution containing 20 ml of the 2·5% ammonium molybdate solution in 10N-H_2SO_4 diluted to 400 ml, and an ascorbic acid solution of 1 g in 100 ml of water were used.

Distilled water and analytical grade reagents were used throughout.

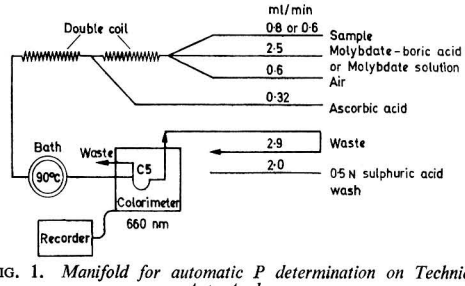


FIG. 1. *Manifold for automatic P determination on Technicon Auto-Analyzer*

Determination

Phosphate in solution was determined by a molybdenum blue method, adapted for automation from the ascorbic acid procedure of Fogg & Wilkinson.[7] The manifold used is shown in Fig. 1. For acid fluoride-soluble P, the standard curve covered the range of 0–8 ppm. For total P, a 0·6 ml/min sample line was used and the working range was 0–10 ppm P.

P determination by vanadate method and effect of iron

Phosphate in the perchloric–sulphuric acid extracts was also determined manually by the phosphovanadate method of Hanson[8] for comparison of the two methods. As varying amounts of ferric iron and other interfering elements are present in perchloric–sulphuric acid extracts, the effects of iron and ascorbic acid concentration, and recovery tests were also investigated.

Results and Discussion

Acid fluoride-soluble P

Analytical results of triplicate sub-samples of 25 soils which were selected to cover the most common range of values are presented in Table I. Also given are results obtained by a manual stannous chloride procedure on the same solutions.

In the majority of cases the deviations of the automated procedure were lower; the mean coefficient of variation of the automated procedure was below 5% and less than that of the manual procedure. At very low concentrations, i.e. less than 5 ppm, the automated method gave higher results, which agrees with the finding of Salt[9] who attributed this to the hydrolysis of organic phosphate during heating in the oil bath.

TABLE I

Comparison of two methods for acid fluoride-soluble P determination

Sample No.	Auto-Analyzer		Manual $SnCl_2$ method	
	Mean, ppm	S.D. (±)	Mean, ppm	S.D. (±)
085/10/68	2·8	0·00	1·9	0·21
213	3·3	0·29	2·6	0·32
150	3·6	0·30	3·1	0·26
137	4·5	0·00	4·2	0·16
197	4·4	0·17	3·5	0·30
C 4083	5·6	0·17	5·0	0·50
130	6·2	0·87	6·4	0·91
144	7·2	0·29	7·1	0·41
093	7·6	0·41	7·9	0·30
217	8·1	0·31	8·3	0·36
113	10·0	0·69	10·7	0·42
013	11·4	0·35	12·3	0·67
C 4091	12·1	0·23	11·9	0·98
107	15·2	0·91	16·2	0·74
041	15·3	0·64	17·4	0·90
215	15·5	0·99	17·1	1·11
233	17·9	0·45	21·2	0·22
C 4062	18·0	1·00	17·6	1·22
C 4295	20·5	0·45	21·9	0·46
C 4041	20·7	0·17	21·1	0·29
019	21·4	0·60	24·8	0·44
050	23·1	0·83	24·3	1·28
095	26·8	0·76	29·8	2·38
135	27·5	1·91	29·9	2·68
C 4020	30·0	0·75	28·7	0·77
Mean coeff. var.	4·3%		5·6%	

For samples with higher phosphate contents, the manual procedure tended to give slightly higher values. These differences, however, were not significant in terms of interpretation for manurial purposes.

The rate of determination was 40/h, or about 250 samples per working day.

$HClO_4/H_2SO_4$ soluble P

Effect of iron and concentration of ascorbic acid

The influence of ferric iron added to standard P solutions, and of the concentration of ascorbic acid are shown in Table II.

With 0·5% ascorbic acid, ferric iron at 2000 ppm in standard phosphate solutions increased transmission readings significantly. However, this interference was satisfactorily eliminated by increasing the ascorbic acid concentration to 1%. In practice, accuracy was not affected if a soil contained less than 30% Fe_2O_3.

P recovery

Table III shows the percentage recovery of known amounts of phosphate added to extracts of 10 different soils. For comparison, the solutions were also analysed by the vanadate procedure. The final concentrations of phosphate measured ranged from 1 to 9 ppm.

The automated procedure gave very good recovery and reproducibility whereas poorer results were obtained by the vanadate method, especially at the lowest concentration.

Ratio of $HClO_4/H_2SO_4$

The effect of increasing the proportion of perchloric acid in the mixture was investigated; no significant differences could be found with the ratio of $HClO_4/H_2SO_4$ ranging from 1:1 to 2:1.

Volume of $HClO_4/H_2SO_4$ mixture

Two volumes, 10 ml and 6 ml of 1:1 perchloric-sulphuric acid mixture were tested and there was no significant difference. The smaller volume was consequently used in routine analysis.

Error of routine analysis

An indication of the error of routine automated determination of total P by perchloric-sulphuric digestion is shown in Table IV. Except for two samples, the errors were larger for the manual vanadate procedure; in the two samples, which had high iron contents, values were considerably lower. The median standard error of the automated determination was a third of that of the manual vanadate procedure.

The precision of the automated procedure was considered satisfactory from the practical viewpoint of routine testing.

Comparison of methods of soil digestion for determining total P

Results of phosphate determined by the automated procedure after three different methods of soil digestion are presented in Table V. In this part of the investigation, 100 mesh top- and sub-soils of six common soil types were used.

The mean coefficients of variation of the three methods were comparable, being less than 5%. For nine samples, the fusion method gave 10–30% higher values than the other methods but for the remaining samples, differences were within

TABLE II

Effect of Fe^{3+} and concentration of ascorbic acid on P standards (% transmission)

P, ppm	0·5% ascorbic acid						1% ascorbic acid.
	0 ppm Fe^{3+}	250 ppm Fe^{3+}	500 ppm Fe^{3+}	1000 ppm Fe^{3+}	1500 ppm Fe^{3+}	2000 ppm Fe^{3+}	2000 ppm Fe^{3+}
0	99·5	99·5	99·5	99·5	99·5	99·0	99·0
	99·5	99·5	99·5	99·5	99·5	99·0	99·0
2	70·0	69·5	69·5	69·5	69·5	71·0	69·0
	69·5	69·5	69·5	69·0	69·5	71·0	69·0
4	48·5	48·0	47·5	47·5	48·5	51·0	47·5
	48·0	48·0	48·0	47·5	48·5	50·5	47·5
5	40·0	40·0	39·5	39·5	40·0	43·5	39·5
	40·0	41·0	41·0	40·0	40·0	43·0	39·5
6	33·0	33·0	33·0	33·0	33·5	37·5	32·5
	33·0	33·5	33·0	33·0	33·5	36·5	32·5
8	23·0	23·0	22·5	23·0	23·5	27·5	22·5
	23·0	23·0	22·5	23·0	23·5	27·5	22·5
10	15·5	15·5	15·5	15·0	16·5	20·0	15·5
	15·5	15·5	16·5	16·0	16·5	19·5	15·5

TABLE III

Percentage recovery of P added to soil extracts

Method	μg P added					
	25		40		80	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Ascorbic acid	99·4	±2·5	99·5	±2·5	99·6	±1·5
Vanadate (manual)	125·4	±12·6	91·7	±12·5	101·9	±6·2

TABLE IV

Mean values and standard errors (S.E.) of P determinations on $HClO_4/H_2SO_4$ extracts

Sample No.	Auto-Analyzer (ascorbic acid method)		Manual vanadate method	
	Mean, ppm	S.E.	Mean, ppm	S.E.
359	130	1·7	133	6·0
360	121	2·1	122	6·0
361	932	51·5	636	33·9
362	153	7·7	167	16·4
363	96	2·0	115	5·8
364	327	3·3	295	12·2
365	110	1·7	117	10·9
366	115	2·4	110	10·4
367	918	43·6	615	31·2
368	112	3·4	122	3·3
369	73	2·8	72	3·3
370	187	3·2	197	9·3
Median S.E.		3·0		9·9

TABLE V

Total soil P estimated by three different methods of digestion but P in solution determined on Auto-Analyzer

Soil series	Parent material	Depth, in	$HClO_4/H_2SO_4$ (mean), ppm	HF/HNO_3 (mean), ppm	Na_2CO_3 fusion (mean), ppm	Min. 5% S.D. between two means, ppm
Batu Anam	Shale	0–6	132	123	163	8·5
		6–12	111	104	149	21·0
Munchong	Shale	0–6	125	112	155	13·2
		6–12	118	106	143	10·6
Kuantan	Basalt	0–6	942	976	925	31·1
		6–12	932	964	940	39·5
Rengam	Granite	0–6	167	183	173	29·2
		6–12	114	112	148	25·1
Sungei Buloh	Local alluvium	0–6	100	101	110	8·0
		6–12	79·7	78·7	93	9·9
Selangor	Marine clay	0–6	335	342	365	16·4
		6–12	191	201	222	17·9

experimental error. Results of perchloric–sulphuric and hydrofluoric–nitric acid digestion were in close agreement.

Both hydrofluoric–nitric acid digestion and sodium carbonate fusion methods require platinum ware and are time consuming. In contrast, perchloric–sulphuric acid digestion is more rapid and an operator can digest 60–70 samples in a normal working day. Although digestion with perchloric acid has been reported to give appreciably lower results than hydrofluoric treatment,[10] this has not been found in this investigation.

Thus, the automated determination of perchloric–sulphuric acid soluble phosphate appears to be a rapid and precise measurement of total phosphorus in Malaysian soils cultivated with rubber.

Acknowledgments

The author thanks the Director of the Rubber Research Institute of Malaya for his permission to publish this paper, Dr. C. Bloomfield for comments, Mr. G. C. Iyer for statistical advice, Mr. K. Ratnasingam and his staff for assistance in chemical analysis and the Soils Division for some of the soil samples.

Rubber Research Institute of Malaya,
P.O. Box 150,
Kuala Lumpur,
Malaysia

Received 19 December, 1969;
amended manuscript 6 March, 1970

References

1. Akhurst, C. G., & Owen, G., *J. Rubb. Res. Inst. Malaya*, 1950, **12**, 167
2. Owen, G., Westgarth, D. R., & Iyer, G. C., *J. Rubb. Res. Inst. Malaya*, 1957, **15**, 29
3. Owen, G., *J. Rubb. Res. Inst. Malaya*, 1953, **14**, 109
4. Arnold, C. Y., *Soil Sci.*, 1947, **64**, 101
5. *Plrs' Bull. Rubb. Res. Inst. Malaya*, 1964, **71**, 41
6. Guha, M. M., & Yeow, K. H., *Plrs' Bull. Rubb. Res. Inst. Malaya*, 1966, **87**, 170
7. Fogg, D. N., & Wilkinson, N. T., *Analyst, Lond.*, 1958, **83**, 406
8. Hanson, W. C., *J. Sci. Fd Agric.*, 1950, **1**, 172
9. Salt, P. D., *Chemy Ind.*, 1968, p. 584
10. Muir, J. W., *Analyst, Lond.*, 1952, **77**, 313

UPTAKE OF MAGNESIUM AND TRACE ELEMENTS BY THE HERBAGE OF A RESEEDED UPLAND PASTURE

By F. C. ARCHER*

Copper sulphate, sodium molybdate, magnesium sulphate, cobalt sulphate and basic slag were applied to the seedbed of a reseeded Welsh upland pasture in conjunction with lime at 1 ton and 4 tons per acre. The higher rate of liming produced a lower uptake of applied Co and a slightly higher uptake of applied Mo. The Cu and Mg contents of the herbage were similar at both rates of liming. Copper sulphate at 20 lb per acre increased the Cu content of the herbage by a factor of about 1·5 during the first growing season, but this effect was not significant during the subsequent seasons. Sodium molydbate at 4 oz per acre increased the Mo content of the herbage by a factor of about 2·0 in the first season after application, but this effect declined and was not observed after 4 years. Cobalt sulphate at 2 lb per acre increased the Co content of the herbage by a factor of about 4·0 in the first season after application, but this effect had declined to a factor of about 1·5 four years later. Basic slag at 6·75 cwt per acre each spring slightly increased the Mo and Mg contents of the herbage, but had no effect on its Co or Cu contents. Magnesium sulphate at 5 cwt per acre produced no significant effects on the Mg content of the herbage.

Introduction

A convenient way of increasing the amounts of Mg, Cu and Co in the diets of grazing animals would be to increase the concentration of these elements in the herbage. Mitchell[1] and Mitchell *et al*[2]. showed that top dressings of cobalt sulphate applied to pastures in Scotland markedly increased the Co content of the herbage, but that copper sulphate treatments raised the Cu content of grasses by only a small amount, although it did increase the Cu content of red clover considerably in certain cases. In Lancashire, Morgan & Clegg[3] found that the application of copper sulphate as a pasture top dressing only slightly increased the Cu content of the herbage. On an upland site in Wales, Williams[4] showed that sodium molybate applied to a reclaimed upland pasture produced an increase in dry matter yield, and also increased the Mo content of the herbage. In Ireland, Walsh *et al.*[5] observed that the incidence of livestock disorders associated with excess Mo intake was often connected with recent heavy dressings of basic slag. Investigations in Warwickshire by Griffiths[6] showed that the Mg content of herbage was increased by application to the soil of magnesite, magnesian limestone, and to a lesser extent, magnesium sulphate. The reclamation and reseeding of upland pastures in Britain almost always involves the application of lime and basic slag. This report describes the effects of the application of copper sulphate, cobalt sulphate, magnesium sulphate, sodium molybdate and basic slag to the soil, on the Cu, Co, Mg and Mo contents of the herbage of a reseeded upland pasture in Wales.

Experimental

Site

The investigation was carried out at a site in Denbighshire at an elevation of about 1500 ft. As far as was known, no lime or fertiliser had ever been applied to the area. The soil was of the Hiraethog[7] series which covers a large proportion of the Welsh uplands. The undisturbed soil profile consisted of a Nardus-dominated root mat over a few inches of peat, covering a stony, yellow-brown loam which merged into angular fragments of Silurian shale. Soil analysis indicated extreme lime and phosphate deficiences. The area was ploughed to a depth of about 10 in. in 1958 and a seedbed was prepared during the early summer of 1959 into which a mixture of perennial ryegrass, timothy and wild white clover seed was sown.

Treatments

Just before the seed was sown the following treatments were applied to the seedbed:

1. Control
2. Copper sulphate – 20 lb per acre $CuSO_4 \cdot 5H_2O$ (5·1 lb per acre Cu)
3. Sodium molybdate – 4 oz per acre $Na_2MoO_4 \cdot 2HO$ (1·6 oz per acre Mo)
4. Magnesium sulphate – 5 cwt per acre $MgSO_4 \cdot 7H_2O$ (55 lb per acre Mg)
5. Cobalt sulphate–2 lb per acre $CoSO_4 \cdot 7H_2O$ (6·7 oz per acre Co)
6. Basic slag – 6·75 cwt per acre.

These treatments were applied to plots 6 ft × 6 ft in size, each plot being separated from the rest by 1·5 ft left untreated. The plots were arranged in 4 blocks with the 6 treatments in each block. Two of the blocks received ground limestone at the rate of 4 tons per acre, and the other two ground limestone at 1 ton per acre. A basal dressing of muriate of potash at 1 cwt per acre (56 lb per acre K) and 'Nitro Chalk' at 2 cwt per acre (47 lb per acre N) was applied to the whole of the experimental area. Superphosphate at 5 cwt per acre (45 lb per acre P) was applied to that part of the experimental area not treated with basic slag, which was applied at a rate calculated to supply an equivalent amount of P. Treatments 1 to 5 and the lime were applied once only, but the basal fertiliser dressings and the basic slag treatments were repeated in the spring of each year. Spectrographic analysis of the basic slag showed that it contained 2·5% Mg, 29 ppm Cu, 6 ppm Mo and < 10 ppm Co.

Sampling

As the primary object of the investigation was to study the effects of the treatments on the trace element content of the

* Present address: Ministry of Agriculture, Fisheries and Food Government Buildings, Kenton Bar, Newcastle upon Tyne, 3

herbage, no estimate was made of dry matter yields but no visual effect on growth was noted. After seeding, the experimental area was permanently fenced and successive sample cuts of herbage were taken each time it had grown 8–12 in tall during the growing seasons of 1960–64. The herbage was cut off about 2 in above ground level with stainless steel shears, and without being allowed to fall on to the ground was transferred to new polyethylene bags for transportation to the laboratory. Three cuts were taken each year in late spring, summer and autumn, but in 1964 samples could only be obtained in May and October. Clover establishment was poor and the material sampled consisted almost entirely of grass.

Analysis

The samples were dried in aluminium trays in a Birmingham–Blackburn oven at 105°. They were then ground in a Christy and Norris mill fitted with an aluminium chute instead of the usual brass one. After wet digestion, Cu was determined by zinc dibenzyldithiocarbamate;[8] cobalt by 2–nitroso 1–naphthol;[9] and molybdenum as thiocyanate.[10] Magnesium was determined spectrochemically. Soil pH was measured in a suspension of 1 part soil to 2·5 parts water.

Results

Although several sample cuts of herbage were taken during each growing season, there was little seasonal variation in their Mg and trace element content and, therefore, the values presented are the means for all cuts during each season.

Effect of lime

The mean Co, Cu, Mo and Mg contents of the oven-dried herbage following the application of compounds containing these elements to the seedbed at two rates of liming are shown in Table I.

At the higher rate of liming, application of cobalt sulphate produced herbage of a lower Co content than at the lower liming rate but the effect was only marked in the first season after application. The uptake of Mo was slightly higher where sodium molybdate had been applied in conjunction with the heavier rate of liming. The Cu and Mg contents of the herbage were very similar at both rates of liming. The effects of lime in reducing the uptake of applied Co and increasing the uptake of applied Mo have been noted by Mitchell *et al.*[2] in Scotland, where the effect of lime on Mo uptake was much greater in the case of clover than in grass. The small effects of lime on Mo uptake found at this site in Denbighshire may be because the material sampled consisted almost entirely of grass.

Effects of cobalt sulphate, copper sulphate, sodium molybdate, magnesium sulphate and basic slag

As the effects of increasing the rate of liming on the composition of the herbage were generally small, results are given in Table II which represent mean values for both rates of liming.

Effect of cobalt sulphate

Cobalt sulphate applied in 1959 increased the Co content of the herbage by a factor of about 4·0 during the 1960 growing season, but this had declined to a factor of about 1·5 four years later. A similar amount of Co applied to a pasture in Scotland[1] continued to affect the Co content of herbage for about 15 months.

Effect of copper sulphate

During the first season after application, copper sulphate increased the Cu content of the herbage by a factor of about 1·6, but this effect was not significant during subsequent growing seasons. This small response is similar in degree to that noted in Scotland[2] in the case of ryegrass. In Lancashire also, Morgan & Clegg[3] recorded only small increases of short duration in herbage Cu content following copper sulphate top dressings.

Effect of sodium molybdate

The Mo content of the herbage was increased by a factor of about 2·0 during the first growing season following its application but this effect declined with time and was not observed after 4 years. Sodium molybdate at the higher rate of 1 lb per acre applied to pastures in Scotland,[2] increased the Mo content of ryegrass by a similar amount, but increased the Mo content of clover to a much greater extent, and the relatively small effect noted here could be another reflection of the absence of clover from the sward.

Effect of magnesium sulphate

Magnesium sulphate produced hardly any significant effects on the Mg content of the herbage. In Warwickshire[6] 3 cwt per acre magnesium sulphate (33 lb per acre Mg) applied annually to grassland produced only small effects on the magnesium content of the herbage; in Cardiganshire[11] magnesium sulphate at 5 cwt per acre (55 lb per acre Mg) gave rise to a small increase in the magnesium content of the herbage, but only in the season of application.

Effect of basic slag

The Mo content of the herbage was increased by application of basic slag. However, this treatment also raised the mean

TABLE I

Mean soil pH values and Co, Cu, Mo and Mg contents of herbage dry matter on treated plots which received two different rates of lime

			Cobalt sulphate		Copper sulphate		Sodium molybdate		Magnesium sulphate	
Ton/acre lime	Soil pH 1	Soil pH 4	ppm Co 1	ppm Co 4	ppm Co 1	ppm Co 4	ppm Mo 1	ppm Mo 4	% Mg 1	% Mg 4
1960	5·0	5·5	0·60	0·43	5·8	5·6	1·2	1·3	0·11	0·12
1961	5·1	5·8	0·19	0·18	6·4	6·1	0·8	1·0	0·11	0·11
1962	5·1	5·8	0·18	0·14	6·4	6·3	0·9	1·4	0·13	0·13
1963	5·1	5·9	0·23	0·14	7·9	7·5	0·9	0·8	0·14	0·15
1964	5·2	5·8	0·13	0·10	6·6	5·5	0·5	0·6	0·11	0·12

TABLE II

Effects on herbage composition of copper sulphate, sodium molybdate, magnesium sulphate, cobalt sulphate and basic slag applied to the soil

(Results expressed on oven-dried material)

	Control	Copper sulphate	Sodium molybdate	Magnesium sulphate	Cobalt sulphate	Basic slag	S.E.
1960 Cu ppm	3·6	5·7	3·5	3·4	3·5	4·1	± 0·29
Co „	0·13	0·11	0·18	0·14	0·51	0·15	± 0·021
Mo „	0·6	0·5	1·2	0·8	0·6	1·0	± 0·09
Mg %	0·10	0·10	0·11	0·12	0·11	0·12	± 0·006
1961 Cu ppm	5·5	6·3	5·2	4·9	5·3	5·6	± 0·35
Co „	0·11	0·09	0·12	0·11	0·18	0·10	± 0·006
Mo „	0·6	0·6	0·9	0·6	0·6	1·1	± 0·04
Mg %	0·10	0·10	0·11	0·11	0·11	0·13	± 0·004
1962 Cu ppm	5·2	6·3	5·1	5·2	5·4	5·0	± 0·30
Co „	0·08	0·08	0·08	0·08	0·16	0·08	± 0·006
Mo „	0·7	0·6	1·1	0·8	0·7	0·9	± 0·08
Mg %	0·11	0·10	0·12	0·13	0·12	0·15	± 0·007
1963 Cu ppm	6·8	7·7	6·8	6·2	6·5	6·5	± 0·34
Co „	0·08	0·08	0·08	0·08	0·18	0·08	± 0·006
Mo „	0·4	0·5	0·8	0·6	0·5	0·8	± 0·10
Mg %	0·14	0·13	0·15	0·14	0·13	0·17	± 0·010
1964 Cu ppm	5·8	6·0	4·7	4·8	5·2	4·8	± 0·34
Co „	0·08	0·07	0·08	0·08	0·12	0·08	± 0·005
Mo „	0·5	0·4	0·5	0·6	0·4	0·8	± 0·11
Mg %	0·12	0·11	0·13	0·12	0·11	0·14	± 0·009

pH value of the soil by about 0·4 pH units above that of the plots receiving the other treatments. It is, therefore, not possible to decide to what extent the effects on herbage Mo content were due to the Mo content of the slag rather than to its action in raising soil pH. Basic slag also increased the Mg content of the herbage to a small extent during each growing season. Pot trials carried out by Heintze[12] demonstrated that some of the magnesium in basic slag was available to plants, although the degree of availability depended on the source of the slag. Basic slag did not significantly affect the Co or Cu content of the herbage, although after 5 growing seasons 40·5 cwt per acre had been applied in annual increments of 6·75 cwt per acre. Similar findings with regard to basic slag have been reported by Reith & Mitchell[13] working in Scotland.

Conclusions

Although copper sulphate at 20 lb per acre produced a small increase in the Cu content of the herbage during the season following its application, this treatment would not seem to be a suitable or economically worthwhile method of increasing the dietary intake of grazing animals under the conditions described.

Sodium molybdate applied to the soil at 4 oz per acre gave a small increase in the Mo content of the herbage which, however, consisted almost entirely of grass. Investigations in Scotland[2] have shown that clover is capable of achieving a much higher Mo content than grass under the same conditions and, therefore, the application of molybdenum salts under the conditions described could be potentially dangerous if the sward contained appreciable amounts of clover, particularly if lime were also applied.

Magnesium sulphate at 5 cwt per acre produced no significant effects on the Mg content of the herbage.

The application of basic slag slightly increased the Mo and Mg contents of the herbage but did not affect its Co or Cu content.

Cobalt sulphate at 2 lb per acre increased the cobalt content of the herbage by a factor of about 4·0 during the first season after application and this effect, though declining, persisted for several years. This treatment, therefore, appears to be a cheap and effective method of increasing the cobalt content of the diet of animals grazing upland pastures under the conditions described.

Acknowledgments

The author wishes to thank Mr. W. Dermott and Mr. Evan Roberts for their help and guidance in conducting the investigation, and also the field and laboratory staff of the N.A.A.S. Sub-Centre at Bangor, for valuable assistance.

Thanks are due to the Soil Science Department, N.A.A.S., Bristol, for spectrochemical analysis of the basic slag for Co, Cu, Mg and Mo, and of the herbage for Mg; and also to the Department of Statistics, Rothamsted Experimental Station, for statistical analysis of the results.

Ministry of Agriculture, Fisheries and Food,
National Agricultural Advisory Service,
Bryn Adda,
Penrhos Road,
Bangor,
N. Wales

Received 13 June, 1969;
amended manuscript 14 January, 1970

References

1. Mitchell, R. L., *Proc. Nutr. Soc.*, 1944, **1**, 182
2. Mitchell, R. L., Reith, J. W. S., & Johnston, I. M., *J. Sci. Fd Agric.*, 1957, **8**, S51
3. Morgan, D. E., & Clegg, A., *N.A.A.S. Q. Rev.*, 1958, **41**, 28
4. Williams, J. H., *Pl. Soil*, 1956, **7**, 327
5. Walsh, T., Neenan, M., & O'Moore, L. B., *J. Dep. Agric. Repub. Ire.*, 1951–52, **48**, 32
6. Griffiths, T. W., *J. Br. Grassld. Soc.*, 1959, **14**, 199
7. Ball, D. F., 1960, *Mem. Soil Surv. Gt. Br.*, (H.M.S.O.)
8. Andrus, S., *Analyst, Lond.*, 1955, **80**, 514
9. Baron, H., *Z. analyt. Chem.*, 1953, **140**, 173
10. Pruvis, E. R., & Peterson, N. K., *Soil Sci.*, 1956, **81**, 223
11. Jones, E., *J. Br. Grassld. Soc.*, 1963, **18**, 131
12. Heintze, S. G., *J. Sci. Fd Agric.*, 1963, **14**, 324
13. Reith, J. W. S., & Mitchell, R. L., *Proc. IV Int. Colloq. Pl. Analysis Fert. Problems* (1962), 1964, 241

COPPER-CATALYSED OXIDATION OF LINOLEIC ACID IN BUFFERED AQUEOUS SOLUTIONS

I.—Effect of ascorbic acid

By W. A. ALLAN* and H. L. WOOD

Ascorbic acid at a concentration of 10^{-3} M was a more effective pro-oxidant in the presence of low copper concentrations than at high concentrations, particularly in the initial stages of oxidation or in the lower pH range (5·5–4·7). This suggested a much more complex role for ascorbic acid than that of copper-reducing agent. Reaction of the ascorbic acid radical with oxygen and consequent formation of free $HO^{\cdot}$ radicals was considered to be the most likely initiation reaction resulting from the oxidation of ascorbic acid to dehydroascorbic acid by copper. Dehydroascorbic acid was also found to be an effective pro-oxidant in the presence of copper.

Introduction

Off-flavours are frequently encountered in dairy products as a result of the chemical oxidation of lipids. Investigation by many workers,[1–3] has indicated that carbonyl compounds are primarily responsible for these oxidised flavours. The chemistry of oxidation of olefins or fatty acids and some of the possible pathways by which volatile carbonyls may be produced has been reviewed by Badings[4] and Lundberg.[5] The major factors operating to control lipid oxidation in aqueous dairy products are believed to be: metallic contamination, particularly copper;[6,7] ascorbic acid;[8–11] sulphydryl compounds;[12] oxygen tension and hydrogen ion concentration;[13,14] and temperature effects.[15,16]

This study was intended to investigate a particular aspect of the chemistry of oxidised flavour production – the role of ascorbic acid in copper-catalysed oxidation of lipid emulsions at low temperature. However, to avoid many of the consequences of the complexity of natural dairy product emulsions and since linoleic acid has been frequently mentioned as a possible source of the more important off-flavour constituents,[12] linoleic acid emulsified in aqueous phosphate buffer has been used as the oxidisable substrate. The effects of variation of ascorbic acid concentration and copper concentration at various pH values within the phosphate buffer range were systematically studied. Unbuffered emulsions at pH of approximately 4·7 were also used. Oxygen uptake was followed manometrically during oxidation, and the extent of fatty acid oxidation at the end of each treatment was estimated by the thiobarbituric acid test.

Experimental

Materials

The linoleic acid (B.D.H. Laboratory Reagent) used in the experiments was stored at 0°. Two different batches of linoleic acid were used during the experiments and it became evident that storage conditions did not provide sufficient protection against oxidation. Effects of ageing were small, however, compared with the differences between treatments. Gas chromatographic analysis of the linoleic acid showed a trace of oleic acid as the only detectable fatty acid impurity.

Emulsions were prepared as follows: 0·564 g of linoleic acid and 0·03 g of Tween 80 were weighed out and shaken in an Erlenmeyer flask with 100 ml of 0·1 M phosphate buffer. Dry nitrogen was bubbled through the liquid for 5–10 min and then, with the nitrogen bubbling, the mixture was treated in the emulsifier (Silverson stainless steel with Teflon bushes) for 4 min. The emulsion was pipetted into the reaction flasks immediately after preparation.

All glassware used was acid-washed (5–10% acetic acid) and all water used was doubly distilled from glass.

Manometry

Double-armed Warburg manometer flasks were used as reaction vessels. A refrigerated Braun rotary Warburg apparatus provided temperature control and shaking. The emulsion was pipetted into the main flask compartment and copper and either ascorbic acid or dehydroascorbic acid into each of the side arms. Reactions were carried out at 5° (± 0·1°). At this temperature it was found necessary to make a thermobarometer reading with each manometer since there were fairly large pressure fluctuations during the course of reading twelve manometers. The reaction was allowed to proceed for 90 h, readings being taken twice daily, with an 18 h (overnight) and a 6 h interval. At the end of the oxidation period the emulsion was tested for amount of oxidation by the thiobarbituric acid (TBA) test.

Thiobarbituric acid test

A slight modification of the method of King[17] was employed. This involved reaction in alcohol under slightly acid conditions at 60° for 1 h. 62% (by vol.) alcohol was used since higher alcohol concentrations precipitated phosphates and lower concentrations would not dissolve the fatty acid. 1 ml of the emulsion to be tested was pipetted into a glass tube having a 10 ml graduation mark. 0·5 ml alcohol, 0·5 ml 1% HCl and 2 ml of TBA reagent (0·15 g 2-thiobarbituric acid in 50 ml 95% ethanol) were then added. The tubes were placed in a waterbath at 60° for 1 h, cooled and made up to the mark with 62% alcohol, and optical densities were read at 532 nm.

Experimental design

The experimental design was a 3 × 3 factorial arrangement of the two variables copper and ascorbic acid which were used

* Present address: Department of Economics, University of Queensland, Brisbane, Queensland, Australia

in the following concentrations: copper at 10(Cu1), 1·0 (Cu2) and 0·1(Cu3) ppm and ascorbic acid at 10^{-3}M(Asc. I), 10^{-4}M(Asc.II) and 10^{-5}M(Asc.III). Treatments at the highest level of copper (Cu1) without ascorbic acid and at the highest level of ascorbic acid (Asc. I) with no added copper were intended as controls. A level of copper contamination (~ 0·02 ppm) was found to be unavoidable in all treatments. Thus the high ascorbic acid treatment with no added copper was effectively a low copper treatment and was continued as such because the results appeared to be of some importance. In preliminary experiments, high oxidation rates were encountered in the low copper, high ascorbic acid treatments. This raised the problem of whether or not ascorbic acid, in the complete absence of copper, was an effective pro-oxidant. Unreplicated trials with copper-chelating agents satisfied this point; ascorbic acid without copper was not effective in promoting fatty acid oxidation. The experimental design outlined above therefore seemed adequate to provide some information on the more important relationships of copper and ascorbic acid. Subsequent experiments[18] confirmed the conclusion that ascorbic acid on its own is not an effective pro-oxidant.

It was decided originally to conduct trials in duplicate at each of the four set points within the pH range of the phosphate buffer used. However, in the majority of cases it was found that some of the buffered emulsion pH values differed between duplicates. For this reason the experiments were carried out in duplicate at pH 6·9 and over the pH ranges 6·45–6·35, 5·75–5·7, 5·6–5·5, respectively.

To compare the oxidative effect of ascorbic acid with that of its first oxidation product (dehydroascorbic acid) the same ascorbic acid treatments as previously used were adopted. The level of dehydroascorbic acid in the comparative treatments was equivalent to the highest ascorbic acid level. These treatments were combined with two levels of copper (Cu1 and Cu3) i.e. (3 + 1) × 2. It seems probable that treatments at two levels of ascorbic acid and two levels of dehydroascorbic acid with two copper concentrations (2 + 2) × 2 would have provided a better balance of information but this was not appreciated at the time. Treatments of ascorbic acid (Asc. I) without added copper, dehydroascorbic acid without copper and copper (Cu1) alone were included in each trial. Duplicate trials were run over the pH ranges 6·95–6·85 and 6·45–6·4.

Two trials were also run using unbuffered linoleate emulsion at ~pH 4·7. All treatments were not duplicated; one trial corresponded to the 3 × 3 design, the other to the (3 + 1) × 2. Sufficient treatments were duplicated to obtain an error estimate for these treatments.

Results

Tables I, II and III show the significant differences between main treatment effects at selected pH values. In Figs 1, 2 and 3, only the results from high ascorbic level treatments are graphically represented. While differences between treatments were more evident at the higher ascorbic acid levels, the pattern shown in the graphs was consistent with that revealed by statistical analysis over the complete range of concentrations.

At all pH values studied there was a strong positive relationship between ascorbic acid concentration and the rate of linoleic acid oxidation in the presence of copper. Even those treatments with low levels of ascorbic acid showed a consistently higher rate of oxidation than the copper only treatment. Oxidation rates of the high ascorbic acid level treatments were significantly greater (usually at 1% level) than those of the copper only or lower ascorbic acid level treatments. The control (Cu1 only) showed an increasing oxidation rate with decreasing pH, with a maximum value at ~pH 5·7.

TABLE I

Significant differences (S.D.) between main treatment effects at pH 6·9

	Ascorbic acid			Copper			Interaction
		I	II		3	1	
Initial oxygen consumption:							Highly significant interaction
S.D. 5% level 3·92	III	14·74	4·12	2	7·37	1·02	
S.D. 1% level 5·58	II	10·62		1	6·35		
				Copper			
					1	2	
Total oxygen consumption:							No interaction
S.D. 5% level 218·2	III	1103·0	373·7	3	231·4	148·9	
S.D. 1% level 310·3	II	729·3		2	82·5		
TBA values:							
S.D. 5% level 0·2118	III	0·8005	0·3851	3	0·2218	0·1478	No interaction
S.D. 1% level 0·3013	II	0·4154		2	0·0740		

TABLE II

Significant differences (S.D.) between main treatment effects at pH 6·35–6·45

	Ascorbic acid			Copper			Interaction
		I	II		3	1	
Initial oxygen consumption:	III	14·92	4·45	2	11·13	0·25	Highly significant interaction
S.D. 5% level 1·06							
S.D. 1% level 2·30	II	10·47		1	10·88		
					Copper		
					1	3	
Total oxygen consumption:	III	1192·3	353·5	2	104·2	77·2	Significant interaction
S.D. 5% level 148·6							
S.D. 1% level 213·4	II	838·8		3	27·0		
					Copper		
					1	2	
TBA values:	III	0·6787	0·2294	3	0·3559	0·0767	No interaction
S.D. 5% level 0·1826							
S.D. 1% level 0·2598	II	0·4493		2	0·2792		

TABLE III

Significant differences (S.D.) between main treatment effects at pH 5·5–5·75

	Ascorbic acid			Copper			Interaction
		I	II		3	2	
Initial oxygen consumption:	III	8·04	2·38	1	5·38	0·51	Significant interaction
S.D. 5% level 1·91							
S.D. 1% level 2·57	II	5·66		2	4·87		
					Copper		
					3	1	
Total oxygen consumption:	III	485·9	160·3	2	72·0	51·4	No interaction
S.D. 5% level 53·1							
S.D. 1% level 71·6	II	325·6		1	20·6		
					Copper		
					1	2	
TBA values:	III	0·4166	0·1687	3	0·1647	0·0194	No interaction
S.D. 5% level 0·0638							
S.D. 1% level 0·0860	II	0·2479		2	0·1453		

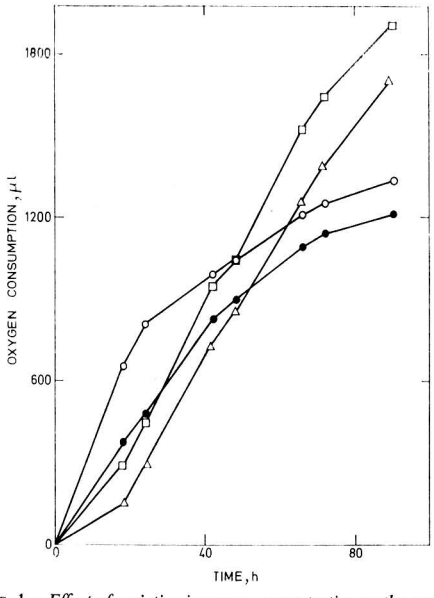


FIG. 1. *Effect of variation in copper concentration on the promotion of linoleic acid oxidation by ascorbic acid at pH 6·9*

All treatments at Ascorbic I level (10^{-3} M)
□ Copper 1 (10 ppm); ○ Copper 3 (0·1 ppm);
△ Copper 2 (1·0 ppm); ● no added copper

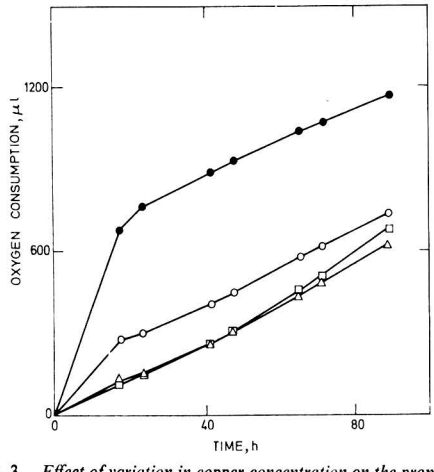


FIG. 3. *Effect of variation in copper concentration on the promotion of linoleic acid oxidation by ascorbic acid at pH 5·6*

All treatments at Ascorbic I level (10^{-3} M)
□ Copper 1 (10 ppm); ○ Copper 3 (0·1 ppm);
△ Copper 2 (1·0 ppm); ● no added copper

In the initial stages (Fig. 1 and Table I) higher oxygen consumption rates were recorded at lower copper concentrations than at higher ones but as oxidation proceeded the pattern changed with total oxygen consumption at 90 h being positively related to copper concentration. Ascorbic acid had a highly significant positive effect on the rate of oxygen uptake. At the initial stages of oxidation, copper appeared to have quite a strong inhibitory effect on the ascorbic acid-induced fatty acid oxidation, while at later stages there was a

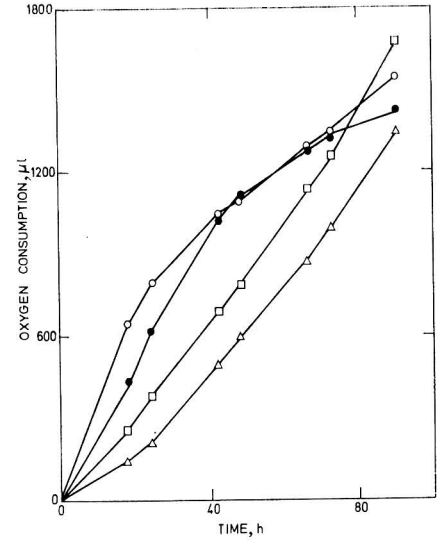


FIG. 2. *Effect of variation in copper concentration on the promotion of linoleic acid oxidation by ascorbic acid at pH 6·45*

All treatments at Ascorbic I level (10^{-3} M)
□ Copper 1 (10 ppm); ○ Copper 3 (0·1 ppm);
△ Copper 2 (1·0 ppm); ● no added copper

rather less strongly marked promotional effect on the oxygen uptake.

Fig. 2 shows the pattern of results to be broadly similar to that at pH 6·9. However the promotional influence of copper on oxygen consumption became evident at a later stage in the oxidation. Table II gives significance of differences between treatments in the factorial analysis. The picture here is again substantially the same as at the higher pH values. TBA values, however, indicate that malonaldehyde production is more emphatically a positive function of copper concentration at pH 6·45 than at pH 6·9. The interactions noted appear to be mainly a result of the difference between the effect of copper at high levels of ascorbic acid and that at low levels of ascorbic acid. The treatment differences are particularly significant at the Asc. I level with high initial oxygen consumption being favoured again by a large excess of ascorbic acid over copper. In the final oxygen consumption figures the significant copper effect shown can be wholly attributed to the difference between treatments at the high level of ascorbic acid. Overall, inhibition of ascorbic acid-induced oxidation of linoleic acid by copper was greater at this pH than at the higher. On the other hand, copper appeared to promote malonaldehyde production more strongly at this pH than at the higher pH value.

Although lower overall rates of oxidation are evident from Fig. 3, it can be seen that their dependence on copper concentration at this pH shows a continuation of the trend which appeared to be emerging when the two higher pH values were compared. Here total oxygen consumption was greater at low copper concentrations than at high copper concentrations. Inhibition of fatty acid oxidation was reflected by the oxygen consumption figures after 90 h, whereas previously it was evident only at earlier stages in the oxidation. It is true that this is emphasised in the graph by the inclusion of the ascorbic acid/no added copper treatment. This inclusion is statisti-

cally valid although the treatment could not be included in the factorial analysis because of the limited number of treatments. The interpretation of the results given is supported, though rather less strongly, by the factorial analysis (Table III).

The dependence of total oxygen consumption on copper concentration has been quite strongly reversed in comparison to that at pH values previously considered. There was again a strong positive relationship between copper concentration and TBA value – evidence of copper-catalysed malonaldehyde production.

There were insufficient replications of treatments for factorial analysis of the main effects in unbuffered emulsions at pH 4·7. Analysis of variance in treatments which were replicated are shown in Table IV. It can be reasonably assumed that the trends which were evident in the buffered emulsions are continued here.

Fig. 4 shows the pattern at high levels of ascorbic acid. Copper had no significant effect in the initial oxidation stages. As the oxidation proceeded, however, copper had a pronounced inhibitory effect on ascorbic acid-induced fatty acid oxidation. Maximum oxidation rates were attained very much later than was the case with the buffered emulsions.

TABLE IV

Means and differences of total oxygen consumption, oxygen consumption at 18 h and TBA values of copper and ascorbic acid in unbuffered emulsions at pH 4·7

Copper and ascorbic acid levels	Total oxygen consumption, μl	Oxygen consumption at 18 h, μl/h	TBA values (optical density)
Asc.I/Cu1	213·0	4·750	0·2100
Asc.I/Cu3	460·0	5·900	0·3660
Asc.I	1094·5	5·400	0·8345
Asc.II/Cu1	130·0	1·300	0·2220
Asc.II/Cu3	91·5	2·000	0·1190
Asc.III/Cu1	97·0	0·900	0·2025
Asc.III/Cu3	43·0	0·650	0·0570
Cu1	80·0	0·750	0·1650
S.E. of diff.	71·0	1·071	0·0778
Nec. diff. 5%	168·0	2·532	0·1839
1%	248·7	3·748	0·2722

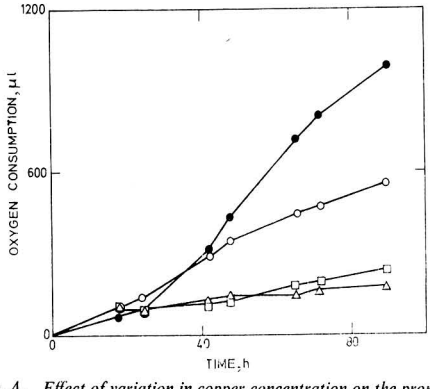


FIG. 4. *Effect of variation in copper concentration on the promotion of linoleic acid oxidation by ascorbic acid at pH 4·7 (unbuffered emulsion)*

All treatments at Ascorbic I level (10^{-3} M)
□ Copper 1 (10 ppm); ○ Copper 3 (0·1 ppm);
△ Copper 2 (1·0 ppm); ● no added copper

It does not seem inconsistent with the other results to suppose that the differences in oxidation behaviour between the buffered and unbuffered emulsions can be largely attributed to pH effects.

The trends shown in Figs 1–4 are summarised in Fig. 5. Different pH values from those used in the preceding graphs were chosen since the purpose of summary was partly for comparison with dehydroascorbic acid-induced oxidation (as shown in Fig.6). Effects of pH change on the pro-oxidant activity of the ascorbic acid, in the presence of high levels of copper, and with no added copper, were contrasted.

When no copper was added total oxygen consumed appeared to vary a little with hydrogen ion concentration. Regression coefficients of TBA and total oxygen consumption against hydrogen ion concentration were not significant (at 5% level) but at high levels of copper the pro-oxidant influence of ascorbic acid was markedly less at low pH than at high pH. A significant negative regression coefficient was obtained for total oxygen consumption against hydrogen ion concentration (though not for TBA values) for the Cu1–Asc. I treatments.

The oxidative effects of dehydroascorbic acid and copper on linoleic acid were not tested so extensively. Tables V, VI and VII give the relevant results. In Fig. 6 the results of the effect of variation in pH and copper on the promotion of linoleic acid oxidation by dehydroascorbic acid at a concentration of 10^{-3}M are graphically illustrated.

At high pH values dehydroascorbic acid had a pronounced pro-oxidant activity with rates of oxygen consumption diminishing as pH values decreased. All dehydroascorbic acid treatments gave significant negative regression coefficients for total oxygen consumption against hydrogen ion concentration: highly significant in the case of Cu1 and no-added-copper treatments. At Cu1 level, the dehydroascorbic acid treatment appeared to be comparable with the corresponding ascorbic acid treatment. However, at lower concentrations

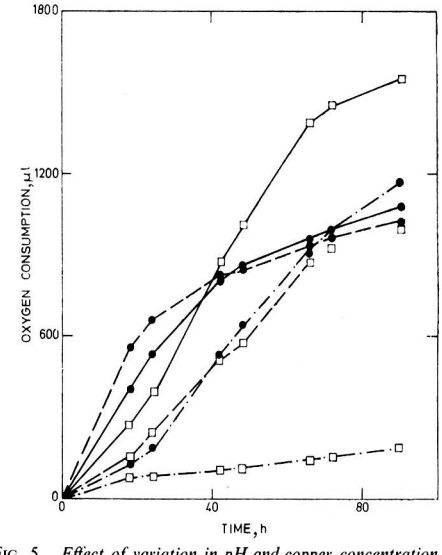


FIG. 5. *Effect of variation in pH and copper concentration on the promotion of linoleic acid oxidation by ascorbic acid*

All treatments at Ascorbic I level (10^{-3} M)
□ Copper 1 (10 ppm); ----- pH 6·45;
● no added copper; —·—·— pH 4·65 (unbuffered);
—— pH 6·85

TABLE V

Means and differences of total oxygen consumption, oxygen consumption at 18 h and TBA values of copper and ascorbic acid and copper and dehydroascorbic acid (DHA) at pH 6·95 – 6·85

Copper, ascorbic acid and dehydro-ascorbic acid levels	Total oxygen consumption, μl	Oxygen consumption at 18 h, μl/h	TBA values (optical density)
Asc.I/Cu1	1624·5	14·85	1·1710
Asc.I/Cu3	1168·5	27·85	0·8750
Asc.I	1085·5	17·90	0·8860
Asc.II/Cu1	744·5	11·15	0·6910
Asc.II/Cu3	560·5	10·25	0·5940
Asc.III/Cu1	313·0	2·95	0·3625
Asc.III/Cu3	252·0	3·50	0·2380
DHA1/Cu1	1440·0	14·80	1·0935
DHA1/Cu3	872·0	8·25	0·6980
DHA1	680·0	6·76	0·7497
Cu1	123·0	1·05	0·1825
S.E. of diff.	41·0	3·90	0·1386
Nec. diff. 5%	92·8	8·83	0·3135
1%	133·3	12·68	0·4504

TABLE VI

Means and differences of total oxygen consumption, oxygen consumption at 18 h and TBA values of copper and ascorbic acid and copper and dehydroascorbic acid (DHA) at pH 6·45 – 6·40

Copper, ascorbic acid and dehydo-ascorbic acid levels	Total oxygen consumption, μl	Oxygen consumption at 18 h, μl/h	TBA values (optical density)
Asc.I/Cu1	1198·0	9·85	0·8610
Asc.I/Cu3	980·5	27·25	0·9185
Asc.I	1127·0	28·90	1·0100
Asc.II/Cu1	481·5	3·95	0·7570
Asc.II/Cu3	497·0	10·45	0·6460
Asc.III/Cu1	228·0	2·45	0·3325
Asc.III/Cu3	277·0	3·90	0·3845
DHA1/Cu1	1235·0	11·25	0·8365
DHA1/Cu3	858·5	7·60	0·9990
DHA1	624·5	9·75	0·7930
Cu1	117·5	1·20	0·2445
S.E. of diff.	140·3	2·64	0·1549
Nec. diff. 5%	312·6	5·88	0·3541
1%	444·6	8·37	0·4908

of copper the differences between the dehydroascorbic acid and the ascorbic acid treatments became more marked. The oxidation rate diminished with decreasing pH at low and at high copper concentrations in contrast to the oxidation pattern in the ascorbic acid treatments. The second notable point of difference was the absence of high initial oxidation rates in the low copper treatments. This was a prominent feature of the ascorbic acid treatments.

It is useful to point out at this stage that the TBA value is essentially a measure of secondary oxidation. The reversal of the tendency shown by oxygen consumption data is not therefore inexplicable. The fact that two distinct patterns of oxidation did emerge despite the conflicting dependencies of TBA value and oxygen consumption on copper concentration is indicative that the pattern of the primary hydroperoxidation process is represented by oxygen consumption figures – though, no doubt, less accurately than by a more direct measure of primary oxidation.

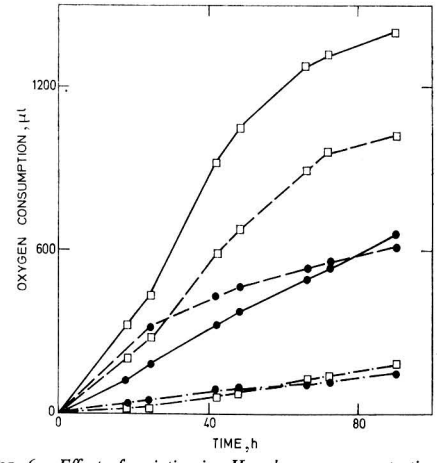


FIG. 6. *Effect of variation in pH and copper concentration on the promotion of linoleic acid oxidation by dehydroascorbic acid*

All treatments at Dehydroascorbic I level (10^{-3} M)
□ Copper 1 (10 ppm); ----- pH 6·45;
● no added copper; —·—·— pH 4·65 (unbuffered);
—— pH 6·85

TABLE VII

Significance of regression of selected figures from various treatments for total oxygen consumption (μl) and TBA values (optical density) against hydrogen ion concentration

	Asc. I/ Cu 1		Asc. I/ Cu 3		Asc. I		DHA/ Cu 1		DHA/ Cu 3		DHA	
	Y^1	Y^2	Y^3	Y^4	Y^5	Y^6	Y^7	Y^8	Y^9	Y^{10}	Y^{11}	Y^{12}
[H⁺]	TBA	O_2	TBA	O_2	TBA	O_2	TBA	O_2	TBA	O_2	TBA	O_2
$1·122 \times 10^{-7}$	1·311	1701	0·903	1220	0·847	1090	1·282	1476	0·689	842	n.d.	n.d.
$1·413 \times 10^{-7}$	1·031	1548	0·847	1117	0·925	1081	0·905	1404	0·707	902	0·740	659
$1·995 \times 10^{-7}$	n.d.	n.d.	0·782	1254	0·837	939	0·628	1354	0·873	1204	0·587	606
$3·548 \times 10^{-7}$	0·669	997	0·762	841	0·955	1028	0·755	1026	0·988	758	0·876	618
$3·981 \times 10^{-7}$	1·053	1399	1·075	1120	1·065	1226	0·918	1444	1·010	959	0·710	631
$5·624 \times 10^{-6}$	0·439	458	0·282	366	0·416	989	0·508	653	0·321	291	0·373	490
$2·239 \times 10^{-5}$	0·245	185	0·295	357	0·739	1177	0·331	181	0·142	191	0·141	152
Significance of regression	n.s.	*	*	*	n.s.	n.s.	n.s.	**	*	*	*	**

Discussion

The ascorbic acid radical,[19] or free cuprous ions[20–22] have been considered as alternative pro-oxidant species in ascorbic acid – copper catalytic systems. In either case it would be expected that the rate of fatty acid oxidation would be positively related to copper concentration in the presence of sufficient ascorbic acid to reduce all copper to the cuprous form. Yamazaki & Piette[23] have shown that ascorbic acid radical concentration is proportional to the rate of oxidation by ascorbic acid oxidase. Rate of ascorbic acid oxidation has been shown to be proportional to copper concentration at $\sim 2 \times 10^{-5}$ mole/l copper.[24] Excess of ascorbic acid would ensure that copper was in the cuprous form. Thus in the present investigation both ascorbic acid radical and cuprous ions would have been proportional to the amount of copper added, at least in the treatments at high levels of ascorbic acid.

Although rates of fatty acid oxidation were very much greater in the presence of copper and ascorbic acid than with either copper alone or ascorbic acid without any reactive metal, it was found that copper showed marked inhibition of ascorbic-induced fatty acid oxidation, particularly at low pH values. It is clear that neither of the above alternatives can, in themselves, explain the mechanism operating the oxidative systems used.

Metallic inhibition of lipid oxidation is usually ascribed to termination of free radical chains by redox reaction between radical and metal, involving intermediate metal–radical complexes.[25] Kharasch & Fono[26] have also suggested that copper salts are capable of deactivating some radicals by trapping them in a loose bound metal–radical complex. Ascorbic acid would presumably compete with free radicals for positions in the co-ordination sphere of the metal ion and would therefore tend to inhibit metallic termination of radical chains and favour radical propagation reactions. High oxidation rates conceivably result from a predominance of propagation reactions over chain termination in a manner similar to that proposed by Haber & Weiss[27] for the catalysis of hydrogen peroxide breakdown by low concentrations of ferrous ion. The implications here are that the reduced metal ion, in a form other than ascorbate complex, is the active pro-oxidant species but, after initiation of oxidation, metallic reactions are unimportant. The function of ascorbic acid would merely be to maintain a low concentration of the reduced ion in an active form (i.e. not complexed with ascorbic acid). The very rapid initial oxidation rates do suggest a more active role for ascorbic acid than this. Although not wholly inconsistent with the model proposed above, particularly in view of the undoubted presence of some oxidation products in the linoleic acid used, high initial rates of oxidation indicate that either ascorbic acid or a metal–ascorbic complex play some direct role in the oxidation process.

Subsequent experiments[18] demonstrate that a low concentration of cuprous ions is not a sufficient condition for high rates of oxidation. Again, experiments with copper chelating agents[18] show that ascorbic acid is ineffective as a pro-oxidant in the complete absence of reactive metal, at least under the experimental conditions. It is probable that an association between metal and ascorbic acid is a necessary step in the pro-oxidation mechanism and further, that this association has an importance beyond the mere reduction of copper.

The work of Weissburger & Luvalle[24] and of Wilkinson[12] suggested that the oxidation of ascorbic acid by copper involves an intermediate copper–ascorbic acid complex. While no published information appears to be available concerning complex formation by copper and dehydroascorbic acid, the arrangement of keto- and hydroxy-groups on the dehydroascorbic acid molecule suggests that such a complex could exist. If it is accepted that copper plays a dual role in lipid oxidation – as initiator and chain terminator – the pH and concentration effects observed in these experiments are consistent with the possible reactions of metal chelates. Thus one could expect the metal to be mainly in the complex form at high ligand concentration/low metal concentration and at higher pH values. These concentration and pH effects could be expected to be pronounced when either the complex had a low stability constant or the chelating group was particularly reactive. Differences in stability constants could possibly explain much of the difference between the pro-oxidant activities of ascorbic and dehydroascorbic acid. Unfortunately there are insufficient data available on stability constants of ascorbic acid–metal complexes and apparently none on metal complexes of dehydroascorbic acid.

However, there are differences between the rates at which copper is reduced by ascorbic acid and by dehydroascorbic acid. A simple qualitive experiment, in which the presence of cuprous ions was detected by 2,9,-dimethyl-1,10-phenanthroline, showed that copper was reduced almost immediately by an excess of ascorbic acid, whereas an excess of dehydroascorbic acid completely reduced copper only after 2–3 h had elapsed. This could provide a basis of explanation for different pro-oxidant activities not because of the short life of the complex but because of the rapidity of cuprous ion production. For reasons which will be discussed later, it is felt that rate of reduction of cuprous ions is not of great importance in the oxidation mechanism.

Copper inhibition of fatty acid oxidation is apparent in the presence of ascorbic acid but not, or at least not markedly, in the presence of dehydroascorbic acid. This, may be due to the transience of the metal–ascorbic complex compared to the metal–dehydroascorbic complex. Rates of oxidation are depressed by increased hydrogen ion concentration. Once again it is considered that the explanation lies in decreasing complex stability with increasing hydrogen ion concentration. However, if the effect of the chelating group is to prevent chain termination reactions, allowing oxidation to proceed through predominantly chain propagation reactions, it could be expected that the more stable complexing agent would be the more effective pro-oxidant, (i.e. dehydroascorbic acid would be a more effective pro-oxidant than ascorbic acid). Such is not the case. The conversion of ascorbic acid to dehydroascorbic acid appears to have a pro-oxidant effect which is not directly related to the capacity of ascorbic acid to lower the rate of metallic chain termination by chelation.

The oxidation of ascorbic acid by copper could provide two possible sources of free radicals by the proposed mechanisms of oxidation, namely the ascorbic acid radical and cuprous ions. Both may react with molecular oxygen to give HO'_2 radicals. It seems probable that both of these reactions can, in fact, occur, and excercise some pro-oxidant effect. However, the difference between the ascorbic acid treatment and the dehydroascorbic acid treatment seems likely to be caused by the ascorbic radical as a source of free radicals rather than by the more rapid reduction of copper by ascorbic acid. The reasons for this are: (i) copper has been shown to inhibit ascorbic acid-induced fatty acid oxidation; and (ii) at the low concentrations of copper required to promote oxidation, it

seems unlikely that the rate of copper reduction would be a rate-limiting step in the oxidation process. Rate of copper reduction of dehydroascorbic acid would be sufficient to maintain a low level of cuprous ion concentration.

The influences of ascorbic acid and dehydroascorbic acid on lipid oxidation that have been considered are: (i) prevention of metallic chain termination; and (ii) a source of free HO'_2 radicals as a by-product of the reaction between copper and ascorbic acid. There is the possibility that a complex between copper and ascorbic and/or dehydroascorbic acid directly catalyses oxidation of fatty acids. In the first place it may be noted that if such a mechanism were of primary significance it could be expected that pro-oxidant activity would bear a positive relationship to both copper concentration and ascorbic acid concentration. Nonetheless it is interesting to consider some work that has been done on model systems of a comparable nature to the systems under present study.

Udenfriend *et al.*[28] have demonstrated the ability of ascorbic acid, dehydroascorbic acid and related chemicals, in the presence of ferrous ions, to hydroxylate aromatic compounds. Hamilton *et al.*[29–31] have suggested that the hydroxylation mechanism involves a radical-like complex of ascorbic acid, ferrous iron and oxygen. One atom of oxygen is transferred to the substrate while the other is reduced. The reaction does not involve a radical chain. In his original work Udenfriend[28] found that copper was effective but only to the extent of 5–10% of the activity of iron. This may be sufficient however to play a small but significant role in lipid oxidation. Oxygen consumption data show, particularly in the low pH, low copper treatments, that the primary oxidation process is autocatalytic. The general low level of dependence on copper concentration indicates a predominantly free radical mechanism for the primary hydroperoxide process. This is not necessarily true of the secondary oxidation reactions. While the inadequacy of the data does not permit any satisfactory statement to be made about secondary oxidation, the model suggested by Hamilton could have considerable value in this context. In particular, the development of oct-1-en-3-one under relatively mildly oxidative conditions[11,12] suggests that an enzyme-like mechanism is worthy of consideration.

The effect of ascorbic acid on fatty acid oxidation is clearly more complex than mere reduction of metal ion catalysts. There is sufficient evidence to suggest two additional functions of ascorbic acid in primary oxidation: (a) chelation of metal ions and prevention of metal radical chain termination reactions; and (b) generation of free radicals by reaction of the ascorbic acid radical with oxygen.

It seems necessary to invoke both functions to explain fully the role of ascorbic acid but it is not possible to assess the relative importance of either or to say with certainty that these are the only functions of ascorbic acid.

In fact, much more detailed study of end products and reaction rates would be required to test the model proposed and allow a more precise formulation of it.

Interpretation of results is further complicated by extensive secondary oxidation reactions. Beyond making an appropriate distinction between primary and secondary oxidation on the basis of dependence on copper concentration, it was not possible to draw any conclusions about the secondary oxidation mechanism. The models of mixed function oxidase systems proposed by Hamilton may be of some importance in this regard, but again the need for more detailed investigation and more comprehensive analysis is evident.

Acknowledgments

The work was done while one of the authors (W.A.A.) was employed at the Queensland Department of Primary Industries. The assistance and help of several staff members is gratefully acknowledged. One of the authors (W.A.A.) is grateful to the Australian Dairy Produce Board whose grant enabled this work to be done.

Department of Agriculture,
University of Queensland,
Brisbane,
Queensland,
Australia

Received 21 November, 1969

References

1. Forss, D. A., Pont, E. G., & Stark, W., *J. Dairy Res.*, 1955, **22,** 91
2. Patton, S., Barnes, I. S., & Evans, L. E., *J. Am. Oil .Chem. Soc.* 1959, **36,** 280
3. Lillard, D. A., & Day, E. A., *J. Dairy Sci.*, 1961, **44,** 623
4. Badings, H. T., *Ned. Melk-en Zuiveltijdschr.*, 1960, **14,** 215
5. Lundberg, W. O., 'Lipids and their Oxidation', (Eds Schultz, H. W., Day, E. A., & Sinnhuber, R. O.) 1962, p. 35 (Westport, Conn: A.V.I.)
6. Hunziker, O. F., & Hosman, D. F., *J. Dairy Sci.*, 1917, **1,** 320
7. Smith, G. J., & Dunkley, W. L., *XVIth. Int. Dairy Congr.*, 1962, **1,** 625
8. Corbett, W. J., & Tracy, P. H., *Fd Res.*, 1941, **6,** 445
9. Brown, W. C., & Olsen, F. C., *J. Dairy Sci.*, 1942, **25,** 1041
10. Rusch, H. P., & Kline, B. E., *Cancer Res.*, 1941, **1,** 465
11. Stark, W., & Forss, D. A., *J. Dairy Res.*, 1962, **29,** 173
12. Wilkinson, R. A., *C.S.I.R.O. Div. Dairy Res. Int. Rep.*, 1964, No. 4,
13. Pont, E. G., Forss, D. A., Dunstone, E. A. & Gunnish, F., *J. Dairy Res.*, 1960, **27,** 205
14. Koops, J., Tarassuk, M., & Pette, J. W., *Ned. Melk-en Zuiveltijdschr.*, 1959, **13,** 279
15. Patton, S., 'Dairy Products', (Eds Schultz, H. W., Day, E. A., & Sinnhuber, R. O.) 1962, p. 190 (Westport, Conn: A.V.I.)
16. Miller, D. D., & Skaggs, S. R., *Proc. west Div. Am. Dairy Sci. Ass.*, 1959,
17. King, R. L., *J. Dairy Sci.*, 1962, **45,** 1165
18. Allan, W. A., Thesis, University of Queensland
19. Kelley, G. G., & Watts, B. M., *Fd Res.*, 1957, **22,** 308
20. Smith, G. J., & Dunkley, W. L., *J. Dairy Sci.*, 1962, **45,** 170
21. Smith, G. J., & Dunkley, W. L., *J. Fd Sci.*, 1962, **27,** 127
22. Smith, G. J., & Dunkley, W. L., *Archs Biochem. Biophys.*, 1962, **98,** 46
23. Yamazaki, I., & Piette, L. H., *Biochim. biophys. Acta*, 1961, **50,** 62
24. Weissberger, A., & Luvalle, J. E., *J. Am. chem. Soc.*, 1944, **66,** 700
25. Ingold, K. U., 'Lipids and their Oxidation', (Eds Schultz, H. W., Day, E. A., & Sinnhuber, R. O.) 1962, p. 93 (Westport, Conn: A.V.I.)
26. Kharasch, M. S., & Fono, I., *J. org. Chem.*, 1959, **24,** 72, 606
27. Haber, F., & Weiss, J., *Proc. R. Soc. (A)*, 1934, **147,** 332
28. Udenfriend, S., Clark, C. T., Axelrod, J., & Brodie, B. B., *J. biol. Chem.*, 1954, **208,** 731
29. Hamilton, G. A., & Friedman, J. P., *J. Am. chem. Soc.*, 1963, **85,** 1008
30. Hamilton, G. A., Workman, R. J., & Woo, L., *J. Am. chem. Soc.*, 1964, **86,** 3390
31. Hamilton, G. A., Friedman, J. P., & Campbell, P. M., *J. Am. chem. Soc.*, 1966, **88,** 5266

PHOSPHOLIPIDS OF MARINE ORIGIN

V.*—The crab – a comparative study of a marine species (*Cyclograpsus punctatus*) and a fresh water species (*Potamon*)

By A. J. DE KONING

A comparison has been made between phospholipids extracted from a marine crab and from a fresh water crab.

Marine crab (body and viscera) contained a phospholipid fraction which liberated 2-aminoethylphosphonic acid upon hydrolysis, while this substance was absent from the fresh water crab. Marine crab phospholipids had a higher content of phosphatidyl choline (57%), a higher content of phosphatidyl serine (5%) but a lower content of phosphatidyl ethanolamine (22%) than phospholipids from the fresh water crab (respective values: 52, 2 and 27%).

Marine crab phospholipids and non-phosphorylated lipids were richer in C20 plus C22 fatty acids but poorer in C18 : 2 acid, than were the corresponding lipids from the fresh water crab.

Introduction

Previous papers in this series have been concerned with the phospholipids of the hake (*Merluccius capensis* Castelnau), the rock lobster (*Jasus lalandii*), the pilchard (*Sardina ocellata* Jenyns) and the abalone (*Haliotis midae*)[1], marine organisms for which there is no fresh water counterpart. The present paper compares the phospholipids of a marine crab (*Cyclograpsus punctatus*) with those of a fresh water crab (*Potamon*). The marine crab used in this work is very common on the South African shores and the fresh water crab, of comparable size, is very common in the rivers of Lesotho and Natal. It should be mentioned that the Lesotho crab of the genus *Potamon* has not been classified but it corresponds very closely to the *Potamon sidneyi* of Natal.[2] A preliminary report of part of this work has already appeared.[3]

Experimental

Extraction and purification of the lipids

The marine crabs were collected on the South African coast at East London and Cape Town, transported live to the laboratory and extracted within 72 h. Fresh water crabs were caught in a nearby stream and also extracted within 72 h.

The two types of crab were comparable in size as is indicated by their average weights, 3·3 g and 5·0 g, and the amounts of lipid extracted per 100g of fresh tissue, 2·2 g and 2·5 g, for the marine and fresh water crabs respectively.

Marine crabs

90 specimens (total 300 g) were extracted three times with 600 ml of chloroform–methanol (2:1, by vol.) in a Waring blender. The combined extracts were filtered and washed three times in a large volume of water according to Folch *et al.*[4] The purified extract was dried over anhydrous sodium sulphate, filtered and the chloroform evaporated *in vacuo* in a rotary evaporator. A brown oil (6·7 g) with a phosphorus content of 1·1% was obtained.

Fresh water crabs

Fresh water crabs were extracted on several occasions and the following is a typical example. 27 specimens (total 135 g) were extracted three times in a Waring blender with 300 ml of chloroform–methanol (2:1, by vol.). The extract was treated as previously described for the marine crabs, and yielded 3·4 g of a brown oil of phosphorus content 1·0%.

Separation and an analysis of the phospholipids

Total lipids (6·7 g) from the marine crabs were separated into a phospholipid fraction (1·74 g; %P 3·9) and a non-phosphorylated lipid fraction (4·5 g; %P 0·03) by chromatography on activated silica gel in light petroleum (b.p. 40–60°).[5] Similarly, lipids from the fresh water crabs (3·4 g) gave 0·94 g of phospholipids (%P 3·7) and 2·17 g of non-phosphorylated lipids (%P 0·03).

Details of the procedures used for the determination of phospholipid composition i.e., the hydrolytic procedure and chromatography on silicic acid have been described in previous papers[1,5] and only some minor improvements and alterations will be mentioned here. The water-soluble components ethanolamine, serine and 2-aminoethylphosphonic acid were quantitatively determined, after separation on the cation-exchange resin Amberlite C.G.120, with the improved ninhydrin reagent of Moore & Stein[6]; a 4M acetate buffer of pH 5·5 was used instead of the citrate buffer of pH 5·0. The composition of the fatty acids liberated from phospholipids and non-phosphorylated lipids was obtained by gas chromatography of their methyl esters on a Perkin Elmer F11 gas chromatograph, with polyethylene glycol succinate as stationary phase at 190° and helium as carrier gas.

Results and Discusssion

Hydrolytic procedure

Table I records the main constituents of the phospholipids from the two types of crab. A striking difference between these phospholipids is the presence of 2-aminoethylphosphonic acid in the marine crab phospholipids and its complete absence in phospholipids of the fresh water crab. This unusual substance was identified by paper chromatography

* Part IV: *J. Sci. Fd Agric.*, 1966, **17**, 460

and by column chromatography on the cation-exchange resin Amberlite C.G.120.[3] It might, however, be argued that since the whole crabs were extracted, 2-aminoethylphosphonic acid was of dietary origin and was therefore only present in the gut. In order to test this, marine crabs were carefully dissected and the edible white muscle separated from the viscera (i.e., gut, liver, heart, gonads and gills). Both portions were extracted separately with chloroform–methanol (2:1 by vol.) and the body lipids as well as the viscera lipids were found to contain 2-aminoethylphosphonic acid in amounts of 0·1% and 0·08%, respectively. It is therefore a genuine constituent of the marine crab and the invertebrate phylum *Arthropoda* can be added to the phyla: *Protozoa*[7–9], *Coelenterata*[10,11] and *Mollusca*[1,11], which have been shown to contain 2-aminoethylphosphonic acid.

Another significant difference is found in the ethanolamine and serine levels, viz., phospholipids extracted from the marine crab contain 1·63% ethanolamine and 0·64% serine whereas the fresh water crab phospholipids contain 2·10% ethanolamine and 0·21% serine. It appears that the sum of the amounts of ethanolamine and serine for the two types of crab is almost identical but the individual values differ markedly. No other similar studies appear to have been made, so that comparisons with other organisms are not possible, but it seems worth investigating whether or not this is a general phenomenon.

The choline content of the marine crab phospholipids is rather higher (9·5%) than that of the fresh water crab phospholipids (8·5%) and this is reflected in a higher phosphatidyl choline content of the phospholipids extracted from the marine crab. (Table II.)

TABLE I

Main constituents of crab phospholipids

Constituents, %	Marine crab	Fresh water crab
P	3·9	3·7
Total choline	9·5	8·5
Lecithin-choline	8·44	7·99
Sphingomyelin-choline	0·79	0·47
Lysolecithin-choline	0·26	0·04
Sphingosine	1·3	1·1
Ethanolamine	1·63	2·10
Serine	0·64	0·21
Myo-inositol	0·88	0·84
2-Aminoethylphosphonic acid	0·17	nil

TABLE II

Composition of marine crab and fresh water crab phospholipids
(Expressed as whole numbers, as % of total phospholipids)

Class	Marine crab	Fresh water crab
Phosphatidyl choline	57	52
Phosphatidyl ethanolamine	22	27
Phosphatidyl serine	5	2
Phosphatidyl inositol	4	4
Lyso phosphatidyl choline	1	1
Sphingomyelin	5	4
Cardiolipins	5	10
Ceramide aminoethylphosphonate	1	nil

It is noteworthy that the *myo*-inositol contents are almost identical.

Column chromatography on silicic acid

The separation of the phospholipids in component fractions is shown in Fig. 1(a), and (b); 1(a) refers to the fresh water crab phospholipids and 1(b) to the marine crab phospholipids. 15 ml fractions were eluted with chloroform–methanol (*C-M*) mixtures of increasing methanol content (Fig. 1) and by combination of the appropriate tubes fractions A–G were obtained for the fresh water crab and fractions A–I for the marine crab phospholipids. The fractions were characterised by chromatography on silicic acid-impregnated paper, followed by staining with the dye Edicol Supra Ponceau 4RS and by paper chromatography of the water-soluble compounds after hydrolysis with 2M-HCl.[5]

The results for the fresh water crab were as follows:

Fraction A consisted of cardiolipins (phosphatidic acids).

Fractions B and C contained ethanolamine, serine and a trace of inositol and therefore consisted largely of phosphatidyl ethanolamine and phosphatidyl serine.

Fraction D contained inositol and traces of ethanolamine and serine and was thus mainly phosphatidyl inositol.

Fraction E was pure phosphatidyl choline, since it gave a single spot on silicic acid-impregnated paper and only choline was found in the water-soluble hydrolysis products.

Fraction F consisted of a mixture of phosphatidyl choline (30%) and sphingomyelin (70%).

Fraction G consisted of a mixture of sphingomyelin (50%) and lysolecithin (50%). Both fractions F and G gave positive reactions for choline and sphingosine while chromatography on silicic acid-impregnated paper revealed the presence of phosphatidyl choline, sphingomyelin and lysolecithin. The ratio of compounds present could be estimated by eluting the stains with a mixture of tert. butanol–1·2M-HCl (1:1) and determining the optical density at 510 nm.

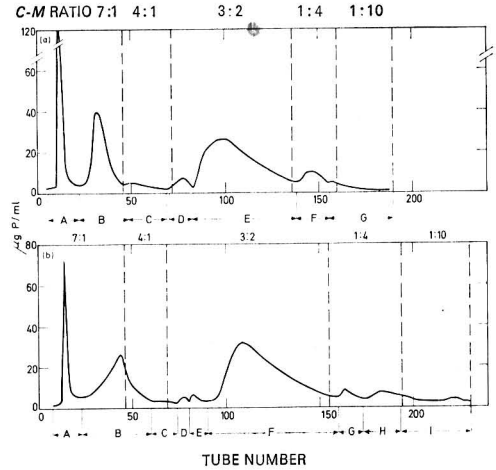


FIG. 1. *Separation of crab phospholipids into component fractions by chromatography on 70 g of silicic acid*

(a) 680mg of fresh water crab phospholipids

(b) 680mg of marine crab phospholipids

Marine crab phospholipids gave fractions A-I whose composition is indicated below:

Fraction A consisted of cardiolipins (phosphatidic acids).

Fraction B consisted of phosphatidyl ethanolamine, phosphatidyl serine and a small amount of phosphatidyl inositol.

Fractions C, D and E contained ethanolamine, serine inositol and 2-aminoethylphosphonic acid and therefore consisted of a mixture of phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol and probably ceramide aminoethylphosphonate.

Fraction D in particular was rich in 2-aminoethylphosphonic acid, confirmed by paper chromatography as well as column chromatography on Amberlite C.G. 120 at 50°.[3] It should also be mentioned that fractions D and E had an unidentified ninhydrin-reactive compound of R_f value 0·72 in the solvent butanol–acetic acid–water (4:1:5, by vol.).

Fractions F and G were pure phosphatidyl choline.

Fraction H consisted of sphingomyelin (80%) and phosphatidyl choline (20%), and fraction I was a mixture of sphingomyelin (84%), phosphatidyl choline (8%) and lysolecithin (8%).

Fatty acids derived from phospholipids and non-phosphorylated lipids

The average equivalent weight and unsaturation of the fatty acids liberated from the phospholipids and non-phosphorylated lipids of both types of crabs is given in Table III. It is clear that phospholipids have higher equivalent weights and degrees of unsaturation than the corresponding non-phosphorylated lipids, as was found in previous work.[5] In addition, the marine crab lipids have higher average equivalent weights and degrees of unsaturation than fresh water crab lipids.

The fatty acid composition of the different lipids, as determined by gas chromatography of their methyl esters, is shown in Table IV. The results indicate that lipids from the marine crab have much lower linoleic acid (C18:2) contents than corresponding lipids of the fresh water crab; this is especially evident in the non-phosphorylated lipids where the figures are 6% and 23%, respectively. On the other hand, marine crab lipids are richer in the fatty acids of carbon chain length 20 and 22. This again is more pronounced in the non-phosphorylated lipids (20% C20 plus C22 acids compared with 12% for the fresh water crabs).

TABLE III

Average equivalent weight and unsaturation of the fatty acids liberated from crab phospholipids and corresponding non-phosphorylated lipids

Source	Equivalent wt.		Unsaturation (double bonds per mol.)	
	phospho-lipids	non-phosphoryl-lated lipids	phospho-lipids	non-phosphory-lated lipids
Marine crabs	317	283	2·5	1·8
Fresh water crabs	303	275	2·2	1·5

Lovern in a series of investigations [12,13] compared the fatty acid compositions of some marine and fresh water fish and also of marine and fresh water plankton. No distinction was made between phospholipids and non-phosphorylated lipids and his results are therefore representative of the total fats. Lovern's conclusions were that lipids of marine and fresh water organisms are of two different types, and, generally speaking, a marine type of fat has more C20 and C22 unsaturated fatty acids and less C16 and C18 unsaturated fatty acids as compared with those of the fresh water type. Present results for the crabs are in agreement with these conclusions, but it is interesting to note that the distinction between marine type and fresh water type is more pronounced with the non-phosphorylated lipids that it is with the phospholipids.

Phospholipid composition

The average phospholipid composition of both types of crab as computed from the values of Table I and III and from the results of the separation on silicic acid is shown in Table II. In general, their composition is similar, although there are interesting differences. In the first place, the detection of ceramide aminoethylphosphonate in the marine crab and the absence of this substance in fresh water crab is noteworthy. In addition, marine crabs phospholipids have a significantly higher content of phosphatidyl choline (57%) than the fresh water crab phospholipids (52%). The phosphatidyl ethanolamine content of marine crab phopholipids (22%) is lower than that of fresh water crab phospholipids (27%) whereas the phosphatidyl serine content is higher (5% against 2%).

Acknowledgments

The author wishes to thank Miss M. Courtenay-Latimer, director of the East London Museum, for identification of the marine crabs and Mrs. S. J. Alexander of the University of Natal, Pietermaritzburg, for identification of the fresh water crabs. The author also thanks Dr. L. Machan for guidance in dissecting the crabs and the University for a research grant.

TABLE IV

Fatty acid distribution in crab phospholipids and the corresponding non-phosphorylated lipids

(Content of each acid is quoted as % of the corresponding methyl ester; % are recorded as whole numbers and those <,1 omitted)

Fatty acid	Phospholipids		Non-phosphorylated lipids	
	Marine crab*	Fresh water crab	Marine crab	Fresh water crab
14 : 0	3	1	5	2
14 : 1				2
15 : 0			1	
16 : 0	14	15	21	17
16 : 1	3	2	11	10
16 : 2 and 17 : 0	2	1	3	1
18 : 0	11	12	4	4
18 : 1	22	23	29	29
18 : 2	4	12	6	23
20 : 0	1	1		
20 : 1	3	2	5	6
20 : 4	8	3	1	
20 : 5	8	11	5	2
22 : 5	4			
22: 6	17	18	9	4

* Traces of C:24 acids were detected in this sample

University of Botswana, Lesotho and Swaziland,
Roma, Lesotho,
Southern Africa

Received 19 January, 1970

References

1. de Koning, A. J., *J. Sci. Fd Agric.*, 1966, **17**, 460
2. Alexander, S. J., Personal communication
3. de Koning, A. J., *Biochim. biophys. Acta*, 1970, **202**, 187
4. Folch, J., Ascoli, I., Lees, M., Meath, J. A., & le Baron, F. N., *J. biol. Chem.*, 1951, **191**, 833
5. de Koning, A. J., *J. Sci. Fd Agric.*, 1966, **17**, 112
6. Moore, S., & Stein, W. H., *J. biol. Chem.*, 1954, **211**, 907
7. Dawson, R. M. C., & Kemp, P., *Biochem. J.*, 1967, **105**, 837
8. Horiguchi, M., & Kandatsu, M., *Nature, Lond.*, 1959, **184**, 901
9. Kandatsu, M., & Horiguchi, M., *Agric. biol. Chem., Tokyo*, 1962, **26**, 721
10. Kittredge, J. S., Roberts, E., & Simonsen, D. G., *Biochemistry*, 1962, **1**, 624
11. Quinn, L. D., *Biochemistry*, 1965, **4**, 324
12. Lovern, J. A., *Biochem. J.*, 1937, **31**, 755
13. Lovern, J. A., *Biochem. J.*, 1935, **29**, 847

BIOCHEMICAL COMPOSITION AND NUTRITIVE VALUE OF KRILL *(EUPHAUSIA SUPERBA* DANA*)*

By G. S. SIDHU, W. A. MONTGOMERY, GWENDA L. HOLLOWAY, A. R. JOHNSON and D. M. WALKER*

Frozen samples of Antarctic krill (*Euphausia superba* Dana) were found to contain 71·4% moisture, and on a dry matter basis 24·6% lipid, 49% protein (N × 6·25), 2·5% chitin, and 9·8% ash. Fatty acids from the crude lipid fraction were made up of 43·8% saturated, 32·8% mono-unsaturated, and 23·4% polyunsaturated types.

Krill protein concentrate prepared as a light, free-flowing powder by isopropanol extraction of krill contained 74·3% crude protein, 15·4% ash, 6% chitin and 0·3% lipid, and was rich in lysine, arginine, tryptophan, and threonine. The protein efficiency ratio of this concentrate was found to be the same as that of casein.

The significance of these results in relation to those obtained for other fish protein concentrates is discussed.

Introduction

Euphausia superba Dana (Antarctic krill) is a small crustacean belonging to the order Euphausiaceae. It is a herbivore representing the first trophic level in Antarctic food chains. It forms the main diet of baleen whales, and is also eaten by other animals such as smaller humpback, sei, minke (or little-piked) whales, crabeater seals, penguins, and petrels.[1] Adult krill attain a length of 4–6 cm and swarm in large numbers in the surface layers of the Antarctic waters during the southern summer months. The dwindling number of whales resulting from over-whaling, the consequent increased abundance of krill in the Antarctic seas, and man's demand for high-quality protein, have created world-wide interest in the exploitation and utilisation of krill. Tentative estimates[2] show that from 100 to 500 million tons of krill could be harvested annually, in contrast to a world total catch of 66 million tons of fish/year.

Of all the zooplankton which have been investigated as sources of high-quality protein, krill seems to provide the best possibility for commercial exploitation because of its swarming behaviour and relatively large size.[3] Although protein and non-protein amino nitrogen of zooplankton constitutes a very high proportion of the total nitrogen,[4] in a limited number of rat-feeding experiments, the small crustacean zooplankton when used alone or in combination with commercial rat feeds, did not give satisfactory growth,[3] apparently due to a poor assimilation of nutrients. On the other hand the largest mammals, whales, and other animals thrive on a diet of krill. Although there have been reports[5–8] on the biochemical composition of krill, more information is required on its nutritive value and the biological value of its protein. This paper describes the biochemical composition of krill and the nutritive value of a protein concentrate prepared from it.

Experimental

Samples

Two samples of krill (A and B) were obtained through the courtesy of the Mawson Institute of Antarctic Research. Sample A was collected south of the Tasman Sea on 31 May, 1968, and sample B north of the Ross Sea on 6 April, 1968, by the crew of the U.S. research ship *Eltanin*. The samples were transported, frozen and held at –20° at the laboratory until required for use. The samples were identified (McWilliam, P., personal communication) as *Euphausia superba* Dana, of adolescent stage, and had an average length of 3·75 cm.

Krill protein concentrate

Krill protein concentrate was prepared on a laboratory scale using a slight modification of a method described by the U.S. Bureau of Commercial Fisheries[9] in which frozen krill in 1–4 kg portions was extracted with isopropanol. The isopropanol was removed from the final product by heating at 70° in a vacuum oven for 16 h, followed by steaming the product and again drying in a vacuum oven at 70°.

Analytical procedures

The moisture content was determined by A.O.A.C.[10] method 22.003 and total volatiles by heating in a vacuum oven at 80°. Total lipids were determined by the method of Smith *et al.*[11] Non-protein nitrogen (*NPN*) (in trichloro-

* Present address: Department of Animal Husbandry, Sydney University, New South Wales 2006, Australia

acetic acid extract), and total N in the whole krill and its protein concentrate were determined by the method of McKenzie & Wallace[12] and chitin was determined by the method of Hackman.[13] The ethanol extract of soluble non-protein primary amino-N was prepared according to the method of Jones[14] and the estimation carried out using the method of Satake *et al.*[15] Ash was estimated by A.O.A.C.[10] method 13.006, P by methods 22.07 and 2.028, and Ca, Na, K, Mg, Fe, Cu, Zn, and Mn by methods 2.087–2.090 using an atomic absorption spectrophotometer. Fluoride was determined by a specific ion electrode manufactured by Orion Research Inc.[16] Methyl esters for fatty acid analysis were prepared from the crude lipid according to the method of Luddy *et al.*[17] Methyl esters were analysed with a Packard Model 7508 gas–liquid chromatograph having a flame-ionisation detector and dual-column oven operating at 170° with a 6 ft × 3/16 in i.d. glass column, packed with Gas-Chrom P support, coated with 25% ethylene glycol succinate liquid phase containing 2% phosphoric acid.[18] Total volatile bases and trimethylamine (TMA) were determined according to the method of Montgomery.[19] Free fatty acids (*FFA*) were determined by a slight modification of A.O.A.C.[10] method 26.060.

Amino acid composition of the concentrate was determined after hydrolysis by the method of Smith & Back[20] in a Beckman amino acid analyser. Tryptophan was determined spectroscopically according to the method of Edelhoch[21] and colorimetrically according to the method of Opieñska-Blauth *et al.*[22] after enzymic hydrolysis of krill protein concentrate with Pronase-P (Kaken Chemical Co. Ltd., Tokyo).

Biological tests

The protein efficiency ratio (*PER*) of protein concentrate from sample B was determined over a period of 4 weeks on weanling (22-day old) rats, 5 males and 5 females in each group, using the A.O.A.C.[10] basal diet and light white casein (B.D.H., Poole, England) as control. The protein level in both the diets was 10%. There was insufficient sample A to prepare protein concentrate for a rat-feeding trial. At the end of the feeding experiment the haemoglobin content of the blood, and the fresh weights of the kidneys and livers of the experimental animals were determined. Microscopic sections cut from the kidneys were examined under an optical microscope for histological changes. The carcasses after removal of the livers, kidneys, and blood samples were dried in a freeze-dryer and subsequently in an oven at 110° to a constant weight to determine the carcass dry matter.

TABLE I

Biochemical composition of krill

	Sample A	Sample B
Moisture, %	72·0	70·7
Total volatiles, %	74·3	73·2
	% on dry matter basis	
Lipid	21·9	27·4
Crude protein (N × 6·25)	48·9	49·0
Non-protein N	3·1	4·5
Non-protein primary amino-N	0·88	1·6
Total volatile bases (N)	0·16	0·26
Trimethylamine (N)	nil	nil
Chitin	2·8	2·3
Ash	10·6	9·0

Results and Discussion

Analytical data on the composition of the two samples of krill are set out in Table I. Sample B contained a higher percentage of lipid, *NPN*, total volatile bases and non-protein primary amino-N than sample A. The levels of the last three constituents suggest that some deterioration in quality had occurred in both samples during transit. Much lower values for lipid content have been reported by Burkholder *et al.*[5] and some Japanese workers,[7,23] although other workers[8,24] have reported values close to those in Table I. Stage of development and season had been observed to have a marked effect on lipid content which was reported[6] to vary from 2 to 26%. The *NPN* in sample A lies between the values of 3·81% reported for *Euphausia pacifica* (whole) and 2·47% for krill muscle.[23] The *NPN* content of sample B cannot be considered representative for fresh krill.

The lipid extracted from the two samples of krill contained 2·2% free fatty acids. The individual fatty acids in these two samples were present in similar amounts and an average composition is given in Table II. The saturated fatty acid constituted 43·8%, and monounsaturated 32·8% of the total. This was in contrast to the 28–30% saturated and 40·1% monounsaturated fatty acids reported by Nonako & Koizumi.[24] The content of polyunsaturated fatty acids (23·4%) in krill was, however, in agreement with their results and was close to the level of these acids in the Antarctic fin whale blubber.[25] The diatom *Fragilariopsis antarctica* on which krill feeds could be a source of these fatty acids or they could arise through synthesis endogenously. Zooplankton crustacea have the capacity to synthesise long-chain polyunsaturated fatty acids by elongation and desaturation of medium-chain fatty acids obtained from dietary plankton.[26,30] Ultimately these polyunsaturated fatty acids are accumulated in the whale through the Antarctic food chain (phytoplankton → *E. superba* → whale).

Only small amounts of 20:1 and 22:1 fatty acids were present in the lipid of krill. This is also true of the lipid of most species of marine plankton[31,32] some of which probably form the diet of krill, and also of Antarctic baleen whale[25] which feeds on krill. However, the zooplankton *Meganyctiphanes norvegica* (M. Sars) has a higher content of these acids[33] as do fin whales from north Atlantic waters.[34]

TABLE II

Fatty acid composition of krill lipids

Fatty acid	Weight, %
12 : 0*	0·2
14 : 0	18·7
14 : 1	0·5
15 : 0	0·6
16 : 0	24·0
16 : 1	11·2
17 : 0	0·9
18 : 0	0·9
18 : 1	20·5
18 : 2	2·3
20 : 0	tr.
20 : 1	0·6
20 : 2	0·6
22 : 1	tr.
22 : 5	13·2
22 : 6	5·9

* Figures represent the number of carbon atoms and the number of double bonds

The protein concentrate in its final form was a free flowing, light, greyish white powder, almost odourless and with a bland taste. The yield was 13·7% and 10·1% of the original frozen krill from samples A and B respectively. The composition is given in Table III. The concentrate from sample A had a higher percentage of protein than that from sample B. When 5–10% of it was added to tortillas and biscuits no change in the taste and flavour could be detected. The chitin prepared from krill had a N content of 6·06%. If allowance is made for chitin nitrogen (which is biologically unavailable) the protein content reported in Table III would be lowered by 2·3%. The protein percentage of krill protein concentrate was lower than that reported[35] for fish protein concentrate prepared from red hake (81%) while the ash and lipid contents were higher (13·5% and 0·18% respectively, for red hake). The higher lipid content of krill and/or the presence of oxidised lipid (due to the prolonged period of storage) which binds strongly to protein could account for less efficient removal of lipid by isopropanol from krill than from red hake. The protein concentrate prepared from herring[36] which is a fatty fish had higher lipid content than that prepared from whole cod which has a low lipid content.

Phosphorus, sodium, and potassium content of krill protein concentrate were within the ranges reported by Hirano *et al.*[6] for whole krill, but values obtained for Ca, Mg, and Cu were much lower, presumably owing to the insolubility of Ca and Mg of the shell in isopropanol and the interaction of Fe and Cu with proteins. The fluoride content (0·0012%) was very low compared with that of fish protein concentrate (0·013–0·020%).[37] The Cu content though higher than that of fish protein concentrate lies within the normal range[38] (0·002–0·04%) of this element in most foods.

The amino acid compositions of the two krill protein concentrates were very similar and the results of analyses are set out in Table IV. The concentrate had a higher content of lysine, threonine, and tryptophan than that from red hake, and is therefore a more suitable material for supplementing cereal diets, which are almost universally deficient in these amino acids. The lower content of methionine in krill protein concentrate is compensated for by a higher content of cysteine. Burkholder *et al.*[5] have reported higher values than those in Table IV for cysteine, methionine, tryptophan, and phenylalanine, expressed as g amino acid/16 g amino nitrogen in the whole krill hydrolysate. The results are not strictly comparable owing to a different basis of calculations and an exceptionally high proportion of non-amino N reported by these authors. On the other hand amino acid composition reported for krill protein by Japanese workers[23] is very similar to that for the concentrate in the present experiment, with the exception of slightly higher values for phenylalanine and tryptophan, and lower values for glutamic acid, alanine, and lysine in krill protein.

The growth data and *PER* values (Table V) indicated that krill protein concentrate was as good as casein and its *PER* lay within the range reported for fish protein concentrates.[35] The differences between adjusted means of carcass dry weights, liver weights and haemoglobin contents of blood of the two groups of rats were not statistically significant. However, the kidneys from the group given the concentrate had signifi-

TABLE III

Proximate composition of krill protein concentrate

	% on dry matter basis	
	Sample A	Sample B
Protein (N × 6·25)	77·5	71·0
Lipids	0·3	0·3
Chitin	5·66	6·36
Ash	14·0	16·75
Ca	3·48	—
P	2·07	—
Mg	0·67	—
Na	1·76	—
K	1·35	—
Fe	0·035	—
Cu	0·023	—
Zn	0·005	—
F	0·0012	—
Mn	tr.	—

TABLE IV

Amino acid composition of krill protein concentrate*

	Krill protein concentrate, g amino acid/16 g N†
Lysine	11·5
Histidine	2·5
Arginine	6·8
Aspartic acid	13·3
Threonine**	5·0
Serine**	4·8
Glutamic acid	18·3
Proline	3·8
Glycine	5·3
Alanine	6·1
Cysteine**	1·5
Valine	5·3
Methionine	2·8
Isoleucine	5·7
Leucine	8·3
Tyrosine**	5·9
Phenylalanine	5·1
Tryptophan:	
colorimetric	1·3
spectroscopic	1·3

* Average of four determinations, two each from samples A & B hydrolysed for 20 h at 110°c
† Chitin N excluded from total N
** Not corrected for loss on hydrolysis

TABLE V

Growth and *PER*

	Casein	Krill protein concentrate	Analysis of variance significance level
Average wt. of rats at start, g	57·9	58·0	—
Average wt. gain in 4 weeks, g	67·4	63·7	—
Feed intake, g	241·4	221·4	—
Adjusted *PER**	3·00	3·09	—
Wt. of liver,** g	5·18	5·47	n.s.
Wt. of kidney,** g	1·08	1·28	1%
Carcass dry matter,† g	45·2	45·0	n.s.
Blood haemoglobin, %	15·2	15·4	n.s.

* Adjusted to casein *PER* of 3·00
** Adjusted means to a body weight of 130 g
† Adjusted means to a body weight of 123 g

cantly higher weights than those from the casein-fed group, and the rats in the former group tended to drink more water, but histological examination of frozen kidney sections under an optical microscope failed to reveal any damage. At the time of the growth experiment information on the detailed mineral composition of krill protein concentrate was not available and it was not possible to adjust the mineral elements in both the diets to the same level, so this may account for enlarged kidneys and the tendency to drink more water.

The present investigation has shown that a protein concentrate rich in essential amino acids and having the same *PER* as that of casein can be prepared from krill. Future refinements in processing could improve the quality further. If suitable technology for harvesting is developed, krill, apart from becoming a source of high-quality proteins could also be a source of oil for man and domestic animals.

Acknowledgments

The authors are grateful to Mr. M. A. Brown and Mr. S. Kozuharov for skilled assistance, to Mr. G. G. Coote for statistical analysis, to Miss Joan Back for amino acid analysis, to Mr. D. W. Gove for histological examination of kidney sections, and to Dr. R. Carrick for supplying the samples of krill.

Division of Food Preservation,
C.S.I.R.O.
P.O. Box 43,
Ryde,
New South Wales 2112,
Australia

Received 17 November, 1969

References

1. Marr, J., '*Discovery*' *Rep.*, 1962, **32,** 33
2. Kreuzer, R., 'Freezing and Irradiation of Fish', 1969, p. 284 (London: Fishing News (Books) Limited)
3. Idyll, C. P., 'Resources of the Sea', 1968, Part II, E/4449/Add. 2 (U.N. Economic and Social Council)
4. Corner, E. D. S., & Cowey, C. B., *Biol. Rev.*, 1968, **43,** 393
5. Burkholder, P. R., Mandelli, E. F., & Centeno, P., *J. agric. Fd Chem.*, 1967, **15,** 718
6. Vinogradova, Z. A., *Dokl. Akad. Nauk SSSR*, 1960, **133,** 680
7. Hirano, T., Kikuchi, T., Ino, K., Tanoue, N., Taguchi, T., & Okada, I., *J. Tokyo Univ. Fish.*, 1964, **50,** 65
8. Saiki, M., Fang, S. C., & Mori, T., *Nippon Suisangaku Kaishi*, 1958–59, **24,** 837
9. *Fishery Leafl. Fish Wildl. Serv. U.S.*, 1966, No. 584
10. 'Official Methods of Analysis', 1965 (10th edn) (Washington, D.C.: Association of Official Agricultural Chemists)
11. Smith, P., jun., Ambrose, M. E., & Knobl, G. N., jun., *Comml Fish. Rev.*, 1964, **26** (7), 1
12. McKenzie, H. A., & Wallace, H. S., *Aust. J. Chem.*, 1954, **7,** 55
13. Hackman, R. H., *Aust. J. biol. Sci.*, 1954, **7,** 168
14. Jones, N. R., *J. Sci. Fd Agric.*, 1955, **6,** 3
15. Satake, K., Okuyama, T., Ohashi, M., & Shinoda, T., *J. Biochem.*, 1960, **47,** 654
16. Instruction Manual for Fluoride Electrode, 1966 (1st Edn) (Cambridge, Mass.: Orion Research Inc.)
17. Luddy, F. E., Barford, R. A., & Reimenschneider, R. W., *J. Am. Oil Chem. Soc.*, 1960, **37,** 447
18. Metcalfe, L. D., *J. Gas Chromat.*, 1963, **1,** 7
19. Montgomery, W. A., in 'Chilling of Fish: Fish Processing Technologists Meeting', 1960, p. 234 (Rotterdam: F.A.O.)
20. Smith, M. B., & Back, J. F., *Aust. J. biol. Sci.*, 1968, **21,** 549
21. Edelhoch, H., *Biochemistry, N.Y.*, 1967, **6,** 1948
22. Opieńska-Blauth, J., Charezinski, M., & Berbec, H., *Analyt. Biochem.*, 1963, **6,** 69
23. Suyama, M., Nakajima, K., & Nonaka, J., *Bull. Jap. Soc. scient. Fish.* 1965, **31,** 302
24. Nonaka, J., & Koizumi, C., *Bull. Jap. Soc. scient. Fish*, 1964, **30,** 630
25. Sango, Y., Aikawa, D., & Murase, K., *Yukagaku*, 1965, **14,** 171
26. Kayama, M., Tsuchiya, Y., & Mead, J. F., *Bull. Jap. Soc. scient. Fish*, 1963, **29,** 452
27. Kelly, P. B., Reiser, R., & Hood, D. W., *J. Am. Oil Chem. Soc.*, 1959, **36,** 104
28. Farkas, T., & Herodek, S., *J. Lipid Res.*, 1964, **5,** 369
29. Jezyk, P. F., & Penicnak, A. J., *Lipids*, 1966, **1,** 427
30. Ackman, R. G., Jangaard, P. M., Hoyle, R. J., & Brockerhoff, H., *J. Fish. Res. Bd Can.*, 1964, **21,** 747
31. Yamada, M., *Bull. Jap. Soc. scient. Fish.*, 1964, **30,** 673
32. Ackman, R. G., Tocher, C. S., & McLachlan, J., *J. Fish. Res. Bd Can.*, 1968, **25,** 1603
33. Ackman, R. G., & Eaton, C. A., *J. Fish. Res. Bd Can.*, 1967, **24,** 467
34. Ackman, R. G., & Eaton, C. A., *Can. J. Biochem.*, 1966, **44,** 1561
35. Stilling, B. R., *Activities Rep.*, 1967, **19,** 109
36. Power, H. E., *J. Fish. Res. Bd Can.*, 1964, **21,** 1489
37. Weinberg, B., *Fishg News int.*, 1966, **6** (1), 16
38. Underwood, E. J., 'Trace Elements in Human and Animal Nutrition', 1962 (2nd edn) p. 89 (New York: Academic Press)

CONDITIONING OF BOVINE MUSCLE
I.—Composition of the proteins of the myofibril

By I. F. PENNY

By selective extraction, precipitation and separation on diethylaminoethyl cellulose the proteins of the myofibril have been divided into a number of fractions which, quantitatively, do not vary in the *longissimus dorsi* muscles of six different beef animals. The fractions have been analysed by starch-gel electrophoresis and some have been found to consist of almost pure proteins while others are complexes. About 80–85% of the myofibrillar protein could be extracted of which 47% was myosin, 12% tropomyosin and 3% troponin. The amount of actin could not be determined since it appeared partly as complexes with α-actinin, with troponin, and with the insoluble residue.

Introduction

When meat is stored *post mortem* at temperatures of 1–4° it becomes progressively more tender. The biochemical changes occurring during this process, usually called conditioning, have not as yet been elucidated. Recently Davey & Gilbert[1] made a major advance when they showed that myofibrils prepared from conditioned meat were cut up during homogenisation into shorter lengths (about 2–4 sarcomeres long) than myofibrils from 1-day old meat (about 10–20 sarcomeres long) and, more important, that the 'Z' bands could not be detected under the light microscope. As a result of their findings, they suggested that the tenderising of meat during conditioning arose from the disruption of the 'Z' bands. Since then, more evidence has been produced which is consistent with this hypothesis. For example, Penny[2] showed that, when myofibrils from conditioned muscle were treated with 1 M potassium chloride or 0·1M pyrophosphate, more protein was extracted than from those prepared from fresh muscle. The increase was shown to be due to increasing extraction of actin, possibly released by disruption of the 'Z' bands. Davey & Gilbert[3] have also shown that more actin can be extracted from conditioned meat, and that this is accompanied by increasing amounts of water-soluble proteins which they suggest come from the disrupted 'Z' band structure.

No detailed identification has, however, been made of the proteins which are broken down or released by conditioning, partly because the exact composition of the myofibril is still not settled. Myosin is usually considered to make up about 54%[4] by wt. of the fibril, actin about 27%, tropomyosin about 12%,[5] troponin about 3%[6] and α-actinin about 6%.[7] Actin has never been isolated in amounts approaching the 27% required on stoicheiometric grounds; for instance, Corsi & Perry[5] and Corsi *et al.*[8] were able to extract only about 15% of the myofibril as actin. The exact composition of the 'Z' band is likewise not known, although electron microscopy evidence shows a complex lattice in which 4 actins from one sarcomere link up with one actin from the next.[9] Tropomyosin is also present in the 'Z' band,[10] together with α-actinin,[11] but troponin has not been found.[10]

It was decided, therefore, to subject the myofibril to various extraction techniques and to examine the extracts by chromatography on diethylaminoethyl (DEAE) cellulose and by starch-gel electrophoresis, to find out how their composition varied with conditioning. In this paper the composition of myofibrils from 1-day old muscle is described.

Experimental

Muscle samples

Longissimus dorsi muscles were removed from between the 8th and 10th ribs of beef animals, slaughtered 24 h previously. Myofibrils were prepared from 100 g portions of the muscles by the centrifuging procedure of Perry & Grey,[12] using 500 ml of ice cold 0·1 M-KCl solution for each washing, the pH of the initial homogenate being adjusted to 7·0 by the addition of 1 M 'Tris' (base).

Extraction of myofibrils

The procedures used for extraction are outlined in Fig. 1. In procedure 1, the myofibrils were first extracted by dialysing at +4° against several changes of 5 mM Tris adjusted with HCl to pH 8·2. The insoluble residue was then extracted with Hasselbach–Schneider (HS)[13] solution (0·5 M-KCl, 1 mM-$MgCl_2$, 10 mM tetrasodium pyrophosphate, 10 mM-KH_2PO_4, pH 6·5). In procedure 2, the order was reversed and the myofibrils were first extracted with HS solution and the insoluble residue was dialysed against 5 mM-Tris chloride, pH 8·2. The figures given in parenthesis in Fig. 1 are used throughout the text to identify the extracts and precipitates.

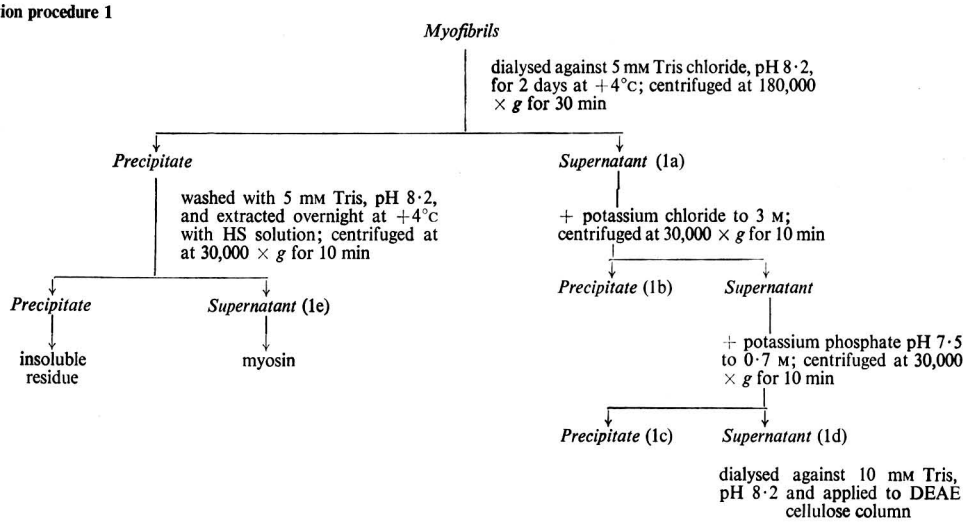
Preparation of proteins for standards

Proteins were prepared from *pre-rigor* rabbit muscle for convenience and because the methods have been standardised for this muscle. With the exception of myosin where the intensity of the bands differed slightly, the starch-gel electrophoresis (s.g.e.) patterns were identical to those obtained from beef muscle and therefore represent the same proteins. Myosin was prepared by the method of Perry,[14] α-actinin and α-actinin-free actin by the method of Serayadin *et al.*,[15] and tropomyosin and troponin by the method of Yasui *et al.*[16]

Chromatography

DEAE cellulose (Whatman DE32) in 0·1 M-KCl and 10 mM Tris-HCl, pH 8·2, was poured into a column (14 × 1 cm dia.) and washed exhaustively with the same solution. The protein solution, containing 20 mg in 20 ml of 10 mM Tris-HCl, pH 8·2, was applied and eluted with 0·1 M-KCl in 10 mM Tris-HCl, pH 8·2. The solution was pumped at 90 ml/h with a Baird & Tatlock Chromapump and the eluting protein was monitored at 280 nm on an Isco UV analyser.

Extraction procedure 1



Extraction procedure 2

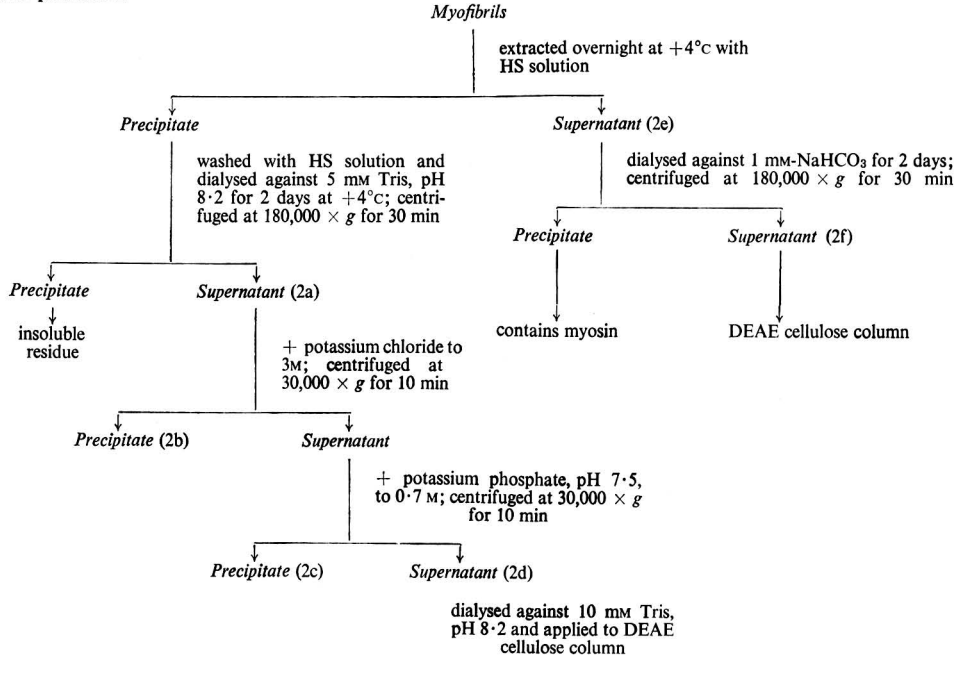


FIG. 1. *Sequence of extractions carried out on myofibrils*

Starch-gel electrophoresis

Samples were first concentrated by dialysis against Aquacide (Calbiochem) and were then dialysed overnight against 6 M urea, 12 mM Tris phosphate, pH 8·2, containing 20 mM mercaptoethanol. The samples were then subjected to starch-gel electrophoresis, using the discontinuous system described by Scopes,[17] except that the inner gel contained 6 M urea. The samples were run for $4\frac{1}{4}$ h at 400 V during which the current fell from an initial 30 mA to 20 mA. The protein bands were stained with a solution of 10 g of Amido Black 10 D and 20 g Nigrosine/1 ml of methanol–acetic acid–water (5 : 1 : 4 by vol.).

Protein determination

The proteins in the extracts were determined by a biuret method[18] in which $E^{0\cdot1\%}_{310\,nm} = 2\cdot2$ for the myofibrillar proteins standardised by the Kjeldahl nitrogen method. Tris interferes with this method, but can easily be removed by dialysis.

Adenosine triphosphatase activity

The Ca^{2+}-activated adenosine triphosphatase (ATPase) activity of the extracts was measured, after dialysing against 0·5 M-KCl to remove the pyrophosphate, by the method described in a previous paper.[2]

Results

Starch-gel electrophoresis of standard proteins

The proteins in the precipitates and supernatants prepared by the two separation procedures were identified by starch-gel electrophoresis. The control preparations of individual proteins were not absolutely pure but the principal bands can be clearly seen in Fig. 2. Tropomyosin usually has one band; although, as in this sample, two very intense bands running close together can sometimes be seen, this is probably an artefact caused by the high concentration since only one band is obtained where the tropomyosin is more dilute. There was also some contaminating troponin and six slower-moving bands of lesser intensity, possibly impurities or aggregated tropomyosin sub-units which readily form in urea if the sulphydryl groups are not protected.[19] Troponin has one main band and three faster moving bands which have been found in all preparations. The slower moving bands are impurities which have not always been observed. The *F*-actin was obviously contaminated with tropomyosin and a trace of troponin (in spite of having removed a considerable amount of these prior to acetone treatment and also of taking the precaution of extracting the acetone-dried powder at 0°). α-Actinin consists of one band and a diffuse area near the slot. The pattern for myosin shows the four bands of the urea sub-units of myosin similar to those described by Parsons *et al.*[20]

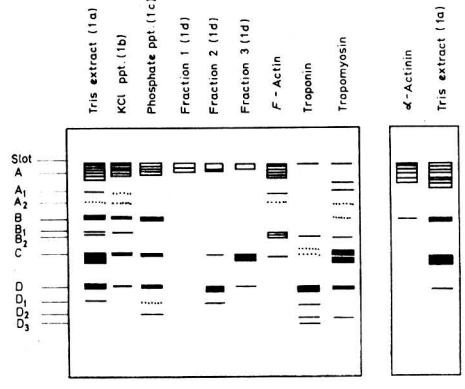


FIG. 2. *Facsimiles of starch-gel electrophoretograms of extracts and precipitates of bovine muscle prepared by procedure 1 of Fig. 1*
Figures in parenthesis refer to coding given to fractions in Fig. 1

Extracts prepared by procedure 1

The amounts of protein in each of the fractions prepared by procedure 1, were calculated as a percentage of the total myofibrillar protein and the means of the results with the standard deviations from six *l. dorsi* muscles are shown in Table I.

Extract with 5mM Tris-HCl, pH 8·2 (1a)

Extraction with 5 mM Tris, pH 8·2, removed 36·5% of the myofibrillar protein (Table I) which is about the maximum obtained by Perry & Corsi[5] and higher than that found by Corsi *et al.*[8] using the same extractant. The myofibrils were well washed during preparation until the amount of protein in the 0·1 M-KCl washings had been reduced to less than 1% of the total. The higher value seems unlikely therefore to be due to contamination by sarcoplasmic proteins. For ease of identification, the major bands found by s.g.e. of the Tris extract (Fig. 2) have been labelled with a letter starting at the insertion slot and the intermediate minor bands with a subscript number. The extract contained tropomyosin (band C), troponin (band D), actin (bands A and B_2) and α-actinin (band B) and also several minor bands.

Precipitates with 3 M-KCl and 0·7 M potassium phosphate, pH 7·5 (1b and 1c)

Ebashi & Ebashi[21] in their preparation of α-actinin added KCl to a concentration of 3 M to precipitate α-actinin. Goll *et al.*[22] also obtained a precipitate by addition of 3 M-KCl to a low ionic strength extract of myofibrils and found it to be partly soluble in 1 mM-$NaHCO_3$. The soluble protein, which they called 'Z' protein, was shown to have similar properties to α-actinin.

In the present work, the addition of potassium chloride to 3 M concentration precipitated from the Tris extract protein amounting to 4·2% of the total, and a further 12·4% was precipitated from the supernatant by addition of potassium phosphate (pH 7·5) to 0·7 M. This latter precipitate contains actin in an inactive form according to Corsi & Perry.[5]

Fig. 2 shows the bands produced by these precipitates on s.g.e., compared with control preparations of *F*-actin,

TABLE I

Analysis of the proteins extracted from myofibrils by procedure 1
Based on total protein = 100 and myosin = 54

Extract with 5 mM Tris-HCl, pH 8·2 (1a)	36·5 ± 1·6
Precipitate in 3 M-KCl (1b)	4·2 ± 0·6
Precipitate in 0·7 M potassium phosphate, pH 7·5 (1c)	12·4 ± 1·9
DEAE cellulose (fraction 1) (1d)	3·8 ± 0·6
DEAE cellulose (fraction 2) (1d)	3·1 ± 0·6
DEAE cellulose (fraction 3) (1d)	13·0 ± 1·4
HS extract (1e):	47·5 ± 2·3
Extracted Ca^{2+} ATPase activity*	87·5 ± 2·3
Myosin in HS extract	47·3 ± 2·3
Actin in HS extract	0·0
Residue	16·3 ± 2·0

* As % of total initial value

tropomyosin, troponin and α-actinin (the latter is shown separately, having been run on a different gel and is included, together with a 5 mM Tris extract, to demonstrate that the B band is coincident with the α-actinin band). The A and B_2 bands of actin can be seen in both precipitates and therefore both contain actin. The B band of α-actinin was found in both precipitates, but not in actin. In the absence of other evidence it must be assumed that this B band is a urea sub-unit of α-actinin and its presence in the Tris extract and the precipitates indicates that α-actinin is a constituent of these fractions. Both precipitates contained some tropomyosin (C band), but the potassium phosphate precipitate contained a relatively greater intensity of the D band, i.e. troponin. This suggests that this precipitate is in fact a complex of actin, α-actinin, tropomyosin and troponin.

Supernatant (1d) after removal of the 3 M-KCl and 0·7 M phosphate precipitates

The supernatant containing about 19·9% of the original total protein was dialysed against 10 mM Tris chloride, pH 8·2, and applied to a DEAE cellulose column. The elution pattern obtained is shown in Fig. 4. Elution with 0·1 M-KCl in 10 mM Tris, pH 8·2, usually gave 3 peaks which were then combined to give fraction 1, containing about 25% of the applied protein. On application of a potassium chloride gradient, another peak (fraction 2) was eluted between 0·2 and 0·25 M-KCl followed by fraction 3 at 0·3–0·5 M-KCl and finally fraction 4 at 0·5 M-KCl. Starch-gel electrophoresis (Fig. 2) revealed that fraction 2 was mainly troponin with some tropomyosin and fraction 3, tropomyosin with a trace of troponin. Thus, although a clear separation of the two fractions was obtained on DEAE cellulose, each fraction was still contaminated with the other. A band was also found, which corresponded to the A_2 band of the original Tris extract. This may represent another protein or an aggregated sub-unit of tropomyosin, since it also appeared in the tropomyosin control. Fraction 1 was not identifiable and fraction 4 was a nucleotide with a $\frac{280\text{ nm}}{260\text{ nm}}$ ratio of 0·78.

Extract of the residue with HS solution (1e)

After removal of the proteins soluble in 5 mM Tris, pH 8·2, the residue was extracted with HS solution when 47·5% of the total myofibrillar protein and 87·5% of the total Ca^{2+}-activated ATPase activity passed into solution. The amount of myosin calculated from the extracted ATPase, and assuming that 100% activity represents 54% of the myofibril, was 47·3% (Table I). This was the same as the amount of protein extracted by the HS solution, and therefore this extract contained only myosin. This was confirmed by s.g.e. (Fig. 3) which showed only the sub-units of myosin. Finally the remaining insoluble residue (16·3% of the original protein) was dissolved in 6 M urea, producing a cloudy solution which was clarified by centrifuging at 30,000 × *g*, and then analysed by s.g.e. As shown in Fig. 3, the solution contained traces of all the proteins.

Extracts prepared by procedure 2

In procedure 2 (Fig. 1), the sequence of extraction was reversed so that myosin was extracted first. The results, showing the average amounts of protein obtained in each fraction, are given in Table II.

HS extract (2e)

The HS solution extracted 56·6% of the total myofibrillar protein and 89% of the Ca^{2+}-ATPase activity. Protein amounting to 7·3% of the myofibril was removed in the supernatant after dialysing the HS extract against 1 mM-$NaHCO_3$ (2f), and its composition is described below. The protein precipitated after dialysis in 1 mM-$NaHCO_3$ represented 49·3% of the myofibril but the amount of myosin, calculated from the extracted ATPase activity, was only 48·2% leaving 1·1% of the protein unaccounted for. The s.g.e. pattern of this material, (Fig. 3) showed that some tropomyosin was present in addition to myosin, and therefore the former was probably responsible for the 1·1% difference.

The analysis by s.g.e. of the proteins soluble in 1 mM-$NaHCO_3$ (2f) showed that the extract was a complex mixture containing 6 bands (Fig. 3). Using the same nomenclature as

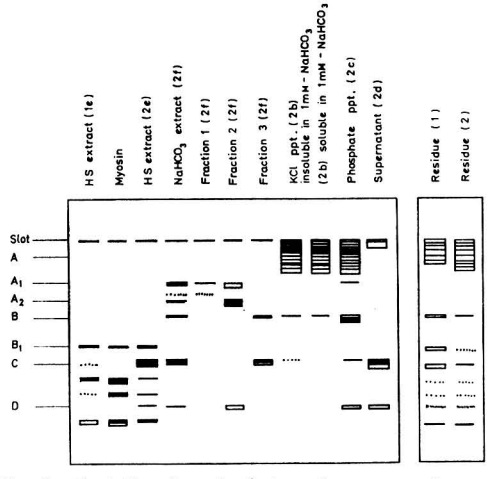


FIG. 3. *Facsimiles of starch-gel electrophoretograms of extracts and precipitates of bovine muscle prepared by procedure 2 of Fig. 1*

Figures in parenthesis refer to coding given to fractions in Fig. 1

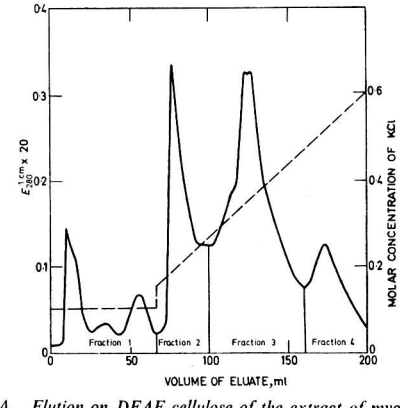


FIG. 4. *Elution on DEAE cellulose of the extract of myofibrils in 5 mM Tris, pH 8·2, after removal of the precipitates formed by the addition of 3 M-KCl and 0·7 M potassium phosphate, pH 7·5 (i.e. supernatant (1d) in Fig. 1)*

The eluant was 10 mM Tris, pH 8·2, with a potassium chloride gradient from 0·1M to 0·6M

———— Absorption at 280 nm; — — — — —KCl concentration of eluant

TABLE II

Analysis of the proteins extracted from myofibrils by procedure 2

Based on total protein = 100 and myosin = 54

HS extract (2e):	56·6 ± 1·6
Extracted Ca^{2+}-ATPase activity*	89·0 ± 1·9
Myosin in HS extract	48·2 ± 1·9
Tropomyosin in HS extract	1·1 ± 0·3
Proteins soluble in 1 mM-$NaHCO_3$ (2f):	7·3 ± 0·5
DEAE cellulose (fraction 1)	2·5 ± 0·3
,, ,, (fraction 2)	2·2 ± 0·4
,, ,, (fraction 3)	2·5 ± 0·3
Extract with 5 mM Tris-HCl, pH 8·2 (2a):	23·5 ± 2·0
Precipitate in 3 M-KCl (2b)	11·2 ± 1·0
Precipitate in 0·7 M potassium phosphate, pH 7·5 (2c)	3·5 ± 0·4
Tropomyosin + troponin (2d)	8·8 ± 1·0
Residue	20·0 ± 3·4

* As % of total initial value

for the 5 mM Tris extract in Fig. 2, these bands correspond to A_1 and A_2 with a minor band in between; and B band of α-actinin; the C band of tropomyosin and the D band of troponin. Elution of the extract from a DEAE cellulose column gave four main fractions as shown in Fig. 5. Analysis by s.g.e. (Fig. 3) showed that fraction 1 contained band A_1 and the minor band, which were not identified; fraction 2 contained a trace of band A_1, but the main components were band A_2 and the troponin band (D). This fraction is noteworthy because unlike the elution of the 5 mM Tris extract, a complete separation from tropomyosin (band C) has been achieved and band A_2 which was considered as a possible aggregated tropomyosin sub-unit has also been separated from tropomyosin and may therefore be a distinct, but unidentified protein. Tropomyosin was found in fraction 3 together with the B band of α-actinin and fraction 4 was again a nucleotide.

Extract of the residue with 5 mM Tris, pH 8·2 (2a)

After removal of the protein soluble in HS solution, the residue was dispersed in 5 mM Tris, pH 8·2, and dialysed for 2 days against several changes of 5 mM Tris, pH 8·2, which extracted a further 23·5% of the total myofibrillar protein. The addition of 3 M-KCl to the extract precipitated protein (2b) amounting to 11·2% of the total protein and 0·7 M potassium phosphate, pH 7·5, precipitated a further 3·5% protein (2c). These precipitates were similar to those obtained from the 5 mM Tris extract by procedure 1 although the amount of tropomyosin was relatively less. As before, the potassium phosphate precipitate contained troponin. The B band of α-actinin was again present in both fractions but was relatively more intense in the potassium phosphate precipitate. The potassium chloride precipitate was dissolved in 1 mM-$NaHCO_3$ to give a solution of the 'Z' protein described by Goll *et al.*[22] About 65% of potassium chloride precipitate passed into solution, but as shown by s.g.e., there was no essential difference between soluble and insoluble portions.

After removal of the potassium chloride and potassium phosphate precipitates, the supernatant (2d) contained 8·8% of the original protein and consisted of tropomyosin and troponin. On applying the supernatant to a DEAE cellulose column, fraction 1, i.e. protein unabsorbed in 0·1 M-KCl in 10 mM Tris, pH 8·2, was not found, possibly because some of this material had already been extracted by the HS solution and was therefore present in fraction 1 of the proteins soluble in 1 mM-$NaHCO_3$. On application of a gradient to the column, two peaks were eluted, corresponding to fraction 2 and 3 of the Tris extract prepared by procedure 1.

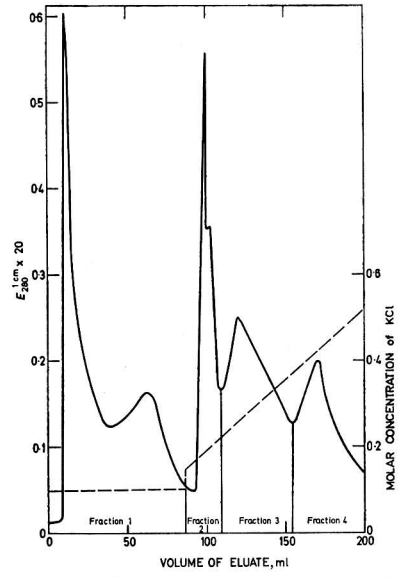


FIG. 5. *Elution on DEAE cellulose of the soluble proteins of the HS extract (2c) after dialysis against 1 mM-$HaHCO_3$ (i.e. supernatant (2f) in Fig. 1)*

The eluant was 10 mM Tris, pH 8·2, with a potassium chloride gradient from 0·1M to 0·6M

———— Absorption at 280 nm; — — — — — KCl concentration of eluant

The residue insoluble in both HS solution and 5 mM Tris, pH 8·2, contained 20·0% of the original protein, and was shown by s.g.e. to contain traces of all the proteins (Fig. 3).

Discussion

The separation procedures used in these experiments have resulted in the isolation of very complex mixtures of proteins. In spite of this, the quantitative results given in Table I and II show that standard deviations of the average values obtained from six different *l. dorsi* muscles were small. This suggests that the proteins separated into the several fractions have an affinity for each other such that they are extracted or precipitated in nearly constant proportions.

The complexity of the fractions makes it difficult to assess the amount of individual proteins present in the fractions themselves or in the myofibril as a whole. For example, fraction 3 prepared from DEAE cellulose by procedure 1 contained 13% of the total protein and was shown by s.g.e. to contain tropomyosin contaminated with some troponin and some minor bands, which may amount to about 1–2%. Since there were also traces of tropomyosin in most of the other fractions and precipitates which may also amount to about 1–2%, the best estimate for the tropomyosin content of the myofibril is 12–14%, in agreement with the value of Perry & Corsi.[5] A similar assessment can be made for the amount of troponin present. Troponin is spread over three fractions and as an approximation based on the visual assessment of the relative densities of the bands on s.g.e., 1% was probably present in the potassium phosphate precipitate, about 2% in fraction 2 from DEAE cellulose and about 0·5%

in fraction 3, making an estimated total of 3·5%. This compares with the 2·5% suggested by Ebashi *et al.*[6] who based their estimate on a very low calculated value for tropomyosin of only 4·2%.

The estimation of the amount of α-actinin is impossible, since it spreads over several fractions. However, an interesting result is obtained by comparing the potassium chloride and potassium phosphate precipitates of procedures 1 and 2; in procedure 1 the amounts of protein precipitated were 4·2% and 12·4% respectively, whereas in procedure 2 the amounts were 11·2% and 7·5% respectively. The difference in the composition of the potassium chloride precipitates prepared by the two extraction procedures is not known but from the reported properties of α-actinin[15,21] the greater proportion of the extracted α-actinin would be expected to be in these precipitates. Therefore, probably more α-actinin has been extracted by procedure 2, and as is also likely, the actin has been altered by the prior extraction of myosin, so that it either complexes with α-actinin and hence co-precipitates with it on the addition of 3 M-KCl, or is by itself now capable of being precipitated by 3 M-KCl.

Assuming both precipitates contain actin, then the amount of actin extracted from the myofibril is less than the combined totals of 16·6% by procedure 1, and 14·7% by procedure 2, because of the other proteins α-actinin and troponin present in the precipitates. These values for actin fall far short of the 27% required on stoicheiometric grounds, but are in line with the values of Corsi *et al.*[8] As the latter found, there is still a considerable amount of firmly bound actin remaining in the residue, together with some myosin.

The other noteworthy fraction contains the group of proteins removed from the HS extract of procedure 2 after dialysis against 1 mM-$NaHCO_3$, which amounts to 7·3% of the myofibril. This value disagrees with the results of Davey & Gilbert,[3] who found that only 1% of their HS extracts from 1-day old muscle was soluble at low ionic strength. In the present experiments a constant amount of tropomyosin and troponin, together with α-actinin and unidentified proteins were extracted by the HS solution and yet a constant amount of tropomyosin and troponin remained in the residue to be extracted later by 5 mM Tris, pH 8·2. This implies that a proportion of the tropomyosin and troponin, in combination with α-actinin and other proteins, is not rigidly bound to the myofibrillar structure and can be readily separated from the other proteins in the myofibril, whereas the remainder of these proteins must be much more firmly bound. It should also be noted that not all the tropomyosin in the HS extract was soluble in 1 mM-$NaHCO_3$, but some was co-precipitated with the myosin.

Acknowledgment

The author wishes to thank Mr. R. A. Clark for technical assistance.

Agricultural Research Council,
Meat Research Institute,
Langford,
Bristol

Received 8 January, 1970

References

1. Davey, C. L., & Gilbert, K. V., *J. Fd Technol.*, 1967, **2,** 57
2. Penny, I. F., *J. Sci. Fd Agric.*, 1968, **19,** 518
3. Davey, C. L., & Gilbert, K. V., *J. Fd Sci.*, 1968, **33,** 343
4. Huxley, H. E., & Hanson, J., in 'Structure and Function of Muscle', (Ed. Bourne, G. H.), 1960, Vol. I, p. 183 (New York: Academic Press)
5. Corsi, A., & Perry, S. V., *Biochem. J.*, 1958, **68,** 12
6. Ebashi, S., Kodema, A., & Ebashi, F., *J. Biochem., Tokyo,* 1968, **64,** 465
7. Ebashi, S., & Endo, M., *Prog. Biophys. mol. Biol.*, 1968, **18,** 123
8. Corsi, A., Ronchetti, I., & Cigognetti, C., *Biochem. J.*, 1966, **100,** 110
9. Knappeis, G. G., & Carlsen, F., *J. Cell Biol.*, 1962, **13,** 323
10. Endo, M., Nonomura, Y., Masaki, T., Ohtsuki, I., & Ebashi, S., *J. Biochem., Tokyo*, 1966, **60,** 605
11. Masaki, T., Endo, M., & Ebashi, S., *J. Biochem., Tokyo*, 1967, **62,** 630
12. Perry, S. V., & Grey, T. C., *Biochem. J.*, 1956, **64,** 181
13. Hasselbach, W., & Schneider, G., *Biochem. Z.*, 1951, **321,** 462
14. Perry, S. V., in 'Methods in Enzymology', (Eds Colwick, S. P., & Kaplan, N. O.), 1955, Vol. II, p. 582 (New York: Academic Press)
15. Seraydarian, K., Briskey, E. J., & Mommaerts, W. F. H. M., *Biochim. biophys. Acta*, 1967, **133,** 399
16. Yasui, B., Fuchs, F., & Briggs, F. N., *J. biol. Chem.*, 1968, **243,** 735
17. Scopes, R. K., *Biochem. J.*, 1968, **107,** 139
18. Itzhaki, R. F., & Gill, D. N., *Analyt. Biochem.*, 1964, **9,** 401
19. Woods, E. F., *J. biol. Chem.*, 1967, **242,** 2859
20. Parsons, A. L., Parsons, J. L., Blanshard, J. M. V., & Lawrie, R. A., *Biochem. J.*, 1969, **112,** 673
21. Ebashi, S., & Ebashi, F., *J. Biochem., Tokyo*, 1965, **58,** 7
22. Goll, D. E., Mommaerts, W. F. H. M., Reedy, M. K., & Seraydarian, K., *Biochim. biophys. Acta*, 1969, **175,** 174

CONDITIONING OF BOVINE MUSCLE
II.*—Changes in the composition of extracts of myofibrils after conditioning

By I. F. PENNY

Myofibrils from *longissimus dorsi* muscles conditioned at 4° for 8 days and 15 days, were extracted by Hasselbach–Schneider solution and 5 mM Tris-HCl, pH 8·2. Both extractants removed increasing amounts of protein from the myofibrils as conditioning proceeded. More myosin, actin, tropomyosin and troponin was extracted by the Hasselbach–Schneider solution and 5 mM Tris, pH 8·2, extracted increasing amounts of a complex mixture containing actin and α-actinin. There was no evidence that any particular protein had been degraded or had disappeared and it is suggested that one of the effects of conditioning may have been an alteration in the binding of some of the proteins to each other in the myofibril.

Introduction

A description has been given in Part I[1] of the composition of extracts of myofibrils from 1-day *post mortem longissimus dorsi* muscle, in Hasselbach–Schneider (HS)[2] solution and in 5mM Tris-HCl, pH 8·2. A number of different fractions were prepared from these extracts by precipitation and separation on diethylaminoethyl (DEAE) cellulose, some of which contained a mixture of proteins, particularly actin and α-actinin, while others consisted of almost pure myosin and tropomyosin. The quantitative values for these fractions were, however, sufficiently consistent to justify the use of the same extraction procedures to examine the changes taking place during conditioning.

Experimental

Sections of *longissimus dorsi* muscles, obtained from beef animals 24 h *post mortem* were sprayed with aureomycin (100 ppm) and then wrapped in 'Saran' (Dow Chemical Co.). They were stored in a refrigerator at +4° and 100 g portions were removed after 8 and 15 days.

The preparation and analytical and extraction methods were identical with those described previously.[1]

The viscosity of the HS extracts, first dialysed against 1 M-KCl to remove pyrophosphate, was measured at 21° in an Ostwald viscometer with an outflow time of 31·5 sec for 1 M-KCl. The adenosine triphosphate (ATP) sensitivity in 1·5 mM ATP and 1·5mM-$MgCl_2$ was determined according to the equation of Weber & Portzehl[3] as:

$$\left[\frac{\log \eta_{\text{rel.}}/C}{\log \eta_{\text{rel.(ATP)}}/C} - 1\right] \times 100$$

where C = concentration of protein in mg/ml.

Results

Figs 1 and 2 show the starch-gel electrophoretograms (s.g.e.) of some of the fractions obtained from extracts prepared by procedures 1 and 2 from a muscle aged 15 days at +4°. No major differences were observed from those for 1-day old muscle, described in Part I,[1] with the exception of the soluble proteins obtained after dialysis of the HS extracts, against 1mM-$NaHCO_3$, which showed only a faint troponin band. The residue, which was insoluble in both the HS and the 5mM Tris solutions showed a comparatively stronger band for troponin, which may be in relatively greater amount because the weight of the residue is less owing to the extraction of other proteins. Tables I and II present the averages with results for six *longissimus dorsi* muscles using the separation techniques described in procedures 1 and 2 as before; the same coding has been used to identify the fractions. Some significant differences were observed when the results for the aged muscles were compared with those for 1-day old muscles. The most significant difference was found in the 3M-KCl precipitate (1b) from the Tris extract in procedure 1, which increased from 4·2% after 1 day, to 7·2% after 8 days and to 10·2% after 15 days. There was a corresponding drop from 12·4 to 7·4%, after 8 days conditioning, in the precipitate with 0·7M potassium phosphate (1c) but this value did not decrease with further conditioning to 15 days.

Differences were also observed in the fractions obtained from DEAE cellulose on elution of the proteins remaining in solution (1d) after precipitation by 3M-KCl and 0·7M potassium phosphate. There was an increase in fraction 1 and a decrease in fraction 3, as shown in Table I but the elution patterns and the s.g.e. patterns were identical to those shown for 1-day old muscle and are, therefore, not illustrated here.

The other significant difference found by procedure 1 was in the amount of protein extracted by HS solution subsequent to extraction in 5mM Tris (1e). There was a steady increase in the amount of myosin as measured by extractable Ca^{2+}-ATPase activity, whereas the amount of actin extracted rose from 0% after 1 day to 6% after 8 days but fell again after 15 days to 3·4%. The latter fall could be partly explained by the increase in the amount of proteins first extracted by 5mM Tris and in particular the fraction precipitated with 3M-KCl. The presence of actin was confirmed by the ATP sensitivity test which gave a value of 28% for the 15-day old sample. The examination of this fraction by s.g.e. (Fig. 2) showed that in addition to myosin and actin, tropomyosin (C band) and troponin (D band) were present, which explains why less tropomyosin was found in fraction 3 from DEAE cellulose. Thus, the values attributed above to actin should be reduced to take into account the tropomyosin and troponin.

As a result of the overall increase in extractability of the proteins, the insoluble residue dropped from 16·7% for 1-day old muscle to 9·0% for 8-day old and finally to 7·4% after 15 days but still contained traces of all the proteins including myosin as shown in Fig. 2.

Extraction by procedure 2 illustrated the now well documented increase,[4–6] in the amount of protein extractable at high ionic strength (2e), as a result of conditioning (Table II). This arose partly from an increase in the percentage of the

* Part I: Preceding paper

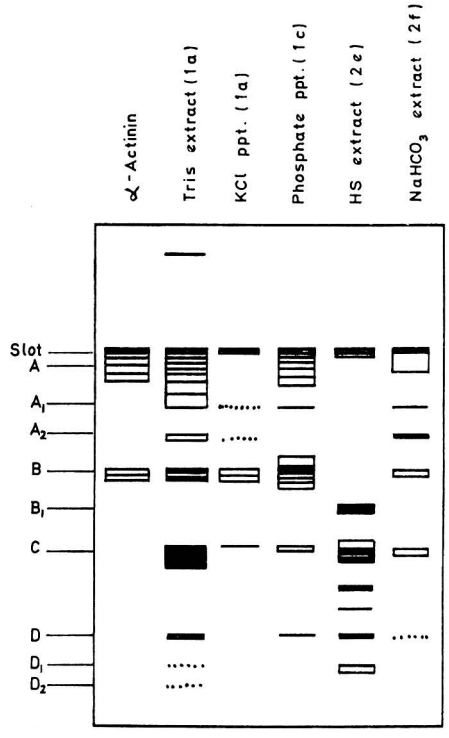


FIG. 1. *Facsimiles of starch-gel electrophoretograms of extracts and precipitates prepared by procedure 1 from the myofibrils of muscle conditioned 15 days at +4°C*

Figures in parenthesis refer to coding given to fractions in Fig. 1 of Part I[1]

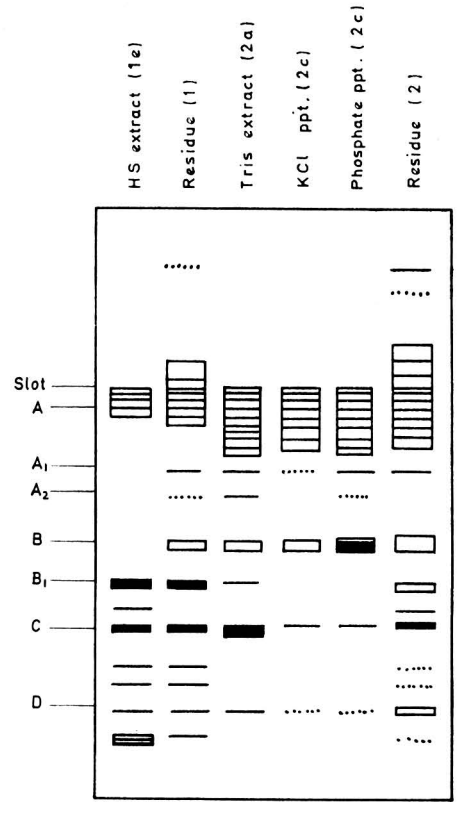


FIG. 2. *Facsimiles of starch-gel electrophoretograms of extracts and precipitates prepared by procedure 2 from the myofibrils of muscle conditioned 15 days at +4°C*

Figures in parenthesis refer to coding given to fractions in Fig. 1 of Part I[1]

Ca^{2+}-ATPase activity extracted, which amounted to 100% after 15 days conditioning; hence all the myosin, i.e. 54% of the myofibril, had then been removed. Part of this HS solution material was also extractable in water and this is described below; the remainder consisted of actin, tropomyosin and troponin, of which the two latter were identified by s.g.e. (Fig. 1) and the actin by the ATP sensitivity test which gave a value of 70%.

The amount of tropomyosin and troponin in the HS extract was calculated on the assumption that the total amount of these two proteins extracted by HS solution and then by 5mM Tris remained constant during conditioning (the contribution of the proteins soluble in 1mM-$NaHCO_3$ has been omitted because, although the amount of troponin decreased slightly, this was balanced by unextracted troponin in the residue). For 1-day old muscle, 1·1% tropomyosin and troponin was found in the HS extract and 8·8% in the 5mM Tris extract making a combined total of 9·9% (Table II). For 8-day old muscle, 6·1% was found in the 5mM Tris extract and therefore 3·8% should be present in the HS extract to give a total of 9·9%. The amount of tropomyosin and troponin in the HS extract from 15-day old muscle was similarly calculated to be 5·4%. The amount of actin extracted by the HS solution was then calculated as the difference between the total protein in the extract and the combined sum of the amount of myosin, of proteins soluble in 1mM-$NaHCO_3$ and that calculated to be tropomyosin and troponin.

The proteins, which remained in solution after dialysing the HS extract against 1mM-$NaHCO_3$, (2f) showed a small decrease on ageing. This was surprising in view of the results of Davey & Gilbert[4] who found that increasing amounts of water soluble proteins, up to 10% of the myofibril, could be extracted from conditioned muscle. Comparison with the 1-day *post mortem* results in Table II showed that it was the quantities of fractions 1 and 2 which were mainly reduced; the latter could be explained entirely by the loss of troponin from this extract as in Fig. 1. One would have expected an increase in these soluble proteins because of the increase in the amounts of tropomyosin and troponin extracted by HS solution, but apparently these cannot be extracted from the actomyosin perhaps because they become tightly bound to actin. Valin[7] found an increase in the amount of tropomyosin that could be extracted from the actomyosin from conditioned muscle but he used a heating step to denature the actomyosin, which presumably released the extra tropomyosin.

Since there was such a large increase in the amount of protein extracted by HS solution it was not surprising to find that the amount of protein subsequently extracted by 5mM

TABLE I

Analysis of the proteins extracted from myofibrils by procedure 1
Based on total protein = 100 and myosin = 54

	Time *post mortem* at +4°C		
	1 day	8 days	15 days
Extract with 5 mM Tris (1a):	36·5 ± 1·6	35·7 ± 1·6	38·1 ± 1·6
3 M-KCl ppt. (1b)	4·2 ± 0·6	7·2 ± 1·7	10·2 ± 0·7
0·7 M phosphate ppt. (1c)	12·4 ± 1·9	7·7 ± 1·8	7·9 ± 1·3
Fraction 1	3·8 ± 0·6	4·8 ± 0·3	5·1 ± 0·6
Fraction 2 (1d)	3·1 ± 0·6	4·0 ± 0·6	3·5 ± 0·6
Fraction 3	13·0 ± 1·4	12·0 ± 1·4	11·5 ± 1·3
HS extract (1e):	47·5 ± 2·7	55·3 ± 2·0	54·6 ± 0·6
Extracted Ca^{2+}-ATPase activity*	87·5 ± 2·7	91 ± 1·6	94·7 ± 0·7
Myosin in HS solution	47·3 ± 2·3	49·3 ± 1·6	51·2 ± 0·3
Actin + tropomyosin + troponin	0	6·0	3·4
Residue	16·3 ± 2·0	9·0 ± 1·8	7·4 ± 1·9

* As % of total initial value

TABLE II

Analysis of the proteins extracted from myofibrils by procedure 2
Based on total protein = 100 and myosin = 54

	Time *post mortem* at +4°C		
	1 day	8 days	15 days
HS extract (2e):	56·6 ± 1·6	70·5 ± 2·1	78·6 ± 5·4
Extracted Ca^{2+}-ATPase activity*	89·0 ± 1·9	94·0 ± 1·6	100 ± 0·0
Myosin	48·2 ± 1·9	50·8 ± 1·6	54·0 ± 0·0
Actin	0·0	9·4	13·3
Tropomyosin + troponin	1·1	3·8	5·4
Water extract (2f):	7·3 ± 0·5	6·5 ± 1·0	5·9 ± 0·3
Fraction 1	2·5 ± 0·3	1·9 ± 0·1	1·9 ± 0·2
Fraction 2	2·2 ± 0·4	1·4 ± 0·2	1·7 ± 0·1
Fraction 3	2·5 ± 0·7	3·0 ± 1·6	2·3 ± 0·5
5 mM Tris extract (2a):	23·4 ± 2·0	15·4 ± 1·8	11·2 ± 3·2
3 M-KCl ppt. (2b)	11·2 ± 1·0	5·6 ± 0·6	4·1 ± 1·5
0·7 M phosphate ppt. (2c)	3·5 ± 0·4	3·7 ± 0·9	2·7 ± 1·8
Tropomyosin + troponin (2d)	8·8 ± 1·0	6·1 ± 0·8	4·5 ± 1·0
Residue	20·0 ± 3·4	14·4 ± 2·0	10·2 ± 2·5

* As % of total initial value

Tris (2a) had fallen from 23·4% of the myofibril to 11·2%. The largest decreases occurred in the potassium chloride precipitate (2b) and in the soluble tropomyosin and troponin (2d); the potassium phosphate precipitate (2c) decreased to a lesser extent. From what has been described above, it seems most likely that a proportion of these proteins have been extracted by HS solution. Between 1 day *post mortem* and 15 days the amount of protein extracted by HS solution increased from 56·6% to 78·6%, a difference of 22%; 12·2% of this must have come from the proteins originally extracted by 5mM Tris from 1-day old samples and the remaining 9·8% from the previously insoluble residue, which had decreased from 20% of the myofibril to 10·2%. This residue, when dissolved in 8 M urea, still contained all the proteins except myosin, as shown in Fig. 1.

Discussion

One of the results of the experiments reported above is the reduction from about 20% of the myofibril to about 10% in the amount of residue insoluble in both HS and 5mM Tris, pH 8·2, solutions after conditioning. This has been shown to be due to the extraction of increasing amounts of myosin, actin and tropomyosin and troponin which were probably bound to the actin. The failure to extract all the myosin and actin from 1-day old muscle suggests that a proportion of these proteins are firmly bound to an insoluble myofibrillar structure. After conditioning, the myofibrils are cut up by the homogenisation procedure into much smaller fragments which would make these proteins much more accessible to the extracting solutions. It is also possible that the insoluble structure has been disrupted so that the proteins become more readily extractable. The nature of this insoluble structure is not known. The starch-gel electrophoretograms in Figs 1 and 2 have shown that when the insoluble residue is dissolved in 8 M urea the bands of the identified proteins are present. In addition however, bands migrating to the cathode were observed which were not found in the residues of the 1-day old muscle as shown previously.[1] These bands may represent breakdown products released from the structure as a consequence of conditioning but further work is required before this can be confirmed.

Tropomyosin and troponin were found in several extracts and precipitates, especially in procedure 2. This has probably no relevance to the effect of conditioning. The simple

explanation, that these proteins are associated with the actin filament in the myofibril and will be found in any fraction containing actin, will suffice, with the exception of the proteins extracted from HS extracts after dialysis against 1mM-$NaHCO_3$. The function of this group of proteins is not known but it appears to be different from the tropomyosin and troponin associated with the actin since it was extracted along with myosin even from unconditioned muscle.

There is no positive evidence that any degradation or loss of any individual protein has occurred during conditioning and therefore the changes in pattern are more probably caused by an alteration of the binding of proteins to each other. The most significant changes occur in the complex fractions containing actin and α-actinin.

The hypothesis put forward by Davey & Gilbert[8] that during conditioning the 'Z' bands are disrupted can be put to the test by the data in Tables I and II. Assuming that one of the proteins of the 'Z' band, α-actinin, is a component of the potassium chloride precipitate—and evidence from other workers[9,10] would support this assumption as well as the s.g.e. results in Figs 1 and 2—then the results for the changes in the composition of the extracts prepared by procedure 2 agree with the hypothesis. From 1-day old muscle, after the myosin has been removed, treatment with 5mM Tris, pH 8·2, extracts some of the remaining actin together with the α-actinin with which it is combined. This complex is precipitated by 3M-KCl and amounts to 11·2% of the myofibril (Table I). After 15 days the affinity of α-actinin for actin is weakened by the effect of conditioning, i.e. the actin is no longer bound firmly to the 'Z' band, and the actin with its associated tropomyosin and troponin is readily extracted along with myosin by the HS solution. There is now not very much actin, if any, left bound with α-actinin to be extracted with 5mM Tris, pH 8·2, and as a consequence the resulting precipitate in 3M-KCl falls to 4·1% of the myofibril.

The hypothesis, however, is not supported by the results from procedure 1 because the amount of protein precipitated by 3M-KCl (1b) increases from 4·2% to 10·2% with conditioning, while the protein precipitated by potassium phosphate (1c) decreases from 12·4% to 7·9%, implying that α-actinin has initially no affinity for the actin with which it is extracted but after conditioning the two proteins combine more readily. This conclusion is, of course, the opposite to that arrived at to explain the results in procedure 2. According to Corsi & Perry[11] the actin extracted by 5mM Tris, pH 8·2, has undergone some change which makes it inactive (i.e. it can no longer be polymerised to *F*-actin). It is also possible that the actin has undergone a further conformational change as a result of conditioning which makes it capable of being precipitated along with α-actinin by 3M-KCl. The 3M-KCl precipitate (1c) from 15-day old muscle may contain more α-actinin but until the exact composition of these complex precipitates is known further suggestions as to the cause of the changes are meaningless.

Acknowledgment

The author wishes to thank Mr. R. A. Clark for technical assistance.

Agricultural Research Council,
Meat Research Institute,
Langford,
Bristol

Received 8 January, 1970

References

1. Penny, I. F., *J. Sci. Fd Agric.*, 1970, **21**, 297
2. Hasselbach, W., & Schneider, G., *Biochem. Z.*, 1951, **321**, 462
3. Weber, H. H., & Portzehl, H., *Adv. Protein Chem.*, 1952, **7**, 211
4. Davey, C. L., Gilbert, K. V., *J. Fd Sci.*, 1968, **33**, 343
5. Weinberg, B., & Rose, *Fd Technol., Champaign*, 1960, **14**, 376
6. Penny, I. F., *J. Sci. Fd Agric.*, 1968, **19**, 518
7. Valin, C., *J. Fd Technol.*, 1968, **3**, 171
8. Davey, C. L., & Gilbert, K. V., *J. Fd Technol.*, 1967, **2**, 57
9. Ebashi, S., & Ebashi, F., *J. Biochem., Tokyo*, 1965, **58**, 7
10. Seraydarian, K., Briskey, E. J., & Mommaerts, W. F. H. M., *Biochim. biophys. Acta*, 1967, **177**, 299
11. Corsi, A., & Perry, S. V., *Biochem. J.*, 1958, **68**, 12

CHLOROPHYLL DEGRADATION AND LIPID OXIDATION IN FROZEN UNBLANCHED PEAS

By K. A. BUCKLE and R. A. EDWARDS

Storage of hand-podded unblanched frozen peas for 20 months at −9·4° resulted in considerable conversion of chlorophylls to pheophytins, the formation of phytol-free derivatives, a decrease in total pigment and the formation of peroxides and TBA-reactive materials. Greater storage stability was found in samples blanched prior to storage, and to a lesser extent in unblanched peas stored in nitrogen or at −23·3°.

Lipoxygenase and chlorophyll bleaching activities of extracts from frozen unblanched peas decreased during storage, lipoxygenase being the more stable.

Model system studies with pea and soya extracts indicated that chlorophyll loss and lipid oxidation during storage were most likely caused by lipoxygenase and a lipohydroperoxide breakdown factor shown to be present in crude extracts.

Introduction

The blanching of chlorophyll-containing vegetables has been universally recognised as an important factor in the retention of colour and flavour. Many workers have shown that the inactivation of enzymes such as peroxidases, lipases and lipoxygenases prevents colour changes associated with the conversion of chlorophylls to pheophytins, chlorophyll destruction,[1–10] and the development of off-flavours in the lipid fraction.[11–17]

Changes in pigments and colour during the storage of unblanched vegetables have been associated with the oxidation of unsaturated fatty acids. Reports have implicated the enzyme lipoxygenase (E.C. 1.13.1.13) in unblanched peas,[2,7,14,18,19] although pigment destruction and fatty acid oxidation have also been observed in blanched beans.[8]

Chlorophyll solutions are rapidly bleached in systems containing lipoxygenase.[7,8,20,21] Holden[21] showed that pure lipoxygenase and unsaturated fatty acids did not cause chlorophyll bleaching without the presence of a bleaching factor present in legume seed extracts. Addition of lipoxygenase and unsaturated acids to legume seed extracts considerably increased the bleaching. Results indicated that bleaching was a result of breakdown of fatty acid hydroperoxides catalysed by the bleaching factor which had properties similar to a lipohydroperoxide breakdown factor found initially in soya extracts,[22–25] and also demonstrated in lower concentration in pea extracts.[21]

The present work was carried out to examine relationships between chlorophyll conversion and destruction, lipid oxidation and the activities of enzymic systems of blanched and unblanched peas during frozen storage. It was recognised that the behaviour of vined peas would be different from that of hand-podded ones, owing to the bruising and spatial disarrangement of enzymes and substrates resulting from the vining process. This paper is concerned with hand-podded peas.

Experimental

Materials

Fresh green peas (*Pisum sativum* var. Edgell Freezer) of mean maturometer index 216[26] were hand-shelled and divided into two lots. Unblanched peas were packed into plain 401 × 411 tinplate containers, evacuated and flushed five times with nitrogen or oxygen, sealed and blast frozen at −40°. The remaining peas were blanched 1 min in boiling water to inactivate peroxidase,[27] cooled in ice water, sealed in tinplate containers in nitrogen or oxygen and blast frozen. Frozen peas were stored at −9·4° and −23·3°.

Anaesthetic grade (B.P.) diethyl ether was purified for spectrophotometry by repeated distillation and treatment with acidified ferrous sulphate,[28] and thoroughly dried. Other solvents were A.R. grade or spectroscopic grade. Chemicals were A.R. grade.

Purified linoleic acid (lot 10-P, > 99·9%), obtained in sealed ampoules from the Hormel Foundation, Austin, Minnesota, was dissolved in ethanol (10 mg/ml) and stored at −23·3° when not in use.

Lipoxygenase (salt-free lyophilised powder, ex soyabean, Batch 22474, activity 80,000 units/mg) and Hemin (crystalline) were from Koch-Light Laboratories. Soyabean meal and 1,1,3,3-tetraethoxypropane were from B.D.H. Ltd. and 2-thiobarbituric acid was from Eastman Organic Chemicals.

Crude chlorophyll solution for bleaching experiments was prepared by exhaustively extracting shredded, blanched spinach leaves with cold acetone, and extracting the pigments into petroleum ether (b.p. 40°–60°) by addition of 10% wt./vol. sodium chloride. Petroleum ether was evaporated under vacuum and the residue dissolved in acetone.

Oxidised linoleic acid was prepared from pure linoleic acid (1 g) dissolved in 10 ml ethanol and 80 ml 0·1M borate buffer (pH 9·0) incubated overnight with 20 mg pure lipoxygenase, with oxygen bubbling continually through the solution. The oxidised acid was made up to 100 ml with buffer and showed an absorbance at 234 nm of approximately 1·0 after ten-fold dilution.

Methods

Pigments

All pigment extractions were carried out in a darkened room, and pigment solutions in flasks were covered with black polyethylene.

Duplicate 10·0 g samples of frozen peas were finely chopped and extracted with 100 ml cold acetone and 1 g of magnesium carbonate powder. Further extraction and washing of the filtered pulp with 80% acetone removed all pigments, and the solution was made to volume (250 ml) to a final concentration of 80% acetone, making allowance for water present in the peas. Pigment concentrations were determined by the methods of Dietrich[29] and Vernon.[30] Concentrations of chlorophylls *a* and *b*, chlorophyllides *a* and *b*, pheophytins

a and *b* and pheophorbides *a* and *b* were also determined by the method of White *et al.*[31] as previously described.[32] Absorbances were measured on a Unicam SP600 or recorded on a Unicam SP800 spectrophotometer and were corrected for absorption at 700 nm. Average determinations were used.

Lipid oxidation

2-Thiobarbituric acid (TBA) method The method adopted was a modification of the procedure of Tarladgis *et al.*,[33] in which 20 g samples were slurried and adjusted to pH 1·5 before distillation over a bunsen flame. This was important for unblanched peas containing lipoxygenase and other enzyme systems to prevent lipid oxidation during blending. Rapid and constant distillation times (10 min) at pH 1·5 were necessary for reproducible results. Malonaldehyde concentrations in the distillates were determined from absorbance readings of the TBA–malonaldehyde complex at 532 nm using a calibration curve for malonaldehyde, derived from 1,1,3,3-tetraethoxypropane by acid hydrolysis.[34] The percentage recovery of malonaldehyde from standard solutions by distillation at pH 1·5 averaged 69·5%, giving a distillation constant K = 3·34. TBA values (mg malonaldehyde/1000 g sample) were obtained from the relationship TBA value = K × absorbance at 532 nm.

Ferric thiocyanate peroxide method Lipid material was extracted from 50 g lots of frozen peas by the method of Bligh & Dyer[35] as modified by Walker.[8] One ml aliquots of acid chloroform extracts were added to 5·0 ml ethanol and 0·2 ml 1 : 1 hydrochloric acid. Freshly prepared 1% wt./vol. ferrous ammonium sulphate (0·1 ml) was added from a calibrated syringe, followed 30 sec later by 1·0 ml 20% wt./vol. ammonium thiocyanate. The absorbance at 480 nm was measured exactly 3 min later. Appropriate blank solutions were prepared to compensate for absorbance at 480 nm due to non-peroxide materials.

Chlorophyll bleaching

Peas were ground with purified sand in a mortar with 0·1 M phosphate buffer pH 6·5 (2 ml/g) and centrifuged for 10 min at 3000 rev/min. Crude soya extracts were prepared by stirring soyabean meal with phosphate buffer (5 ml/g) for 30 min at room temperature, and centrifuging. Supernatants were stored in ice.

Enzyme extract (0·5 ml) was added to 22·0 ml buffer, 2·0 ml crude acetone solution of chlorophyll and 0·5 ml ethanol solution of fatty acid (5 mg) in a 50 ml volumetric flask covered with black polyethylene. The flask was continually shaken and 2·0 ml aliquots withdrawn at different reaction times and added to 8·0 ml acetone. The solutions were centrifuged for 5 min at 3000 rev/min and absorbances read at 662 and 700 nm. Control solutions containing heat-inactivated enzyme, fatty acid and chlorophyll were run and percentage chlorophyll bleaching calculated.[21] The absorbance of unbleached solutions was 0·75–0·80 at 662 nm.

Lipoxygenase activity

Absorbance of conjugated linoleic acid hydroperoxides was measured at 234 nm after incubation of 0·2 ml enzyme extract with 5 mg linoleic acid and 24·3 ml 0·1M phosphate buffer (pH 8·0) for 2 min.

Thin-layer chromatography

Pigments from stored frozen peas were dissolved in diethyl ether and chromatographed on thin layers of cellulose (0·5 mm).[36]

Results

Pigment changes during frozen storage

Formation of magnesium-free derivatives

Storage of unblanched frozen peas for 20 months at −9·4° resulted in substantial conversion of chlorophylls to the magnesium-free compounds (Table I). Conversion was greater in blanched peas stored at −9·4° than in unblanched peas stored at −23·3°, and samples stored in oxygen showed slightly greater conversion than those in nitrogen. Adequately blanched peas stored at low temperatures showed little pigment degradation. In all cases, chlorophyll *a* degraded faster than chlorophyll *b* to the respective magnesium-free compounds.

Formation of chlorophyllides and pheophorbides

Phytol-free pigments were not found in any blanched samples, and this was substantiated by chromatography of pigment extracts on cellulose thin layers. Considerable quantities of chlorophyllides and pheophorbides were found in unblanched peas, particularly after storage at −9·4° (Table II). Pigment changes in nitrogen at −9·4° are shown in Fig. 1. Chlorophylls *a* and *b* decreased rapidly during storage, while chlorophyllide *a* initially increased but then decreased as conversion to pheophorbide *a* progressed. Pheophytin *a* decreased after 8 months storage while pheophorbide *a* increased continually after 3 months. Pheophorbide *b* slowly increased throughout the storage period. Attempts to show the presence of chlorophyllase in unblanched peas were inconclusive.

The major chlorophyll-derived pigments isolated from acidified extracts from unblanched peas stored 20 months at −9·4° were pheophorbides *a* and *b* with absorption maxima in diethyl ether at 409, 466, 506, 534, 564, 610 and 667 nm (pheophorbide *a*) and 412, 434, 526, 558, 600 and 654 nm (pheophorbide *b*). The hydrochloric acid numbers[37] were 14–15 and 19–20 respectively. Pheophytins *a* and *b* were found in much lower concentrations.

TABLE I

Chlorophyll conversion to magnesium-free derivatives in frozen blanched and unblanched peas

Sample	Storage temp. (°C)	Chlorophyll conversion, % — Storage time, months				
		0	3	8	13	20
Blanched, nitrogen*	− 9·4	4·0	9·6	13·0	18·2	22·3
Blanched, nitrogen	−23·3	4·0	3·2	5·1	6·0	8·3
Blanched, oxygen*	− 9·4	4·0	8·3	13·7	20·0	25·8
Blanched, oxygen	−23·3	4·0	6·2	4·6	4·2	7·8
Unblanched,** nitrogen	− 9·4	6·4	26·0	48·9	69·0	83·1
Unblanched,** nitrogen	−23·3	6·4	7·0	10·3	8·1	13·9
Unblanched,** oxygen	− 9·4	6·4	31·3	56·8	70·4	87·0
Unblanched,** oxygen	−23·3	6·4	7·3	14·4	12·1	18·8

* Storage atmosphere

** Conversion of (chlorophylls plus chlorophyllides) to (pheophytins plus pheophorbides) in these samples

TABLE II

Formation of phytol-free pigments in frozen unblanched peas during storage

Storage atmosphere	Storage temp, °C	Chlorophyll as chlorophyllide, %*					Pheophytin as pheophorbide, %*				
		Storage time, months									
		0	3	8	13	20	0	3	8	13	20
Nitrogen	− 9·4	0·0	7·2	21·5	22·9	37·6	0·0	26·5	48·0	71·0	86·6
Nitrogen	−23·3	0·0	0·0	0·0	3·7	4·7	0·0	0·0	13·3	26·4	28·9
Oxygen	− 9·4	0·0	0·0	10·4	21·7	65·9	0·0	23·3	59·9	67·7	81·7
Oxygen	−23·3	0·0	0·0	0·0	3·3	5·8	0·0	0·0	4·8	17·1	24·8

* Includes *a* and *b* pigments

Decrease in total pigment

The total pigment content of unblanched peas decreased during frozen storage at −9·4° and −23·2° (Table III). Losses of total pigment in samples stored at −9·4° and −23·3° in oxygen for 20 months amounted to 35·6% and 14·4% respectively. In nitrogen, the losses were 29·6% and 6·0% respectively. Total pigment contents of blanched peas showed no change during storage.

Lipid oxidation

2-Thiobarbituric acid (TBA) values of unblanched peas increased throughout the storage period (Fig. 2). Samples stored at −9·4° showed higher TBA values than those stored at −23·3°, and storage in oxygen gave higher TBA values than storage in nitrogen. TBA values of samples stored in nitrogen at both temperatures increased at a slower rate over the last few months of storage indicating possibly a lowering of the oxygen tension, and hence a reduction in hydroperoxide production, or a decreased rate of hydroperoxide destruction. Blanched peas showed only a slight increase in TBA-reactive materials during frozen storage, much less than in unblanched peas.

Peroxide values in unblanched peas (Fig. 3) increased during 8 months storage and then decreased, except for peas stored in nitrogen at −23·3° which decreased slightly after 13 months storage. Samples stored in nitrogen had lower peroxide values at each storage temperature compared with samples stored in oxygen. Peroxide values in samples at −9·4° were two to three times higher than those at −23·3°. Increases in peroxide values of the lipids of blanched peas were negligible compared with the changes in unblanched peas.

Chlorophyll bleaching and lipoxygenase activities in pea and soya extracts

Preliminary experiments were carried out to test crude extracts from peas and soyabean meal for chlorophyll bleaching and lipoxygenase activities as reported by Holden.[21]

The chlorophyll bleaching activity of crude pea and soya extracts was determined over the range pH 4·5 to 9·0. Distilled water extracts made from fresh peas and soyabean meal showed maximum bleaching at approximately pH 6·5 in the presence of linoleic acid, and at a slightly lower pH in the absence of linoleic acid (Fig. 4). The bleaching activity of pea extract dropped sharply above pH 6·5, and without linoleic acid gave less bleaching compared with soya extract.

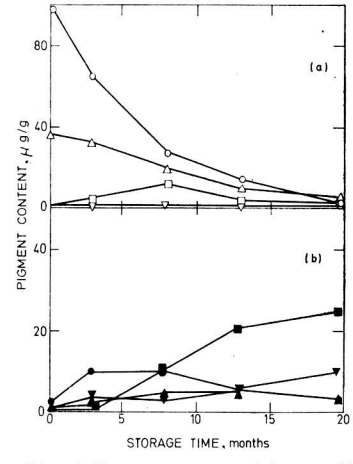


FIG. 1. *Chlorophyll pigment content of frozen unblanched peas stored in nitrogen for* 20 *months at* −9·4°C

(a) ○ chlorophyll *a* □ chlorophyllide *a* △ chlorophyll *b* ▽ chlorophyllide *b*

(b) ● pheophytin *a* ■ pheophorbide *a* ▲ pheophytin *b* ▼ pheophorbide *b*

TABLE III

Total pigment content of unblanched frozen peas

Storage atmosphere	Storage temperature, °C	Total pigment content, μg/g				
		Storage time, months				
		0	3	8	13	20
Nitrogen	− 9·4	145·7	143·8	121·8	112·5	102·5
Nitrogen	−23·3	145·7	143·2	137·8	137·6	136·9
Oxygen	− 9·4	145·7	135·9	111·1	106·0	93·7
Oxygen	−23·3	145·7	147·6	143·7	135·0	124·3

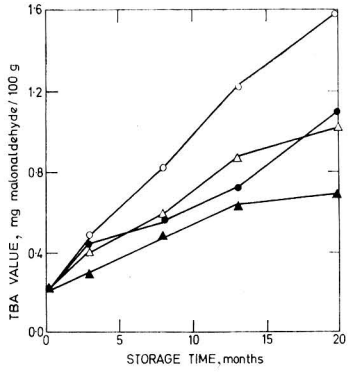


FIG. 2. *TBA values of frozen unblanched peas*

○ stored in oxygen at — 9·4°C △ stored in nitrogen at — 9·4°C
● stored in oxygen at —23·3°C ▲ stored in nitrogen at —23°3°C

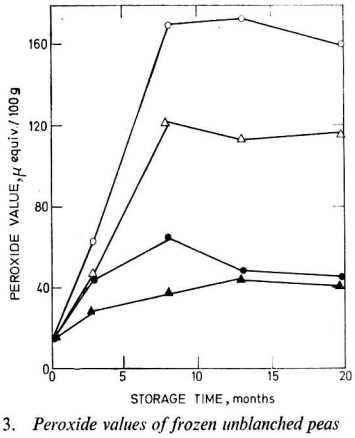


FIG. 3. *Peroxide values of frozen unblanched peas*

○ stored in oxygen at — 9·4°C △ stored in nitrogen at — 9·4°C
● stored in oxygen at —23·3°C ▲ stored in nitrogen at —23·3°C

The addition of 1 mg purified lipoxygenase to a system containing 0·2 ml crude soya extract and 5 mg linoleic acid caused 81% bleaching in 2 min at pH 6·5 compared with 54·4% bleaching with extract and linoleic acid. Bleaching with extract alone (6·4%) was greater than that produced by linoleic acid and 2 mg lipoxygenase (3·4%). Addition of 1 mg lipoxygenase to soya extract increased the bleaching to 8·9% but was still less than that produced by extract and linoleic acid.

The addition of long-chain fatty acids that are not substrates for lipoxygenase gave less bleaching than acids containing the *cis*, *cis*-1, 4-pentadiene system of double bonds. While 5 mg pure linoleic, linolenic (> 99·8%) and arachidonic (> 90%) acids produced 76·3%, 81·4% and 67·8% bleaching respectively with 0·5 ml soya extract at pH 6·5, stearic (> 95%), elaidic (> 95%) and oleic (> 99%) acids produced only 26·2%, 24·0% and 25·4% bleaching respectively after 2 min incubation. In the absence of fatty acids, 12% bleaching occurred.

Chlorophyll bleaching activity in peas was completely destroyed by acetone extraction. An acetone powder of

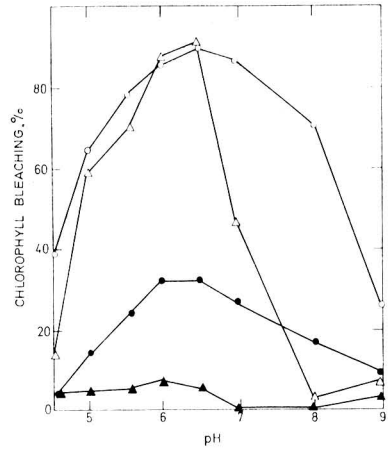


FIG. 4. *Effect of pH and linoleic acid on chlorophyll bleaching with soya and pea extracts*

○ soya extract and linoleic acid △ pea extract and linoleic acid
● soya extract ▲ pea extract

fresh peas, however, retained 64% of the lipoxygenase activity initially present. The greater ease of destruction of bleaching activity was also found in unblanched frozen peas during storage. This is described in detail later. Preliminary fractionation studies on crude soya extract have verified that most of the material responsible for bleaching is precipitated from ammonium sulphate solutions between 20% and 35% by wt., and is readily distinguished from lipoxygenase activity.[38]

Breakdown of preformed hydroperoxides in oxidised linoleic acid was indicated by an increase in the absorption at 280 nm at increasing enzyme levels and incubation times. Hydroperoxide level as indicated by absorbance at 234 nm or peroxide values measured by the ferric thiocyanate method generally decreased slightly at high enzyme levels and extended incubation periods, but not to the same extent as reported by other workers.[21,23,25]

Chlorophyll was bleached in the presence of pea extract (4 ml pH 6·5 buffer/g) and 5 mg oxidised linoleic acid to almost the same extent as with unoxidised acid (Table IV). The addition of hemin (5×10^{-4} M) initially increased chlorophyll bleaching after 1 min and 2 min incubation, but on further incubation showed very little increase in bleaching. Hemin and oxidised linoleic acid bleached chlorophyll up to 1 min incubation, but little further bleaching occurred up to 10 min. The addition of pea extract to oxidised linoleic acid and hemin initially showed bleaching similar to that produced by unoxidised linoleic acid, but the increase in bleaching during further incubation was less in the presence of oxidised acid. Chlorophyll was not bleached when incubated with linoleic acid and hemin, or with oxidised linoleic acid, in the absence of pea extract.

Chlorophyll bleaching and lipoxygenase activities in frozen unblanched peas

Enzyme activities in frozen unblanched peas decreased during storage for 20 months at −9·4° and −23·3° (Fig. 5).

TABLE IV

Effect of oxidised linoleic acid and hemin on chlorophyll bleaching by pea extract

Contents	Chlorophyll bleaching, %			
	Incubation time, min			
	1	2	4	10
5 mg linoleic acid, 0·5 ml boiled pea extract	0·0	0·0	0·0	0·0
5 mg linoleic acid, 0·5 ml pea extract	25·2	49·6	69·3	79·3
5 mg linoleic acid, 0·5 ml hemin	0·0	0·0	0·0	0·0
5 mg linoleic acid, 0·5 ml hemin, 0·5 ml pea extract	41·1	62·7	72·2	82·7
5 mg oxidised linoleic acid, 0·5 ml boiled pea extract	0·0	0·0	0·0	0·0
5 mg oxidised linoleic acid, 0·5 ml pea extract	22·3	41·8	61·9	69·3
5 mg oxidised linoleic acid, 0·5 ml hemin	27·4	32·6	31·2	30·3
5 mg oxidised linoleic acid, 0·5 ml hemin, 0·5 ml pea extract	44·8	59·5	69·5	71·5

Chlorophyll bleaching activity decreased more rapidly than lipoxygenase, and losses in samples stored in oxygen were greater than in those stored in nitrogen, at either temperature. Blanched peas showed no enzymic activity at any storage period.

Discussion

Considerable changes occurred in unblanched peas during frozen storage compared with peas that were adequately blanched. Not only was there a greater conversion of chlorophylls to pheophytins, which is consistent with an increase in free fatty acids as a result of lipase activity,[13,39] but this was accompanied by the formation of chlorophyllides and pheophorbides and also a decrease in total chlorophyll. Unblanched, brined cucumbers have been shown to contain phytol-free pigments,[31] but their presence in frozen unblanched peas has not previously been reported. The enzyme chlorophyllase (E.C. 3.1.1.14) is widely distributed and although it is now believed that the final step in the biosynthesis of chlorophyll *a* is the esterification of chlorophyllide *a* at C-7,[40] the activity of this enzyme in the degradation of chlorophyll is not well understood.[41] The liberation of active enzyme induced by cellular damage during freezing and storage is possible, but attempts to demonstrate the presence of chlorophyllase were inconclusive.

The absence of these changes in blanched peas, except for a relatively slow conversion of chlorophylls to pheophytins, indicates that the reactions are principally enzymic. Chlorophyll degradation and lipid oxidation in frozen unblanched peas have been attributed to lipoxygenase[2,7] but other factors are also involved. Holden[21] examined the chlorophyll bleaching and lipoxygenase activities of pea, soya and other legume seed extracts and showed that chlorophyll degradation appeared to be coupled to the breakdown of fatty acid hydroperoxides catalysed by a heat-labile bleaching factor. The results of the present work are in agreement with those findings. That lipoxygenase alone is not responsible is shown by the following factors: the pH optimum for chlorophyll bleaching in pea and soya extracts was somewhat lower than that for lipoxygenase;[42–46] purified lipoxygenase did not bleach chlorophyll in the absence of crude extracts and linoleic acid, but increased bleaching in their presence; extraction of peas with acetone destroyed all chlorophyll bleaching activity, but 64% lipoxygenase activity remained; breakdown of preformed linoleic acid hydroperoxides by hemin resulted in chlorophyll bleaching when no crude extract was present, while in the presence of extract, hemin

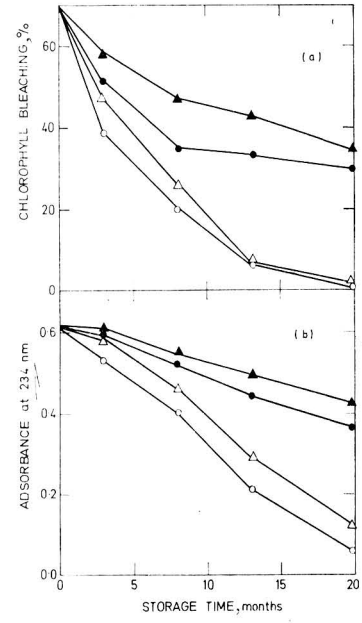


FIG. 5. *Chlorophyll bleaching and lipoxygenase activities of frozen unblanched peas*

(a) Chlorophyll bleaching; (b) Lipoxygenase
○ stored in oxygen at — 9·4°c △ stored in nitrogen at — 9·4°c
● stored in oxygen at —23·3°c ▲ stored in nitrogen at —23·3°c

increased bleaching although it did not induce any hydroperoxide formation; crude extracts incubated with linoleic acid produced increasing quantities of materials absorbing at 280 nm, while oxidation of fatty acids with purified lipoxygenase showed increases in absorbance at 234 nm with little or no absorption near 280 nm; and bleaching activity was destroyed more rapidly than lipoxygenase activity during storage of frozen unblanched peas.

The increases in TBA and peroxide values of frozen unblanched peas were consistent with lipoxygenase activity, although Walker[8] showed that after a lag period of 12 months considerable quantities of peroxides accumulated from heat-induced lipid oxidation reactions, and chlorophylls were destroyed, in samples of blanched French beans stored a

further 10 months at −17·8°. The gradual loss of lipoxygenase activity during storage observed in the present study was in agreement with the findings of Rhee & Watts,[15] who showed that 31% of the lipoxygenase present in raw Blackeye peas was destroyed during 100 days storage at −17·8°.

TBA values increased throughout the storage period, but were low compared with values for rancid animal products.[15] Peroxide values decreased after 8 months except for peas stored in nitrogen at −23·3°. This could be explained by a more rapid rate of peroxide formation compared to peroxide destruction up to 8 months storage, at which time the supply of substrate (e.g. linoleic acid, oxygen) may have diminished sufficiently while allowing peroxide destruction to continue with an increase in the level of TBA-reactive materials.

The decrease in total chlorophyll content is in agreement with a scheme involving peroxide formation by lipoxygenase, and its subsequent destruction with coupled chlorophyll breakdown by a factor(s) similar to the lipohydroperoxide breakdown factor observed in pea and soya extracts, although it is possible that other mechanisms may also be involved.

The use of the term 'carotene oxidase' to describe lipoxygenase activity in soya extracts in terms of coupled carotene destruction,[47,48] has recently been questioned. Blain *et al.*[49] examined the carotene bleaching activity of tomato extracts in the presence of unsaturated fats and concluded that the characteristics of the water-soluble factor(s) responsible for bleaching were unlike those shown by crude soya lipoxygenase, and more closely resembled hematin compounds. Kies *et al.*[50] have recently shown that carotene oxidase activity in a partially purified soyabean lipoxygenase preparation is more susceptible to heat inactivation than lipoxygenase activity, indicating that some factor other than lipoxygenase is also involved in the coupled bleaching of carotene. The homogeneous crystalline soyabean lipoxygenase preparation of Theorell *et al.*[51] was ineffective in destroying carotene in the coupled reaction with methyl linoleate, but did produce linoleate hydroperoxide.

In view of some similarities and differences between these reports and some of the results of the present work, the characteristics of the enzyme systems responsible for the coupled destruction of carotene, and chlorophyll bleaching in pea and soya extracts, are to be investigated in more detail.

Acknowledgments

The authors thank Mr. P. C. Thompson, Gordon Edgell Pty. Ltd., Sydney for supplying the raw materials, and General Motors-Holden Pty. Ltd., and the University of New South Wales for Postgraduate Scholarships to K.A.B.

Department of Food Technology,
University of New South Wales,
P.O. Box 1,
Kensington,
N.S.W. 2033,
Australia

Received 27 November, 1969

References

1. Lindquist, F. E., Dietrich, W. C., & Boggs, M. M., *Fd Technol., Champaign*, 1950, **4,** 5
2. Wagenknecht, A. C., Lee, F. A., & Boyle, F. P., *Fd Res.*, 1952, **17,** 343
3. Dietrich, W. C., Lindquist, F. E., Bohart, G. D., Morris, H. J., & Nutting, M. D., *Fd Res.*, 1955, **20,** 480
4. Dietrich, W. C., Lindquist, F. E., Miers, J. C., Bohart, G. S., Neumann, H. J., & Talburt, W. F., *Fd Technol., Champaign*, 1957, **11,** 109
5. Dietrich, W. C., Lindquist, F. E., & Boggs, M. M., *Fd Technol., Champaign*, 1957, **11,** 485
6. Boggs, M. M., Deitrich, W. C., Nutting, M. D., Olson, R. L., Lindquist, F. E., Bohart, G. S., Neumann, H. J., & Morris, H. J., *Fd Technol., Champaign*, 1960, **14,** 181
7. Wagenknecht, A. C., & Lee, F. A., *Fd Res.*, 1956, **21,** 605
8. Walker, G. C., *J. Fd Sci.*, 1964, **29,** 383
9. Walker, G. C., *J. Fd Sci.*, 1964, **29,** 389
10. Last, J. H., & Shipton, J., *C.S.I.R.O. Fd Preserv.* Q., 1966, **26,** 44
11. Lee, F. A., & Wagenknecht, A. C., *Fd Res.*, 1951, **16,** 239
12. Lee, F. A., *Fd Res.*, 1954, **19,** 515
13. Lee, F. A., Wagenknecht, A. C., & Hening, J. C., *Fd Res.*, 1955, **20,** 289
14. Wagenknecht, A. C., & Lee, F. A., *Fd Res.*, 1958, **23,** 25
15. Rhee, K. S., & Watts, B. M., *J. Fd Sci.*, 1966, **31,** 675
16. Whitfield, F. B., & Shipton, J., *J. Fd Sci.*, 1966, **31,** 328
17. Chow, L., & Watts, B. M., *Fd Technol., Champaign*, 1969, **23,** 973
18. Siddiqi, A. M., & Tappel, A. L., *Archs Biochem. Biophys.*, 1956, **60,** 91
19. Rhee, K. S., & Watts, B. M., *J. Fd Sci.*, 1966, **31,** 664
20. Mapson, L. W., & Moustafa, E. M., *Biochem. J.*, 1955, **60,** 71
21. Holden, M., *J. Sci. Fd Agric.*, 1965, **16,** 312
22. Blain, J. A., & Styles, E. C. C., *Nature, Lond.*, 1959, **184,** 1141
23. Dillard, M. G., Henick, A. S., & Koch, R. B., *J. biol. Chem.*, 1961, **236,** 37
24. Blain, J. A., & Barr, T., *Nature, Lond.*, 1961, **190,** 538
25. Gini, B., & Koch, R. B., *J. Fd Sci.*, 1961, **26,** 359
26. Lynch, L. J., & Mitchell, R. S., *C.S.I.R.O. Bull.*, 1953, No. 273
27. Masure, M. P., & Campbell, H., *Fruit. Prod. J.*, 1944, **23,** 369
28. Vogel, A. I., 'Practical Organic Chemistry', 1961, (London: Longmans, Green & Co. Ltd.)
29. Dietrich, W. C., *Fd Technol., Champaign*, 1958, **12,** 428
30. Vernon, L. P., *Analyt. Chem.*, 1960, **32,** 1144
31. White, R. C., Jones, I. D., & Gibbs, E., *J. Fd Sci.*, 1963, **28,** 431
32. Buckle, K. A., & Edwards, R. A., *J. Fd Technol.*, in press
33. Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan, L. R., *J. Am. Oil Chem. Soc.*, 1960, **37,** 44
34. Kwon, T. W., & Watts, B. M., *J. Fd Sci.*, 1963, **28,** 627
35. Bligh, E. G., & Dyer, W. J., *Can. J. Biochem. Physiol.*, 1959, **37,** 911
36. Buckle, K. A., & Edwards, R. A., *Phytochemistry* 1969, **8,** 1901
37. Willstatter, R., & Stoll, A., 'Investigations on Chlorophyll', 1928, (translated by Shertz, F. M., & Merz, A. R.), p. 24 (Lancaster, Pennsylvania: Science Printing Press)
38. Goswami, R. C., & Buckle, .K A., 1969, Unpublished observations
39. Bengtsson, B., & Bosund, I., *J. Fd Sci.*, 1966, **31,** 474
40. Bogorad, L., in 'The Chlorophylls', 1966 (Ed. Vernon, L. P., & Seely, G. R.), p. 481 (New York: Academic Press Inc.)
41. Holden, M., *Rep. Rothamsted exp. Stn for* 1966, 1967, 310
42. Koch, R. B., Stern, B., & Ferrari, C. G., *Archs Biochem. Biophys.*, 1958, **78,** 165
43. Dillard, M. G., Henick, A. S., & Koch, R. B., *Fd Res.*, 1960, **25,** 544
44. Surrey, K., *Pl. Physiol.*, 1964, **39,** 65
45. Ames, G. R., & King, A. T., *J. Sci. Fd Agric.*, 1966, **17,** 301
46. Allen, J. C., *Europ. J. Biochem.*, 1968, **4,** 201
47. Blain, J. A., Hawthorn, J., & Todd, J. P., *J. Sci. Fd Agric.*, 1953, **4,** 580
48. Tookey, H. K., Wilson, R. G., Lohmar, R. L., & Dutton, H. J., *J. biol. Chem.*, 1958, **230,** 65
49. Blain, J. A., Patterson, J. D. E., & Pearce, M., *J. Sci. Fd Agric.*, 1968, **19,** 713
50. Kies, M. W., Haining, J. L., Pistorius, E., Schroeder, D. H., & Axelrod, B., *Biochem. Biophys. Res. Commun.*, 1969, **36,** 312
51. Theorell, H., Holman, R. T., & Axelrod, A., *Acta chem. scand.*, 1947, **1,** 571

VARIATIONS IN THE VITAMIN C CONTENT OF IMPORTED TOMATOES

By D. G. TWOMEY and JANE GOODCHILD*

An analysis was made of the vitamin C content of late winter tomatoes.

A range of 6·8 to 31·2 mg reduced ascorbic acid per 100 g of fruit was observed in the batches of fruit examined in mid-March 1969. The mean value was 12·6 mg ascorbic acid per 100 g of fruit.

It is emphasised that this mean figure was considerably lower than the 20 mg per 100 g given in McCance & Widdowson's (1967) 'Tables of the Composition of Foods' which are widely used in dietary surveys.

Introduction

The annual report of the National Food Survey Committee[1] for 1964 calculated that fresh tomatoes contributed 8·4% of the total vitamin C content of household food consumption, a proportion exceeded by only two other single items of the diet; potatoes 31·8% and oranges 11%, with cabbages, Brussel sprouts and cauliflower together providing 10·7%, and liquid milk 8·2%.

During the early spring months, when the vitamin C content of potatoes is low and fresh green vegetables are not plentiful it is usual to regard tomatoes as a valuable source of vitamin C, especially for people who do not eat citrus fruit or take blackcurrant or similar vitamin C supplements.

Bender[2] reported a range in vitamin C concentration of freshly pressed tomato juice from 1·8 to 29·3 mg per 100 g of fruit. Tomatoes grown in direct sunlight were shown by Hassan & McCollum[3] to contain significantly more ascorbic acid than fruit grown in the shade, and the work of Clutter & Miller[4] indicated that storage and transport conditions after harvesting will also affect the ascorbic acid content of the tomato picked before ripening. During a survey of vitamin C consumption during the winter of 1968–1969 a wide variation was observed in ascorbic acid content of tomatoes imported from the Canary Islands and purchased from retail shops during a two-day period in mid-March 1969.

Experimental

Materials

Random samples of sound ripe imported tomatoes were examined within two days of purchase, 450 g samples being cut into segments and ground with sand and 4% metaphosphoric acid in a large pestle and mortar. The macerate was kept covered by acid to extract the ascorbic acid and to prevent its oxidation. After maceration, the whole volume of extracted juice was measured, and the solids were removed by centrifugation and filtration.

Method

Aliquots of the filtrate were analysed for vitamin C colorimetrically, with 2,6-dichlorophenol–indophenol, according to the method of Hughes.[5]

Results

It was found that the reduced ascorbic acid content of the tomatoes purchased in March in several towns in England and Wales varied from 6·8 to 31·2 mg per 100 g of fruit with a mean value of 12·6 mg (standard error +2·6), compared with the value of 20 mg per 100 g recorded by McCance & Widdowson.[6] The tomatoes of each purchase tended to be of uniform size, but there was no apparent relationship between size of fruit and ascorbic acid content.

Only the reduced ascorbic acid is reported here so that a comparison may be made with the figures of McCance & Widdowson, because their values for fresh fruit were based on a 2,6-dichlorophenol–indophenol titration, which does not measure dehydroascorbic acid. A study of the proportions of ascorbic acid to dehydroascorbic acid has been made and will be reported at a later date.

Discussion

Any assumption that the ascorbic acid content of tomatoes is reasonably consistent throughout the year, regardless of season or source, is disproved by the present results. During March, imported tomatoes are a much less rich source of ascorbic acid than the single value for fresh tomatoes published by McCance & Widdowson implies.

These observations emphasise the limitations of published tables of ascorbic acid content and underline the importance of making laboratory determinations on food samples, whenever possible, when attempting dietary assessment of vitamin C intake.

Acknowledgments

The authors thank Dr. R. E. Hughes, University of Wales Institute of Science and Technology, for helpful advice during the preparation of the manuscript and Dr. M. Brook for encouragement during the course of the study.

Basic Research Department,
Beecham Products (U.K.),
Brentford,
Middlesex

Received 12 January, 1970

References

1. National Food Survey Committee for 1964 (Minist. Agric., Fish. Fd natn. Fd Surv. Comm., 1966)
2. Bender, A. E., 'Dietetic foods', 1967 (London: Leonard Hill Books)
3. Hassan, H. H., & McCollum, J. P., Bull. 573, 1954 (Univ. of Illinois agric. expl Stn)
4. Clutter, M. E., & Miller, E. V., *Econ. Bot.*, 1961, **15**, 218
5. Hughes, R. E., *Biochem. J.*, 1956, **64**, 203
6. McCance, R. A., & Widdowson, E. M., 'The composition of foods', 1967 (London: H.M.S.O.)

* Present address: Department of Biology, Ewell County Technical College, Surrey

NOTE ON L-ASCORBIC ACID CONTENT OF ENGLISH EARLY TOMATOES

By D. G. TWOMEY and B. D. RIDGE*

An analysis was made of the vitamin C content of English early tomatoes harvested in the spring and early summer.

As the season progressed, the reduced ascorbic acid content was observed to increase from 12·3 mg/100 g in May to 22·1 mg/100 g in July.

The 20 mg ascorbic acid/100 g tissue for tomatoes quoted in McCance & Widdowson's 'Tables of the Composition of Foods' probably applies only to tomatoes ripened on the plant in midsummer and overestimates the content of the earlier crop.

Introduction

English glasshouse tomatoes enter the retail market during early spring and are continuously available until the late autumn. Hammer *et al.*[1] found that American tomatoes maturing in autumn and winter contained approximately half the ascorbic acid concentration of fruits maturing in summer. Fryer *et al.*[2] reported that when American glasshouse tomatoes were harvested red ripe there was an essentially linear increase in ascorbic acid concentration with increasing cluster number up the plant.

The first tomatoes harvested are normally those from the lowest trusses, and a preliminary study of the ascorbic acid content of English tomatoes during the early season of 1969 was carried out by the authors.

Experimental

English tomatoes, not identified by variety, were purchased in 2-lb batches as they appeared in the retail shops in Gloucestershire during the spring of 1969. Within 24 hours of purchase, 8 to 10 sound ripe tomatoes from each batch were prepared for analysis by removing the calices and bisecting the fruit longitudinally. The halves were selected so that one half of each tomato was placed in one of two jars, resulting in approximately 250 g of halved fruit in each jar. Fruit was homogenised for one minute in a stainless steel, electrically driven macerator, whilst submerged under a measured volume of freshly prepared 4% metaphosphoric acid, the head space being flushed with a gas mixture of 95% nitrogen and 5% carbon dioxide. This method has been shown previously to prevent oxidation of ascorbic acid during maceration. The whole volume of macerate was measured before removal of the solids by centrifugation, followed by filtration.

The method of Hughes[3] was used to determine the vitamin C content photometrically with 2,6-dichlorophenol–indophenol.

Results and Discussion

Results are given in Table I.

An increase in reduced ascorbic acid concentration of tomatoes purchased from shops as the season progressed was observed. A concentration of 12·3 mg/100 g fruit, at the beginning of May, increased to 22·1 mg/100 g fruit during July. This is consistent with the findings of Fryer *et al.*[2] and Clutter & Miller[4] who suggested from observations on American fruit that a seasonal variation occurred in ascorbic acid content.

The present measurements on fruit purchased later in July agree with those reported by the Government Chemist in 1967,[5] and with the 20 mg/100 g fruit given in McCance and Widdowson's Tables.[6]

TABLE I

Ascorbic acid content of English tomatoes

Date of purchase	Reduced ascorbic acid, mg/100g (mean of two determinations on 500 g macerated fruit)
1/5/69	12·3
20/5/69	15·0
12/6/69	15·2
30/6/69	16·9
3/7/69	15·3
9/7/69	22·1
18/7/69	20·3
	Mean 16·7

The fruit examined in spring, however, did not supply the quantity of vitamin C that reference to McCance and Widdowson's Tables would suggest. These findings confirm the earlier observation[7] on imported tomatoes in winter, that reliance on published tables will lead to an overestimate of the vitamin C contribution made by tomatoes during winter and spring, a period of the year when dietary vitamin C intake is at its lowest. It would appear that the figure of 20 mg/100 g content of ascorbic acid in tomatoes applies only during the summer months.

Acknowledgments

The authors wish to thank Dr. R. E. Hughes, of the University of Wales Institute of Science and Technology, for guidance on the preparation of the manuscript and Dr. M. Brook for his advice and encouragement during the course of this study.

Basic Research Department,
Beecham Products (U.K.),
Brentford,
Middlesex

Received 12 January, 1970

References

1. Hammer, K. C., Lyon, C. B., & Hamner, C. L., *Bot. Gaz.*, 1942, **103**, 583
2. Fryer, H. C., Ascham, L., Cardwell, A. B., Frazier, J. C., & Willis, W. W., *Proc. Am. Soc. hort. Sci.*, 1954, **64**, 360
3. Hughes, R. E., *Biochem. J.*, 1956, **64**, 203
4. Clutter, M. E., & Miller, E. V., *Econ. Bot.*, 1961, **15**, 218
5. *Rep. Govt Chem., Lond.*, 1967, **49** (London: H.M.S.O.)
6. McCance, R. A., & Widdowson, E. M., 'The composition of foods', 1967 (London: H.M.S.O.)
7. Twomey, D. G., & Goodchild, J., *J. Sci. Fd Agric.*, 1970, **21**, 313

* Present address: Department of Biology, Ewell County Technical College, Surrey

PINK DISCOLORATION IN CANNED PEARS
I.—Role of tin in pigment formation

By B. V. CHANDLER and K. MARY CLEGG*

The major pigment in discoloured canned pears is identified as a purple-pink insoluble tin–anthocyanin complex from the effect of reagents for anthocyanins on the spectra of solid and syrup fractions of the product. This characterisation is supported by significant correlations between the intensity of reflected colour and the insoluble tin content of the solid fraction. It is suggested that formation of a tin complex is essential for the occurrence of pink discolorations in canned pears, and experiments are described designed to vary the intensity of discoloration by modifying the conditions controlling complex formation.

Introduction

The pink discoloration which sometimes occurs during the commercial processing of canned pears results from decomposition of the leucocyanidins in the fresh fruit, and the pigment has been assumed to be cyanidin, the principal identifiable product when acidified pear extractives are heated.[1,2] However, whereas cyanidin is water-soluble, the pigment in canned pears adheres strongly to the pear segments,[1] and it has been found that nearly colourless syrups are obtained on filtration of even extremely discoloured products. Reddish pigments also form in canned apples and gooseberries and it has been suggested[3] that these discolorations are in some way dependent on the presence of tin, since they do not occur in lacquered cans. Similar, but less detailed observations relating to discoloured canned pears had been made 30 years earlier by Russian workers.[4]

This paper presents evidence that the pigment in canned pears is a tin–anthocyanin complex, and reports some preliminary experiments designed to inhibit the discoloration by preventing complex formation.

Experimental and Results

Examination of pink pears

William's Bon Chrétien (Bartlett) pears in plain tinplate cans from a commercial batch showing discoloration were further heated in the can for 80 min at 110° to accentuate the discoloration. Syrup from these cans was filtered twice through Whatman No. 541 and No. 1 filter papers to give 'pink pear syrup'. The halves were blended with twice their weight of acidified water (pH 2·5) and the solid fraction was separated by centrifugation. After four such treatments the sediment was transferred in $\frac{1}{2}$ in. thick layers onto Whatman No. 541 filter paper and drained for 1 h at full water pump vacuum to give 'pink pear debris'. The wash waters in this preparation showed no absorption at 530 nm.

Treatment of moist or lyophilised debris with neutral or acidified ethanol, butanol or amyl alcohol in a ratio of debris to solvent of 5:1 by wt. gave extracts of absorbance at 500–550 nm of less than 0·02 in 1 cm cells. Attempts to obtain coloured extracts from pink pear syrup were similarly unsuccessful with a ratio of syrup to butanol or amyl alcohol of 10:1 by vol.

The reflectance spectrum of pink pear debris had a minimum at 530–540 nm recorded on a Beckman Model DU Spectrophotometer against a standard magnesium oxide block, while normal pear debris gave a featureless spectrum (Fig. 1). Irreversible absorption of cyanidin chloride onto normal pear debris resulted in material which also gave a featureless spectrum against magnesium oxide, but a broad minimum appeared at 500–525 nm when the spectrum was measured against pear debris of normal colour; when the spectrum of pink pear debris was measured against normal pear debris the minimum still appeared at 530–540 nm but was greatly accentuated (Fig. 2).

Treatment with strong acids, e. g. 1:1 (by vol.) ethanol–1N hydrochloric acid, led to loss of colour from both debris and syrup, and the colour was not regenerated on careful addition of alkali. Direct treatment with alkali resulted in transient

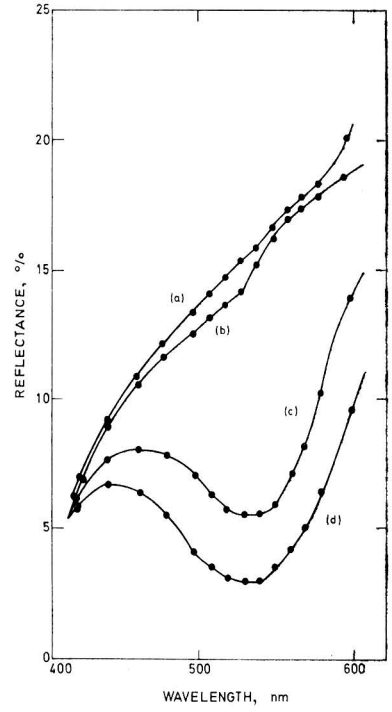


FIG. 1. *Reflectance spectra against magnesium oxide of* (a) ***debris*** *from normal canned William B.C. pears,* (b) ***debris from normal*** *processed Packham pear purée,* (c) ***debris from discoloured canned*** *William B.C. pears, and* (d) ***debris from Packham Pear purée*** *processed with stannous chloride added*

* Present address: Department of Food Science, University of Strathclyde, Glasgow, C.1.

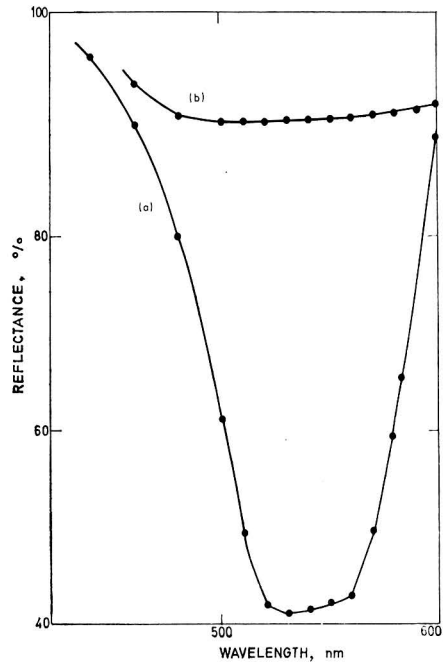


FIG. 2. *Reflectance spectra against debris from normal canned William B.C. pears of* (a) *debris from discoloured canned William B.C. pears and* (b) *debris from normal canned William B.C. pears irreversibly dyed with cyanidin chloride*

green, then brown colorations in both fractions, at about pH 8·5. Peroxide caused slow decolorisation in both debris and syrup, but sulphurous acid had no effect. The spectrum of the syrup was unaltered by the addition of aluminium chloride, but the maximum was shifted from 530–535 nm to 515 nm on addition of hydrochloric acid; the acidified syrup had an absorbance approximating to that of a solution of 2 mg cyanidin chloride/l.

A solution of cyanidin chloride in 15% sucrose solution had an absorption maximum at 520 nm, unchanged by addition of acid, but shifted to 537 nm by addition of stannous or aluminium chloride, the shift being reversed by addition of acid. Solutions of both cyanidin chloride and its tin complex turned green on addition of alkali, the colour fading rapidly. Both peroxide and sulphurous acid decolorised cyanidin chloride solutions, but only peroxide decolorised the cyanidin–tin complex.

Tin and iron contents of pink pear halves

Canned William B.C. pear halves from the same source as above were overprocessed for 80 min at 110°. The drained halves in each sample were blended, and total tin and total iron contents were determined[5] on selected samples. Pink pear debris was prepared as above from all samples and the reflectances of the thoroughly drained material were measured at 530 nm against a standard magnesium oxide block. The debris was also used for analyses of insoluble tin and insoluble iron; initially the debris was dried at 80° for 16 h before analysis but, because drying rates for the samples varied, subsequent samples of the debris were analysed immediately after draining. Tables I(a) and I(b) show the results of these examinations.

TABLE I (a)

Reflectance measurements and tin and iron analyses of pears with varying degrees of discoloration

Reflectance, %	mg/100 g purée		mg/100 g dry debris	
	Total Fe	Total Sn	Insol. Fe	Insol. Sn
27·1	—	—	10·0	80·0
26·5	0·37	12·3	6·1	92·5
23·4	0·40	7·0	8·2	55·0
19·8	0·42	12·0	8·5	66·5
19·1	—	—	8·6	66·5
19·1	—	—	10·8	153·5
18·7	0·49	11·5	8·6	111·5
18·4	0·29	6·8	4·7	63·0
18·1	0·34	8·0	12·5	95·0
17·9	—	—	13·0	80·5
17·9	0·32	12·0	4·0	125·0
16·1	—	—	9·5	107·5
15·9	0·30	7·6	8·2	149·0
15·9	—	—	7·5	108·0
15·7	—	—	9·8	88·0
15·6	0·36	11·8	15·5	178·0
15·1	—	—	6·1	135·0
15·1	—	—	7·9	155·0
14·3	0·37	7·1	4·8	146·0
14·3	—	—	8·2	116·5
14·2	—	—	8·0	146·0
14·0	0·28	7·8	6·5	133·5
12·9	—	—	7·1	127·5
12·9	—	—	4·6	192·5
12·1	0·31	7·1	5·5	112·0
11·1	—	—	10·8	73·5
8·2	—	—	8·7	169·5

TABLE I (b)

Reflectance measurements and tin and iron analyses of pears with varying degrees of discoloration

Reflectance, %	mg/100 g purée		mg/100 g moist debris	
	Total Fe	Total Sn	Insol. Fe	Insol. Sn
22·0	—	—	0·17	2·4
16·5	—	—	0·10	1·6
13·3	0·38	9·2	0·17	3·6
13·2	—	—	0·52	3·5
13·1	—	—	0·27	1·5
12·5	0·48	12·5	0·19	3·9
11·9	—	—	0·64	3·0
11·8	—	—	0·22	4·3
11·8	—	—	0·23	4·4
11·5	0·41	9·8	0·10	2·9
11·5	0·35	11·5	0·22	3·9
11·1	—	—	0·44	3·3
10·3	0·45	40·1	0·09	2·6
10·2	0·36	11·4	0·16	4·0
10·0	0·47	11·9	0·24	4·4
9·9	0·40	9·9	0·20	3·0
9·9	—	—	0·19	6·0
8·9	0·35	11·9	0·20	5·2
8·2	0·43	10·5	0·13	3·0
8·2	—	—	0·22	5·6
7·1	—	—	0·17	5·9
5·9	—	—	0·16	6·0

TABLE II

Effect of processing time and tin addition on colour, insoluble tin content, and leucocyanidin content of canned pears (representative results)

	With 200 ppm tin				Without tin			
	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
Reflectance of pink pear debris at 530 nm against MgO, %	19·6	15·3	12·0	8·7	20·2	18·8	14·8	10·6
Insoluble tin content of moist pink pear debris, ppm	7·1	13·2	33·4	76·2	3·1	6·1	9·9	31·0
Absorbance in leucocyanidin test[1]	0·25	0·36	0·33	0·17	0·35	0·48	0·25	0·18

Effect of added tin on pear discoloration

Peeled and cored Packham pear halves were packed into plain tinplate cans in 25% sugar syrup with and without the addition of 100 ppm tin as stannous chloride. Three cans from each treatment were processed in boiling water for 30, 60, 90, and 120 min, the drained halves were puréed, and pear debris was prepared as above for insoluble tin analyses and reflectance measurements at 530 nm. In addition, leucocyanidin determinations were made on the debris by the method of Luh *et al.*[1] The results of a typical examination are shown in Table II.

Experiments reproducing abnormal processing conditions

Simulation of excessive blanching or exhausting

A purée was prepared from peeled and cored Packham pears (1·8 kg) and a syrup (1·1 kg) containing 25% sucrose and 3% sodium chloride (to inhibit enzymic browning). The purée was heated in nylon pouches in boiling water for 1 h. The light grey-pink purée was then re-packed into pouches with the addition of 0, 50, and 100 ppm tin as stannous chloride and re-heated in boiling water. Within 1 h the grey-pink discoloration in the tin-free sample merely deepened slightly while in tin-treated samples it changed to an intense purple-pink; further heating increased the intensity of both discolorations, especially in the tin-treated samples.

The pigment in the tin-treated samples was insoluble but some of the pigment in the untreated sample could be extracted by acidified amyl alcohol to give a solution with a weak absorption maximum at 535 nm; paper chromatographic examination showed cyanidin to be a component of this solution.[1] The reflectance spectra of pear debris prepared from untreated samples had a very slight inflexion at 515 nm in the range 420–600 nm, whereas that from tin-treated pears had a marked minimum at 530–540 nm (Fig. 1).

Blanching in citric acid solution

Peeled and cored Packham pears were divided into two batches; one batch was held at room temperature in 3% brine and the other at 100° in 25% syrup containing 0·2% citric acid for 3 min. The fruit in each batch were then quartered, and one-quarter from each pear was taken to provide four groups from each pre-treatment for processing in plain cans at 110° for 30, 60, 90, and 120 min. The occurrence of discoloration was similar, increasing with processing time in samples from both pre-treatments; where differences occurred between pre-treatments, the samples held in brine were slightly less discoloured.

Accelerated corrosion prior to the heat process

Purée prepared as above was packed into nylon pouches with and without the addition of 1·5% citric acid. Six discs of tinplate (dia. 1 in) were added to each pouch in half the acidified samples and some of these were held for 2 h at 60° before processing. After 2 h in boiling water, samples without additives showed brown or grey discolorations, acidified samples without tinplate were light pink or orange-pink, and samples treated with tinplate and acid were deep purple-pink. Purées receiving a preliminary incubation with tinplate and citric acid were not as intensely discoloured as comparable unincubated samples, although the tin contents were about 65 ppm and 45 ppm respectively.

Processing at controlled pH

Packham pear quarters were distributed one from each pear into four batches for processing in nylon pouches – a control pack in 25% syrup, and packs to which stannous chloride (200 ppm Sn), or sodium citrate (0·20%), or both salts had been added. After 1 h in boiling water the control sample showed negligible pinking, the sample with sodium citrate alone showed a slight blush, and those with stannous chloride showed intense pinking. Of the two stannous chloride treatments, the buffered sample (final pH 5·2) was only slightly less pink than the unbuffered sample (final pH 3·8). Blending and standing overnight did not alter the colour of the samples, indicating that adequate equilibriation of component ions had occurred during processing.

In another experiment Packham pear quarters were separated into two batches; one was held 3 min in boiling water before canning in 25% syrup, and the other was held 3 min in boiling 0·5% bicarbonate before canning in 25% syrup containing 0·1% bicarbonate. After a 2 h process in boiling water the pH-adjusted pack (final pH 5·0) was only very slightly less pink than the control pack (final pH 4·2).

Heat stability of tin–cyanidin complex

A solution of cyanidin chloride in citrate buffer at pH 4·0 was treated with 100 ppm tin as stannous chloride. After 15 min at 100° the mauve solution deposited a reddish precipitate, leaving a colourless supernatant liquid. The precipitate was initially soluble in acidified butanol but further heating for 1 h rendered it insoluble. An acidified butanol extract of the reaction mixture after 15 min had an absorbance maximum at 545 nm in a 1 cm cell of 0·70, compared with 0·49 for the extract of an identical cyanidin solution heated in the absence of tin. After 1 h at 100° the tin-free solution was decolorised and gave no pigment on acidification, whereas the tin treated-solution still contained pigment in the form of an insoluble tin complex.

Discussion

The pink pigment in discoloured canned pears resembles the anthocyanidins in certain spectral and chemical properties.

Its ultra-violet spectrum exhibits a distinct peak in the region of anthocyanin absorption, and its reactions with acid, alkali, and peroxide (but not sulphurous acid) are similar to those of cyanidin in low concentration. However, the pink pear pigment differs from the anthocyanidins in its extreme insolubility in the higher alcohols under conditions effective for the extraction of anthocyanins not otherwise readily removed from plants. There is also a marked difference in the stabilities of anthocyanidins and the pink pear pigment to heat, most clearly demonstrated by the fact that discoloured canned pears maintain or increase their pink colour under heating conditions that would cause loss of anthocyanin pigments from other processed foods. Finally, comparative visual and instrumental examinations clearly demonstrated that the colour of the pigment in canned pears was not a true pink but rather a purple-pink, the position of maximum absorption being closer to the blue region of the spectrum than that of the anthocyanins themselves.

These seeming inconsistencies can be explained if the pink pear pigment is related to a tin–cyanidin complex, similar to the tin–anthocyanin complexes reported in anthocyanin-containing canned foods.[6] In both its spectral and chemical properties, the pink pear pigment, especially when examined in the syrup fraction, more closely resembled a synthetic tin–cyanidin complex than cyanidin itself. Moreover, addition of tin ions was found to decrease colour loss from heated cyanidin solutions at pH 4·0, an effect analogous to the inhibition of the natural fading of cyanidin-3-glucoside solutions in the pH range 3·0–4·0 by the formation of aluminium complexes.[7] Thus, the formation of a tin–cyanidin complex would account for the retention of the pink colour in pears and other leucocyanidin-containing fruits processed in plain cans whereas grey or brown products with little or no pink discoloration are obtained when lacquered cans are used.[3] On the other hand, processed strawberries, whose pigments do not contain a complex-forming group,[6] quickly lose their red colour even when packed in plain cans.

Support for this suggestion is supplied by the results in Table I where there is a significant linear correlation between the intensity of reflected colour and the insoluble tin content of pink pear debris; the levels of significance were $P<0{\cdot}01$ and $P<0{\cdot}05$ when the tin analyses were carried out on moist and dry tissues respectively. No significant correlations were found between total tin or insoluble iron contents and the colour intensity of pear tissue. Furthermore, as the results in Table II show, the intensity of discoloration in canned pears increases with increasing tin content, whether added or derived from can corrosion, and experiments with pear purée in nylon pouches clearly demonstrate that tin is necessary for development of intense pigmentation under processing conditions.

However, other pear constituents besides anthocyanins form insoluble tin compounds, and the association between discoloration and tin content might be indirect, since discoloration is symptomatic of high total polyphenolic contents as well as high leucocyanidin contents.[1] In addition, the high insoluble tin content of discoloured pears, increasing during the heat treatment (Table II), might be accounted for by the formation of insoluble tin complexes in increasing amounts with greater corrosion of the tinplate and more extensive penetration of stannous ions into the pears. Moreover, other polyphenolics besides cyanidins may be produced from leucocyanidin breakdown to form increasing amounts of insoluble tin salts simultaneously with pigment production.

Nevertheless, these findings strongly indicate that the formation of a stable pigment in commercial canned pears depends on the formation of an insoluble and stable complex between cyanidin-type molecules produced from leucocyanidin degradation and tin ions produced from can corrosion. Variations in the rates of tin dissolution and leucocyanidin degradation under various processing conditions could then account, at least in part, for random occurrences of discoloration in commercially processed pears. Processes which promote leucocyanidin breakdown or retard tin corrosion could thus circumvent the pear discoloration problem; the use of lacquered cans with apple and gooseberries[3] indicates the application of this principle but with pears the product shows other colour defects.

Two pre-processing treatments were tested which aimed at degrading the pear leucocyanidins before the introduction of tin. In the first procedure, pear purée was held for 1 h at 100° prior to treatment with tin salts. In the second procedure, which has already been recommended for prevention of pink discoloration,[8] pears were blanched in citric acid before the usual canning process. In neither case was discoloration prevented, and the results cast doubt on the practicality of avoiding the problem by accelerating leucocyanidin degradation in a blanching operation. This conclusion conforms with the observation that leucocyanidins persist in pears which have been heated in boiling water for 2 h (Table II). An experiment to simulate accelerated corrosion of tinplate prior to the heat process gave results opposite to those expected: a purée in which tin corrosion was promoted was less pink after a heat treatment than the control sample, possibly because the pigments initially formed were chemically reduced during the corrosion process.

Furthermore, characterisation of the pigment as related to a tin–cyanidin complex would account for contradictory reports on the variation of discoloration with pH.[9,10] Since fruit acids accelerate both tin corrosion and leucocyanidin breakdown and also provide ions which compete with cyanidin in tin complex formation, the actual processing conditions could determine whether high acid contents increased or inhibited pigment production. The ability of citrate ions at pH 5·2 to rupture complexes involving anthocyanins and metallic ions has been demonstrated by Jurd & Asen,[11] and the procedure recommended by Opris[12] for preventing discoloration by blanching and processing in the presence of bicarbonate may be effective because it establishes a pH unfavourable for complex formation. However, in the present experiments treatment of Packham pears with citrate or bicarbonate did not inhibit discoloration in the processed product.

Some of the trial processes reported in this paper produced a slight reduction in pigment formation with Packham pears, a variety very susceptible to pink discoloration. Since the basic factor in pear discoloration is the leucocyanidin content (low in William B.C., high in Packham pears), these procedures may provide a method to control the problem in those William B.C. pears with sufficiently low leucocyanidin conrents, but cannot be guaranteed to be successful in all cases. The most significant reduction was obtained by promoting tin corrosion in pear purées before degradation of the leucocyanidins, a procedure which might be expected to intensify complex formation. However, studies which will be reported in a subsequent paper have shown that it inhibits discoloration by preventing the formation of the stable purple-pink tin complex, which the present paper identifies as the essential factor in canned pear discoloration.

C.S.I.R.O. Division of Food Preservation,
P.O. Box 43,
Ryde,
New South Wales 2112,
Australia

Received 10 December, 1969

References

1. Luh, B. S., Leonard, S. J., & Patel, D. S., *Fd Technol., Champaign*, 1960, **14**, 53
2. Nortje, B. K., *S. Afr. J. agric. Sci.*, 1966, **9**, 681
3. Anthistle, M. J., & Dickinson, D., 'Natural colouring matters in canned fruits.' *Res. Leafl. Veg. Cann. and quick Freez. Res. Ass. No. 4*, 1959
4. Saburov, N. V., Kalebin, M. I., & Antonov, M. V., *Konserv. Prom.*, 1935, No. 6, 10; *Chem. Abstr.*, 1936, **30**, 1454d
5. Board, P. W., & Elbourne, R. G. P., *Fd Preserv. Q.*, 1964, **24**, 53
6. Heintze, K., *Dt. LebensmittRdsch.*, 1960, **56**, 194
7. Asen, S., Norris, K. H., & Stewart, R. N., *Phytochemistry*, 1969, **8**, 653
8. Khan, S. A., & Khan, S., *W. Pakist. J. agric. Res.*, 1965, **3**, 48
9. Ingalsbe, D. W., Carter, G. H., Neubert, A. M., & Blodgett, E. C., *Proc. Wash. St. hort. Ass. 57th A. Mtg*, 1961, p. 158
10. van der Merwe, H. B., *Fd Inds S. Afr.*, 1963, **15**, 56
11. Jurd, L., & Asen, S., *Phytochemistry*, 1966, **5**, 1263
12. Opris, St., *Industria Aliment., Buc.*, 1964, **15**, 289

PINK DISCOLORATION IN CANNED PEARS
II.*—Measurement of potential and developed colour in pear samples

By B. V. CHANDLER and K. MARY CLEGG†

Procedures are described for the comparative assessment of the leucocyanidin content of pears, which is the principal factor determining their susceptibility to discoloration on processing, and for measuring the shade and depth of discoloration in the processed material. Application of these procedures to pears of different varieties, storage treatment, and processing treatments lead to useful conclusions relevant to the discoloration problem, and establish relations between the three parameters under certain defined processing conditions. The results emphasise the difficulties associated with the assessment of the susceptibility of large consignments of pears to discoloration under normal canning operations.

Introduction

For studies on the pink discoloration that sometimes develops in canned pears, a need has arisen for comparative assessments of both the susceptibility of pears to discoloration, and the shade and depth of the discoloration in processed material.

It is now accepted[1] that the major factor in the susceptibility of pears to discoloration is their leucocyanidin content, usually assessed by extraction of pear flesh and heating the acidified extracts to convert the colourless leucocyanidin to the coloured cyanidin for spectrophotometric measurement. The many problems encountered with this procedure were discussed by Smathers & Charley[2] who could extract no more than two-thirds of the total leucocyanidin in pears and found marked variations in the amount of cyanidin produced depending on the solvent used for colour development. This paper now reports that a modification of the earlier procedure of Luh *et al.*[1] gives satisfactory and reproducible results that can be used for comparative purposes provided precautions are taken in the preparation of the extracts.

Nortje[3] was the first to replace the subjective assessment of discoloration in processed pears used by earlier workers[1,4] with an instrumental method using green and amber filters to give a single reflectance measurement of pink colour. However, since the discoloration in canned pears is due to a purple-pink tin complex,[5] a procedure was developed involving a tristimulus reflectance photometer which provided two parameters to measure separately the depth of pink and purple discolorations.

This method is now described, the results obtained with processed material in relation to the leucocyanidin contents of fresh pears are discussed, and the applicability of such measurements to the assessment of the susceptibility of pears to pink discoloration is assessed.

Experimental and Results

Determination of leucocyanidin number

Pear material (peeled and cored) was rapidly blended with 27·5% (by wt.) sucrose syrup containing 3% sodium chloride, in a flesh to syrup ratio of 180:110 parts by wt. An aliquot (10 g) of the freshly blended purée was stirred mechanically for 10 min with butanol (50 ml), 10N hydrochloric acid (2 ml), and sodium chloride (1·5 g), and the butanol extract of soluble pear leucocyanidin was separated by centrifugation. The extract was heated at 100° for 15 min and the absorbance of the resultant solution was measured at 530 nm in 1 cm cells; the result, multiplied by 1000, was recorded as the leucocyanidin number (*LN*) to provide a measure of the susceptibility of the pear sample to discoloration. Ten replicates of the determination of the *LN* for three purée samples by this method gave mean values of 246 ± 5, 329 ± 7, and 471 ± 9. Application of this procedure to purées which had already been extracted as above gave only pale yellow solutions with negligible absorbance at 530 nm.

* Part I: Preceding paper

† Present address: Department of Food Science, University of Strathclyde, Glasgow, C.1.

Fig. 1 shows typical results of the application of this test to purées of mature and partly mature Packham pears which had been allowed to stand for various periods after blending. Butanol extracts allowed to stand 1 h before colour development gave *LN* values for three purées of 215, 341, and 389, whereas comparable fresh extracts gave respective values of 271, 397, and 473. Butanol extracts allowed to stand 24 h after colour development gave *LN* values for three purées of 224, 283, and 315, compared with respective values of 241, 308, and 347 when readings were taken immediately after colour development.

Three purée samples blended without salt gave *LN* values of 35, 43, and 67 after standing 10 min, whereas comparable purées blended with salt gave values of 241, 357, and 318 respectively; the purées blended without salt were brown, while those blended with salt were creamy white. When the extraction with butanol was performed without addition of acid (which was subsequently added before colour development), values of 241, 362, and 665 were obtained for three purées which gave *LN* of 300, 491, and 875 when acidified butanol was used for the extraction.

Three purée samples heated for 1 h before extraction with butanol gave *LN* values of 550, 763, and 1250 while the comparable unheated samples gave values of 490, 678, and 870; determinations on ten samples of the same purée separately heated gave 576 ± 19. Fig. 2 shows typical results for two pear purées which had been heated for varying periods at 100° in nylon pouches.

Application of the method

The method for *LN* determination was applied individually to samples of randomly purchased fruit. Values of 202, 241, 427, 645, and 895 were obtained for Packham pears; 429, 495, 525, 590, and 630 for Winter Cole pears; 107, 121, 151, 207, and 265 for William B.C. pears; and 1800 and 2500 for quinces.

Immature late season Packham pears from one orchard were held at −1° and 83% R.H. for varying periods before being ripened by storing several days at 20° and 70% R.H. Determinations of *LN* were made on purées from 15 six-pear samples, 5 samples ripened after no more than 10 days, 5 samples after 11–20 days, and 5 samples after 21 days cool storage; *LN* values ranged from 255 to 409 (mean 318), from 190 to 258 (mean 215), and from 173 to 195 (mean 184) for each of the above three storage periods, respectively.

In the following year, immature early season Packham pears from the same source were similarly treated, and *LN* determinations again made on 15 six-pear samples, 5 samples ripened after 7 days, 5 samples after 8–14 days, and 5 samples after 28 – 35 days cool storage; *LN* values ranged from 442 to 840 (mean 662), from 382 to 720 (mean 516), and from 300 to 525 (mean 422) for each of the above three storage treatments, respectively. Determinations were also made on unripened pears cool stored 35–42 days; 9 six-pear samples gave values between 520 and 765 (mean 628).

Five Packham pears from another consignment gave individual *LN* values after 42 days cool storage ranging from 725 to 1075 (mean 925), while five pears ripened after the same treatment gave values between 468 and 1150 (mean 712).

Assessment of discoloration by reflectance measurements

Tristimulus readings from a Zeiss Elrepho reflectance photometer were used to measure the shade and depth of colour developed in processed pear purée and quarters, the quarters being pulped before examination. Two values, x and y, corresponding to the two ordinates of the C.I.E.

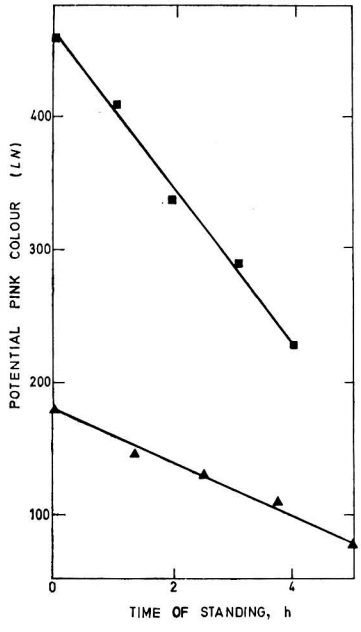


FIG. 1. *Change in leucocyanidin content* (LN) *of pear purées with time*

▲ Mature pears; ■ partly mature pears

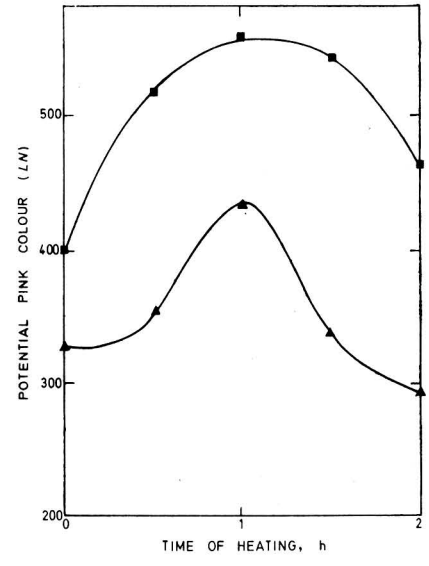


FIG. 2. *Change in leucocyanidin content* (LN) *of pear purées with heating*

▲ Mature pears; ■ partly mature pears

chromaticity diagram,[6] were calculated from the three reflectance readings, R_x, R_y, y_z, such that:

$$x = \frac{782\ R_x + 198\ R_z}{782\ R_x + 1000\ R_y + 1379\ R_z} \times 10^3$$

$$\text{and } y = \frac{1000\ R_y}{782\ R_x + 1000\ R_y + 1379\ R_z} \times 10^3$$

Values for x increased with increasing pink discoloration in the sample, and values for y decreased with increasing purple discoloration; purple-pink samples gave low y values and had smaller x values than pure pink samples of the same visual depth of colour. Examination of over 200 experimental packs processed during this work confirmed the limits of the C.I.E. chromaticity diagram.[6] Thus, pink discoloration only became apparent when x values exceeded 365, below which discoloration was limited to the light buff shades associated with over-processing; purpling, on the other hand, only became apparent in purées with y values below 290. Tests showed that there was little change in x or y values of purées allowed to stand 24 h.

Application of the method

Samples of quince purée (*LN* 1800) were heated with 100 ppm of various chemicals in nylon pouches for 75 min at 100° to develop pure pink and purple-pink discolorations. The same sequences were obtained when the treatments were arranged visually in order of decreasing pinkness (aluminium chloride, control, metabisulphite, stannous chloride, stannous citrate, stannic chloride) and in order of decreasing x value (427, 421, 415, 391, 377); and similarly, the visual arrangement in order of decreasing purple (stannous citrate, stannous chloride, stannic chloride, aluminium chloride, control, metabisulphite) was the same as the order of increasing y value (299, 302, 318, 346, 348, 362).

Two batches of Packham pears and one batch of William B.C. pears were canned as quarters in 25% syrup and processed at 100°. Cans were withdrawn at regular intervals and the degree of discoloration evaluated visually by scoring 2 for each quarter markedly discoloured and 1 for each quarter slightly discoloured. Reflectance measurements were also used to obtain x and y values for each can and in Fig. 3 these values are plotted against time of heating and in Fig. 4 against the scores for visual assessment of discoloration; the William B.C. pears are not represented in Fig. 4 because they showed no visible discoloration.

Correlation between discoloration and leucocyanidin content

From examination of 32 samples of pear purée heated 2 h at 100° in nylon pouches in the absence of tin ions, a significant correlation was established ($r = 0{\cdot}682$) between the *LN* of the fresh purée and the x value of the product. The regression equation: $LN = 9{\cdot}688\ x - 3324$, was used to obtain a fair prediction of the discoloration which would be developed in pear purée heated under these conditions. Furthermore, an estimate of the susceptibility of purée to pink discoloration was obtained, based on the observation that x must reach 365 for pink discoloration to be apparent; by calculation, a purée would have to have a *LN* greater than 220 before it would

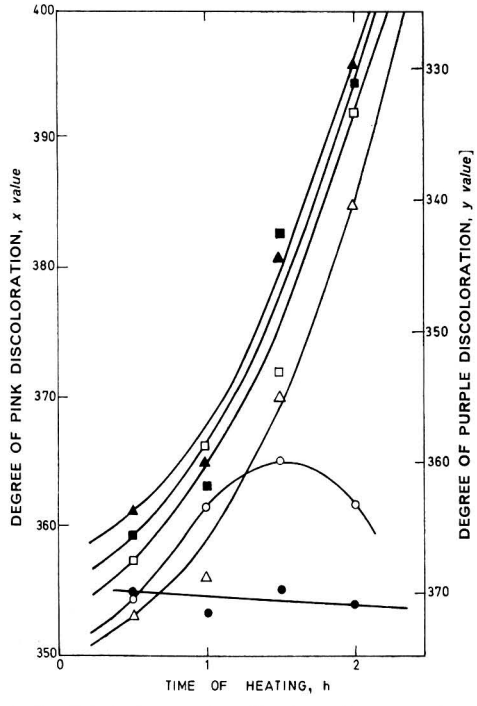


FIG. 3. *Relation between time of heating and instrumental assessment of pear discoloration*

△ Pink discoloration in Packham pears (first sample); □ pink discoloration in Packham pears (second sample); ○ pink discoloration in William B.C. pears; ▲ purple discoloration in Packham pears (first sample); ▲ purple discoloration in Packham pears (second sample); ● purple discoloration in William B.C. pears

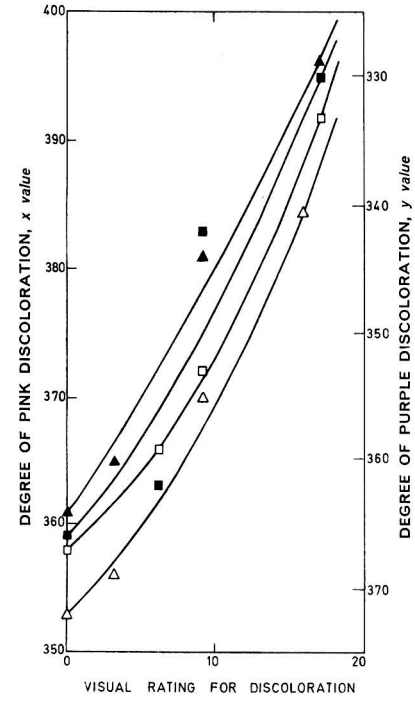


FIG. 4. *Relation between instrumental and visual assessments of discoloration in Packham pears*

△ Pink discoloration in Packham pears (first sample); □ pink discoloration in Packham pears (second sample); ▲ purple discoloration in Packham pears (first sample); ■ purple discoloration in Packham pears (second sample)

turn pink in a two-hour process. This result was supported by experience over two seasons with Packham, Winter Cole, and William B.C. pears in that no sample with *LN* less than 220 showed discoloration, while all but a few samples with *LN* above 220 became discoloured on processing.

There was no direct correlation between *LN* and *y* value, since purpling is dependent on the presence of tin ions which, as discussed in a subsequent paper,[7] enhance or suppress discoloration according to their effective concentration at the time of leucocyanidin degradation. For instance, purées heated 2 h at 100° in the presence of 100 ppm tin did not discolour if the *LN* was less than about 475; above this value, purple-pink discoloration occurred, and in ten such samples processed under these conditions the extent of purpling, as measured by the *y* value, showed a very significant correlation ($r = -0{\cdot}859$) with the *LN* of the fresh purée according to the regression equation: $LN = 2844 - 7{\cdot}034y$. In addition to affecting the *y* value, the presence of tin also affected the relationship between *LN* and the *x* value, and in the above ten purées heated for 2 h at 100° with 100 ppm tin there was a highly significant correlation ($r = 0{\cdot}892$) between *LN* and extent of pink discoloration according to the regression equation: $LN = 24{\cdot}65\,x - 8532$.

Discussion

The procedure described for the measurement of the leucocyanidin content is essentially that of Luh *et al.*[1] which is found to give satisfactorily reproducible results provided measurements are completed as rapidly as possible. The addition of salt is essential to inhibit enzymic browning which is accompanied by a considerable reduction in leucocyanidin content (over 80% in 10 min), but even in the presence of salt the *LN* values will decrease if the purée or its extract stands for quite short periods, a reduction of 10–20% occurring within 1 h. On the other hand, the developed colour is quite stable, losing less than 10% of its initial absorbancy within 24 h.

A single extraction effectively removes all butanol-soluble leucocyanidins from pear purée, since negligible colour is produced on a second treatment. The current procedure differs from those studied by Smathers & Charley[2] mainly in incorporating hydrochloric acid into the extraction medium, thus increasing the amount of leucocyanidin extracted by at least 25%. The mild acid hydrolysis occurring under these conditions either depolymerises the leucocyanidins or ruptures their linkages to other pear constituents such as structural polysaccharides, and a similar process apparently occurs initially on heating. Heat-catalysed hydrolysis is, however, less controllable and gives less reproducible results than acid-catalysed hydrolysis, at least partly because continued heating results in well-recognised polymerisation reactions[8] with loss of leucocyanidin and little colour formation. The high values attainable in heated purées cannot be achieved by other procedures, and the conclusion of Smathers & Charley[2] that an accurate measure of the total leucocyanidin content of pears is not possible by present methods was confirmed. Nevertheless the method outlined in this paper is quite suitable for comparative purposes.

Another difficulty facing investigations involving pear leucocyanidins is the considerable differences in their concentration among individual pears even when obtained from the same source and subjected to the same storage treatments, a fact not adequately recognised by some previous investigators in this field. When purées were made from samples of six fruits from the same batch of comparable pears, the leucocyanidin contents varied by as much as 40% on both sides of the mean value, and this variation could explain some of the conflicting reports[3,4,9] on the effect of storage treatments and ripening procedures on leucocyanidin content. Although the possibility of seasonal variations prohibits a definite conclusion, the present results indicate that fruit picked early in the season are higher in leucocyanidin content than fruit picked later in the season and retain their comparatively higher figures despite a decrease that occurs in storage, especially during the ripening process. Thus, the wide range of *LN* values obtained for Packham pears reflects the fact that the season for this variety as a dessert fruit is extended to about nine months by judicious harvesting and storage procedures.

The above observations could provide useful information to processors faced with the problem of pink discoloration in canned pears arising from high leucocyanidin contents in the raw material, and the determination of the *LN* offers a means of screening fruit for this factor. In agreement with its recognised superior retention of colour during heat processing by virtue of its low leucocyanidin contents, the William B.C. pear gave *LN* values ranging from 105 to 265, while two varieties susceptible to pink discoloration, the Packham and the Winter Cole, gave values ranging from 200 to 1150 and from 430 to 630 respectively. Quince, a fruit closely related to the pear and very susceptible to pink discoloration, gave values as high as 2500.

The relation between *LN* and degree of discoloration on processing was satisfactorily established for pear purées through tristimulus reflectance measurements. Separate parameters for the extent of pinking and purpling were obtained as *x* and *y* values on the C.I.E. chromaticity diagram; and these values were used to follow the variations in discoloration with time of heating and with addition of various chemicals. For instance, the degree and the shade of discoloration was found to be profoundly influenced by tin ions which inhibit the discoloration or produce purple-pink pigments according to their concentration.[7] In the present work, relations were established between the *LN* of pear purées and the degree of the discoloration developed after heating 2 h at 100° in nylon pouches, both for untreated purées (pink only) and for purées containing 100 ppm tin ions (pink and purple). Calculations from the regression equations using the limits imposed by the C.I.E. chromaticity diagram (no pink below $x = 365$, no purple above $y = 290$) gave *LN* values of 220 below which no discoloration would occur under either condition, 465 below which discoloration would not occur in the presence of 100 ppm tin, and 805 above which the otherwise pink discoloration developed a purple shade in the presence of 100 ppm tin. It may be noted that two equations relating *x* to *LN* give almost identical intercepts (345 ± 1) corresponding to *x* values for an imaginary purée of zero leucocyanidin content.

The effect of tin ions on the extent and shade of discoloration in heated pear products increases the difficulty of assessing the susceptibility of large consignments of pears to discoloration on canning. The leucocyanidin content, its variation from pear to pear, the extent of can corrosion, and the oxidation/reduction status of the product all come into question[7] and make it impossible to suggest a screening procedure satisfactory in all circumstances. As indicated above, a figure of 220 provides a limit for the *LN* below which purées will not discolour on heating for 2 h at 100° in nylon pouches;

this figure would provide a considerable safety margin for pears canned as halves which are normally heated for 20–30 min at about 100°, and it would inevitably exclude a considerable number of pears that would not discolour under commercial processing.

The experimental techniques outlined above, therefore, have more value in the study of the variation in the leucocyanidin content of pears and in the comparison of their behaviour on heating than in the selection of raw material for canning purposes.

C.S.I.R.O. Division of Food Preservation,
P.O. Box 43,
Ryde,
New South Wales 2112,
Australia

Received 10 December, 1969

References

1. Luh, B. S., Leonard, S. J., & Patel, D. S., *Fd Technol., Champaign*, 1960, **14**, 53
2. Smathers, R. U., & Charley, H., *J. Fd Sci.*, 1967, **32**, 310
3. Nortje, B. K., *S. Afr. J. agric. Sci.*, 1966, **9**, 681
4. van der Merwe, H. B., *Fd Inds S. Afr.*, 1963, **15** (11), 56
5. Chandler, B. V., & Clegg, K. M., *J. Sci. Fd Agric.*, 1970, **21**, 315
6. Judd, D. B., *Circ. U.S. natn. Bur. Stand No.* **478**, 1950
7. Chandler, B. V., & Clegg, K. M., *J. Sci. Fd. Agric*, 1970, **21**, 323
8. Jurd, L., & Lundin, R., *Tetrahedron*, 1968, **24**, 2653
9. Ingalsbe, D. W., Carter, G. H., Neubert, A. M., & Blodgett, E. C., *Proc. Wash. St. hort. Ass. 57th A. Mtg*, 1961, p. 158

PINK DISCOLORATION IN CANNED PEARS III.*—Inhibition by chemical additives

By B. V. CHANDLER and K. MARY CLEGG†

Despite the identification of the pigment responsible for discoloration in canned pears as a tin-cyanidin complex, addition of stannous ions to susceptible pear purée prior to processing partly or completely inhibits the discoloration. This effect cannot be obtained with canned pear halves, but other reducing agents, such as sulphur dioxide, are effective inhibitors of the discoloration. By such treatment canned pears of acceptable quality can be produced from varieties which are normally avoided for processing because of their susceptibility to discoloration. A mechanism whereby reducing agents prevent the formation of pink pigments is described, and an explanation is given of the differences in the effect of stannous ions on pear halves and pear purées.

Introduction

It was previously reported[1] that the 'pink' pigment in discoloured canned pears is a purple-pink insoluble tin–anthocyanin complex derived from reaction between stannous ions from can corrosion and cyanidin-like compounds from heat-catalysed degradation of pear leucocyanidins. However, during attempts to prevent discoloration by creating conditions unfavourable to complex formation, there were indications that a preliminary treatment to promote can corrosion prior to leucocyanidin break-down might control discoloration in borderline cases, even though the introduction of stannous ions into the sample by tin corrosion should favour complex formation. This paper confirms the inhibition of discoloration by tin ions under certain circumstances, indicates a possible mechanism for the inhibition by such reducing agents, and presents a method for preparing canned pears of satisfactory colour and flavour from varieties which normally undergo considerable discoloration during processing.

Experimental and Results

In this work the use of pear purée and the distribution of pear quarters among four processing treatments eliminated as far as possible variations arising from the considerable differences in the leucocyanidin content of individual pears.[2] Peeled and cored pear quarters were processed in a 27·5% (by wt.) sucrose syrup with a flesh to syrup ratio of 180:110 parts by wt. In experiments with purée, the flesh from peeled and cored pears was rapidly blended in the above proportion with similarly prepared syrup to which 3% sodium chloride had been added. Processing was carried out in nylon pouches whenever it was necessary to ensure absence of tin ions from the control pack.

In experiments with chemical additives, the concentration of tin salts was expressed as ppm tin, and sulphur dioxide and hydrogen sulphide were added as sodium metabisulphite and sodium sulphide, respectively.

The colour-generating potential of pear material, i.e. the leucocyanidin number (*LN*), and the shade and depth of 'red' and 'purple' colours developed on processing (*x* and *y* values) were measured by methods already described.[2]

Effect of tin ions on developed and potential colour in heated pear purée

Packham pear purée (*LN* 236) was processed in nylon pouches for 1 h at 100° with the addition of varying amounts of stannous chloride. The control sample without tin turned a grey pink and samples with less than 50 ppm tin turned bright purple-pink; samples with more than 50 ppm tin retained their creamy yellow colour, and even on heating for 4 h were only discoloured a light brown. As previously observed,[2] there was an increase of *LN* on heating, but addition of tin ions reduced the colour potential of the heated purées, which reached a limiting value with the addition of 100 ppm tin (Fig. 1).

* Part II: Preceding paper

† Present address: Department of Food Science, University of Strathclyde, Glasgow, C.1.

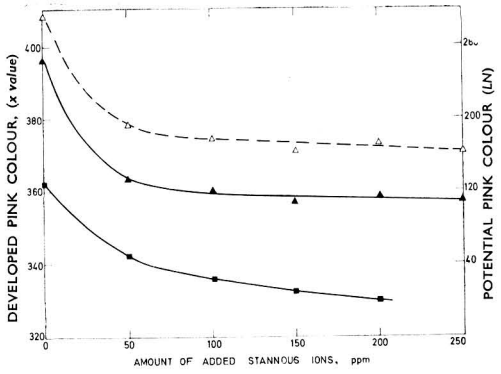


FIG. 1. *Potential and developed colour of pear purées heated with varying amounts of stannous ions*

△ Potential colour in purée (*LN* 236) heated 1 h;
▲ developed colour in purée (*LN* 515) heated 2 h;
■ developed colour in purée (*LN* 382) heated 1 h

Two purées of *LN* 382 and 515 were heated for 1 and 2 h respectively with varying amounts of stannous chloride; the *x* values are recorded in Fig. 1. In the purée with the lower *LN*, pinking was inhibited at 50 ppm tin, and there were negligible differences in the *y* values (356 ± 2). In the other sample, pinking was reduced but the *x* values tended to remain constant after 100 ppm, while the *y* values (335 ± 3) of the tin-treated samples were lower than that of the control (351) but still not low enough for the purée to appear purple.[2] From similar experiments the efficiency of known levels of stannous ions in preventing colour development was found to depend on leucocyanidin content. Thus, 100 ppm tin prevented pigmentation in purées of *LN* < 350 heated for 2 h at 100° while 250 ppm controlled discoloration in similarly treated purées of *LN* < 750.

Packham pear purée was processed in nylon pouches at 100° for 1 h and re-packed into nylon pouches with and without the addition of stannous chloride before a second processing at 100° for 2 h. The heated purée was initially grey pink; the colour merely darkened slightly in the absence of tin, but intense purple-pink discolorations developed in the presence of tin at 50 ppm and at 100 ppm. When tin (100 ppm) was added to the purée before any heat treatment, discoloration was limited to the light buff shade of overprocessed pears.

Inhibition of colour formation in pear extractives by tin ions

A salt-free Packham pear purée which gave an immediate *LN* of 312 was treated with 25 and 50 ppm tin and allowed to stand for 4 h. The purées were then light brown and creamy yellow and gave *LN* of 260 and 286 respectively, while the untreated control was very dark brown and gave a *LN* of 148

Determinations of *LN* were carried out on a Packham pear purée with combinations of three variations on the procedure: heating the purée prior to extraction with butanol, addition of tin to the purée, and addition of tin to the extract. A significant reduction in developed colour only occurred if heat was applied after the addition of tin (Table I).

Effect of tin ions on discoloration in processed pears

Packham pear quarters were processed in plain tinplate cans at 100° with and without the addition of 250 ppm tin; samples were withdrawn at 30 min intervals for determinations of *LN* and *x* and *y* values. In Fig. 2 these values are plotted against time of process for one representative experiment. The tin contents of the drained quarters from these packs increased with processing time from 6 to 14 ppm in the control samples and from 20 to 32 ppm in the tin-treated samples.

Packham pear quarters were held 4 h in 3% sodium chloride at 20° with and without the addition of 200 ppm tin; the untreated samples were canned only in plain syrup, while the tin treated-samples were canned in both plain syrup and syrup containing 200 ppm tin. Samples were withdrawn after 30, 60, 90, and 120 min at 100° for visual comparison of colour. The discoloration in the control samples was marginally less than that in the tin-treated samples canned in either tin-free or tin-treated syrup. The tin contents of the drained halves from these packs increased with the processing time from 1 to 8 ppm, 31 to 43 ppm, and 56 to 69 ppm, respectively.

TABLE I

Effect of adding tin at various stages in the determination of the potential colour (*LN*) of pear purées

Tin added to purée, ppm	Time of heating of purée prior to extraction with butanol, min	Reading for *LN*	
		Tin not added to butanol extract	Tin (500 ppm) added to butanol extract
0	0	212	116
100	0	197	
0	60	290	152
100	60	185	

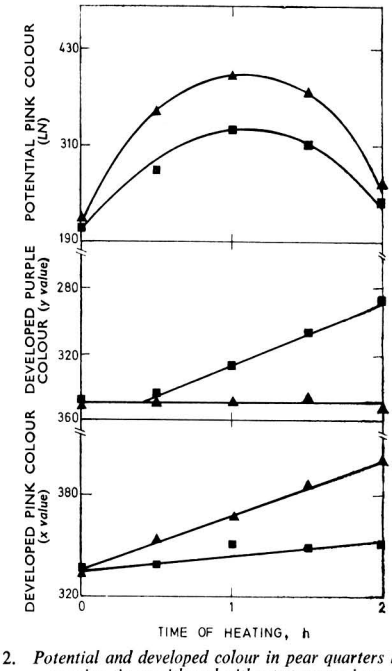


FIG. 2. *Potential and developed colour in pear quarters heated for varying times with and without stannous ions*

▲ No stannous ions added; ■ 250 ppm stannous ions added

TABLE II

Effect of tin on discoloration of pears processed in nylon pouches

Pre-processing bath	Tin content of added syrup, ppm	Degree of discolor-ation	Tin content of quarters or purée, ppm
Pear quarters:			
3% brine	—	*	—
3% brine	30	**	20
3% brine + 300 ppm tin	—	***	32
300 ppm tin	—	****	27
300 ppm tin	100	****	63
Pear purée:			
300 ppm tin	—	†	67

† No discoloration
**** Extreme discoloration

Packham pear quarters were divided into three batches, held separately at 20° for 15 min with gentle agitation in baths of 3% brine, water containing 300 ppm tin, and 3% brine containing 300 ppm tin. The quarters were then processed as such or as purée in nylon pouches, with and without the addition of tin. After 2 h at 100° samples were examined for appearance and tin content; significant results only are shown in Table II.

Packham pear quarters were divided into two batches, one of which was subjected to ultrasonic vibrations for 30 min in 3% sodium chloride before both were gently agitated 15 min in a bath containing 300 ppm tin. When processed at 100° for 2 h in nylon pouches with syrup containing 100 ppm tin, the two batches showed extreme discoloration with negligible differences in shade or depth of colour or in the tin contents (69 ppm) of the drained halves.

Effect of various chemical additives on discoloration in pear purée

William B.C. pear purée (*LN* 181) was treated separately with stannous chloride, stannous citrate, stannic chloride, and aluminium chloride at a metallic ion concentration of 100 ppm, and with sulphur dioxide at 100 ppm. After processing for 150 min at 100° in nylon pouches, the sulphited sample remained creamy yellow (x, 355; y, 356), while that treated with aluminium chloride showed the greatest discoloration (x,383; y, 369); intermediate results were obtained with stannous chloride and stannous citrate (x, 366; y, 379 for both) and with stannic chloride, cupric chloride, and no additive (x, 379; y, 372 for each).

The effect of four other reducing agents was tested in a similar experiment with Packham pear purée (*LN* 465). The products from treatments with 100 ppm of oxalic acid (x, 390; y, 357), and arsenious oxide (x, 395; y, 356) were only very slightly less discoloured than the control (x, 404; y, 344). Ascorbic acid inhibited discoloration at 200 ppm but only in samples heated less than 60 min, and eventually the discoloration was more pronounced than in the control (Fig. 3) even though analyses showed that the pack still contained 85% of its original ascorbic acid. Hydrogen sulphide prevented pink discoloration at 50 ppm (x, 360; y, 375) but the samples developed definite yellow discolorations, especially those with more than 100 ppm hydrogen sulphide, probably as the result of formation of molecular sulphur.

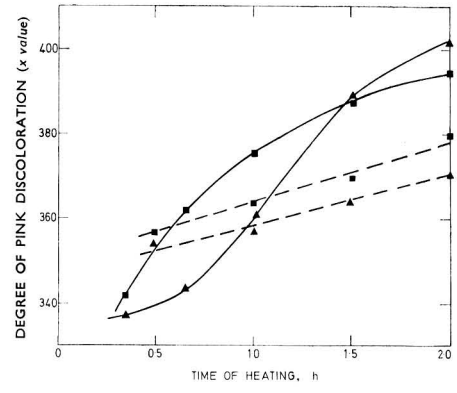


FIG. 3. *Effect of ascorbic acid and sulphur dioxide on discoloration in processed pears*

■——■ Pear purée heated without ascorbic acid;
▲——▲ pear purée heated with ascorbic acid (200 ppm);
■- - -■ pear quarters heated without sulphor dioxide;
▲- - -▲ pear quarters heated with sulphur dioxide (200 ppm)

Effect of decreasing headspace oxygen on discoloration in canned pears

The following procedures aimed at lowering the concentration of headspace oxygen were found to have no effect on the discoloration in canned Packham pears: sealing under high vacuum, flushing several times with nitrogen before sealing, and addition of oxidase and catalase enzyme systems with subsequent incubation for 4 h before processing. Analysis of the headspace gases in these packs showed that very low levels of residual oxygen had been achieved in some cases.

Inhibition of pear discoloration by sodium metabisulphite

Packham pear purée (*LN* 795) was processed at 100° in nylon pouches for 30, 60, and 120 min with and without the addition of 250 ppm sulphur dioxide to the syrup. The samples with sulphur dioxide showed no discoloration after 120 min, giving x and y values of 359 and 351, respectively; untreated samples showed a marked increase in x value from 329 to 363, 372, and 413 and a slight decrease in y value from 349 to 351, 347, and 343 over this period.

Packham pear purée (*LN* 660) was processed at 100° for 2 h in nylon pouches with the addition of varying amounts of sulphur dioxide to the syrup. The degree of pinking was measured by the x value with the results shown in Fig. 4; the samples showed no purpling, with little change in y value (350 ± 5).

Packham pear quarters were divided into three groups; one was processed without special treatment, another was canned in sulphited syrup (200 ppm sulphur dioxide), and the third was held in the sulphited syrup for 2 min at 100°, then drained and canned in normal syrup. The cans were processed at 100° and samples were withdrawn at 30 min intervals; the quarters were drained and were homogenised for examination. As expected, because the samples were packed in lacquered cans, discoloration was very slight and limited to processing times of 60 min and above; the discoloration was a grey pink with no purpling (y values 363 ± 5). In Fig. 3 the x values are plotted against processing time for the most and the least discoloured samples which were, respectively, the control sample and the sample canned in sulphited syrup. Sulphur

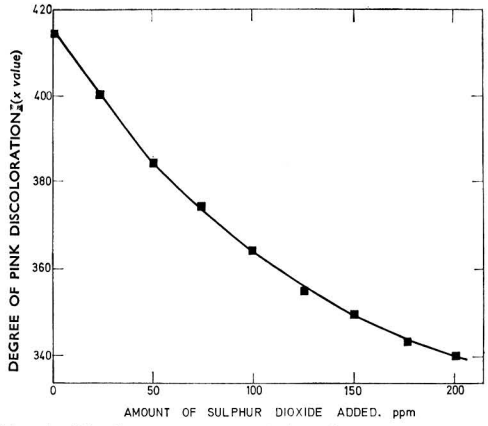


FIG. 4. *Discoloration in pear purée heated with varying amounts of sulphur dioxide*

dioxide was present at about 50 ppm in the samples canned in sulphited syrup, but could not be detected in the samples which had only been blanched in sulphited syrup.

Winter Cole pear halves showing pink discoloration (x, 425; y, 338) after processing in nylon pouches were repacked in nylon pouches with and without the addition of 500 ppm sulphur dioxide to the syrup. After 1 h at 100° the discoloration in the sulphited sample (x, 426; y, 349) differed in shade but not in total visual intensity from the halves heated without sulphite (x, 435; y, 335).

Six Packham pear purées of varying *LN* (296–795) were processed in nylon pouches for 2 h at 100° with the addition of 100, 125, 150, 175, 200, 225 and 250 ppm sulphur dioxide in the syrup. A highly significant correlation ($r = 0{\cdot}9944$) existed between the *LN* of the purée and the lowest concentration of sulphur dioxide [*S*] required to prevent pink discoloration, according to the regression equation: $LN = 3{\cdot}32\,S - 32{\cdot}5$.

Organoleptic examination of pears canned with sulphur dioxide

Winter Cole pear quarters were separated into four batches, one-quarter to each batch, and held in 3% brine until processed. Two of the batches were canned in syrup to which 200 and 250 ppm sulphur dioxide had been added; these batches were filled into lacquered cans, while both plain tinplate and lacquered cans were used for the two unsulphited batches. The cans were sealed under 20 in. vacuum and processed for 60 min at 100°. The products were held at room temperature for 5 days and submitted for organoleptic examination to a panel of 63 experienced tasters who were asked to rank the four samples for colour, flavour, and general preference, and to comment on any off-flavours.

Statistical analysis of the taste test results (Table III) showed average treatment differences that were very highly significant for colour, highly significant for general preference, and not significant at $P = 0{\cdot}05$ for flavour. There were no significant differences between the controls processed in plain tinplate and lacquered cans, and significant differences only for colour between the two sulphited treatments. The sulphited pears were greatly preferred for their pale cream colour, their flavour was not impaired, and overall the sulphited samples were considered more acceptable than the untreated samples.

TABLE III

Mean ranks* recorded by a 63-member panel for pears canned with and without sulphur dioxide

	Plain tinplate cans without SO_2	Lacquered cans		
		Without SO_2	200 ppm SO_2	250 ppm SO_2
Colour	3·39[a]	3·34[a]	1·76	1·51
Flavour	2·47[b]	2·71[b]	2·52[b]	2·31[b]
General preference	2·70[c]	2·88[c]	2·31[d]	2·11[d]

* Lowest mean ranks correspond to most preferred product

[a–d] Any two figures not represented by the same letter are significantly different at the 5% level

Only five tasters reported off-flavours in the sulphited products that could be ascribed to the treatment, using the terms 'slight sulphur taste', 'as if sulphur dioxide present', 'sulphurous', 'sulphury', and 'sulphur dioxide'; only one taster noted an off-flavour that could be associated with sulphur dioxide in the product with the lower level of additive. Otherwise, comments on off-flavour were applied indiscriminately to sulphited and unsulphited samples. Analyses showed that the samples from the lower and the higher sulphite treatments contained 58 ppm and 75 ppm sulphur dioxide, respectively. Further storage for 12 months at room temperature did not affect the colour and flavour of either treated or untreated samples.

Discussion

A reduced level of 'pink' discoloration was previously reported[1] in heated purées to which tinplate discs had been added, the result of either an electrochemical reduction of the pigment by the dissolving metal or a reaction involving tin ions; the tin ions could have reduced the pigment or its precursors chemically, or prevented their production by complex formation. Experiments with stannous chloride as an additive showed that stannous ions were the active agent, inhibiting 'pink' discoloration in pear purées heated in nylon pouches, provided their concentration reached a critical level which was dependent on the leucocyanidin content of the purée. If the concentration of stannous ions was too low, the purple-pink tin complex was formed (Fig. 1).

Despite the results with pear purée, attempts to control the discoloration in canned pear halves by addition of stannous ions were entirely without success. Instead stannous ions produced an intensification of the discoloration in pear halves (Fig. 2), even at five times the concentration that prevented discoloration in comparable purées; the colour-generating potential of the treated halves had been lowered but not enough to prevent discoloration. Tin analyses indicated that inadequate penetration of stannous ions into pear halves could account for the difference in discoloration of halves and purée, but attempts to increase the penetration (by, for example, blanching baths and sonic treatment before processing) did not increase the tin contents enough to reduce the discoloration. Even when the final tin contents of the halves were the same as the final tin contents of comparable undiscoloured purée, the halves were intensely discoloured, suggesting that penetration had not been quick enough to interfere with colour development.

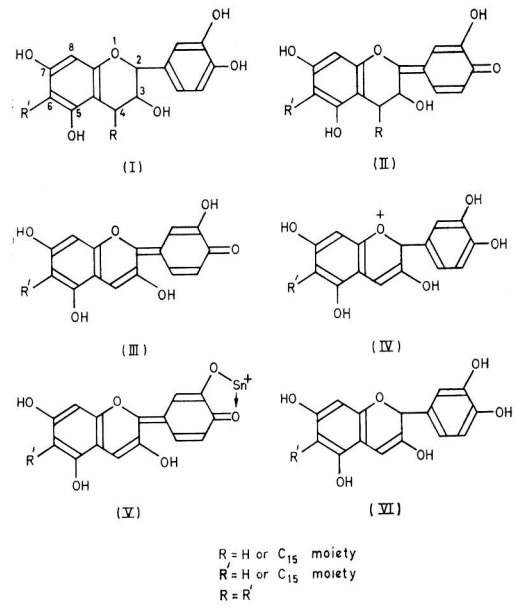
Replacement of stannous ions by a more efficient inhibitor of 'pink' discoloration required knowledge of the mechanism

of inhibition. Addition of stannous ions to discoloured purée demonstrated that their inhibition of discoloration was not due to chemical reduction of pigments already formed from leucocyanidin but to some reaction prior to pigment formation. Reactions of leucocyanidin with stannous ions seem limited to colourless salt formation since its currently accepted structure[3,4] contains no complex-forming group, the *o*-dihydroxy group in the *β*-ring having no chelating ability in non-ketonic flavonoids.[5] Catechin, with a structure very similar to leucocyanidin, has been reported[6] to form insoluble complexes with stannous ions, but, since no colour change is involved, the product is probably a simple insoluble salt similar to the soluble sodium and aluminium salts whose spectra also show negligible differences from that of catechin.[5,7] Such salts are unlikely to reduce discoloration in processed pear purées, and indeed it was found that aluminium ions produced intense discolorations and cupric ions had no effect whatever under conditions whereby stannous ions inhibited the discoloration.

Furthermore, addition of tin salts to pear purée did not significantly reduce its colour-generating potential unless heat was applied after the addition, whereas addition of tin salts greatly decreased the amount of pigment produced by extracted pear leucocyanidins, irrespective of whether or not the purée had been heated prior to extraction (Table I). These results may be explained by a reaction of stannous ions with an intermediate in the conversion of leucocyanidin to pigments, but not with leucocyanidin itself, and addition of various chemicals to purées processed in nylon pouches clearly demonstrated that the stannous ion behaves here as a reducing agent. Other strong reducing agents (sulphur dioxide, hydrogen sulphide, and ascorbic acid) were all effective in inhibiting discoloration, but not weaker reducing agents such as arsenious oxide and oxalic acid. A preprocessing bath with sulphur dioxide (3000 ppm) was part of a canning procedure previously suggested for certain pear varieties,[8] but its function was apparently to inhibit enzymic browning in the holding tanks since pink discoloration was still encountered.

Identification of a reduction as the reaction inhibiting pear discoloration conforms with the previous observation that leucocyanidin, which contains no readily reducible groups,[3,4] does not itself react with the inhibitor. Furthermore, it was shown that neither sulphur dioxide nor stannous chloride chemically reduce pigments already formed from degradation of leucocyanidin (**I**), but prevent or reverse this degradation by reducing intermediates in the breakdown. Quinone methines of the type **II** may be proposed as the intermediates concerned; they could be formed by enzymic or atmospheric oxidation of the leucocyanidin, they could be converted by heat via the anhydrobase (**III**) to cyanidin (**IV**), or in the presence of tin to the purple-pink tin complex (**V**), and their formation could be reversed or inhibited by the inhibitors of 'pink' discoloration in processed pears. It may be noted that the suggested mechanism requires the positions of polymerisation of the leucocyanidin molecule to be the 4 and 6 (or 8) positions: if they were the only other presently accepted alternatives,[3,4] the 2 and the 6 (or 8) positions, the quinone methine structure could not be formed. These observations therefore have a bearing on this point of obscurity in leucocyanidin chemistry.

The first step in the process of 'pink' discoloration is therefore seen as an oxidation of leucocyanidin (**I**) to the corresponding quinone methine (**II**) which may be reduced back to the *o*-dihydroxy compound (**I**). In the absence of reducing



agents, heat converts **II** into the conjugated anhydrobase (**III**) which would be a strong complexing agent for stannous ions, forming the purple-pink chelate (**V**), only one of whose many resonating structures is shown. In the absence of stannous ions, the anhydrobase would be converted principally to the colourless and unstable pseudobase (**VI**) in the weakly acidic conditions existing in canned pears, with smaller amounts of the pigment, cyanidin (**IV**), which requires pH levels below 3·0 for optimum stability.

Thus, whether stannous ions act to promote or inhibit discoloration in pears is determined not only by the amount of tin added but also by the time at which it becomes available to react with the products of leucocyanidin breakdown; if the degradation has proceeded only to the quinone methine (**II**), stannous ions will reverse the initial oxidation step, but if it has proceeded to the anhydrobase (**III**), stannous ions promote discoloration by the formation of the stable purple-pink tin complex (**V**). Stannous ions fail to inhibit discoloration in canned pears, but not in canned purée, because quinone methines are formed and degraded in pear halves before stannous ions sufficiently penetrate the tissue. Furthermore, this mechanism, which does not involve the formation of free cyanidin, accounts for discoloration occurring under conditions which normally lead to fading in cyanidin solutions and in foods containing low concentrations of cyanidin glycosides, such as certain cherry products.

Reducing agents are believed[9] to improve the extraction of polyphenols from plant tissues by suppressing their oxidation to *o*-quinones, either by atmospheric or enzymic oxidation. For instance, addition of sulphur dioxide[10] increased the yield of total polyphenolics in persimmon extracts, but at the same time it decreased their conversion to pigments on heating with acid. Similarly, it has been found that stannous ions and sulphur dioxide, although inhibiting polyphenolic oxidation in pear purées, lowered their colour-generating potential. The suggestion is thus supported that reducing agents decrease

the pink discoloration in heated pears by reversing or inhibiting the oxidation of leucocyanidin to the quinone methine. This theory is not invalidated by discolorations in canned pears with reduced headspace oxygen, since oxidative reactions could still have occurred in the products.

To test the practical application of these findings, a pear variety highly susceptible to pink discoloration, the Winter Cole, was canned with and without the addition of sulphur dioxide. An alternative procedure using ascorbic acid may also have given satisfactory results, but it is a more expensive and less effective inhibitor of the discoloration than sulphur dioxide. The quality of the sulphited product was highly satisfactory; it had the attractive creamy-yellow colour normally associated with William B.C. pears processed in unlacquered cans and there was negligible off-flavour production, especially at the lower level of the additive (200 ppm). The concentration of residual sulphur dioxide in the processed product was low (less than 80 ppm) and did not present difficulties, except for prohibiting the use of unlacquered cans for the process. Of course, the use of lacquered cans would, by itself, obviate the problem of pink discoloration in William B.C. pears but a dull-looking product usually results. Because the presence of stannous ions normally enhances the colour of the product to a creamy yellow, only occasionally giving rise to pink discolorations when pears of abnormally high leucocyanidin content are canned the canned pear industry has been based on William B.C. pears, which have a low colour-generating ability,[2] processed in plain tinplate cans.

It is unlikely that the occasional occurrence of pink discoloration in canned William B.C. pears would justify a change to a sulphited pack in lacquered cans, even though the small amount of sulphur dioxide needed would lead to less than 40 ppm remaining in the product. However, if it is desired to can pear varieties now avoided because of their susceptibility to discoloration, such as Packhams, Winter Coles, Josephines, and Keiffers, a satisfactory process could be developed from these findings. Provided that food legislation permits, similar procedures using low levels of sulphur dioxide could also be used in the processing of other leucoanthocyanidin-containing foods subject to undesirable pink discoloration, such as lychees, gooseberries, okra, and chestnuts.

C.S.I.R.O. Division of Food Preservation,
P.O. Box 43,
Ryde,
New South Wales 2112,
Australia

Received 10 December, 1969

References

1. Chandler, B. V., & Clegg, K. M., *J. Sci. Fd Agric.*, 1970, **21**, 315
2. Chandler, B. V., & Clegg, K. M., *J. Sci. Fd Agric.*, 1970, **21**, 319
3. Nortje, B. K., *J. Fd Sci.*, 1966, **31**, 733
4. Geissman, T. A., & Dittmar, H. F. K., *Phytochemistry*, 1965, **4**, 359
5. Jurd, L., & Geissman, T. A., *J. org. Chem.*, 1956, **21**, 1395
6. Heintze, K., *Dt. LebensmittRdsch.*, 1960, **56**, 194
7. Jurd, L., in 'The Chemistry of Flavonoid Compounds', 1962, Ch. 5 (T. A. Geissman, Ed.) (Oxford: Pergamon Press)
8. Szechenyi, L., *Élelm. Ipar*, 1955, **9**, 121
9. Khanna, S. K., Viswanathan, P. S., Krishnan, P. S., & Sanwal, G. G., *Phytochemistry*, 1968, **7**, 1513
10. Joslyn, M. A., & Goldstein, J. L., *J. agric. Fd Chem.*, 1964, **12**, 511

ERRATA

In the paper by Bottrill & Hawker, *J. Sci. Fd Agric.*, 1970, **21**

Page 193, Table I, *for* pheopharbide *read* pheophorbide

Page 195, Fig. 4 caption *for* (a) *dipped and* (b) *undipped*
read (a) *undipped and* (b) *dipped*
In part (a) (iii) of the diagram the upper 'C' refers to 'C_a' and the lower 'c' to 'C_b'

Page 196, right-hand column, line 8, *for* Table II *read* Table III.

In the paper by Booth & Ewart, *J. Sci. Fd Agric.*, 1970, **21**

Page 188, Table I, bottom of Ponca $\gamma_3{}^7$ column *for* 10·5 *read* 1·05
bottom of Amino acid column should read $\frac{\text{Polar} + \text{ionic}}{\text{non-polar}}$

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JUNE, 1970

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JUNE, 1970

1.—AGRICULTURE AND HORTICULTURE

Soils and Fertilisers

Soil Formation, Classification, Constituents

Soil genesis as related to parent material and climate. I. Morphology, physical, chemical and physico-chemical properties. R. L. KARALE, R. V. TAMHANE and S. C. DAS (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 227–239. Engl., 10 ref.).—Soil description profiles of 7 soils derived from basalt and 3 from granite, are given. Phys. and chem. characteristics are listed. It is concluded that under low to moderate rainfall, soils retain the character of the parent rock, but in heavy rainfall, climatic factors are most important. M. T. Rawnsley.

Phosphorus transformations in a chronosequence of soils developed on wind-blown sand in New Zealand. II. Inorganic phosphorus. J. K. SYERS and T. W. WALKER (*J. Soil Sci.*, 1969, **20** (2), 318–324. 13 ref.).—In a chronosequence of sandy soils ranging up to 10,000 yr, total P in the profile decreased considerably with time, and this was accounted for mainly by decrease in acid-extractable Ca-P. Org. P increased somewhat and non-occluded P to a moderate extent with time. Residual inorg. P (probably occluded apatite) decreased, whilst occluded P was little affected by time. A. H. Cornfield.

Soil chemical characteristics of recent volcanic ash. E. BORNEMISZA and J. C. MORALES (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 528–530. 9 ref.).—Fresh ash and that emitted 2 yr previously from a volcano (Costa Rica) had pH of 5·1 and cation exchange capacity of 1·6–2·1 mequiv. per 100 g; 0·5 M-$NaHCO_3$-extractable P was high, but exchangeable Mn^{2+} was low. Lysimeter tests showed that the ash did not fix P or retain cations against leaching. Chem. compn. of the ash is compared with values for ash ejected at other volcanic sites. A. H. Cornfield.

Poorly drained soils with permafrost in interior Alaska. R. J. ALLAN, J. BROWN and S. RIEGER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 594–599. 11 ref.).—Phys., chem. and mineralog. characteristics are presented for 5 soils. A. H. Cornfield.

Regional distribution of potassium in the B horizon clay of some prairie loess soils of the Midwest (U.S.A.). K. L. WELLS and F. F. RIECKEN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 582–587. 24 ref.).—The total clay content of soils with comparable landscapes and age decreased from west to east and was related to av. annual rainfall and depth at which clay had accumulated in the profile. Montmorillonite was the dominant clay mineral in all profiles, but illite decreased from west to east as total K content decreased and av. annual rainfall increased. A. H. Cornfield.

Influence of chemical weathering on basal spacings of clay minerals. E. S. CONYERS, L. P. WILDING and E. O. MCCLEAN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 518–523. 20 ref.).—The extent of K removal and changes in basal spacings of 5 clays were detd. after treatment with H- and OH-resin, Na tetraphenylboron + resin, and boiling N-HNO_3 + resin. A. H. Cornfield.

Heterogeneity in silicon–iron mixed hydroxides. A. J. HERBILLON and J. TRAN VINH AN (*J. Soil Sci.*, 1969, **20** (2), 223–235. 34 ref.).—Several mixed hydroxides were prep. by co-pptg. $FeCl_2$ or $FeCl_3$ with Na_2SiO_3 at pH 8, followed by ageing and purification; their properties are discussed. A. H. Cornfield.

Apparent and partial molal volumes of cation-saturated kaolins. A. K. HELMY, F. F. ASAAD, M. N. HASSAN and H. SADEK (*J. Soil Sci.*, 1969, **20** (2), 274–277. 8 ref.).— A. H. Cornfield.

Physical Properties of Soils

Building up of soil structure by phosphate fertilisation of legume in a crop rotation. III. Evaluation and expression of soil structure by various methods. K. S. PHARANDE, B. L. JAIN and T. D. BISWAS (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 241–248. Engl., 14 ref.).—Expt. showed that choice of soil index depends on type of soil and intended use of soil. For a loam soil of Delhi, soil aggregate stability indices (mean wt. dia.; log geometric mean dia. etc.) were comparable with soil structure index expressed as % of water stable aggregates > 0·25 mm. Correlation coeff. of structural indices and related soil properties are presented. M. T. Rawnsley.

Effect of tillage, no tillage, and mulch on soil water and plant [maize] growth. J. N. JONES, JUN., J. E. MOODY and J. H. LILLARD (*Agron. J.*, 1969, **61** (5), 719–721. 7 ref.).— A. H. Cornfield.

Rapid method for testing soil-surface mulches. L. F. LIPPERT, J. M. LYONS and F. H. TAKATORI (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 552–559. 4 ref.).—A method of testing the efficiency of 6 mulching materials (emulsions of asphalt resins, thermoplastic petroleum resins, paraffin wax and latex, lignin sulphonate and colloidal suspensions of oxidised carbonaceous materials), in reducing soil moisture loss and increasing soil temp. is described. A summary of the effects of the treatments on germination and early growth of 6 crop species is presented. A. H. Cornfield.

Effects of adsorbed cations on physical properties of tropical red earths and tropical black earths. I. Plastic limit, percentage stable aggregates, and hydraulic conductivity. S. AHMED, L. D. SWINDALE and S. A. EL-SWAIFY (*J. Soil Sci.*, 1969, **20** (2), 255–268. 22 ref.).—Similar relative effects were obtained for plastic limits (*PL*), % stable aggregates (*SA*), and hydraulic conductivities (*HC*) when the red earth (mainly kaolin and Fe oxide) and the black earth (mainly montmorillonite) were saturated with Ca^{2+}, Mg^{2+}, K^+, or Na^+ or combinations of these. *PL* were independent of the nature of the cations, but were dependent upon clay type. Both *SA* and *HC* were lower where K^+ or Na^+ than where Ca^{2+} or Mg^{2+} were the dominant cations. Heavy K manuring without liming may lead to structural deterioration of these soils. A. H. Cornfield.

Steady-state method for determining diffusion coefficients in soil. P. B. TINKER (*J. Soil Sci.*, 1969, **20** (2), 336–345. 15 ref.).—The method, which uses cation-exchange papers as sources and sinks, was tested on the inter-diffusion of Ca^{2+} and Mg^{2+} in a clay soil. A. H. Cornfield.

Rootzone salt profiles and lucerne growth as influenced by irrigation water salinity and leaching fraction. C. A. BOWER, G. OGATA and J. M. TUCKER (*Agron. J.*, 1969, **61** (5), 783–785. 2 ref.).—At const. salinity of irrigation water applied at different rates soil salinity was lowest at the soil surface and had the same value irrespective of the leaching fraction [proportion of water which passed out of the rooting zone (1 m)]. Soil salinity increased more with depth as the leaching fraction decreased. Lucerne yields decreased with increasing av. salinity of the rootzone when electrical conductivity of the saturation extract (*EC*) exceeded 3 mmho per cm^3. Yields were decreased 50% when soil *EC* was 11 mmho per cm^3. With increasing salinity of irrigation water more water was required to prevent damage to lucerne. A. H. Cornfield.

Potassium and magnesium in irrigation water and their effects on the physico-chemical properties of soil. J. S. KANWAR and R. DEO (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 217–226. Engl., 7 ref.).—One light (**I**) and one heavy (**II**) soil were irrigated in 50 × 22·5 cm cylinders with soln. of Na, K, Ca and Mg in various proportions. The treated soils were analysed for pH, hydraulic conductivity (*HC*), dispersion coeff. (*DC*), and exchangeable cations (*EC*). *HC* was always higher in **I** than in **II** at all levels of K. As K/Ca or K/Mg increased, *HC* decreased, but increased with K/Na. For *DC* only **I** was affected. For *EC*, it was found that K absorbed some 9 times faster than Na, but this varied with concn. In many cases, similar trends are noted for both **I** and **II**, allowing for different cation exchange capacity. M. T. Rawnsley.

Making extract for salinity appraisal of soils. I. P. ABROL and D. K. GUPTA (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 249–251. Engl., 2 ref.).—A modification of the method by Longenecker and Lyerly (*Soil Sci.*, 1964, **97**, 268) is described and its advantages are discussed. M. T. Rawnsley.

Comparison of potassium-activity ratios derived from equilibration procedures and from measurements on displaced soil solution. P. MOSS (*J. Soil Sci.*, 1969, **20** (2), 297–306. 32 ref.).—Direct and indirect methods of determining cation ratios of natural soil soln. are reviewed. There was excellent agreement between K activity ratios derived from measurements on displaced soil solution (EtOH method) and those determined by Q/I equilibration. A rapid Q/I procedure using two K–Ca soln. gave results very similar to those obtained by the full Q/I procedure. A. H. Cornfield.

Physical properties of peats as related to degree of decomposition. D. H. BOELTER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 606–609. 6 ref.).—Water retention, water yield coeff., and hydraulic conductivity of samples from different peat deposits were related to their degree of decomp. as measured by fibre % and bulk density. A. H. Cornfield.

Rapid method for determining the surface area of aluminosilicates from the adsorption dynamics of ethylene glycol vapour. R. A. G. RAWSON (*J. Soil Sci.*, 1969, **20** (2), 325–335. 3 ref.).—Surface areas of soils and clays are determined by adsorbing ethylene glycol (I) vapour under vac. ($\sim 10^{-2}$ cm Hg). The adsorption of a monolayer of I is inferred from the dynamics of pressure changes in the adsorption chamber. A. H. Cornfield.

Separation of the light fraction from soil by ultrasonic dispersion in halogenated hydrocarbons containing a surfactant. G. W. FORD, D. J. GREENLAND and J. M. OADES (*J. Soil Sci.*, 1969, **20** (2), 291–296. 2 ref.).—Max. recovery of the light fraction (non-humified plant and faunal remains, and phytoliths) from soils was obtained by ultrasonic dispersion with bromoform–light petroleum (d 2·0 g cm^{-3}) or Nemagon (1,2-dibromo-3-chloropropane, d 2·06 g cm^{-3}) containing 0·1% Aerosol OT-100. Apparatus for a continuous-flow procedure for treatment of soil is described. Compn. of the ash of the light fractions obtained from a no. of soils is presented. A. H. Cornfield.

Electron microprobe analysis of thin sections of soil to observe loci for cation exchange. D. E. HILL and B. L. SAWHNEY (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 531–534. 16 ref.).—The analysis on Cs^+-saturated soil demonstrated the relation between micromorphology and the loci of cation exchange in natural soil. The Cs^+ absorption capacity of clay-enriched microstructures, silty matrices, and weathered biotite sand grains was determined by *X*-ray scanning and Cs^+ activity measurements. A. H. Cornfield.

Method for treatment of clay soils for thin-section fabric studies. R. B. SINGH (*J. Soil Sci.*, 1969, **20** (2), 269–273. 5 ref.).—The method involves replacing the pore water of the soil specimen with styrene monomer using a two-step diffusion process, followed by impregnation with an Araldite resin mixture and curing at 55°. This method has given better thin sections than 3 other methods described in the literature. A. H. Cornfield.

Biological Aspects, Available Nutrients, Soil Analysis

Isolation and identification of phenolic lignin decomposition products in woodland soil. C. KUNZE (*Pl. Soil*, 1969, **31** (2), 389–390. Ger., 5 ref.).—Five phenolic acids and 2 phenolic aldehydes were identified by chromatography in hot water extracts of the soil. A. H. Cornfield.

Acidic properties of plant lignins and humic materials of soils. S. O. THOMPSON and G. CHESTERS (*J. Soil Sci.*, 1969, **20** (2), 346–356. 16 ref.).—The acidic properties of lignins from a number of plant species growing on a virgin loamy sand, of humic acids from the soil, and of the 1,4-dioxane-extractable humic fraction and residual humic acids from virgin and cultivated soils were investigated by determining Cu^{2+} and Ca^{2+} cation-exchange capacities and by non-aq. potentiometric titration, The lignins and dioxane extracts were titrated in pyridine, and the humic acids in $HCONMe_2$. A. H. Cornfield.

Crop rotation and biological soil disinfection. H. ZOGG (*Qualitas Pl. Mater. veg.*, 1969, **18** (1–3), 256–273. Engl., 22 ref.).—Plant pathological aspects concerning decomp. of root parasites, e.g., *Ophiobolus graminis* (cause of take-all disease of wheat), in the soil are discussed. W. J. G.

Concentration and distribution of *Ceratocystis paradoxa* (de Seynes) Moreau in soil. J. E. C. ABERDEEN and B. C. PATIL-KULKARNI (*Aust. J. agric. Res.*, 1969, **20** (5), 843–856. 7 ref.).—Sugar-cane discs were used as selective substrates for *C. paradoxa*. Four methods of sampling were used, dry-measured, wet-measured, rolling (% cover), and single contact. Interference from other fungi was negligible. The equiv. random concn. of spores was 0–148/g soil at 4–8 in. below the surface. Most were at 3–6 or 6–10 in. M. T. Rawnsley.

Effects of addition of copper, manganese, zinc and chromium compounds on ammonification and nitrification during incubation of soil. P. R. PREMI and A. H. CORNFIELD (*Pl. Soil*, 1969, **31** (2), 345–352. 14 ref.).—The effects of 100–10,000 ppm Cu, Mn, Zn, and Cr as SO_4^{2-} and of Cu and Zn as CO_3^{2-} on ammonification and nitrification during incubation (3 weeks) of an initially neutral soil were studied. Some stimulating, but more usually inhibitory, effects of trace elements on both processes were found, varying considerably with level and type of cation added, on whether incubation conditions were aerobic or anaerobic, and on degree of pH change. A. H. Cornfield.

Nitrification in soil: systems approaching a steady state. A. D. MCLAREN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 551–556. 12 ref.).—Equations are set up for the consecutive reactions $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$ in an idealised soil column and solved, for an open system with a const. rate of entry of aq. NH_4^+, for concn. of ions as functions of time of flow and distance within the column. The equations take into account growth of nitrifying organisms but it is assumed that diffusion and ion exchange within the soil and fixation of nutrients by the nitrifiers are of secondary importance. A. H. Cornfield.

Applied nitrogen losses in relation to oxygen status of soils. B. D. MEEK, L. B. GRASS and A. J. MACKENZIE (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 575–578. 5 ref.).—There were considerable losses of NO_3^- as N_2 when a calcareous silty clay was incubated at saturation moisture content or slightly below this content, but not when incubated at field capacity. The redox potential (*Eh*) decreased with increasing soil moisture content. Incubation after addition of barley straw increased the extent of denitrification. The NO_3^- content and redox potential of soil in the field were high near the surface, but decreased at depths approaching the water table. A. H. Cornfield.

Effect of some common insecticides on the availability of plant nutrients in black cotton soil. P. A. VARADE and D. K. BALLAL (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 255. Engl.).—None of the insecticides DDT, BHC, endrin and chlordane affected the availability of N and P. M. T. Rawnsley.

Phosphorus soil tests based upon correlations with inorganic phosphorus fractions and greenhouse tests. N. P. DATTA and M. S. KHERA (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 191–196, Engl., 17 ref.).—Seventeen different extractant soln. were used to determine available P in 22 soils, neutral to alk. in reaction. The extractants, 0·3 N-NaOH–0·5 N-$Na_2C_2O_4$, 0·025 N-HCl–0·03 N-NH_4F (1 : 2), 0·5 M-NH_4HCO_3 (pH 7·8) and 0·5 M-$NaHCO_3$ (pH 8·5) gave significant correlation coeff. with P-uptake values by tomato plants. M. T. Rawnsley.

Phosphorus adsorption sites in soils. R. D. HARTER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 630–632. 10 ref.).—Regression analysis of phosphate adsorption by 15 soils as a function of 5 soil characteristics indicated that org. matter was important in the initial bonding of phosphate by soils. It is suggested that applied phosphate is bonded initially to anion exchange sites on org. matter and is then transformed into less sol. Fe and Al phosphates. A. H. Cornfield.

Utilisation of ^{32}P-tagged phosphate by rice in different soils. S. T. GAIKWAD and S. PATNIAK (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 257–258. Engl., 5 ref.).—Published methods and correlations were examined, but no precise results can be derived. Better methods are needed to determine relations between dry wt., utilisation of applied P, and 'A' values (Fried and Dean, *Soil Sci.*, 1952, **73**, 263), and also to give specific recommendations for certain soils. M. T. Rawnsley.

Correlation of available molybdenum values obtained by different methods to molybdenum uptake by alfalfa [lucerne]. A. N. PATHAK, H. SHANKAR and R. V. MISRA (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 151–153. Engl., 5 ref.).—Lowe and Massey's method (*Soil Sci.*, 1965, **100**, 238) was found to be more reliable than that of Grigg (*J. Sci. Technol.*, 1953, No. 2, **34**, 405) at least for the 13 different Indian soils examined. M. T. Rawnsley.

A comparison of several procedures for estimating the sulphur status of rice soils. J. VENKATESWARLU and B. V. SUBBIAH (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 155–159. Engl., 5 ref.).—The S status of 13 different soils was estimated by 16 different methods. Poor correlations were obtained, mainly because some extract inorg. S while others extract only org. S. An isotopic diln. technique was found to be the most reliable method for detn. of native available S. M. T. Rawnsley.

Effects of soil and crop management practices on the removal of ^{90}Sr by plant uptake, leaching, runoff and erosion. F. HAGHIRI (*Agron. J.*, 1969, **61** (5), 793–796. 11 ref.).—Sod crops were more effective than cultivated crops in decreasing the loss of ^{90}Sr in runoff and leachate water from a silt loam, but both cropping systems decreased Sr loss compared with a gravel mulch. Over 5 yr the % loss of Sr in runoff water and sediment decreased, whilst that in leachate water increased, with time. Lucerne removed more Sr than did maize. High rates of CaO decreased uptake of Sr by crops and its removal by leachate water. A. H. Cornfield.

Cation-exchange properties of micas. I. Relation between mica composition and potassium exchange in solutions of different pH. A. C. D. NEWMAN (*J. Soil Sci.*, 1969, **20** (2), 357–373. 37 ref.).—For a given soln concn. and pH (3–9), the quasi-equil. concn. of K^+ in NaCl soln. in contact with mica was largely independent of the fraction of the mica-K removed. The soln. concn. ratio C_K/C_{Na} ranged from 1×10^{-5} for dioctahedral micas to $1{\cdot}2 \times 10^{-3}$ for a very reactive biotite. In the phlogopite–biotite series the F content of the micas was the most important single constituent determining K release. Micas that lost much negative charge when the K was exchanged released K most readily to acidic soln. It appears that micas may act as K-buffers in soil. A. H. Cornfield.

pH-dependent bonding of potassium by a spodosol. R. J. BARTLETT and J. L. MCINTOSH (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 535–539. 15 ref.).—Liming a spodosol, high in extractable Al^{3+} and pH-dependent cation-exchange capacity (*CEC*), caused K deficiency in tomato seedlings. Treatment of the soil with $CaCO_3$, $CaSiO_3$, $CaHPO_4$, or CaF_2 resulted in decreases in K activity and extractable Al^{3+} and concomitant increases in $pK^+ - 0{\cdot}5\,p(Ca^{2+} + Mg^{2+})$ and soil *CEC*. A. H. Cornfield.

Fertilisers

Use of certain residues from the textile industry as fertilisers. I. POPESCU and M. DIMA (*Bul. Inst. politeh. Iaşi*, 1968, **14**, (18) (3–4), 281–286. Fr., 11 ref.).—Contents of macro-components and micro-elements of textile-industrial residues were studied, e.g., vegetable fibres (**I**) decomp. by micro-organisms or vegetable fibre ash (**II**). Their application as fertilisers to wheat, maize, sunflower and sugar-beet resulted in a moderate increase in yield ranging from 6·1% for wheat to 10·2% for sunflower. Most efficient dosages were found to be 10–20 t/ha of **I** and 0·5–1 t/ha of **II**. These residues are relatively high in Ca, Cu, Mo, Co, etc. content and show a beneficial influence on quality and yield of crops. (From Rom. summ.) S. Fritz.

Ammonia volatilisation from various ratios of formamide and urea solutions in soils. J. R. LOFTIS and C. E. SCARSBROOK (*Agron. J.*, 1969, **61** (5), 726–727. 10 ref.).—Soils were treated in the surface 1·5-cm with various mixtures of $HCONH_2$ and urea (112–336 kg N per ha). Over 14 days loss of NH_3 accounted for 13–18% of the N applied to a sandy loam (pH 5·9) and a fine sandy loam (pH 5·7), but there was no loss from a calcareous clay. Urea was nitrified twice as rapidly as was $HCONH_2$ in the clay, whilst in the two other soils the N of both sources was nitrified at the same rates. The % loss of applied N as NH_3 increased with rate of N application. NH_3 loss was similar with all ratios of $HCONH_2$/urea (0 : 1 to 1 : 0). A. H. Cornfield.

Urea – agronomic applications. T. E. TOMLINSON (*Fertil. Soc.*, 1970, 51 pp. 507 ref.).—The expanding use of urea as a fertiliser is discussed. Hydrolysis to NH_3 and CO_2 is of great importance but in some circumstances may lead to development of alk. micro-sites and loss of NH_3 from surface dressings or damage to seedlings. It is readily leached through salts but in practice behaves similarly to NH_4 salts owing to rapid hydrolysis. The acidifying effect is similar to that of NH_4NO_3 and less than that of an equiv. dressing of $(NH_4)_2SO_4$. E. G. Brickell.

Comparison of sulphate of ammonia and slow-release nitrogen fertilisers for rice in Trinidad. N. AHMAD and P. T. S. WHITEMAN (*Agron. J.*, 1969, **61** (5), 730–734. 6 ref.).—A slow-release pelletted N fertiliser (EAP 3033, 18% N, 63 days to release 75%) increased rice grain yields to a greater extent than did $(NH_4)_2SO_4$ or a medium-release pelletted N source (EAP 3032, 21 days to release 75%) when all materials were applied at 70 kg N per ha. With the slow-release materials only 50% of final dry wt. was attained when 50% of the final N uptake was absorbed. A. H. Cornfield.

Evaluation of the influence of absence or presence of superphosphate in nursery and evaluation of the most effective time of application of superphosphate in the transplanted area in rice cultivation. KHIN WIN, AUNG KHIN and THEIN MAUNG (*Un. Burma J. Life Sci.*, 1969, **2** (1), 1–6).—Radioactive superphosphate was used. Rates of application in nursery did not show much difference in uptake of phosphate fertiliser in transplanted plants. Single phosphate fertiliser application, just before primordial initiation, appeared unsuitable in rice cultivation. M. J. Rawlins.

Correlation of phosphorus soil test with the response of alfalfa [lucerne] grown in goradu soil. B. S. TRIVEDI and B. V. MEHTA (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 127–131. Engl., 4 ref.).—Expt. showed that available soil P is more efficient than broadcast superphosphate. Bray's modification of Mitscherlich's equation (*Soil Sci.*, 1944, **58**, 305) was used to correlate results from soil tests; a table for application of P at optimum levels to various soils growing lucerne is presented. M. T. Rawnsley.

Correlation of phosphorus soil test analyses with wheat yield responses. M. S. KHERA and N. P. DATTA (*J. Indian Soc. Soil Sci.*, 1969, **17** (2), 133–140. Engl., 4 ref.).—Alluvial soils were tested, using Cate and Nelson's method (*Int. Soil Testing Bull.*, **1**, 1965) to correlate soil test analyses with plant responses. Less P was needed to maintain the critical levels of NP 823 and 824 wheat varieties than for Sonora 64. Actual yields for the latter agreed with predicted yields. M. T. Rawnsley.

Hydrolysis and sorption of ortho-, pyro-, tripoly-, and trimetaphosphate in 32 midwestern (U.S.) soils. R. W. BLANCHAR and L. R. HOSSNER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 622–625, 5 ref.).—Na trimetaphosphate (TMP) hydrolysed to tripolyphosphate (TPP) in 1 day in 30 of 32 soils. TPP hydrolysed to pyrophosphate (PP) and orthophosphate (OP) within 7 days in 30 soils, but with further time the conversion of PP to OP varied considerably among the different soils. The Na salts of OP and PP were equally effective as sources of P for maize. TMP added to soil remained water sol. TPP and PP were sorbed to a greater extent than was OP. A. H. Cornfield.

Reactions of fertiliser phosphate. E. O. HUFFMAN (*Outl. Agric.*, 1968, **5** (5), 202–207. 48 ref.).—Properties of fertiliser materials such as NH_4 phosphates, high analysis granular mixed fertilisers and polyphosphates (**I**), are reviewed. Behaviour of complex fertiliser mixtures during dissolution, and the properties of soln. obtained at different stages of dissolution are discussed. Complex orthophosphates (**II**) of Fe, Al, Ca, NH_4 and K are among the initial fertiliser–soil reaction products discussed. **I** and **II** are compared and hydrolysis of **I** to **II** is considered in detail. W. J. G.

Soil acidification with sulphur in a forest tree nursery. R. E. MULLIN (*Sulphur Inst. J.*, 1969, **5** (1), 1–3).—Use of powdered S resulted in a reduction in soil pH from 7·0 to 5·0–6·0. It was found that the use 900 lbs/acre of S reduced pH by one unit. C. V.

Effect of ammonium sulphate fertiliser on the sulphur content of tea garden soils in Malawi. P. M. GRANT and T. F. SHAXSON (*Trop. Agric. Trin.*, 1970, **47** (1), 31–36. Engl., 11 ref.).—Standard applications of $(NH_4)_2SO_4$ fertiliser resulted in a rapid increase in adsorbed SO_4^{2-} and org. S, the soil being enriched down to 2 ft after 30 yr. S deficiency in established tea can be prevented by the use of $(NH_4)_2SO_4$ once every 3–6 yr. E. G. Brickell.

Sulphur and molybdenum interaction in plant nutrition. U. C. GUPTA (*Sulphur Inst. J.*, 1969, **5** (1), 4–6. 10 ref.).—Discusses the interaction of S and Mo as reported for clover, pea vines, Brussels sprouts, beans, lucerne, spinach and other crops. In soils where the concn. of Mo is limiting, high S fertilisers may aggravate Mo deficiency. Conversely, concn. of Mo (> 10 ppm) toxic to livestock, in pasture plants, can be reduced by application of SO_4^{2-} to the soil. C. V.

Fate of applied molybdate in acidic soils. B. H. SMITH and G. W. LEEPER (*J. Soil Sci.*, 1969, **20** (2), 246–254. 14 ref.).—Less than 4% of the Mo (10 ppm as Na_2MoO_4) added to 7 soils (pH 5·1–6·5) and stored moist for 3–10 months was leached out by the equiv. of 25 cm rain but 36–91% was extractable by

0·1 M-NaOH. Up to 40% resisted treatment with H_2O_2 and NaOH, and most of this was extractable with dithionite + citrate. It is concluded that applied molybdate is not lost by leaching from acidic soils other than sands, but is adsorbed on sesquioxide surfaces and slowly changed to less sol. forms. A. H. Cornfield.

Availability of zinc, copper and iron in fusions with sulphur. K. W. SHARPEE, A. E. LUDWICK and O. J. ATTOE (*Agron. J.*, 1969, **61** (5), 746–749. 7 ref.).—Application of Zn in granular form as fusions of S with ZnO or $ZnCO_3$ to soils of pH 7·0–7·8 resulted in increased yields of maize and uptake of Zn compared with those from $ZnSO_4$, ZnO, or $ZnCO_3$. A fusion of CuC_2O_4–$CuSO_4$–S decreased yields slightly but increased Cu uptake compared with $CuSO_4$. A FeS–S fusion was ineffective. A. H. Cornfield.

Zinc seed treatment as a source of zinc for beans, *Phaseolus vulgaris*. P. E. RASMUSSEN and L. C. BOAWN (*Agron. J.*, 1969, **61** (5), 674–676. 10 ref.).—Treatment with ~900–1800 μg Zn per bean or 0·2–0·4 kg Zn per ha was not effective beyond the 3-compound-leaf stage, but when used together with soil treatment seed treatment eliminated the temporarily scattered Zn deficiency symptoms which occurred when only soil treatment was used. Seed + soil treatment did not hasten maturity or increase bean yields. A polyflavonoid Zn complex was superior to $ZnSO_4$ or ZnEDTA for seed treatment. A. H. Cornfield.

Leaching of added selenium from alkaline soils as influenced by sulphate. M. J. BROWN and D. L. CARTER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 563–565. 18 ref.).—Leaching of Se from $BaSeO_4$ applied to soils (pH 7·7–7·8) was increased by mixing $BaSO_4$ with the $BaSeO_4$ or by adding $CaSO_4$ to the leaching water. A. H. Cornfield.

Stability of different forms of selenium applied to low-selenium soils. E. E. CARY and W. H. ALLAWAY (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 571–574. 8 ref.).—In some acid-to-neutral soils Se added as $NaHSeO_3$ (**I**) was less available to lucerne than that added as co-pptd. $Fe(OH)_3$–$HSeO_3$ (**II**), whilst in other soils, or those limed to neutrality, availability of Se was similar from both sources. The availability of Se° was less than that of Se in **I** or **II**. Oxidation of Se° to SeO_3^{2-} was initially rapid, but varied between soils, and was not pH-dependent. A. H. Cornfield.

Granulation of ammonium nitrate for use as a fertiliser. OFFICE NATIONAL INDUSTRIEL DE L'AZOTE (Br. Pat. 1,174,811, 5.1.67. Fr., 6.1.66).—From 0·01 to 10·0 (0·1–5·0)% of powdered (< 500 μm) natural or synthetic fibrous or laminar magnesium mineral (e.g., pyroxene, amphibole, mica, steatite) is mixed with the soln. of NH_4NO_3 before the granulation process. Up to 0·5% of a wetting agent (e.g., Na naphthylmethylsulphonate) may also be added. J. A. Sugden.

Plant Physiology, Nutrition and Biochemistry

Light, Air and Water Relationships

Effect of light, culture solution, and growth period on growth and chemical composition of hydroponically produced oat seedlings. C. R. TRUBEY, C. L. RHYKERD, C. H. NOLLER, *et al.* (*Agron. J.*, 1969, **61** (5), 663–665. 10 ref.).—Oat seedlings receiving light were higher in β-carotene (**I**), but lower in fresh wt., crude protein (**II**) and water-sol. carbohydrates (**III**), than were those grown in the dark over 6 days. Addn. of complete nutrients to the culture, compared with water, decreased **III**, but did not affect dry matter, **II**,**I** or ash contents. A. H. Cornfield.

Biochemical basis for plant competition. C. C. BLACK, T. M. CHEN and R. H. BROWN (*Weed Sci.*, 1969, **17** (3), 338–344. 58 ref.).—A survey of biochem. characteristics of plants showed that they could be divided into two groups on the basis of efficiency of photosynthesis. Results are discussed in relation to plant breeding, development of herbicides to inhibit specific biochem. reactions, ecology, and increasing food production. A. H. Cornfield.

Effect of oxygen, carbon dioxide, and ethylene on the ripening of pears at ambient temperatures. G. D. BLANPIED and E. HANSEN (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 813–816. 1 ref.).—Ripening of preclimacteric pears at 70°F was stimulated by 500 ppm C_2H_2 in atm. containing 1–10% air and 50% O_2, in air, and in air containing 2·5–10% CO_2. Chlorophyll loss was linearly related to concn. of O_2 and CO_2, and respiration rate to concn. of CO_2. Firmness loss was linearly related to concn. of CO_2 but not to O_2. A. H. Cornfield.

Effects of spraying plants with suspensions of inert dusts. D. W. EVELING (*Ann. appl. Biol.*, 1969, **64** (1), 139–151. 9 ref.).—Spraying excised leaves of 3 species with suspensions of kaolin, talc, SiO_2 or TiO_2 in water increased subsequent water loss and penetration of NH_3. Increased permeability persisted for at least 4 weeks even after partial removal of deposits. With each type of material permeability increased with increasing concn. and with decreasing particle size. Spraying potted *Coleus* plants with kaolin suspensions increased transpiration and decreased fresh wt. of shoots. Some of these inert dusts are used in pesticide formulations. A. H. Cornfield.

Root development and phosphorus uptake by tomato plants under controlled soil moisture conditions. R. M. THORUP (*Agron. J.*, 1969, **61** (5), 808–811. 9 ref.).—Studies with tomato plant using the split-root technique showed that root length and wt. and P uptake increased considerably with increasing soil moisture content (from below the permanent wilting % to 1–5 atm. tension). Moisture was transferred through the plant root system from zones of low to zones of high tension within the soil. A. H. Cornfield.

Drought dormancy in *Astrebla lappacea*, *Chloris acicularis* and *Stipa aristiglumis*. R. D. B. WHALLEY and A. A. DAVIDSON (*Aust. J. agric. Res.*, 1969, **20** (6), 1035–1042. 19 ref.).—It is suggested that the named plants, when stressed for moisture, are in a dormant state similar to that found in perennial grasses. M. T. Rawnsley.

Plant Nutrition and Metabolism

Growth and foliar nutrition of white pine (*Pinus strobus*) seedlings as influenced by simultaneous changes in moisture and nutrient supply. C. E. SCHOMAKER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 614–618. 9 ref.).—Dry matter yields of white pine seedlings in sand–perlite medium decreased with decreasing concn. of nutrients (major and trace elements) from Hoagland full-strength to 0·1 full-strength. Dry wt. decreased with increasing time (7–28 days) between application of nutrients. Needle N, K, and P % increased, whilst Ca and Mg % were unaffected, by decreasing level of water supply. Foliar levels of major elements were generally negatively correlated with those of trace nutrients. A. H. Cornfield.

Effect of *Calothrix* inoculation on vegetable crops. K. S. DADHICH, A. K. VARMA and G. S. VENKATARAMAN (*Pl. Soil*, 1969, **31** (2), 377–379. 8 ref.).—In pot tests inoculation of soil with *Calothrix anomala* (N_2-fixing blue-green algae) increased dry matter yields and N % of chili (*Capsicum annum*) and lettuce. The responses to *Calothrix* inoculation were obtained whether or not urea was applied to the soil. A. H. Cornfield.

Assimilability by higher plants of nitrogen fixed by blue-green algae from the atmosphere. E. M. PANKRATOVA and A. S. VAKHRUSHEV (*Mikrobiologiya*, 1969, **38** (6), 1080–1084. Russ., 17 ref.).—The blue-green algae *Nostoc muscirum* and *Anabaena cylindrica* grown in an atm. contg. $^{15}N_2$, took up ~17–63 μg of isotope per 0·13 g of dry algae. Barley could assimilate the N_2 fixed by the algae, but showed preference for the products of algal destruction over extra-cellular products released by viable algae Viable cultures of the algae provided 4·1–5·8% of the N required by the barley at 21 days. Combined cultivation of algae and plants stimulated root growth in the latter, arising from improved N-nutrition and the effect of active bodies synthesised by algal coenosis. L. A. Haddock.

Absorption and distribution of nitrogen after floret initiation in wheat. H. M. RAWSON and C. M. DONALD (*Aust. J. agric. Res.*, 1969, **20** (5), 799–808. 14 ref.).—The possible use of sterile tillers as sources of nutrients was examined by use of ^{15}N on plants in controlled environments. Results suggest that most of the plant-N is taken up via the root system of the main stem and primary tillers, with only a small uptake by roots of secondary tillers, and almost nil by tertiary tillers. Most of the latter will probably be sterile. At senescence these appear to lose most of their N to the fertile tillers. M. T. Rawnsley.

Effect of nitrogen on phosphorus absorption by excised barley roots. G. D. HUMBLE, A. EL LEBOUDI and V. V. RENDIG (*Pl. Soil*, 1969, **31** (2), 353–364. 22 ref.).—The extent of absorption of ^{32}P-labelled PO_4^{3-} from soln. cultures by excised barley roots varied with form (NH_4^+ or NO_3^-) and concn. of mineral-N present, and time of exposure. A. H. Cornfield.

Effect of soil depth and plant age on ^{32}P uptake by maize and sorghum. T. L. LAVY and J. D. EASTIN (*Agron. J.*, 1969, **61** (5), 677–680. 9 ref.).—Studies with ^{32}P-labelled PO_4^{3-} injected into a silty clay loam showed that by 23 days of age most of the P absorbed by roots of maize and sorghum came from the upper 15 cm of soil. When plants were older than 59 days sorghum absorbed more ^{32}P than did maize. A. H. Cornfield.

Volatiles from apple fruits as affected by phosphorus fertilisation. D. S. BROWN, J. R. BUCHANAN, J. R. HICKS, *et al.* (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 705–715. 4 ref.).—Release of ester volatiles from freshly-harvested apples was higher from high-P than from low-P fruit, but the reverse was true after storage for 4 months. In general the more mature the fruit the lower were the production rates of esters and the higher those of an alcohol. All volatiles were produced at a much higher rate from fresh than from stored fruit. Results are discussed in relation to the effects of P on synthesis of esters and alcohols in the fruit. A. H. Cornfield.

Effect of potassium on the growth and chemical composition of some tropical and temperate pasture legumes. I. Growth and critical percentages of potassium. II. Potassium, calcium, magnesium, sodium, nitrogen, phosphorus and chloride. C. S. ANDREW and M. F. ROBINS (*Aust. J. agric. Res.*, 1969, **20** (6), 999–1007. 19 ref.; 1009–1021. 36 ref.).—I. Eight tropical legumes; *Phaseolus lathyroides, P. atropurpureus, Desmodium intortum, D. uncinatum, Stylosanthes humilis, Lotononis bainesii* and four temperate pasture legumes, *Medicago sativa, M. truncatula, Trifolium repens* and *T. fragiferum* were used. All were grown in pots of a K-deficient soil with varying addn. of KCl. Growth responses and chem. compn. of the plant tops were recorded and used to determine critical % of K in the plant tops.

II. Data and discussion are presented for nutrient groups, cations (K^+, Ca^{2+}, Mg^{2+}, Na^+) and N, P and Cl^-. From the analyses, (*i*) the effect of KCl treatment was assessed, (*ii*) nutrient sufficiency at all levels of K treatment could be detd. and (*iii*) a partial mineral characterisation of the species was carried out.
M. T. Rawnsley.

Influence of silicic acid on uptake of manganese, aluminium, zinc, and copper by tomatoes grown on an acid soil. D. E. PEASLEE and C. R. FRINK (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 569–571. 10 ref.).—Addition of H_2SiO_3 (1·1 g/kg soil) to a fine sandy loam (pH 4·6) decreased the concn. and total uptake of Mn and Al in tomato tissues in a pot test, but had no effect on uptake of Cu and Zn or on dry matter yields. A. H. Cornfield.

Redistribution of calcium in *Phaseolus vulgaris* L. D. W. GREENE and M. J. BUKOVAC (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 368–378. 22 ref.).—The movement of root-absorbed $^{45}Ca^{2+}$ in the bean plant was studied in the presence and absence of Ca in the nutrient. Most of the Ca was transported from the root and lower stem to the upper stem and leaves. Movement was markedly increased by subjecting the plant to Ca stress. ^{45}Ca deposited in the primary leaves was redistributed to newly formed tissue, and this redistribution was independent of the Ca status of the plant.
A. H. Cornfield.

Variable selectivity for strontium by maize hybrids which accumulate different concentrations of strontium, calcium, magnesium and potassium. R. R. BRADFORD and D. E. BAKER (*Agron. J.*, 1969, **61** (5), 766–768. 15 ref.).—Four maize hybrids in glasshouse tests with a silt loam treated with 100–400 ppm Sr^{2+} differed in their ability to absorb Sr^{2+} in relation to absorption of Ca^{2+} and Mg^{2+}. Accumulation of Sr^{2+} was predominantly a function of the availability of Sr^{2+} in the soil and the accumulation characteristics for Sr^{2+} and Ca^{2+} by the hybrids. It seems unlikely that hybrids can be developed which can accumulate Ca^{2+} in preference to Sr^{2+}.
A. H. Cornfield.

Leaf symptoms of manganese deficiency in avocado trees. E. F. WALLIHAN (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 141–144. 8 ref.).—Symptoms of Mn deficiency in the avocado leaf are described and illustrated. Mild symptoms occurred with 16 ppm Mn in the leaf (dry basis) and severe chlorosis with 1·3 ppm leaf Mn. Foliar Mn^{2+} sprays virtually eliminated the symptoms.
A. H. Cornfield.

Effect of iron and manganese deficiency on the chlorophyll, amino acid, and organic acid status of macadamia (*Macadamia tetraphylla*). I. M. GILLFILLAN and W. W. JONES (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 210–214. 11 ref.).—Leaf chlorophyll % was highly correlated with leaf Fe % in macadamia leaves ranging in degree of chlorosis from severe to none. Leaf chlorophyll was well correlated with leaf Mn, but only in the deficient range (< 15 ppm Mn on dry leaf basis). Fe-deficient leaves accumulated arginine and citric acid, in particular, and homoserine and lysine. Mn-deficient leaves accumulated isocitric acid and were lower in homoserine than were normal leaves. A. H. Cornfield.

Foliar absorption of iron by [detached] chrysanthemum [leaves] as influenced by lime-induced chlorosis. R. B. RUTLAND and M. J. BUKOVAC (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 569–575. 17 ref.).—
A. H. Cornfield.

Mechanism of the differential response of Wf9 and Oh40B maize seedlings to iron nutrition. S. O. ODORUKWE and D. N. MAYNARD (*Agron. J.*, 1969, **61** (5), 694–697. 27 ref.).—Sand culture tests with two inbred lines receiving varying levels of Fe showed that Oh40B, which showed the most chlorosis, also accumulated the most P and Mn. With high levels of Fe toxicity symptoms occurred in Wf9 but not in Oh40B. Difference in susceptibility to chlorosis is probably due to the lower Fe/P and Fe/Mn ratios in the susceptible than in the tolerant line resulting in less 'available' Fe in the former. A. H. Cornfield.

Restoration of protein levels in detached leaves by supply of sulphate. P. J. RANDALL (*Pl. Soil*, 1969, **31** (2), 385–388. 7 ref.).—When young detached leaves of subterranean clover were placed in a complete nutrient (including SO_4^{2-}) for 4 days, 80% EtOH-insol. N in the leaves from plants grown with adequate SO_4^{2-} supply was unaffected, but that in leaves from S-deficient plants was increased. The test may be useful in indicating S-deficiency in plants. A. H. Cornfield.

Protein, amino acid and chlorophyll metabolism during the ontogeny of snap bean. T. C. HALL (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 379–387. 14 ref.).—Analyses of protein, total amino acids and chlorophyll in the root, stem, leaf, pod and seed of snap bean from 10 to 80 days of age are presented. A. H. Cornfield.

Free amino acids of some plant tissues dependent on ecological factors. E. SCHWERDTFEGER (*Qualitas Pl. Mater. veg.*, 1969, **18** (1–3), 152–170. Ger., 12 ref.).—The acids react sensitively to metabolic changes introduced by internal and external factors, and can therefore be used as detectors for the intensity of interactions caused by these factors. It is shown that the total content of free amino acids is influenced by fertilisation (esp. N-fertilisation) as well as by light intensity and seasonal climate. (From Engl. summ.) W. J. G.

Influence of temperature and relative humidity of the air on the activity of tobacco orthodiphenol oxidase. D. G. DEVDARIANI (*Pishch. Tekhnol.*, 1969, [**2** (69)], 90. Russ.).— C. V.

Germination, Growth Regulation, Senescence

Germination and morphology of red pine (*Pinus resinosa*) seeds and seedlings in contact with EPTC, CDEC, CDAA, 2,4-D and picloram. T. T. KOZLOWSKI and S. SASAKI (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 655–662. 16 ref.).—The effects of various concn. of the herbicides placed in direct contact with red pine seeds and seedlings on germination, cotyledonary form, and shoot and root elongation were studied. A. H. Cornfield.

Effect of sea-water on the germination and early seedling growth of certain field crops. T. KURIAN (*Salt Res. Ind.*, 1969, **6** (3/4), 84–85).—Amongst the crop seeds studied were sesame, groundnut, linseed, mustard and safflower. All were very tolerant when sea-water was applied during germination. In very low concn., stimulation of the plumule and radicle growth occurred, but above 10,000 ppm there was reduced growth. C. V.

Effects of kinetin in overcoming high-temperature dormancy of lettuce seed. O. E. SMITH, W. W. L. YEN and J. M. LYONS (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 444–453. 16 ref.).—Dipping lettuce seed for 3 min in kinetin (0·1 g/l) was the most effective method for overcoming the poor germination of lettuce seed occurring at high temp. (30–35°). A. H. Cornfield.

Interaction of photoperiod and auxin metabolism in rooting of *Chrysanthemum morifolium* cuttings. Y. LESHEM and M. SCHWARZ (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 589–594. 16 ref.).—A short-day photoperiod resulted in more and longer roots from chrysanthemum cuttings than did a long-day period. 100 ppm indolebutyric acid, exogenously applied, increased rooting under both light regimes. Rooting was inhibited by dichlorophenol, tyrosine, and tri-iodobenzoic acid, all of which normally interfere with auxin metabolism. Endogenous levels of auxin were somewhat higher under short than under long-day periods. Increased rooting due to short-day periods may be auxin mediated. A. H. Cornfield.

Biosynthesis of ethylene and the ripening of fruit. L. W. MAPSON (*Endeavour*, 1970, **29** (106), 29–33. 28 ref.).—This review covers: biosynthesis of C_2H_4 in fruit; synthesis of C_2H_4 from methionine and linolenic acid in tissue slices and in cell-free systems; action of CO_2 in the ripening process; control of ripening by use of low concn. of O_2 and high concn. of CO_2. C_2H_4 initiates the chain of chem. reactions that cause the changes associated with ripening of fruit. By control of its synthesis *in vivo*, onset of ripening can be inhibited or retarded and storage life of the fruit can be controlled.
J. N. Ashley.

Some effects and potential uses of Ethrel on ornamental crops. J. B. SHANKS (*HortScience*, 1969, **4** (1), 56–59).—Ethrel, a mixture of 2-chloroethylphosphonic acid and related compd., shows activity in dwarfing, inducing abscission, stimulating lateral or basal branching and increasing the no. of branchings following topping. C. V.

Effect of Ethrel [2-chloroethylphosphonic acid] on the ripening of Montmorency sour cherries. J. L. ANDERSON (*HortScience*, 1969, **4** (2), 92–93).—This compd. is a water-sol. plant growth regulator, thought to be degraded within the plant cells with liberation of ethylene which is probably responsible for the biological activity. C. V.

A comparison of the effects of Cycocel and tipping on fruit set in *Vitis vinifera* L. K. G. M. SKENE (*Aust. J. biol. Sci.*, 1969, **22** (6), 1305–1311. 18 ref.).—Cycocel [(2-chloroethyl)trimethylammonium chloride] (**I**) (200 mg/l) was added to nutrient soln. when rooted cuttings were transplanted or at 2 weeks before anthesis. Fruit set was increased from 20 to 41%. Plants from which shoot tips were removed at anthesis, either with or without **I** treatment, showed similar fruit set. It is suggested that **I** treatment and tipping increase fruit set by reducing competition between developing leaves and ovaries for available metabolites.
M. T. Rawnsley.

Other Aspects

Routine analysis of ^{15}N in plant material by mass spectrometry. G. PROKSCH (*Pl. Soil*, 1969, **31** (2), 380–384. 5 ref.).—Separation of N by the Dumas method (ignition with CaO–CuO mixture in sealed tubes) gave slightly but significantly higher results for ^{15}N in plant tissue than did separation of N by Kjeldhal digestion, distillation of NH_3 and its conversion to N_2 by NaOBr before analysis by mass spectrometry. A. H. Cornfield.

Effect of bruising on discoloration and concentration of phenolic compounds in apple fruit tissue. M. INGLE and J. F. HYDE (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 738–745. 22 ref.).—About 50% of the browning, measured by reflectance of tissue at 600 nm, occurred within 30 min of impact bruising and max. bruising discoloration occurred within 2 h of bruising. Discoloration increased with temp., but the effect varied with cultivar. Bruising decreased the concn. of total phenols, chlorogenic acid, and, in particular, flavanols. A. H. Cornfield.

Effect of temperature on the composition of grapes grown under field and controlled conditions. W. M. KLIEWER (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 797–806. 23 ref.).—Total sol. solids, pH, total titrable acidity, sugars, malic acid and malates, and tartaric acid and tartrates were determined in the fruit of two varieties of grape during the ripening period of plants grown in the field and in a phytotron at controlled temp. A. H. Cornfield.

Temperature effects on the fatty acid composition of the seed oil of wild species of flax. D. M. YERMANOS, S. H. PATIL and S. HENSTREET (*Agron. J.*, 1969, **61** (5), 819–820. 4 ref.).—Three wild species of flax, *Linum angustifolium*, *L. grandiflorum*, and *L. schiedeanum*, differing in chromosome numbers, showed significant differences in changes in fatty acid compn. of the seed oil due to varying temp. of growth. A. H. Cornfield.

Growth of young orchardgrass (*Dactylis glomerata* L.) plants in different environments. W. C. TEMPLETON, JUN., J. L. MENEES and T. H. TAYLOR (*Agron. J.*, 1969, **61** (5), 780–782. 12 ref.).—Leaf development, plant wt. and shoot/root ratios in young orchardgrass plants were studied in a factorial expt. involving 2 temp. regimes, 2 photoperiods, 3 levels of N, and 4 levels of P in a fine sandy loam mixed with sand. A. H. Cornfield.

Composition of essential oil constituents of the carrot root in response to cultivar and site. W. SCHUPHAN (*Qualitas Pl. Mater. veg.*, 1969, **18** (1–3), 44–70. Ger., 25 ref.).—Physiol. studies in tap roots of two carrot cultivars provided data on the formation of essential oils and other constituents essential to nutrition. (From Engl. summ.) W. J. G.

Composition of the essential oil of leaves of the strawberry cultivar Citation. T. R. KEMP, L. P. STOLTZ, W. T. SMITH, JUN. and C. E. CHAPLIN (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 334–339. 8 ref.).—Twelve org. compd. were identified in the essential oil of the leaves of strawberry by use of g.c. and mass spectrometry.
A. H. Cornfield.

Rôle of water content in the decay of radiation induced oxygen-sensitive sites in barley seeds during post-irradiation hydration. B. V. CONGER, R. A. NILAN and C. F. KONZAK (*Radiat. Res.*, 1969, **39** (1), 45–56. 30 ref.).—Himalayan barley seeds (H_2O content 1·2–12·8%) were exposed *in vacuo* to 25 krad of ^{60}Co γ-rays and then hydrated in oxygenated or O_2-free water for various periods before final examination. Data suggest that hydration of macromol. within the cell may be the rate determining process in the decay or quenching of the radiation-induced O_2-sensitive sites; intermediate products or processes may be involved in the development of O_2-dependent damage. C. V.

Metabolic changes in sweetpotato roots induced by γ-radiation in response to cutting. M. OGAWA and I. URITANI (*Radiat. Res.*, 1969, **39** (1), 117–125. 22 ref.).—Using ^{60}Co γ-rays, sweetpotato roots were irradiated at 5–120 krad, then cut into discs and incubated aerobically. Polyphenol (**I**) content and activity of *o*-diphenoloxidase (**II**), peroxidase (**III**) and phenylalanine ammonia-lyase (**IV**) increased greatly in both irradiated and non-irradiated samples after cutting, but following irradiation with > 60 krad the **I** content and **III** activity was significantly stimulated above the non-irradiated sample; formation of lignin-like substances was inhibited and no significant effect on **II** was noted; at > 90 krad, increase in **IV** was correlated with increase in **I**. C. V.

Effect of air pollution on agricultural produce. F. H. F. G. SPIERINGS (*Landbouwvoorlichting*, 1969, **26** (10/11), 377–381. Dut.).—The different effects of HF, SO_2, O_3, Cl_2 and various olefins on plants were discussed. Distinction was made between acute damage (influence of high concn. of poisonous gas for a few hours) and very gradual damage (influence of low concn. of poisonous gas for several days). J. C. T. Niewenhuis.

Chlorotic dwarf of Eastern White Pine caused by ozone and sulphur dioxide interaction. L. S. DOCHINGER, F. W. BENDER, F. L. FOX and W. W. HECK (*Nature, Lond.*, 1970, **225**, (5231) 476. 6 ref.).—In the affected plantations in Ohio, the daily 2 h peak concn. of SO_2 were up to 7·8 pphm with av. of 5 pphm on 25% of days; max. hourly av. of O_3 were > 5·5 pphm for 60% of days (concn. > 10 pphm were common); max. combination was O_3 12 and SO_2 7·8 pphm. Expt. show that SO_2 and O_3 (10 pphm of each), acting separately or synergistically, promote initial symptoms of current needle mottling and premature defoliation and that SO_2 is the more reactive. This continued decomp. of chlorophyll and loss of leaves eventually, by blocking photosynthesis, seriously retards growth. W. J. Baker.

Crops and Cropping

Field Crops

Chemical composition and yield of oats and peas separated from a forage mixture at successive stages of growth. L. J. KLEBESADEL (*Agron. J.*, 1969, **61** (5), 713–716. 12 ref.).—Mineral elements, nutritive value and yields of oats and peas grown in mixed stand and sampled 9 times from 36 to 115 days of age are reported.
A. H. Cornfield.

Yield–protein relationships in wheat grain, as affected by nitrogen and water. G. L. TERMAN, R. E. RAMIG, A. F. DREIER and R. A. OLSON (*Agron. J.*, 1969, **61** (5), 755–759. 17 ref.).—The chief effect of N applied with adequate irrigation water was to increase grain yields, whilst the chief effect of N with severe water deficit was to increase protein %. With intermediate water supply, N increased both yields and protein %. A. H. Cornfield.

Effect of nitrogen-fixing blue-green algae on certain cultivated crops. G. S. VENKATARAMAN and S. K. GOYAL (*Mikrobiologiya*, 1969, **38** (4), 709–713. Russ., 13 ref.).The algae grown with free access to air consisted of *Cylindrospermum musciola* and *Nostoc* sp. applied at a concn. of 1 kg/ha. The use of algae had a beneficial effect on the growth of rice; the dry mass and the N content of *Capsicum annum* also increased, especially when added urea was employed. A similar effect was noted with *Lactuca sativa*.
L. A. Haddock.

Nitrogen fertilisation of irrigated hybrid maize on sandveld soils. E. D. ALVORD (*Rhodesia agric. J.*, 1969, **66** (6), 139–143. 12 ref.).—Calcium ammonium nitrate was used in 3 expt. to determine the most efficient time of application of N as a nutrient, when growing maize on sandveld soil. Plant population was also studied and SR52 (single hybrid) and SR14 (double hybrid) were compared. The former was more productive when planted in October. For application of N > 190 lb/acre, the time and splitting of treatment differed from those where the application was < 190 lb.
M. J. Rawlins.

Sugar production from sweet sorghum as affected by planting date, after-ripe harvesting, and storage. D. M. BROADHEAD (*Agron. J.*, 1969, **61** (5), 811–812. 5 ref.).—Sugar yields per ha from sweet sorghum, *Sorghum bicolor*, planted mid-April or mid-May, were higher than from mid-June planting. Delay in harvesting for 1–4 weeks after the ripe stage resulted in slightly decreased yields. Delay in processing for 24–48 h after harvesting had little effect on sugar yields. Some differences in Brix°, sugar % and purity due to treatments were noted. A. H. Cornfield.

Effect of potassium deficiency and topdressing after crop analysis on yield of potatoes from diluvial sandy soils. J. PRUMMEL (*Versl. landbouwk. Onderz. Ned.*, 1969, (733), 17 pp. Dut., 24 ref.).—K deficiency reduced yield. The critical value for K_2O in leaves depended on the date of sampling. With 6% N on dry matter of leaves, the critical value for tuber yield was 7·5 to 8% K_2O on dry matter. To prevent losses, leaf samples should be taken for analysis before mid-July. J. C. T. Niewenhuis.

Patterns of spring growth in swards of different grass varieties. A. DAVIES and D. M. CALDER (*J. Br. Grassld Soc.*, 1969, **24** (3), 215–225. 16 ref.).—The patterns of winter and spring growth were compared in varieties of ryegrass and cocksfoot over 3 yr.
A. H. Cornfield.

Legume contributions to yields and compositions of *Desmodium* spp.–Pangolagrass mixtures. A. S. WHITNEY and R. E. GREEN (*Agron. J.*, 1969, **61** (5), 741–746. 12 ref.).—Dry matter production per ha over 2 yr. by *Desmodium canum*–Pangola grass mixture was approx. twice, whilst that by *D. intortum*–Pangola-grass was approx. 3 times, that of Pangola grass in pure stand. Inoculation of legume seed before sowing increased dry matter more with *D. intortum* than with *D. canum*. N fixation by *D. canum* was 17–96 kg and by *D. intortum* 213–264 kg/ha/yr.; ~ 33% of the N fixed was transferred to the grass. A. H. Cornfield.

Influence of nitrogen fertilisers on Washington creeping bentgrass, *Agrostis palustris* Huds. I. Growth and mineral composition. F. E. MARKLAND and E. C. ROBERTS and (II) L. R. FREDERICK. **II. Incidence of dollar spot, *Sclerotinia homoeocarpis*, infection.** (*Agron. J.*, 1969, **61** (5), 698–700. 9 ref.; 701–705. 12 ref.).—I. Forage yields and major and trace element contents of the forage were studied in relation to level and form of N [$NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, urea, activated sewage sludge (**I**), urea-form, and processed tankage].

II. High levels of available N reduced dollar spot injury in field tests, the lowest incidence occurring where **I** was applied.
A. H. Cornfield.

Long-term fertility requirements of Coastal Bermuda grass, *Cynodon dactylon*. W. W. WOODHOUSE, JUN. (*Agron. J.*, 1969, **61** (5), 705–708. 15 ref.).— A. H. Cornfield.

Effect of waterlogging on the growth and isoflavone concentration of *Trifolium subterraneum* L. C. M. FRANCIS and A. C. DEVITT (*Aust. J. agric. Res.*), 1969, **20** (5), 819–825. 15 ref.).—Three subspp., *T. yanninicum* (**I**), *T. brachycalycinum* (**II**) and *T. subterraneum* (**III**), were waterlogged. Leaf growth in **I** was unaffected, in **II** was reduced by 46% and in **III** by 26%. Root growth was retarded most in **II** and least in **I**. Total isoflavones increased in all subspp. but there were variations in quantities of individual isoflavones. Winter waterlogged clover can develop very high concn. of isoflavones, making it dangerous for breeding sheep. Some varieties of **I** were found to be low in formononetin.
M. T. Rawnsley.

Functions for cotton production from irrigation and nitrogen fertilisation variables. I. Yield and evapotranspiration. D. W. GRIMES, H. YAMADA and W. L. DICKENS. **II. Yield components and quality characteristics.** D. W. GRIMES, W. L. DICKENS and W. D. ANDERSON (*Agron. J.*, 1969, **61** (5), 769–773. 13 ref.; 773–776. 16 ref.).—Yields and quality of cotton lint and other plant parts, evapotranspiration and efficiency of water use are reported in relation to quantity of irrigation water and level of applied N on a clay loam and a fine sandy loam.
A. H. Cornfield.

Differential tolerance of cotton varieties to excess manganese. C. D. FOY, A. L. FLEMING and W. H. ARMIGER (*Agron. J.*, 1969, **61** (5), 690–694. 14 ref.).—Six varieties of cotton varied considerably in their tolerance to Mn when grown in acid soils and nutrient soln. high in Mn. Decreased growth, high Mn uptake, and crinkle leaf symptoms, typical of Mn toxicity, occurred in most unlimed soils. Tolerance was not related to Mn % in the tops. High Mn affected top growth more than root growth. Cotton varieties showing the greatest tolerance to Mn were not necessarily those which were tolerant to high Al. A. H. Cornfield.

Horticultural Crops

Citrus in Rhodesia. I. Cultural requirements of citrus. MIN. AGRIC. **II. Diseases of citrus.** J. O. WHITESIDE. **III. Pests of citrus and their control.** C. J. HODGSON (*Rhodesia agric. J. Tech. Bull.*, 1969, No. 7, 1–10. 2 ref.; 11–17. 4 ref.; 18–29. 4 ref.).—
W. J. G.

Effect of phosphorus and chicken manure on yield, fruit quality and leaf composition of grapefruit trees. A. BAR-AKIVA and J. PATT (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 145–152. 18 ref.).—Over 5 yr grapefruit trees on a black calcareous loam receiving 60–160 kg N/ha/yr and no P developed severe P deficiency symptoms and produced low yields, of poor fruit. Application of 16 kg P + 160 kg N/ha/yr or of 8 m^3/yr chicken manure gave high yields, of good fruit, increased leaf P, Ca and Mn and decreased leaf N and Cu. Fruit yields were high when leaf P (dry basis), sampled from fruit-bearing terminals, was > 0·075%. A. H. Cornfield.

Preharvest defoliation of apple nursery stock using Ethrel [2-chloroethylphosphonic acid]. J. N. CUMMINS and P. FIORINO (*HortScience*, 1969, **4** (4), 339–341. 12 ref.).—Two-year-old apple trees growing in the nursery row were defoliated by sprays of Ethrel at concn. of 2000, 3750 and 5000 ppm. At 2000 ppm, early spring growth was somewhat delayed but not unacceptably so. This appears to be a promising commercial practice. C. V.

Nitrogen nutrition of the peach tree. IV. Storage and mobilisation of nitrogen in mature trees. B. K. TAYLOR and B. VAN DEN ENDE (*Aust. J. agric. Res.*, 1969, **20** (5), 869–881. 15 ref.).—Results of tests on 8-yr old trees on a quant. basis, agreed closely with results obtained by Taylor (*Aust. J. biol. Sci.*, 1967, **20**, 379–387). The level of storage N in mature trees could be altered significantly with N supplement in a single season, suggesting that extra N could be used each yr. Concn. of arginine in roots is a sensitive indicator of storage N concn. M. T. Rawnsley.

Peach thinning with ethylene. G. C. MARTIN and M. NELSON (*HortScience*, 1969, **4** (4), 328–329).—Ethrel (2-chloroethylphosphonic acid) (**I**) was studied. Fruit thinning caused by ethylene resulted from abscission (*A*) between fruit and receptacle. This is also the major zone of *A* during natural 'June drop' and when chemical thinners such as **I** or 3-chlorophenoxy-α-propionamide are applied. C. V.

Ethylene-induced ripening of pears in relation to maturity and length of treatment. E. HANSEN and G. D. BLANPIED (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 807–812. 7 ref.).—Pears picked at a premature stage required 48 h in an atm. contg. 500 ppm C_2H_4 for induction of ripening, whilst fully mature fruit required only 24 h. Ripening of fruit picked in the post-mature stage proceeded without exposure to C_2H_4, but was stimulated by a 12-h treatment. Mature pears treated for 48 and 160 h ripened at equal rates.
A. H. Cornfield.

Influence of Alar [succinic acid mono-(2,2-dimethylhydrazide], Ethrel [2-chloroethylphosphonic acid] and gibberellic acid on browning of peaches. D. W. BUCHANAN, C. B. HALL, R. H. BIGGS and F. W. KNAPP (*HortScience*, 1969, **4** (4), 302–303).—Ethrel (**I**) at 30 and 50, gibberellic acid (**II**) at 50 and Alar (**III**) at 2000 ppm were applied as whole tree sprays. **I** and **II** consistently prevented browning of purée and sliced peaches for 24 h but the results with **III** were inconsistent. C. V.

Chemical promotion of tomato fruit ripening. R. W. ROBINSON, H. WILCZYNSKI, F. G. DENNIS, JUN. and H. H. BRYAN (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 823–830. 14 ref.).—Detached green tomato fruit ripened more rapidly and uniformly when treated with 1000 ppm and, particularly, with 10,000 ppm Ethrel (2-chloroethylphosphonic acid), and stored at 75°F. Field sprays applied 2 weeks before harvest increased the proportion of ripe fruit without affecting total yield. Very immature and mature green fruit responded to the treatment, both on and off the plant. Treated green tomatoes did not respond when stored at 50°F but did so when the temp. was subsequently raised. A. H. Cornfield.

Phosphorus and magnesium fertiliser studies with pepper. H. Y. OZAKI and J. R. ILEY (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 462–469. 15 ref.).—Yields of peppers on a fine sandy soil were increased in two successive crops by an initial application of 220 lb P (superphosphate), but only in the first crop by 22 lb P/acre. Side dressings of $MgSO_4$ (15–60 lb Mg/acre) did not affect growth or pepper yields. Application of Mg as $MgSO_4$ or $MgNH_4PO_4$ sometimes increased early growth. A. H. Cornfield.

Fertilisation inter-relationships in yield, nitrate, and oxalic acid content of spinach. W. S. REGAN, V. N. LAMBETH, J. R. BROWN and D. G. BLEVINS (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 485–491. 20 ref.).—Increasing N rate (100–300 lb/acre as NH_4NO_3) on a silt loam (pH 5·2) resulted in decreasing yields of spinach, particularly where the K and/or P supply was low. Liming to pH ~6 increased yields 2–6 fold. Yields were increased by P or K, but to the greatest extent by P + K. Nutrient imbalances owing to low P – high K – high N levels of fertilisation contributed to high leaf concn. of both NO_3^- and oxalic acid. Liming increased leaf NO_3^- when K was low but not when K was high. A. H. Cornfield.

Response of *Dianthus caryophyllus* L. (carnation) to synthetic abscisic acid. H. M. CATHEY (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 560–568; 13 ref.).—The effects of spray applications of abscisic acid to carnation plants on growth and flowering habit in relation to dosage, kind and duration of photoperiod, and cultivar were studied. A. H. Cornfield.

Residual effect of Cycocel [(2-chloroethyl) trimethylammonium chloride] in poinsettia control. R. A. LARSON and M. L. MCINTYRE (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 667–672. 7 ref.).—The most effective decrease in height was obtained when both the parent and second generation plants were treated with Cycocel. A. H. Cornfield.

Response of some ornamental plants to synthetic abscisic acid. H. M. CATHEY (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 693–698. 7 ref.).— A. H. Cornfield.

Effect of foliar applications of 3-indolebutyric acid (IBA) on rooting of ornamental plants. J. J. MCGUIRE, L. S. ALBERT and V. G. SHUTAK (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 699–704. 20 ref.).—Terminal (foliar) application of IBA by dipping stimulated root initiation of 14 species of woody ornamental plants. A. H. Cornfield.

Plantation Crops

Effects of fertilisers on growth of young cacao. M. WESSEL (*Trop. Agric. Trin.*, 1970, **47** (1), 63–66. Engl., 4 ref.).—Although cacao seedlings are very sensitive to toxic effects of even small dressings esp. when applied in the planting hole, monthly applications of urea (5 g per seedling in the 1st year and 10 g in the 2nd yr after planting) had a beneficial effect on growth and stimulated early jorquetting. E. G. Brickell.

Correction of boron deficiency in cacao in Ecuador. S. MESTANZA S. and J. LAINEZ C. (*Trop. Agric. Trin.*, 1970, **47** (1), 57–61. Engl., 12 ref.).—B treatments (foliar spray of Solubor) led to an increase in leaf dry wt. and an increase in N and B as % of dry wt. Pollen germination, growth of pollen tube, fruit set and fruit persistence were all increased. E. G. Brickell.

Variability in mineral nutrition and production of Robusta coffee clones. J. FORESTIER and J. BELEY (*Café-Cacao-Thé*, 1969, **13** (4), 290–296. Fr., 3 ref.).—A fertiliser trial was carried out to determine clone response to changes in mineral nutrition. It consisted of 12 replications, each contg. one control plot, one plot receiving a moderate and one a high amt. of nitrogeneous fertiliser. N plots also received bicalcic phosphate, KCl and $MgSO_4$. The effects on trace elements, production and marketable coffee/fresh coffee weight yield ratio are discussed. W. J. G.

A sulphur deficiency in sugar-cane. J. M. GOSNELL and A. C. LONG (*Proc. S. Afr. Sug. Technol. Ass., 43rd Ann. Congr.*, 1969, 26–29. Engl., 8 ref.).—Gypsum, $MgSO_4$, rock S, $CuSO_4$, $ZnSO_4$, $Na_2B_4O_7$ (14·3%), and Na_2MoO_4 were used as fertilisers on a sandy loam soil. High S application resulted in a sucrose yield up to 2·5 times that of the control. Improved yields were also found with smaller amt. of S. Deficiency symptoms were: overall yellowish appearance of leaves; reduction in leaf size and plant height. There is a possibility that Al toxicity may occur when S is added. M. T. Rawnsley.

Causes of early flowering in tobacco. J. M. HOPKINSON (*Aust. J. agric. Res.*, 1969, **20** (6), 1061–1071. 14 ref.).—Misjudgment of transplanting time, and effects of low temp. were investigated as causes of early flowering of tobacco. M. T. Rawnsley.

Effect of nitrogen, phosphorus and potassium fertilisation on the mineral composition of tobacco. L. A. PETERSON, S. G. DOLAR and G. CHESTERS (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 560–563. 11 ref.).—Application of N in field trials increased tobacco leaf Mg and Mn levels and decreased leaf B. P application increased leaf Ca, Mg, and B levels and decreased leaf Zn and Cu. K application decreased leaf Ca, Mg, B, Zn and Cu levels. A. H. Cornfield.

Forest Crops

Critical foliage concentrations of nitrogen and phosphorus as a guide to the nutrient status of *Araucaria cunninghamii* (hoop pine) underplanted to *Pinus*. B. N. RICHARDS and D. I. BEVEGE (*Pl. Soil*, 1969, **31** (2), 328–336. 9 ref.).—Critical foliar N levels for suitable growth of hoop pine underplanted to *Pinus* varied with foliar P levels and *vice versa*. For practical purposes suitable critical levels were 1·35% for N and 0·11% for P (dry basis). Foliar N and P levels accounted for 81% of the variation in height growth. A. H. Cornfield.

Animal Husbandry

Feedstuffs

Animal feeds. M. GUTCHO (*Fd Process. Rev.*, 1970, (10), 353 pp.).—The review is based on over 270 U.S. patents, since 1960, relating to production of animal feeds. It is divided into sections: (1) **Forage and fodder,** which includes prepn. and preservation of forage, drying forage, pelleted foods, feed licks and blocks, and detoxifying plant material. (2) **Fats and oils.** This deals with refining processes, soapstocks in feed products, high fat oilseed meals, and vegetable oil emulsions etc. (3) **Molasses and flavourings,** prevention of gel formation in molasses – H_3PO_4 supplements is discussed together with ammoniated sugars and flavourings. (4) **Oestrogens as growth stimulators,** dealing with compd. from gibberella cultures, coumarine compd. etc. (5) **Antibiotics as anabolic stimulators.** This covers stabilising bacitracin and tetracycline feedstuffs, antimicrobials etc. (6) **Antioxidants in feeds,** e.g., activated dihydroquinolines. (7) **Minerals and vitamins.** (8) **Growth-promoting chemical additives,** for poultry, ruminants, non-ruminants etc. (9) **Poultry, ruminant and pet feeds** (3 sections). (10) **Utilisation of industrial waste and by-products.** This deals with feed supplements from poultry waste and products from the meat industry etc. W. J. G.

Cultivation of fodder yeast in the foam phase while adding fodder biomycin (?). A. S. VECHER, G. F. PROKAZOV and L. A. YURCHENKO (*Pishch. Tekhnol.*, 1969, [4 (71)], 96. Russ.).— C. V.

Carrying capacity of pastures. S. J. COWLISHAW (*J. Br. Grassld Soc.*, 1969, **24** (3), 207–214. 25 ref.).—Data from all published results of grazing trials in which 3 or more rates of stocking were compared showed negative correlations between stocking rate/acre and live-wt. gain/animal/day. In general, the longer the grazing season the steeper was the slope of the regression line. Application of the results is discussed in agronomic and economic terms. A. H. Cornfield.

Relative occurrence of toxic concentrations of cyanide and nitrate in varieties of Sudan grass and sorghum–Sudan grass hybrids. J. T. GILLINGHAM, M. M. SHIRER, J. J. STARNES *et al.* (*Agron. J.*, 1969, **61** (5), 727–730. 12 ref.).—The NO_3^--N% in 5 varieties increased considerably with rate of application of NH_4NO_3 (28–112 kg N per ha). CN^- % increased with rate of applied N, but also varied with height of forage and variety. NO_3^- and CN^- levels indicated that poisoning of cattle was more likely from CN^-. A. H. Cornfield.

Kenaf, *Hibiscus cannabinus* L., a multi-purpose crop. G. B. KILLINGER (*Agron. J.*, 1969, **61** (5), 734–736. 20 ref.).—The crude protein content of 55-day-old kenaf leaf ranged from 24–29%, and of the whole plant, excluding roots, averaged 16–17%. The plant yielded 20,000 kg dry stem per ha. Kenaf silage was accepted by cattle, but not by sheep. A. H. Cornfield.

Digestibility of structural carbohydrates of grasses by rumen micro-organisms *in vitro*. J. M. A. TILLEY, R. A. TERRY, R. E. DERIAZ and G. E. OUTEN (*J. Br. Grassld Soc.*, 1969, **24** (3), 238–243. 17 ref.).—The *in vitro* digestion of grass samples showed that the amount of digestible cellulose, hexosan or pentosan was not greatly

affected by species or by stage of maturity. A survey of published data on crude fibre and cellulose digestibility confirmed these findings, although there was a marked difference between grasses and legumes. A. H. Cornfield.

Sources of variation in the *in vitro* digestibility of tropical grasses. M. N. McLeod and D. J. Minson (*J. Br. Grassld Soc.*, 1969, **24** (3), 244–249. 14 ref.).—The *in vivo* dry matter digestibility of 4 tropical and one temperate pasture grass species was affected by fineness of grinding, sample size, pH of original rumen fluid and size of rumen fluid inoculum. Different relations were found between *in vivo* and *in vitro* results for each species, with a max. predicted difference of 3·5 digestibility units. This method can accurately predict the *in vivo* digestibility of tropical grasses provided similar samples of known *in vivo* digestibility are tested concurrently. A. H. Cornfield.

Digestion of pasture plants by sheep. IV. The digestion of *Phalaris tuberosa* at different stages of maturity. J. P. Hogan, R. H. Weston and J. R. Lindsay (*Aust. J. agric. Res.*, 1969, **20** (5), 925–940. 27 ref.).—Results showed that increased maturity was associated with increase in energy for chewing, decline in digestibility of org. matter and structural carbohydrates, decrease in food intake and decline in quantities of amino acids etc. made available. However, other digestion factors remained unchanged. Implications of these results are discussed in detail. M. T. Rawnsley.

Relation between metabolisable and digestible energy in sheep and cattle. N. McC. Graham (*Aust. J. agric. Res.*, 1969, **20** (6), 1117–1122. 27 ref.).—A statistical evaluation of published data shows that metabolisable energy is not a const. fraction of digestible energy. A linear equation which represents most diets or feeding levels, is described. M. T. Rawnsley.

Effect of altering the physical form of corn silage on utilisation by dairy cattle. C. N. Miller, C. E. Polan, R. A. Sandy and J. T. Huber (*J. Dairy Sci.*, 1969, **52** (12), 1955–1960. 22 ref.).—Results of feeding trials with lactating cows indicated that there is little advantage in chopping maize silage sufficiently fine to ensure breakage of all the kernels. Digestibility of starch is increased by fine chopping but that of crude fibre is decreased. Finely chopped silage feeds had no consistent effect on improving milk production and caused some depression in milk fat. M. O'Leary.

Effect of level of protein in silage on the intake and production of dairy cows. M. E. Castle and J. N. Watson **[Appendix. The composition of the non-protein nitrogen fraction of the two silages.** A. D. Hughes] (*J. Br. Grassld Soc.*, 1969, **24** (3), 187–194. 17 + 9 ref.).—The low-protein silage (8·2% digestible crude protein *DCP* in its dry matter) was higher in dry matter digestibility and starch equiv. than was the high protein silage (15·9% *DCP*). Dry matter intake and av. daily milk yields were higher with the low- than with the high-protein diet with or without a groundnut supplement. The digestibility of the silage dry matter was a better indicator of quality than was its protein content. A. H. Cornfield.

Feed intake of sheep supplemented with varying quantities of wheat while grazing pastures differing in herbage availability. J. P. Langlands (*Aust. J. agric. Res.*, 1969, **20** (5), 919–924. 9 ref.).—Flocks of 5 sheep were grazed at 5 stocking rates and received 0, 100, 200, 300 or 400 g of wheat/day. Herbage intake decreased when supplementary feeding or stocking rate increased, and the nutrient intake was increased by an av. of 54% of the nutrient content of the wheat. M. T. Rawnsley.

Utilisation of sulphur and nitrogen by ruminants. R. J. Moir, M. Somers and A. C. Bray (*Sulphur Inst. J.*, 1967–8, **3** (4), 15–18. 31 ref.).—It appears that the N : S ratio in many fodders is > 10 : 1 while the ratio of total N recycled to the rumen to sulphur recycled is 70–80 : 1, thus the animal cannot profit from the N-recycling because of S deficiency. Therefore supplements of non-protein N (urea), are useless unless S is added. C. V.

Comparative feeding value of silages made from wilted and unwilted grass and grass/clover herbage. F. E. Alder, D. St. L. McLeod and B. G. Gibbs (*J. Br. Grassld Soc.*, 1969, **24** (3), 199–206. 11 ref.).—The intake, by cows and steers, of silage made from wilted herbage was higher than that made from unwilted herbage. There were no significant differences in milk yields between cows fed on wilted and unwilted silage only. Live-wt. gains were higher with wilted than with unwilted silage in 2 of 3 yr. A. H. Cornfield.

Nutritive value of soyabean oil meal in comparison with dried skimmed milk. J. M. van Leeuwen, H. J. Weide and C. C. Braas (*Versl. landbouwk. Onderz. Ned.*, 1969, (732), 19 pp. Dut., 28 ref.).—Quality of the (modified) soya protein was compared with protein in dried skimmed milk. The effects of treatments of soyabean oil meal (*SOM*) on its nutritive value were measured in fattening trials with veal calves. Soya protein, isolated from toasted *SOM* followed by a chem. (hydrolysation) or heat (autoclave) treatment, improved slaughter quality. J. C. T. Niewenhuis.

Chemical composition and nutritive value of four grass hays and their component parts. C. R. Krueger, R. I. Hamilton, J. M. Scholl and B. R. Baumgardt (*Agron. J.*, 1969, **61** (5), 659–663. 17 ref.).—Chem. compn. and nutritive value (dairy goats) were studied in orchardgrass, timothy and 2 varieties of smooth bromegrass harvested at 50% inflorescence emergence and artificially dried. Chem. compn. and *in vitro* dry matter digestibility of various plant parts are also presented. A. H. Cornfield.

Assessment of the nutritive value of silage by determination of *in vitro* digestibility of homogenates prepared from fresh undried silage. R. H. Alexander and M. McGowan (*J. Br. Grassld Soc.*, 1969, **24** (3), 195–198. 11 ref.).—A good prediction of the *in vivo* digestibility of 18 silage samples was obtained from data for *in vitro* digestibility of org. matter and % dry matter of the silages. Bivariate regression equations are presented. A. H. Cornfield.

Nutritional problems of livestock production from semi-arid grasslands in the tropics. J. J. Topps (*J. Br. Grassld Soc.*, 1969, **24** (3), 250–257. 29 ref.).—A critical review. A. H. Cornfield.

Nutrition of the milk-fed calf. II. Intake of calves fed on milk containing increasing concentrations of dry matter and supplemented with iron, copper and manganese. R. H. Khouri and F. S. Pickering (*N.Z. Jl agric. Res.*, 1969, **12** (3), 509–518. 24 ref.).—Supplementation of spray-dried whole-milk powder with Fe and Cu at levels of 46·7 and 5·1 mg/kg resp., maintained blood haemoglobin concn. Fortification with 0·25 g of Mg/kg did not prevent, but appeared to delay, the onset of progressive hypomagnesaemia. E. G. Brickell.

Effect of physical and chemical treatment of grains on growth and feed utilisation by the chick. II. Effect of water and acid treatments of grains and grain components on chick growth, nitrogen retention and energy utilisation. O. L. Adams and E. C. Naber (*Poult. Sci.*, 1969, **48** (3), 922–928. 6 ref.).—Treatment of wheat flour, wheat gluten and hard wheat with water or 0·1 N-HCl (20 h soak), before inclusion in diets, improved growth rate of chicks. The treatments did not improve the feeding value of soft wheat or commercial maize and wheat starches. The treatments increased metabolisable energy of the diets, but did not affect N retention. A. H. Cornfield.

Metabolisable energy of rapeseed meal for growing chickens and laying hens. G. N. Lodhi, R. Renner and D. R. Clandinin (*Poult. Sci.*, 1969, **48** (3), 964–970. 20 ref.).—The av. metabolisable energy (*ME*) of samples of prepress-solvent and solvent-processed rapeseed meal increased with age of bird from 1203 kcal/kg for 4-week old chicks to 1782 kcal/kg for hens. *ME* of rapeseed meal for 4-week-old chicks and hens increased when the measurements were made 3–4 weeks after the birds had been receiving the meal in their diets. A. H. Cornfield.

Relative value of rapeseed and soyabean oils in chick starter diets. R. E. Salmon (*Poult. Sci.*, 1969, **48** (3), 1045–1050. 10 ref.).—Replacing all or part of the 10% soyabean oil in the diets by rapeseed oil decreased wt. gains of chickens to 41 days of age, but usually did not affect feed efficiency. There were little changes in fatty acid compn. of depot fat from 6 to 41 days of age. Abdominal depot fat contained relatively more palmitic, palmitoleic, stearic and oleic acids, and less linoleic and linolenic acids than did the dietary fat. A. H. Cornfield.

Effect of ascorbic acid on the carbohydrate metabolism of vitamin A-deficient chicks. M. Perek and J. Kendler (*Poult. Sci.*, 1969, **48** (3), 1101–1104. 16 ref.).—Withholding vitamin A (**I**) from chicks resulted in decreased wt. gains, increased blood glucose levels and decreased liver glycogen levels. Addn. of 25–100 ppm ascorbic acid (**II**) to the **I**-deficient diet prevented these effects to 19 days of age, but was less effective to 33 days of age. 100 ppm **II** in the diet decreased blood glucose and increased liver glycogen levels in chicks fed 700 I.U. **I**, but not in those fed 7000 I.U. **I**. A. H. Cornfield.

Provision of selenium to sheep by means of heavy pellets. R. E. Kuchel and R. A. Buckley (*Aust. J. agric. Res.*, 1969, **20** (6), 1099–1107. 24 ref.).—Pellets contg. Se as Ca selenate, Ba selenate

or elemental Se, were fed to sheep grazing pastures of normal Se content, and enhanced blood and tissue Se levels for periods up to 12 months. Optimum results were obtained using pellets contg. finely divided metallic Fe and elemental Se in ratio 20 : 1.
M. T. Rawnsley.

Requirement for cobalt of sheep grazed on cobalt-deficient pastures. H. J. Lee and H. R. Marston (*Aust. J. agric. Res.*, 1969, **20** (5), 905–918. 28 ref.).—Relatively large amt. of Co every few weeks maintained growth and reproductive capacity, but did not supply total Co requirement; wide variations in response occurred. Weekly supply of 1 mg of Co/day was sufficient in most cases. The Co must be given either orally or by injection in the flank.
M. T. Rawnsley.

Efficacy of cobalt pellets for providing cobalt for penned sheep. D. W. Dewey, H. J. Lee and H. R. Marston (*Aust. J. agric. Res.*, 1969, **20** (6), 1109–1116. 18 ref.).—Expt. over 5 yr show that active Co pellets, either singly, doubly or accompanied by a steel grinder, are an effective and labour-saving method of giving Co at the equiv. of 1 mg/day. The amt. of Co released varies widely. No pellets were rejected, and no surface accumulation of Ca phosphate occurred to restrict release of Co. Amt. of Co in various organs and in association with vitamin B_{12} are given.
M. T. Rawnsley.

Method for sizing forage cell wall particles. L. W. Smith and D. R. Waldo (*J. Dairy Sci.*, 1969, **52** (12), 2051–2053. 7 ref.).—A method is described for separating and describing cell wall particles by size, from ground, chopped (< 5·0 cm), ground and pelleted or wafered forages, gastro-intestinal contents, and faeces. Neutral detergent extraction of sufficient sample to give 3–5 g of dry cell walls is followed by dry sieve analysis. M. O'Leary.

Effects of Diet and Environment on Livestock

Ryegrass varieties in relation to dairy cattle performance. IV. Milk production from cows grazing ryegrass pastures at two stages of growth and receiving two levels of nitrogen fertiliser. V. Influence of starch and peanut [groundnut] oil supplements on the yield and composition of milk produced by cows grazing perennial ryegrass. G. F. Wilson and R. M. Dolby (*N.Z. Jl agric. Res.*, 1969, **12** (3), 489–499. 16 ref.; 500–508. 13 ref.).—IV. Cows grazing short pasture produced significantly more milk than those grazing long pasture. Milk yields were unaffected by N fertiliser levels but the milk contained significantly lower % of solids-not-fat (*SNF*) and protein (*P*).

V. The cows receiving starch (900 g/day) produced milk contg. higher % of *SNF* and *P*. Groundnut oil (440 ml/day) produced milk with a higher % of fat and a lower % of *P*.
E. G. Brickell.

Implantation of intensively-fed beef cattle with hexoestrol. A. MacDearmid and T. R. Preston (*Anim. Prod.*, 1969, **11** (3), 419–422. 6 ref.).—Steers receiving a single implant of 60 mg hexoestrol grew 24% faster than those receiving none and 7% faster than steers receiving 30 mg. Splitting of the implant did not cause significant differences. In another trial, the 60 mg implant caused a 23% growth increase over control and a 13% increase in feed conversion efficiency, bringing about a saving of 131 kg feed per animal. The tenth rib joints of steers given the 60 mg implant contained more crude protein and less fat than did those of control steers. M. Long.

Effect of melengestrol acetate on synchronisation of oestrus, subsequent fertility and milk constituents of lactating dairy cows. J. D. Roussel and J. F. Beatty (*J. Dairy Sci.*, 1969, **52** (12), 2020–2023. 4 ref.).—Results of expt. with 40 lactating dairy cows showed that MGA can be used successfully for oestrus synchronisation without adversely affecting milk yield or milk constituents and with a slight increase in fertility. M. O'Leary.

Energy requirements of weaned lambs. T. J. Forbes and J. J. Robinson (*Anim. Prod.*, 1969, **11** (3), 389–397. 16 ref.).—Two trials were carried out on lambs aged 12 months in one trial and 7 months in the other. In both trials half the lambs were fed 900 g air-dry feed/day and the other half 540 g. The trial with the older lambs lasted 100 days and the other 47 days. Digestibility trials were carried out at each level to obtain estimates of digestible org. matter intake (*DOMI*). The mean *DOMI* for maintenance for a 45-kg lamb was estimated at 400 g or 1500 kcal *ME*/day. This value was unaffected by age. The *DOMI* for production rose from 1·55 kg per live-wt. gain for the younger animal to 1·71 kg for the older. M. Long.

Zinc nutrition in sheep. I. Relation of zinc to growth, testicular development and spermatogenesis in young rams. E. J. Underwood and M. Somers. **II. Influence of zinc deficiency in ram lambs upon digestibility of dry matter and utilisation of nitrogen and sulphur of the diet.** M. Somers and E. J. Underwood (*Aust. J. agric. Res.*, 1969, **20** (5), 889–897. 26 ref.; 899–903. 18 ref.).—I. A diet including 17·4 ppm of Zn was adequate for normal wt. gain etc., but not for testicular development. A diet contg. 2·4 ppm of Zn, fed over 20–24 weeks, caused many deficiency symptoms, including cessation of spermatogenesis, but this could be remedied in 20 weeks by Zn supplement. A level of 32·4 ppm of Zn gave complete development. These expt. were carried out in isolation, and thus conclusions cannot safely be applied to field conditions.

II. Zn deficiency was shown to impair N and S utilisation.
M. T. Rawnsley.

Nutritive value of the diet selected by grazing sheep. IV. Variation in the diet selected by sheep differing in age, breed, sex, strain and previous history. V. Further studies of the relationship between digestibility estimated *in vitro* from oesophageal fistula samples and from faecal and dietary composition. J. P. Langlands (*Anim. Prod.*, 1969, **11** (3), 369–378. 12 ref.; 379–387. 11 ref.).—IV. No difference in the ability to digest a lucerne ration was found between wethers aged 6 and 66 months. Neither strain nor sex had any influence on selection, and no reason could be suggested for the occasional differences found between groups in the N content or digestibility of the diet selected.

V. Digestibility of grazed *Phalaris tuberosa–Trifolium repens* pastures was estimated. Corresponding estimates of the N content of the faeces and of the fistula samples, the live wt. and faecal output of the sheep and of herbage availability were taken. Observations were stratified according to stocking rate, digestibility, month, herbage availability or org. matter intake. Results are discussed and it is concluded that faecal N is not a satisfactory index of digestibility of the diet selected by grazing sheep.
M. Long.

Relative value for wool growth and nitrogen retention of several proteins administered as abomasal supplements to sheep. W. F. Colebrook and P. J. Reis (*Aust. J. biol. Sci.*, 1969, **22** (6), 1507–1516. 27 ref.).—Protein supplements were whole egg protein (*EP*), egg albumen (*EA*), maize gluten (*MG*), casein (*C*) and gelatin (*G*). Wool growth rates increased substantially with *EP*, *EA* and *C*. Supplements of *MG* were only half as effective while *G* supplements had little effect. All supplements except *G* stimulated body wt. gain and enhanced N retention. M. J. Rawnsley.

Intravenous infusion of cysteine and wool growth of Romney sheep. G. M. Dryden, G. A. Wickham and F. Cockrem (*N.Z. Jl agric. Res.*, 1969, **12** (3), 580–587. 11 ref.).—Cysteine infusion counteracted the winter depression in wool growth. In wethers fed at two dietary protein levels, a significant protein level × cystein infusion interaction was observed. The sheep on high protein grew more wool than those on the low protein diet. E. G. Brickell.

Single cereal diets for bacon pigs. I. Effects of diets based on barley, wheat, maize meal, flaked maize or sorghum on performance and carcass characteristics. D. J. A. Cole, E. G. Clent and J. R. Luscombe (*Anim. Prod.*, 1969, **11** (3), 325–335. 30 ref.).—Two trials were carried out. Generally differences in feed utilisation for live-wt. gain were related to differences in the digestible energy content of the cereals used. Flaked maize was consistently inferior, possibly due to its low lysine and trytophane content, and poorer amino acid balance. Differences between treatments for killing-out % and predicted lean % were slight. All diets gave satisfactory growth, performance and carcass quality. M. Long.

Digestibility of hard and soft wheat offals in growing pigs. D. L. Frape, J. Wilkinson and L. G. Chubb (*Anim. Prod.*, 1969, **11** (3), 429–432. 8 ref.).—The apparent digestibility of diets fell with increasing proportions of wheat offal replacing glucose, although the digestibility of the offals did not vary with level of inclusion. Offals from hard and soft wheat did not, in general, differ in digestibility. M. Long.

Protein supplementation of rations based on whey for growing pigs. I. Rate of gain, efficiency of food utilisation, and carcass quality. II. Digestibility and nitrogen retention. J. R. Carr and A. C. Dunkin (*N.Z. Jl agric. Res.*, 1969, **12** (3), 519–532. 27 ref.; 533–542. 20 ref.).—I. On average, gilts grew significantly faster than castrates, utilised the whey consumed more efficiently and produced leaner carcasses. Pigs fed at 2·5 lb daily meal allowance

grew 10% faster than those at 1·5 lb per day but did not differ significantly in carcass characteristics.

II. Pigs fed 2·5 lb meal daily, compared with those receiving 1·5 lb, consumed slightly (but significantly) less apparent digestible N per 5 days and yet retained both a greater wt. and % N per 5 days. The apparent digestibility energy intakes of the two groups were almost identical. E. G. Brickell.

Physiological response of domestic fowl to abrupt changes of ambient air temperature. P. C. Harrison and H. V. Biellier (*Poult. Sci.*, 1969, **48** (3), 1034–1045. 22 ref.).— A. H. Cornfield.

Influence of environmental temperature on plasma proteins of domestic fowl. T. M. Huston and T. Subhas (*Poult. Sci.*, 1969, **48** (3), 997–1000. 6 ref.).— A. H. Cornfield.

Effect of level and source of phosphorus and different calcium levels on productivity and phosphorus utilisation by laying hens. A. J. Salman, M. S. Ali and J. McGinnis (*Poult. Sci.*, 1969, **48** (3), 1004–1009. 20 ref.).—Plant P (wheat mixed feeds) was as available for egg production, egg shell formation, bone ash and body wt. maintenance as was a mixture of plant P and $CaHPO_4$. Performance was no different with 2·75 or 4·5% Ca in the diet. 0·3% plant P was adequate for egg production and overall performance of pullets with both levels of Ca. A. H. Cornfield.

Response of two breeds of chickens to graded levels of dietary phosphorus. E. E. Gardiner (*Poult. Sci.*, 1969, **48** (3), 986–993. 13 ref.).—There were significant differences due to dietary P level, breed (broiler crossbreds and Single Comb White Leghorns) and battery type on body wt., % bone ash, plasma inorg. P, and % livability to 4 weeks of age. A. H. Cornfield.

Whole body counter studies on the absorption of ^{60}Co, ^{59}Fe, ^{54}Mn and ^{65}Zn by chicks as affected by their dietary levels and other supplemental divalent elements. F. A. Suso and H. M. Edwards, jun. (*Poult. Sci.*, 1969, **48** (3), 933–938. 11 ref.).—Feeding high levels of labelled Co^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} generally resulted in lower absorption and reduced biological half-life of the isotopes in chicks. Zn^{2+} absorption was affected by Fe^{2+}, Cd^{2+} and Hg^{2+}, and Fe^{2+} absorption was affected by Zn^{2+}. A. H. Cornfield.

Effect of hake meal in broiler rations. G. H. Arscott and D. L. Crawford (*Poult. Sci.*, 1969, **48** (3), 1123–1125. 3 ref.).—Up to 7·5% hake meal could replace a similar amount of soyabean meal protein without affecting wt. gains of broilers to 8 weeks of age, but causing improved feed efficiency. 5·0–7·5% herring meal improved both wt. gains and feed efficiency. A. H. Cornfield.

Effects of maize oil and lysine on growth, fatty acid composition and palatability of Large Broad White turkeys. C. W. Carlson, E. Guenthner, K. C. Schneider *et al.* (*Poult. Sci.*, 1969, **48** (3), 1027–1033. 15 ref.).—Addn. of 4% maize oil, 0·1% L-lysine, or maize oil + lysine to the diet of turkeys from 12 to 24 weeks of age slightly increased wt. gains in only 1 of 2 expt. The treatments had no marked or consistent effects on flavour, tenderness or juiciness, but the control and lysine-fed turkeys were preferred. The maize oil treatments resulted in increase in linoleic acid of carcass fatty acids, with concomitant decreases in palmitic, palmitoleic and oleic acids. A. H. Cornfield.

Selection of zinc-supplemented diets by turkey poults. P. Vohra and J. R. Heil (*Poult. Sci.*, 1969, **48** (3), 1118–1121. 2 ref.).—When given free choice between Zn-deficient and Zn-supplemented diets from hatch, poults favoured the consumption of Zn-supplemented diets by the time they were about 2 weeks old. A. H. Cornfield.

Performance of adult female Japanese quail on linoleic acid-deficient diets. C. C. Calvert (*Poult. Sci.*, 1969, **48** (3), 975–978. 5 ref.).— A. H. Cornfield.

Analysis and Other Aspects

Evaluation of the Orion cyanide electrode for estimating the cyanide content of forage samples. J. T. Gillingham, M. M. Shirer and N. R. Page (*Agron. J.*, 1969, **61** (5), 717–718. 7 ref.).—Values for CN^- in 270 samples of forage as determined by the electrode were highly correlated with values obtained by the colorimetric alkaline picrate method. The electrode method overestimated CN^- content by ~40%, but a regression equation for correcting this is presented. A. H. Cornfield.

Increases of extractable β-carotene in haylage during *in vitro* digestion. R. Almendinger and F. C. Hinds (*J. Dairy Sci.*, 1969, **52** (12), 2044–2046. 5 ref.).—Release of β-carotene (I) from haylage in an *in vitro* digestion system, corresponding to bovine *in vivo* conditions, was found to be double that obtained with conventional assay methods. Major increase in extraction occurred as a result of rumen and pepsin–HCl incubation and was mainly due to a pH–water effect on the haylage, with a pH 7·0 presoak increasing the rate of acid release of I. Various proteolytic enzymes did not enhance I release, and so protein is not considered to be the primary binder. However, protein integrity or the presence of taurocholic acid was necessary to retard the destruction of pigment during incubation. M. O'Leary.

Effect of ethyl alcohol on the vitamin A status of Holstein heifers. R. W. Miller, R. W. Hemken, D. R. Waldo and L. A. Moore (*J. Dairy Sci.*, 1969, **52** (12), 1998–2000. 13 ref.).—Addn. of 3% EtOH to the ration (lucerne hay and vitamin A-fortified grain) of Holstein heifers, resulted in a 37% increase in blood plasma vitamin A concn. 4 h after feeding, persisting to 12 h. after feeding. 1% EtOH in the feed did not increase plasma vitamin A concn. After a 21-day trial period, final liver vitamin A concn. were higher than the initial ones, with animals receiving 0, 1, or 3% EtOH in the ration. The increase for heifers fed 3% EtOH was 13, for those fed 1% EtOH, 37, and for those fed no EtOH, 50%. M. O'Leary.

Thyroid function as measured by ^{131}iodine release rate, weight and RNA/DNA in growing lambs, and its relation to growth rate. S. A. Draper, N. B. Haynes, I. R. Falconer and G. E. Lamming (*Anim. Prod.*, 1969, **11** (3), 399–407. 33 ref.).—A highly significant relationship was found between the rate constant for ^{131}I release and growth rate. The RNA/DNA ratio and thyroid size were both found to be uncertain as for estimating thyroid activity. This is suggested as a reason for the contradictory results obtained when attempts have been made to alter the thyroid status of animals. M. Long.

Effects of food intake on numbers of salmonellae and *Escherichia coli* in rumen and faeces of sheep. F. H. Grau, L. E. Brownlie and M. G. Smith (*J. appl. Bact.*, 1969, **32** (1), 112–117).— C. V.

Colostral and milk whey proteins in the sow. I. The transition of mammary secretion from colostrum to milk with natural suckling. II. The effect of delayed suckling on colostrum and milk whey proteins. F. J. Bourne (*Anim. Prod.*, 1969, **11** (3), 337–343. 15 ref.; 345–349. 4 ref.).—I. A marked fall in colostral whey proteins was found with Wessex sows in the 24 h after the birth of the first piglet. Colostral whey electrophoretic fractions, especially the γ-globulin fraction, showed a similar fall.

II. When suckling was prevented, colostral whey protein levels did not fall during the first 4 h after parturition. Removal of the piglets from the sow did not affect farrowing. M. Long.

[Differences in] lactate dehydrogenase [values] in the blood plasma of Pietrain and Large White pigs. J. C. M. Hessel-de Heer (*Anim. Prod.*, 1969, **11** (3), 423–427. 7 ref.).— M. Long.

Teaching poultry management principles. I. Determining optimum broiler weights in the growing operation. II. Evaluation of broiler contracts in the growing operation. R. K. Noles (*Poult. Sci.*, 1969, **48** (3), 910–917. 3 ref.; 918–922. 3 ref.).—Some economic aspects of poultry management are presented. A. H. Cornfield.

***In vitro* calcium [^{45}Ca] transport in laying fowl intestine: characterisation of the system and medium composition.** A. Bar and S. Hurwitz (*Poult. Sci.*, 1969, **48** (3), 1105–1113. 22 ref.).— A. H. Cornfield.

Influence of steam pelleting on the utilisation of phosphorus by the laying hen. W. F. Pepper, J. D. Summers, E. T. Moran and H. S. Bayley (*Poult. Sci.*, 1969, **48** (3), 1055–1060. 11 ref.).—Steam pelleting was ineffective in improving the availability of P or org. P sources to the laying hen. High egg production from hens receiving only plant P indicates high availability of this source of P. A dietary level of 0·39% plant-P was sufficient for good production. A. H. Cornfield.

Effect of a limited time feeding system on reproductive performance of heavy breed pullets. G. Schumaier and J. McGinnis (*Poult. Sci.*, 1969, **48** (3), 949–953. 11 ref.).—Limiting the daily feeding time to 1–3 h, beginning at 8 weeks of age, controlled body wt. gain during the growing and laying periods. Egg size was decreased, but egg production, % fertility and hatchability of eggs, chick wt. and growth were unaffected. A. H. Cornfield.

Controlled egg production in poultry. A. H. Sykes (*Outl. Agric.*, 1968, **5** (5), 223–227. 9 ref.).—The need for control of the onset of egglaying in the pullet, and the timing and duration of the first

moult are discussed with respect to economic advantages. Methods of control include: variation of day length; nutrition; specific feed additives, esp. Methallibure which prevents the onset of laying and inhibits established ovulation. W. J. G.

Response of broiler chicks to γ-radiation exposures. Changes in early growth parameters. I. L. BRISBIN JUN. (*Radiat. Res.*, 1969, **39** (1), 36–44. 15 ref.).—The details of the observations carried out on 90 chicks at 2 days old are given; body size was less affected than body wt. C. V.

Dry-ashing method for determination of total arsenic in poultry tissues. J. L. MORRISON and G. M. GEORGE (*J. Ass. off. analyt. Chem.*, 1969, **52** (5), 930–932. 6 ref.).—A shorter modification of Stone's method (*ibid.*, 1967, **50**, 1361–1362) is described; it is sensitive to < 0·1 ppm of As. Tissue samples were dry-ashed in the presence of MgO and $Mg(NO_3)_2$ and the As was detd. colorimetrically with Ag diethyldithiocarbamate. The As was detd. from standard curves prep. by adding 0–2 ppm of As to the tissue. Calculation is corrected for losses during the ashing procedure. D. I. Rees.

[Additive for] animal feed compositions. UGINE KUHLMANN (Inventors: G. PAUL and M. H. LOISEAU) (Br. Pat. 1,174,951, 13.2.67. Fr., 11.2.66).—Addn. of Na-phosphocreatinine.$5H_2O$ to animal feeds accelerates the rate of growth of young livestock, and is non-toxic. S. S. Chissick.

Growth promotion of livestock animals. WILLOWS FRANCIS LTD. and A/S. FERROSAN (Inventors: A. A. MCKENZIE MORRISON and S. A. THIEL) (Br. Pat. 1,174,564, 10.3. and 11.7.66).—Genebilic acid (or salts), when added to animal feeds, accelerates the growth of young animals, including poultry. S. S. Chissick.

2.—FOODS AND CROP CONVERSION

Cereals, Flours, Starches, Baking

Wheat drying at reduced temperatures. M. I. R'YAZANTSEVA, A. G. KIRZHNER, K. P. RAKUTOV *et al.* (*Pishch. Tekhnol.*, 1969, [4(71)], 38. Russ.).— C. V.

Denaturation rate of fresh harvested maize grain proteins. F. D. BRATERSKY (*Pishch. Tekhnol.*, 1969, [4(71)], 11. Russ.).— C. V.

Amino acid composition of the proteins of crumb and crust of wheat bread. G. M. MEL'KINA, V. M. GIL'ZIN and L. Y. AUERMAN (*Pishch. Tekhnol.*, 1969, [4(71)], 23. Russ.).— C. V.

Influence of a gene causing hardness on the milling and baking quality of two wheats. K. J. SYMES (*Aust. J. agric. Res.*, 1969, **20** (6), 971–979. 7 ref.).—Falcon, a hard wheat, and Heron, a soft wheat, differed only in the gene which determines hardness (according to particle size index). Falcon was found to be superior to Heron. Reasons for the influence of one gene are discussed. M. T. Rawnsley.

Photometric method for determining amylase activity. K. K. GORBATOVA, V. A. SOLOMAKHINA and G. N. POGUDINA (*Pishch. Tekhnol.*, 1969, [4(71)], 169. Russ.).— C. V.

Baked goods production processes. M. GUTTERSON (*Fd Process. Rev.*, 1969, (9), 353 pp.).—The review is based on a survey of over 190 U.S. patents from 1960 onwards. It is divided into sections: (1) **Bread.** This covers continuous and fermentation processes, enhancement of shelf life, modified breads, rope and mould inhibiting agents and dusting compn. (2) **Yeast leavened products.** E.g., crackers, sweet goods and yeast raised processes. (3) **Chemically leavened products.** E.g., cakes, shortening and non-shortening compn., biscuits, baking acids etc. (4) **Air leavened products.** (5) **Non-leavened products.** (6) **Refrigerated doughs.** Includes biscuits and cake batters. (7) **Emulsifiers and dough improvers.** This includes monoglycerides, emulsifier processes etc. W. J. G.

Powder for use as starting material for pastry dough. K. KAUTZ (Br. Pat. 1,175,595, 15.2.67).—Prepn. is by mixing melted edible fat, flour and a lipoid (e.g., lecithin), emulsifying the mixture in water, opt. in presence of an emulsifier (glyceride ester), and then heating to between 65° and the b.p. prior to (spray) drying. The total fat content should be 0·1–60% of the total wt. S. S. Chissick.

Sugars, Syrups, Confectionery

Diffusion of beet and cane. H. BRÜNICH-OLSEN (*Sug. Technol. Rev.*, 1969, **1** (1), 3–42. 9 ref.).—Aspects reviewed are, (*i*) diffusion through beet or cane tissue, including effect of cell walls, speed of diffusion, detn. of extraction coeff. and effect of varying extract concn., (*ii*) technical extraction of beet and cane, including modes of extraction, evaluation of losses, effects of mixing and juice transfer, milling-diffusion and effects of cane fibre-content, (*iii*) chem. and microbiol. aspects, including quality of extract, overall recovery etc. W. J. G.

Aspects of flotation clarification of mixed juice. M. MATIC (*Proc. S. Afr. Sug. Technol. Ass., 43rd Ann. Congr.*, 1969, 199–205. 16 ref.).—Removal of impurities by vac. flotation improved as pH increased from 7 to 11; pH 9 was optimum for starch, phosphate, SiO_2 and protein. However, a much more voluminous ppt. was formed. The suggestion that evaporator scaling and ash content could be reduced by flotation at high pH followed by carbonatation was shown to have no concrete advantages. Bubble size and type of floc are important in dispersed air flotation. M. T. Rawnsley.

Raw sugar clarification. J. G. DAVIES (*Sug. Technol. Rev.*, 1969, **1** (1), 77–83. 30 ref.).—The problems of adding milk of lime and heating cane juice, are discussed together with the possible use of other chemicals, e.g., MgO. Separation of the ppt., from clarified juice, is also considered, including subsidation, flotation and centrifugation techniques. W. J. G.

Potentiometric determination of chlorides in molasses. G. W. COMRIE (*Proc. S. Afr. Sug. Technol. Ass., 43rd Ann. Congr.*, 1969, 151–155).—The automatic apparatus consisted of a Metrohm Potentiograph, and a Multi-Titration set which comprised: a Ag rod indicator, kept free of grease and AgCl; a general all purpose calomel electrode as reference, with a 1 N-KNO_3 electrolyte; and a U-tube filled with KNO_3—agar gel as salt bridge. The sample was 40 g molasses in 1000 ml water. Good results were obtained, but always lower than from volumetric methods, probably because of the amt. needed to complete the colour change. CO_3^{2-}, PO_4^{3-}, SO_3^{2-}, SO_4^{2-} do not interfere, but Br does. M. T. Rawnsley.

Microelements in granulated and refined sugar. A. A. GERASIMENKO, E. A. GRIVTSEVA and L. A. ORLOVA (*Pishch. Tekhnol.*, 1969, [5(72)], 39. Russ.).— C. V.

Determination of water in raw sugar using the Karl Fischer method. D. M. OOSTHUIZEN (*Proc. S. Afr. Sug. Technol. Ass., 43rd Ann. Congr.*, 1969, 146–150. Engl., 15 ref.).—McComb's method (*Analyt. Chem.*, 1957, **29**, 1375) involving dissolving sugar in formamide, was used and the moisture in the resulting soln. was titrated directly with Karl Fischer reagent. Results for the Karl Fischer method were higher than those for standard drying at 105°. Reasons for this are discussed, and include the possibility that moisture within the sugar crystal is not evaporated during drying. M. T. Rawnsley.

Determination of the thermal effects in the crystallisation of sugar. A. V. SUBCHENKO, S. E. KHARIN and S. Z. IVANOV (*Pishch. Tekhnol.*, 1969, [4(71)], 161. Russ.).— C. V.

Saccharifying starch into hydrolysate. NIPPON SHIRYO KOGYO CO. LTD. (Br. Pat. 1,175,900, 25.5.67. Jap., 7.10.66 and 21.1.67. Addn. to Br. Pat. 1,121,100).—A starch paste, obtained by heating a starch slurry of > 20% concn. at 120–160° so as to evaporate off part of the water, is cooled and treated with a source of β-amylase and partially hydrolysed recycled starch liquor. High yields of maltose are obtained. S. S. Chissick.

Malting, Brewing and Alcoholic Beverages

Determination of carbon dioxide in still and sparkling wines. E. CAPT, J.-F. SCHOPFER and A. DUFOUR (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1969, **60** (2), 114–120. Fr., 6 ref.).—A closed-circuit air entrainment apparatus is described together with detailed procedures for its use. After oxidn. of SO_2 with H_2O_2 ($CuSO_4$ catalyst) and acidification of the wine with acid phosphate buffer, CO_2 is removed by forced circulation of air, absorbed in standard baryta and detd. by back-titration. For sparkling wines, CO_2 is initially fixed by addn. of 50%-NaOH soln. to the refrigerated wine before sampling. The method is simple, reproducible to

within 0·1 g/l and can be carried out at cellar temp. thus avoiding release of combined CO_2 or interference by volatile acids. E. C. Apling.

Alteration in the content of colouring and tannin (?) substances in grapes and wine. G. G. VALUYKO and L. M. GERMANOVA (*Pishch. Tekhnol.*, 1969, [5(72)], 111. Russ.).— C. V.

Determination of nitrogen of melanoidins in wine. G. G. AGABAL'YANTS, S. P. AVAK'YANTS and V. S. GUL'YAYEVA (*Pishch. Tekhnol.*, 1969, [4(71)], 164.) Russ.).— C. V.

Gas chromatographic determination of methanol in wines, cognac alcohols and cognacs. B. V. LIPIS, Z. A. MAMAKOVA and V. Y. YAZLOVETSKAYA (*Pishch. Tekhnol.*, 1969, [5(72)], 168. Russ.).— C. V.

Accelerated determination of copper and iron in cognacs and wines. Y. E. FALKOVICH, I. A. SIDORENKO and V. A. SAFONOV (*Pishch. Tekhnol.*, 1969, [5(72)], 171. Russ.).— C. V.

Compounds identified in whisky, wine and beer: a tabulation. J. H. KAHN (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1166–1178. 54 ref.).—A list of components identified in the beverages is presented. D. I. Rees.

[A] **Producing beers of low carbohydrate content,** [B] **Sweetening of beers.** A.B.M. INDUSTRIAL PRODUCTS LTD. (Inventor: J. W. GREEN) (Br. Pat. 1,174,618–619, 13.12.67).—[A] The beers, especially suitable for, e.g., diabetics, are prep. by adding to the yeast-fermenting beer wort a quantity of amyloglucosidase and allowing the fermentation to continue to a predetermined carbohydrate level, at which stage the enzyme is deactivated by heat (180°F, 15 sec). [B] The process of [A] produces sufficient glucose to make it possible to avoid the use of priming sugar. S. S. Chissick.

Removal of diacetyl from beer. FORSCHUNGSINSTITUT FÜR DIE GÄRUNGSINDUSTRIE, ENZYMOLOGIE UND TECHNISCHE MIKROBIOLOGIE (Inventors: P. LIEBS, H.-C. WOLTER and M. KRÜGER) (Br. Pat. 1,177,310, 9.10.68).—The beer is treated with yeast (*Saccharomyces carlsbergensis* or *S. cerevisiae*), which is subsequently removed when the diacetyl (**I**) content is at its min. possible value. E.g., the **I** content of beer is reduced from 1200 to 0·134 mg/l by treatment with beer yeast for 1 h. S. S. Chissick.

[A] **Treatment of alcoholic beverages.** [B] **Stabilisation of fermented beverages.** ARTHUR GUINNESS & CO. (DUBLIN) LTD. (Inventors: R. J. A. POLLOCK and [A] M. J. WEIR (Br. Pat. 1,177,126–7, 10.7.67 and [B] 10.6.68).—[A]. The beverage is separated, by passing it through a semipermeable membrane under pressure, into a low EtOH–high solids fraction (**I**) and a high EtOH–low solids fraction (**II**). The **I** and **II** from different sources (e.g., wine and beer) can be mixed to give beverages of unusual flavour, or **I** can be shipped as a concentrate. [B]. Formation of haze during storage is inhibited by, e.g., chilling the **I** to ppt. haze-forming compd., clarifying the concentrate and combining it with **II**, with aq. EtOH or with water. S. S. Chissick.

Fruits, Vegetables and Their Products

The effect of *N*-dimethylaminosuccinamic acid on the ripening of apple fruits. M. J. C. RHODES, P. J. HARKETT, L. S. C. WOOLTORTON and A. C. HULME (*J. Fd Technol.*, 1969, **4** (4), 377–387. 30 ref.).—Six trees were given two applications of *N*-dimethylaminosuccinic acid (**I**). Fruit from these trees was stored at 12° in desiccators through which CO_2-free air passed. CO_2 from the apples was measured and samples of the air stream were analysed for C_2H_4 by gas chromatog. The amt. of C_2H_4 produced were still sufficient to initiate enzyme processes of ripening. **I** probably modifies the balance of the various hormones. M. J. Rawlins.

Acoustic vibration for detecting quality of apples. J. A. ABBOTT, N. F. CHILDERS, G. S. BACHMAN *et al.* (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 725–737. 13 ref.).—Acoustic 'spectra' of apples were obtained by suspending and mechanically vibrating individual fruit at frequencies ranging from 20 to 4000 Hz. Resonance frequency, 'stiffness' coeff. (derived from fruit mass and frequency) and other physical measurements were sometimes correlated with harvest dates, firmness and sol. solids. The method also identified apples which had been bruised. Acoustic measurements should be suitable for determining textural suitability of whole apples for harvest, storage and consumption. A. H. Cornfield.

Adhesion of dried apple slices. E. EPSTEIN, A. I. NELSON, M. P. STEINBERG and L. S. WEI (*Fd Technol., Champaign*, 1969, **23** (12), 1593–1596. 11 ref.).—Ripening did not appreciably affect level of adhesion. None of the different drying conditions of temp. and humidity was effective in reducing adhesion to an acceptable level. Leached apple pulp caused adhesion but apple juice did not. A process developed for producing a dehydrated product with little or no adhesion involved pre-drying slices in a pilot rotary drier for 30 min, dusting the slices with hydrophobic or regular corn starch and then completing the drying. I. Dickinson.

Improved dried pears. D. MCG. MCBEAN (*Fd Preserv., Q., C.S.I.R.O. Aust.*, 1969, **29** (3), 49–52).—Pears were washed, halved and cored, exposed to S fumes and placed in direct sunlight. Treatment with citric acid, ginger, passion fruit juice and cloves, prior to sulphuring, imparted insufficient improvement to warrant adoption. The dried pears had a more pronounced flavour than those canned from the same fresh fruit. This method could prove an outlet for surplus pears, and those unsuitable for canning. M. J. Rawlins.

Anthocyanin pigments in Trousseau grapes. R. CARRENO-DIAZ and B. S. LUH (*J. Fd Sci.*, 1969, **34** (5), 415–419. 26 ref.).—Isolation, purification and identification of anthocyanins as monoglucosides in Trousseau grape, support its classification as a cultivar of *Vitis vinifera*. These pigments were extracted with 0·1%-HCl in MeOH, and purified with cation exchange resin and by paper chromatog. Identification was based on R_F values in various solvents. M. J. Rawlins.

Measurement of strawberry texture with an Instron machine. D. K. OURECKY and M. C. BOURNE (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 317–325. 14 ref.).—The use of the machine for measuring skin toughness and flesh firmness in strawberry fruit is described. Firmness and toughness were affected by stage of maturity, size of fruit, and temp. of the fruit at time of measurement, and varied considerably among 64 strawberry cultivars. A. H. Cornfield.

Serendipidy berries—source of a new intense sweetener. G. E. INGLETT and J. F. MAY (*J. Fd Sci.*, 1969, **34** (5), 408–411. 6 ref.).—Water extracts of the berries were separated by chromatog. using G50 and G200 Sephadex. The sweetener was found to be bound to fruit protein. Degradation of the protein fraction, using a proteolytic enzyme bromelain, gave a low mol. wt. material with intense sweetness of excellent quality. M. J. Rawlins.

Physical, chemical, and organoleptic attributes of 'Charleston Gray' watermelons at different stages of maturity. W. K. NIP, E. E. BURNS and D. R. PATERSON (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 547–551. 14 ref.).—Surface colour, texture, sugar content, % sol. solids, and sugar: acid ratio of the flesh were good indices of the eating quality of the flesh of watermelons. The colour of the ground spot of the rind, measured by the ratio of redness to yellowness, determined by reflectance, was significantly correlated with the colour and sweetness of the fruit. A. H. Cornfield.

Effect of maleic hydrazide and 2,4,5-trichlorophenoxy propionic acid on ripening and quality of mango fruit. S. KRISHNAMURTHY and H. SUBRAMANYAM (*Pestic. Sci.*, 1970, **1** (2), 63–65. 20 ref.).—Dipping in hot water accelerated the ripening process and reduced fungal spoilage in *Mangifera indica* cv. Pairi mangoes. Accelerated ripening was counteracted by incorporation of maleic hydrazide (MH) in the dip water, thereby extending the storage life with minimum fungal spoilage. 2,4,5-Trichlorophenoxy propionic acid (2,4,5-TP) also delayed the ripening process, but did not improve skin colour. Hot water treatment, with or without MH and 2,4,5-TP, increased the carotene content of the flesh. These compd. did not significantly alter the chem. compn. or quality of the fruit. W. J. G.

Vapour analysis of fermented Spanish-type green olives by gas chromatography. H. P. FLEMING, J. L. ETCHELLS and T. A. BELL (*J. Fd Sci.*, 1969, **34** (5), 419–422. 14 ref.).—Chromatog. of head-space vapour (*HSV*) of pure culture fermented olives, separated 5 major components. Three were identified as CH_3CHO, EtOH and Me_2S, and were verified by syringe reaction and vac. distillation methods. The same compd. (in addn. to others) were present in the *HSV* of unfermented olives. *HSV* profiles may be a rapid method for detecting volatile end products, resulting from metabolism of various micro-organisms. M. J. Rawlins.

Chemical and microbiological changes in stored uncured peanuts. W. Y. COBB, S. E. GILLILAND and E. B. WILLIAMS (*Fd Technol., Champaign*, 1969, **23** (12), 1586–1589. 19 ref.).—During storage of uncured groundnuts at 2°–50% r.h. and 2°–70% r.h., decreases were noted in moisture content, peroxide value, free fatty acid content and free monocarbonyls. Bacterial and mould counts also exhibited downward trends. Samples were organoleptically

acceptable after 60 days; 2°–70% r.h. samples were slightly preferable. Loss of typical raw groundnut flavour during storage may be associated with monocarbonyl decrease. Samples stored at ambient external temp., developed pod surface mould, but flavour was acceptable after 60 days. Bacterial and mould counts in these externally-stored nuts showed a rapid initial increase followed by a gradual loss as moisture values fell. Aflatoxin levels did not exceed 12 ppb in any sample tested. I. Dickinson.

Determining the content of 'oxymethylfurfural' in canned apple juice. A. F. Fan-Yung and M. E. Soloid (*Pishch. Tekhnol.*, 1969, [4(71)], 174. Russ.).— C. V.

Gas chromatographic determination of phenolic amines. D. E. Coffin (*J. Ass. off. analyt. Chem.*, 1969, **52** (5), 1044–1047. 12 ref.).—Phenolic amines tyramine (**I**), octopamine (**II**), synephrine (**III**), β-methoxyoctopamine (**IV**) and β-methoxysynephrine (**V**) were isolated on a cation exchange resin and quant. detd. by g.l.c. of their trifluoroacetyl deriv. Recoveries of 80–85 for **I** and 90–100% for **II**, **III**, **IV** and **V** were obtained from standards and from standards added to apple and orange juices. All commercial orange juice samples contained **III** at levels of 12·5–23·4 mg/l, and also contained another component, identified as a phenolic amine. Phenolic amines were not detected in commercial apple juice. D. I. Rees.

Proanthocyanidins as related to apple juice processing and storage. G. Johnson, B. J. Donnelley and D. K. Johnson (*Fd Technol., Champaign*, 1969, **23** (10), 1312–1316. 7 ref.).—Enzymic oxidn. during milling and pressing can result in complete disappearance of lower mol. wt. proanthocyanidins. Oxidised products remaining after the clarification process, contribute to juice colour and can further polymerise to produce increased colour and sediment formation. Prevention of oxidn. during milling gives a lighter coloured juice of higher astringency and flavour, but lower stability than regular processed juice. Decrease in proanthocyanidins, as affected by higher storage temp., generally parallels increase in colour. Fortification of apple juice with ascorbic acid has a tendency to reduce sediment formation but results in nonenzymic browning at higher storage temp. I. Dickinson.

Chemical composition of Florida orange juice. K. M. Floyd, G. R. Rogers, J. E. Harrell and P. S. Wilkes (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1150–1152. 14 ref.).—A statistical treatment is presented of the analysis of 55 samples of juice for sol. solids, acidity, polyphenolics (by u.v. spectrophotometry), *l*-malic acid (polarimetrically), amino acids (by formol titration) and betaine (by ion exchange and pptn. of the reineckate salt). D. I. Rees.

Presence and origin of 2-pyrrolidone-5-carboxylic acid in processed Concord grape juice and its concentrate. P. Markakis and A. Amon (*Fd Technol., Champaign*, 1969, **23** (11), 1463–1465. 8 ref.).—Presence of the acid was demonstrated by ion exchange column chromatog. and paper chromatog. Using radioactivity tests, the origin was traced chiefly to the glutamine of the juice and to a small extent glutamic acid. I. Dickinson.

Copper–flavonoid complexes in acidic solutions. K. A. Harper (*J. Fd Technol.*, 1969, **4** (4), 405–407. 14 ref.).—A study of the interaction between Cu^{2+} and several pure flavonoids under acidic conditions, such as exist in natural blackcurrant juice, was made. Amperometric titration was used. M. J. Rawlins.

Microelements in canned berry juices. V. D. Malina and Y. A. Kl'yachko (*Pishch. Tekhnol.*, 1969, [4(71)], 32. Russ.).— C. V.

Impedance method for the evaluation of quality in vegetable products. N. A. Golovkin and A. I. Tsvetkov (*Pishch. Tekhnol.*, 1969, [3(70)], 165. Russ.).— C. V.

Changes in moisture content of some vegetables when cooked by infra-red rays. A. N. Mal'sky and N. Y. Savina (*Pishch. Tekhnol.*, 1969, [4(71)], 79. Russ.).— C. V

Sulphur dioxide effect on lipid content of potatoes. N. I. Mondy, E. Pettersen Sonoff and L. R. Mattick (*Fd Technol., Champaign*, 1969, **23** (12), 1597–1599. 13 ref.).—Total crude lipid decreased significantly in both cortex and pith sections of Ontario potatoes which had keen exposed for 1 h to an atm. of SO_2 and stored at 40°F for 4 weeks following treatment. The phospholipid fraction followed the same trend as crude lipid. Free fatty acid content of pith and cortex increased after treatment. The neutral fat fraction decreased immediately after treatment and then increased after four weeks of storage. pH of both sections decreased following treatment, but the most rapid decrease occurred in the cortex section. I. Dickinson.

Sapid components in carrot. Hitoshi Otsuka and Tsuneko Take (*J. Fd Sci.*, 1969, **34** (5), 392–394. 9 ref.).—The relationship between substances present in carrot to its taste was studied. From hot water extracts, large amt. of amino acids and carbohydrates were detected, and very small amt. of nucleic acid deriv. and org. acids. The taste of carrot was a result of the presence of glutamic acid and the buffer action of some amino acids. M. J. Rawlins.

Morphological changes produced in cauliflower skins during pickling, and their relationship to texture parameters. C. A. Saxton and G. G. Jewell (*J. Fd Technol.*, 1969, **4** (4), 363–375. 21 ref.).—Florets of cauliflowers were packed into 1-gal jars of brine (13·25% w/w NaCl); the brine was replaced with fresh brine after 24 h and left for 7 weeks, agitating daily. The freshened cauliflowers were then studied by electron microscopy for texture and electron histochemistry. Evidence showed changes in hardness, elasticity and cohesion, during the initial 24 h in brine, correlated with plasmolysis and re-organisation of cell wall materials. M. J. Rawlins.

Antimicrobial and antithyroid compounds in some edible vegetables. A. I. Virtanen (*Qualitas Pl. Mater. veg.*, **18** (1–3), 8–28. Ger., 53 ref.).—The secondary compd. which are formed by rapid enzymic reactions of some org. compd., found in some edible vegetables, often have specific physiologic, antibiotic effects etc. Org. S compd. in *Allium* and *Brassica* species and in cresses were examined. (From Engl. summ.) W. J. G.

Sulphur components of *Allium* species as flavouring matter. R. A. Bernhard (*Qualitas Pl. Mater. veg.*, 1969, **18** (1–3), 72–84. Engl., 14 ref.).—Aliphatic disulphides, responsible for aroma and flavour in *Allium* (onions) species were identified by g.c. as Pr_2, Pr allyl, Me Pr, Me allyl, Me_2 and diallyl sulphides. Procedure for their qual. and quant. detn. is described. Disulphide compn. of various species and of fresh and dehydrated onion samples are compared. W. J. G.

Relationships between flavour of canned onions and their chemical components as separated by gas chromatography. G. K. Chua, L. J. LaCroix, R. Levy and A. M. Unrau (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 817–822. 8 ref.).—12 components were detected in light petroleum extracts of canned onions (18 varieties); 3 components were important in determining flavour as shown by multiple regression analysis of organoleptic rating vs. peak height. Only one of 6 components detected in headspace samples was of significance in relation to organoleptic rating. A. H. Cornfield.

Non-alcoholic Beverages

Volatile substances in a black tea infusion. L. G. Kharebava and V. P. Starodubtseva (*Pishch. Tekhnol.*, 1969, [3(70)], 38. Russ.).— C. V.

Spectrophotometric determination of caffeine in leaf tea. J. M. Newton (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1133–1134. 9 ref.).—The u.v. spectrophotometric method used previously for instant tea (*ibid.*, 1969, **52**, 653–656) was applied, with slight modification in the extraction procedure, to leaf tea. Standard deviation, found for caffeine content of 2·14–3·95%, ranged from 0·08 to 0·20%. D. I. Rees.

[A] **Preparing a particulate coffee product,** [B] **Making a dehydrated tea product, and** [C] **Preparing a coffee product.** Struthers Scientific & International Corp. (Br. Pat. 1,178,772, 773 and 775, 19.1.67. U.S., 28.1.66. B and C div. out of A).—An aq. tea/coffee extract is partially frozen, the ice removed and the resulting soln. freeze dried. The ice is washed and the washings are, e.g., added to fresh extract. S. S. Chissick.

Cocoa compositions. American Cyanamid Co. (Inventor: K. Whelan) (Br. Pat. 1,177,860, 4.8.67. U.S., 31.8.66).—Dietetic, reconstitutable chocolate drink compn. are prep. by adding ≤ 0·025% by wt. of dioctyl Na sulphosuccinate, as a dispersing agent, to cocoa. S. S. Chissick.

Milk, Butter, Other Dairy Products, Eggs

Electrochemical method for detecting hydrogen peroxide–catalase-treated milk. E. J. Siegenthaler and F. V. Kosikowski (*J. Dairy Sci.*, 1969, **52** (12), 1922–1927. 13 ref.).—Electrochem. detn. of O_2 in milk was shown to be an effective and rapid means of detecting hydrogen peroxide–catalase treated milk. It is suggested that a fresh raw milk contg. > 15 ppm of O_2 should be viewed with suspicion. M. O'Leary.

Method for recovery of progesterone added to milk. R. S. THOMPSON, J. F. DICKEY and D. M. HENRICKS (*J. Dairy Sci.*, 1969, **52** (12), 2048–2050. 12 ref.).—A modification of the method of Short (*J. Endocrinol.*, 1958, **16**, 415) is described for extracting progesterone from milk. The milk sample was subjected to total lipid extraction followed by two solvent partitioning procedures. The final residue was redissolved in 95%-EtOH. Scintillation counting of the final extract indicated 72·9 (± 3·8)% recovery of the added hormone. M. O'Leary.

Dry-ash spectrophotometric method for determination of phosphorus in milk. R. V. BEDESSEM, P. ALIOTO, and R. J. MOUBRY (*J. Ass. off. analyt. Chem.*, 1969, **52** (5), 917–920. 11 ref.).—A modification of the method in the 'Lab. Manual of Milk Industry Foundation' (1959, 457–461) is described. The milk, after addn. of $Mg(NO_3)_2$, was dry-ashed and, after reaction with bisulphite–hydroquinone and NH_4 molybdate, P was detd. Recoveries were 95–105% of P. D. I. Rees.

Polarographic determination of riboflavin in milk. A. L. MARKMAN and S. I. VIT'YAYEVA (*Pishch. Tekhnol.*, 1969, [2(69)], 153. Russ.).— C. V.

Rheological and electrical properties of cream. U. P. ANDRIANOV and G. V. TVERDOKHLEB (*Pishch. Tekhnol.*, 1969, [2(69)], 53. Russ., 14 ref.).— C. V.

Physico-chemical properties of milk fat and the thermal stability and consistency of butter. S. S. GUL'YAYEV-ZAITSEV and R. P. KOLESNIKOVA (*Pishch. Tekhnol.*, 1969, [4(71)], 61. Russ., 11 ref.).— C. V.

Contribution of milk fat to the flavour of milk. A. TAMSMA, F. E. KURTZ, R. S. BRIGHT and M. J. PALLANSCH (*J. Dairy Sci.*, 1969, **52** (12), 1910–1913. 3 ref.).—A series of beverages, contg. 1–3% fat, were prep. using whole milk concentrate (**I**), milk fat (**II**) and vegetable fats (**III**) as the source of fat. **II** and **III** were deodorised under two sets of conditions, one more effective than the other in removing volatile compd. Flavour evaluation tests indicate that the desirable flavour characteristics of **II** are attributable partly to non-volatile compd. and also to volatile compd. unique to **II**. M. O'Leary.

Concentrated dairy cultures. V. W. CHRISTENSEN (*Fd Engng*, 1969, **41** (5), 104–106).—These cultures, frozen in liquid N_2 (− 320°F), can eliminate starter failures in cheese processing. They retain original activity during shipping and prolonged storage and, when thawed, they will set bulk starter media directly. Other applications are discussed. W. J. G.

Characteristics and biological properties of the milk coagulating enzyme obtained from the mould *Byssochlamys fulva*. A. REPS, S. POZNAŃSKI and W. KOWALSKA (*Bull. Acad. pol. Sci. Sér. Sci. biol.*, 1969, **17** (9), 535–541. 11 ref.).—Optimum temp. for milk coagulation of a milk coagulating protease, produced by *Byssochlamys fulva*, was found to be 64–66°. The enzyme was less sensitive than rennet to changes in mild acidity within the range pH 6·65–5·25. The kinetics of an increased acidity in milk coagulated both by protease and by rennet was similar. The proteolytic effect of the enzyme on milk was similar to that of rennet. M. O'Leary.

Cheesemaking from concentrated milk. E. J. MANN (*Dairy Inds*, 1969, **34** (4), 223–224. 12 ref.).—The review briefly considers: effects on cheese yield; fat retention; cold renneting; white pickled cheese manufacture; Svecia cheese manufacture. W. J. G.

Processed cheese. E. J. MANN (*Dairy Inds*, 1969, **34** (2), 87–88. 19 ref.).—A brief review covers: continuous processes; emulsifying agents; fat cryst; physico-chem. aspects; processed cheese standard; microbiol. aspects. W. J. G.

An aureomycin—Rose Bengal agar for enumeration of yeast and mould in cottage cheese. W. W. OVERCAST and D. J. WEAKLEY (*J. Milk Fd Technol.*, 1969, **32** (11), 442–447. 13 ref.).—A peptone dextrose agar (**I**) contg. 20 ppm of aureomycin and 20 ppm of Rose Bengal was compared with an acidified potato dextrose agar (**II**) for the enumeration of yeast (*Y*) and mould (*M*). Isolated *M* and *Y*, as well as *M* and *Y* from cottage cheese, were compared on the two agars. Results showed no significant difference between mean counts on the agars; bacterial growth of 5 species was negative. Advantages of **I** are outlined. W. J. G.

Influence of crystalline carotene on the quality and stability of butter. E. R. STAVROVA, L. S. KREMNEVA and A. G. VEINER (*Pishch. Tekhnol.*, 1969, [5(72)], 68. Russ.).— C. V.

Amino acid composition of hen eggs under different conditions of storage. G. B. CHIZHOV and R. A. DIDENKO (*Pishch. Tekhnol.*, 1969, [5(72)], 77. Russ.).— C. V.

Effect of dry heat on the internal quality of eggs. D. V. VADEHRA, R. C. BAKER and H. B. NAYLOR (*Poult. Sci.*, 1969, **48** (3), 1051–1054. 6 ref.).—Heating eggs in a forced-air incubator at 50–100° before storage at room temp. for 12 days resulted in better internal quality (Haugh units) than in controls. The most effective treatment, heating at 60° for 4 h, greatly decreased bacterial spoilage during storage. Heating decreased the vol. of foam produced and increased the whipping time. A. H. Cornfield.

Salted egg yolks. I. Viscosity and performance of pasteurised and frozen samples. II. Viscosity and performance of acidified pasteurised and frozen samples. H. H. PALMER, K. IJICHI, S. L. CIMINO and H. ROFF (*Fd Technol., Champaign*, 1969, **23** (11), 1480–1485. 14 ref.; 1486–1488. 6 ref.).—Pasteurisation over temp. 144–148°F had little, if any, effect on either η or performance and did not modify the effects caused by frozen storage. Specific effects of freezing temp. and time of frozen storage are discussed. Homogenisation or milling after pasteurisation had only minor effects on η of thawed salted yolks and did not affect their performance in mayonnaise or cream puffs.

II. Yolks were subjected to various freezing and storage conditions ranging from −20–35°F and were tested for η and for emulsifying performance in mayonnaise and cream puffs. Acidification of the salted yolks from pH 6·2 to 5·0 and 4·6 increased η and damaged the performance of salted yolks; damage was accentuated by pasteurisation, freezing and storage. I. Dickinson.

Sugared egg yolks: Effects of pasteurisation and freezing on performance and viscosity. H. H. PALMER, K. IJICHI, H. ROFF and S. REDFERN (*Fd Technol., Champaign*, 1969, **23** (12), 1581–1585. 5 ref.).—Pasteurisation at temp. 140–148°F had no important effect on performance or η of commercial sugared yolks. Performance was detd. in lab. and commercial scale prepn. of unleavened sponge cakes and in commercial leavened sponge cakes, orange chiffon cakes and yellow cakes. Freezing and storage conditions had a marked effect on η and some effect on performance. I. Dickinson.

Rheological characteristics of processed whole egg. S. J. CORNFORD, T. L. PARKINSON and J. ROBB (*J. Fd Technol.*, 1969, **4** (4), 353–361. 9 ref.).—The relationship between egg viscosity and its bakery performance was studied. Flow curves, detd. with a rotational viscometer, indicated that unfrozen raw and pasteurised liquid whole egg are Newtonian fluids and thawed frozen whole egg is pseudo-plastic. M. J. Rawlins.

Cause of discoloration of hard cooked egg rolls. P. G. SCHNELL, D. V. VADEHRA and R. C. BAKER (*J. Fd Sci.*, 1969, **34** (5), 423–426. 6 ref.).—Storage under u.v. light caused discoloration. Peroxides and/or hydroperoxides, produced by water, caused hydrolysis of the peptide chain, resulting in an increase of non-protein N and aromatic amino acids, and caused oxidn. of tryptophan. Discoloration could be prevented by addn. of reducing agents or using a coloured packing film. M. J. Rawlins.

Process for enriching milk with proteins. PHARMACIA FINE CHEMICALS AB, (Br. Pat. 1,175,914, 21.6.67. Swe. 22.6.66).—Skimmed milk (**I**) is divided by gel filtration into a first fraction containing the proteins (**II**) and 10–90% by wt. of the low mol. wt. substances (**III**), and a second **II**-free fraction containing the balance of **III**. The first fraction is mixed with whole milk and/or **I** and evaporated. (3 fig.). S. S. Chissick.

Stabilisation of evaporated milk. ALLIED CHEMICAL CORP. (Br. Pat. 1,177,427, 11.9.67. U.S., 14.9.66).—0·02–0·1% of sterile Na, K or NH_4 polyphosphate is added to the cold milk which has been previously sterilised by heating to a high temp. for a short time. S. S. Chissick.

Cheese production. UNILEVER LTD. (Inventor: H. P. TOOLENS) (Br. Pat. 1,175,472, 1.3.68. Lux., 1.3.67).—Colourless proteolytic micro-organisms, especially mutant strains of *Brevibacterium linens* and *Arthrobacter sp.*, useful in making cheese of good colour and flavour, are obtained by exposing the culture to u.v.- or γ-radiation of wavelength < 320 nm for 2–6 min. S. S. Chissick.

Edible Oils and Fats

Lipid research. B. J. F. HUDSON (*Chemy Ind.*, 1970, (8), 252–259).—The review covers recent trends in supplies and uses of oils

and fats, methodology (chromatog.; glyceride compn.; d.t.a.; n.m.r.), flavour and autoxidation, and induced changes in compn. of raw materials (e.g., synthetic fatty acids and crop selection for compn. or nutrition), The possibility of future research in the interaction of lipids with other biol. materials and the formation of lipoprotein mol. complexes, is discussed. W. J. Baker.

Fats, emulsifiers and emulsions. Practical fat aspects for cereal chemists. K. J. KEARNEY (*Fd Technol. Aust.*, 1969, **21** (11), 585–588, 593).— I. Dickinson.

Liquid extraction of acetone solutions of vegetable oils. E. F. BUKHTAR'YOVA and V. V. BELOBORODOV (*Pishch. Tekhnol.*, 1969, [2(69)], 117. Russ.).— C. V.

Influence of soyabean oil bleaching on its oxidative stability and tocopherol change. R. KH. KHAFIZOV, N. K. NADIROV and L. A. TOLCHINA (*Pishch. Tekhnol.*, 1969, [4(71)], 51. Russ., 10 ref.).— C. V.

Determination of carotenes and tocopherols in millet oil. L. G. SALOMATINA (*Pishch. Tekhnol.*, 1969, [3(70)], 168. Russ., 13 ref.).— C. V.

Carotene and tocopherol contents in barley oil. A. I. DEMCHENKO (*Pishch. Tekhnol.*, 1969, [5(72)], 18. Russ., 17 ref.).— C. V.

Accelerated mineralisation of margarine in photocolorimetric determination of nickel with dimethylglyoxime. K. MEDRZYCKA and H. NIEWIADOMSKI (*Chemia analit.*, 1969, **14** (4), 771–776. Pol., 10 ref.).—Conditions for mineralisation of samples were studied. When the sample, contg. 0·02–0·2 mg of Ni/kg of margarine is dried at 125–130°, ignited, calcined at 650° for 30 min, and dissolved in 0·1 N-HCl, recovery of Ni is 90%. The detn. is completed according to the procedure of Rudnicki and Niewiadomski (*ibid.*, 1968, **13**, 755). The precision was 0·004–0·009 mg of Ni/kg of margarine. One detn. takes 5–7 h. B. Kamiński.

Meat, Poultry, Fish

Emulsification in comminuted meat systems. I and II. A. GORDON (*Fd Process. Ind.*, 1969, **38** (458), 54–56; (459), 50–52. 39 ref.).—Recent research is reviewed. Difficulties associated with fat separation, water separation, variable colour intensity and stability, and textural problems arising from mushiness are discussed. A procedure is discussed, involving extraction of protein material, followed in several cases by isolation of specific fractions which are then tested for effectiveness in terms of emulsifying capacity and/or emulsion stability. Methods of measuring emulsifying capacity are discussed. Results obtained have been applied in a linear programming system and the possibility of application to sausage formulation is considered. Evidence relating to a variety of parameters concerned with emulsification is described. I. Dickinson.

Alteration of proteins during storage of meat in cooled condition. N. A. GOLOVKIN and L. A. MELUZOVA (*Pishch. Tekhnol.*, 1969, [5(72)], 71. Russ., 14 ref.).— C. V.

Changes of some protein fractions of beef muscle *postmortem*. H. GUENTHER and F. TURBA (*J. Fd Sci.*, 1969, **34** (5), 469–470. 9 ref.).—Myosin and the major degradation product were isolated by a tissue press and gel filtration. Both proteins were characterised by peptide maps. Myosin did not change in viscosity following addn. of ATP and was free of actin. The degradation product exhibited reduced ATP-ase activity and SH-groups content, to values approx. half those of myosin after ageing for 6 days. The sedimentation const. decreased from 6·2 to 4·1. Comparison of the peptide maps showed 18 of the 75 peptide spots missing and the appearance of 8 new peptides in the degradation product. M. J. Rawlins.

Sensory differentiation of beef tenderness and juiciness components over short intervals of cooking time. P. ROGERS and S. J. RITCHEY (*J. Fd Sci.*, 1969, **34** (5), 434–435. 8 ref.).—Steaks were cooked at 350°F for 20, 23, 26 and 29 min, and tested by judges for tenderness and sensory evaluation. Changes occurred rapidly between 20 and 26 min and then decreased, probably representing change in heat penetration. M. J. Rawlins.

Effect of thawing procedures on cooking and palatability of lamb cuts. P. R. WOODHAMS and R. A. SMITH (*Publs Meat Ind. Res. Inst. N.Z.*, 1965, No. 99, 16 pp.).—Effects of temp. and length of thawing period on thawing losses, cooking times, cooking losses, cooked yields and palatability, were studied. Various lamb cuts were cooked, (*i*) from the frozen state, (*ii*) after thawing at room temp., for > 12 h, (*iii*) after thawing at refrigeration temp. (5°) for 24 and 72 h. W. J. G.

Influence of type of poultry and carcass part on the extractability and emulsifying capacity of salt-soluble proteins. A. J. MAURER, R. C. BAKER and D. V. VADHERA (*Poult. Sci.*, 1969, **48** (3), 994–997. 7 ref.).—Influence due to type of poultry (light and heavy fowl, broilers, and mature turkeys) and carcass part (whole carcass, leg, thigh, etc.) were studied. Results are related to suitability for preparing poultry 'emulsion' products. A. H. Cornfield.

Origin and nature of aroma in fat of cooked poultry. E. L. PIPPEN, E. P. MECCHI and M. NONAKA (*J. Fd Sci.*, 1969, **34** (5), 436–442. 31 ref.).—Fat, separated from raw solid tissue, washed in water at < 40° and then cooked, does not contain poultry aroma. Fat, separated from cooked poultry contains characteristic aroma, apparently caused by compd. dissolved in it during cooking; the compd. are derived from lean portions of the meat. Migration of S contg. compounds into the fat during cooking was observed. Protein, amino acids, sugars and other water sol. components appear to be involved in formation of the aroma. M. J. Rawlins.

Hydrogen sulphide, a direct and potentially indirect contributor to cooked chicken aroma. E. L. PIPPEN and E. P. MECCHI (*J. Fd Sci.*, 1969, **34** (5), 443–446. 10 ref.).—H_2S, in freshly prep. broth and meat of freshly cooked chicken, greatly exceeds the 10 ppb H_2S odour threshold in water. Freezing and reheating broth reduces the H_2S to subthreshold levels. H_2S was passed through molten chicken fat contg. 5%-CH_3CHO. After expulsion of excess H_2S and CH_3CHO, there was a fixed S content in the odorous fat. Thus H_2S may contribute to aroma through the formation of secondary products. M. J. Rawlins.

Influence of heating on decomposition of fish muscle protein substances. KAMAL ABD EL LATIF KHAMMADI (*Pishch. Tekhnol.* 1969, [3(70)], 85. Russ.).— C. V.

Reflectance characteristics of canned tuna. II. Relationship of pigment concentration to luminous reflectance and colour evaluation. III. Observations on physical and chemical properties of the pigment system. A. C. LITTLE (*Fd Technol., Champaign*, 1969, **23** (11), 1466–1468. 4 ref.; 1468–1472. 7 ref.).—**II.** A method for evaluation of the change in chromaticity, on reduction of canned tuna with Na dithionite, is presented. Magnitude and direction of the chromaticity shift is related inversely to the level of luminous reflectance of the sample and to the visual scores, and is directly related to concn. of haemochrome pigment. The method can be employed in quality evaluation of canned tuna, useful as a supplement to the Y value detn.

III. The pigment in canned tuna is intermediate between ferro- and ferri-haemochrome states. Homogenisation of samples in an inert atm., resulted in obliteration of spectral definition. A hypothesis involving the relationship between background light scattering and absorption coeff. is presented with supporting evidence. A two stage oxidn. of the haemochrome system is postulated. Evidence that antioxidants may block the oxidation sequence at Fe^{3+} is presented. I. Dickinson.

Formation of a green pigment from tuna myoglobins. O. K. GROSJEAN, B. F. COBB, N. MEBINE and W. D. BROWN (*J. Fd Sci.*, 1969, **34** (5), 404–407. 23 ref.).—A green pigment was produced when tuna myoglobins, trimethylamine oxide (TMAO) and cysteine were heated in phosphate buffer, pH 5·7. This did not occur when mammalian myoglobins, which contain no cysteine residues, were used. Denaturation of myoglobin exposed a sulphydryl group. TMAO promoted formation of a disulphide bond between cysteine and the sulphydryl group. The green colour could be reversed by Na_2SO_4. M. J. Rawlins.

Uptake of salt in kippering of herring. A. AITKEN and C. R. BAINES (*J. Fd Technol.*, 1969, **4** (4), 389–398. 5 ref.).—Factors controlling salt uptake during brining were studied. Fresh and frozen herrings, and those iced for 2 days, were brined in stainless steel tanks. Brine agitation, time and salt concn. were adjusted as required. The kippers were smoked and frozen until analysis. The important factors were brine concn., time and rate of stirring. Orientation of fish, oil content and fish size were less important. M. J. Rawlins.

Storage life extension of refrozen silver salmon steaks. T. C. YU, M. K. LANDERS and R. O. SINNHUBER (*Fd Technol., Champaign*, 1969, **23** (12), 1602–1604. 15 ref.).—Quality of refrozen silver salmon steaks was retained, after storage for 14 months at 0°F, by vac. packaging the fish to which antioxidants had been added. Salmon steaks packed without vac. were of acceptable quality,

after the same storage period, when they were coated prior to the second freezing with a starch soln. contg. antioxidants and sodium tripolyphosphate. I. Dickinson.

Correlation of pH and quality of fresh New Zealand oysters (*Ostrea lutaria*). J. THOMAS (*N.Z. Jl Sci.*, 1969, **12** (4), 784–788. 9 ref.).—Quality was judged by appearance and odour characteristics. Oysters were shucked and stored on ice. pH measurements were made at intervals for 33 days; there was no seasonal variation of pH either initially or during storage but pH decreased with storage while odour increased. Oysters remained in good condition for 4–5 days at pH > 6·1. M. J. Rawlins.

Food Additives

Preservatives, Colouring Matter

Sulphite: an important food preservative. A. J. KIDNEY (*Sulphur Inst. J.*, 1967–8, **3** (4), 10–14. 23 ref.).— C. V.

Sorbic acid. R. E. J. LISHMUND (*Fd Process. Ind.*, 1969, **38** (458), 51–53).—The non-toxicity and acceptability of sorbic acid in the framework of the current U.K. food regulations, is discussed. I. Dickinson.

Sorbic acid in food preservation. E. LÜCK (*Fd Process. Ind.*, 1969, **38** (459), 53–54.)—The uses of sorbic acid and its salts in foods such as cooking fats, protein foods, vegetable products, fruit, fruit juices, soft drinks, wines, confectionery and bakery products, are discussed. I. Dickinson.

Organoleptic evaluation of the effectiveness of antioxidants in milk fat. L. M. HILL, E. G. HAMMOND, A. F. CARLIN and R. G. SEALS (*J. Dairy Sci.*, 1969, **52** (12), 1917–1921. 12 ref.).—The ability of antioxidants to protect milk fat was detd. in an accelerated stability test at 40°. There were considerable variations in the effectiveness of antioxidants with different batches of milk fats but in general the most effective were thiodipropionic acid, propyl gallate, and butylated hydroxytoluene. Least effective were nordihydroguaiaretic acid and carboxymethyl-mercaptosuccinic acid. M. O'Leary.

Effect of antioxidants and synergists on peroxide decomposition in milk fat. L. M. HILL, E. G. HAMMOND and R. G. SEALS (*J. Dairy Sci.*, 1969, **52** (12), 1914–1916. 7 ref.).—Antioxidants were shown markedly to accelerate milk fat peroxide breakdown in vac. at 40°. Rate of breakdown increased with increasing antioxidant concn., except with nordihydroguaiaretic acid, where the reverse applied. Synergists, citric acid and isopropyl citrate, decreased the rate of peroxide breakdown compared with controls and significantly inhibited the acceleration of peroxide decomp. caused by addn. of antioxidants. M. O'Leary.

Detection of chloramine T in dairy products. W. F. VAN GILS (*Analyst, Lond.*, 1970, **95** (1126), 91–94. 5 ref.).—After appropriate pretreatment of the sample, e.g., milk, ice cream etc., chloramine T is hydrolysed to toluene-*p*-sulphonamide (**I**) with N-HCl in presence of 2% aq. Na_2SO_3. After extraction of **I** into diethyl ether, a 0·3% ethanolic soln. of phenothiazine (**II**) and 0·3% aq. NaOCl are added to the extract and the mixture shaken with benzene for ~30 min. A violet coloration of the benzene layer indicates presence of chloramine T in the sample. Colour standards permit an approx. estimate of concn. to be made. Sensitivity is ~25 mg per kg, but can be improved to 1 mg if necessary. The method is independent of the loss of active Cl from chloramine T; **I** reacts with ethanolic **II** only when free Cl and O are present. W. J. Baker.

Method for determination of monosodium glutamate in food products. E. FERNANDEZ-FLORES, A. R. JOHNSON and V. H. BLOMQUIST (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1131–1132).—The method used previously for protein hydrolysates (*ibid.*, 1969, **52**, 1744) was studied collaboratively on soup products. Av. recovery of 0·3–10% of monosodium glutamate present was ~102% with a standard deviation of 3·6%. D. I. Rees.

Synthetic colours for the food industry. T. A. BORODA and Y. S. ROZUM (*Pishch. Tekhnol.*, 1969, [3(70)], 27. Russ.).— C. V.

Spices, Flavours, Other Additives

Differentiation of geographic origin of spices. II. Oregano by gas chromatography and thin layer chromatography. W. H. STAHL, J. N. SKARZYNSKI and W. A. VOELKER (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1184–1189. 6 ref.).—For g.l.c., the ground sample was injected by means of a solid sampler into a column of 20% of Carbowax 20M on Chromo WAW, temp. programmed from 70 to 200° at 4°/min, with flame ionisation detection. For t.l.c., an EtOAc extract was spotted on a SiO_2 gel G layer, developed with C_6H_{14}–EtOAc–tetrahydrofuran (72 : 25 : 3·5); spots were revealed under u.v. light and also by charring with H_2SO_4; a two-dimensional t.l.c. was also carried out. G.l.c. analysis gave the main means of differentiation; Greek oregano had a ratio of concn. of carvacrol to thymol of 7 : 1 whereas in Mexican oregano, its ratio was 1 : 1. D. I. Rees.

Pungent principles of ginger and their importance in certain ginger products. D. W. CONNELL (*Fd Technol., Aust.* 1969, **21** (11), 570–575. 8 ref.).—The major pungent principle was isolated from a commercially prep. sample of ginger oleoresin and, by usual spectroscopic and chem. techniques, was identified as shogaol (**I**). Processing and storage of oleoresin (**II**) derived from ginger, can result in chem. conversion of > 50% of the product to other substances. This is a result of the conversion of gingerol (**III**) to **I** and zingerone (**IV**). It is suggested that **I** and **IV** do not occur naturally in fresh rhizomes. Chem. changes of **II** at acid pH can occur at five times the rate observed at neutral pH and can be accelerated by elevated temp. Conversion of **III** to the less pungent **I** and thence to non-pungent residues is undesirable because of loss of pungency and an accumulation of non-pungent residues. Decomp. of **III** to **IV** and aliphatic aldehydes must be avoided. I. Dickinson.

Synthesis of 2,5- and 2,6-dialkylpyrazines. H. G. PEER and A. VAN DER HEIJDEN (*Recl Trav. chim. Pays-Bas*, 1969, **88** (11), 1335–1336. Engl., 8 ref.).—Condensation of isopropylglyoxal (3-methyl-2-oxobutanal) with 1,2-diaminopropane in methanolic NaOH, followed by passage of O_2 through the mixture, gave a 1 : 1 mixture of 2-isopropyl-5-methylpyrazine (**I**) and its 2,6-isomer. Complete separation of the isomers was effected by column chromatog. on Al_2O_3 with $CHCl_3$ as eluent. The compd. are flavour constituents of roasted foods, e.g., coffee contains **I**. J. I. M. Jones.

Sensory effects of hydrocolloid sols on sweetness. M. VAISEY, R. BRUNON and J. COOPER (*J. Fd Sci.*, 1969, **34** (5), 397–400. 14 ref.).—A trained sensory panel assessed sweetness and texture interaction in cornstarch, guar and carboxymethylcellulose. Viscosity (η) curves, over a range of sucrose levels, were detd. using a Brookfield viscometer. Relationship between these curves and sweetness perception indicated that gums with less η drop as shear rates increase, tend to mask sweetness perception. M. J. Rawlins.

Detection and quantitative analysis of cyclohexylamine [in cyclamates]. S. W. GUNNER and R. C. O'BRIEN (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1200–1202. 12 ref.).—Cyclohexylamine (**I**) is extracted from an alk. soln. contg. a known amt. of 3,3,5-trimethylcyclohexylamine (and EDTA for Ca cyclamate) into $CHCl_3$. Excess reagent ($CHCl_3$ soln. contg. 1 mg/ml of picryl chloride) is added, the soln. is worked up and the **I** content is found by g.l.c. with electron capture detection. Quant. recoveries of **I** were obtained. D. I. Rees.

Carcinogenicity testing and permitted lists. P. GRASSO (*Chemy Britain*, 1970, **6** (1), 17–22. 37 ref.).—Tests for carcinogenicity of additives in food processing (e.g. colourings) are considered, particularly discrepancies in test results. Subcutaneous sarcoma can be produced by ordinarily non-carcinogenic materials due to the reactive response of the tissues of rats and mice around a solid implant, not due to chem. carcinogenesis. Other phys. properties (viz. surface activity and amphipathy) produced tumours by the same mechanism. Test results using new-born mice and bladder implantation are also suspect. P. M. Gooch.

Monoglyceride phosphoric acid and its salts. EASTMAN KODAK Co., Assee of J. D. CAWLEY and M. P. O'GRADY (Br. Pat. 1,174,789, 23.12.66. U.S., 29.12.65).—Lysophosphatidates (phosphorylated monoglycerides) suitable for preventing rancidity in oils and fats, are prep. by treating a glycidyl ester of a 6–22C hydrocarbyl carboxylic acid with anhyd. phosphoric, pyrophosphoric or polyphosphoric acid. E.g., glycidyl stearate, dehydrated polyphosphoric acid and hexane are refluxed for 45 min. and the mixture worked up to yield monoglyceride phosphoric acid. S. S. Chissick.

Food Processing, Refrigeration, Packaging and Storage

Freeze concentrates–coffee extract. F. K. LAWLER (*Fd Engng*, 1969, **41** (4), 73–75).—A method of freeze concn. comprising (*i*) 'slush-freezing' involving formation of ice crystals and (*ii*) centrifugation to remove the ice, is described. W. J. G.

Continuous vacuum drying of whole milk foam. III. Optimisation operations. J. C. CRAIG, JUN., N. C. ACETO, E. F. SCHOPPET and T. F. HOLDEN (*J. Dairy Sci.*, 1969, **52** (12), 1948–1954. 15 ref.).—Acceptable operating conditions for vac. foam-drying of whole milk over a wide range of milk foam stabilities were derived from a mathematical model and found operationally feasible in a pilot plant. Only markedly stable (slow-drying) milks have not yet been tested. M. O'Leary.

Influence of heat treatment of condensed-milk type emulsion before freezing, upon stability in storage. N. I. KOZIN and G. T. PONOMAR'YEV (*Pishch. Tekhnol.*, 1969, [5(72)], 64. Russ.).— C. V.

Freeze-drying of dairy products. E. J. MANN (*Dairy Inds*, 1969, **34** (5), 298–299. 17 ref.).—The review covers: continuous plant; liquid milk; coagulated products; process for heat-sensitive products; yoghurt; cottage cheese; caseinate complex; ice cream. W. J. G.

Freezer burn of animal tissue. VII. Temperature influence on development of freezer burn in liver and muscle tissue. G. KAESS and J. F. WEIDEMANN (*J. Fd Sci.*, 1969, **34** (5), 394–397. 6 ref.).—Liver or muscle samples were frozen at different rates with and without wt. loss, and stored at − 4 and − 20°. At − 20°, low wt. loss initiated burn; at − 4°, a condensed layer formed but no burn appeared. Prior treatment with glycerol or NaCl soln. reduced burn in liver and longitudinal cuts of muscle. Fat content had little influence on burn. M. J. Rawlins.

Hot-room for incubation of canned foods. R. ATKINS (*Fd Preserv. Q., C.S.I.R.O. Aust.*, 1969, **29** (3), 43–48. 4 ref.).—A simple insulated and shelved construction of timber or metal, to accelerate shelf-life studies, is a useful adjunct to quality control in a cannery. Two main temp. ranges, 86–95°F for general spoilage, and 122–130°F for thermophilic organisms, are important to detect changes in product and corrosion of containers. Conditions are maintained by a thermostat and fan. M. J. Rawlins.

Estimating the central temperatures of canned food during the initial heating or cooling period of heat process. KAN-ICHI HAYAKAWA (*Fd Technol., Champaign*, 1969, **23** (11), 1473–1477. 27 ref.).—A method for estimation of the initial portion of heating or cooling curves is discussed. A series of temp. response curves, for finite cylinders, were obtained by using a digital computer. I. Dickinson.

Corrosion resistance of steel under conditions of canning production. Y. A. KL'YACHKO and A. S. CHEDAYEV (*Pishch. Tekhnol.*, 1969, [3(70)], 35. Russ.).— C. V.

Aseptic packaging of milk and milk products. E. J. MANN (*Dairy Inds*, 1969, **34** (1), 30–31. 20 ref.).—A brief review is given covering: packaging and processing tests; cream and other products; new packaging systems; aseptic bottling. W. J. G.

Practical guides on water activity and storage stability. L. B. ROCKLAND (*Fd Technol., Champaign*, 1969, **23** (10), 1241–1251. 24 ref.).—Phys., chem. and thermodynamic evidence is presented in support of the 'local isotherm' concept of moisture sorption. Isotherms are considered as composites of local isotherms, each of which is dominated by a specific type of bound water. Stability of foods is related to the influence of bound water. Explanations are given for the optimum moisture phenomenon and other anomalous reactions which contribute to deterioration of food products during storage. It is suggested that both moisture content and equil. r.h. co-ordinates of an established moisture sorption isotherm be employed for specifying optimum stability conditions. I. Dickinson.

Water activity and enzyme activity. L. W. ACKER (*Fd Technol., Champaign*, 1969, **23** (10), 1257–1270. 23 ref.).—Relationships of enzyme activity to water, water mobility, substrate mobility are discussed together with other factors important for reaction in low-moisture solid or liquid foods. The sorption isotherm enables prediction of enzyme reaction. Enzyme reactions in low-moisture foods show significant dependence on water activity, not water content. Oxidases which do not use water as a reactant, show the same dependence as hydrolases. Water serves as a medium for enzyme reactions and as vehicle for substrate transport. For a non-aqueous liquid substrate that can move to the enzyme, water needs to act only as reactant and enzymic changes can take place at low water activity. Other factors important for the degree of enzymic action are mol. wt. and mobility of substrate. I. Dickinson.

The xerophilic mould, *Xeromyces bisporus*, as a spoilage organism. H. DOLLYN and J. R. EVERTON (*J. Fd Technol.*, 1969, **4** (4), 399–403. 7 ref.).—Growth characteristics of *Xeromyces bispora* were studied in relation to pre-packaged foodstuffs. It grew under conditions where other moulds were inhibited or restricted. Ascospores of *X. bisporus* were resistant to heat; growth was very tolerant of high CO_2 concn. M. J. Rawlins.

Physiological studies of gamma-irradiated tomato fruit. II. Effects on deterioration and shelf life. A. S. ABDEL-KADER, L. L. MORRIS and E. C. MAXIE (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 831–842. 17 ref.).—Irradiation damage (100–700 krad) was less in fruit treated at the ripe stage than in fruit treated at the mature-green stage. Shelf life was not affected by treatment at the mature-green stage, but was extended 4–12 days when ripe fruit was treated with 250–350 krad. A. H. Cornfield.

Physiological studies of gamma-irradiated tomato fruit. III. Effects on ascorbic acid content, acidity and texture. A. S. ABDEL-KADER, L. L. MORRIS and E. C. MAXIE (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 843–853. 32 ref.).—γ-irradiation (50–600 krad) decreased the ascorbic acid content of ripe fruit more than that of mature-green fruit. The treatment decreased total acidity and firmness, increased pH, and had no effect on total sol. solids. A. H. Cornfield.

Effect of ozone on storage of cranberries. J. S. NORTON, A. J. CHARIG and I. E. DEMORANVILLE (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 792–796. 4 ref.).—The extent of rot of cranberries stored at 40°F for 8 weeks was decreased slightly by the presence of 0·27 ppm O_3. In another test at 60°F for 5 weeks, 0·6 ppm O_3 doubled the extent of rot and also increased wt. loss. A. H. Cornfield.

Respiration of Dougherty apples in controlled atmosphere storage conditions. R. W. FOSTER and B. MCDONALD (*N.Z. Jl Sci.*, 1969, **12** (4), 840–850. 2 ref.).—Measurements were made on Dougherty apples to determine CO_2 production and O_2 usage in controlled atm. storage conditions. M. J. Rawlins.

Microbiology of fresh apple and potato plugs preserved by gas exchange. J. G. KAFFEZAKIS, S. J. PALMER and AMIHUD KRAMER (*J. Fd Sci.*, 1969, **34** (5), 426–429. 21 ref.).—Inoculated samples were treated with various gases (anhyd. SO_2, CO, and 10% C_2H_4O + 90% CO_2). Death kinetics of micro-organisms *Escherichia coli*, *Clostridium botulinum* and *Staphylococcus aureus* were detd., together with the effects of gas on the browning enzyme systems of products. M. J. Rawlins.

Preparation and storage of dehydrated carrot flakes. T. S. STEPHENS and T. A. MCLEMORE (*Fd Technol., Champaign*, 1969, **23** (12), 1600–1602. 9 ref.).—Fresh unpeeled carrots were cooked, comminuted, made into purée and dried for 35 sec on a double drum drier. Flakes prepd. from the dried product and canned in a N_2 atm., retained their initial amt. of ascorbic acid, decreased only slightly in β-carotene and retained a typical carrot flavour after 2 yr storage at 0 and 68°F. Flakes canned in air, decreased in ascorbic acid and in β-carotene content and developed a pronounced off-flavour by the end of the second month of storage. Salt, added during prepn. of the flakes, did not affect flake quality during storage. I. Dickinson.

Artificial curing of onions for control of neck rot (*Botrytis allii* Munn). K. M. HARROW and S. HARRIS (*N.Z. Jl agric. Res.*, 1969, **12** (3), 592–604. 14 ref.).—Suitable curing temp., resistance of onions to airflow, moisture loss, and heating times at different airflows are discussed. A modulating heat source is desirable to maintain the drying air at 36–38° as ambient temp. fluctuates. E. G. Brickell.

Sorption and diffusion of water in dry soyabeans. G. D. SARAVACOS (*Fd Technol., Champaign*, 1969, **23** (11), 1477–1479. 12 ref.).—Water sorption isotherms and diffusivities of water in dry soyabeans were detd. at 30° with a vac. sorption apparatus. Defatted soyabeans sorbed more water at a faster rate than full-fat beans. Diffusivity of water in full-fat soyabeans increased significantly at higher moisture content. Sorption characteristics are important in the processing and use of dehydrated soyabean products. I. Dickinson.

A study of factors affecting the frozen storage life of New Zealand deep sea oyster. (*Ostrea Putaria*). J. THOMAS (*N.Z. Jl Sci.*, 1969,

12 (4), 777–783. 15 ref.).—Oysters were treated the day after dredging and stored at 15°F for 3 months. They were frozen rapidly at − 45°F and slowly at 0°F. Packaging films were of low or high *d* polyethylene. Some samples were pretreated and glazed prior to packing. To prevent flavour deterioration for up to 3 months, pretreatment with 1% glucose or 0·1% ascorbic acid/0·5% sodium tripolyphosphate, rapid freezing, low storage temp. and packaging which reduced contact with air, proved advantageous. M. J. Rawlins.

Filling. J. R. KITCHEN (*Fd Process. Ind.*, 1969, **38** (458), 37–50).—Greater speeds in production lines have led to a demand for high speed filling machines for a range of products such as liquids, powders, pastes and irregular shaped objects, e.g., crisps, sweets, cakes and pies. Recent developments are reviewed in four articles: **Volumetric filling.** (37–39); **Filling by weight.** (42–43); **Filling viscous and semi-viscous materials.** (45–46); **Form fill and seal.** (49–50). I. Dickinson.

Nutrition, Proteins, Amino Acids, Vitamins

Protein nutritional value of wild plants used as dietary supplements in Natal. B. M. G. SHANLEY and O. A. M. LEWIS (*Pl. Fds hum. Nutr.*, 1969, **1** (4), 253–258. 14 ref.).—Total protein content and lysine, tryptophan, cystine and methionine contents of plant leaves, from eleven wild plants, were detd. Value of the protein as a supplement to maize meal protein was then detd. In all cases, protein value of maize meal–leaf mixtures was higher than for maize meal alone. W. J. G.

Leaf protein as a human food. N. W. PIRIE (*Pl. Fds hum. Nutr.*, 1969, **1** (4), 237–246. 34 ref.).—Equipment which extracts 60–70% of protein from leaf crops has been designed. The protein is coagulated by suddenly heating the extract to 80°, filtering and then washing it at pH 4. The product contains 60–70% of protein and 20–30% of highly unsatd. fat. Protein quality, yield and costs are discussed. W. J. G.

School milk and mental alertness. ANON (*Dairy Inds*, 1969, **34** (3), 132–133. 2 ref.).—The nutritional value of milk and the effects of malnutrition on learning ability are discussed. W. J. G.

Utilisation of amide nitrogen by the young rat. M. WOMACK and J. E. WILSON, JUN. (*J. Fd Sci.*, 1969, **34** (5), 430–433. 11 ref.).—Amide-N contributed to body N stores less efficiently than an equal amt. of α-amino-N. No difference in N utilisation was found between two groups of rats fed different levels of amide-N as wheat. In the usual wheat-contg. diet, failure to use part of the amide-N would not limit protein synthesis. M. J. Rawlins.

Microbial production of amino acids from hydrocarbons. IV. L-Glutamic acid production by *Corynebacterium hydrocarboclastus* R-7. I. SHIIO and R. UCHIO (*J. gen. appl. Microbiol., Tokyo*, 1969, **15** (1), 65–84).—This organism is an excellent utiliser of hydrocarbon and produces a large amount of glutamic acid (**I**) from various n-alkanes by the addition of penicillin (**II**). Influence of time, concn., pH, presence of metal ions, S-containing amino acids and $(NH_4)_2HPO_4$ on **I** production were studied. Under optimum conditions when the culture medium contained 61·7 g/l of n-hexadecane (**III**) together with 20 units/ml of **II**, the highest concn. of **I** was attained after 48 h at 20 g/l of medium. At 14 h, after addition of **II**, a yield of 72% w/w of **III** consumed was attained. C. V.

Vitamin D and cardiovascular, renal and brain damage in infancy. M. S. SEELIG (*Ann. N.Y. Acad. Sci.*, 1969, **147** (15), 537–582. 160 ref.).— C. V.

Method and apparatus for making a protein product. RALSTON PURINA CO. (Br. Pat. 1,174,906, 15.12.66. U.S., 15.12.65).—A meat-simulating product may be obtained from protein-containing vegetable materials (**I**) (especially oil-free soyabean meal) by adding a sulphur-containing substance (e.g., S, K_2S, Na_2S), capable of altering the cystine bond structure between the polypeptide chains, to the moistened **I**. The process is carried out at pH ~ 8·6, at 270–310°F and at 300–600 lb/in² pressure and the material extruded through orifices. A suitable apparatus is described. S. S. Chissick.

Unclassified, Tobacco

Holm-oak fruit (*Quercus ilex*). V. Industrial production of acorn meal and extraction of the oil. J. A. FIESTAS ROS DE URSINOS, F. RAMOS AYERBE and F. MAZUELOS VELA (*Grasas Aceit., Seville*, 1969, **20** (5), 227–230. Span., 8 ref.).—Industrial processes for drying the acorns and extraction of oil, either direct or after lamination and granulation of the meal, are examined. The extracted meal (~ 40% of the dried acorns) contains 6% of protein. The oil (~ 9% of the dried meal) is dark and cloudy and contains ~ 4% of wax. L. A. O'Neill.

Cottonseed protein isolates: two-step extraction procedure. L. C. BERARDI, W. H. MARTINEZ and C. J. FERNANDEZ (*Fd Technol., Champaign*, 1969, **23** (10), 1305–1312. 21 ref.).—A two-step selective extraction of cottonseed flours with water and then with 0·015N-NaOH for 30 min at 25° with 1 : 15 ratio (flour : solvent) is described. Two protein isolates were recovered, isolate I (13% N, 16% of total N) pptd. at pH 4, isolate II (16% N, 52% of total N) pptd. at pH 7. Variation in temp. between 25 and 60°, and ratio between 1 : 15 and 1 : 10, had no effect. Gossypol in flour lowered yields of the isolates. Isolate I and II differed in compn., mol. wt. and solubility characteristics. They accounted for slightly more of the total N and wt. of the flour than an isolate obtained from a single step, alk. extraction. I. Dickinson.

Rennin production by mould fungi of the *Aspergillus* genus. N. S. PALEVA (*Mikrobiologiya*, 1969, **38** (6), 1002–1005. Russ., 14 ref.).—Rennin production was studied in 73 members of mould fungi of the *Aspergillus genus*. Active rennin production arose with *A. flavus* f-26, *A. oryzae* 269/3801 and *A. oryzae* f-46 grown on wheat brans and with *A. parasiticus* 320 and *A. oryzae* 3-9-15 grown submerged in liquid media. Unit activity for rennin synthesised per g of dried substance varied from 13000–8000 for the above members to nil for 17 members. L. A. Haddock.

Quantitation of flavourful food components using isotope dilution. W. Y. COBB (*J. Fd Sci.*, 1969, **34** (5), 466–469. 22 ref.).—Flavour compd., labelled with ^{14}C were added to a food system prior to reduced-pressure distillation, during which conversions occur. Recovered material was analysed quant. by u.v. spectroscopy. The concn. of native compd. were calc. by isotope diln. M. J. Rawlins.

Cherries in spreads and cottage cheese sauce. T. I. HEDRICK, P. MARKAKIS and S. WAGNITZ (*J. Dairy Sci.*, 1969, **52** (12), 2057–2059).—Procedures for the manufacture of cherry-flavoured dairy spreads and related development information are presented. M. O'Leary.

Cadmium content of cigarettes. M. NANDI, H. JICK, D. SLONE, *et al.* (*Lancet*, 1969, **11**, (7634), 1329–1330. 12 ref.).—Analysis of whole cigarettes, ash and filters after smoking of six brands showed that ~ 70% of the Cd content of the tobacco passes into the smoke. C. V.

Recovery of nitrogenous material from micro-organisms. BRITISH PETROLEUM CO. LTD. (Inventor: P. CORTEEL) (Br. Pat. 1,175,912, 15.4.66).—The micro-organism, e.g., a straight chain, paraffinic hydrocarbon-containing yeast (Cryptococcaceae or Cryptococcoideae), is treated with a proteolytic enzyme (pepsin, trypsin, etc.), which is then removed or deactivated; a second enzyme, capable of attacking the carbohydrate present in the cell wall (a cellulase) is added and finally an aq. soln./suspension containing the nitrogenous compd. is recovered. S. S. Chissick.

Tobacco. H.-J. FROHLING (Br. Pat. 1,175,441–442, 3.6.67. Ger., 5.3.66. [B] div. out of [A]).—The flavour and aroma of tobacco are improved by adding [A] 5–500 ppm of a 3-R^1-4R^2-styrene where R^1 and R^2 are OH or Me or R^1R^2 is methylenedioxy, e.g., 1-hydroxy-2-methoxy-4-vinylbenzene, or [B] 2·5-750 ppm of a 3-R^3-4-R^4-maleimide where R^3 and R^4 are H, Me or Et. Both compd. are isolated from tobacco steam distillate. R. J. M.

3.—PEST AND DISEASE CONTROL, SANITATION

Plant Diseases, Pests and Weeds

Automated esterification plant for manufacture of pesticides. A. HILLIARD and V. BANG (*Chem. Process.*, 1969, **15** (10), 50–54. 7 ref.).—The fully automated plant, designed for the manufacture of pesticides based on P and S as principal raw materials, is described in detail. The production cycle is described with emphasis on production efficiency achieved. Products include organo-P insecticides, e.g., Et parathion, Me parathion, malathion, fenitrothion etc. W. J. G.

Thin layer chromatography identification of pesticides using dip-coated presensitised microscope slides. N. V. FEHRINGER and J. D. OGGER (*Solutions*, 1969, **1** (3), 27–30. 9 ref.).—The use of t.l.c. for qual. analysis of pesticides using microscope slides as plates is described. Incorporation of hydroquinone, 2′,7′-dichlorofluoroscein and $AgNO_3$ in the adsorbent layer is discussed. Microscope slides are prep. by immersing in an EtOH–$CHCl_3$ slurry of Al_2O_3 adsorbent. W. J. G.

Herbicides

Accurately predicting a herbicide's potential. J. W. WHITWORTH and W. P. ANDERSON (*Weed Sci.*, 1969, **17** (3), 290–295. 3 ref.).—Reliable, accurate field screening methods were developed for assessing the potential of 8 herbicides for weed control in 8 crop species during the first year of testing. Data from the 1-yr test was almost as reliable as data averaged over 5 yr, with respect to weed control and phytoxicity to the crop plants.
A. H. Cornfield.

Technique for measuring the relative movement of herbicides in soil under leaching conditions. T. CHAPMAN, P. A. GABBOTT and J. M. OSGERBY (*Pestic. Sci.*, 1970, **1** (2), 56–58. 3 ref.).—A technique for determining the relative leaching of herbicides in soil is described. This consists of coating a glass plate with a uniformly thin layer of soil, applying the herbicide as a streak to the bottom of the plate and then eluting it with water. This is analogous to t.l.c. After elution, the position of the herbicide is detd. by a bioassay with browntop grass (*Agrostis tenuis*). This provides a speedy and economical method which is particularly useful as a screen for experimental herbicides. W. J. G.

Effect of soil water stress and soil temperature on translocation of diuron. R. H. SEDGLEY and L. BOERSMA (*Weed Sci.*, 1969, **17** (3), 304–306. 10 ref.).—The rate of photosynthesis of wheat plants whose roots had been treated with diuron decreased to a greater extent at 0·3 than at 2·5 bar soil moisture content and to a greater extent in soil at 23·9° than at 10°. Transpiration was decreased by diuron treatment at 0·3 bar at both temp. but not at 2·5 bar. This work was carried out to investigate damage done to wheat where diuron has been used for weed control.
A. H. Cornfield.

Herbicidal activity of certain bisquaternary ammonium salts and their surface active properties. G. S. SUPIN, Z. S. SIDENKO, L. D. STONOV *et al.* (*Zh. obshch. khim.*, 1969, **37** (12), 2651–2653. Russ., 9 ref.).—Fifteen salts were studied. It was shown polarographically, from a fall in the maxima and from surface tension detn. in H_2O, that the polarographic procedure registers slight differences in surface active properties at low concn., not shown by the stalagmometric method. Herbicidal properties of a given series rise with increased surface activity, measured from the fall in the polarographic max. of Mn. L. A. Haddock.

Effect of pyrazon on growth, photosynthesis, and respiration. R. FRANK and C. M. SWITZER (*Weed Sci.*, 1969, **17** (3), 344–348. 10 ref.).—Tests *in vitro* and *in vivo* showed that pyrazon is an inhibitor of photosynthesis and the Hill reaction. Pyrazon accumulated much more rapidly in *Chenopodium album*, a susceptible species, than in sugar-beet, a tolerant species. Pyrazon had no effect on respiration of leaf discs or root sections of either species.
A. H. Cornfield.

Injury to sugar-cane by 2,4-D formulations. F. E. RICHARDSON (*Proc. S. Afr. Sug. Technol. Ass., 43rd Ann. Congr.*, 1969, 122–129. 5 ref.).—Phenoxyacetic acid formulations (Shellamine 7·2, Fernimine Selective 7, Fernimine Selective 5, Fernesta 7, Shell Weed-killer D concentrate, and Fernimine 4) were applied at 3 and 18 lb acid equiv./acre, to three sugar-cane varieties (N : Co. 376, N : Co.382, N : Co.310) pre- and/or post-emergent. Post-emergent sprays caused the most damage. Ester formulations may cause more damage than amines, but effects vary according to susceptibility of the sugar-cane variety. M. T. Rawnsley.

Effects of simazine alone and in combination with hay or plastic mulch on 'McIntosh' apple trees and accumulation of simazine residues. W. J. LORD, R. A. DAMON, JUN. and B. GERSTEN (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 62–70. 23 ref.).—Application of simazine to the soil (for weed control) over 3 yr had no effect on apple tree growth, leaf nutrient levels, or fruit colour. Hay mulch alone or with simazine increased leaf N and K in 1–2 years and decreased leaf Ca and Mg in one year. Black sheet polyethylene mulch had little effect on tree performance. Residual simazine was concentrated in the upper 3-in. soil layer whether applied in granular or wettable powder form. Residues were greatest under plastic and least under hay mulch, but the hay mulch itself retained considerable residues. A. H. Cornfield.

Effects of four herbicides on growth and yield of orange trees. G. F. RYAN and D. W. KRETCHMAN (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 159–165. 13 ref.).—Yields from young orange trees over 5 yr were increased by annual or semi-annual application of diuron (3·2–6·4 lb) or simazine (6·4–9·6 lb per acre) applied for weed control. Dalapon (4·5 lb per acre) did not affect growth of or yields from young or mature trees over 5 yr. EPTC (6 lb per acre twice a year for 5 yr) reduced growth but did not affect fruit yields. A. H. Cornfield.

Phytotoxicity of demethylated analogues of diphenamid. W. A. GENTNER (*Weed Sci.*, 1969, **17** (3), 284–285. 6 ref.).—Diphenamid was much more toxic to tomato plants and 3 species of weed than was 2,2-diphenylacetic acid. *N*-methyl-2,2-diphenylacetamide and 2,2-diphenylacetamide showed intermediate toxicity.
A. H. Cornfield.

Absorption and penetration of picloram in potato tuber discs. C. R. SWANSON and J. R. BAUR (*Weed Sci.*, 1969, **17** (3), 311–314. 7 ref.).—Absorption of picloram (**I**) by the discs, from buffered solutions, decreased with increasing pH (4–8) and increased with concn. of **I** and with temp. (4–37°). Most of the absorbed **I** leaked out within 12 h when the treated discs were placed in **I**-free buffers.
A. H. Cornfield.

Influence of herbicides on plant growth and agronomic characteristics of soyabean varieties. B. J. JOHNSON (*Agron. J.*, 1969, **61** (5), 791–793. 9 ref.).—Pre-emergence herbicides applied at normal rates had similar effects on four soyabean varieties ranging in maturity from early to late. Effects on early growth, final height, seed yields, and incidence of lodging are discussed.
A. H. Cornfield.

Weed control with paraquat in plantation crops. I. E. DARTER (*Outl. Agric.*, 1968, **5** (5), 208–214. 24 ref.).—The use of paraquat in orchard and plantation crops including rubber, oil palm, coconut, coffee, cocoa, tea, bananas, citrus fruits, vines, apples, pears, peaches and sugar-cane, is discussed extensively. W. J. G.

Response of tropical vegetation to herbicides. R. W. BOVEY, C. C. DOWLER and J. D. DIAZ-COLON (*Weed Sci.*, 1969, **17** (3), 285–290. 7 ref.).—Cacodylic acid controlled guava, and diquat controlled vegetation in a mixed tropical forest within 2 weeks of treatment. Mixtures of 2,4-D, 2,4,5-T, and picloram effectively controlled vegetation for 6 months. A. H. Cornfield.

Translocation of assimilates and dalapon in established johnsongrass, *Sorghum halapense*. R. J. HULL (*Weed Sci.*, 1969, **17** (3), 314–320. 18 ref.).—Translocation of assimilated ^{14}C-labelled CO_2 and 2-^{14}C-labelled dalapon applied to the leaves of established johnsongrass followed a similar pattern. A. H. Cornfield.

Antagonistic responses with combinations of carbamate and growth regulator herbicides. C. N. PRENDEVILLE, C. S. JAMES, G. F. WARREN and M. M. SCHREIBER (*Weed Sci.*, 1969, **17** (3), 307–309. 9 ref.).—The effects of combinations of carbamate and growth regulator herbicides compared with individual materials were studied on two grasses and 4 dicotyledonous species.
A. H. Cornfield.

Use of minimum tillage and herbicide for establishing legumes in Kentucky bluegrass swards. T. H. TAYLOR, E. M. SMITH and W. C. TEMPLETON, JUN. (*Agron. J.*, 1969, **61** (5), 761–766. 20 ref.).—Lucerne and white clover were successfully established in Kentucky bluegrass swards when the seed was sown (0·6–1·2 cm deep) in shallow disked strips (1·9 cm deep and 0·6–5·7 cm wide) and paraquat (1·11 kg active ingredient/ha) was applied in bands 5·1–10·2 cm wide over the seeded rows. Spring plantings were more successful than late summer ones, whilst mid-summer plantings were unsuccessful. A. H. Cornfield.

Effects of growth regulators and herbicides on germination of *Hydrilla* turions. K. K. STEWARD (*Weed Sci.*, 1969, **17** (3), 299–301. 15 ref.).—Germination and growth of subterranean turions (vegetative propagules) of *Hydrilla* (aquatic weed) were enhanced by gibberellic acid, and of axillary turions by indoleacetic acid and 2,4-D. The use of these materials in releasing propagules from dormancy before applying herbicides is discussed in relation to control of the weed. A. H. Cornfield.

Influence of picloram granules and sprays on whitebrush, *Aloysia lycoides*. R. E. MEYER and T. E. RILEY (*Weed Sci.*, 1969, **17** (3), 293–295. 3 ref.).—Whitebrush was effectively controlled by picloram at 3–4 lb per acre (2% granules), particularly in the cooler months and when application was followed by rainfall.

Spray applications of picloram were effective even at 1–2 lb per acre. A. H. Cornfield.

Longevity of dodder control by soil-applied herbicides in the greenhouse. J. H. Dawson (*Weed Sci.*, 1969, **17** (3), 295–298. 11 ref.).—When compared with chlorpropham (**I**) as the standard treatment for controlling germination and emergence, trifluralin, DCPA, BIPC, dinoseb, dichlobenil and 4 expl. herbicides were as effective or superior in longevity of control. The period of control by **I** (6 lb per acre) was doubled when *p*-chlorophenyl *N*-methylcarbamate (1·5 lb) was applied with it. A. H. Cornfield.

Effects of EPTC (*S*-ethyl dipropylthiocarbamate) on petiole fatty acids of sicklepod, *Cassia obtusifolia*. R. E. Wilkinson and W. S. Hardcastle (*Weed Sci.*, 1969, **17** (3), 335–338. 16 ref.).— A. H. Cornfield.

Light intensity and the absorption and translocation of 2,4,5-T by woody plants. H. A. Brady (*Weed Sci.*, 1969, **17** (3), 320–322. 9 ref.).—Absorption of foliar-applied 2,4,5-T (iso-octyl-ester) increased with light intensity (40–4000 ft candles) in 2 species of evergreen woody plants; on 2 deciduous species absorption increased with intensity up to 2680 ft candles, but decreased at higher intensity. Translocation of 2,4,5-T in the evergreens differed little with light intensity; in one of the deciduous species it increased and in the other decreased with increasing intensity. A. H. Cornfield.

Fungicides

Acquired resistance to fungicides. J. Dekker (*Wld Rev. Pest Control*, 1969, **8** (2), 79–85. 19 ref.).—The occurrence of resistance in fungi to fungicides is discussed together with possible mechanisms operative in fungi, e.g., decreased permeability, detoxification, decreased conversion into fungitoxic compd., decreased affinity for inhibitor at the reactive site. W. J. G.

Fungicides for deciduous top fruit: a survey in 1968. A. H. M. Kirby (*Wld Rev. Pest Control*, 1969, **8** (1), 45–58).—Recommended treatments are presented for apple and pear diseases, e.g., scab, powdery mildew, rusts, bitter and black rot etc., stone fruit diseases, e.g., brown rot, Coryneum blight, leaf spot, leaf curl, black knot etc., grape diseases and nut diseases. Side effects of various fungicides are discussed. W. J. G.

***N*-Acetyl-*N*-trichloromethylsulphenylarene sulphonamides.** R. Brans and R. J. Cremlyn (*J. chem. Soc. C*, 1970, (2), 225–226. 23 ref.).—In quant. studies on the acetylation of Na sulphonamides by AcCl, Ac_2O–pyridine or Ac_2O–H_2SO_4 (**I**) it was found that **I** was the most effective. Condensation of the appropriate *N*-acetylsulphonamide with trichloromethanesulphenyl chloride produced an *N*-acetyl-*N*-trichloromethylsulphenylarene sulphonamide (**II**). Fungicidal activity was shown by the following deriv. of **II**: *p*-Me, *p*-chloro, 5-bromo-2-methoxy and 2,5-dimethoxy. V. Rolfe.

Control of stem end rot (*Gloeosporium musarum*) of banana fruit. P. G. Long (*Trop. Agric. Trin.*, 1970, **47** (1), 9–15. Engl., 5 ref.).—Benlate [(1-butylcarbamoyl)-2-benzimidazole carbamate] gave better control of stem end rot than did thiabendazole and could be used at lower concn.; a 5 sec immersion in the fungicidal suspension (0·025%) was sufficient even when there was a delay of up to 36 h between inoculation and dipping the fruit. E. G. Brickell.

Control of fruit rot in boxed bananas by thiabendazole (TBZ). C. A. Phillips (*Trop. Agric. Trin.*, 1970, **47** (1), 1–7. Engl., 4 ref.).—Trials indicate that relatively low concn. of TBZ (100 ppm) were very effective in controlling fruit decay. Diln. of the fungicide during dipping of wet fruit may be prevented by periodic addn. of fungicide; spray application at higher concn. may be feasible. External appearance and eating qualities of the fruit are not affected. E. G. Brickell.

Insecticides and Others

Temperature coefficient of insecticidal action using radioactive DDT. M. Yameen Zubairi (*Pak. CSIR Bull. Mono. no.* **6**, 1969, 19 pp. 35 ref.).—*Aedes aegypti* larvae were subjected to ^{14}C-DDT suspensions in a continuous treatment expt. at different temp. A negative temp. coeff. of DDT toxicity was obtained at low concn. (0·001–0·004 ppm). At high concn. (0·002 ppm), DDT showed a positive temp. coeff. between 10 and 20°. The amt. of ^{14}C-DDT detd. in the treated larvae (whole, and in three parts, head, thorax and abdomen) was greater with higher temp. The thorax picked up greater amt. of ^{14}C than did the head or abdomen at 20 and 30°; but at 10° the head contained the largest amt. It is suggested that the nervous system is the site of action of DDT. W. J. G.

The synthesis of potential insecticides. II. Carbamic esters of 4-alkylthiopyrazolones. I. T. Kay, D. J. Lovejoy and S. Glue (*J. chem. Soc. C*, 1970, (3), 445–448. 13 ref.).—Prepn. of 2-(alkylthio)acetoacetic esters (**I**) from the reaction of alkyl 2-chloroacetoacetate with alkane sulphenyl chloride is described. **I** was used for prepn. of a series of 4-alkylthio-2-pyrazolin-5-ones which were then converted into their Me- and Me_2-carbamic esters (**II**). Detailed syntheses are described. **II** gave good control of the green pea aphid, *Acyrthosiphon*, on broad bean plants by a systemic action (concn. in soil ~ 30 ppm). V. Rolfe.

Tricholomic acid. III–VIII. T. Kamiya *et al.* (*Chem. pharm. Bull., Tokyo*, 1969, **17** (5), 866–872. 16 ref.; 873–878; 879–885. 12 ref.; 886–889. 11 ref.; 890–894. 10 ref.; 895–900. 13 ref.).—The amino acid is found in the mushroom *Tricholoma muscarium*. It is insecticidal to flies and has a pleasant taste. C. V.

Effect of a systemic insecticide on the spread of mosaic and on certain sugar-cane insects. J. Dick and G. M. Thomson (*Proc. S. Afr. Sug. Technol. Ass., 43rd Ann. Congr.*, 1969, 72–74. 4 ref.).—Dimethoate did not significantly affect the spread of mosaic in N : Co.339 and N : Co.376. However, some reduction in no. of eggs laid was found for *Numicia* and *Perkinsiella*. The cost of dimethoate is almost prohibitive for regular use. M. T. Rawnsley.

Accumulation and loss of organochlorine insecticide residues by beetles, worms and slugs in sprayed fields. B. N. K. Davis and M. C. French (*Soil Biol. Biochem.*, 1969, **1** (1), 45–55. 11 ref.).—Comparison at four sites showed large differences in uptake, retention and metabolism. Live beetles (*B*) caught in traps after DDT application did not contain > 4 ppm of *p,p'*-DDT (**I**), but if exposed to artificial spraying, 70 ppm was found on their body surfaces. Metabolism to *p,p'*-DDE (**II**) was rapid in one species, resulting in 58–75 ppm of residues, which declined to 5 ppm in 4 months. Worms (*W*) and slugs (*S*) acquired much higher **I** levels which were much more persistent; breakdown in *W* was chiefly to **II**, but in *S* TDE resulted. *W* from an aldrin (**III**)-treated site contained both dieldrin and **III**, the proportions changing with time. C. V.

Relative efficacy of endrin as a prophylactic spray for control of the jute stem-weevil, *Apion corchori* Marsh., (Curculionidae). R. L. Tripathi and S. P. Bhattacharya (*Trop. Agric. Trin.*, 1970, **47** (1), 81–83. Engl.).—Seven applications of 0·04% at intervals of 10 days gave highest fibre yield but 0·03% at 15-day intervals was economically more sound. E. G. Brickell.

Review of insects that attack sugar-cane setts and impair germination. B. E. Hitchcock (*Tech. Commun. Bur. Sug. Exp. Stn*, 1968, (1), 24 pp. 86 ref.).—The review deals with moth borers, Dynastidae, Melolonthidae (white grubs), Elateridae (wireworms), termites and other miscellaneous pests. P. C. W.

***Oxalis acetosella*, a new host of the coffee root lesion nematode in South India.** A. C. Kumar, H. V. Shamanna and G. I. D'Souza (*Indian Coff.*, 1969, **33** (7), 227).— C. V.

Pathogenicity of *Hemicriconemoides* sp. to coffee. A. C. Kumar and G. I. D'Souza (*Indian Coff.*, 1969, **33** (7), 225–226).—An unidentified species was found to be a potential parasite inducing stunting of stem and root and reducing root wt. The nematodes multiplied nearly six times on Robusta and approx. four times on Arabica. C. V.

Assay of newer insecticides in the control of green bug on Arabica coffee seedlings. G. H. Venkataramaiah and G. I. D'Souza (*Indian Coff.*, 1969, **33** (1), 9–12. 16 ref.).—Folidol (parathion) gave the most effective control when applied as a foliar spray. Several granular insecticides were also studied. C. V.

Insecticide trials in tobacco seedbeds. 1962–1965. I. C. Cunningham (*Queensland J. agric. Anim. Sci.*, 1969, **26** (1), 99–106).—Azinphos-ethyl caused lamina puckering and vascular thickening and distortion in tobacco seedlings. Fenitrothion and trichlorophon treated plants were stunted and had dark circular leaves. Isobenzan caused intervascular and marginal distortion of the leaves while endrin applications had no phytotoxic effects. C. V.

Control of cyclodiene-resistant onion maggots in organic soils of south-western Quebec. J. P. Perron (*Phytoprotection*, 1968, **49** (2), 61–79).—Diazinon remained the most effective organophosphorus compound against *Hylemiya antiqua* (Meig.), whether applied as seed, furrow-granular or post-emergence treatment

(1 oz/lb of seed for wettable powder or 2 lb/acre for granular or emulsifible concentrates). Phytotoxicity of seed treatment was reduced by use of thiram 75 (0·4 oz/lb seed). Use of an adhesive carrier instead of water contributed to reduction of the phytotoxic effect. Treatment of seed three weeks before sowing was beneficial, but with longer times effectiveness decreased. C. V.

Biological studies of spider mites attacking cotton plants in west Tennessee. S. D. CALDWELL and S. E. BENNETT (*J. Tenn. Acad. Sci.*, 1968, **43** (2), 57–59).—The life history of *Tetranychus urticae* and other species is briefly suveyed, this being the first time that *Eotetranychus* has been found on cotton. *T. atlanticus* causes greater damage than any other species since it defoliates the plants. Mite defoliation could be beneficial if delayed until cotton is mature and the bolls open. Natural predators are very helpful as control agents. Indiscriminate insecticide application is considered to be partly responsible for population increase. Phorate, Kelthane and ethion are equally effective in the control of all stages of the mites. C. V.

Plants infected with root-knot nematodes in Rhodesia, Malawi and Zambia. G. C. MARTIN (*Rhodesia agric. J. Tech. Bull.*, 1969, No. 8, 14 pp. 21 ref.).—The galling response and host values of ~ 850 plants are tabulated. W.J. G.

New pimento fruit disorder associated with the feeding of the leaf-footed bug, *Leptoglossus phyllopus*. H. L. COCHRAN (*Proc. Am. Soc. hort. Sci.*, 1968, **93**, 470–475. 6 ref.).—Characteristics of the disorder are described and illustrated. A. H. Cornfield.

Potato production using resistant varieties on land infested with potato cyst-eelworm, *Heterodera rostochiensis* Wolf. F. G. W. JONES and D. M. PARROTT (*Outl. Agric.*, 1968, **5** (5), 215–222. 22 ref.).—Eelworm-resistant varieties of potato, which owe their resistance to a dominant gene from *S. tuberosum* sub-species *andigena*, provide an effective and economic method of pest control, superior to the previous method of crop rotation. In the roots of the potato, the majority of larvae of the eelworm develop into males, thus reducing possible reproduction. The effects of various sequences of rotation of resistant and susceptible potatoes, and other crops, on the eelworm population are reported in detail. W. J. G.

Control of *Aphis fabae* on broad beans (*Vicia faba*) by the systemic action of gamma-BHC, thionazin and aldicarb. R. F. SKRENTNY and J. A. ELLIS (*Pestic. Sci.*, 1970, **1** (2), 45–48. 20 ref.).—The in-row application of aldicarb (I) granules at 2 lb active ingredient (a.i.)/acre at planting gave complete control of *Aphis fabae* Scop. on broad beans (*Vicia faba* L. cv. Seville) up to 7 days before harvest and resulted in a three-fold increase in yield compared with a similar thionazin (II) treatment. Bean plants grown from seeds which were soaked in γ-BHC (III) soln. at 20 ppm for 24 h prior to planting were protected from this aphid for most of the growing season almost as effectively as with II. T.l.c. was used for detn. of I, and g.l.c. for detn. of II and III in plants and soils. Simultaneous bioassays were made with *A. fabae*. Sensitivity of *A. fabae* to II, III, I and its major metabolites (sulphone and sulphoxide) was detd. Toxicity studies with *A. fabae*, *Acyrthosiphon pisum* and *Megoura viciae* showed increasing sensitivity in that order to III at 1 ppm in bean plants. W. J. G.

New virus disease of the coconut rhinoceros-beetle in Western Samoa. K. J. MARSCHALL (*Nature, Lond.*, 1970, **225** (5229), 288–289. 6 ref.).—Damage to coconut palms was greatly decreased by infecting populations of *Oryctes rhinoceros* with the virus *Rhabdionvirus oryctes* by distributing a mixture of virus and sawdust over rotten coconut logs, in which the beetles breed. Of all beetle-grubs collected during January–May, ~ 58% died from the virus and ~ 36% from the entomophagous fungus *Metarrhizium anisopliae*; some synergistic effect is possible. Further trials, involving large-scale release of the virus, are in progress. W. J. Baker.

Phosphoric esters and compositions containing them. RHONE-POULENC S.A. (Inventor: M. SAULI) (Br. Pat. 1,175,634, 3.7.68. Fr., 3.7.67, 8.4.68).—Esters with insecticidal and acaricidal properties have formula $R^{II}CH_2SPS(OR^I)_2$ wherein R^I is alkyl of 1–4 C; R^{II} is 4-R-5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl; and R is H, alkyl or cycloalkyl of > 5 C, or Ph which may contain 1–2 halogen. An example is *OO*-Et_2 *S*-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl) methyl thiothionophosphate, obtained by stirring a mixture of $(OEt)_2PS_2NH_4$ and 2-chloromethyl-5-oxo-4,5-dihydro-1,3,4-oxadiazole in acetone for 8 h. F. R. Basford.

Diseases and Pests in Livestock;

Veterinary Treatments

Control of Exogenous Pests

Infra-red, ultra-violet, and visible spectrophotometric determination of Imidan [phosmot] in animal dips and sprays. F. P. CZECH, M. D. MACK and G. EVANS (*J. Ass. off. analyt. Chem.*, 1969, **52** (5), 1017–1026. 9 ref.).—The i.r. procedure is recommended as a general method for dips and sprays at levels of 0·05–0·9%-phosmot (*O,O*-dimethyl *S*-phthalimidomethyl phosphorodithioate. The aq. soln, satd. with NaCl is extracted with CS_2 and the extract is analysed using 11 bands at 1900–700 cm^{-1}. The more sensitive, but slower u.v. method is recommended for analysis (at 263 nm) of aq. emulsions contg. ~ 0·5% of I. The colorimetric method is suitable for trace analysis of 10–300 μg of I in filth-laden dips and sprays. It is based on the oxidn. of I to PO_4^{3-} which is detd. by the phosphomolybdate blue method. D. I. Rees.

Other Treatments

Effect of subclinical helminthosis on nitrogen metabolism in beef cattle. J. E. VERCOE and P. H. SPRINGELL (*J. agric. Sci., Camb.*, 1969, **73** (2), 203–209. 13 ref.).—When compared with Brahman × Hereford/Shorthorn cross steers, British (Hereford/Shorthorn cross) steers showed lower dry matter and N digestibilities, lower N balances and higher dialysable faecal N. Gastro-intestinal leakage (I), plasma content (II), total plasma protein (III), plasma protein half-life (IV), and plasma urea (V) were unaffected by breed differences. With helminths removed, I was reduced and II and III were increased. The Brahman crosses had lower values for III and higher for V; IV was unaffected by parasites. M. Long.

Grass tetany I. Influence of starch and peanut [groundnut] oil supplements on plasma magnesium, calcium and phosphorus levels in grazing dairy cows. G. F. WILSON, C. S. W. REID, L. F. MOLLOY *et al.* (*N.Z. Jl agric. Res.*, 1969, **12** (3), 467–488. 47 ref.).—No cases of clinical tetany were observed. Depression of plasma Mg was lessened by starch and increased by oil supplementation. E. G. Brickell.

Bloat in cattle XXXII. Attempts to prevent legume bloat in dry and lactating cows by partial or complete elimination of the rumen holotrich protozoa with dimetridazole. R. T. J. CLARKE, C. S. W. REID and P. W. YOUNG (*N.Z. Jl agric. Res.*, 1969, **12** (3), 446–466. 7 ref.).—Although dimetridazole (1,2-dimethyl-5-nitroimidazole) (I) can reduce or eliminate holotrichs in the rumen, bloat may still occur in some cows. This fact, combined with a reduction in food intake and milk production, indicates that I is of little practical value as a prophylactic. E. G. Brickell.

Chromatographic separation and fluorometric analysis of coccidiostat nequinate (Statyl) in feed mixtures. G. J. KROL and N. G. NASH (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1141–1146. 4 ref.).—Nequinate (methyl-4-hydroxy-6-butyl-7-benzyloxy-3-quinoline carboxylate) (I) was separated from interfering material by partition column chromatog. with use of Sephadex LH-20. Methanesulphonic acid (which enhanced fluorescence by 50-fold) was added and I was detd. fluorometrically. An av. recovery of 99·5% was obtained for ~ 20 ppm of I with standard deviation of 3·8%. D. I. Rees.

Biological properties of avian *Pasteurella*, its prophylaxis and therapeutic control. SAW BROWN E MAUNG and Y. M. EISSA (*Un. Burma J. Life Sci.*, 1969, **2** (1), 17–21. 11 ref.).—The main topics studied were (*i*) biol. properties of *Pasteurella multocida* strains isolated from fowls, (*ii*) the mode of entry, (*iii*) effect of vaccines and (*iv*) effect of therapeutic treatment on infected chicks. M. J. Rawlins.

Effects of 5-vinyl-2-oxazolidinethione (goitrin) on the radioiodine metabolism in growing chicks. T. MATSUMOTO, H. ITOH and Y. AKIBA (*Poult. Sci.*, 1969, **48** (3), 1061–1069. 20 ref.).—The comparative effects of goitrin and propylthiouracil on ^{131}I metabolism in male chicks is reported. A. H. Cornfield.

Balances of sodium and potassium in normal and bluecomb-diseased turkeys. H. E. DZUIK and O. A. EVANSON (*Poult. Sci.*, 1969, **48** (3), 961–963. 4 ref.).—Studies with fasted and unfasted normal and diseased turkeys showed that differences in balances and body concn. of Na^+ and K^+ between normal and diseased birds were caused by fasting. There were no indications that there

was a particular need for K^+ which would account for the beneficial effects of KCl treatment on bluecomb disease.
A. H. Cornfield.

Influence of several histomoniastats on performance of Large White turkeys. P. W. Waldroup, J. F. Maxey, R. E. Ivy *et al.* (*Poult. Sci.*, 1969, **48** (3), 978–985. 25 ref.).—Feeding 0·025% Histotat (4-nitrophenylarsonic acid) decreased wt. gains and feed efficiency to 24 weeks of age. 0·025–0·050% Carb-O-Sep (*p*-uredobenzenearsonic acid) had no adverse effects on the performance of poults to 24 weeks of age. 0·0075% Nifursol (3,5-dinitrosalicyclic acid-5-nitrofurfurylidene hydrazide) and 0·015–0·060% Emtrymix (1,2-dimethyl-5-nitroimidazole) increased body wt. to 6 weeks of age in some trials, but treatment effects disappeared by 24 weeks of age. A. H. Cornfield.

Reduction of corticosteroid side effects. Unimed Inc. (Br. Pat. 1,175,482, 15.1.68. U.S., 9.3.67).—The animal is treated simultaneously, or before or after administration of the steroid, with at least one of the following: β-(2 or 4-pyridyl)alkyl alkyl- and dialkylamines; 1-(2′- or 4′- pyridyl)-2,3-*cis* or *trans*-dicarboxylic acid cyclobutanes; α-picolinic acid dialkyl amides; and their non-toxic salts. S. S. Chissick.

Sanitation, Hygiene and Safety

General Sanitation, Pollution

Resistance potential of anopheline and culicine mosquitoes to cholinesterase inhibitors. G. P. Georghiou (*Wld Rev. Pest Control*, 1969, **8** (2), 86–94. 38 ref.).—Investigation was carried out into (*i*) extent of selection of anophelines by cholinesterase inhibitors and insect response to such selection, (*ii*) parallel cases in related genera, e.g., *Culex* and *Aedes*, and (*iii*) possible ecological and biochem. differences between *Anopheles*, *Culex* and *Aedes* which would explain differences in resistance in these genera.
W. J. G.

Salmonella excretion by pet terrapins. A. E. Jephcott, D. R. Martin and R. Stalker (*J. Hyg., Camb.*, 1969, **67**, (3) 505–510. 19 ref.).— C. V.

Salmonellae in sewage: A study in latent human infection. R. W. S. Harvey, T. H. Price, D. W. Foster and W. C. Griffiths (*J. Hyg., Camb.*, 1969, **67** (3), 517–523. 14 ref.).—A residential estate of 4000 persons containing neither industry nor retail butchers shops regularly showed the presence of this organism in the sewage. The range of serotypes was wide and some suggested an exotic origin. No overt infection was reported during the survey. C. V.

Brucellosis in Northern Ireland: a serological survey. D. G. McDevitt (*J. Hyg., Camb.*, 1969, **67** (3), 409–416. 29 ref.).—Sera from blood donors from various groups occupationally exposed to cattle were serologically examined. The findings suggest that transmission is by occupational exposure rather than by drinking. C. V.

Some rodenticidal properties of coumatetralyl. J. H. Greaves and P. Ayres (*J. Hyg., Camb.*, 1969, **67** (2), 311–315).—3-(α-Tetralyl)-4-hydroxycoumarin (**I**) is an anti-coagulant used extensively in Germany. The present study was designed to investigate the toxicity in Warfarin (**II**)-resistant and -susceptible rats (*Rattus norvegicus* Berk.) and compare its palatability with **II** acetonylbenzyl deriv. Medium oatmeal + 0·1% **I** was not markedly less palatable than the control but **II** at 0·05% was less readily eaten. **I** at 0·05% and 0·005% was about as toxic as **II** at 0·005% with non-resistant rats. **II**-resistant rats were significantly less susceptible to **I**. It is considered that **I** may give good results in **II**-resistant infestations but it is possible that eventually there may be an increase in the incidence of resistance to both **I** and **II** anticoagulants. C. V.

Acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. V. M. Brown, D. H. M. Jordan and B. A. Tiller (*J. Fish Biol.*, 1969, **1** (1), 1–9. 22 ref.).—Initially it was considered that the effect of a mixture of similar poisons in water was the summation of the individual toxic fractions and this could be extended to include poisons with dissimilar chem. and pharmacolog. properties. It was found that concn. of NH_3, Zn and NH_3 + Zn which were lethal to 50% of a batch of trout within 48 h were similar whether fish were immersed in const. concn. or in concn. fluctuating within ± 50% of the 48 h LC_{50} at equal intervals of time, as long as the periodicity of the fluctuations did not exceed the resistance time (the period of exposure after which irreversible changes occur) for the particular poison involved. The cause of observed variations is discussed. C. V.

Tobacco, a sensitive monitor for photochemical air pollution. W. W. Heck, F. L. Fox, C. Stafford Brandt and J. A. Dunning (*Natn. Air Pollut. Control Admin.*, 1969, AP-55, 23 pp.).—A technique is described in which the sensitive tobacco variety, Bel W3 is used as a monitor for photochem. air pollution. The plant can be used as an indicator of the oxidant complex in both urban and rural areas. Two pilot studies were carried out over a 3-yr period to determine, (*i*) sensitivity of tobacco to oxidant concn. in urban areas, (*ii*) the possibility of estimating frequency of injury, (*iii*) relationship between oxidant concn. and quant. estimate of injury, (*iv*) best soil mix and nutrient combination for max. plant sensitivity, (*v*) growth interactions and shade factors.
W. J. G.

Food Hygiene and Safety

Food-borne infections and intoxications: A four-year (1965–1969) summary report. Issued by the Food Protection and Toxicology Center, University of California, Davis, November 1969.
P. P. R.

Microbiological standards for food: public health aspects. B. C. Hobbs and R. J. Gilbert (*Chemy Ind.*, 1970, (7), 215–219. 16 ref.).—A critical review is presented of tests for detecting, identifying and measuring the no. of micro-organisms in foods, viz. colony, coliform and streptococcal counts, control of pathogens (salmonellae, staphylococci and anaerobes) tests for cross-contamination and efficiency of cleaning. Problems in ensuring safety and quality through microbiol. standards are indicated.
W. J. Baker.

Microbiological quality control in the food industry. D. A. A. Mossel (*J. Milk Fd Technol.*, 1969, **32** (6), 155–171. 163 ref.).— C. V.

Rôle of preliminary dilution in the quantitative bacteriological examination of foodstuffs. E. Novel (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1969, **60** (2), 121–138. Fr., 18 ref.).—Dilution (*D*) procedures are critically examined and an exptl. investigation of the effects of *D* on calc. bacterial count is reported. For low microbial populations the calc. initial count increases progressively with successive tenfold *D*, finally dropping suddenly to zero at high *D*; for high populations, the initial count calc. from successive *D* rises to a max. and then progressively falls to zero. It is concluded that the apparent increase in count is mainly detd. by the min. vol. of nutrient medium necessary to permit one organism to develop into a colony; diminution in calc. initial count with successive *D* is ascribed to retention of organisms on surfaces of vessels, pipettes, etc. E. C. Apling.

Surveillance methods for viruses in foods. D. O. Cliver and J. Grindrod (*J. Milk Fd Technol.*, 1969, **32** (11), 421–425. 27 ref.).—Methods reviewed are (*i*) inoculation of living host, e.g., tissue cultures or lab. animals, by both direct testing and testing after concn. and (*ii*) removal of enteroviruses from food surfaces.
W. J. G.

Investigation of volatile reducing substances as an indicator of decomposition for raw and processed foods. B. R. Moorhouse and H. Salwin (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1135–1141. 15 ref.).—The volatiles are stripped from an aq. extract of the sample by aeration, and then passed through an alk. $KMnO_4$ soln.; the unconsumed $KMnO_4$ is detd. by titration with $Na_2S_2O_3$ after addn. of KI. The method was applied to ground beef, shrimp and peaches. Content of volatile reducing substances in raw samples increased as the food decomposed; they were partly lost during cooking and completely lost during freeze-drying. The method is considered suitable as an index of decomp. of raw foods only. D. I. Rees.

Faecal streptococci in industrially processed food – An incidence study. N. F. Insalata, J. S. Witzeman and F. C. A. Sunga (*Fd Technol., Champaign*, 1969, **23** (10), 1316–1318. 18 ref.).—Data from 5719 samples of processed food are presented. Enterococci were recovered from 10·6, coliforms from 8·5 and *Escherichia coli* from 0·3% of the samples; 4·8% of the samples contained both enterococci and coliforms. Frozen foods showed the highest frequency of occurrence for both faecal streptococci and coliforms. Results indicate that the value of enterococci as indicators of faecal contamination may depend on type of product and/or process being examined. I. Dickinson.

Detection of micro-organisms in foods – *Clostridium perfringens*. H. E. HALL (*J. Milk Fd Technol.*, 1969, **32** (11), 426–430. 30 ref.).—Cultural methods for detection and enumeration of the organism, which may cause illness if present in sufficient amt., are briefly reviewed. Alternative methods, involving cultural or serological examination of faeces, or direct examination of food, are discussed. W. J. G.

Microbiological standards for dairy products. W. A. COX (*Chemy Ind.*, 1970, (7), 223–229. 31 ref.).—Routine microbiol. tests on dairy products are reviewed, together with new and recently modified standards. Specific applications of these tests and standards to milk, cream, butter, cheese, conc. or sterilised milk products and milk powder are discussed critically, with respect to bacteriological data and process control during production. W. J. Baker.

Microbiology of imitation and dairy products and their components. C. A. SMUTZ, V. D. FOLTZ and R. MICKELSEN (*J. Milk Fd Technol.*, 1969, **32** (11), 446–447. 13 ref.).—84 imitation and filled milk products, and 31 components used in their manufacture, were examined for total plate count, coliform count and the incidence of salmonellae and staphylococci. W. J. G.

Bacteriological quality of pasteurised milk from retail sources. R. M. MCLARTY and J. ROBB (*Dairy Inds*, 1969, **34** (3), 134–138. 22 ref.).—Samples of pasteurised milk obtained through various retail sources, i.e., shop, doorstep supply, vending machine, canteen etc., were subjected to various tests: (*i*) mesophilic and psychotrophic counts, (*ii*) coliform tests, (*iii*) thermoduric counts and (*iv*) phosphatase test. Only 1·2% failed the phosphatase test and 13% failed the coliform test. Other tests showed the presence of considerable post-pasteurisation contamination. W. J. G.

Resazurin test paper method for determining the sanitary quality of raw milk. G. OTSUKA and T. NAKAE (*J. Dairy Sci.*, 1969, **52** (12), 2041–2044. 3 ref.).—Expt. results showed a high correlation between the standard plate count of raw milk and the resazurin reduction time, using resazurin-impregnated filter paper. M. O'Leary.

Source of bacteria in fresh cream and the methylene blue reduction test as a guide to hygienic quality. H. R. JENKINS and R. J. HENDERSON (*J. Hyg., Camb.*, 1969, **67** (3), 401–408. 13 ref.).—129 samples were examined: 60 (46·5%) had counts > 100,000 bacteria/ml, the commonest being *Bacillus* spp. (aerobic spore formers), Gram-negative bacilli, staphylococci and micrococci. As most creams had been either pasteurised as cream or produced from pasteurised milk, it was probable that these organisms resulted from unsatisfactory or unhygienic premises, unsuitable equipment or manual handling during filling and capping. Methylene blue tests related well with bacterial counts but there were seven (5·4%) anomalous results. C. V.

Salmonellae and the fluorescent-antibody technique. J. M. GOEPFORT and N. F. INSALATA (*J. Milk Fd Technol.*, 1969, **32** (12), 465–473. 35 ref.).—Applications of the direct method, involving staining of the salmonellae with fluorescein-conjugated salmonella serum, and the indirect method, involving first exposure of the smear to untagged salmonella serum and secondly application of a fluorescein-conjugated antiglobulin, are reviewed, considering esp. the application to food and feed analysis. Problems, advantages and disadvantages encountered are discussed in detail. W. J. G.

Bacteriological control of ice cream manufacture. I. Introduction and raw materials. II. Processing and finished products. III. Interpretation of results—plant and personal hygiene. T. P. LLOYD (*Dairy Inds*, 1969, **34** (4), 199–202; (5), 271–274; (6), 363–366. 10 ref.).— W. J. G.

Effects of time and temperature on salmonellae in inoculated butter. J. E. SIMS, D. C. KELLEY and V. D. FOLTZ (*J. Milk Fd Technol.*, 1969, **32** (12), 485–488. 10 ref.).—Three batches of butter, made from commercial cream, were contaminated with *Salmonella typhimurium* by inoculation of the cream and wash water. The contaminated butter was kept at 77, 40, 32, 0 and −10°F for 10 weeks. An increase in *Salmonella* was observed at 77° and a decrease at ⩽ 40°. The effect of variation of salt content was also examined. W. J. G.

Factors affecting survival of *Salmonella* in Cheddar and Colby cheese. R. E. HARGROVE, F. E. MCDONOUGH and W. A. MATTINGLY (*J. Milk Fd Technol.*, 1969, **32** (11), 480–484. 10 ref.).—Seven samples of Colby and 65 samples of Cheddar cheese were manufactured using artificially contaminated milk. The effects of milk pasteurisation, size of starter inoculum, titratable acidity and cheese pH, type of lactic culture, chem. additives, salt and moisture content, supplemental cheese micro-organisms and curing temp. were examined. W. J. G.

[Salmonella] contamination of Ceylon desiccated coconut. K. MEEDENIYA (*J. Hyg. ,Camb.*, 1969, **67** (4), 719–729).—The source was animal contamination on the mill premises which passed through the successive stages of preparation and into the cutter which became the focal point. Some organisms survived the drying stage. Two strains of *S. seftenberg* were the most frequent contaminants. C. V.

Salmonellas, shigellas and enteropathogenic *Escherichia coli* in uncooked food. T. VELAUDAPILLAI, G. R. NILES and W. NAGARATNAM (*J. Hyg., Camb.*, 1969, **67** (2), 187–191. 10 ref.).—Centrifuged deposits of washings of vegetables and fruits were examined. The extent of contamination of vegetables growing to a height ~ 1 ft from ground was greater than in the taller varieties. Degree of contamination amongst fruit was almost negligible. Sources of contamination (irrigation, manure, handling) are briefly discussed. C. V.

Estimation of aflatoxin in corn by 'dockage' assay. R. M. JOHNSON, W. T. GREENWAY and W. P. DOLAN (*J. Ass. off., analyt. Chem.*, 1969, **52** (6), 1304–1306. 7 ref.).—Dockage, i.e., broken maize, foreign matter and chaff, was separated by aeration from the contaminated maize sample; analysis of dockage and dockage-free maize showed levels of ~ 130 and 20 μg/kg of aflatoxin B_1, resp. Samples of maize to which contaminated maize had been added, were analysed and in all cases, except one, only traces of aflatoxin were found in dockage-free maize whilst appreciable amt. were found in dockage; the exception contained 61% of dockage. Analysis of dockage is thus recommended as a sensitive means of detecting aflatoxin in maize samples. D. I. Rees.

Collaborative study on a method for detection of aflatoxin B_1 in green coffee beans. C. P. LEVI (*J. Ass. off. analyst. Chem.*, 1969, **52** (6), 1300–1303. 5 ref.).—Green coffee beans contg. from 0–79 μg/kg of added aflatoxin B_1 were analysed by the Florisil column clean-up procedure of Scott (*ibid.*, 1968, **51**, 609), followed by the visual comparison of spots after t.l.c. on a SiO_2 gel layer. No aflatoxin was detected in the blank sample but recoveries of added aflatoxin ranged from 50–75%. Low recoveries were thought to be due to insufficient deactivation of the Florisil. D. I. Rees.

Stimulation of gas production and growth of *Clostridium perfringens* Type A (No. 3624) by legumes. L. B. ROCKLAND, B. L. GARDINER and D. PIECZARKA (*J. Fd Sci.*, 1969, **34** (5), 411–414. 21 ref.).—Dry beans contain an unidentified factor which stimulates (*a*) growth, and (*b*) gas production by the intestinal anaerobe, *C. perfringens* Type A. Both (*a*) and (*b*) were inhibited by antibiotics which block flatulence in higher animals. An assay was developed to estimate the response of micro-organisms to various substrates, at four times their natural levels, in a synthetic basal medium. These had no effect, but dry bean homogenates caused prolific gas production. M. J. Rawlins.

Cross-contamination by cooked-meat slicing machines and cleaning cloths. R. J. GILBERT (*J. Hyg., Camb.*, 1969, **67** (2), 249–254).—Counts were made of contaminating organisms, these being recorded for the 1st to the 50th successive slices of chopped pork, corned beef, brisket and salami. The importance and method of cleaning the slicing machine and using preferably disposable paper is stressed. C. V.

Bacteriological standards for fish and fishery products. J. M. SHEWAN (*Chemy Ind.*, 1970, (6), 193–199. Engl., 76 ref.).—Factors involved in establishing standards, specifications or limits to ensure high edible quality, long shelf-life, and freedom from toxins, etc., of products reaching the consumer are discussed extensively. The possible incidence of poisoning through *Staphylococcus aureus, Salmonella, Clostridium botulinum and Vibrio parahaemolyticus*, etc., is examined on the basis of quant. data. Suggested standards, based mainly on viable plate counts at 35–37°, are evaluated with respect to different products; the need for more rigorous standards for precooked products is emphasised. W. J. Baker.

Necessary degree of sterility in canned foods. B. L. FLAUMENBAUM (*Pishch. Tekhnol.*, 1969, [5 (72)], 93. Russ.).— C. V.

Peculiarities of thermophilic obligate-anaerobic micro-organisms causing swell damage in canned foods. M. V. ZALASHKO and I. Y. OVRUTSKAYA (*Pishch. Tekhnol.*, 1969, [5 (72)], 97. Russ., 11 ref.).— C. V.

Contamination by Pesticides, Pesticide Toxicity

Analysis of multiple pesticide residues—chemical identity and confirmation of results. K. A. McKULLY (*Wld Rev. Pest Control*, 1969, **8** (1), 59–74. 125 ref.).—Methods of residue analysis are reviewed and include: spectrophotometry; spectrometry; paper chromatog.; t.l.c.; liquid/liquid partition; column chromatog.; chem. reaction; and bioassay. W. J. G.

Biological effects of pesticides. (*Ann. N.Y. Acad. Sci.*, 1969, **160** (1), 1–422).—Apart from a series of papers (6) on the general aspects, the following are specially noted: **Summary on instrumentation in pesticide analysis.** H. P. BURCHFIELD. **Current status of pesticide residue methodology.** F. A. GUNTHER (53 ref.). **Non-metabolic decomposition of pesticides.** D. G. CROSBY (53 ref.). **Metabolism of insecticides in plants and animals.** T. R. FUKUTO and R. L. METCALF (80 ref.). **Metabolism of fungicides and related compounds.** R. G. OWENS. (70 ref.). **Metabolism of herbicides.** V. H. FREED and M. L. MONTGOMERY (31 ref.). **Pesticide residues in the atmosphere.** Z. JEGIER (24 ref.). **Pesticide residues in soils, water and crops.** E. P. LICHTENSTEIN (14 ref.). **Pesticides in wild animals.** E. H. DUSTMAN and L. F. STICKEL (82 ref.). **Pesticide residues in foods.** R. E. DUGGAN (22 ref.). **Body burden of pesticides in man.** W. F. DURHAM (53 ref.). **Phosphorylation and carbamylation of cholinesterase.** R. D. O'BRIEN (25 ref.). **Alkylating chemicals and the reproductive cells.** H. JACKSON and A. W. CRAIG (25 ref.). **Genetic effect of the biological alkylating agents with reference to pesticides.** O. G. FAHMY and M. J. FAHMY (36 ref.). **Influence of chlorinated hydrocarbons and organophosphate insecticides on metabolism of steroids.** D. KUPFER (27 ref.). **Insecticides and microsome enzymes.** J. C. STREET (61 ref.). In a further 12 papers, nutritional interactions, the teratogenic effects and mutagenic effects of captan, haematological effects, the occurrence, diagnosis and treatment of organophosphate pesticide poisoning in man, etc., are discussed. C. V.

Fate of diazinon in field-sprayed agricultural crops, soil and olive oil. D. O. EBERLE and D. NOVAK (*J. Ass. off. analyt. Chem.*, 1969, **52** (5), 1067–1074. 22 ref.).—MeOH extracts of crops and soil, to which H_2O and NaCl had been added, were extracted with CH_2Cl_2. Cyclohexane soln. of olive oil were extracted with MeCN and washed with C_6H_{14}. The diazinon present was purified on Al_2O_3 and analysed by g.l.c. using a packed column contg. SE-30, XE-60 or QF-1 as stationary phase, and various detectors. Limits of detection were 0·01–0·02 ppm. Possible metabolites with strong cholinesterase-inhibiting activity were detected by t.l.c. at the 0·002 ppm level. The only metabolite detected in crops at harvest was oxodiazinon at the ~0·002 ppm level.
D. I. Rees.

Cucumber bioassay test for soil residues of certain herbicides. C. C. DOWLER (*Weed Sci.*, 1969, **17** (3), 309–310. 8 ref.).—A cucumber bioassay test based on assessing visual injury ratings after 28 days of growth in test soils could detect 0·01 ppm bromacil, dicamba, diuron, fenac, picloram or prometone in soils. Visual injury scores increased with level of soil herbicide up to ~2 ppm. There were high negative correlations between injury ratings and fresh plant wt. A. H. Cornfield.

Trifluralin persistence as affected by depth of soil incorporation. K. E. SAVAGE and W. L. BARRENTINE (*Weed Sci.*, 1969, **17** (3), 349–352. 5 ref.).—Persistence in soil increased with depth of incorporation; rate of loss by volatilisation decreased with increasing depth of application of the herbicide.
A. H. Cornfield.

Behaviour of pyrazon in soil. R. FRANK and C. M. SWITZER (*Weed Sci.*, 1969, **17** (3), 323–326. 4 ref.).—Pyrazon decomposed in soil at an exponentially decreasing rate characteristic of the activity of soil micro-organisms. There was negligible loss through chem. decomp. or leaching. The 0–3 in. layer of soils treated with pyrazon was slightly toxic to turnip seedlings even 120 days after application. Pyrazon residues in sugar-beet plants receiving post-emergence applications of the weedicide were < 0·1 ppm (dry basis) 6 weeks after application of 8 lb pyrazon per acre.
A. H. Cornfield.

Vapour density of soil-applied dieldrin as related to soil-water content, temperature, and dieldrin concentration. W. F. SPENCER, M. M. CLIATH and W. J. FARMER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 509–511. 9 ref.).—In a silt loam containing more than one mol. layer of water, the vapour density (v.d.) of dieldrin increased with soil dieldrin concn. until a saturation v.d. equal to that of dieldrin without soil was reached at ~25 ppm. The v.d. of dieldrin fell to very low values when water content fell below one mol. layer, but increased again as water was added to dry soil. This study relates to dieldrin persistence in soils.
A. H. Cornfield.

Herbicide residue in soil when applied to sorghum in a winter wheat–sorghum–fallow rotation. G. A. WICKS, C. R. FENSTER and O. C. BURNSIDE (*Agron. J.*, 1969, **61** (5), 721–724. 7 ref.).—When atrazine and propazine (1·12–8·96 kg/ha) were applied at 11 locations over 7 yr, grain yields from winter wheat sown 15 months after treatment were generally unaffected, compared with hand-weeded sorghum. A. H. Cornfield.

Extraction of hydroxyatrazine from soil. R. J. HANCE and G. CHESTERS (*Analyst, Lond.*, 1970, **95** (1126), 106. 4 ref.).—A radiochem. procedure for recovering ~90% of the hydroxyatrazine (**I**) is described. After addn. of ^{14}C-labelled **I**, the soil is set aside for ≮ 3 days and then treated with 0·1 N-HCl. After centrifugation, the soln. is added to 1,4-dioxan plus the Bruno–Christian scintillation mixture (cf. *Analyt. Chem.*, 1961, **33**, 1216); ^{14}C-activity is detd. with a liquid scintillation spectrometer. The soil residue is heated under reflux first with MeCN–H_2O (9 : 1) and then, after filtration, with MeCN–H_2O-conc. aq. NH_3 (90 : 9 : 1). The two filtrates are combined, conc. to remove MeCN, and radioactivity is detd. as before. Degradation of chlorotriazine herbicides in soil is believed to result in the corresponding **I**.
W. J. Baker.

Spectrophotometric determination of propoxur residues on vegetable matter. W. F. VAN GILS (*Analyst, Lond.*, 1970, **95** (1126), 88–90. 16 ref.).—Alk. hydrolysis of the active ingredient 2-isopropoxyphenyl-*N*-methylcarbamate yields 2-isopropoxy-phenol (**I**), which is then coupled with 2,6-dibromo-*p*-benzoquinonechloroimide at pH 7 to form a blue indophenol deriv., the extinction of which is measured against a blank at 595 nm; down to ~0·1 mg of propoxu per kg of, e.g., lettuce, can be detd. from a calibration graph.
W. J. Baker.

Artifacts of dieldrin in gas chromatographic analysis of green plant material. D. E. GLOFELTY and J. H. CARO (*Analyt. Chem.*, 1970, **42** (2), 282–284. 14 ref.).—The artifacts, probably xanthophyll esters, are not easily detected under normal g.l.c. conditions and are resistant to common clean-up procedures for plant material. Possible methods of: (*i*) separating single artifacts, e.g., by g.l.c. using DC-200 and QF-1 on 100–120 mesh Gas Chrom Q at 160° or 220° and at a flow rate (Ar:CH_4=95:5) of 145 or 30 ml/min, with a ^{63}Ni electron capture detector and (*ii*) removing both artifacts, e.g., by saponification clean-up, are discussed but neither appears completely satisfactory. S. S. Chissick.

Metabolism of pyrazon in sugar-beet and soil. G. R. STEPHENSON and S. K. RIES (*Weed Sci.*, 1969, **17** (3), 327–331. 7 ref.).—Studies with ^{3}H- and ^{14}C-labelled (at 4 and 5 positions) pyrazon applied to soil showed that total radioactivity recoverable by sugar-beet plants decreased with time, and there was a greater loss of ^{3}H- than of ^{14}C-labelled compd. from soil. One metabolite of pyrazon was identified in the soil and 3 in the plant.
A. H. Cornfield.

Hazards to wildlife from the use of DDT in orchards. S. BAILEY, P. J. BUNYAN, D. M. JENNINGS and A. TAYLOR (*Pestic. Sci.*, 1970, **1** (2), 66–69. 13 ref.).—An investigation into the poisoning of birds with 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) on a fruit farm in South-East England is described. Pesticide levels in wild birds were sufficient to be held responsible for deaths. It is suggested that levels in samples of soil, earthworms, sludge, eggs, etc. did not indicate the source of the poisoning. Examination of caged birds following exposure to normal spraying indicated little hazard from this type of operation. Assessment of levels of intoxication is discussed. W. J. G.

Rapid gas-liquid chromatographic method for determining residues of fenthion, disulfoton and phorate in corn, milk, grass and faeces. M. C. BOWMAN and M. BEROZA (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1231–1237. 10 ref.).—The parent compd. and its metabolites are oxidised to a single compd. with *m*-chloroperbenzoic acid. Oxygen analogue sulphones of the titled compd. are analysed by g.l.c. on a packed column contg. 10% of OV-101 as stationary phase, at 215° with flame photometric detection. Recoveries of ~96% were obtained for fenthion and 74 to 82% for disulfoton and phorate. D. I. Rees.

Effect of stage of gestation during contamination on storage and excretion of dieldrin by dairy heifers. D. G. BRAUND, L. D. BROWN, J. T. HUBER *et al.* (*J. Dairy Sci.*, 1969, **52** (12), 1988–1997. 24 ref.).—Pregnant Holstein heifers were contaminated for 60 days

with 0·1 mg of dieldrin/kg of body wt. beginning either 60, 120, or 180 days before the expected calving date. Body fat of the 120- and 180-day groups contained two and three times, resp., more 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene (HEOD) than the 60 day group. Very little HEOD was eliminated from pregnant heifers between contamination and parturition. Three different dietary treatments, fed *postpartum*, had no effect on rate of residue decline. In all cases, residue levels decreased to one-half the initial level after three weeks and to one-third after five weeks. About 2·5% of the daily intake was excreted as unchanged HEOD in the faeces, during contamination, but only traces in the urine. The presence of HEOD in faeces, 30 days after cessation of contamination, is considered to indicate metabolic origin and recycling.
M. O'Leary.

Excretory pathway of terbacil (sinbar) in lactating cows. W. H. Gutenmann and D. J. Lisk (*J. Lisk* (*J. agric. Fd Chem.*, 1969, **17** (5), 1011–1013. 5 ref.).—At terbacil (**I**) levels of 5 and 30 ppm in the feed, **I** was excreted in the milk at levels up to 0·03 and 0·08 ppm, resp. No residues of **I** were found in urine or faeces. **I** was stable when incubated with fresh rumen fluid, beef liver homogenate and the 10,000-*g* supernatant microsomal fraction of beef liver. I. Dickinson.

Pesticide residues in fat of cattle treated with backrubbers impregnated with coumaphos, methoxychlor or DDT. E. M. Burnett (*Pestic. Sci.*, 1970, **1** (2), 70–72. 13 ref.).—Cattle were treated thrice weekly for 12 weeks with simulated backrubbers impregnated with 1% soln. of pesticides in mineral oils. Both coumaphos and methoxychlor, applied in S.A.E. 50 oil, induced max. residues of 0·21 ppm in omental fat after 12 weeks treatment. Application of DDT in S.A.E. 50 oil or in used crankcase oil induced residues of 0·5–7 ppm DDT in omental fat after 12 weeks treatment. Where diesel fuel oil was the vehicle, DDT residues in omental fat were in the range 17–26 ppm. W. J. G.

Gas–liquid chromatographic determination of dichlorvos in milk, eggs and various body tissues of cattle and chickens. M. C. Ivey and H. V. Claborn (*J, Ass. off. analyt. Chem.*, 1969, **52** (6), 1248–1251).—Extraction and clean-up procedures are described. Hexane extracts were analysed on a conventional glass column contg. 10% of DC-200 at 170° with flame photometric detection. Down to ~ 0·002 ppm could be detected and recoveries of 79–87% were obtained from fat, muscle and chicken skin, 93–97% from milk and 77% from eggs. Very low recoveries were obtained for **I** added to liver and kidney tissues which indicated that it was not present *per se* in these tissues. D. I. Rees.

Effect of high levels of dietary DDT on egg production, mortality, fertility, hatchability and pesticide content of yolks, for Japanese quail. S. I. Smith, C. W. Weber and B. L. Reid (*Poult. Sci.*, 1969, **48** (3), 1000–1004. 8 ref.).—Addn. of 100–200 ppm DDT to the diet had no effect on egg production or mortality, or on hatchability or fertility of eggs, but yolk DDT% increased with level of dietary DDT. 400 ppm dietary DDT caused 50% mortality in the adult quail and markedly decreased fertility and hatchability. Day-old chicks exhibited marked ataxia and spasms. Yolk DDT and DDE contents increased with time of feeding of the dams with DDT. A. H. Cornfield.

Planimetric determination of *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD in milk fat. H. Gertig, K. Laskowski, W. Nowaczyk *et al.* (*Chemia analit.*, 1969, **14** (4), 853–859. Pol., 30 ref.).—Residues of DDT, DDE and DDD were detd. planimetrically after t.l.c. separation from milk fat. The sample of butter was melted, filtered and dried, the fat was decomposed with oleum, and DDT and its metabolities were then extracted into light petroleum. The org. extract was conc., subjected to t.l.c. on Al_2O_3 contg. 10–15% of gypsum, and the chromatograms were developed with n-heptane. Spots of DDT, DDE and DDD were located by u.v. and the Morley–Chiba reagent (*J. Ass. off. analyt. Chem.*, 1964, **47**, 306). 0·2 μg of the compd. can be detected on the chromatogram; this value corresponds to 0·08 mg content per kg of the fat. Recovery was DDT 93, DDE 91 and DDD 88%.
B. Kaminski.

Head space gas procedure for screening food samples for dithiocarbamate pesticide residues. H. A. McLeod and K. A. McCully (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1226–1230. 20 ref.).—10N-H_2SO_4 was added to the food sample in a container closed with a serum cap; after standing for 30 min at 66°, the CS_2 evolved was analysed by g.l.c. on a glass column of 10% of SE-30 on 80–100 mesh Chromosorb W at 60° with N_2 carrier gas and flame photometric detection. Ethylene bisdithiocarbamates, e.g., maneb, failed to give the above reaction, but CS_2 evolution was successful when the acid was replaced by a 1·5% soln. of $SnCl_2$ in 4N-HCl. Recoveries of > 80% were obtained at 3·5 and 7 ppm except for nabam (44 to 83%). D. I. Rees.

Uptake of chlordane vapours by foodstuffs of low moisture content. Chee Tee Yeo and A. Benvenue (*J. Ass. off. analyt. Chem.*, 1969, **52** (6), 1206–1213). 10 ref.).—Wheat flour, polished rice and cane sugar were exposed to chlordane (**I**) vapours for up to 90 days. Flour and rice, in bulk form, adsorbed 3 times more **I** than did the sugar. Flour packaged in Kraft paper and polyethylene bags contained only $\frac{1}{3}$ and $\frac{1}{18}$ of the amt. found in bulk samples, resp. **I** residues were isolated from flour and rice by CH_3CN–H_2O extraction and from sugar by a petroleum ether extraction from aq. soln.; both extracts were cleaned up on Florisil columns before g.l.c. analysis. D. I. Rees.

Acute oral toxicity of chlordane in albino rats. E. M. Boyd and F. I. Taylor (*Ind. Med. Surg.*, 1969, **38** (12), 42–49. 29 ref.).—Lab. animals fed on a low protein diet, developed toxicity to chlordane (*C*) more readily than those on a normal diet; death occurred 4 days after administration. Compared with other pesticides, *C* did not induce the degree of susceptibility caused by other pesticides, notably captan, carbaryl and parathion, and consequently might not present a particular hazard in low-diet countries. C.V.

Combating micro-organisms. Allied Chemical Corp. (Br. Pat. 1,175,180, 13.9.68. U.S., 15.9.67).—The use of diketene (**I**) as a fumigant for treating rooms, animal quarters, fabric etc., is claimed. It can be introduced as a liquid and allowed to evaporate, mixed with a propellant and sprayed, or carried in a stream of inert gas, and is active against the vegetative spore forms of *S. aureus*, *B. globigii*, etc. **I** is most effective in atm. of r.h. > 90%, and can be used as a mixture with e.g., ethylene oxide. S. S. Chissick.

4.—MISCELLANEOUS

DMSO: Agricultural solvent, penetrant carrier and antivirial agent. B. C. Smale (*Sulphur Inst. J.*, 1969, **5** (3), 2–6. 45 ref.).—A review of dimethylsulphoxide is given which includes examples of its use with indicated agricultural toxicants. C. V.

Use of kaolin-coated slides for collection of agricultural-spray deposits. J. R. Lake (*Chemy Ind.*, 1970, (7), 233–236. 6 ref.).—A critical study of dye-impregnated kaolin-coated targets was made with respect to kaolin thickness (500–1400 μm), stain dia., drop velocity and general validity of the method. The slides are of limited value in estimating drop-size of deposits from nozzles used normally in ground crop-spraying. Under field conditions, provided that the largest drops are small enough (< ~ 1000 μm) to prevent shattering on impact, the technique is virtually confined to sampling, e.g., aircraft-application, where drops are travelling at near terminal velocities at ground level. In the lab., twin-flash photography of drops in flight (for subsequent measurement) is preferable to the coated-slide technique. W. J. Baker.

Determination of sulphur in biological materials. P. R. Bird and R. D. Fountain (*Analyst, Lond.*, 1970, **95** (1126), 98–102. 14 ref.).—For dry samples complete oxidn. of all S to SO_4^{2-} with $NaHCO_3$–Ag_2O at 550°, is followed by reduction to H_2S with HI–formic acid–H_3PO_2. The evolved H_2S is absorbed in N-NaOH and titrated with Hg(II) acetate in presence of dioxan–acetone as visual indicator. For material in soln., H_2S is released by addn. of HCl. Precautions necessary for samples with high SO_4^{2-} content are indicated and specific procedures are described for detn. of total S, total SO_4^{2-}-S, ester SO_4^{2-}-S, neutral-S and inorg. SO_4^{2-}-S, in., e.g., urine, faeces, blood, etc. The only serious interference is from Se. The method can be used in combination with liquid scintillation counting of ^{35}S. W. J. Baker.

Lead accumulation in roadside soil and grass. T. J. Chow (*Nature, Lond.*, 1970, **225** (5229), 295–296. 4 ref.).—Concn. of Pb in grasses alongside U.S. motorways were 20–60 ppm and were max. in grass adjacent to the road edges. Contents (290–825 ppm) of Pb in grass ashes were 2- to 12-fold those in corresponding soils at 5–15 cm depth. The different isotopic compn. of Pb at surface and at depth, and the similarity in compn. of Pb in the grass, on

the surface-soil and in the regional petrol, indicate that the excess Pb derives from motor-vehicle exhaust. Part of the Pb could be due to direct fall out of Pb aerosols. W. J. Baker.

Gas chromatography collection syringe for volatile compounds [from food products]. R. E. HURST (*Chemy Ind.*, 1970, (3), 90–92. Engl.).—The combined collection/injection syringe described permits rapid and reproducible trapping of volatiles, from, e.g., fish products, in sufficient concn. for spectral analysis after separation by g.l.c. The volatiles are drawn in a stream of N_2 into the bore of the syringe which is immersed in liquid N_2 in a polyurethane-insulated Al reservoir. After insertion of the plunger, the syringe is sealed, withdrawn from the cold-jacket and then heated to vaporise the condensate for injection on to the g.l.c.
W. J. Baker.

Pulse irradiated bacterial survival in the presence of nitric oxide. D. L. DEWEY and B. D. MICHAEL (*Radiat. Res.*, 1969, **39** (1), 82–89. 16 ref.).—NO, like O_2, sensitises biol. material to ionising radiation and is used up during radiation. Unlike O_2 there is a post-NO treatment effect both at high and low doses and a high concn. of NO fails to sensitise at low dose rates but possesses the power to sensitise at high ones. C. V.

5.—RECENT BOOKS AND JOURNALS

Isotopes and radiation in soil organic matter studies. INTERNATIONAL ATOMIC ENERGY AUTHORITY. 1968, 584 pp. (Vienna: International Atomic Energy Authority).—Proceedings of Symposium held in Vienna, July 1968. S. C. H.

Physiology of plant growth and development. Ed. M. B. WILKINS. 1969, 695 pp. (New York, London, etc.: McGraw-Hill). S. C. H.

The biogenesis of starch granules in higher plants. N. P. BADENHUIZEN. 1969, 121 pp. (New York: Appleton-Century-Crofts). S. C. H.

Advances in corn [maize] production: principles and practice. Ed. W. H. PIERRE, S. R. ALDRICH and W. P. MARTIN. 1967, 2nd printing, 476 pp. (Ames, Iowa: Iowa State University Press).—Results of Corn Congress held at Delevan, Wisc., Oct. 1964. Contains 16 papers on various aspects of growing maize. S. C. H.

Population genetics in animal breeding. F. PIRCHNER (Trans. by F. PIRCHNER and M. VON KROSIGK). 1970, xii + 274 pp., 33 illustrations, 51 tables. Price 74/-. (San Francisco: W. H. Freeman and Co.).—**1. Review of basic concepts of genetics and statistics. 2. Genetic structure of populations.** The Hardy–Weinberg Law and linkage are explained. Analysis of inheritance and the estimation of gene frequencies are made. **3. Change of gene frequencies.** This chapter shows how mutation, migration, selection and random drift influence gene frequencies. **4. Inbreeding. 5. Quantitative variation.** Gene effects, variances and repeatability are discussed. **6. Heritability.** This includes a section on estimating heritability from the likeness among relatives as well as the variance. **7. Genetic correlations.** Some examples are given and some genotype–environment interactions are mentioned. **8–11.** These chapters cover theoretical concepts of **selection** including one dealing with experimental results for poultry and dairy examples. **12. Threshold characters. 13. Inbreeding depression. 14. Heterosis.** This is reviewed and explained. **15. Breeds and breed formation. 16. Biochemical polymorphism and population genetics. 17. Final considerations.** This is followed by a useful bibliography and index. C. J. R.

Poultry: Feeds and nutrition. P. J. SCHAIBLE. 1970, 623 pp. and Appendix of 18 pp. Approx. 40 fig. $22.50 (U.S.), $23.50 (Foreign). (Westport, Connecticut: Avi Publishing Co.).—After six chapters dealing with the industrial aspects of poultry and feeds and with the historical background to the subject, the following three chapters describe the anatomy, physiology and metabolism of poultry birds. The next part of the book deals with nutrition and feeds and includes chapters on minerals, proteins and amino acids, fats, carbohydrates, water, feed additives and supplements and nutritional inter-relationships. After two short chapters on mouldy feeds and disease and parasite control, feed formulation is discussed in detail (40 pp.). Further aspects covered include poultry management, feed manufacture, feeding systems, an interesting chapter on poisons and three chapters on poultry other than the chicken. A glossary, and feed and processing definitions, are included in the Appendix. P. P. R.

The pork industry: problems and progress. Ed. D. G. TOPEL. 1968, 236 pp. (Ames, Iowa: State University of Iowa Press). S. C. H.

Insecticide and fungicide handbook for crop protection. Ed. H. MARTIN. 1969, 3rd edn., 387 pp., 60/- (Oxford & Edinburgh: Blackwell Scientific Publications). S. C. H.

Medical and veterinary chemicals. R. SLACK and A. W. NINEHAM. 1968, vol. 1, parts I & II, 254 pp.; vol. 2, part III, 208 pp., £6 per set. (Oxford, etc.: Pergamon Press). S. C. H.

Lichens and air pollution: study of cryptogamic epiphytes and environment in the Stockholm region. E. SKYE. 1968, 137 pp. (Uppsala: Almqvist & Wiksells Boktryckeri AB).—[*Acta phytogeogr. suec.*, 52].— S. C. H.

Chemical fallout: current research on persistent pesticides. Ed. M. W. MILLER and G. C. BERG. 1969, 531 pp. (Springfield, Ill.: Charles C. Thomas Publisher).—**Distribution, accumulation and removal in nature.** (125 ref.); **Impact on natural populations other than man.** (160 ref.); **Mechanisms of action: doses to individuals and their relation to acute toxic dose.** (98 ref.); **Mechanisms of action: effects in individuals.** (179 ref.); **Estimation of effect on human populations and evaluation of control of pesticides.** (85 ref.). S. C. Haworth.

Methods in cell research. A. RUTHMANN. 1970, 368 pp., 70/-. (London: G. Bell & Sons Ltd.). S. C. H.

Cell and tissue culture. J. PAUL. 1970, 4th edn., 430 pp., 45/-. (Edinburgh & London: E. & S. Livingstone). S. C. H.

Handbook of molecular cytology. Ed. A. LIMA-DE-FERIA. 1969, 1508 pp. (Amsterdam, London: North-Holland Publishing Co.).—North-Holland Research Monographs, Frontiers of Biology, vol. 15 S. C. H.

The cytology and life history of bacteria. K. A. BISSET. 1970, 3rd edn., 147 pp., 50/-. (Edinburgh: E. & S. Livingstone). S. C. H.

Biological stains: handbook on the nature and uses of the dyes employed in the biological laboratory. R. D. LILLIE. 1969, 8th edn., 498 pp. (Baltimore: Williams & Wilkins Co.). S. C. H.

Marine algae: a survey of research and utilisation. T. LEVRING, H. A. HOPPE and O. J. SCHMID. 1969, 421 pp. (Hamburg: Cram, de Coruyter & Co.).—Botanica Marina Handbooks, vol. 1. S. C. H.

Sugar Technology Review. 1969, **1** (1), annual. (Amsterdam: Elsevier Publishing Co.).—All aspects of sugar manufacture and refining, covering beet sugar as well as cane industry. W. J. G.

AUTHOR INDEX

J. Sci. Fd Agric., 1970, Vol. 21, June

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

CONTENTS

Printed in England by Staples Printers Limited at their Kettering, Northants, establishment