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

Published by the Society of Chemical Industry

Volume 16 | No. 9 | September, 1965

SOCIETY OF CHEMICAL INDUSTRY

FOUNDED IN 1881 INCORPORATED BY ROYAL CHARTER 1907

Offices of the Society: 14 Belgrave Square, S.W.1

Telephone: BELgravia 3681/5

Annual Subscription to the *Journal of the Science of Food and Agriculture*

£15 post free, single copies £1 17s. 6d. post free

CHLORINATED PESTICIDE RESIDUES IN LAMB AND MUTTON FAT FOLLOWING DIPPING AND OTHER TREATMENT

By H. EGAN

Fat from sheep slaughtered at various intervals following treatment with dieldrin (1958) or with γ-BHC (1961) were examined for residues of these respective pesticides. From the results the approximate rates of diminution in residue during the weeks following the treatment are calculated. Results are also presented for the residue levels found in a limited survey of carcass fat taken from slaughter houses during 1962 in the normal course of marketing in England and Wales: in this survey the previous history of individual animals was carefully traced and comments on the residue levels found are related to this.

Introduction

This paper describes three studies, the first two being trials on sheep dipped with dieldrin and γ-BHC, respectively, in order to ascertain the levels of these pesticides which in practice are likely to occur in the fat, together with information on the rate of decay of such residues. The third study took the form of a limited survey in which fat taken at slaughter-houses throughout England and Wales was examined for residues. In this latter study arrangements were made in advance to ensure that the dipping history and other pertinent information was available for each carcass, with the object (1) of comparing the results (and any conclusion which might be drawn from them) with the results and conclusions obtained in the dipping trials and, in particular, (2) of making a preliminary assessment of the residue pattern in slaughter-house meat. There was no experimental control of the dipping process in the survey.

Together, the three studies have extended over a number of years, as indicated in the detailed account below. Many different individuals, from several organisations took part, the present account being drawn up by one of the participants.

(1) 1958 Dieldrin Trial

Animals were reared and the actual treatment carried out at the Cooper Field Research Station, Berkhamsted, Herts. All the sheep were born in the spring of 1957 at Berkhamsted to Scotch Blackface/Border Leicester ewes tupped by Suffolk/Hampshire rams.

Forty-two animals were arranged in four groups, one group of sixteen to be dipped and another to be sprayed, one group of six to be dusted and a control group of four sheep. Each treated group consisted of five wethers and eleven ewes. In the event, dusting was not done, as three sheep had to be removed for treatment against blowfly before advice on the technique for dusting had been received from New Zealand. The remaining three sheep were added to the controls, making seven in all. The sheep were shorn on 27 May, 1958, and treatment was made on 4 July, that is $5\frac{1}{2}$ weeks later. The weather during treatment was changeable, the morning being dull but the afternoon fine with a light breeze. The minimum temperature was 55° F, maximum 70° F; rainfall in the 24-h. period was 0·03 in. and relative humidity at 9 a.m. was 95%.

Three hundred gallons of dipping wash containing 0·05% of dieldrin were prepared from a 15% dieldrin emulsion concentrate. Each sheep was kept in the dip wash for 30 sec., during which time the head was immersed at least once. The dipping removed 40 gal. from the bath, corresponding to an average of about 20 pints per animal. After being dipped, the sheep were kept in a concrete draining pen for 4–5 h. before being returned to pasture; during this time much wash dripped from the sheep, but this was not returned to the bath. Sheep of the type and fleece-length used were estimated normally to retain approximately 2·6 pints of dip wash each after drainage.

For spraying, a Cooper–Allman Spray Race was set to project 10 gal. of a 0·25% dieldrin wash per minute at a pressure of 12–15 p.s.i. The 16 sheep passed through the sprayer in 20 sec. so that each was on average exposed to 1·6 pints of spray and, it was estimated, each

retained 1·3 pints. The animals were returned to pasture with the dipped animals after being confined in a paddock for 4–5 h.

The treated sheep were slaughtered, in batches of four, at intervals of 4½ weeks (32 days), 9½ (67), 13½ (94) and 17½ weeks (122 days), and the controls 5½ (39) and 15½ weeks (108 days) after treatment. The knives used for preparing the carcasses were washed with a fat solvent and wiped after use on each sheep. Twenty-four hours after hanging, samples consisting of perirenal fat and meat from the right shoulder were taken from each sheep and each sample was placed in a polythene bag, labelled and weighed. Details of the individual animals are given in Table I.

Table I

Details of sheep in dieldrin treatment trial

Ear No.	Sex	Weight in lb. (alive)		Date of slaughter	Sample weight in g.			Pooled sample No.	
		Pre-treatment	Pre-slaughter		Kidney	Fat	Muscle	Fat	Muscle
Controls									
264	F			12.8.58	148	631	550	1	11
283	M			12.8.58	138	238	507		
286	M			12.8.58	130	517	620		
300	M			12.8.58	139	378	516		
304	M		121	20.10.58	138	804	587	2	12
320	F		138	20.10.58	136	913	596		
332	F		122	20.10.58	128	740	554		
Dipped									
282	F	103	122	5.8.58	125	70 50*	520	3	13
303	M	128	152	5.8.58	150	400	505		
309	F	112	132	5.8.58	145	410	510		
322	F	94	121	5.8.58	145	150	500		
273	M	115	122	8.9.58	139	270	587	4	14
310	F	104	120	8.9.58	126	418	588		
311	F	121	135	8.9.58	132	350	587		
331	F	121	133	8.9.58	138	355	525		
280	F	120	136	6.10.58	137	318	667	5	15
281	M	123	136	6.10.58	160	532	595		
297	F	96		6.10.58	138	200	502		
349	F	100	112	6.10.58	125	584	554		
285	F	100	119	3.11.58	132	493	525	6	16
301	F	111	126	3.11.58	143	300	516		
313	M	120	137	3.11.58	125	337	550		
314	M	115	131	3.11.58	158	275	520		
Sprayed									
271	F	113	138	5.8.58	130	385	500	7	17
292	M	148	168	5.8.58	195	200	500		
315	F	132	154	5.8.58	145	850	530		
346	F	112	139	5.8.58	145	415	515		
270	F	123	123	8.9.58	126	365	607	8	18
287	M	105	110	8.9.58	134	300	596		
334	F	91	98	8.9.58	118	445	500		
352	F	107	104	8.9.58	119	347	552		
267	F	108	126	6.10.58	133	755	538	9	19
268	M	116	130	6.10.58	135	439	544		
318	F	124	125	6.10.58	129	389	509		
351	F	115	129	6.10.58	136	362	520		
276	M	119	131	3.11.58	157	470	515	10	20
279	M	97	110	3.11.58	126	262	534		
326 **	F	110	130	3.11.58	172	475	515		

* 50 g. of brisket fat were included with this sample
** One sheep destroyed in October after an accident

Samples were held at first in cold store at a temperature of −5° C and, after pooling, in deep freeze at −20° C until required for analysis. An equal amount from each of the four samples from a group of animals was pooled, diced and minced and then divided into three sub-samples of approximately equal weight. Pooling was first carried out in mid-October on the August and September samples from treated animals and the August controls. The August samples showed considerable autolysis, the August controls less and the September samples no evident autolysis. The October and November samples and October controls were pooled in late November. In this way, ten fat and ten lean pooled samples, each (with one exception) divided into three parts, were obtained.

Separate analyses were carried out at three Laboratories. One set of fat and meat samples was analysed at the Laboratory of the Government Chemist, by two methods, namely total chloride[1] and phenylazide[2] methods. Pooled samples of fat, taken 9½ and 13½ weeks after treatment were analysed by ' Shell ' Research Ltd. at the Woodstock Agricultural Research Centre by two methods, namely the phenylazide method and a bioassay method based on that of Sun & Sun[3] using *Drosophila melanogaster*. The third set of pooled samples was sent by air for analysis to the Wallaceville Animal Research Station of the New Zealand Dept. of Agriculture, who used the phenylazide method. Woodstock used 10-g., the Government Laboratory 50-g. and Wallaceville 100-g. aliquots of lean meat and 25-g. aliquots of fat for both sample analysis and recovery determinations.

At Woodstock a recovery of 115% was obtained at 2 p.p.m., 87% at 3 p.p.m., 86% at 4 p.p.m. and 95% at 6 p.p.m. using the phenylazide method; the overall mean recovery from 16 determinations in the dieldrin concentration range 2–6 p.p.m. averaged 98% with a mean deviation of ±13%. At the Laboratory of the Government Chemist recoveries were 75% and 70% for fat and muscle meat respectively by the total chlorine method, 85% and 70% respectively by the phenylazide method. At Wallaceville recoveries were 65% and 70% at an added dieldrin level of 0·5 p.p.m. in muscle and 91·5% at 2 p.p.m. level in fat. The results, in all cases corrected for both control and reagent blank values and for recovery, are summarised in Table II. A reasonably linear relationship exists between the interval between dipping and slaughter and the logarithm of the dieldrin residue level in the fat; extrapolation of the graph obtained when the residue levels above are so plotted shows that the level falls to 0·25 p.p.m. in 4–5 months for spray treatment and in from 5–6 months for dipping treatment. Fat content of the lean meat samples varied from 3·2 to 5·7%, the average for all the samples being 4·6%. This proportion is very similar to that for the respective residues in the fat and muscle of corresponding samples, which varies from 20 to 1 (for the highest fat residues) to 10 to 1 approximately.

(2) **1961 BHC Trial**

Unshorn sheep (45), not recently dipped, were selected by the Central Veterinary Laboratory, Ministry of Agriculture, Fisheries & Food in May 1961. Five sheep were set aside as untreated controls and the remainder divided into two groups of 20 sheep each. Three control sheep were slaughtered on 3 July: all three had received trichlorphon (200 mg./kg. orally) on 29 May and it had been necessary in addition to spray two with 0·025% γ-BHC on 26 June. Samples of kidney fat and thigh muscle were taken from each sheep for analysis; these samples were also used for recovery experiments and to investigate the clean-up procedure in the analysis. The 40 sheep to be dipped were given a 0·75% fenchlorphos spray treatment on 18 May; one group of 20 sheep was sheared on 21 August. The sheep were dipped in 0·025% γ-BHC (Cooper's ' Lice and Mange Liquid ' diluted 1 : 300) at Weybridge on 28 August. Two shorn and two unshorn sheep were slaughtered 1, 2, 4 and 6 weeks after dipping; two unshorn sheep were also slaughtered after 8, 12, 18, 24, 30 and 40 weeks. From each, samples of kidney fat and thigh muscle were taken for analysis. From 9 October (6 weeks), fat sheep were, so far as possible, selected. Certain fleece samples were also examined. All fat and muscle samples were minced and packed into closed polythene bags for transmission to the Laboratory of the Government Chemist, where on receipt they were placed in deep-freeze storage pending analysis.

After being thawed overnight, each sample was mixed thoroughly in a glazed dish, separate portions of muscle tissue being set aside for fat analysis. For γ-BHC analysis, 10 g. of sample

Table II

Residues found (p.p.m.) in dieldrin treatment trial by chlorine and phenylazide methods or bioassay

	Sample	Government Laboratory		Woodstock		Wallaceville	Mean	Weeks*
		Chlorine	Phenyl-azide	Phenyl-azide	Bioassay	Phenyl-azide		
Fat								
Dipped	3	8·8	9·6	—	—	No sample	9·2	4½
	4	2·9	4·1	2·7	1·4	3·0	3·0	9½
	5	1·6	2·1	2·0	1·8	1·8	1·9	13½
	6	1·1	0·8	—	—	0·4	0·8	17½
Sprayed	7	4·2	4·8	—	—	3·7	4·6	4½
	8	1·2	1·8	1·9	1·6	1·2	1·6	9½
	9	0·9	1·5	1·0	0·7	1·2	1·1	13½
	10	0·0	0·5	—	—	0·2	0·2	17½
Meat								
Dipped	13	0·50	0·48	—	—	0·45	0·48	4½
	14	0·24	0·14	—	—	0·24	0·21	9½
	15	0·17	0·13	—	—	0·21	0·17	13½
	16	0·31	0·07	—	—	Nil	0·13	17½
Sprayed	17	0·37	0·51	—	—	0·31	0·40	4½
	18	0·24	0·17	—	—	0·12	0·18	9½
	19	0·24	0·11	—	—	0·09	0·15	13½
	20	0·0	0·08	—	—	0·04	0·04	17½

* after treatment

were ground with sand and anhydrous sodium sulphate, tumbled for 1 h. with 150 ml. of hexane, filtered, and the combined filtrate and hexane washings treated with 1 : 1 concentrated fuming (40% SO_3) sulphuric acid (30 ml. for fat, 10 ml. for muscle tissue samples). The clear hexane phase was passed through a Davidow column, combined with subsequent hexane washings, reduced in volume to a few ml. and made up to a known volume. The whole extract or an aliquot was then taken to dryness, dissolved in 20 μl. of dioxan and 4–12 μl. of this solution were measured and transferred to prepared Whatman No. 3 chromatographic paper. Chromatograms were developed with 70% v/v acetone–water (liquid paraffin immobile phase) and assessed by the area method, standard series being run with each sample.[4] Towards the end of the trial, certain fat samples were also examined by gas chromatography, using hexane extraction followed by dimethylformamide partition clean-up.[5]

With 10 g. of control sample no residue, i.e. less than 0·1 p.p.m. γ-BHC, could be detected in either the thigh muscle or kidney fat. In consequence, no correction for blank value was applied to the results. Samples from the same sheep were used to determine recovery levels, known amounts of γ-BHC being added at the initial extraction stage of the method. The mean recovery values varied with the level of γ-BHC added, from 70% at 10 p.p.m. to 50% at 2 p.p.m. for fat samples and from 80% at 20 p.p.m. to 50% at 1 p.p.m. for muscle samples. The main losses occurred in the clean-up stage of the method, but the repeatability of even the lowest recovery levels was good. Corrections for recovery were made on a sliding scale and are included in the results. The repeatability of the results may be taken as $\pm$10% of the level concerned, with $\pm$0·2 p.p.m. for results of 2 p.p.m. or less. The γ-BHC residues found in the two 'pre-trial' sheep sprayed with 0·025% γ-BHC and slaughtered 8 days after treatment were: thigh muscle, less than 0·1 p.p.m., kidney fat, 1·6, 2·3 p.p.m.

The results, corrected as described above, for sheep subsequent to the dipping on 28 August are shown in Table III. The relatively high residue in the muscle of one unshorn sheep is probably associated with the high fat content of the tissue. The residual γ-BHC in each type of sample falls as the interval between dipping and slaughter increases. Fig. 1 shows that an approximately linear relationship exists between time elapsing after dipping and the logarithm of the residue level. Residues for thigh muscle from shorn sheep were too low to show any clear relationship. Approximate average intervals between dipping and for the attainment of different residue levels can be deduced and are given in Table IV. This for kidney fat is

Table III

Residues of γ-BHC found in kidney fat and thigh muscle (1961 dipping trial)

Interval, weeks	Residual γ-BHC, p.p.m.		Fat in muscle, %	Interval, weeks	Residual γ-BHC, p.p.m.		Fat in muscle, %
	Kidney fat (F)	Thigh muscle (M)			Kidney fat (F)	Thigh muscle (M)	
Shorn				*Shorn*			
1	1·9	0·2	6·3	4	0·3	0·1	7·7
1	2·8	0·2	6·9	4	0·3	0·1	8·7
2	0·7	0·2	8·9	6	0·1	0·1	7·8
2	1·6	0·2	7·0	6	0·1	0·1	7·5
Unshorn				*Unshorn*			
1	8·9	1·0	9·2	12	2·2	0·1	—
1	9·3	1·0	7·4	12	2·2	0·1	—
2	4·7	0·6	8·9	18	1·2	not examined	—
2	6·0	1·6	12·0	18	1·2	not examined	—
4	2·6	0·6	10·4	24	0·9	not examined	—
4	7·1	0·2	8·3	24	0·5	not examined	—
6	3·8	0·4	6·2	30	1·0	not examined	—
6	3·6	0·6	7·7	30	0·3	not examined	—
8	2·6	0·3	6·6	40	0·1	not examined	—
8	2·7	0·3	5·4	40	0·2	not examined	—

approximately seven times greater for unshorn sheep than for shorn sheep. Biological and chemical examinations showed that after dipping, the fleece of unshorn sheep carried a considerably higher residue of γ-BHC than the fleece of shorn sheep: results are given in Table V. Comparison with the results for kidney fat and thigh muscle suggests that the unshorn fleece acts as a ' body reserve ' for residual γ-BHC over a considerable period.

The intervals for unshorn sheep given in Table IV are rather greater than those found subsequently in New Zealand by Collett & Harrison.[6] However, these authors used a dipping bath containing only 0·0125% γ-BHC; maximum residues (mean values 5·0 p.p.m. unshorn, 2·5 p.p.m. shorn) were observed within 2 weeks of dipping and decreased to the pre-trial level of about 0·2 p.p.m. after 12 and 8 weeks, respectively. Residues in cattle, hogs, sheep and goats dipped or sprayed with lindane preparations have been reviewed by Claborn & Radeleff.[7] Jackson *et al.*[8] dipped sheep (and goats) in 0·025% lindane suspension: eight shorn yearling Delaine wethers were used and omental fat samples were taken by biopsy. The latter results are included in Fig. 1, and are intermediate between the findings in the present trial for the fat from the unshorn sheep and the fat from the shorn sheep; the slope of the approximate decay relationship shown is thus intermediate between those for the unshorn and shorn samples in the present trial. Whilst direct comparison of the results of the two trials is not possible, the above

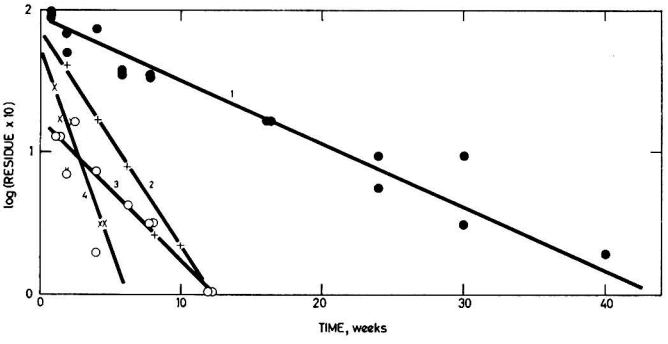

FIG. 1.—*Logarithmic decay of residual γ-BHC in sheep fat and muscle tissue after dipping*

curve 1 unshorn, fat curve 2 fat (Jackson *et al.*[8])
curve 3 unshorn, muscle curve 4 shorn, fat

Table IV

Calculated average γ-BHC residues after dipping

Residual γ-BHC, p.p.m.	Average time from dipping, weeks		
	Unshorn, fat	Shorn, fat	Unshorn, muscle
2·5	10–12	(0–1)	—
1·0	4–5 months	2–3	(0–1)
0·5	~6 months	3–4	3
0·1	9–12 months	6	12

Table V

Residues found in fleece in 1961 BHC trial

Interval from dipping, weeks	Fleece residue, p.p.m. γ-BHC	
	Shorn sheep	Unshorn sheep
6	2, 3	500, 760
8	2, 20	110, 710
12	1, 2	320, 485
18	—	325, 430
30	—	1, 16
40	—	45, 80

observations suggest that (other things being equal) the fleece condition of the American sheep at the time of dipping was intermediate between that for the two groups of sheep in the BHC trial.

(3) 1962 Survey of Home-Killed Mutton

Arrangements were made with Divisional Veterinary Officers of the Ministry of Agriculture, Fisheries & Food for samples of kidney fat from the carcasses of lambs or sheep, known to have been dipped, to be received from 21 slaughterhouses (each located in a different administrative county) during August, September and October 1962. Three individual samples were received from 19 of the slaughterhouses and two samples only from the others. The total number of separate samples received was thus 61. On receipt, the fats were placed in deep-freeze storage pending analysis.

Representative sub-samples from all the samples were examined for the presence of the principal chlorinated insecticides by gas chromatography, using the method of de Faubert Maunder *et al.*[5] with two independent columns. Those found to contain appreciable residues were also examined qualitatively by paper chromatography, using the same clean-up method, followed by alumina column treatment (5% v/w added water) and the chromatographic system described by Evans,[4] in order to confirm the identity of residues. In addition, four of the samples showing high dieldrin residues were saponified, extracted with hexane and the extract treated by the dimethylformamide partition process,[5] further purified by column chromatography, taken to dryness, the residue dissolved in carbon disulphide and examined by infra-red spectrophotometry using a micro-cell technique.

Recoveries by the gas chromatographic method following the addition of pure insecticide in solution to mutton fat control samples before dispersion in hexane were: HEOD (dieldrin), 80%; α-BHC, 75%; and γ-BHC, 70%. Each of these recoveries was the mean of values obtained by four workers, the mean deviation from the mean for each insecticide being of the order ±4%. The limit of sensitivity of the method was approximately 0·1 p.p.m. for dieldrin and 0·05 p.p.m. for either α- or γ-BHC.

The results (uncorrected for recovery) obtained by gas chromatography, together with certain information received with the samples, are set out in Table VI. The repeatability for individual dieldrin results is assessed as ±20% or better, with ±10% in most cases. The high dieldrin residues were all confirmed by paper chromatography, the highest being also confirmed by infra-red spectrophotometry. DDT (and DDE) residues were detected only in the two cases in which a DDT dip had been used; the total residues were of the order of 1 p.p.m. A brief analysis of the distribution of insecticide levels is given in Table VII, the distribution for dieldrin

Table VI

Dieldrin and other chlorinated insecticide residues found in 1962 survey (p.p.m.)

County	Sex	Dieldrin (R)	α-BHC	γ-BHC	Interval,[a] days	Insecticide in prepared dip, %			Log (10 R)
						Dieldrin	γ-BHC	Other BHC	
Berkshire	M	5·4	0·0	0·8	81	0·10	0·030	nil	1·73
	M	3·4	0·0	0·1	81	0·10	0·030	nil	1·53
	F	10·0	1·3	0·4	77	0·06	0·018	0·027	2·00
Brecon	(M)	0·1	0·0	0·0	79	0·05	0·016	nil	0·0
	M	0·6	0·0	0·0	79	0·05	0·016	nil	0·78
	(M)	0·1	0·0	0·0	79	0·05	0·016	nil	0·0
Caernarvon	F	0·0	0·05	0·8	33	nil	0·016	0·050	—
	F	0·1	0·0	0·3	33	nil	0·016	0·050	0·0
	F	0·1	0·05	0·4	33	nil	0·016	0·050	0·0
Cardigan	M	1·7	0·4	0·0	40	0·10	0·030	nil	1·23
	M	1·8	0·0	0·0	46	0·10	0·030	nil	1·26
	M	0·6	0·6	0·05	55	0·03	0·016	0·014	0·78
Carmarthen	M	10·0	0·0	1·4	18	0·10	0·030	nil	2·00
	F	6·0	0·0	1·5	18	0·10	0·030	nil	1·78
Cumberland	F	3·1	0·4	0·4	39	0·05	0·017	0·010	1·49
	F	1·5	0·4	0·4	39	0·05	0·017	0·010	1·18
	F	1·7	0·4	0·7	39	0·05	0·017	0·010	1·23
Denbigh	M	0·0	0·1	0·0	66	nil	0·020	0·010	—
	F	1·9	0·05	0·2	(60)	0·10	0·030	nil	1·28
	M	0·2	0·05	0·0	51	0·06	0·018	0·027	0·30
Derby	M	0·0	0·3	0·1	30	nil	0·017	0·026	—
	M	6·2	0·6	0·1	60	0·06	0·018	0·027	1·79
	F	3·4	0·5	0·3	64	0·03	0·016	0·014	1·53
Isle of Ely	M	0·9	0·0	0·05	46	0·10	0·030	nil	0·95
	F	9·3	0·05	2·0	46	0·10	0·030	nil	1·97
Glamorgan	M	2·8	0·05	0·0	38	0·05	0·016	nil	1·45
	F	0·4	1·2	0·1	50	nil	0·020	0·040	0·60
	F	0·0	0·1	0·0	66	0·10	0·030	nil	—
Hereford	M	5·1	0·3	0·05	(50)	0·06	0·018	0·027	1·72
	M	3·2	0·7	0·15	67	0·03	0·016	0·037	1·51
	M	2·6	0·7	0·2	43	0·05	0·018	0·010	1·41
Huntingdon	M	4·9	0·05	0·05	76	0·10	0·030	nil	1·69
	M	4·3	0·05	0·1	82	0·10	0·030	nil	1·63
	M	2·8	0·05	0·05	82	0·10	0·030	nil	1·45
Lancashire	?	0·0	0·0	0·0	37	0·10	0·030	nil	—
	(M)	0·1	0·0	0·0	37	0·10	0·030	nil	0·0
	?	0·7	0·0	0·0	37	0·10	0·030	nil	0·85
Lincs (L'sey)	F	2·3	0·1	0·15	60	0·05	0·016	nil	1·36
	F	3·3	0·0	0·1	78	0·03	0·016	0·037	1·52
	M	0·3	4·5	0·2	42	nil	0·020	0·040	0·48
Merioneth	F	0·9	0·2	0·05	63	0·10	0·030	nil	0·95
	F	1·9	0·05	0·05	63	0·10	0·030	nil	1·28
	M	1·4	0·1	0·0	63	0·10	0·030	nil	1·15
Norfolk	M	3·7	1·5	0·3	82	0·06	0·018	0·027	1·57
	M	2·4	0·0	1·0	85	0·03	0·016	0·037	1·38
	?	0·6	0·0	0·05	80	0·10	0·030	nil	0·78
Radnorshire	F	0·6	0·05	0·0	78	0·05[b]	0·017	nil	0·73
	F	1·5	0·0	0·0	78	0·05[b]	0·017	nil	1·18
	(M)	0·8	0·0	0·0	78	0·05[b]	0·017	nil	0·90
E. Suffolk	?	4·7	1·8	0·4	39	0·06	0·018	0·027	1·67
	?	2·2	0·0	0·05	39	0·06	0·018	0·027	1·34
	?	0·3	0·1	0·0	54	0·06	0·018	0·027	0·48
W. Suffolk	F	4·1	0·0	0·1	44	0·05	0·016	nil	1·61
	M	5·4	0·0	0·4	44	0·05	0·016	nil	1·73
	F	6·9	0·0	0·2	44	0·05	0·016	nil	1·84
Yorks N.R.	M	0·3	0·0	0·0	85	0·10	0·030	nil	0·48
	F	0·8	0·3	0·05	34	0·03	0·016	0·014	0·90
	F	0·0	0·3	0·0	69	0·32[c]	0·020	0·045	—
Yorks W.R.	F	3·7	1·0	0·1	62	0·06	0·018	0·027	1·57
	M	2·9	0·7	0·15	62	0·06	0·018	0·027	1·46
	M	0·1	0·0	0·0	70	0·32[c]	0·018	nil	0·0

[a] between dipping and slaughter [b] aldrin [c] DDT

Table VII

Distribution of residues found in 1962 survey

Insecticide, p.p.m.	No. containing dieldrin			Number containing	
	Dieldrin-treated	Others	Total	α-BHC	γ-BHC
* 0·0— 1·0	17	9	26	56	58
1·1— 2·0	8	—	8	4	3
2·1— 3·0	7	—	7	—	—
3·1— 4·0	7	—	7	—	—
4·1— 5·0	4	—	4	1	—
5·1— 6·0	4	—	4	—	—
6·1— 7·0	2	—	2	—	—
7·1— 8·0	—	—	—	—	—
8·1— 9·0	—	—	—	—	—
9·1—10·0	3	—	3	—	—
* of which not more than 0·1	5	7	12	41	37
not more than 0·3	8	8	16	45	48

being illustrated in Fig. 2. Of the 61 samples, 52 were from animals which had been treated with dieldrin (or, in three cases, aldrin) dips. Of the nine not treated, only two appear to contain significant traces of dieldrin, 0·3 and 0·4 p.p.m. respectively. It may be concluded from Fig. 2 that the residue distribution is largely independent of sex. Dieldrin, but not aldrin, residues were found in the three samples from animals which had been treated with aldrin dips.

All 61 animals had also been treated with either lindane (33) or technical BHC (28) dips. Small but analytically significant residues of α-BHC were found in 21 samples (concentration of α-BHC exceeding 0·1 p.p.m.), all of them from samples associated with technical BHC treatment with two exceptions (0·2 and 0·4 p.p.m. of α-BHC respectively). With a single exception (4·5 p.p.m. of α-BHC), no individual BHC isomer residue exceeded 2 p.p.m. by a significant amount: the same is true for the combined BHC isomer residues. No β- or δ-BHC isomer was found in any sample.

In Fig. 3 the various dieldrin residue levels found are distinguished according to the dieldrin concentration in the prepared dipping bath when prepared in the manner recommended by the dip manufacturer. The straight line X–Y represents an enveloping curve which (ignoring such obvious features as the time elapsing between dipping and slaughter and the period of immersion

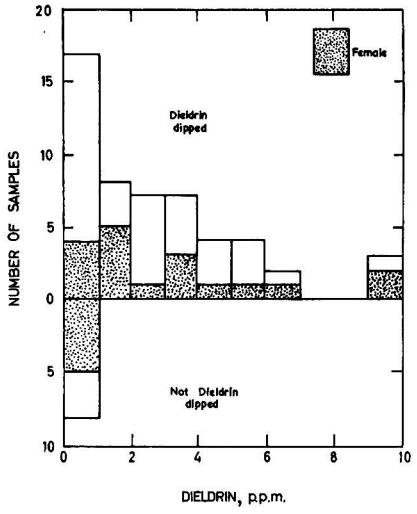

FIG. 2.—*Distribution of dieldrin residues in home-killed mutton fat*

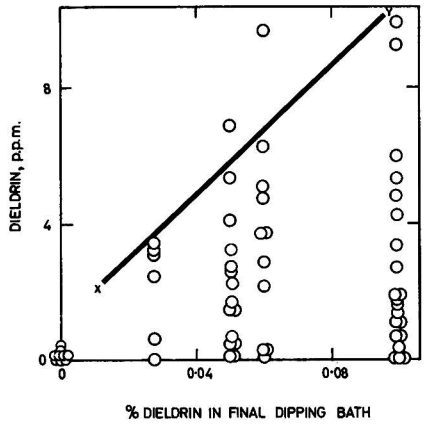

FIG. 3.—*Variation of residue with dieldrin concentration in the dipping bath*

in the dipping bath) indicates approximately the maximum residue likely to occur. In Fig. 4 the logarithm of the various dieldrin residues (final column of Table VI) is plotted against the number of days elapsing between dipping and slaughter. No evident simple relationship between these quantities can be discerned, although there is some suggestion of a linear correlation in the case of the nine shorn samples included.

Discussion

From the approximately linear relationship between the logarithm of the residual dieldrin level and the interval from dipping observed in the 1958 dieldrin trial, the interval necessary for the average fat residue to fall to, for example, 0·25 p.p.m., was estimated to be from 4–5 months for spraying or 5–6 months for dipping. Precise fleece conditions were not taken into account in that trial but, from the same linear relationship, it can be deduced that the average residue level to be expected in the fat 8 weeks after dipping (i.e., the average interval for the 61 samples in the 1962 survey) is of the order of 3 p.p.m. Having regard to the fact that in the survey

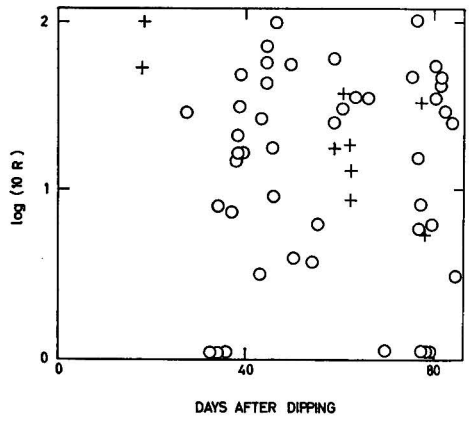

FIG. 4.—*Logarithmic decay of dieldrin residues with time*

+ shorn ○ unshorn

dipping conditions (such as type and brand of dip, individual manner of preparing the bath and handling the flock, weather, fleece condition) were known to vary over the very large area of country concerned, the observed average residue level of 2·4 p.p.m. dieldrin corresponding to an *average* interval of 56 days is in reasonable accord with the level deduced from the 1958 trial. It seems clear, however, that for unshorn sheep, residues in fat within 1–4 weeks only of dipping may be as high as 5 p.p.m., whether of γ-BHC or of dieldrin.

Since the information obtained by the officers of the Ministry of Agriculture in the 1962 survey included a record of the history of each animal, it was possible to obtain further details of samples subsequent to the analysis. Thus, it was found that of the four samples for which residues were found to exceed 5 p.p.m. dieldrin (including three in excess of 9 p.p.m.), three at least had a dipping history which was unrepresentative of general practice and which would largely account for the exceptionally high levels. The samples examined all came from animals marketed at comparatively short intervals after dipping, ranging in fact from 18 to 85 days (average interval, 56 days). Nevertheless, the monthly slaughter of sheep as a percentage of the annual total for the months of August, September and October 1962 were 10·1, 11·2 and 14·8 respectively, making a total for the three months of some 36%. Corresponding figures for 1963 were 8·5, 10·6, 15·8, and about 35% respectively.

Acknowledgments

The author freely acknowledges the substantial contributions of the following: 1958 dieldrin trial, Mr. D. L. Harrison (Animal Research Station, Wallaceville, New Zealand), Mr. D. W. Jolly (then with Messrs. Cooper McDougall & Robertson Ltd.), Mr. J. G. Reynolds (' Shell ' Research Ltd., Woodstock); 1961 BHC trial, Dr. S. B. Kendall and Dr. W. N. Beesley (Central Veterinary Laboratory, Ministry of Agriculture, Fisheries & Food) and Dr. J. M. Barnes (Medical Research Council). Acknowledgment is also due to many other officers of these and other organisations, including in particular Divisional Veterinary Officers of the Ministry of Agriculture, Fisheries & Food in the 1962 survey; and to a number of the author's colleagues in the Laboratory of the Government Chemist, in particular to Dr. W. H. Evans, Mr. J. Roburn and Mr. N.McI. Soutar. Finally, the author's special thanks are recorded to Dr. E. J. Miller who acted as Secretary or organiser to the various Committees associated at different times with the work described; and to the firms and Departments concerned.

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Received 16 February, 1965

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WHEAT PROTEINS

II.*—Changes in the Protein Composition of *Triticum vulgare* during the Life Cycle of the Plant

By C. B. COULSON† and A. K. SIM

Changes in the wheat protein system, particularly endosperm proteins, during the life cycle of the plant have been followed using a starch gel electrophoresis technique. Fractions of low electrophoretic mobility were progressively degraded during germination and progressively synthesised towards the end of the ripening period, indicating their probable role as storage proteins of the endosperm. In contrast, fractions of higher mobility were less affected during germination and were utilised at a later stage. Similarly, these components were synthesised first during ripening and remained at a relatively constant level during the build-up of apparently high molecular weight material. On the information available it was not possible to determine whether the fast-moving fractions represented the enzymes of the system or were precursors of the larger components.

Introduction

Recent investigations of the protein composition of wheat flour and isolated gluten have indicated a rather complex system of up to 32 components.[1-4] The solubility characteristics of these fractions do not permit an entirely satisfactory classification in terms of Osborne's classical nomenclature[5] of gliadin, glutenin, albumins and globulins.[4]

It has been recognised for some time, however, that these fractions collectively play an important role in the ultimate rheological properties of the isolated gluten complex,[6,7] although many workers have regarded such fractions as the soluble albumins as ' contaminants ' within the gluten system.[8]

An alternative approach to the classification of wheat proteins in terms of biochemical functionality has been reported from another laboratory.[9-16] Electron microscope examination of ripening wheat endosperm has indicated an initial period of rapid synthesis of protein material, particularly within enclosed osmophilic bodies.[14,16] This material appears to correspond to those components of low electrophoretic mobility in starch gel separations and is thought to represent the storage protein of the endosperm.[14]

Studies by Jennings & Morton[9] of the changes in nucleic acid/protein content of the endosperm during ripening indicated that some of the more ' soluble ' protein fractions of higher electrophoretic mobility may represent intermediates in the formation of the storage proteins, but this could not be confirmed by the tracer experiments of Graham & Morton.[15]

The present paper describes an investigation of the changes in the protein constituents of the wheat plant throughout the life cycle, using the starch gel electrophoresis technique described in a previous paper.[17] By following these changes, particularly during germination and ripening, it was hoped to gain a better understanding of their possible function and interrelationship.

Experimental

(1) *Materials*

All fresh material was obtained from wheat (*Triticum vulgare* cv. Als) grown in the same experimental plot (11 acres) (Seed Testing Station, Dept. of Agriculture for Scotland, East Craigs, Edinburgh).

(2) *Experimental techniques*

(*a*) *Preparation of protein solutions*

All protein solutions were prepared as described in the previous paper[17] except that dilute acetic acid solution (0·01N) was used as solvent in place of distilled water. When required,

* Part I: *J. Sci. Fd Agric.*, 1965, **16**, 458

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protein extracts were concentrated by dialysis against polyethylene glycol (Carbowax 20M, G. T. Gurr Ltd., London) contained in dialysis tubing (Union Carbide International Co., New York; $\frac{18}{32}$ in.), by partial or complete freeze-drying or by ultrafiltration where appropriate.

Solutions were normally stored at −15°. Continual freezing and thawing, however, tended to cause significant protein precipitation, hence samples which were to be retained for long periods were freeze-dried before storage. Where possible, protein extracts were fractionated immediately after extraction, particularly for comparative studies.

(b) *Ultrafiltration*

Free amino-acids and peptides were removed from protein solutions by ultrafiltration through evacuated dialysis tubing (Visking, $\frac{18}{32}$ in.) supported on a slotted nylon frame (LKB—Produkter, Sweden). Porous polyethylene tubing (cf. Siegelman & Firer[18]) was also found to be an effective supporting medium, but was less suitable for small volumes of solution.

(c) *Two-dimensional paper chromatography*

Two-dimensional ascending paper chromatography was carried out on a modified Datta frame system (Messrs Aimer Ltd., London) allowing five papers (Whatman No. 1; 10 in. × 10 in.) to be run concurrently. For more detailed separations, a combination of descending and ascending techniques was used. Separations were carried out on Whatman No. 1 paper (16 in. × 9 in.) using the descending technique along the long axis. For ascending runs, papers were rolled in a cylindrical shape and allowed to stand in a tray of solvent placed in the base of the chromatography tank.

Solvent systems employed were as follows:

(i) (1st) n-Butanol/glacial acetic acid/water (12:3:5; 7 h.)
(2nd) Phenol/ammonia (500 g. in 125 ml. of water 1 ml. of aqueous ammonia, sp. gr. 0·88)

(ii) (1st) n-Butanol/glacial acetic acid/water (12:3:5; 7 h.)
(2nd) Phenol/ethanol/ammonia (150:40:10; 12 h.)
Stain: Ninhydrin (0·2%) in acetone.

(d) *Starch gel electrophoresis*

Electrophoretic separations, etc., were carried out exactly as described in a previous paper.[17]

(3) *Biological techniques*

The investigation was conveniently divided into the following stages: (A) dormancy; (B) vernalisation; (C) germination; (D) growth; (E) ripening; (F) post-harvest.

(A) *Dormancy*

Ripe wheat seeds obtained from field plots were separated as far as possible by hand dissection into various anatomical parts. The protein components of these sections were extracted and examined mainly under the conditions developed for the fractionation of endosperm proteins and associated subcomponents.

Husks were removed by hand threshing and ground to a fine powder in liquid air. A sample (5 g.) was dispersed in acetic acid solution (0·01N, 30 ml.), the extract concentrated by ultrafiltration to $\frac{1}{8}$ of the original volume, and portions of the ultrafiltrate and residual solution were examined by two-dimensional chromatography and starch gel electrophoresis in an aluminium lactate buffer.

Embryos (including scutellum) were then removed from the ripe seeds (approx. 100) by hand dissection and milled to a fine powder in liquid air. A portion of the material (0·01 g.) was dispersed in acetic acid solution (0·01N, 5 ml.) and the extract concentrated by ultrafiltration. Samples of the ultrafiltrate and residual solution were examined as before. Extracts of two commercially available samples of wheat germ were similarly examined (Bemax, Vitamins Ltd., London; and isolated wheat germ, Scottish Co-operative Wholesale Society, Leith).

The remaining portion of the seeds were milled by hand and the flour removed by sieving

(100 mesh). The residue, containing mainly testa and aleurone cells, was washed several times in distilled water to remove the bulk of the adhering endosperm material and then finally ground to a fine powder in liquid air. A sample (2 g.) was dispersed in acetic acid solution (0·01N, 10 ml.) and the extract examined as before after ultrafiltration.

A portion of the isolated endosperm material (2 g.) was dispersed in acetic acid solution (0·01N, 6 ml.) and the extract examined as above before and after ultrafiltration. A large number of different varieties of *T. vulgare* have been examined and results are reported in the previous paper.[17]

(B) *Vernalisation*

Ripe wheat seeds were evenly spread on moist paper towelling and stored for 7 days at 40°. After 4–5 days, however, some of the seeds began to germinate, thus preventing an accurate assessment of any changes which might be attributable to the vernalisation procedure. A second sample was then stored in a dry state at −15° for 7 days. During this period, no germination was observed.

Embryos were removed from the dry seeds (approx. 100) by hand dissection, dispersed in acetic acid solution (0·01N, 5 ml.) and the extract examined by starch gel electrophoresis.

The remainder of the seeds was then ground and acetic acid extracts of the isolated flour examined as above. Extracts of unvernalised samples were used as controls.

(C) *Germination*

The early stages of growth of the plant were readily studied by germinating samples in the laboratory, thus avoiding possible attack by soil bacteria which might disturb the natural protein degradation pattern. Seeds were initially germinated by soaking for 24 h. at room temperature followed by incubation at 25° in a thermostated bath. After 3 days, however, the seeds developed a rancid smell and were rejected. The following method was then adopted.

Vernalised seeds were evenly spread on moist filter paper (Whatman No. 3) in a photographic tray (10 in. × 15 in.). A second layer of moist paper was placed on top of the seeds, and the samples sandwiched between two thick pads of moist cellulose wadding.* Several layers of seeds were similarly prepared. Finally, the tray was covered with a sheet of polythene and placed in a darkened cupboard at room temperature.

Samples (approx. 100) were removed daily and immediately frozen in liquid air. After removal of embryonic material, both portions of the seed were ground to a fine powder and extracted in acetic acid solution as in the previous section. Samples of each extract were examined by starch gel electrophoresis and chromatography after ultrafiltration as already described.

Sampling was continued for 20 days, i.e., until complete exhaustion of endosperm material. The total nitrogen content of each sample was determined by the micro-Kjeldahl technique.

(D) *Growth*

Young wheat seedlings were harvested from field plots shortly after the first appearance of green leaves until flowering as shown in Table I. All samples were frozen in liquid air immediately after harvesting.

Three weeks after planting.—At this stage the aerial parts of the plant contained only young leaves. These were removed by hand dissection and ground in liquid air. A sample (10 g., fresh weight) was dispersed in acetic acid solution (0·01N, 30 ml.) and the extract freeze-dried. The small amount of dried material was taken up in acetic acid solution (0·01N, 0·5 ml.) and examined by starch gel electrophoresis and two-dimensional chromatography.

A similar extraction was carried out in sodium tetraborate solution (0·05M, 30 ml.) and the extract again freeze-dried. A portion of the residual material (0·5 g.) was taken up in distilled water (6 ml.) and examined by starch gel electrophoresis in a tris–citric acid buffer.

24–34 *weeks after planting.*—After 24 weeks, immature spikelets approx. 1mm. in length were visible at the growing tip of the young seedling. These were removed by hand dissection (approx. 100 seedlings), ground in liquid air and dispersed in acetic acid solution (0·01N, 6 ml.).

* Moist paper towelling used for this purpose caused significant growth of moulds.

Table I

Field harvesting data for growing wheat samples

Date	Weeks after planting	Field data	Dissection data
15th October, 1961	0	Seeds planted	—
7th November, 1962	3		All aerial parts examined (leaves only)
10th April, 1962	24		Immature spikelet removed
29th May, 1962	31		,,
18th June, 1962	33½	Date of shooting (appearance of spikelet above uppermost leaf)	—
21st June, 1962	34		Immature spikelet removed
25th June, 1962	34½	Date of flowering (first appearance of anthers)	—
3rd July, 1962	36	Estimated date of pollination	Immature seed removed from spikelet
11th July, 1962	37		,,
18th July, 1962	38		,,
25th July, 1962	39		Milky endosperm removed from seeds
1st August, 1962	40		,,
8th August, 1962	41		,,
15th August, 1962	42		Embryos and endosperm removed
22nd August, 1962	43		,,
29th August, 1962	44		,,
31st August, 1962	44 approx.	Date of ripening	—
5th September, 1962	45		Embryos and endosperm removed
10th September, 1962	46	Date of harvest (field)	,,
24th September, 1962	48		,,
22nd October, 1962	52		,,

After freeze-drying, the residual material was taken up in acetic acid solution (0·5 ml.) and examined by starch gel electrophoresis in an aluminium lactate buffer and by chromatography as before.

Acetic acid extracts of leaves were again examined as for the previous sample. Examination of leaves was discontinued from this stage.

Samples were harvested 31 and 34 weeks after planting and acetic acid extracts of the young spikelet similarly examined.

(E) *Ripening*

36–38 *weeks after planting.*—At this stage it was possible to remove the young seeds from the wheat head. It was not possible, however, further to dissect the seed except perhaps by manipulation under a microscope which was not considered practical in this investigation.

Spikelets were frozen in liquid air and then lightly ground with a pestle or similar heavy roller. In this way, the husks were quickly removed and the young seeds isolated with a pair of forceps.

The immature seeds (approx. 100) were then ground and extracted in acetic acid solution. The extract was freeze dried, taken up in acetic acid solution and examined by starch gel electrophoresis and chromatography as in the previous section.

Samples harvested 37 and 38 weeks after planting were similarly examined.

39–41 *weeks after planting.*—After 39 weeks the immature seeds contained sufficient endosperm material to allow removal of the testa and associated tissues. At this stage, however, the embryonic parts were not sufficiently developed to merit removal, and could be detected only under magnification.

The endosperm material was removed from the green testa by hand dissection and dispersed in acetic acid solution (0·01N, 20 ml., approx. 100 seeds). A portion of the extract was examined by starch gel electrophoresis as before.

Acetic acid extracts of samples obtained 40 and 41 weeks after planting were similarly examined.

42–46 *weeks after planting.*—Immature seeds were isolated from the spikelets as described in the previous section. At this stage, the embryonic parts had developed sufficiently to allow their removal. The endosperm material was isolated as already described. A sample (0·1 g.)

of the isolated embryos was dispersed in acetic acid solution (0·01N, 5 ml.) and a portion of the extract examined by starch gel electrophoresis as above. An acetic acid extract (0·01N, 6 ml.) of the endosperm material (10 g.) was similarly examined.

Samples were harvested 43, 44, 45 and 46 weeks after planting, i.e., until full ripening and examined as above.

(F) *Post-harvest*

Samples obtained 2 and 6 weeks after crop harvesting and threshing were examined as above

Results

(A) *Dormancy*

Acetic acid extracts of husks from ripe wheat seeds did not appear to contain any protein material as judged by starch gel electrophoresis. Chromatographic separation of ninhydrin-positive substances showed the presence of the usual plant amino-acids (17–19)with glutamic acid, alanine, glutamine, γ-amino-n-butyric acid, leucine and probably citrulline as major constituents, judged on the basis of colour intensities. Assessments were based on at least four separate extractions, chromatographed in triplicate.

Embryonic material contained a large proportion of acetic acid-soluble proteins, readily fractionated by starch gel electrophoresis. At least 13 fractions were observed of intermediate to high mobility, roughly corresponding to regions F and G of the endosperm protein pattern (Fig. 1). All extracts contained one major fraction in very high concentration of similar mobility to the major fraction of region F.

Comparisons of embryonic and endosperm components over several separations indicated that the protein composition of each extract was quite different.

Similar fractionations were obtained for acetic acid extracts of commercial wheat germ samples.

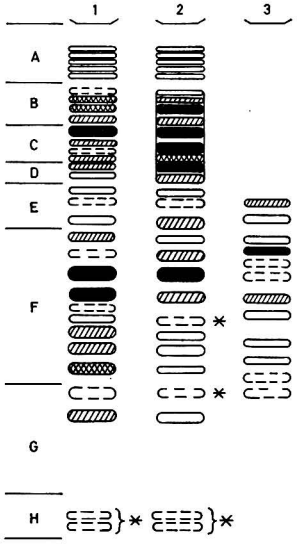

FIG. 1.—*Diagram of starch gel electrophoresis patterns of wheat protein fractions*

1. Endosperm—distilled water (pH 5·6)
2. Endosperm—acetic acid (0·01N)
3. Embryo—acetic acid (0·01N)

*Fractions visible only after concentration.

Glutamic acid, alanine, glutamine and γ-amino-n-butyric acid appeared to be the major ninhydrin-positive constituents of embryo ultrafiltrates.

Isolated testa material appeared to contain a small amount of acetic acid-soluble proteins, all of which corresponded to fractions previously observed in corresponding flour extracts. Most of these components were of intermediate electrophoretic mobility and may possibly represent a contamination by endosperm material during the isolation procedure.

Ultrafiltrates again contained the usual amino-acids, the major components being glutamic acid, asparagine, glutamine, γ-amino-n-butyric acid and probably citrulline.

Endosperm extracts contained at least 30 separate fractions as judged by starch gel electrophoresis. Some of the minor, fast-moving components were visible only after concentration of the solution, but this led to serious overloading with respect to many of the major components of low mobility with a consequent reduction in resolution. Patterns were very similar to those already reported for aqueous extracts except for some variations in concentration of individual components. A comparison of aqueous and acetic acid extracts is shown in Fig. 1.

Chromatographic separation of ninhydrin-positive substances of ultrafiltrates revealed the presence of glutamic acid, asparagine, glutamine, proline and probably citrulline as major constituents.

(B) *Vernalisation*

Storage of dormant seeds for 7 days at −15° did not appear to cause any significant alteration of the acetic acid-soluble protein components of either endosperm or embryo.

(C) *Germination*

Although significant degradation of endosperm proteins during the early stages of germination was indicated by nitrogen analyses (Fig. 2) no corresponding alteration of the acetic acid-soluble components was observed by starch gel electrophoresis. Patterns identical with those of ungerminated controls were obtained during the first 5 days of germination. After 6 days, however, significant weakening of the slow-moving fractions (Fig. 1, regions A–D) was observed. These fractions became progressively weaker throughout the 6th to 9th days, after which only traces of protein material were observed in this region.

Fractions of higher electrophoretic mobility appeared to be unaltered throughout this period, up to approximately the 13th day of germination. After this stage, these components also became progressively weaker, until after 17 days, only traces of protein material remained. No protein material was observed in subsequent extracts. Few new components of higher mobility were observed throughout the degradation of slow-moving fractions, although a small amount of unresolved material of higher mobility (region E) was noted between the 6th and 9th

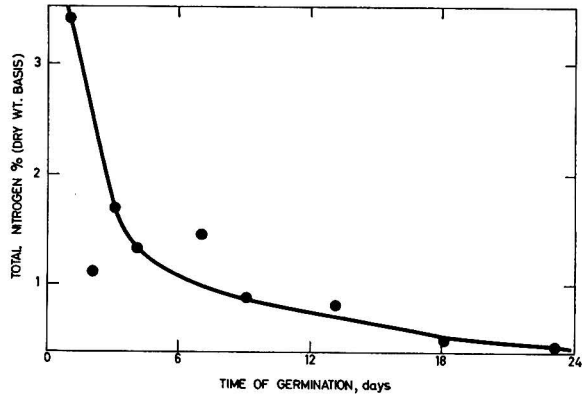

FIG. 2.—*Changes in the total nitrogen content of wheat endosperm during germination*

days. An accumulation of material with relatively small molecular weight, similar to that produced by *in vitro* proteinase degradation,[4] did not however occur.

Examination of incised embryos indicated that unaltered acetic acid-soluble protein material could be extracted only during the first or second day of germination, after which there appeared to be a change-over to material of different solubility characteristics. Attempts to determine more accurately the point of change-over were hindered by the difficulty in determining which seeds were in the process of germination.

All subsequent samples of embryonic material appeared to contain no acetic acid-soluble components as judged by starch gel electrophoresis.

No qualitative changes were observed in the free amino-acid contents of endosperm and embryo ultrafiltrates throughout germination. The major free amino-acids of endosperm ultrafiltrates appeared to be alanine, glutamine, proline, γ-amino-n-butyric acid, valine, methionine and leucine. Embryo ultrafiltrates contained glutamic acid, alanine, asparagine and glutamine as major constituents. Some variation in the levels of glutamic acid, asparagine, glutamine and γ-amino-n-butyric acid was detected in all samples throughout germination.

(D) *Growth*

Immature leaves harvested 3 weeks after planting appeared to contain no acetic acid-soluble protein material. Chromatographic separation of the extracts, however, showed a relatively high proportion of ninhydrin-positive material. Most of the components corresponded to the usual plant amino-acids, including glutamic acid, serine, alanine, asparagine, glutamine, and γ-amino-n-butyric acid as major constituents. Sodium tetraborate extracts contained at least eight distinct protein components (Fig. 3), although some of the fractions were poorly resolved.

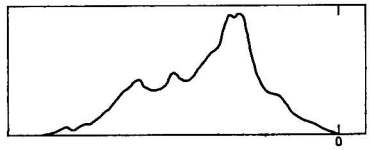

FIG. 3.—*Starch gel electrophoresis of sodium tetraborate-soluble proteins of young wheat leaves* (densitometric scan)

Immature spikelets removed from the plant before the date of flowering (31 and 34 weeks after planting) appeared to contain no acetic acid-soluble protein material as judged by starch gel electrophoresis. Fractionations of sodium tetraborate-soluble leaf proteins were essentially similar to those already obtained.

The free amino-acid content of all acetic acid extracts was similar to that above, except for a lower level of serine and γ-amino-n-butyric acid.

(E) *Ripening*

Extracts of intact immature seeds harvested 36 and 37 weeks after planting showed no indication of acetic acid-soluble protein components. Major ninhydrin-positive constituents were glutamic acid, alanine, asparagine, glutamine, probably citrulline, and proline.

After 38 weeks, however, a small amount of acetic acid-soluble material was extracted from the intact seeds. These components, although extremely weak, appeared to correspond to components of ripe endosperm of intermediate electrophoretic mobility (Fig. 1, region F). No components of low mobility were observed. Direct chromatographic separations of acetic acid-soluble material were unsatisfactory presumably because of protein interference. Improved results were obtained after ultrafiltration, the major components being similar to those of the previous samples.

Acetic acid extracts of endosperm material obtained 39 weeks after planting appeared to contain the full complement of protein components present in similar extracts from ripe seeds. The general distribution of protein material was, however, somewhat different, the slow-moving fractions (Fig. 1, regions A–D) being extremely weak. A progressive increase in concentration

of these fractions occurred after 40 and 41 weeks, while the concentration of the fractions of higher mobility (region F) apparently remained constant (Fig. 4).

After 42 weeks, the immature seeds developed sufficiently to allow removal of the embryonic tissues. This did not, however, appear to alter the electrophoresis patterns of acetic acid-soluble endosperm components. Again an increase in concentration of slow-moving components was observed, with no significant alteration of other fractions. After 43 weeks, little variation in protein pattern was observed, fractionations being similar to those of control samples.

No acetic acid-soluble protein components were observed in embryonic extracts until after 43 weeks. At this stage only traces of protein were present. All subsequent samples were, however, identical to control extracts.

(F) *Post-harvest*

No changes were observed in the acetic acid-soluble protein fractions of endosperm or embryo after harvesting.

Discussion

Studies in the proteins of wheat throughout the life cycle of the plant produced some interesting results, although no drastic changes, except perhaps in the case of the embryonic proteins, were observed. Results obtained indicated the progressive degradation and utilisation of storage protein material during germination and its gradual resynthesis during ripening.

Analysis for total nitrogen (dry weight basis) of germinating wheat endosperm appeared to indicate the rapid utilisation of protein material during 4–5 days of germination, compared with that of other constituents, e.g., starch. This is presumably indicative of protein degradation and translocation in view of the relatively low levels of non-protein nitrogen in the dormant wheat endosperm. Results presented in Fig. 2 can also be explained in terms of a slower rate of utilisation of starch and other components over the initial period with an increase after the 5th or 6th day.

This rapid removal of nitrogen-containing material was not, however, detected by starch gel electrophoresis until after the 6th day of germination. Over the period from the 6th to the 9th day, components of low electrophoretic mobility (Fig. 1, A–D) became progressively weaker, presumably due to protein degradation and translocation. It is of interest to note that although

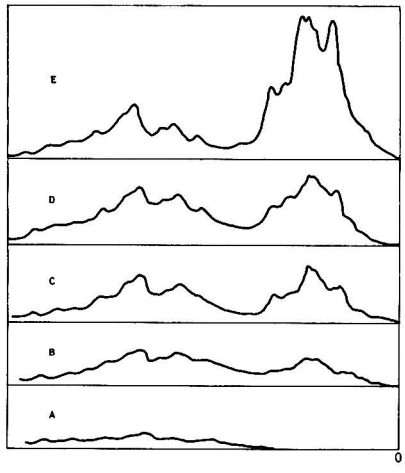

FIG. 4.—*Starch gel electrophoresis of dilute acetic acid-soluble endosperm proteins of wheat extracted at various stages during ripening* (densitometric scans)

(A) 38 weeks after planting
(B) 39 weeks after planting
(C) 40 weeks after planting
(D) 41 weeks after planting
(E) 42 weeks after planting

the degradation pattern resembled to some extent that produced by proteolysis,[4] no significant build-up of new components of smaller molecular weight was observed. This would seem to indicate, as expected, a rapid utilisation of the degraded material.

In contrast, components of intermediate electrophoretic mobility (Fig. 1, F) remained essentially unaltered throughout this period. Similar patterns were obtained up to the 13th day, after which these components became progressively weaker until the 17th day when only traces remained. A similar degradation pattern was observed by Danielsson[19] in pea seeds. In this case the breakdown of reserve protein was greatest from 5 to 10 days after germination, while albumins appeared to be degraded at a slower, more constant rate. Similarly, Bagley *et al.*[20] reported that the major period of protein alteration in germinating groundnuts occurred between the 4th and 9th days, while most of the storage protein appeared to have been utilised by the 15th day.

From these results, it would appear that the components of low electrophoretic mobility are more intimately involved in the germination procedure and possibly represent the true storage proteins of the endosperm. Components of higher mobility may well represent some of the enzyme systems of the plant which are involved in the metabolism of storage material. This conclusion is further substantiated by evidence from the ripening process discussed below, although it is supposed that entirely different sets of enzymes would be involved in both processes,

Embryo proteins, which have been shown to be quite distinct from endosperm components. appear to perform a very different role during germination. These components were shown to disappear quite dramatically during the very early stages, to be replaced by components of different solubility characteristics. The normal complement of acetic acid-soluble protein components present in the dormant embryo was observed only during the 1st and 2nd days of germination. After this period, only components soluble in solutions of higher pH, e.g., sodium tetraborate, could be extracted. This would seem to imply the involvement of these acetic acid-soluble components in one of the first stages in the germination process, perhaps as initiators of other processes which lead to endosperm protein degradation. A more detailed examination of these components, particularly during vernalisation, would be of interest.

The synthesis of acetic acid-soluble protein components of ripening wheat endosperm seems to substantiate further the view that the components of low electrophoretic mobility may represent the storage proteins of the dormant grain. It was of interest to note that these components were only produced at detectable levels very late in the ripening process. Acetic acid-soluble fractions were observed only for the last 5 weeks before final ripening of the grain (Fig. 4B). The concentration of components of intermediate mobility (Fig. 1, F) as judged by stain intensities, appeared to remain relatively constant over this period, in contrast to the progressive build up of slower-moving fractions (Fig. 1, A–D). After the first appearance of fractions of low mobility, no further qualitative changes in protein composition were observed, each extract containing the full complement of components present in the ripe endosperm.

Accurate assessment of the appearance of acetic acid-soluble embryonic components was extremely difficult due to the small amounts of material available. Results suggest, however, that these fractions also appear towards the end of the ripening period.

These results seem essentially similar to those reported by Graham & Morton.[15] The major difference between their report and the present findings would seem to be in the time factor involved for the ripening process. Although the precise date of wheat ripening is not given, the period from flowering to ripening in Australia would appear to be approximately 33–40 days. The corresponding period in this study was approximately 67 days.

By following throughout the ripening process the changes in proteins soluble in dilute acetic acid and in sodium pyrophosphate, it was shown[15] that from the 19th day after flowering until maturity, there was a rapid increase in the amount of acetic acid-soluble endosperm proteins (slow-moving fractions) compared with pyrophosphate-soluble proteins (intermediate mobility). This appears to be in agreement with the present results, since similar observations were made from the 31st day after flowering (39 weeks after planting) (cf. Fig. 4) which is roughly equivalent on the Australian time scale.

No protein components of low electrophoretic mobility were observed in pyrophosphate

extracts of their initial field sample (13 days after flowering), in contrast to all subsequent extracts. They did, however, detect a small amount of these components in dilute acetic acid extracts. This date of sampling roughly corresponds, in the present study, to the date of first appearance of protein fractions of intermediate mobility, when no slow-moving fractions were observed. The differences in these observations may be due, however, to their selection of stages: Graham & Morton did not examine any stages before the 13th day.

Acknowledgments

The authors wish to thank Mr. R. Seaton, Seed Testing Station, Dept. of Agriculture for Scotland, East Craigs, Edinburgh, for kindly supplying the wheat samples, and the Hercules Powder Co. Inc. for financial support for the project.

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Received 5 March, 1965

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NITROGEN REDISTRIBUTION DURING ENSILAGE AT LOW MOISTURE LEVEL

By C. J. BRADY*

The redistribution of nitrogen which occurs during the wilting of immature ryegrass to moisture contents of 51% and 40%, and during its ensilage in air-tight containers at these moisture levels was measured. The extent of protein hydrolysis was less at the lower moisture level, and evidence was also gained that metabolism of the amino-acids released was less extensive in the drier samples. A passage of nitrogen into proline residues which occurred rapidly during wilting, did not proceed when the grass of low moisture content was stored anaerobically.

Introduction

The rapid hydrolysis of protein in detached plant leaves has been well documented, and studies of nitrogen redistribution in a variety of plants during ensilage and haymaking have been reported.[1–4] Rapid wilting to a high dry matter content obviously limits the extent of

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protein breakdown,[4] but an increase during storage in non-protein nitrogen (NPN) in hay samples containing 86–89% of dry matter has been reported.[5] Greenhill *et al.*, [6] however, found no increase in the content of nitrogen soluble in hot 90% (v/v) ethanol during prolonged storage of hay of 83% dry matter content. That some enzymic activity can occur in biological material at these moisture levels seems probable.[7, 8]

The work of Kemble & Macpherson[2, 3] demonstrated that, besides the hydrolysis of protein, a considerable redistribution of nitrogen between amino-acid residues occurred during the wilting and ensilage of ryegrass. This conclusion is also clear from results,[4, 9] which show little or no change in the content of NPN not estimated as amide-, volatile-, or free or bound α-amino-N in wilting grass and clover, although some such increase would result from the release of the basic amino-acids of the protein. In ensiled grass and clover, on the other hand, the increases in this unaccounted NPN fraction, appeared to be greater than calculated for the release of the basic acids.[3, 4, 9] From analyses of the amino-acids in a series of silage samples, Kirchmeier & Kiermeier[10] concluded that amino-acids and ammonia together accounted for almost all the protein degradation products, and that, of the non-protein amino-acids found, only γ-aminobutyric acid was present in any quantity in silages of good quality. Their results included no analyses of samples before ensilage, so that recovery of individual amino-acids is not apparent. Maslowski *et al.*,[11] by quantitative paper chromatography, followed the changes in the content of a number of amino-acids in free and bound form in smooth brome grass ensiled for 35 days. Their results showed an overall loss of a number of individual amino-acids during ensilage period, and confirmed other reports[3, 12] of a considerable net gain in alanine. In detached, wilting plant material a very considerable net gain of proline has been observed,[2, 13] and a similar accumulation of proline may occur in the leaves of growing plants when subject to water stress.[14]

In the preparation of low-moisture silage (or haylage as it is called), plants are wilted to a dry matter content of about 50%, and then ensiled in gas-tight silos. In these circumstances, fermentation by plant enzymes or by bacteria is limited in extent, and the product may retain much of the soluble carbohydrate initially present.[15] Hydrolytic processes which occur in silage, such as the hydrolysis of hemicellulose[16] and of protein, can be expected to occur in the low moisture material, unless the removal of moisture in itself has imposed some limitation on these reactions. Previously,[4] it was shown that, in silage made from ryegrass wilted before ensilage to a dry matter content of about 40%, the breakdown of protein was as great as in silage of high moisture content. In the present work, the change in nitrogen distribution in ryegrass silage made with dry matter contents of about 50% and 60% was investigated. Consideration was given to changes in the unaccounted NPN fraction, and the contribution to this fraction of the nitrogen of γ-aminobutyric acid and of the basic amino-acids in the protein was determined. As a further measure of the metabolism of the free amino-acids, the recovery of proline and of alanine in the silage was estimated.

Experimental

Materials and procedure

The plant material used was short rotation ryegrass (*Lolium* sp., N.Z. Hl variety) grown in the laboratory grounds and harvested a few days prior to head emergence. Wilting was in the dark, in a forced circulation drying cabinet at 35°. The wilting times used were 12 h. to give a moisture content of 51%, and 24 h. to give a moisture content of 40%, of test weight. The method of ensilage, the losses of weight and of dry matter recorded, and the changes in the buffer index values observed have been reported separately.[17] Each ensilage treatment included duplicate silos, and from all silos, duplicate samples were taken for analysis. From unwilted, and wilted grass prior to ensilage, triplicate samples for analysis were drawn.

Analytical methods

Dry matter was estimated by drying for 16 h. in a fan-ventilated air oven at 80° (± 1°).

Total nitrogen (including nitrate) was estimated by a Kjeldahl method.[17a]

NPN was extracted into aqueous alcohol,[4] and then partitioned in a biphasic chloroform–

ethanol–water system after much of the ethanol in the original extract had been removed by concentration *in vacuo* at 40°. Interphasal material was rejected, and nitrogen in the washed chloroform phase (' lipid N ') and in the aqueous phase plus washings (' water-soluble NPN ') was determined by a semi-micro Kjeldahl procedure.[18]

In the aqueous phase, *volatile basic-N* and *amide-N* were determined by the methods of Pucher *et al.*, [19] *amino-N* with the reagent of Matheson *et al.*[20] using norleucine as a standard and correcting for the volatile base present, and *carboxyl-N* by the Synge modification[21] of the method of Van Slyke *et al.*,[22] but with pH 2·5 buffer in a volume of 1 ml. Bound carboxyl-N was estimated as the increase in carboxyl-N after hydrolysis in 6N–hydrochloric acid at 105° for 16 h. in sealed, evacuated tubes. Unaccounted NPN was calculated as the difference between the total water-soluble NPN and the sum of volatile basic-, amide-, total carboxyl- and the excess of amino- over free carboxyl-N (i.e., α -amino–N).

The content of phenylalanine plus tyrosine, of γ-aminobutyric acid, ornithine, histidine and lysine was determined on a 50-cm. column, and the content of arginine on a 15-cm. column using the method of Moore *et al.*[23] Proline and alanine in the extracts were converted to their 2,4-dinitrophenyl derivatives and separated on Celite columns buffered at pH 7·45.[24] Added alanine was recovered to the extent of 99·1 ± 1·5%, and added proline to 98·4 ± 1·3%.

Results

Observations, which are reported in detail elsewhere,[17] showed that only slight changes in pH, and in the buffer index value at pH 3·85 occurred in the ryegrass silages, ensiled with 51% or with 40% of moisture; the changes were a little greater in the moister silages. These results, together with the slight losses of dry matter noted, indicated that bacterial activity during storage was quite limited. Changes in nitrogen distribution during the wilting and ensilage periods are recorded in Table I.

An increase in the content of water-soluble NPN occurred during the wilting and especially during the ensiling periods of treatment. The extent of protein hydrolysis was greater with the higher moisture content, while at the higher but not at the lower moisture level, a secondary proteolytic effect occurred during extended storage. Volatile basic-N, which appeared as ammonia during ion-exchange chromatography, increased during storage, but after storage for 4 months, the content of volatile basic-N was not as great as that found in good quality silage of higher moisture content.[4] At the lower moisture level, a loss of amide-N occurred. It was noteworthy that while, at the higher moisture level, the content of amino-N consistently exceeded the carboxyl-N content, this was not so in the silage having 40% moisture. It seemed possible that this difference was due to a higher proline content in the more severely wilted

Table I

Nitrogen redistribution during ryegrass ensilage

Initial moisture content, % of test wt.	Ensiling period, days	Total N nitrogen, % of dry matter	% of total nitrogen							
			Lipid N	Water-sol. NPN	Volatile N	Amide N	Amino N	Carboxyl N	Bound carboxyl N	Un-accounted NPN
85	Unwilted	3·15	2·6	14·1	0·0	3·1	5·6	6·4	0·9	3·7
	0	3·07	2·7	18·5	0·0	2·5	7·7	6·6	1·9	6·4
	7	3·15	3·5	42·1	0·6	2·6	22·7	18·9	5·7	10·5
51	31	3·21	2·8	41·4	1·0	2·1	20·9	20·0	3·4	14·0
	133	3·25	2·9	47·2	4·0	2·6	23·9	22·1	2·9	13·8
	0	2·92	2·7	21·6	0·7	2·3	8·4	9·4	3·0	6·2
40	7	2·97	2·8	36·0	1·7	2·3	15·5	15·5	3·9	12·6
	133	3·06	2·6	37·4	3·1	0·9	16·7	18·5	2·6	12·3
Significance of treatment differences		>1%	1–5%	>1%	>1%	>1%	>1%	>1%	>1%	
S.E. of sample means		0·028%	0·26%	0·92%	0·14%	0·11%	0·91%	0·35%	0·65%	
95% Confidence limit		0·06%	0·6%	2·0%	0·3%	0·2%	1·9%	0·8%	1·4%	

sample. Bound carboxyl-N increased during the wilting treatment, and increased and then decreased during ensilage. The unaccounted NPN figure, which of course is subject to the accumulated errors of the analyses, increased sharply during ensilage.

In Table II are reported the contribution of various free amino-acids to the unaccounted NPN fraction. In this Table, the nitrogen of γ-aminobutyric acid is subtracted as unaccounted NPN, although some account of this N has been made in Table I as the excess of amino-over free carboxyl-N. The uncertainty of such accounting, however, is clear from the analyses of the 40% moisture samples, when the amino-N estimates do not exceed those for free carboxyl-N despite a quite high content of γ-aminobutyric acid. Because of this effect, the residual unaccounted NPN may be underestimated for the 51% moisture samples.

The results obtained show that, when account is made of the nitrogen of amino-acids which is not estimated as carboxyl-N, a considerable unaccounted NPN fraction remains, and this fraction increased during ensilage. Each of the amino-acids has been recovered in better than 97% yield by the analytical methods used, so that poor recovery of the amino-acids does not account for much of the residual unaccounted NPN. The non-carboxyl-N of the bound amino-acid fraction has not been included, and a high content of basic amino-acids in this fraction would influence the balance. However, analyses of hydrolysates of the 7-day and 133-day samples of 51% moisture content showed no bound histidine, 0·12% and 0·20% respectively of the total N as guanidino-N of bound arginine and 0·38% and 0·16% respectively as ε-N of bound lysine. The contribution of basic amino-acids in bound form to the residual unaccounted NPN does not then appear to be large. Two-dimensional paper chromatograms of the extracts gave no evidence of any significant amount of ninhydrin-positive material, other than γ-amino-butyric acid, which would not be accounted for in Table I.

In Table III, the contribution of free arginine, histidine and lysine, together with that of free alanine and proline, and of tyrosine plus phenylalanine to the water-soluble NPN is listed. Included in Table III are estimates of what the content of these amino-acids would be, assuming that the composition of the protein hydrolysed during wilting and ensilage was that of the total protein, and that the composition of the latter was that found by Chibnall *et al.*[25] for the leaf proteins of *Lolium perenne*.

During the wilting stage, marked losses of arginine and lysine, and some loss of histidine and the aromatic amino-acids, occurred. The content of alanine and more especially that of proline exceeded that calculated.

The excess of proline increased markedly during the 12-h. period when the plant moisture content was decreasing from 51 to 40%. In this same period, there was a loss of free alanine.

When consideration is given to the content of bound lysine, this amino-acid was recovered in about the expected amount during the ensilage phase. Losses of arginine, histidine, proline and the aromatic amino-acids occurred, however, and these losses were more marked at the

Table II

Composition of unaccounted non-protein nitrogen fraction

Initial moisture content, % of test wt.	Ensiling period, days	% of total nitrogen						
		Un-accounted NPN	γ-Amino-butyric acid-N	ε-N of lysine	Ring N of histidine	Guani-dino-N of arginine	δ-N of orni-thine	Residual un-accounted NPN
85	Unwilted	3·7	0·40	0·08	0·05	0·10	0·00	3·1
	0[1]	6·4	0·64	0·15	0·13	0·19	0·00	5·3
51	7	10·5	1·12	0·81	0·40	1·23	0·00	6·9
	31	14·0	1·10	1·05	0·41	1·25	0·10	10·1
	133	13·8	1·75	1·31	0·55	1·15	0·26	8·8
	0[2]	6·2	0·73	0·15	0·20	0·23	0·00	4·9
40	7	12·6	1·45	0·83	0·41	1·55	0·00	8·4
	133	12·3	1·05	0·89	0·30	1·45	0·00	8·5

[1] Wilting time, 12 h. [2] Wilting time, 24 h.

Table III

Free amino-acids in wilted and ensiled ryegrass

Initial moisture % of test wt.	Ensiling period, days		Amino-acid N (% of total nitrogen)					
			Arginine	Histidine	Lysine	Proline	Alanine	Tyrosine + phenyl-alanine
85	Unwilted		0·10	0·08	0·17	0·22	0·35	0·05
51	0[1]	Expected[3]	0·73	0·26	0·57	0·40	0·64	0·29
		Found	0·25	0·20	0·30	0·45	1·10	0·25
	7	Expected	4·10	1·26	2·69	1·34	2·20	1·59
		Found	1·64	0·60	1·62	1·06	2·62	1·00
	31	Expected	4·01	1·23	2·63	1·31	2·15	1·55
		Found	1·67	0·62	2·09	1·07	3·08	0·89
	133	Expected	4·84	1·47	3·15	1·55	2·54	1·87
		Found	1·55	0·89	2·68	1·23	3·32	0·80
40	0[2]	Expected	1·17	0·40	0·85	0·52	0·85	0·46
		Found	0·30	0·31	0·31	1·03	1·05	0·34
	7	Expected	3·23	1·00	2·14	1·09	1·80	1·26
		Found	2·08	0·64	1·65	1·23	2·00	0·81
	131	Expected	3·43	1·06	2·27	1·15	1·89	1·33
		Found	1·97	0·47	1·77	1·51	2·03	0·75

[1] Wilting time, 12 h. [2] Wilting time, 24 h.
[3] Recovery of amino-acid N expected, assuming the composition of the protein hydrolysed was that of the total protein.

higher moisture content. Particularly at this higher moisture level, the content of alanine exceeded the calculated figure.

Discussion

The analyses in Table I afford evidence that, within the moisture content range used in haylage production, the extent of protein breakdown is related to the amount of moisture present. With 51% moisture in the grass, 29% of the protein present at the time of ensilage was hydrolysed within the first 7 days, compared with 18% at 40% moisture. The results in Table III, particularly those for arginine and alanine, also indicate more limited metabolic changes in the drier silage. Although convincing work with silage on ruminal nitrogen metabolism is lacking, it appears likely, from other work on nitrogen retention by ruminant animals,[26,27] that a higher content of soluble carbohydrate and reduced content of readily soluble nitrogen in haylage would make it a more effective nitrogen source for the ruminant than is high-moisture silage. That the wilting of grass prior to ensilage favours high dry-matter intake by cattle has been well demonstrated.[15]

In the samples ensiled with 51% moisture, a further hydrolysis of protein occurred in the period between 1 and 4 months. The evidence of a change in pH and of buffer index value indicated some bacterial activity in these samples,[17] and it is probable that this secondary proteolytic activity was due to bacterial proteases. The appearance of ornithine in these samples after 1 month and 4 months of ensilage (Table II), also suggests bacterial activity, since enzymes producing ornithine from arginine via citrulline have been demonstrated in a number of lactic acid-producing bacteria.[28,29]

Evidence supporting the conclusion that proteins broken down in starving leaves have a composition similar to that of the total leaf protein was presented by Kemble & Macpherson.[30] In Table III, the assumption is made that this conclusion applies also to the basic amino-acids

which were not estimated by these authors. That this assumption is justifiable appears likely from a comparison of analyses of isolated grass[25,31] and grass-hay[32] proteins.

The passage of nitrogen into proline during wilting[2,13] was well demonstrated in these samples (Table III). It was also apparent that this marked accumulation of proline did not continue during ensilage undertaken at a low moisture level, and it must be concluded that it is an aerobic process. The marked loss of lysine during the aerobic phase was completely curbed in the anaerobic environment, but the loss of arginine continued anaerobically, but to a lessened extent. A loss of arginine during ensilage has been reported previously,[33] and is to be expected when a range of lactic acid bacteria develop. Of the changes in the content of other amino-acids during the early stages of ensilage, most arise from the action of plant enzymes,[3,34] and the results shown in Table III are consistent with that conclusion.

Macpherson & Slater[34] have demonstrated a rapid increase in the content of γ-aminobutyric acid in ryegrass during the early stages of wilting and of ensilage. In the experiment reported here, nitrogen as γ-aminobutyric acid increased both during the wilting and ensilage phases. While the concentrations reached in these silages were notably less than found by Macpherson & Slater, this may not be due to the lowered moisture level, for after 7 days, more nitrogen was recovered as γ-aminobutyric acid in the drier silage.

An attempt to account for the decided increase in the unaccounted NPN fraction of the ensiled grass (Table II), left about half of the increase in this fraction, not determined as nitrogen of the basic amino-acids. Separation of the lipophilic portion of the ethanolic extracts showed no change during conservation in this portion (Table I). The use of group methods of analysis for amino-N is necessarily subject to some uncertainty as all amino-acids do not give the same product yield;[21] any decided change in the proportions of amino-acids present may then give a misleading response. Confirmation of the increase in the unaccounted NPN fraction should be sought using quantitative analysis of the amino-acids in hydrolysates of the extracts.

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Received 8 February, 1965

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THE PYRETHRINS AND RELATED COMPOUNDS
VII.*—New Pyrethrin-like Compounds with Ester and Ketonic Groups in the Alcoholic Side Chain

By C. CORRAL† and M. ELLIOTT

Pyrethrin-like esters of a new type were obtained by reaction of the 4-bromo derivatives of esters (Et, Pr, Ph) of 3-methylcyclopent-2-enone-2-acetic acid with silver chrysanthemates. The structure of the intermediate bromo compounds and hence of the esters was deduced from the ease with which hydrogen bromide was eliminated by tertiary amines to give cyclopentadienones. 2-Acetonyl-3-methylcyclopent-2-enone was prepared and by similar reactions gave a chrysanthemyl derivative.

Introduction

The unsaturated side chain CH_2X (X is *cis*-CH:CH·CH:CH_2 or *cis*-CH:CHMe) in the keto-alcohols of the natural pyrethrins is a centre important for high insecticidal activity.[1] This investigation was made to discover if compounds in which X contained non-olefinic unsaturation (e.g., COOR, COMe or CN) would also be active insecticidally, because these should be more easily synthesised than esters (such as allethrin, where X is $CH:CH_2$) and the natural pyrethrins (in which X is alkenyl). It was anticipated that esters of 3-methylcyclopent-2-enone-2-acetic acid, like 2-alkyl-3-methylcyclopentenones, would give 4-bromo compounds with *N*-bromosuccinimide, from which the required esters could be obtained by reaction with silver chrysanthemates. The syntheses and proofs of structure of these esters are described here.

Experimental

(Melting points and boiling points are not corrected.)

Ultra-violet spectra were measured in ethanol on a Unicam S.P. 500 spectrophotometer, Infra-red spectra were determined for liquid films on a Perkin-Elmer Infracord spectrometer. model 137. The absorption bands noted are those significant for structural assignments or identification.

Esters of 3-methylcyclopent-2-enone-2-acetic acid.—Furfurylideneacetone[2] was converted to 4,7-dioxo-octanoic[3] and thence to 3-methylcyclopent-2-enone-2-acetic acid,[3] m.p. 110–113° (lit.[3] 108·5–110·5°); λ_{max} 237 mμ (ε 13,500) in water.

The acid (20 g.) was esterified by refluxing with ethanol (100 ml.) and toluene-4-sulphonic acid (0·2 g.) for 10 h. to give *ethyl 3-methylcyclopent-2-enone-2-acetate* (**Ib**) (18·6 g., 79%), b.p. 100°/4 × 10^{-3} mm., n_D^{20} 1·4863 (Found: C, 66·0; H, 7·6. $C_{10}H_{14}O_3$ requires C, 65·9; H, 7·7%), λ_{max} 235 mμ (ε 13,400), ν_{max} 1700s, 1730s (C=O), 1645s (C=C) cm.$^{-1}$. The ester was characterised as the *2,4-dinitrophenylhydrazone*, red plates from ethanol, m.p. 150·5–151° (Found: C, 52·9; H, 4·8; N, 15·6. $C_{16}H_{18}N_4O_6$ requires C, 53·0; H, 5·0; N, 15·5%) and *semicarbazone* (from ethanol) m.p. 203–204° (Found: C, 55·4; H, 7·3; N, 16·9. $C_{11}H_{17}N_3O_3$ requires C, 55·2; H, 7·2; N, 17·6%).

The *propyl ester* (**Ic**), prepared similarly (75%), had b.p. 100–103°/2 × 10^{-3} mm., n_D^{20} 1·4794 (Found: C, 66·6; H, 8·2. $C_{11}H_{16}O_3$ requires C, 67·3; H, 8·2%), λ_{max} 231 mμ (ε 11,000), ν_{max} 1700s, 1730s (C=O), 1645s (C=C) cm.$^{-1}$; *2,4-dinitrophenylhydrazone* (red plates from propanol), m.p. 159–60° (Found: C, 54·3; H, 5·4; N, 14·9. $C_{17}H_{20}N_4O_6$ requires C, 54·1; H, 5·7; N, 14·8%).

Phenyl 3-methylcyclopent-2-enone-2-acetate (**Id**).—Purified thionyl chloride[4] (8·0 ml.) was added to 3-methylcyclopent-2-enone-2-acetic acid (15·4 g.) in benzene (250 ml.) and pyridine (8·0 ml.) at 0°. After 1 h. the clear liquid was decanted from the cake of pyridine hydrochloride and phenol (20 g.) was added. The product was poured into water after 8 h. and the organic

* Part VI: *J. chem. Soc.*, 1965, p. 3097

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layer was diluted with ether, separated and washed with sodium hydroxide solution and water. Distillation of the product after drying (Na_2SO_4) and evaporation of solvents gave the *phenyl ester*, b.p. 155–160°/1·21 × 10^{-3} mm., which solidified. After two recrystallisations (ethyl acetate) the product (5 g.) had m.p. 104–105° (Found: C, 72·7; H, 6·0. $C_{14}H_{14}O_3$ requires C, 73·0; H, 6·1%); λ_{max} 229 mμ (ε 14,600).

Allyl 3-methylcyclopent-2-enone-2-acetate (**Ie**).—A procedure similar to that used for the phenyl ester (but the washing was with sodium carbonate solution instead of sodium hydroxide) gave the *allyl ester*, b.p. 107°/3 × 10^{-3} mm., n_D^{20} 1·4990 (Found: C, 67·7; H, 7·5. $C_{11}H_{14}O_3$ requires C, 68·0; H, 7·3%). λ_{max} 230 mμ (ε 13,400); ν_{max} 1705s, 1745s (C=O), 930m, 988m (C=CH_2) cm.$^{-1}$ The ester (n_D^{20} 1·4982) was also made from the acid and allyl alcohol in the presence of toluene-4-sulphonic acid. It was characterised as the *2,4-dinitrophenylhydrazone* (red plates from allyl alcohol), m.p. 172–172·5° (Found: C, 54·1; H, 4·7; N, 14·9. $C_{17}H_{18}N_4O_6$ requires C, 54·5; H, 4·8; N, 15·0%) and *semicarbazone* (from ethanol) m.p. 186–187° (Found: C, 58·1; H, 6·7; N, 17·1. $C_{12}H_{17}N_3O_3$ requires C, 57·4; H, 6·8; N, 16·7%).

3-Methylcyclopent-2-enone-2-acetamide (**If**).—Ethyl 3-methylcyclopent-2-enone-2-acetate (11·6 g.) was set aside for 4 days at room temperature with aqueous ammonia (sp. gr. 0·88; 25 ml.). The ester went into solution after 1 day and crystals separated after 2 days. The *amide* (6·8 g.) was filtered off and the mother liquor was concentrated to dryness *in vacuo* to give a second crop of amide (total, 8·4 g., 86%). The amide, colourless needles (from acetone), had m.p. 152–154°. (Found: C, 62·9; H, 7·1; N, 8·9. $C_8H_{11}NO_2$ requires C, 62·7; H, 7·2; N, 9·1%); λ_{max} 233 mμ (ε 13,000). The same compound was obtained, but in lower yield, by the action of ammonia on the acid chloride (from the acid, with thionyl chloride in pyridine). The *semicarbazone* (colourless needles from ethanol) had m.p. 227° (Found: C, 51·3; H, 6·6; N, 25·4. $C_9H_{14}N_4O_2$ requires C, 51·4; H, 6·7; N, 26·7%).

3-Methylcyclopent-2-enone-2-acetonitrile (**Ig**).—The amide (5·93 g.) was refluxed with acetic anhydride (250 ml.) under a fractionating column for 2 h. whilst acetic acid was removed (distillation temperature rose from 118 to 136°). After evaporation of excess acetic anhydride the residue was distilled and a fraction (2·4 g., 46%), b.p. 95–98°/1·75 × 10^{-2} mm., n_D^{20} 1·5050 (Found: C, 70·1; H, 6·8; N, 10·5. C_8H_9NO requires C, 71·0; H, 6·7; N, 10·4%) was isolated by two successive fractionations, a white solid that sublimed initially and a higher boiling residue being rejected; λ_{max} 228 mμ (ε 13,500); ν_{max} 2260 m (C≡N), 1705s (C=O) cm.$^{-1}$ When the amide was heated at 140–150° for 3 h. with 1·1 mol. of acetic anhydride, only a trace of white solid sublimed initially and the *nitrile* (30%) was isolated more easily. The *2,4-dinitrophenylhydrazone* (red needles from ethanol) had m.p. 210–211°. (Found: C, 53·6; H, 4·4; N, 22·5. $C_{14}H_{13}N_4O_5$ requires C, 53·3; H, 4·2; N, 22·2%) and the *semicarbazone* (colourless needles from ethanol), m.p., decomp. above 200°. (Found: C, 56·1; H, 6·0; N, 28·7. $C_9H_{12}N_4O$ requires C, 56·2; H, 6·3; N, 29·1%).

2-Acetonyl-3-methylcyclopent-2-enone (**Ih**).—2,5,8-Trioxononane[5] (m.p. 54–56°; lit.[5] 59–60°) (130 g.) was set aside for 24 h. at room temperature with 5% aqueous sodium hydroxide (700 ml.) (intermittent agitation). The product was extracted with ether (10 × 200 ml.), the extracts were washed with dilute sulphuric acid and water, dried (Na_2SO_4), evaporated and distilled. *2-Acetonyl-3-methylcyclopent-2-enone* (36 g., 31%), b.p. mainly 90–94°/1·75 × 10^{-2} mm., n_D^{20} 1·4960 (Found: C, 71·3; H, 7·9. $C_9H_{12}O_2$ requires C, 71·0; H, 7·95%); λ_{max} 234 mμ (ε 10,600), unchanged in the presence of alkali; ν_{max} 1705s (broad, C=O and conjugated C=O), 1650s (C=C) cm.$^{-1}$ *Bis-2,4-dinitrophenylhydrazone* (red plates from chloroform), m.p. 241° (Found: C, 49·0; H, 4·1; N, 22·0. $C_{21}H_{22}N_8O_8$ requires C, 49·0; H, 4·3; N, 21·8%).

Reactions with N-bromosuccinimide.—(a) Ethyl 3-methylcyclopent-2-enone-2-acetate (10 g.) was heated in boiling carbon tetrachloride (50 ml.) with *N-bromosuccinimide* (9·8 g.) for 10 min., when reaction was complete (separation of succinimide at surface of solvent). After cooling to 0°, succinimide was filtered off and triethylamine (5 ml.) was added to the filtrate which was set aside at 0° for 10 h. Triethylamine hydrobromide was filtered off and dissolved in 5% sodium hydroxide (150 ml.). The solution was acidified (dil. nitric acid) and excess silver nitrate was added. The precipitate of silver bromide (8·53 g., after drying to constant weight at 130°) was equivalent to 84% of the bromine in the *N*-bromosuccinimide used up.

The mother liquor after separation of amine hydrobromide was evaporated and distilled at 3×10^{-2} mm. After rejection of a fraction b.p. 90–102° (1·1 g.), evolution of carbon monoxide took place at a bath temperature of 150° and then *diethyl 3,5-dimethylindan-1-one-2,4-diacetate* distilled (5·7 g., 58%) b.p. 180–190°, n_D^{20} 1·5235. (Found: C, 67·8; H, 7·2. $C_{19}H_{24}O_5$ requires C, 68·6; H, 7·3%); λ_{max} 212, 255, 301 mμ (ε 30,200, 12,600, 3300) [Optika CF4 spectrometer]; indan-1-one has λ_{max} 244, 292 mμ (ε 13,100, 2910) and 2,3,4,5-tetramethylindan-1-one, λ_{max} 213, 253, 298 mμ (ε 24,900, 12,800 and 2200).[6]

In another experiment, the undistilled residue after filtration of triethylamine hydrobromide gave the 2,4-*dinitrophenylhydrazone* of *dimeric ethyl 3-methylcyclopenta-2,4-dienone-2-acetate* (**IVb**), m.p. 181–182° (from ethanol–nitrobenzene) (Found: C, 53·7; H, 4·9; N, 15·3. $C_{32}H_{32}N_8O_{12}$ requires C, 53·3; H, 4·5; N, 15·5%) and the *semicarbazone* of (**IVb**), m.p. 225·5–226·5° decomp. (from acetic acid) (Found: C, 54·3; H, 6·4; N, 18·2. $C_{22}H_{30}N_6O_6$ requires C, 55·7; H, 6·4; N, 17·7%).

Ethyl 3-methylcyclopent-2-enone-2-acetate (7·0 g.) in carbon tetrachloride (40 ml.) was brominated with *N*-bromosuccinimide (6·8 g.) as before. After removal of succinimide, silver (±)-*cis-trans*-chrysanthemate[7] (11·5 g.) was added to the carbon tetrachloride solution of the bromo compound which was set aside at room temperature overnight and then warmed on steam for 2 h. Silver bromide and unreacted silver salt were then filtered off (using Celite filter aid) and the residue was distilled and redistilled to give the (±)-cis-trans-*chrysanthemate* of *ethyl 4-hydroxy-3-methylcyclopent-2-enone-2-acetate* (**IIIb**) (7·0 g.), b.p. 170–180°/3 × 10^{-2} mm., n_D^{20} 1·4989 (Found: C, 68·5; H, 7·9. $C_{20}H_{28}O_5$ requires C, 68·9; H, 8·1%); λ_{max} 225 mμ (ε 18,800).

(*b*) Similarly, the propyl ester (**Ic**) (3·0 g.), *N*-bromosuccinimide (3 g.) and silver (±)-*trans*-chrysanthemate (7·0 g.) gave the (±)-trans-*chrysanthemate* of *propyl 3-methyl-4-hydroxycyclopent-2-enone-2-acetate* (3·0 g.), b.p. 195–197°/1 × 10^{-2} mm., n_D^{20} 1·4970. (Found: C, 69·4; H, 8·4. $C_{21}H_{30}O_5$ requires C, 69·6; H, 8·3%); λ_{max} 224 mμ (ε 16,800).

(*c*) The phenyl ester (**Id**) (3·55 g.) with *N*-bromosuccinimide (2·72 g.) gave the bromo compound (**IId**) after 3 h., in the usual way, and thence the (±)-cis-trans-*chrysanthemate* of *phenyl 3-methyl-4-hydroxycyclopent-2-enone-2-acetate* (**IIId**) (3·0 g.) b.p. 205–220°/1 × 10^{-2} mm., m.p. 125–126° (from ethyl acetate) (Found: C, 72·7; H, 7·1. $C_{24}H_{28}O_5$ requires C, 72·7; H, 7·1%); λ_{max} 222 mμ (ε 22,600). In a separate experiment, 98% of the bromine introduced with *N*-bromosuccinimide was eliminated with trimethylamine.

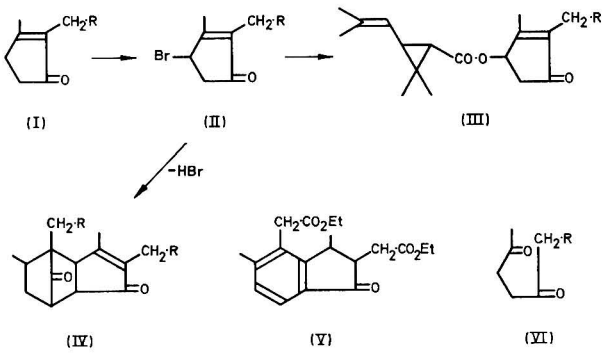
(*d*) 2-Acetonyl-3-methylcyclopent-2-enone (**Ih**) (3·1 g.) with *N*-bromosuccinimide (3·6 g.) (reaction complete in 10 min.) and then silver (±)-*cis-trans*-chrysanthemate gave the (±)-cis-trans-*chrysanthemate* of *2-acetonyl-3-methyl-4-hydroxycyclopent-2-enone* (**IIIh**) (2·9 g.) b.p. 160–180°/1 × 10^{-2} mm., n_D^{20} 1·5079 (Found: C, 70·9; H, 8·7. $C_{19}H_{26}O_4$ requires C, 71·6; H, 8·2%) λ_{max} 225 mμ (ε 15,700). A forecut of (±)-*cis-trans*-chrysanthemic acid (1·9 g.) was obtained. Of the bromine introduced, 97% was available for elimination with trimethylamine.

(*e*) The allyl ester (**Ie**) (2·6 g.) with *N*-bromosuccinimide (2·6 g.) in carbon tetrachloride (20 ml.) required 8 h. and addition of benzoyl peroxide for completion of reaction. The (±)-*trans*-chrysanthemate (b.p. 190–192°/1·21 × 10^{-2} mm., n_D^{20} 1·5087) obtained from silver salt (6·0 g.) contained bromine (Found: C, 68·3; H, 8·0; Br, 2·8. Calc. for $C_{21}H_{28}O_5$: C, 70·0; H, 7·8%); λ_{max} 224 mμ (ε 18,900). The esters from other runs contained 6·8 and 7·3% of bromine respectively.

(*f*) The nitrile (**Ig**) (2·0 g.) reacted to completion with *N*-bromosuccinimide (2·62 g.) in carbon tetrachloride (20 ml.) in 2 h. An oil and succinimide floated on the surface. Carbon tetrachloride was evaporated *in vacuo* and the product was taken up in benzene and filtered from succinimide. When the benzene solution was treated with silver (±)-*trans*-chrysanthemate (5 g.), the usual procedure (and modifications) gave only recovered chrysanthemic acid (2·3 g., 92%) and a trace of high boiling product from which the band at 2260 cm.$^{-1}$ (C≡N) was absent. In a repetition of the above preparation, the benzene solution of the bromo compound (**IIg**) gave trimethylamine hydrobromide that contained 97% of the bromine introduced.

Results and discussion

The ethyl (**Ib**), propyl (**Ic**) and phenyl (**Id**) esters of 3-methylcyclopent-2-enone-2-acetic acid (**Ia**) (obtained by cyclisation of acetonyl-laevulic acid,[3,8] produced when furfurylacetone is



cleaved with hydrochloric acid in ethanol) reacted smoothly with *N*-bromosuccinimide to give monobromides (**IIb, c, d**) without significant evolution of hydrogen bromide. This was similar to the behaviour of 3-methylcyclopent-2-enones containing a 2-alkyl group but different from the situation with a 2-alkenyl side-chain when hydrogen bromide was evolved and polymerisation occurred.[7] The mono-bromides when treated with the silver salts of chrysanthemum monocarboxylic acids gave the (bromine-free) esters (**IIIb, c, d** respectively) required.

Proof was necessary that bromine was introduced at $C_{(4)}$ on the ring and thus that the derived esters had the required structures. When the monobromides (e.g., **IIb**) reacted with trimethylamine or triethylamine at 0° in carbon tetrachloride, amine hydrobromide containing 80–95% of the bromine in the *N*-bromosuccinimide used was precipitated. This was not a quaternary bromide derived from the monobromo compound, for the mother liquors contained a dehydrobrominated product that was recognised as a dimeric cyclopentadienone (**IVb**) by the formation of a bis-2,4-dinitrophenylhydrazone and a bis-semicarbazone and because, on distillation it eliminated carbon monoxide and rearranged to an indanone (**V**). This behaviour is characteristic of 3-methyl-2-alkyl- and -alkenylcyclopentadienones.[9] (Earlier[7] it was assumed that 2,3-dialkylcyclopentadienones would be dimeric, but give monomeric derivatives; there is now spectroscopic evidence that both derivatives are also dimeric.[10]) As hydrogen bromide was eliminated under such mild conditions, when rearrangement was very unlikely, bromine must have been at $C_{(4)}$ or $C_{(5)}$ on the cyclopentenone ring, for no other allylic or activated position had adjacent hydrogen. It was rigorously established[7] that substitution in 3-methyl-2-alkylcyclopentenones was at $C_{(4)}$ and the corresponding position in the compounds now examined would be equally preferred.

The allyl ester of 3-methylcyclopent-2-enone-2-acetic acid (**Ie**) reacted more slowly with *N*-bromosuccinimide and the chrysanthemate (**IIIe**) derived from the intermediate bromo compound still contained bromine, so some substitution must also have occurred at an alternative site in the allyl ester group.

3-Methylcyclopent-2-enone-2-acetonitrile (**Ig**) was obtained by dehydration of the amide (**If**) with acetic anhydride. Couvreur & Bruylants[11] found that aliphatic nitriles (e.g., propionitrile) reacted relatively slowly with *N*-bromosuccinimide in carbon tetrachloride in the absence of benzoyl peroxide, and Bailey & Bello,[12] from investigations with vinylacetonitrile and crotonitrile, found that the cyano group attached directly to the α-carbon inhibited allylic substitution. It was expected, therefore, that $C_{(4)}$ in the nitrile (**Ig**) would again be the most reactive position for substitution by bromine and, in agreement, 83% of the bromine introduced was eliminated easily with trimethylamine. However, the main product isolated after reaction of the bromo compound (in this instance not soluble in carbon tetrachloride) with silver chrysanthemate was chrysanthemic acid. In all reactions with the silver salt, including those with the ethyl and propyl esters, and with the 3-methyl-2-alkylcyclopentenones,[7] a small

forerun of acid was isolated and it was concluded that, depending on the ease with which hydrogen bromide was eliminated from the bromide, more or less ester was obtained, the competing process being removal of silver chrysanthemate by reaction with liberated hydrogen bromide. In the nitrile (**IIg**) this reaction predominated and with the acetone derivative (**IIh,** below) it diminished the yield of ester, but not below an acceptable level.

Alder & Schmidt[5] made 2,5,8-trioxononane (**VI**) by acidic cleavage of the addition product of sylvan and methyl vinyl ketone. Aqueous sodium hydroxide cyclised this triketone to 2-acetonyl-3-methylcyclopent-2-enone (**Ih**) which reacted with *N*-bromosuccinimide to give a bromide, from which nearly all the bromine was removed with trimethylamine, and which gave the (±)-*cis-trans*-chrysanthemate (**IIIh**).

The detailed description of the biological activities of the new compounds will be given elsewhere; it was found that the unsaturated functions in these esters were less effective in producing high insecticidal activity, than were the olefinic substituents in the natural esters.

Acknowledgment

This work was supported by a grant (to C. C.) by the Fundacion ' Juan March ', which is gratefully acknowledged.

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Received 16 February, 1965

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SOURCES OF ENERGY FOR THE LACTATING DAIRY COW*

By A. W. A. BURT

The overall efficiency of energy conversion by the lactating cow is briefly discussed, together with some effects of variation in the amount of concentrates fed due to variation in productivity. The diurnal changes in the concentration and ratios of rumen volatile fatty acids, associated with a particular feeding routine, were shown to have considerable effects upon the relative caloric contributions of the different volatile fatty acids. Changes in management may therefore be important, and the difficulties of measuring these are discussed.

Additions of orthophosphoric acid to diets containing urea depressed the digestibility of dietary organic matter and crude fibre, but had little effect upon rumen pH. Subsequent rumen-ammonia levels, reflecting rate of release from these diets, were directly related to rumen pH at the time of feeding.

Introduction

This paper is divided into three parts. The first deals with some aspects of changes in the supply of energy to the cow at different stages of lactation and different rates of production under winter feeding conditions, in situations where diets are based on roughages for maintenance and concentrate mixtures for production. In the second part, diurnal fluctuations in the production of rumen metabolites within a normal feeding system are discussed and the third part deals with some unexplained effects of added chemicals in the diet.

(1) The energy balance of the lactating cow

Table I shows the energy intake and output of a cow receiving a typical winter diet and giving 4 gallons of milk. The diet consists of hay and sugar beet pulp for maintenance and concentrates for all production, and provides around 53 Mcal. of gross energy daily. The energy produced in 4 gallons of milk is around 13—14 Mcal. daily, representing an efficiency of conversion of 25%. The same Table summarises the energy losses which occur between intake and production and shows that the two major sources of loss are faecal energy and heat output. The data were derived by calculation from the results of digestibility and balance trials at Colworth and are very similar to the direct measurements of Møllgard.[1] Because of the technical difficulties involved there are all too few direct measurements of energy metabolism in the lactating cow.[2] No allowance was made in this calculation for energy storage or loss due to changes in body reserves. This varies, but is generally likely to be small in cows adequately fed, relative to the daily output of milk.

In normal farm practice the productivity of the cow varies with stage of lactation and individuality and this variation results in a considerable alteration in total energy requirements. Under controlled winter feeding conditions this is usually met by alteration in the amounts of concentrates fed. This is illustrated in Fig. 1 which shows total starch equivalent and dry matter intake

Table I

Intakes, outputs and energy losses in a cow giving 4 gallons of milk per day

Food intake Hay 11 lb., Sugar beet pulp 5 lb., Concentrates 16 lb., Water 120 lb. Total 52·71 Mcal.

Milk output 40 lb. (3·79% fat, 4·46% lactose, 3·02% protein) 13·04 Mcal.

Energy Loss as faeces 39·67

	Mcal.
Faeces	16·10
Urine (estimated)	1·50
Methane (estimated)	4·22
Heat (estimated)	17·85
Total	39·67

* Read at meeting of Agriculture Group, 22 October, 1963

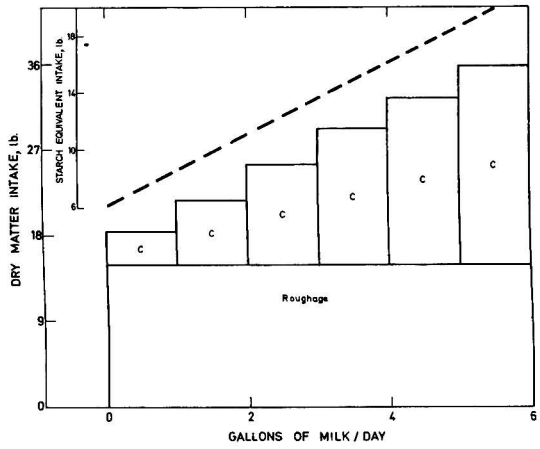

FIG. 1.—*Concentrate and roughage dry-matter intake at different milk yields*
– – – starch equivalent intake ——— dry matter intake c = concentrates

and the distribution of dry matter intake between concentrates and roughage in cows fed on such a system. This increase in concentrate intake with production has the effect of increasing the digestibility of the total energy intake and allows the energy requirements of the cow to be met within the limits of its appetite for dry matter at different levels of production.

These changes also alter the dietary ratio of roughage to concentrates very considerably, as the cow giving 2 gallons daily may be receiving 8 lb. of concentrates with 16 lb. of roughage dry matter, while one giving 5 gallons may be eating 20 lb. of concentrates with the same amount of roughage. This represents a change in ratio of concentrates to roughage from 1:2 to 1:0·8. Many studies have shown that changes of this order have marked effects upon the digestibility of the total energy intake, for instance Bloom *et al.*[3] found changes in dry-matter digestibility from 54% to 63% on four different ratios of concentrates: hay, when replacement was based on estimated net energy values. Similar effects were found by Elliott & Loosli,[4] who also showed that the productive utilisation of digested energy improved with increasing proportions of concentrates in the diet.

This increase in digestibility of the total ration as the percentage of concentrates rises is probably due to the increasing percentage of more digestible dry matter from concentrates in the ration. There is no evidence to suggest that at this level of concentrate intake, concentrates improve the digestibility of the roughage fed. Indeed, the evidence reviewed by Reid[5] suggested that the digestibility of rations declines more with increasing intake, as the ratio of concentrate roughage is increased.

Two other effects would be expected from this wide variation in concentrate/roughage ratios within the normal lactation. First, it might be thought that there would be considerable variation in the molar ratios of volatile fatty acids produced in the rumen and absorbed, since it is well recognised that a higher ratio of concentrates to roughage in the diet produces a fermentation in the rumen yielding larger proportions of propionic and butyric acids relative to acetic acid. It might be expected that this would give rise to appreciable variation in the composition of milk due to stage of lactation, including possibly, increases in the ratio of solids-not-fat to butterfat at the higher levels of concentrate intake, but this is not generally apparent, since there are declines in both fat and solids-not-fat percentages at peak of lactation.[6,7] At this time the demands of the cow for milk synthesis may be considerable, as is shown in Table II. The 6 Mcal. of apparently digested protein are balanced by an output of 3 Mcal. of milk protein, while the amount of digested fat is small relative to fat output in milk. The demand for rumen volatile fatty acid precursors of milk fat and lactose is therefore high. This high demand at peak lactation

Table II

Intake, digestion and production of energy in the different organic constituents

	Crude protein	Carbohydrates (NFE* + crude fibre)	Fat	Total
Intake (Mcal./day)	9·19	41·17	2·35	52·71
Apparently digested (Mcal./day)	6·05	29·49	1·09	36·63

	Protein	Lactose	Fat
Milk production (Mcal./day)	3·10	3·46	6·48

* nitrogen-free extract

may well counterbalance the increased input from the diet and the variation in concentrate/roughage ratio may therefore not vary the proportions of the individual rumen volatile fatty acids sufficiently to bring about any marked change in milk composition.

Secondly, the variation in concentrate/roughage ratio might be expected to have important effects upon the reaction of the cow to variations in composition of the concentrate. While the quantitative response varies, for instance the response per pound of additional starch equivalent (S.E.) fed can vary from 0·6 lb. of milk/lb. S.E. at a yield of 2 gallons daily to 1·4 lb. milk/lb. S.E. at 4 gallons daily;[8] the response to qualitative changes in the diet, e.g., the change in milk fat percentage due to feeding different fats and oils[9] shows surprisingly little variation at milk yields ranging from 1½ to 4 gallons with corresponding alterations in the ratio of roughage to concentrates resulting from the normal practice of feeding for production.

(2) The daily intake

In practice, the daily diet of the cow is fed as a series of discrete meals, usually of different foods, and the size, distribution and nature of the foods involved varies from farm to farm. Fig. 2 shows a typical feeding routine and some of the changes in rumen liquor pH and the concentration of total volatile fatty acids that result therefrom. These results are the mean of four separate observations on each of two cows fitted with rumen fistulae. The diet consisted of 10 lb. of concentrates at two feeds and 11 lb. of hay and 20 lb. of grass silage at two other feeds. After fasting overnight, rumen liquor pH at 7 a.m. was around 6·6 and volatile fatty acid (V.F.A.) concentration around 86 mequiv./litre. After feeding 5 lb. of concentrates pH fell by 0·5 units and V.F.A. concentration rose to 126 mequiv./litre 2 h. later. Rumen V.F.A. and pH do not show equivalence because of the buffering effects of the large quantities of saliva secreted[10] and of the breakdown products of some of the dietary constituents, particularly protein. The noticeable drop in rumen pH after the 11.0 a.m. silage feed was not associated with any increase in the

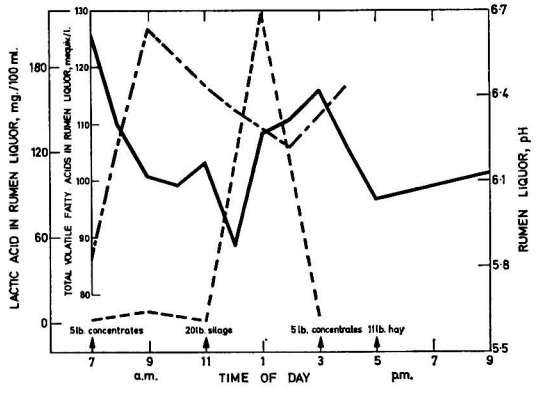

FIG. 2.—*Diurnal changes in rumen pH and volatile fatty acid and lactic acid concentrations*
— — — total volatile fatty acid - - - - lactic acid ——→ rumen pH

concentration of total V.F.A., although lactic acid concentration increased tremendously. It can be calculated that the increase in lactic acid was approximately equivalent to the 24 g. fed in 20 lb. of silage. This change in pH may be due to lower rates of saliva secretion after feeding silage compared with other foods.[11] The rapid disappearance of lactic acid, the concentration of which had dropped to its usual low level in rumen fluid by 3.0 p.m., reflects its rapid dissimilation by the rumen microflora by well recognised metabolic routes.[12]

The relative molar concentration of the main rumen volatile fatty acids at different times of day under this system of feeding are shown in Table III. The molar percentage of acetic acid was reduced from 70% at 7 a.m. to 66% 2 h. later, with corresponding increases in propionic and butyric acids. Propionic acid increased further from 19 to 22%, after the silage had been fed, probably due to conversion of lactic acid to propionic acid. Between 2.0 p.m. and 4.0 p.m. there was practically no change in the relative percentages of rumen V.F.A. in spite of feeding a further 5 lb. of concentrates at 3.0 p.m.

At first sight, these changes in the percentage of rumen volatile fatty acids are relatively small. They have been expressed in Table IV in terms of their total caloric contribution at 7.0 a.m. and 4.0 p.m. When the results are expressed in this way, the contribution of the higher acids, particularly butyric, to the V.F.A. energy available to the cow becomes much more important, and a marked diurnal change in the relative importance of acetic compared with propionic and butyric becomes apparent. Between 7.0 a.m. and 4.0 p.m. not only is there an increase in the total caloric density of the rumen liquor of around 50%, but the percentage contributions of the different acids change markedly. At 7.0 a.m. 52% of the V.F.A. calories are in the form of acetic acid and 41% as propionic and butyric acids, while by 4.0 p.m. these percentages are reversed. If, over these relatively short periods, it can be assumed that the uptake of V.F.A. into portal blood is proportional to its concentration in rumen liquor, then these diurnal changes could have pronounced effects upon the intermediary metabolism of the cow. This is an aspect about which we know very little, but it is clear that these changes are of the same order as the differences that might be induced by the different ratios of roughage to concentrates fed at different stages of lactation. While the latter may reflect long-term changes in the rumen microbial

Table III

Effect of time of day on the molar percentages of the major volatile fatty acids (V.F.A.) in rumen fluid

Time	Total V.F.A., mequiv./l.	Molar percentage			
		Acetic	Propionic	*n*-Butyric	Isobutyric + *n*-valeric + isovaleric
7 a.m.	86·7	70·1	17·9	8·7	2·3
8 a.m.	107·9	67·0	19·8	10·1	3·2
9 a.m.	126·2	66·2	19·6	10·4	3·7
11 a.m.	116·9	65·8	19·3	10·8	4·0
12 noon	112·8	64·2	20·0	11·8	4·0
2 p.m.	105·2	61·0	21·9	13·8	3·4
4 p.m.	116·6	60·8	21·1	14·0	4·1

Table IV

Calorific contribution of the major rumen volatile fatty acids at 7 a.m. and 4 p.m

Time	Total calorific value, kcal./litre	Calorific contribution (kcal./litre)			
		Acetic	Propionic	Butyric	Valeric
7 a.m.	24·2	12·7	5·7	4·3	1·5
4 p.m.	35·4	14·8	9·0	9·1	2·5
		Percentage of total calories			
7 a.m.	100	52·4	23·6	17·8	6·2
4 p.m.	100	41·8	25·4	25·7	7·1

Heats of combustion (kcal./mole)

Acid		*Acid*	
Acetic	209·4	Butyric	524·4
Propionic	367·4	Valeric	681·8

population, do the diurnal changes described arise from a similar change in the types of bacteria present or do they largely reflect metabolic adaptation of a particular rumen microflora? It might be that the increased proportions of the longer chain volatile fatty acids at lower pH which have often been found, could be a mechanism for restricting further reduction in pH which has adverse effects upon bacterial activity, by restricting the amount of hydrogen ion produced per calorie metabolised to volatile fatty acid.

From these marked changes in rumen V.F.A. level and distribution, it would be expected that altering the distribution of the dietary intake could have marked effects upon productivity. Changing the pattern of dairy cow diets from a small number of discrete feeds of different materials to a system of frequent feeding of all the dietary constituents might be expected to have a major effect. However Mochrie *et al.*[13] found no appreciable effect upon productivity when lactating heifers were fed a mixed hay and concentrate ration at 2, 4 or 8 times per day. It is undoubtedly true that more frequent feeding levels out fluctuations in the concentration of rumen metabolites; for instance, rumen ammonia, V.F.A. and pH have been found to be much more stable with more frequent feeding.[14]

The assessment of response to changes in management routines is fraught with difficulty as illustrated by some trials carried out at Colworth. When the whole daily ration was divided into 10 daily feeds and compared with the normal feeding system (already described) in cows housed in adjacent standings, the only appreciable or significant effect was a slight increase in the lactose content of the milk. However, in a second experiment, with one control group housed adjacently and another control group housed in a separate shed, but milked by the same staff, there was more evidence of a difference between the two sets of controls. This may have been due to the more frequent feeding of adjacent cows stimulating productivity of adjoining animals in some way, or it may be due to a difference between sheds, but the latter is rather unlikely. This illustrates the extreme difficulty of making experimentally valid assessments of the effects of changes in management practice upon the dairy cow. In passing, it is of interest to note that Harrison & Hill[15] reported that increasing feeding frequency in sheep had a marked effect in increasing the rate of flow of digesta from the abomasum, so that stimulation of intestinal activity by feeding more frequently, or indeed psychological stimulation from feeding adjacent cows, is not beyond the bounds of possibility.

(3) The effects of phosphoric acid

Mixtures of orthophosphoric acid and ethanol with molasses and urea have been used to some extent in practice to meet the need of ruminants for nitrogen, and it has been claimed that the ethanol and orthophosphoric acid have substantial beneficial effects upon the utilisation of non-protein nitrogen from urea. We have consistently found that the limited substitution of urea and cereals for vegetable protein in dairy cow diets depresses the output of milk and milk constituents as illustrated in Table V. These results are in accord with those published by Bartlett & Blaxter[16] when the use of urea was being considered under the pressure of postwar shortages of vegetable protein.

Balch & Campling[17] found that the addition of ethanol to mixtures of urea, molasses and orthophosphoric acid had no effect and other workers have shown that ethanol is rapidly metabolised in the rumen to volatile fatty acids, largely acetic acid.[18] It was possible that the addition of orthophosphoric acid at rather higher levels than those used by other workers might inhibit ammonia release from urea and thereby enhance its utilisation. We therefore compared concentrate mixtures containing 2% of urea with the same mixture to which was added 2% of 66% w/w phosphoric acid, suitably diluted. The diets fed are shown in Table V. Two fistulated cows were used to compare these diets in a changeover design. There was some slight reduction in the evolution of ammonia when orthophosphoric acid was added, although ammonia concentrations in rumen liquor were still appreciably higher than those found in other experiments using vegetable protein. The amount of ammonia released (assuming that this is directly proportional to the concentration of ammonia in the rumen) was directly associated with rumen liquor pH as shown in Fig. 3. This shows that the effect of orthophosphoric acid upon rumen pH and ammonia release was relatively slight compared with the effects of diurnal and other fluctuations in pH at

Table V

Effects of phosphoric acid on urea utilisation

(1) *Diets used for phosphoric acid experiment*

	kg./cow/day
Concentrates (including 2% urea)	5·00
Hay	4·55
Sugar beet pulp	2·45

Orthophosphoric acid added: 66 g. or 0·55% of the whole diet as pure acid.

(2) *Effects upon milk output of substituting urea and cereals for vegetable protein*

Treatment	Yield (lb./week)		
	Milk	Fat	Solids-not-fat
Control (vegetable protein)	206·7	8·89	17·46
2% Urea + cereals	191·0	8·23	16·16
Significant difference (P = 0·05)	7·4	0·46	0·68

(3) *Effects on digestibility*

Factor	Urea diet	Urea diet + orthophosphoric acid	Significant difference (P = 0·05)
Organic matter	76·0	73·4	2·1
Nitrogen-free-extract	83·7	81·6	4·4
Crude fibre	61·4	58·2	2·1
Nitrogen	68·0	65·0	5·2

time of feeding. This is illustrated further in Fig. 4 which shows mean diurnal pH curves for the two treatments.

At the same time as these studies were being made, the digestibility of the whole diet was determined, and it is clear from Table V that a significant reduction in the digestibility of organic matter occurred and this was paralleled by significant reductions in crude-fibre digestibility with some reduction in the digestibility of the nitrogen and nitrogen-free extract fractions. In other studies we have shown that the addition of phosphoric acid at 1½ or 3% restricts milk output on normal diets not containing urea.

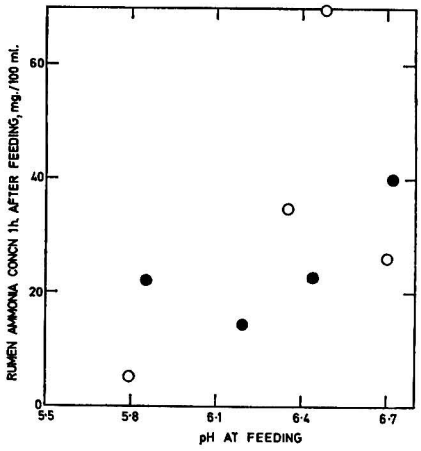

FIG. 3.—*Effect of rumen pH on ammonia released after feeding*

○ urea ● urea + phosphoric acid

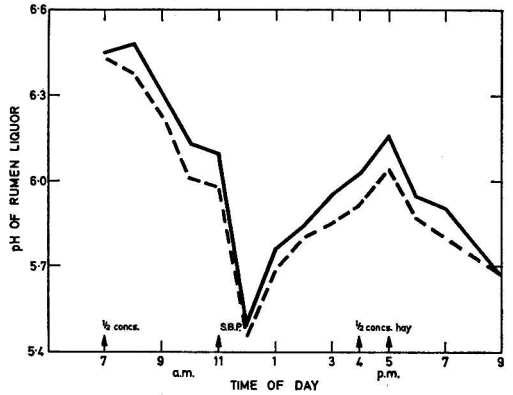

FIG. 4.—*Urea and phosphoric acid in dairy cow diets: pH of rumen liquor*

S.B.P. = sugar beet pulp concs. = concentrates
——— urea – – – urea+phosphoric acid

In view of the negligible effect of the additional acid upon pH in the rumen it is difficult to account for these results. In the rat prolonged addition of up to 0·75% orthophosphoric acid to the diet had no harmful effect upon growth or nitrogen metabolism.[19] This intake, as a percentage of total dry matter fed, is of the same order as that used in the cow trial. In view of the important place of organic acids in rumen metabolism it is not clear why these effects should occur, unless it is postulated that the slight reduction in rumen pH observed is sufficient to reduce the activity of the rumen microflora. This is very unlikely and it may be that we are faced with the paradox that the ruminant is more sensitive to mineral acids than the non-ruminant. Recent unpublished observations of Dr. R. M. Forbes at the University of Illinois tend to support this suggestion.

It would appear that several aspects mentioned in this paper are well deserving of further study, particularly the relationship between systems of feeding and the response of the cow to different diets, and the physiological mechanisms whereby the peculiar effects of phosphoric acid are produced. However, the answers to the more practical problems wait on the further development of techniques which allow the proper assessment of changes in bovine metabolism under conditions more akin to normal herd management.

Acknowledgments

The author wishes to acknowledge the help of his colleagues, particularly Mr. C. R. Dunton who supervised the conduct of these experiments, Mr. D. C. Thomas for chemical analysis, Mr. J. Taylor for statistical analysis of the data and Dr. K. J. Hill who fistulated the cows used.

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Received 27 July, 1964; amended manuscript 10 March, 1965

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STUDIES ON THE QUALITY OF SOME IMPROVED VARIETIES OF INDIAN WHEATS

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

A number of techniques, including milling and baking, were employed to assess the quality of seven improved varieties—NP-710, -718, -797, -799, -823 and -824, and C591—of Indian wheats which have shown several distinctive features. The yields of straight-grade flour were high in all cases, ranging from 74·2% (NP-799) to 77·1% (NP-824). The maximum extensograph curve area (155 cm.^{2}) and 100-g. flour loaf volume were given by the variety NP-824. The doughs are tight and short as indicated by the poor extensibilities and steep heights of the extensograms. The varieties showed practically no response to the improver potassium bromate. The variety NP-823 rated as good as NP-824 in a number of tests, but its loaf volume was significantly lower than that of NP-824. The only abnormal feature of the flour of this variety was its paste viscosity which was the lowest in the whole series. The protein content of C591 varied considerably when grown in different locations, but it maintained its milling and baking qualities, the loaf volume per unit protein being the second highest among the varieties tested.

Introduction

India is an important wheat-growing country, the annual estimated production being 11·8 million tons.[1] First detailed study of the quality of Indian wheats was carried out by Rattan Singh & Bailey[2] at the University of Minnesota, during 1938–39. Studies on the quality of Indian wheats, confined mainly to the assessment of milling and baking quality and surveys of the protein content of the wheats, were undertaken at the Punjab Agricultural College, Lyallpur, and the results were published in the annual reports[3] for 1942–47. The water absorptions of the flours were determined by the method of kneading the dough by hand but, for lack of equipment, the physical properties of the doughs were not assessed. The programme of studies was interrupted in 1947 as a consequence of the partition of the Indian continent. In this paper, the results of an integrated study of the quality of some vulgare types of Indian wheats carried out at the Grain Research Laboratory, Winnipeg, are reported. It is shown that the Indian wheats have certain distinctive features: they give high yields of straight-grade flours, but yield doughs which are tight and short.

Experimental

The samples of New Pusa (NP) varieties, viz., NP-710, -718, -797, -799, -823 and -824 developed by the Division of Botany, Indian Agricultural Research Institute, New Delhi, were obtained through the courtesy of the Director. Four samples of C591 grown at different

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Experimental Stations were obtained from the Economic Botanist for Cereals and Pulses, Punjab. The samples were free from insect infestation. To assess the quality of these wheats, the tests described below were employed.

Tests on grain

Moisture, protein content, and wheat meal fermentation tests were performed in accordance with the A.A.C.C. procedures.[4]

Grading was effected according to the Canadian grain standards.[5]

Kernel weight, i.e. the weight of 100 kernels.

Bushel weight, i.e. the weight of grain filling an Imperial pint container multiplied by 64.

Pentosans

These were calculated from the furfural contents of hydrochloric acid distillates,[6] furfural being estimated by the potassium bromate–potassium bromide method of Hughes & Acree[7] with the modification of Vernon & Metzner.[8]

Lipoxidase activity was determined by the method of Irvine & Anderson.[9] The results are expressed as μl. of O_2/min./g.

Berliner turbidity values[10]

Doughs were prepared from 10 g. of ground wheat and 5 ml. of 2% solution of sodium chloride in distilled water; samples were prepared in duplicate. The gluten was immediately washed out in a Theby gluten washer; a second sample was rested for 30 min. before washing out the gluten. Wet gluten (0·5 g.) was weighed and divided into 15 equal pieces for determination of the turbidity in 0·02 N-lactic acid solution in test tubes arranged in a rotary thermostat (Type-MCP-Buhler) according to the method of Berliner. After 30 min. the tubes were removed from the thermostat, and set aside for 10 min. before the turbidity was recorded in a photoelectric-colorimeter. The values Q_0 and Q_{30} signify the swelling power of gluten recovered immediately after kneading the dough and after 30 min. resting of the dough, respectively.

Milling quality

The cleaned samples of grain were tempered at 15·5% moisture content and kept overnight at room temperature, before being milled. Portions weighing 2 kg. were milled in the Allis Chalmers Experimental mill. The bran was dressed in the Bran Finisher,[11] and the recovered fraction was added to the straight grade flour.

Tests on flour

Moisture, protein, wet gluten, ash, yellow pigment, diastatic activity (mg. maltose/10 g. flour/h.), and gassing power of flours using the modified pressuremeter[12] were determined according to the A.A.C.C. procedures.[4]

Pentosans were determined as above.

Colour grade

The determination was made with the Kent-Jones & Martin photo-electric colour grader.[13,14]

Sedimentation values were obtained by the methods of Pinckney *et al.*[15] and of Zeleny.[16]

Dough expansion volume was obtained by the method of Miller *et al.*[17]

Berliner turbidity values

For flours, the gluten pieces were allowed to react with the lactic acid solution in the test tubes for 40 min. as against 30 min. in the case of glutens derived from whole wheat meals. The rest of the procedure was the same as already described.

Farinograph curves

Fifty g. of flour were mixed in the small stainless steel bowl of the farinograph to a consistency centred around 500 B.U. The speed of the mixing arm was 63 r.p.m. Dough development time refers to the time taken for mixing the dough to a consistency of 500 B.U., using the appropriate water absorption.

Extensograph curves

Flour (300 g.) and salt (6 g.) were mixed in the farinograph bowl using the respective farinograph water-absorptions reduced by 3% in each case. The extensograph was set at 100 B.U./100 g. load, and the extensograph curves developed at 45 and 135 min. after mixing the doughs. The doughs were rounded and shaped at 90 min. also. The extensograms have been interpreted[18] on the basis of curve area, ' Ratio figure ' and extensibility of the doughs.

Amylograph curves[19]

Flour (50 g.) was dispersed in 450 ml. of distilled water and then placed in the visco-Amylograph bowl for developing the paste viscosity curve; the instrument was adjusted for a 1·5° C rise in temperature per minute.

Baking quality

Loaves were prepared from 100 g. of flour, using the malt–phosphate–potassium bromate modification of the A.A.C.C. pup loaf baking formulae. The ' Remix ' procedure developed by Irvine & McMullan[20] was adopted for ascertaining the differences in the baking quality and dough-handling properties of the various flours. The water absorptions were adjusted to suit proper handling of the dough at the mixing, remixing and panning stages in the baking operation. The doughs were mixed for 3 min. in the G.R.L.[21] mixer, fermented for 2 h. 45 min. at 86° F, remixed for 2½ min., allowed to recover for 25 min., sheeted, moulded, proofed for 55 min. and then baked for 25 min. at 450° F. The loaf volume was measured half an hour after baking by the rapeseed displacement method,[22] whereas the loaves were scored for appearance, crumb texture and crumb colour, the next day. The maximum score for each of these factors was ten.

Results and discussion

Results regarding the quality of grain, flour and yields of flour are presented in Tables I-III.

Table I

Grain characteristics of the different varieties of Indian wheats

Variety	Moisture, %	100-kernel wt., g.	Bushel wt., lb.	Protein (N × 5·7), %	Pento-sans, %	Lipoxidase activity, $\mu l.O_2$/min./g.	Grade
NP-710	9·6	4·04	64·00	10·9	6·9	81	2
NP-718	9·7	4·30	64·25	10·7	6·7	86	2
NP-797	10·1	4·30	60·00	10·4	6·7	80	2
NP-799	10·1	3·88	58·00	9·7	6·8	81	3
NP-823	9·6	5·33	63·75	12·0	6·1	78	2
NP-824	9·9	3·92	64·25	11·3	6·7	93	2
C591-*Expt. Stations*							
Jullundur	10·0	3·80	65·00	10·5	6·9	85	2
Gurdaspur	10·5	3·92	65·75	8·9	6·9	81	2
Abohar	10·1	4·06	65·25	10·9	6·6	80	1
Gurgaon	9·5	4·22	65·25	8·8	6·7	88	2

Table II

Milling characteristics of Indian wheats

Variety	Flour yield, %	Bran, %	Shorts, %	Feed flour, %	Bran flour, %	Remarks
NP-710	76·2	17·7	3·1	2·3	1·7	Milled freely
NP-718	76·0	17·2	3·7	2·2	1·4	Fine-sized bran
NP-797	76·4	17·5	3·2	1·4	2·1	Large flaky bran
NP-799	74·2	19·1	3·1	1·7	1·9	Large flaky bran
NP-823	76·5	16·5	2·7	2·5	1·6	Good-sized bran
NP-824	77·1	16·7	2·7	2·0	1·5	Good-sized bran
C591-*Expt. Stations*						
Jullundur	74·3	17·8	4·4	2·3	1·4	Fine-sized bran
Gurdaspur	74·9	17·5	4·0	2·3	1·7	Fine-sized bran
Abohar	74·2	18·0	3·7	2·1	1·6	Fine-sized bran
Gurgaon	75·6	17·6	3·4	2·3	1·8	Fine-sized bran

Table III

Quality characteristics of the flours of Indian wheats

Variety	Protein, %	Wet gluten, %	Gassing power, mm./10g./6h.	Diastatic activity,* mg./10g./h.	Pentosans, %	Ash, %	Yellow pigment,† p.p.m.	Colour grade
NP-710	10·0	30·4	315	239	2·42	0·57	4·4	3·7
NP-718	9·8	27·7	335	239	2·42	0·53	4·0	3·8
NP-797	9·3	26·7	295	274	2·54	0·51	4·4	3·3
NP-799	8·4	24·7	270	179	2·54	0·52	4·1	2·9
NP-823	10·9	30·8	340	245	2·42	0·45	4·6	3·8
NP-824	10·2	28·7	340	217	2·78	0·52	3·9	3·0
C591-*Expt. Station*								
Jullundur	9·8	27·0	495	378	2·87	0·68	3·2	4·2
Gurdaspur	8·2	21·5	460	366	2·87	0·68	3·7	3·3
Abohar	10·0	28·6	430	358	2·78	0·64	3·1	4·1
Gurgaon	7·6	21·4	445	322	2·99	0·68	3·1	2·6

* As maltose † As β-carotene

The varieties secured fairly high grades on the basis of Canadian grain standards. The kernels had attractive appearance and were of amber hue. The initial moisture contents of the samples were low. The weights per bushel were generally high, that for C591 being considerably higher than for any of the NP-varieties. The protein contents of NP-wheats ranged from 9·7 to 12·0% and that of C591 from 8·8 to 10·9% when the latter was grown in different locations. The differences in the pentosan contents of the varieties were negligible, but the lipoxidase activity of NP-824 was found to be slightly higher than the rest of the varieties.

The yields of straight-run flour ranged from 74·2% (NP-799) to 77·1% (NP-824) and the percentage of shorts was higher for C591. NP-718 and C591 produced fine-sized bran which indicated the relatively harder nature of the grains of these varieties. The colour grades of NP-flours ranged from 2·9 to 3·8 and that of C591 from 2·6 to 4·2, *vis-a-vis* a range of 1·7 to 2·1 reported[23] for flours of the upper grades of the Canadian wheats. The high flour yields shown by these varieties conform with the earlier literature reports[2, 3, 24, 25] about this attribute of the Indian wheats.

Flour characteristics

The flour of C591 showed higher diastatic activity and gassing power which averaged 356 mg., and 457 mm. pressure, respectively. The flour of NP-799 had the lowest diastatic activity, viz., 178 mg. The gassing power of NP-wheats ranged from 270 mm. (NP-799) to 340 mm. (NP-823 and 824), and these wheats had lower ash and pentosan contents than the C591 flours. The yellow pigment contents of the various flours were generally high, ranging

from 3·1 to 4·6 p.p.m., and resembled those of durum wheats rather than the vulgare types. The low values reported previously for 31 samples of Punjab wheats by Rattan Singh & Bailey[2] might be ascribed to the nature of the solvent used in the extraction of the pigments.[26]

Ancillary quality tests

The Q_{30} Berliner turbidity values (Table IV) indicated that the gluten quality of NP-718 and -824 and C591 was superior to others when the whole meals were made into dough and rested for 30 min. before the gluten was recovered for the test. The corresponding values for the meals of NP-710, -797, -799 and -823 were much lower. However, the glutens derived from the experimentally milled flours of these latter varieties showed considerably higher Q_{30} values. Since wheat in India is consumed largely in the form of whole meal, the importance of this test in the evaluation of the quality of wheat is emphasised.

Tests such as the Berliner, wheat meal fermentation, and dough expansion volumes rated NP-823 and NP-824 equal and superior to the rest of the varieties, but NP-718, although rated high by the Berliner, wheat meal fermentation, and Zeleny sedimentation tests, was low in dough expansion value. When the values of various test are expressed per unit protein, the over-all ranking of the varieties was much the same.

Farinograph curves

The farinograph curves titrated to 500 B.U., are illustrated in Fig. 1. For the amounts of protein that the various flours contained, water absorptions were high. The dough development times ranged from 1½ to 5½ min. The flours of NP-718 and 824 took the longest times to knead to the 500 B.U. consistency. The results in Tables III and IV indicate that the mixing time generally increased with the protein content of the flours, but the behaviour of NP-718 and 823 flours was slightly different, there being no correspondence between the mixing time and the respective protein contents.

Extensograph curves

There was marked variation in the pattern of the extensograph curves which is illustrated in Fig. 2 and Table V. The flour of NP-824 was the strongest as measured by extensograph area. The ' Ratio figures ' of the extensograph curves varied widely, viz., 2·9 to 13·7. Judged by this criterion, the doughs of NP-718, -799, -823 and -797 were very tight, the ' Ratio figures ' being 13·7, 11·2, 9·8 and 8·0, respectively. The ' Ratio figures ' pertaining to the doughs of C591 ranged from 2·9 to 5·6 when grown in different locations. Unlike the doughs of Indian wheats, the ' Ratio figures ' for Canadian wheat[23] grade No. 1 and 2 appear to fall in the range 1·15 to 1·26.

Table IV

Ancillary quality tests data on ground wheat and test flours of Indian wheats

Variety	Berliner turbidity values				Dough rupture time, min.	Sedimentation value, c.c.	Dough expansion, c.c.	Max. paste viscosity, B.U.	Dough mixing time, min.
	Ground wheat		Flour						
	Q_0	Q_{30}	Q_0	Q_{30}					
NP-710	3·2	2·5	19·2	17·5	56	25·0	12·1	785	3·25
NP-718	20·0	14·5	23·3	23·0	201	26·9	11·5	845	5·50
NP-797	9·2	3·6	19·3	14·1	73	19·8	11·7	660	2·00
NP-799	11·6	9·8	24·6	21·5	57	16·5	8·6	695	1·50
NP-823	11·6	7·1	23·1	21·8	243	30·1	17·4	480	4·50
NP-824	23·6	23·2	25·7	24·6	206	30·1	18·7	810	5·50
C591-*Expt. Stations*									
Jullundur	22·6	18·0	23·6	24·5	61	18·8	8·8	700	2·75
Gurdaspur	20·0	14·6	24·0	23·6	56	17·1	9·0	580	1·75
Abohar	24·2	23·4	24·4	21·8	62	21·3	9·9	780	2·75
Gurgaon	24·4	22·0	24·4	20·2	61	10·8	10·4	630	1·50

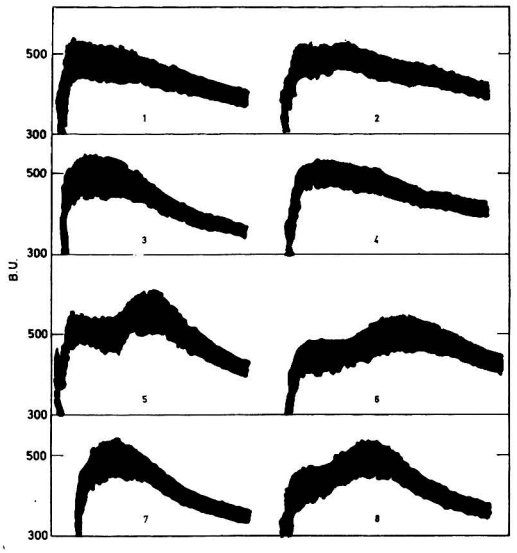

FIG. 1.—*Farinograph curves of flours of different varieties*

(curves above 300 B.U. line)

1 NP-799 2 C591 Gurdaspur 3 NP-793 4 C591 Jullundur
5 NP-718 6 NP-824 7 NP-710 8 NP-823

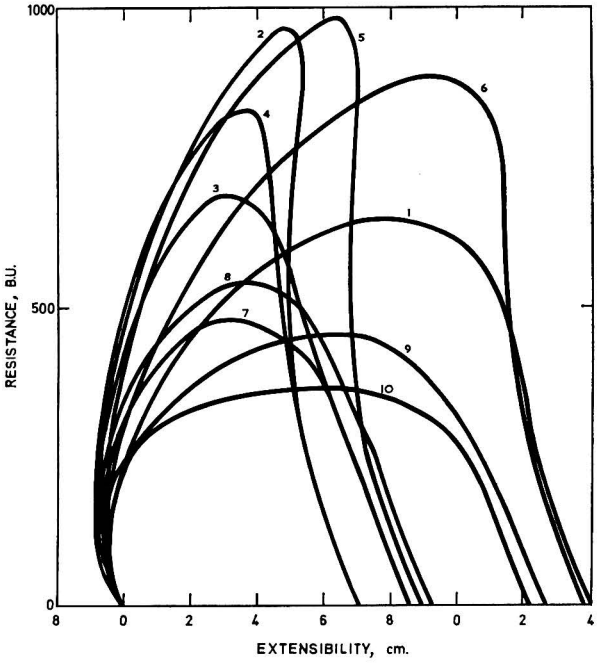

FIG. 2.—*Extensograph curves of Indian flours*

curves 1–6 NP-710, -718, -797, -799, -823, -824, respectively
curves 7–10 C591 Jullundur, Gurdaspur, Abohar, Gurgaon stations, respectively

Table V

Extensograph curve characteristics of the doughs of flours of Indian wheats

Variety	Water absorption,* c.c.	Extensibility, cm.	Height at 5 cm. extension, B.U.	Maximum height, B.U.	Area, cm.²	'Ratio figure'
NP-710	62·7	14·5	500	637	103·2	3·45
NP-718	64·2	7·0	960	965	74·4	13·70
NP-797	55·9	8·6	682	682	72·0	8·0
NP-799	52·8	7·3	820	825	66·7	11·2
NP-823	63·0	8·9	880	995	104·8	9·8
NP-824	63·2	13·8	610	880	155·1	4·4
C591-*Expt. Stations*						
Jullundur	64·3	8·6	480	480	56·0	5·6
Gurdaspur	61·2	9·8	525	540	69·5	5·4
Abohar	63·5	12·7	395	439	77·0	3·1
Gurgaon	59·2	12·2	350	370	64·5	2·9

* Farinograph water-absorption less 3% in each case

Amylograph curves

Figs. 3 and 4 illustrate the paste characteristics of the different flours when the temperature of the system was increased gradually. The peak viscosity was shown by the flour of NP-718, and the lowest viscosity in the case of flour of NP-823 (480 B.U.). The paste viscosities of C591 flours ranged from 580 to 780 B.U., depending on the location in which it was grown.

Baking quality

The results of baking tests are presented in Tables VI and VII. The first set of loaves were prepared using the farinographic water-absorptions which were reduced by 4% in each case.

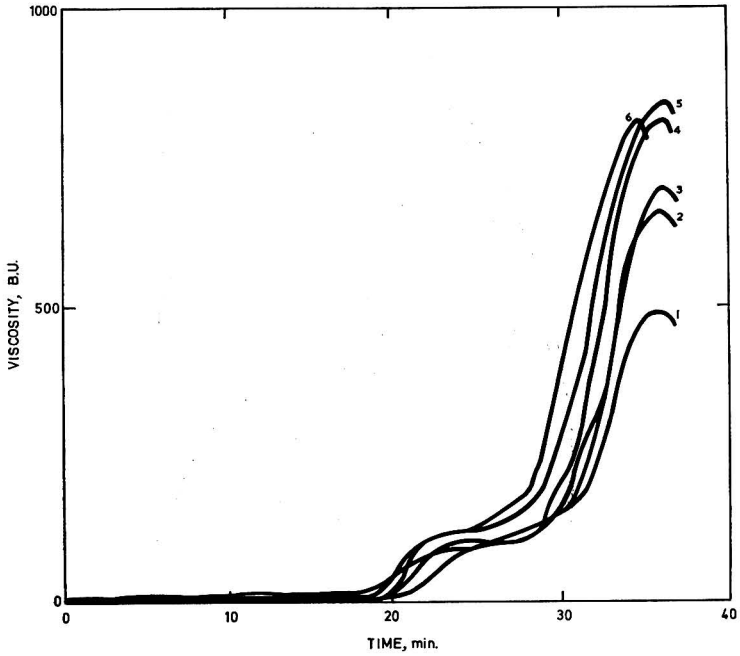

FIG. 3.—*Amylograph curves of the flours of different varieties*
curves 1–6 NP-823, -797, -799, -824, -718, -710 respectively

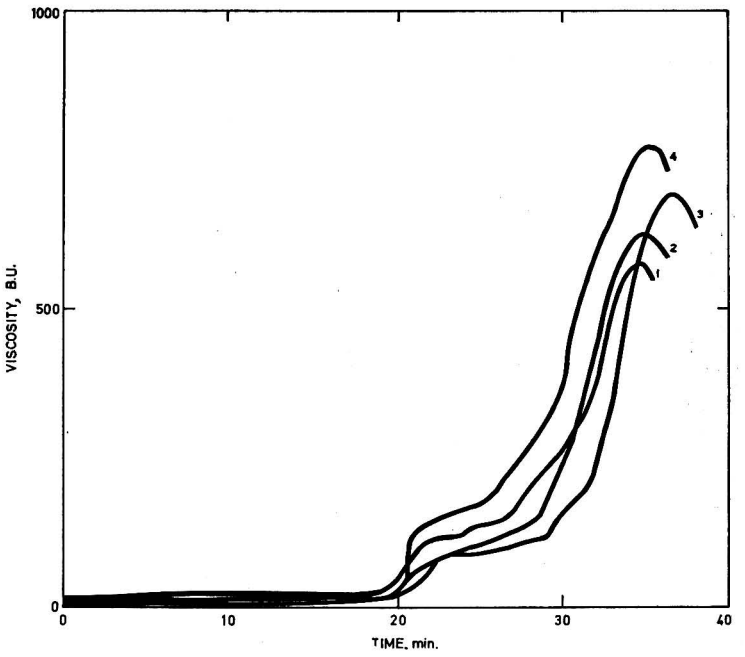

FIG. 4.—*Amylograph curves of C-591*
curves 1–4 for wheat grown at Gurdaspur, Gurgaon, Jullundur and Abohar, respectively

The dough of NP-824 handled satisfactorily at different stages in the baking operation and its loaf volume was the highest in the lot. The average loaf volume of C591 exceeded that of the remaining NP-varieties. The crusts of C591 loaves were dark brown as compared with those of the loaves of NP-varieties. Probably this was due to the relatively higher diastatic activity of C591 flours pointed out earlier. The loaf of NP-710 appeared green, that of NP-718 had a shelled top, and the loaf of NP-823 was better looking than the loaves of NP-710, -797 and -799. The dough of NP-710 was slack at the mixing stage and became still more slack and sticky at the remixing and panning stages. The dough of NP-718 was superior in this respect, that of 797 being slack. The flours of C591 made satisfactory doughs except for the sample obtained

Table VI

Baking quality of Indian wheats using the farinograph water-absorptions reduced by 4% in each case

Variety	Water absorption, c.c.	Loaf characteristics	
		Volume, c.c.	Total score (max. 30)
NP-710	61·7	400	8·5
NP-718	63·2	445	14·0
NP-797	54·9	420	9·5
NP-799	51·8	420	11·5
NP-823	62·0	485	12·0
NP-824	62·2	740	20·0
C591-*Expt. Stations*			
Jullundur	63·3	470	9·0
Gurdaspur	60·2	555	14·0
Abohar	62·5	565	14·0
Gurgaon	58·2	555	15·0

Table VII

Effect of adjusting the water absorption and potassium bromate on the baking quality of Indian wheats

Variety	Adjusted water absorption,* c.c.	Loaf characteristics			
		15 p.p.m. bromate		No bromate	
		Volume, c.c.	Score (max. 30)	Volume, c.c.	Score (max. 30)
NP-710	51·7	400	8·0	425	10·0
NP-718	56·7	500	17·5	500	17·0
NP-797	49·9	400	9·0	425	11·0
NP-799	49·8	400	11·0	415	11·0
NP-823	58·0	485	13·5	490	17·0
NP-824	60·2	745	24·5	720	22·0
C591-*Expt. Stations*					
Jullundur	58·2	495	7·5	435	5·5
Gurdaspur	56·7	545	16·0	535	14·0
Abohar	59·0	570	12·5	550	13·0
Gurgaon	53·7	490	10·5	510	12·5

* Adjustment of Farinograph absorption to suit proper handling of the doughs

from the Experimental Station, Jullundur, which gave slack and sticky dough at the remixing and panning stages. With regard to appearance, the loaf of NP-824 was rated superior to all and that of C591 as the next best.

On reduction of the water absorption further (Table VII), fairly stiff doughs were obtained which handled well, there being no adverse effect on the loaf volumes, but the exterior appearance of the loaves of NP-710, -797, and -799 was unsatisfactory. There was practically no response by any of the flours to the addition of potassium bromate improver. By adjustment of the water absorption again in the case of C591 flours, the loaf volume results indicated no response to the improver. In a study of the baking quality of Indian wheats, Rattan Singh & Bailey[2] employed farinograph water-absorptions which were fairly high but adopted shorter mixing and fermentation times. The ' Remix ' procedure employed by us entailed faster and longer mixing in the G.R.L. mixer, and subsequent longer fermentation. This technique seems to bring out clearly intrinsic differences in the handling properties of the dough when farinograph water-absorptions reduced by 4% in each case were used in the baking test. All the doughs, with the exception of NP-824, were slack. The water absorptions found optimum were considerably less than those indicated by the farinograph and those used by Rattan Singh & Bailey[2] and by others[3] whose findings are based on the hand-kneading method of determining the water absorptions. However, the tight and short doughs made with considerably reduced water absorptions mixed at high speed and for longer times and also subjected to longer fermentation, produced satisfactory loaves without prejudice to the volume of the loaves. The average loaf volume of C591 obtained by us by using restricted water absorption, was of the same order as that reported by Rattan Singh & Bailey.[2]

Acknowledgment

Grateful acknowledgment is made to the Colombo Plan Administration of Canada for the award of a residential fellowship to one of the authors (G. S. B.) at the Grain Research Laboratory, Board of Grain Commissioners for Canada. The authors express their gratitude to Dr. J. A. Anderson, former Director, for his encouragement and interest in the project. Thanks are also due to the Milling and Baking departments for technical assistance.

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Received 4 March, 1965

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THE MINERAL COMPOSITION OF APPLES. IV.*—The radial distribution of chemical constituents in apples, and its significance in sampling for analysis

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

The distribution of the major mineral elements and some organic constituents about the longitudinal axis of Cox's Orange Pippin apples has been investigated. Although surface colouring is sometimes indefinite potassium, phosphorus, nitrogen and calcium concentrations generally, although not invariably, reach maximum levels at the 'blushed' side of the fruit and fall to minimum levels at the 'unblushed' side. Large errors can therefore result from the mineral analysis of one section only, instead of the whole fruit. Reduction in sample size can be achieved by the combination of opposite pairs of sections for mineral analysis and the results are usually within ±5% of those for the whole fruit.

Introduction

It was shown in previous papers that there were large variations in concentrations of mineral elements between individual apples picked from one tree[1] and that the variations of concentrations of minerals (and other chemical constituents) were even greater within a single apple.[2] Later work showed that it was necessary to remove seeds and stems from samples before analysis

* Part III: *J. Sci. Fd Agric.*, 1965, **16,** 438

for some elements.[3] It was concluded that analytical results could be misleading if a sample contained too few apples, and unless the whole of each apple, with the seeds and stems removed, was homogenised for subsampling.

Tests of ashing and analytical techniques showed that the method of homogenising apple samples by freezing and grating was very satisfactory when determinations of minerals were to be made.[4] This procedure, however, requires cold storage facilities; and a considerable amount of space is needed because of the large numbers of apples comprising each sample. A reliable method of reducing sample size by taking representative sections of each apple before freezing would be very valuable.

In addition to the variations noted above, Archbold & Barter[5] showed a radial variation in dry weight, sugar and acid concentrations about the calyx eye to stem axis of apples and Bishop[6] found a similar, though larger, variation in potassium concentrations. The higher concentrations were usually on the ' blushed ' side of the fruit. Archbold & Barter showed that when diagonally opposite quarters of apples were combined for analysis, errors were less than when adjacent quarters (i.e., one half of an apple cut through half of the ' blushed ' and half of the ' unblushed ' sides) were analysed and amounted to less than 1% of the value of the whole apples. It seemed probable, therefore, that for mineral analysis a considerable reduction of sample size would be achieved by combining opposite sections, of equal size, of apple even though variations in concentrations between sections were higher. With this in view, the following investigation was made of the radial variation of concentrations of some of the major elements occurring in apple ash, and also of nitrogen and some of the other chemical constituents in the apple.

Materials and methods

All the apples used were of the variety Cox's Orange Pippin. Those for Experiments I and V were taken at random from orchard boxes in store, while apples of varying size for Experiments II and III were from one tree. The samples for Experiment IV were from a time of picking trial and were taken from store after a water-loss experiment.

The apples were washed in distilled water and their stems were removed before sectioning. Seeds were carefully removed from each section after it had been cut. Details of sectioning are given below, but in general the sections were cut relative to the ' blushed ' and ' unblushed ' surfaces of the apple although these were not always clearly defined.

Sections which were to be chopped by scalpel were treated in the following way. Fine cuts were made across the hard tissue that had been at the centre of the apple—as in Fig. 1 (c)—before each section was frozen. When each section was frozen the surface of the peel was scored with a scalpel as in Fig. 1 (d). This resulted in a more homogeneous chopped sample.

Whole sections, chopped and grated subsamples, were extracted or ashed, and analysed for minerals by methods previously described[4,7] and indicated below. Sulphur, as sulphate, was determined by the method of Obermer & Milton.[8] Other constituents were determined by the

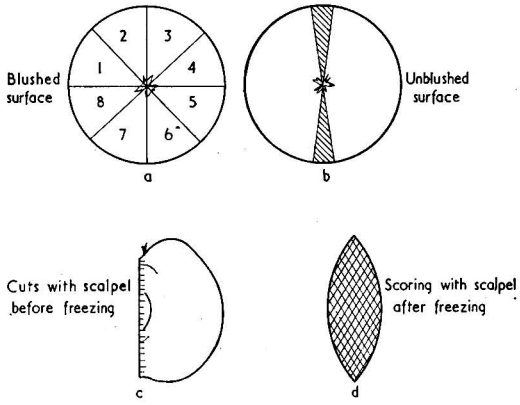

FIG. 1.—*Method of sectioning apples*

methods described for their determination in rings cut from apples[2] with the exception of nitrogen which was determined by the method of Williams.[9] The same sub-sample had to be used for dry weight and nitrogen and similarly alcohol-insoluble matter and pectin were determined on one subsample.

Experimental and results

I. The distribution of potassium

Each of five apples was cut into eight equal longitudinal sections about the calyx eye to stem axis as shown in Fig. 1 (a) and each section was weighed. After being frozen and partially thawed the whole of each section was extracted with cold water and potassium was determined by flame photometer.[7] The results are shown in Table I.

Table I

Potassium concentrations in sections of apples

Sections shown in Figure 1a; mass in g, potassium as mg. per 100g. fresh weight.

Apples	a		b		c		d		e	
Section	Mass	K	Mass	K	Mass	K	Mass	K	Mass	K
1	9·9	121	11·9	151	13·7	122	13·0	131	18·4	170
2	9·8	112	9·1	147	13·4	122	14·4	142	20·8	171
3	9·9	111	12·1	147	13·9	116	14·4	139	20·4	160
4	10·6	107	11·2	148	13·4	116	13·2	135	22·1	156
5	9·5	107	12·9	150	12·7	118	16·9	136	24·6	148
6	8·9	108	13·1	154	12·8	120	17·3	133	18·3	153
7	9·8	118	13·2	159	13·9	128	16·8	132	15·8	170
8	9·1	115	16·3	156	14·0	129	17·1	131	17·0	181
Whole apple	77·5	112	99·8	152	107·8	122	123·1	135	157·5	163

II. The distribution of mineral elements

Following the results of Experiment I, analyses were extended to include the major elements occurring in apple ash. Two apples were sectioned as described above, and the whole of each section was ashed with nitric acid and analysed for potassium, sodium, calcium, magnesium, phosphorus and sulphur. The results are given in Table II and these values have been used to calculate the largest possible errors which would have been obtained had one or one pair of opposite sections been analysed instead of the whole apple. These calculations are shown in Table III.

Table II

Mineral concentrations in sections of apples

Sections shown in Figure 1a; mass in g., minerals as mg. per 100g. fresh weight

Apple	Section	Mass	K	Na	Ca	Mg	P	S
B	1	12·02	198	2·4	6·3	7·0	12·1	7·8
	2	10·99	199	1·9	6·3	7·1	11·7	8·0
	3	10·70	188	1·8	6·2	7·3	11·1	8·1
	4	11·45	172	1·4	5·7	7·2	9·9	6·9
	5	11·68	172	1·7	5·2	6·8	10·4	6·2
	6	13·62	184	1·6	5·6	7·1	11·0	6·7
	7	12·00	183	1·8	6·3	7·2	11·5	7·0
	8	11·01	183	1·9	6·9	7·2	11·3	7·0
	Whole apple	93·47	185	1·8	6·1	7·1	11·1	7·2
C	1	15·39	185	2·4	4·7	5·0	9·3	6·7
	2	13·59	194	2·0	4·6	5·0	10·3	6·5
	3	13·84	195	2·1	5·0	5·2	10·8	7·2
	4	16·64	207	2·3	5·1	5·2	11·4	6·9
	5	13·67	211	2·3	5·2	5·3	11·4	7·0
	6	14·50	199	2·3	5·6	5·4	10·1	7·5
	7	14·70	186	2·1	4·8	5·0	9·5	6·6
	8	16·59	174	2·2	4·3	4·9	9·3	6·8
	Whole apple	118·92	194	2·2	4·9	5·1	10·2	6·9

III. The distribution of minerals and other constituents

Two more apples were sectioned as described above, but after they had been weighed the sections were frozen at −15° and chopped with a scalpel. It was then possible to subsample for the determination of nitrogen and other constituents in addition to minerals so that a general distribution pattern could be obtained. Owing to shortage of material it was impossible to duplicate many of the determinations. Minerals were determined after ashing by the combustion technique.[4] Results are given in Table IV. Approximate errors which would have resulted from the analysis of one, or one pair of opposite sections instead of the whole apple are shown in Table V.

IV. Test of the proposed sampling technique for minerals in ash

Opposite pairs of thin sections from between the ' blushed ' and ' unblushed ' surfaces of apples, as shown in Fig. 1 (b), were cut from two samples of 20 apples, D3/5 and D3/6. The mass of each pair of sections amounted to about 1/16 that of the whole fruit. All the sections from each sample were put into a flask and ashed with nitric acid. The remainders of the apples in each sample were combined and frozen and grated. Subsamples of the grated material were then ashed with nitric acid. Analytical results for these sections and remainders, corrected for water loss in store, are shown in Table VI. Results for the analysis of other samples of 25 whole, grated, apples picked on the same days are also included for comparative purposes. A slight loss of water (about 1·5%) occurs when apple samples are frozen,[4] so the remainder and whole apple

Table III

Largest percentage errors possible if one section only or pairs of opposite sections of a single apple were analysed instead of the whole fruit

(Calculated from results in Table II)

Apple	Greatest % errors		K	Na	Ca	Mg	P	S
B	One section	+	7·6	33	13·1	2·8	9·0	12·5
		−	7·0	22	14·7	4·2	10·8	13·9
	Opposite pairs	+	3·2	13·2	3·3	2·1	1·8	4·2
		−	3·8	9·9	4·9	2·8	4·5	2·8
C	One section	+	8·8	9·1	14·3	5·9	11·8	8·7
		−	10·3	9·1	12·2	3·9	8·8	5·8
	Opposite pairs	+	2·1	5·9	4·1	2·0	1·5	1·5
		−	2·1	5·4	4·1	1·0	1·0	1·5

Table IV

Concentrations of minerals and other constituents in sections of apples

Sections shown in Fig. 1a; mass in g., minerals as mg., and other constituents as g. per 100g. fresh weight

Apple	Number of section	Mass	K	Ca	Mg	P	N	pH	Titratable acid	Dry weight	Alcohol-insoluble matter (A.I.M.)	Alcohol-soluble matter (A.S.M.)	Starch	Pectin
A	1	6·74	221	8·1	6·9	13·9	130	3·31	0·99	14·4	2·8	11·6	—	0·60
	2	8·23	216	6·7	7·3	13·7	107	3·29	0·98	12·5	3·3	9·2	—	0·63
	3*	8·12	227	6·6	6·8	13·5	113	3·27	0·93	15·6	3·2	12·4	—	0·63
	4*	7·87	216	5·8	6·0	13·7	58	3·25	0·95	14·8	4·1	10·7	—	0·67
	5	8·03	220	5·6	6·3	14·6	71	3·24	0·96	14·0	3·2	10·8	—	0·54
	6*	9·33	216	5·8	5·7	15·4	95	3·28	0·95	13·4	3·5	9·9	—	0·56
	7	7·71	222	7·0	5·6	14·9	119	3·26	0·93	13·4	3·4	10·0	—	0·77
	8*	7·25	228	9·8	6·6	16·2	146	3·36	1·02	14·0	3·3	10·7	—	0·62
	Whole apple	63·28	221	6·9	6·4	14·5	105	—	0·96	14·0	3·4	10·7	—	0·63
D	1*	16·50	218	6·4	7·6	15·0	113	3·23	0·92	18·7	3·5	15·2	0·24	0·62
	2*	14·93	228	5·7	8·2	14·3	111	3·31	1·00	20·5	4·1	16·4	0·23	0·68
	3*	18·01	200	5·2	7·6	12·6	93	3·18	0·98	18·8	3·5	15·3	0·19	0·63
	4*	18·96	187	4·5	6·6	12·7	97	3·26	0·97	18·3	3·2	15·1	0·11	0·59
	5	14·21	200	5·8	5·9	12·6	100	3·27	0·88	17·7	3·4	14·3	0·10	0·68
	6*	16·40	204	6·2	7·3	12·8	87	3·32	0·98	17·6	3·6	14·0	0·12	0·68
	7*	13·93	224	6·2	7·5	14·9	103	3·28	0·99	19·0	3·6	15·4	0·16	0·55
	8*	17·08	233	6·5	7·9	15·4	116	3·30	0·99	18·4	3·1	15·3	0·15	0·61
	Whole apple	130·02	212	5·8	7·3	13·8	103	—	0·96	18·6	3·5	15·1	0·16	0·63

* Combustion duplicated

Table V

Largest percentage errors possible if one section only or pairs of opposite sections of a single apple were analysed instead of the whole fruit

(Calculated from results in Table IV)

Apple	Greatest % errors		K	Ca	Mg	P	N	Dry wt.	A.I.M.	A.S.M.	Starch	Pectin	Titratable acid
A	One section	+	3	42	14	12	39	11	21	16		22	6
		−	2	19	13	7	45	11	18	14		14	3
	Opposite pairs	+	2	13	3	3	10	4	10	5		11	3
		−	2	9	3	2	4	7	10	10		10	3
D	One section	+	10	12	12	12	13	10	17	9	50	8	4
		−	11	22	19	9	15	5	11	7	40	13	8
	Opposite pairs	+	2	5	6	2	3	2	10	2	9	8	3
		−	1	5	8	2	5	2	10	2	19	6	6

Table VI

Comparison of samples of frozen, grated apples with sections cut from apples and the remainder of the same apples frozen and grated

(Results as mg. per 100g. fresh weight)

Sample	No. of apples, preparation and mass ashed	K	Na	Ca	Mg	P	S
D3/5	25 apples, grated, 100g.	143	1·9	4·5	5·2	11·7	7·0
D3/5 (c+d)	20 apples, opposite sections, 134g.	145	1·6	4·4	5·8	12·5	7·3
	20 apples, remainders, grated, 100g.	147	1·7	4·3	5·6	12·4	7·4
D3/6	25 apples, grated, 100g.	146	1·9	4·4	5·2	12·0	7·1
D3/6 (c+d)	20 apples, opposite sections, 128g.	141	2·0	4·1	5·6	11·8	7·6
	20 apples, remainders, grated, 100g.	146	1·9	4·3	5·4	11·9	7·2

results are subject to an error which would slightly increase or decrease some of the differences between these and the results for the sections, which were not frozen.

V. Experimental test of the proposed sampling method for nitrogen.

Opposite pairs of sections were taken, as in the previous experiment, from 20 apples. It was necessary to freeze and grate these sections in order to sub-sample for nitrogen determinations, so for technical reasons they had to be larger and the mass of the pairs amounted to about $\frac{1}{4}$ that of the whole fruit. The remainder of the apples were also frozen and grated. Sub-samples of about 5 g. were digested for nitrogen determination. The results are shown in Table VII.

Discussion

Distribution

The radial distribution of most minerals varied from section to section in a rather irregular manner, but there was a suggestion in several of the experiments that potassium concentrations rise and fall progressively from one side of the fruit to the other. Examples of a regular rise and fall were the values for apples a, b, c and e (Table I), apples B and C (Table II), and apple D (Table IV). A similar pattern was demonstrated earlier by Bishop, also in this laboratory, who

Table VII

Total nitrogen in subsamples of grated pairs of opposite sections and remainders of 20 apples

Sample	Mass, g.	Mass digested, g.	N, mg./100g. fresh weight	
Sections	469	4·95	65·0	65·1
		5·50	65·2	
Remainders	1579	5·05	64·6	64·7
		5·83	64·8	
Whole fruit (calculated)	2048			64·8

made potassium determinations on apple sections of similar shape and size. In most instances, in the present analyses, potassium concentration was high in sections from the red or ' blushed ' side of the fruit (section 1 and 8) and low on the green side (sections 4 and 5). In most of the samples calcium and phosphorus concentrations showed the same trend, but with more variation from section to section; so also did nitrogen in the two experiments in which it was determined. In fact the nitrogen pattern in apple A (Table IV) showed the biggest mineral gradient recorded, the concentration of nitrogen being twice as high on the red side of the fruit as it was on the green side. There was also a large calcium gradient in the same direction.

In apple C (Table II) there was a reversal of the ' colour ' pattern in that potassium was higher in sections 4 and 5 than in sections 1 and 8; the same was true for calcium and phosphorus. This may have been an apple with indefinite colouring which was therefore difficult to orientate.

Magnesium concentrations showed no particular trend, nor did those of sodium and sulphur.

The concentrations of organic constituents shown in Table IV were also distributed in an irregular manner, and no significance can be attached to the patterns. They could of course be different if the apples were sampled at different times, because the organic composition changes during maturation and senescence. For this reason the fact that starch was low on the green side of apple D (Table IV) may not necessarily be associated with the low nitrogen and calcium figures.

Sampling for minerals

The results shown in Table II are considered accurate for two reasons. There were no sub-sampling errors and there was sufficient ash solution to replicate determinations of minerals when necessary.[7] Hence the greatest possible errors for single sections and pairs of opposite sections could be calculated accurately. These results, given in Table III, show that even though errors for some elements may be large if one section only is analysed, they may be reduced (with the exception of those for sodium) to below 5% by the combination of any pair of opposite sections for analysis. The large errors for sodium arise from the irregular radial distribution of this element and the exceptionally high concentration on the ' blushed ' side of apple B.

Results for the chopped sections of apples A and D, shown in Table V were subject both to sub-sampling errors and larger analytical errors. Sub-samples had to be smaller than usual because of the limited amount of material and, except when some combustions were duplicated, determinations could not be repeated. Conclusions about sampling errors must therefore be viewed with caution and there is little point in calculating the greatest possible errors, given in Table V, very accurately. With the exception of errors for calcium in the small apple A, these rough calculations of errors for ash minerals confirm those given in Table III. It will be seen from Table IV that combustions were duplicated for sections of apple A with the highest and some of the lowest calcium concentrations, so it seems that the large errors when pairs of opposite sections are combined for analysis are genuine for this particular apple and not a result of faulty sampling and analytical techniques. Large errors are possible if one section only is taken for determination of nitrogen and these can be reduced by combination of pairs of opposite sections. Errors for nitrogen again appear to be large in apple A but it was not possible to confirm this result.

The experimental tests of the method of combining opposite sections of bulk samples for analysis gave results (Tables VI and VII) which were generally better than expected from the previous work. This was probably because sections were cut midway between the ' blushed ' and ' unblushed ' sides of the fruit where errors in taking one section only would usually, though not always, be smaller. The errors for magnesium, however, in both the D3 samples were about double the expected value and may have been due to the small size of the sections. Sodium results were better than expected. With the exception of sulphur in the D3/6 sample, errors for all minerals were less than $\pm 5\%$ and were no greater than those involved in taking 20 or 25 apples to make a sample (Table VI and previous work[1]).

Conclusion

Reduction of sample size by taking one section to represent the whole fruit can lead to large errors in mineral analysis. These may be even larger for calcium, and possibly nitrogen, in small apples. Diminution of these errors by cutting the section from midway between the ' blushed ' and ' unblushed ' sides of the fruit seems theoretically possible for some elements, but this is not practicable for Cox's Orange Pippins because of the indefinite marking and irregular shape of many apples. Archbold & Barter came to the same conclusion when working with Bramley's Seedling apples.[5]

Mineral concentrations derived from analysis of pairs of opposite sections differ only slightly from those in the whole fruit and the actual size of the sections appears to make little difference providing they are cut through the centre of the fruit. There is an obvious saving in storage space when samples are taken in this way; but more time is required to cut the sections, without subsequent homogenisation, than for grating whole apples *and* removing their seeds and stems before freezing them.

It seems probable that a reduction of sample size for determination of dry weight and titratable acid could be made in the same way, but this would need confirmation by analysis of large subsamples and tests with bulk samples. Reduction of sample size before determination of alcohol-soluble and -insoluble fractions, starch and pectin cannot be recommended from the few results available. In addition to the necessity of obtaining more results based on bigger subsamples, an investigation of the effects of time of picking and time in store on the radial distribution of these constituents should be made.

Acknowledgments

Mrs. J. M. Martin and Mr. R. D. Jones assisted with the chemical analyses.

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Received 16 March, 1965

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EFFECTS OF GIBBERELLIC ACID AND (2-CHLOROETHYL)-TRIMETHYLAMMONIUM CHLORIDE ON POTATO GROWTH AND DEVELOPMENT*

By P. W. DYSON

The effects of gibberellic acid (GA) and (2-chloroethyl)trimethylammonium chloride (or Chlorocholine chloride, CCC), on growth of potatoes in pots were measured by growth analysis. GA was applied to the ' seed pieces ' and CCC to the soil at emergence.

GA accelerated emergence and stem growth but delayed leaf and tuber growth. CCC decreased growth of stems, leaves and stolons and caused tubers to form earlier. CCC did not modify the effects of GA. The initial effects of both regulators were reversed in the later stages of growth, so that yield and leaf area duration were unchanged.

Introduction

Gibberellic acid (GA) has a profound effect on growth and development of the potato plant. When applied to tubers it accelerates bud and stem extension,[1-7] either by breaking dormancy[8,9] or by an effect on bud extension,[10] producing earlier emergence after planting. When applied as a spray to foliage, GA increases leaf area,[5,11,12] and stem growth[5] especially when the nitrogen supply is small.[11,12] It increases stolon length,[1,2] delays tuberisation [1-5,13] and causes malformation of tubers.[1,4,14] Total dry matter may increase,[11,12] because of the increase in leaf area, but only temporarily. A larger fraction of dry matter is directed to haulm growth.[5]

The growth-regulating compound CCC has an effect in many respects opposite to that of GA. It decreases stem length, stolon length and leaf area,[15] makes the leaves darker green and the plant better able to tolerate water stress.[16] The inhibitory effect of CCC on growth is transitory, and decreases steadily over a 42-day period.[15]

The only practical use made of GA is to treat tubers in order to speed the emergence of shoots, which may increase yields in short-growth periods. Tsukamoto[1] reports yield increases when whole tubers were soaked in GA solutions of concentration up to 50 p.p.m. Cutting tubers also causes earlier emergence,[6] but Tsukamoto found that treatment with GA and cutting decreased yield. Timm *et al.*[3] found 5 p.p.m. was optimal for increasing yields in a growth period of 108 days, with no benefit in 154 days. Larger concentrations hastened stem growth but retarded leaf development.

The purpose of the present experiment was to investigate by growth analysis the effects of GA and CCC alone or together. It seemed possible that the combined treatment might induce early emergence and avoid the excessive stem and stolon growth caused by GA.

Experimental

The treatments were: (*a*) 2 rates of GA, 0 (G0) and 50 p.p.m. (G1) and (*b*) 3 rates of CCC; C0, 790 p.p.m. or 5×10^{-3} M (C1) and 3160 p.p.m. or 2×10^{-2} M (C2) applied in all factorial combinations: (–), C1, C2, G1, G1C1, G1C2. There were 5 sample harvests of 6 plants for each treatment at each harvest—a total of 180 plants. Fifteen extra seed pieces were planted after treatment with GA at 1000 p.p.m. (G2).

Seed pieces with a single eye were cut from Majestic tubers with a 1·5 cm. dia. cork borer, and the inner tissue cut away until each piece weighed 5 g. The seed pieces were cut on 15 March, 1963, and placed in damp sand to sprout and form callus. After 7 days, 500 seed pieces were selected with sprouts of 1–2 mm. long; half were soaked for 1 h. in a 50-p.p.m. solution of GA and half in distilled water. After 4 days in sand all shoots were measured, and 100 pieces with uniform shoot length were selected for planting from each treatment. The seed pieces were planted, 4 in. deep on 27 March, in 7 in. plastic pots containing 3·25 kg. of clay loam soil with N 1·05 g, K 1·75 g. and P 0·92 g. per pot (as NH_4NO_3, K_2HPO_4 and KH_2PO_4). To each pot were

* Read at meeting of Agriculture group, 16 February, 1965

added 0·2 and 0·4 g. of N on 21 May and 6 June respectively. CCC was applied to the soil at 50 ml. of solution per pot when all control plants had emerged on 18 April.

The first harvest (H1) was on 3 May, the second (H2) on 17 May, the third (H3) on 31 May and the fourth (H4) 28 June. The remaining plants were allowed to mature fully and were harvested when all foliage was dead, on 2 August (H5). Leaf area was measured by rating[17] at each harvest. Additional leaf-area rating of all intact plants was made on 19 April, of plants for H4 on 14 June and plants for H5 on 17 July. Stem and stolon lengths were measured at H1, H2, H3 and H4; all stems were measured on 19 April. Dry weights of leaves, stems, stolons and tubers were determined at each harvest. Tuber number and size distribution were noted at H2, H3, H4 and H5. Nitrogen was determined in stems, leaves and tubers at H2.

Results

Shoot growth and emergence

Mean shoot lengths at planting were 9, 17 and 32 mm., respectively, with increasing concentration of GA. The treated shoots were more slender and pointed than the untreated. Shoots of 90% of the plants treated with 1000 p.p.m. GA emerged from the soil 4 days after planting, with 50 p.p.m. GA after 8 days and without treatment after 18 days. CCC was applied on 18 April when all control plants had emerged. On 19 April all plants were rated and stem length measured (Table I).

Table I

Leaf area and shoot length—19 April

Treatment	Leaf area per plant, cm^2.	Stem length, cm.
None	23·3	3·3
50 p.p.m. GA	11·1	14·4
1000 p.p.m. GA	14·8	19·8

GA increased stem growth and decreased total leaf area (Table I). The shoots of GA-treated plants were very pale and the lower leaves were smaller than on control plants, which formed a dark green rosette with little stem extension.

Stem growth (Table II)

At Harvest 1, 15 days after treatment, CCC had no effect on stem length but at Harvest 2 its effect was large, particularly with 50 p.p.m. GA. Only the upper internodes of plants treated with GA were shortened by CCC and this made the plants top heavy.

At Harvest 3, the larger concentration of CCC still affected plants that also received 50 p.p.m. GA. There were no effects of CCC at Harvest 4. Between Harvests 3 and 4 plants treated with either concentration of CCC continued to grow in length, but plants without CCC had stopped

Table II

Results on plants at various times with different treatments

Time of measurement	Stem length, cm.				Stem dry weight per plant, g.			
Treatment	H1	H2	H3	H4	H1	H2	H3	H4
Nil	14	24	31	30	0·23	0·82	1·3	1·6
C1	13	22	27	33	0·21	0·66	1·2	2·1
C2	11	18	23	25	0·18	0·50	1·2	2·3
G1	27	42	46	46	0·23	1·13	1·9	2·3
G1C1	26	38	43	43	0·23	1·01	1·7	2·6
G1C1	28	30	38	40	0·25	0·77	1·3	2·3
L.S.D. (p = 0·05)	5·2	3·6	8·1	7·1	0·071	0·16	0·22	0·66

growing at Harvest 3. More prolonged stem extension of the CCC-treated plants compensated for the earlier decrease, but no compensation occurred in plants treated with GA.

The treatments had no effect on stem dry weight at Harvest 1. GA decreased weight per unit length, thus compensating for greater extension. At Harvests 2 and 3, CCC decreased stem weight, whereas GA increased it, but at Harvest 3 the CCC effect occurred mainly in combination with GA. By Harvest 4, all treated plants had more stem dry weight than the controls, plants with CCC had more weight per unit length and both GA- and CCC-treated plants had more lateral growth.

Leaf growth

The larger concentration of CCC decreased leaf area of plants without GA at Harvest 1 and GA decreased leaf area in plants not treated with CCC (Table III). At Harvest 2, CCC decreased

Table III

Leaf area of plants at various times after different treatments

	Leaf area per plant (dm^2.)						
Time of measurement / Treatment	H1	H2	H3	14 June	H4	17 July	Leaf area duration dm^2. weeks
Nil	2·9	6·5	10·7	11·1	11·3	0·1	58
C1	2·4	5·4	10·2	10·8	11·7	1·4	60
C2	1·9	5·0	9·4	11·1	11·2	3·5	67
G1	1·9	6·9	10·7	12·0	12·1	2·0	65
G1C1	1·9	5·6	11·2	11·3	11·9	1·4	61
G1C2	2·2	5·7	10·4	11·9	12·1	0·8	60
L.S.D. (p = 0·05)	1·0	0·6	1·0	1·3	1·3	1·6	

leaf area with or without GA. Plants with treatment GA recovered from the effects of either CCC concentration at Harvest 3, but without GA only plants given the smaller concentration of CCC had recovered. There were no significant differences between treatments in leaf area on 14 June or at Harvest 4. The rates of senescence of leaves tended to be opposite to the initial rates of increase in leaf area. On 17 July, CCC-treated plants without GA had more leaf area, but those with 50 p.p.m. GA had less, than comparable plants without CCC. Because of this compensation of slower initial leaf expansion by delay in senescence, leaf area duration between emergence and complete sensecence was not decreased by GA or CCC.

The lower leaves on the main stem of GA-treated plants had smaller areas and the largest leaf was at node 12, whereas it was at node 10 in the control plants (Fig. 1). The larger concentration of CCC decreased the area of all leaves, but especially after leaf 6. CCC and GA together decreased the area of the first 11 leaves only. At Harvest 3, when the main stem leaves had almost fully expanded, compensatory effects are apparent. (Fig 2). All treated plants had smaller leaves

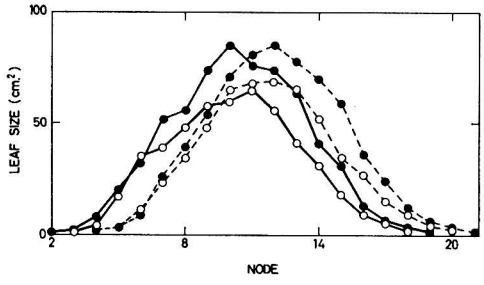

FIG. 1.—*Size of leaves at different positions on main stem of potato plants at Harvest 2 after different treatments*

●—● no treatment ○—○ C2 ● - - ● G1 ○ - - ○ G1 C2

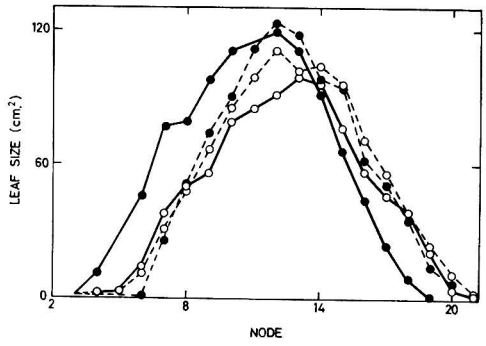

FIG. 2.—*Size of leaves at different positions on main stem of potato plants at Harvest 3 after different treatments*
● —● no treatment ○—○ C2 ● - - ● G1 ○ - - ○ G1 C2

at some earlier stage but produced larger leaves on nodes 14–20. Leaf number varied but the differences between treatments were not significant.

Leaf dry weight was closely related to leaf area, irrespective of treatment, except at Harvest 1, when CCC increased, and GA decreased weight per unit area (Table IV). At Harvest 1, but not after, GA decreased the weight of main stem leaves. The weight of lateral leaves was increased at all harvests. CCC decreased the dry weight of main stem leaves at Harvests 1, 2 and 3. At Harvest 4, there was no significant effect because of extra growth of the later-formed leaves on plants given CCC. The largest amount of CCC increased the dry weight of lateral leaves at Harvest 3 of plants not given GA and at Harvest 4 independently of GA treatment.

Table IV

Dry weight of leaves at various times after different treatments

Time of measurement	Main stem leaves				Lateral stem leaves		
Treatment	H1	H2	H3	H4	H2	H3	H4
Nil	99	230	406	459	3	16	54
C1	95	204	387	450	4	25	95
C2	82	187	384	408	4	37	134
G1	56	241	412	429	9	47	107
G1C1	58	198	405	413	9	41	142
G1C2	67	199	386	437	10	39	127
L.S.D. (p = 0·05)	29	19	24	194	4	17	56

Stolon growth

CCC shortened stolon growth at Harvest 2, but not at Harvest 1. CCC had a more immediate effect on plants without GA (see Table V). 50 p.p.m. GA increased stolon length but the stolons were very thin and their total weight was decreased. Plants with CCC had thicker stolons that weighed more per unit length than those of untreated plants. After Harvest 1 weight per unit length of stolons varied.

Tuberisation and tuber growth

As is seen from Table VI, at Harvest 1, three plants given the larger concentration of CCC and one with the smaller had formed tubers, but other plants had not. At Harvest 2, plants with either concentration of CCC had a few even-sized tubers, whereas control plants had more tubers differing more in size. Only three plants with GA had tubers at Harvest 2 but all six plants with GA and the larger amount of CCC had formed tubers. At Harvest 4, tuber number was increased by the larger amount of CCC. At Harvest 5, the late-formed tubers had died and CCC again decreased tuber number. Total tuber dry weight was decreased by GA at Harvest 2, by CCC at Harvest 3 but was not changed by any treatment at Harvest 4 or Harvest 5.

Table V

Length and weight of stolons at various times and after different treatments

Time of measurement	Total stolon length per plant, cm.				Total stolon dry weight per plant, mg.			
Treatment	H 1	H2	H3	H4	H1	H2	H3	H4
Nil	20	519	451	378	32	367	323	235
C1	21	249	309	297	34	144	220	208
C2	12	198	182	212	21	94	173	122
G1	39	511	479	586	16	170	217	248
G1C1	33	407	558	469	15	215	224	220
G1C2	33	473	363	344	19	122	142	134
L.S.D. (p = 0·05)	11	160	215	199	14	157	95	118

Table VI

Results for tubers at various times after different treatments

Time of measurement	Tubers per plant					Tuber dry weight per plant, g.			
Treatment	H1	H2	H3	H4	H5	H2	H3	H4	H5
Nil	—	11·0	12·7	11·5	9·0	1·95	11·2	38·6	51·9
C1	0·2	6·0	9·2	11·2	6·5	1·95	8·6	40·5	45·7
C2	1·3	6·3	8·7	15·8	5·5	2·23	7·2	35·7	49·6
G1	—	4·8	11·2	14·0	7·2	0·25	8·0	39·2	52·1
G1C1	—	7·0	10·5	10·3	6·0	0·16	7·5	31·8	49·5
G1C2	—	7·5	10·2	9·7	5·7	0·79	8·6	35·3	48·4
L.S.D. (p = 0·05)	—	5·2	5·0	4·5	1·9	0·64	1·8	6·0	6·0

Total dry matter

Total dry matter was determined at the first 4 harvests but not at Harvest 5 because parts of stems and leaves had decayed. Top dry weight constituted only 18% of total dry matter at Harvest 4 and presumably less at Harvest 5 (Table VII). GA decreased total dry weight at Harvests 1 and 2. At Harvest 3 GA alone decreased total dry matter. CCC alone also decreased total dry matter but in combination with GA it did not further decrease total dry matter.

Net assimilation rate

Net assimilation rate (E) was decreased by GA in the interval between Harvest 1 and Harvest 2 (see Table VIII). Mean E increased from Harvest 1 to Harvest 4.

Nitrogen content

Nitrogen determinations were done only on duplicate samples (Blocks 3 and 4) of three treatments at Harvest 2 (see Table IX). At this stage the plants with CCC were darker green

Table VII

Total dry weight per plant (g.) at various times after different treatments

Time of measurement	H1	H2	H3	H4
Treatment				
Nil	1·2	5·5	17·0	45·6
C1	1·2	4·8	14·1	48·3
C2	1·0	4·7	12·8	43·6
G1	0·8	4·1	14·7	47·3
G1C1	0·8	3·5	13·9	40·3
G1C2	0·9	3·8	14·3	43·6
L.S.D. (p = 0·05)	0·4	0·8	1·9	6·2

Table VIII

Net assimilation rate after different treatments (g./m²/week)

Time of measurement / Treatment	H1–H2	H2–H3	H3–H4*
Nil	58	69	
C1	60	62	
C2	58	58	
G1	43	62	
G1C1	40	64	
G1C2	50	67	
Mean	52	64	69*
L.S.D. (p = 0·05)	22	14	

* There were no consistent trends: the mean value includes estimated value for leaf fall and individual values are not available.

Table IX

Nitrogen values at Harvest 2 after different treatments

	Nitrogen, % of dry matter			Nitrogen per plant, mg.			
Treatment	Stem	Leaf	Tuber	Stem	Leaf	Tuber	Total
Nil	1·04	3·69	1·31	10·2	93	23	126
C2	1·69	4·36	1·47	9·5	88	38	136
G1	1·27	3·21	1·96	18·4	83	12	113
G1C2	1·74	4·14	1·46	14·3	86	10	110
L.S.D. (p = 0·05)	0·34	0·67	1·79	4·0	23	15	3

than the controls, and had more nitrogen % of dry matter in leaves and stems. GA increased stem nitrogen % and per plant. CCC alone increased tuber nitrogen, and hence total nitrogen, per plant. GA decreased tuber nitrogen and total nitrogen per plant.

Discussion

Large amounts of GA affected growth as Timm *et al.* also found;[3] 1000 p.p.p. had more effect than 50 p.p.m. and affected more internodes and leaves. Probably the smaller concentrations used by Timm (5 p.p.m.) affected enough basal internodes to hasten emergence and did not decrease the size of the lower leaves. The CCC failed to decrease the length of basal internodes when applied to plants treated with GA. CCC acted faster on plants without GA, suggesting that GA had an immediate effect on the shoot apex at the time of application not reversible by CCC. This was confirmed by a separate trial in which CCC was ineffective when applied to the seed pieces immediately after GA. CCC slowed stem and stolon extension and leaf expansion, and a larger proportion of the photosynthates was used in earlier tuber formation. GA had an opposite effect and a larger proportion of the photosynthates were used in stem and, later, in leaf growth.

Compensatory effects reversed the initial effects of GA and CCC by Harvest 4. In the final stages of growth, only the size and distribution of lower internodes and leaves and leaf persistence were changed. Most of the effects of GA are undesirable and the only potentially useful one is to accelerate emergence.

CCC shows several useful initial effects: it causes earlier formation of more uniform tubers, decreases stem growth and diverts a larger proportion of photosynthates to tuber growth despite increased nitrogen uptake.

According to Ivins[18] increase in leaf area index (L) of potato crops above 3 does not accelerate tuber growth; increase in nitrogen supply increases tuber yield, not by producing a larger maximum L, but by prolonging the life of the foliage and so lengthening the growth period. If CCC, or a similar growth regulator, were applied to a field crop when L had reached about 3, it might limit leaf area and increase tuber yield by prolonging the life of the leaves. This possibility is being tested in pot and field experiments.

According to Milthorpe[19] the net assimilation rate (E) of a potato crop after tuber formation is controlled by the rate tubers grow. The increase of E after Harvest 2 (Table VIII) may therefore be a consequence of tuber formation. E is also decreased by GA from Harvests 1–2. Thus, there is a relationship between net assimilation rate and the ratio of shoot/tuber growth rates. For any given time interval, the rate increase of dry matter per unit area of leaf can be divided into the rate it accumulates in the tubers T_A and the rate in the shoots S_A, thus, $S_A + T_A = E$. In Fig. 3, E is plotted against S_A/T_A and illustrates the coincident increase in E and decrease in

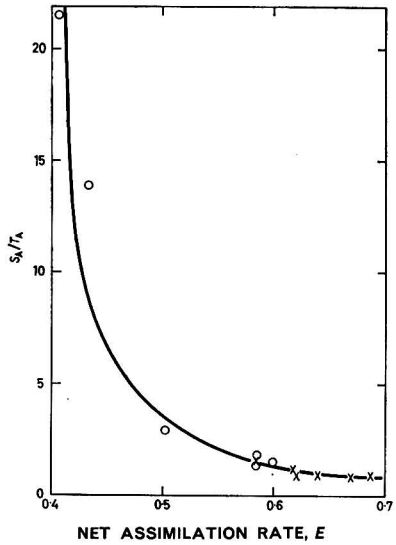

FIG. 3.—*Relation between ratio of rate of increase of dry matter in shoots/rate of increase of dry matter in tubers* (S_A/T_A) *and net assimilation rate* (E)
○ $H_1 - H_2$ × $H_2 - H_3$

S_A/T_A. If E controls S_A/T_A, a decrease in E caused by decreased incident radiation should increase shoot growth relative to tuber growth. On the contrary, according to Milthorpe[19] tuber growth increases relative to shoot growth at the end of the growth period when incident radiation is decreasing. Moss[20] demonstrated that removing fruits from tomatoes or ears from maize decreases E, and Burt[21] and Nösberger[22] decreased E in potatoes by removing tubers: hence it is concluded that S_A/T_A influences E.

Plotting S_A against T_A (Fig. 4) shows that an increase in T_A only slightly depresses S_A. The relationship is defined by:

$$S_A = -0{\cdot}287\ T_A + 0{\cdot}409$$

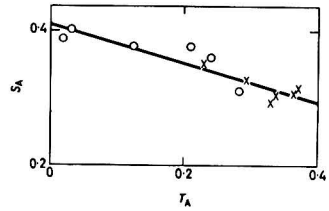

FIG. 4.—*Relation between rate of increase of dry matter in shoots* (S_A) *and in tubers* (T_A)
○ H1–H2 × H2–H3 $y = 0{\cdot}409 - 0{\cdot}287x$ $r = 0{\cdot}91$

Hence, a unit increase in T_A means a decrease in S_A of only 0·287 units. It is, therefore, concluded that, in a young potato plant, an internal factor associated with surplus carbohydrate restricts photosynthesis until tubers develop to form a sink for carbohydrate and remove the restriction on E.

Acknowledgments

The author thanks Dr. E. C. Humphries for help and advice, and the Potato Marketing Board for a scholarship which made this work possible.

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Received 18 February, 1965; amended manuscript 8 June, 1965

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FERMENTATION STUDIES ON RED CLOVER

By P. McDONALD, ANNA C. STIRLING, A. R. HENDERSON and R. WHITTENBURY

Two experiments were carried out to study the fermentation changes occurring during the ensilage of red clover (*Trifolium pratense*). In the first experiment red clover containing 11·8% of water-soluble carbohydrates (WSC) was bruised and ensiled, the treatments being: control, molassed, inoculated, and wilted. All silages were well preserved (pH 3·9–4·0), the gaseous losses being lowest in the control and inoculated herbages.

In the second experiment the effect of bruising red clover before ensiling was studied. Two silos were filled with long material (WSC—9·7%) and two with bruised material (WSC—8·0%). The bruised silages were well preserved (pH 4·0–4·1) and the bruising treatment resulted in lower gaseous losses than those which occurred in the production of the non-bruised silages (pH 4·3).

The results show the advantages of bruising clover and indicate that wilting, addition of molasses and inoculation of the clover may be advantageous. Feeding trials, using sheep, were carried out to determine digestibilities and intakes and the importance of the results is discussed.

Introduction

The advantages of legumes as rich sources of protein and essential mineral elements, especially, calcium and magnesium, in the diets of ruminants are well known. Unfortunately legumes have usually been regarded as difficult crops to conserve, especially as silage. This difficulty has

been attributed to their low content of water-soluble carbohydrates (WSC)[1,2] and to their relatively high buffering capacity compared with grasses.[3-5] Of the legumes lucerne has been studied fairly extensively[6,7] and the use of molasses as an additive in silage making has been recommended. In other studies with unwilted legumes, the addition of molasses improved the fermentation process but resulted in high seepage losses of sugars.[8]

Recent studies on different varieties of red clover (*Trifolium pratense*) have shown that yields of up to 12,000 lb. of dry matter/acre can be obtained in S.E. Scotland,[9] and it is suggested that this crop may have considerable value in grassland economy under Scottish conditions.

The purpose of the present investigation was to study the effect of a number of treatments upon the biochemical changes and losses during the ensilage of red clover.

Experimental

Procedure

The silo unit used in these studies consisted of four metal silos (153 cm. dia. × 182 cm. high) each suspended from a weighing device which enabled daily measurements of weight changes to be recorded. Each silo was fitted with ten thermocouples which were sited at different levels within the herbage during filling. A detailed description of the silo unit and the techniques of sampling, digestibility and bacteriological methods have been given elsewhere.[10,11] The technique used for the separation and determination of organic acids was that developed by Lessard.[12] The acids are eluted from a silica gel column with benzene-butanol, collected in 4-ml. fractions and titrated with 0·005N-sodium hydroxide. Dry-matter contents of silages were determined by a toluene distillation technique.[13] Water-soluble carbohydrates were determined after extraction by a modified Somogyi technique.[14] The buffering capacity to lactic acid of the herbage samples was determined as described by McDonald & Henderson[5] and the results were designated ' lactic buffer capacity (LBC) ', i.e., mg. of lactic acid required to bring 1 g. of dried, milled herbage to pH 4. Cellulose was determined by the Crampton & Maynard method.[15]

In order to assess the true losses occurring during the formation of surface waste, a technique based on that described by Wittwer *et al.*[16] was employed. When the silos were three-quarters full, a sheet of fine mesh Terylene netting was placed on the surface of the herbage to act as a marker. Above this were placed four perforated polythene bags containing weighed samples (4 kg.) of herbage representative of the mass. The silos were filled as before and the weight of herbage above the net recorded. When the silos were emptied, the waste material, polythene bags and well-preserved silage above the netting were separately weighed. From these recordings, together with dry-matter values, the losses occurring during the production of the waste material could be estimated.

First experiment.—Broad red clover (*Trifolium pratense*) was used and the treatments were as follows:

Silo A: clover 1678 kg. (238·4 kg. of dry matter)
Silo B: clover 1500 kg. (213·2 kg. of dry matter) + molasses solution 72·6 kg. (33·9 kg. of dry matter)
Silo C: clover 1671 kg. (237·4 kg. of dry matter) + inoculum 4 kg. (0·6 kg. of dry matter)
Silo D: partially wilted clover 1551 kg. (312·4 kg. of dry matter)

In the first three treatments, the clover was cut and bruised on 24 June, 1963, with a flail-type forage harvester (Lundell 60, rotor speed 1,200 r.p.m.) and ensiled the same day. In treatment D, the clover was cut with a mower on the morning of 24 June, wilted for 27 hours, then lifted and bruised with the flail-type forage harvester.

The molasses used in treatment B consisted of 48·4 kg. of molasses diluted with 24·2 kg. of water and this was sprayed on to the clover from a pressure spray during filling. The molasses contained 70·1% of dry matter, 0·27% of total nitrogen, and 44·8% of WSC.

The inoculum consisted of a suspension of lactobacillus strains selected for their ability to grow rapidly in silage and to ferment all of the available carbohydrates.

In addition to the large silo experiments, a number of small laboratory silos were filled with similar material and were subsequently examined at intervals for bacteriological changes.

After filling, the contents of silos A, B and C occupied 3·02 cu.m., and silo D, 3·40 cu.m. The consolidation weight applied to the surface of the ensiled material was 400 kg., equivalent to a pressure of 22 g./sq.cm.

Effluents were collected daily, or when they appeared, and stored at −18° until subsequently analysed. The silos were opened on 20 August, 58 days after filling.

Digestibility trials and intake measurements were carried out on the fresh clover in triplicate and on the silages in duplicate using Cheviot wether sheep.

Second experiment.—This was designed to study the effect of bruising. The experiments were carried out in duplicate as follows:

Silo A: long clover 910 kg. (151·6 kg. of dry matter)
Silo B: long clover 910 kg. (151·6 kg. of dry matter)
Silo C: bruised clover 910 kg. (140·0 kg. of dry matter)
Silo D: bruised clover 910 kg. (140·0 kg. of dry matter)

The clover was a second cut taken on 18 September, 1963, from the same field as that used in the first experiment, but whereas the June cut consisted almost entirely of red clover, the material used in the second experiment contained about 34% of grasses, mainly *Lolium perenne*.

The long herbage was cut with a mower and hand loaded. The bruised material was cut and bruised with a flail-type forage harvester similar in make and type to that used in the first experiment. All four silos were filled with the fresh herbage on the same day.

The contents of silos A and B occupied 3·02 cu.m. while the bruised material occupied 2·27 cu.m. after filling. The consolidation weight applied was 400 kg. as in the first experiment. Similar procedures to those described above were adopted for measurements of waste and digestibility. The silos were opened on 19th November, 63 days after filling.

Results

(1) *Experiment* 1

Volume changes

The changes in volume occupied by the silages are shown in Fig. 1. The volume of the molassed herbage fell most rapidly, while that of the wilted material remained consistently higher throughout the ensiling period; the latter had however a greater weight of dry matter per unit volume at the end of the experiment.

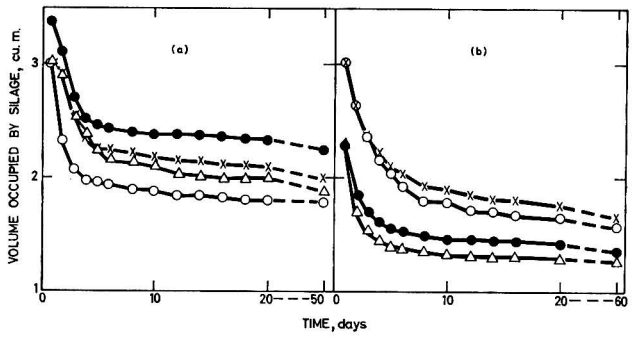

FIG. 1.—*Volumes occupied by silages at various times*
(a) Expt. 1 (b) Expt. 2 Treatment: A × B ○ C △ D ●

Composition

The chemical composition of the clover and silages is given in Table I. The fresh clover was of low dry-matter content (14·21%). At the time of cutting, wilting conditions were poor and, after 27 h., the dry matter content was only 20·14%.

Table I

Composition of clovers and silages (% of true dry matter)

	Experiment 1						Experiment 2					
	Clover		Silages				Clover		Silages			
	Fresh	Wilted	A	B	C	D	Long	Bruised	A	B	C	D
Dry matter[a]	14·21	20·14	17·72	18·73	17·48	19·66	16·66	15·38	16·56	16·97	18·89	18·95
Organic matter	89·3	87·3	88·8	90·5	90·3	86·3	90·6	85·6	89·8	89·9	81·2	83·7
Crude protein	15·1	13·7	14·7	13·9	15·6	14·8	14·9	15·2	18·3	17·3	15·8	14·9
Ether extract	2·7	2·4	2·5	2·0	2·3	2·3	2·2	2·1	2·9	2·9	2·4	2·2
Crude fibre	24·3	23·8	27·3	26·6	26·8	25·0	25·1	23·1	26·4	26·7	24·2	24·8
N.F.E.[b]	47·3	47·5	44·3	47·9	45·6	44·2	48·3	45·3	42·2	43·0	38·8	41·9
Total N	2·41	2·19	2·35	2·23	2·49	2·37	2·39	2·43	2·93	2·77	2·53	2·38
Protein N	1·88	1·76	1·07	1·00	1·17	1·23	1·99	2·03	1·57	1·51	1·71	1·59
Non-protein N	0·53	0·43	1·28	1·23	1·32	1·14	0·40	0·40	1·36	1·26	0·82	0·79
Volatile N	0·02	0·02	0·20	0·15	0·18	0·19			0·26	0·26	0·12	0·12
Water-soluble carbohydrates	11·8	11·2	0·7	2·9	0·7	0·6	9·7	8·0	1·6	1·4	1·4	1·2
Cellulose	28·9	28·5	31·8	30·1	32·3	29·4	28·6	27·6	29·9	30·5	28·8	29·2
Lignin	7·0	6·9	7·9	7·1	8·7	7·8	9·5	9·5	9·8	9·9	9·5	10·1
Lactic acid			9·3	12·4	10·4	10·5			6·7	6·8	7·9	7·6
Formic acid			nil	nil	nil	nil			nil	nil	nil	nil
Acetic acid			3·6	2·7	2·5	2·9			2·5	2·7	2·5	2·4
Propionic acid			nil	0·49	0·26	0·20			nil	nil	nil	nil
Butyric acid			0·08	nil	nil	nil			nil	nil	nil	nil
Succinic acid			0·14	0·30	nil	0·21			0·40	nil	nil	nil
pH[a]	5·7	5·7	4·0	3·9	3·9	4·0	6·1	6·0	4·3	4·3	4·1	4·0

[a] = determined on fresh material [b] N.F.E. = Nitrogen-free extractives

The WSC content of the fresh herbage was 11·8% and that of the wilted material slightly lower (11·2%). The four silages were well preserved, the molassed and inoculated materials being of slightly lower pH value than the control and wilted silages. The lactic acid (9·3–12·4%) and acetic acid (2·5–3·6%) contents were relatively high, the highest lactic value occurring in the molassed silage. Butyric acid was detected, in trace amounts, in the control.

The LBC value of the fresh clover was 63 and that of the wilted 58.

The effluent pH values for the control and wilted materials are given in Fig. 2. The values for the effluents obtained from the molassed and inoculated herbages followed a similar pattern to those of the control.

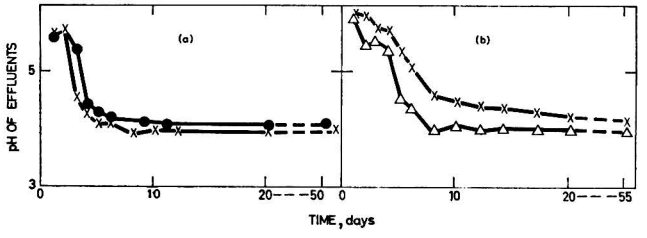

FIG. 2.—*pH values of effluent after various times*

(a) Expt 1. (b) Expt. 2 Treatment: A × C △ D ●

Temperature changes

The temperature in all four silos remained relatively low, the maximum values reaching 20° (A), 22° (B), 21° (C) and 22° (D). The temperatures in silo D remained slightly higher than those in the other three silos during the whole of the ensiling period.

Losses

The total weights of fresh silage removed from the silos and the total losses of dry matter and its components are shown in Table II.

Table II

Percentage losses during ensilage

	Experiment 1								Experiment 2							
	A		B		C		D		A		B		C		D	
	Total	Effluent	Total	Effluent	Total	Effluent	Total	Effluent	Total	Effluent	Total	Effluent	Total	Effluent	Total	Effluent
Total fresh	30·0	29·1	33·2	32·2	29·9	29·3	11·1	10·4	14·2	13·1	15·3	14·1	25·3	24·6	25·3	25·0
Dry matter	13·9	9·0	21·1	14·0	14·4	9·4	13·9	3·2	16·1	2·6	16·3	2·8	9·2	7·2	9·5	6·9
including gaseous dry matter	4·9		7·1		5·0		10·7		13·5		13·5		2·0		2·6	
dry matter in waste material	36·5		38·2		43·6		51·5		35·2		33·4		37·8		23·8	
dry matter in well-preserved material	11·6		19·9		11·9		11·3		14·2		13·4		4·4		7·6	
Crude protein	16·0	10·3	17·5	11·9	11·6	11·5	6·6	4·2	+2·9	3·7	3·1	4·0	5·4	7·0	12·9	7·0
Ether extract	21·3		31·9		28·5		19·0		+7·4		+10·7		+3·1		5·2	
Crude fibre	3·4		nil		5·6		9·2		11·7		11·1		4·9		2·6	
N.F.E.[a]	19·2		28·0		17·4		20·0		26·7		25·5		22·1		16·3	
Total N	16·0	10·3	17·5	11·9	11·6	11·5	6·6	4·2	+2·9	3·7	3·1	4·0	5·4	7·0	12·9	7·0
Protein N	50·9		58·1		46·8		39·8		33·8		36·4		23·6		29·2	
Water-soluble carbohydrates	94·7	10·5	88·0	23·5	95·0	8·5	95·5	3·0	86·3	2·7	88·1	2·3	83·9	17·3	86·9	17·2
Cellulose	5·0		4·5		4·1		11·2		12·3		10·8		5·3		4·1	
Lignin	2·1		7·4		+6·3		1·9		13·5		12·8		8·9		3·4	
Water	32·7	32·4	35·5	35·6	32·4	32·6	10·5	12·2	13·8	15·2	15·2	16·4	28·2	27·8	28·2	28·3

[a] see Table I + means increase instead of loss

The quantities of surface waste material removed from the silos were A, 94; B, 64; C, 71; and D, 59 kg. From the buried bag and marker technique the partition of losses of dry matter between this waste material and the well preserved material were estimated and these are also given in Table II.

Nutritive value

The digestibility coefficients and digestible nutrients for the clover and silages are given in Table III. During the trials the sheep were fed at voluntary intake levels. The daily dry matter intakes (g./kg.$W^{0.73}$) are also shown in Table III.

Bacteriological studies

Most of the bacteria on the fresh clover were Gram-negative types but the number of lactobacilli was appreciable, 90,000/g. In the small laboratory-made silages, examined after 4 days, the count of lactic acid bacteria was highest in the molassed and inoculated silages and lowest in the wilted material. After 9 days, lactobacilli were dominant in all the silages by which time the count on the wilted material had risen to the level found in the inoculated silage at the four-day examination. The pH values were all low; A (control), 4·2; B (molassed), 4·1; C (inoculated), 3·8 and D (wilted), 4·1. After 46 days, with lactobacilli the surviving organisms, the pH values were, A, 4·3; B, 4·0; C, 4·1 and D, 4·2. The most rapid drop in pH occurred in the inoculated material. The possibility that the tendency for a rise in pH might be due to a limiting sugar content is suggested by the continued decrease in pH value found in the molassed silage. As would be expected, bacterial action in the wilted material was slow, although the number of naturally occurring lactobacilli on the fresh crop was sufficient to ensure that they became dominant in all the laboratory silages.

When the large experimental silos were opened, lactobacilli were again found to be dominant in all silages.

(2) *Experiment 2*

Volume changes

The long herbage was more difficult to compact than the bruised material and the volumes in silos A and B remained consistently higher than those in silos C and D (Fig. 1).

Composition

It can be seen from Table I that the dry matter contents of the clover were again low (15·38, 16·66%). The WSC values were 9·7 and 8·0% for the long and bruised herbages respectively. All silages were well-preserved, but the bruised materials were of lower pH value and of slightly higher lactic acid content than the long herbage. Butyric acid was not detected in any of the samples.

The LBC value of the long herbage was 54 and that of the bruised material 55.

Analysis of the effluents indicated that the pH values of the bruised silages fell more rapidly than those of the long material. The results of treatments A and C are shown in Fig. 2.

Temperature changes

The temperature in the silos again remained low, the maximum values being A, 22°; B, 18°; C, 20° and D, 19°.

Losses

The total weights of fresh silage removed from the silos and the total losses of dry matter and its components are shown in Table II.

The quantities of surface waste material removed from the silos were A, 76; B, 110; C, 73; and D, 77 kg. As in the previous experiment, the partition of losses of dry matter between the waste and the well preserved material were estimated from the buried bag and marker technique and the results are shown in Table II.

Table IIIA

Percentage digestibility (D) *and percentage of digestible nutrients* (DN) *in true dry matter*

	Clover		Silages							
			A		B		C		D	
	D	DN	D	DN	D	DN	D	DN	D	DN
Experiment I										
Organic matter										
1.	72·7	65·0	67·9	60·3	73·6	66·6	70·6	63·8	68·4	59·0
2.	73·7	65·8	72·0	63·9	70·0	63·4	70·2	63·4	70·0	60·4
3.	70·2	62·7								
Mean	72·2	64·5	70·0	62·1	71·8	65·0	70·4	63·6	69·2	59·7
Crude protein										
1.	65·5	9·9	61·2	9·0	64·4	9·0	65·9	10·3	62·0	9·2
2.	69·6	10·5	69·8	10·3	61·8	8·6	66·5	10·4	66·8	9·9
3.	66·8	10·1								
Mean	67·3	10·2	65·5	9·7	63·1	8·8	66·2	10·4	64·4	9·6
Ether extract										
1.	57·5	1·6	54·6	1·4	47·0	1·0	49·4	1·1	57·1	1·3
2.	62·8	1·7	66·0	1·6	45·0	0·9	52·8	1·2	59·1	1·3
3.	58·0	1·6								
Mean	59·4	1·6	60·3	1·5	46·0	1·0	51·1	1·2	58·1	1·3
Crude fibre										
1.	68·3	16·6	65·4	17·9	73·5	19·6	67·7	18·2	64·0	16·0
2.	66·9	16·3	69·4	19·0	68·2	18·2	66·6	17·9	64·9	16·2
3.	60·8	14·8								
Mean	65·3	15·9	67·4	18·5	70·9	18·9	67·2	18·1	64·5	16·1
N.F.E.[a]										
1.	78·2	36·9	72·5	32·1	77·5	37·1	75·0	34·2	73·6	32·5
2.	79·1	37·4	74·6	33·1	74·5	35·7	74·4	33·9	74·6	32·9
3.	76·8	36·3								
Mean	78·0	36·9	73·6	32·6	76·0	36·4	74·8	34·1	74·1	32·7
S.E.[b]										
1.	—	58·7	—	53·1	—	59·2	—	56·4	—	52·3
2.	—	59·7	—	56·9	—	55·9	—	56·0	—	53·8
3.	—	56·5								
Mean	—	58·3	—	55·0	—	57·6	—	56·2	—	53·1
Experiment II										
Organic matter										
1.	67·7	58·0	65·8	59·1	66·5	59·8	65·5	53·2	67·4	56·4
2.	68·4	58·5	64·5	57·9	67·6	60·8	67·5	54·8	67·3	56·4
3.	69·3	59·3								
Mean	68·5	58·6	65·2	58·5	67·1	60·3	66·5	54·0	67·4	56·4
Crude protein										
1.	62·3	9·5	67·9	12·4	67·4	11·7	63·5	10·1	60·4	9·0
2.	64·6	9·8	68·1	12·5	66·7	11·6	62·2	9·9	60·1	9·0
3.	63·3	9·6								
Mean	63·4	9·6	68·0	12·5	67·1	11·7	62·9	10·0	60·3	9·0
Ether extract										
1.	66·8	1·4	59·3	1·7	65·8	1·9	71·2	1·7	67·2	1·5
2.	67·6	1·4	64·0	1·8	60·3	1·8	68·9	1·6	64·4	1·4
3.	62·6	1·3								
Mean	65·7	1·4	61·7	1·8	63·1	1·9	70·1	1·7	65·8	1·5
Crude fibre										
1.	62·2	14·3	63·4	16·8	62·7	16·7	62·6	15·1	66·0	16·4
2.	62·0	14·3	58·9	15·6	67·4	18·0	66·0	15·9	65·9	16·3
3.	65·4	15·1								
Mean	63·2	14·6	61·2	16·2	65·1	17·4	64·3	15·5	66·0	16·4
N.F.E.[a]										
1.	72·4	32·8	66·9	28·2	68·5	29·5	67·8	26·3	70·6	29·6
2.	72·9	33·0	66·4	28·0	68·6	29·5	70·5	27·4	70·9	29·7
3.	73·5	33·3								
Mean	72·9	33·0	66·6	28·1	68·6	29·5	69·2	26·9	70·8	29·7
S.E.[b]										
1.	—	52·0	—	52·3	—	53·1	—	47·1	—	50·0
2.	—	52·5	—	51·2	—	54·0	—	48·7	—	49·9
3.	—	53·2								
Mean	—	52·6	—	51·8	—	53·6	—	47·9	—	50·0

[a] N.F.E. = See Table I [b] S.E. = starch equivalent

Table IIIB

Daily intakes of dry matter
(g./kg.$W^{0.73}$ where W = weight of sheep)

	Clover	Silages			
		A	B	C	D
		Experiment I			
1.	88·8	86·4	63·3	71·9	75·7
2.	93·3	69·7	71·9	74·2	65·0
3.	83·2				
Mean	88·4	78·1	67·6	73·1	70·4
		Experiment II			
1.	82·4	54·8	68·4	68·4	55·5
2.	92·1	60·7	58·3	56·2	59·3
3.	82·9				
Mean	85·8	57·8	63·4	62·3	57·4

Nutritive value

The digestibilities and daily dry matter intakes (g./kg. $W^{0.73}$) of the clover and silages are shown in Table III.

Bacteriological studies

The initial count of lactic acid bacteria on the long herbage was 210/g. of fresh material, but, as might be expected through the release of plant juices and distribution of the organisms by mechanical action, it was considerably higher (18,000/g.) on the bruised material. When the silos were emptied, lactobacilli were dominant in all silages. The majority were homofermentative types which were able to decarboxylate malate. The growth of anaerobes was not extensive and the greatest number of proteolytic types were found in silo A.

Discussion

The WSC contents of the clovers, although low compared with those of *Lolium* spp.,[10,11] did not appear to be limiting in achieving a satisfactory type of fermentation in the experimental silos.

It is clear from the results of the first experiment that the addition of molasses had a beneficial effect in increasing the lactic acid content of the silage, although the inoculation treatment also resulted in material of similar pH value. The addition of molasses did increase the effluent production, however, and the total loss of dry matter from this treatment was higher than that from the other three. This confirms the findings of Wittwer *et al.*[8] It appears that, in practice, the addition of molasses to a very wet crop is liable to be wasteful because of the high effluent flow.

The buffering capacity of herbage is another important factor in ensilage. The LBC values were 58–63 and 54–55 in Experiments 1 and 2 respectively. These values are similar to those reported for legumes in an earlier publication[5] and are much higher than the values found for grasses.

Of great importance are the changes occurring during the early stages of fermentation, in which buffering and neutralising substances are increased through decarboxylation of organic acids such as malic acid. Legumes are richer in organic acids[17] than grasses and it is the breakdown of these substances by bacteria which adversely affects preservation.

The relatively low dry-matter content of the crop is one of the obvious disadvantages of ensiling red clover, and, in order to reduce losses of dry matter in the effluent, wilting seems to be an important pretreatment. This is clear from the effluent-loss figures for the partially wilted material ensiled in Experiment 1. Ideally, a dry-matter content of 25%, and preferably 30%, would be desirable. One fact, however, which is apparent from the first experiment is that the highest loss of gas was found with the wilted material. This may occur readily with wilted crops unless they are adequately consolidated. Wilting appears to be beneficial in reducing the buffering capacity. In Experiment 1, the LBC value of the wilted clover was 58 compared with a value of 63 for the fresh material. Smith[18] and Playne[19] have also commented on this reduction in buffering capacity after wilting herbage.

The benefit of bruising herbage before ensilage has been stressed by many workers[20,21] and this is clearly evident from the results of the second experiment.

The pH, as judged from the effluent samples (Fig. 2) fell more rapidly in the bruised than in the long material and the losses of dry matter were markedly lower in the forage harvested clover than in the long clover in spite of the higher effluent flow.

In these experiments the buried bag and marker technique, as described earlier, was used to measure losses of dry matter. It is clear from the calculated figures that the production of waste material leads to excessive losses of dry matter which are difficult to assess in farm practice based solely on surface waste measurement. The total losses of dry matter in the formation of waste material ranged from 36·5 to 51·5% in the first experiment and 23·8 to 37·8% in the second experiment.

The digestibilities of the silages are very similar to those of the fresh clovers. In Experiment II the digestible crude-protein figures of silages A and B are notably higher than those obtained for the other silages and the clover. These figures are a reflection of the crude protein percentages of these silages, which have increased as a result of the higher losses of gas during ensilage.

The intake figures are extremely variable and illustrate the need for carrying out feeding trials of this type with large numbers of animals. The values obtained, however, suggest that the silages were not consumed as readily as the fresh clovers.

The conclusions of these experiments indicate that red clover can be ensiled satisfactorily provided the herbage is bruised as a pretreatment. Because of the relatively high moisture content of this crop, wilting is also beneficial. The addition of molasses to red clover is not as important as when lucerne is ensiled, but may be an additional insurance against a butyric-type fermentation. Inoculation of clover crops, low in WSC, with homofermentative lactobacilli may be beneficial. A rapid fermentation would make the maximum use of the soluble carbohydrates as a potential source of lactic acid, cutting down sugar wastage by other bacteria which may dominate the microflora in crops with low numbers of homofermentative lactobacilli.

Acknowledgments

The authors would like to thank Prof. S. J. Watson for his interest in this work, Dr. H. T. Macpherson for supplying details of the nitrogenous fractions and Mr. W. G. Davie for technical assistance. They also acknowledge the help of the Agricultural Research Council in providing a grant towards the construction of the silo unit used in these studies.

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Received 20 January, 1965; amended manuscript 21 April, 1965

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COLORIMETRIC DETERMINATION OF CHLORATE IN SOIL AND PLANT EXTRACTS

By A. BANDERIS

A colorimetric method, using *o*-tolidine, for the determination of small amounts of chlorate in soil and plant extracts is presented. Substances known to interfere in the colorimetric procedure are unlikely to be present in significant amount in soil and plant extracts, but if present, compensation for their effects can be made. Examination of different parts of the plant showed that conducting tissue was the most suitable for chemical analysis, although the lamina or the whole plant can be used provided the extract is shaken with activated charcoal. Some observations on several plant species grown in pots containing soil contaminated with chlorate are given.

The method is useful for confirming visual diagnosis of chlorate toxicity in plants and for measuring the residual concentration in the soil.

Introduction

The accidental contamination of arable and horticultural soils or equipment where sodium chlorate has been used as a weed killer results in crop and plant failures. Very often, the cause of the condition may not be associated directly with chlorate because of distance from the source or because its use was not known or overlooked.

In the diagnosis of mineral toxicities or deficiencies in plants it is frequently necessary to analyse both the soil and the plant. In the case of chlorate toxicity it has been customary in this laboratory to assess the amount of chlorate in the soil and to note the effect on the plant by visual observation. In the past few years many plants and soils have been analysed and the presence of chlorate established. Unfortunately, most of the current methods are qualitative or only semi-quantitative. So far as the author can ascertain no really quantitative method for determination of chlorate as applied to soils and plants, has been published. As sodium chlorate is still employed as a weedicide, it is desirable that a method capable of measuring microgram quantities of chlorate in soil and plant extracts should be developed.

Methods which could probably be applied to extracts of soils and plants include the polarographic method of Meites & Hofsass,[1] the iodide-thiosulphate method[2] or the *o*-tolidine method of Urone & Bonde.[3] It has been stated that the polarographic method, even when modified,[3] is rather time-consuming and requires a high degree of skill, and the iodide–thiosulphate procedure is not sufficiently sensitive. The method of Urone & Bonde,[3] for the determination of chlorate in well water, is based on the well known reaction of chlorate ion with chloride ion in highly acidic solution to yield chlorine:

$$NaClO_3 + 6HCl = NaCl + 3Cl_2 + 3H_2O$$

The chlorine released forms a yellow colour with *o*-tolidine, with a maximum absorption at 448 mμ. This method appeared the most promising and its application to soil and plant extracts was investigated.

Ferric iron and nitrite form yellow colours with *o*-tolidine. Strong oxidising agents which release chlorine from chloride will also react with the reagent.[2,3] Of these, only small amounts of iron and manganese are likely to be present in soil extracts, the amount varying with the nature of the extractant, being greater with acid than with water extracts and with the acidity of the soil. Nitrites will very rarely be present but could be under certain soil conditions, and therefore consideration must be given to this ion. As far as dilute acid extracts of plants are concerned none of the above substances are likely to be present in sufficient amounts to interfere.

In acid concentrations below 2M-hydrochloric acid and temperatures between 17 and 22°, chlorate in the range 0–5 μg. per ml. produces no colour with *o*-tolidine within 30 min. but with acidities between 5M and 6M full colour development occurs inside 10 min. and remains stable for 30 min. On the other hand the interfering substances mentioned previously form colours with the reagent in 0·5–6M-acid practically instantaneously. In strong hydrochloric acid ferric iron

will also form a yellow colour. Further the soil and plant extracts may be coloured. However, these interfering substances can be compensated for in the colorimetric procedure.

Method

Reagents

Use deionised water throughout.

(1) *o-Tolidine reagent* (0·05% in approximately 3M-hydrochloric acid)

Dissolve completely 0·25 g. of *o*-tolidine hydrochloride in 350 ml. of water, slowly, and with constant stirring, add 125 ml. of conc. hydrochloric acid. Cover the clear, warm and yellow coloured solution with a watch glass and set it aside at room temperature until the solution becomes colourless. To the colourless solution add, with stirring, 1 drop of dilute standard chlorate solution at 1-min. intervals until the solution just develops a yellow tinge. Dilute to 500 ml. with water, transfer to a dark coloured bottle and leave for 24 h. After this time the reagent should be colourless and ready for use. Protected from direct sunlight and stored at room temperature it should keep indefinitely.

(2) *Concentrated hydrochloric acid* (zero chlorate demand and adjusted to 10·5M).

Add 2 ml. of conc. hydrochloric acid to 2 ml. of dilute chlorate standard solution (4B, below), mix and after 10 min. add the mixture to 2·5 litres of concentrated hydrochloric acid in a clear glass bottle. Mix thoroughly. Adjust the normality of the acid to $10{\cdot}5 \pm 0{\cdot}1$M and keep the treated acid in full daylight for 2 days. Check for free chlorine by adding 5·0 ml. of this acid to a mixture of 4·0 ml. of water and 1·0 ml. of *o*-tolidine reagent. If no yellow colour develops in 10 min., the acid is ready for use; if colour develops leave a day longer and recheck.

(3) *Dilute hydrochloric acid* (compensating solution)

Dilute 125 ml. of reagent (2) to 500 ml. with water.

(4) *Chlorate standards*

(*A*) *Stock solution.*—Dissolve 1·276 g. of reagent-grade sodium chlorate in water and dilute to 1 litre. (1 ml. of this solution contains 1·0 mg. of chlorate)

(*B*) *Dilute solution.*—Immediately before use, dilute 10·0 ml. of stock solution to 200 ml. with water. (1 ml. of this solution contains 50 μg. of chlorate)

(5) *Nitric acid* (approx. 0·02M)

Add 100 ml. of 70% w/w nitric acid to 200 ml. of water. Boil until the volume is reduced to about 100 ml. Cool and make up to 1 litre with water. Dilute 20 ml. of this acid to 1 litre with water. The resulting acid is approximately 0·02M.

Preparation of standard curve

From the standard chlorate solution 4B prepare solutions containing from 0 to 5 μg. of chlorate per ml. Transfer 4·0 ml. of each standard to 6 in. × 1 in. test tubes previously cleaned with hydrochloric acid and rinsed with water. Add 1·0 ml. of *o*-tolidine reagent and mix. Rapidly, and with shaking, add 5·0 ml. of 10·5M-hydrochloric acid. Protect the tubes from direct sunlight. Measure the optical density of the colours produced at 448 mμ in 1·0 cm. cells 10–20 min. after the addition of the acid, using water as the reference solution. Prepare a graph of optical density against chlorate concentration.

Sample preparation and chlorate extraction

(a) *Soils*

Sieve the fresh soil through a 3 mm. sieve as soon as possible after receipt. Mix thoroughly and immediately weigh 50 g. into a clean 4-oz. shaking bottle. Add 50 ml. of water and close with a polythene stopper. Shake on an end-over-end shaker for 30 min. Decant the soil–water

suspension into a centrifuge tube and centrifuge until clear and filter to free the extract from floating organic matter. If dry soil is used increase the extraction time to 1½–2 h.

Determine the moisture content on a separate portion of the soil if results expressed on a dry-matter basis are required.

Plants

Free the plant sample from any adhering soil particles. Cut into small pieces and dry at 105° overnight. Extract 0·1–0·2 g. samples of the dry tissue with 20 ml. of 0·02M-nitric acid as for soils. Add 0·25 g. of activated charcoal and shake for 10 sec. and filter. If the plant sample is extracted in the fresh state increase the extracting time to 1 h.

Colour development

Transfer 4·0-ml. aliquots of clear soil or plant extracts containing less than 20 μg. of chlorate to each of two tubes, A and B. To tube A add 1·0 ml. of reagent (1) and 5·0 ml. of reagent (2), mixing well after each addition. (This gives the colour due to chlorate and any interfering substances that may be present.) To tube B add 1·0 ml. of reagent (1) and 5 ml. of reagent (3). (This gives the colour due to interfering substances only.) After 10–20 min. measure the optical densities of the solutions, the difference being equivalent to the chlorate concentration of the extracts. If the concentration exceeds 20 μg. of chlorate in 4·0 ml., use smaller volumes of extract and dilute to 4·0 ml. with water before adding any reagent.

If the soil extract is highly coloured (which will most likely be the case with peaty or highly organic soils), it will be necessary to allow for the difference between the colours of the extract in 5·5M and 1·5M acid. This can be done by adding 6·0 ml. of reagent (2) and 6·0 ml. of reagent (3) to 4·0-ml. aliquots of the extract and adding the difference in optical density to that already obtained.

Experimental and results

(1) *Soils*

Type of extractant.—Weighed amounts of a sandy loam and a clay loam which had been dried and sieved, and a compost, all of which were known to contain chlorate were shaken with an equal weight of extractant on an end-over-end shaker for 2 h. The extracts were centrifuged until clear and any fragments of organic matter filtered off. Aliquots of 4·0 ml. were taken for determination of chlorate. The results in Table I show that water and dilute acids extracted virtually the same amount of chlorate. The solutions containing sodium phosphate generally extracted too much colour for the determination to be made directly. The sandy loam was an exception but, even in this case, and in those of the dilute phosphoric acid, the colours were intense. The use of dilute neutral sodium and calcium chloride solutions on the sandy loam gave results (not shown) identical with those obtained on the water extracts. Therefore, while any of the solutions, except those containing phosphate, could be used as an extractant, water was selected because of its analytical convenience.

Table I

Chlorate extracted by various reagent solutions

Extractant	Chlorate, p.p.m.		
	Sandy loam	Potting compost	Clay loam
Water	2·3	5·5	228
0·02M-HCl	2·3	5·4	232
0·10M-HCl	2·3	5·5	218
0·01M-HNO_3	2·3	5·5	230
0·02M-HNO_3	2·3	5·6	224
0·3M-H_3PO_4	2·2	5·5	212
0·3M-NaH_2PO_4	2·1	C	C
0·3M-Na_3PO_4 (pH 7·0)	2·1	C	C

C = solution coloured.

Soil to water ratio.—A high soil to water ratio is desirable so as to give as high a concentration of chlorate in the extract as possible. It was found that with heavy or peaty soils, at a 2:1 ratio, the soil often absorbed all the water. With 1:1 and lower ratios a clear extract was always obtained in sufficient quantity and gave consistent results. The maximum volume of extract permitted by the colorimetric procedure is 4·0 ml., so a 1:1 ratio was preferred which allowed lower concentrations of chlorate to be determined in the soil.

Time of extraction.—Table II gives results of increase in time of shaking on the extraction of chlorate from soils of different texture and of composts. These results show that in the light textured soils all the extractable chlorate has been brought into solution in half an hour, while on heavy soils and composts some increase takes place with time. In all cases extraction could be considered complete in 2 h.

Interfering substances.—Chlorides, nitrates and phosphates do not interfere. Ferric ion gives a weak colour with *o*-tolidine which at 448 mμ has an absorption approximately one hundredth that of the same concentration of chlorate.[3] Iron is present in all soils in a variety of forms and compounds. Although the effect of this element could probably be ignored, its presence in water extracts is compensated for in the proposed method.

Nitrite also produces a colour with *o*-tolidine. However, as nitrite and chlorate react they cannot both be present in the same extract. Although nitrite is not normally present in soils, its presence cannot be ignored, for tests on soils containing nitrite in the absence of chlorate would give rise to a wrong diagnosis. The colour development at the two acidities eliminates this possibility.

Free chlorine and strong oxidising substances, which release chlorine from chloride, will react with *o*-tolidine and of these, manganese is the most likely to be present in soils. It is most improbable that manganese would be present in water extract of soils, but, again, colour development at the two acidities will compensate for the effect of any that may be present.

Water extracts of soils are nearly always coloured to a greater or less extent and the colour of any single extract becomes less intense in strong than in dilute acid. Interference from coloured extracts is also allowed for in the method.

It was observed that, when fresh contaminated soil was extracted and analysed repeatedly, a decrease in chlorate concentration occurred. To obtain more information on this aspect, fresh and dried soil extracts, filtered and unfiltered, were analysed for chlorate on different days. The results are shown in Table III. It is apparent from these results that, as long as moist soil or an unfiltered extract contains chlorate, the concentration of the latter decreases with time, whereas any chlorate present in the dried soil or filtered extract remains constant up to at least 14 days. Since there is also a loss of chlorate on drying it is advisable that the soil be extracted and filtered immediately on arrival in the laboratory.

Recovery of chlorate added to filtered extracts.—When chlorate is added to filtered extracts of contaminated and uncontaminated soils interesting results were obtained. It is seen from Table IV that, when the initial concentration of chlorate in the extract is high, virtually complete recovery is obtained throughout the range of chlorate addition. When the concentration in the extract is 1 p.p.m. or less, recovery is not complete. It is noticeable, however, that the loss of added chlorate is nearly the same throughout the series and that after the first addition of chlorate the difference between successive values is, within experimental error, equal to the incremental addition. This is also shown by the results on adding chlorate to the uncontaminated soil

Table II

Chlorate extracted (p.p.m.) at different extracting times

Soil	Time of shaking, h.					
	½	1	1½	2	3	24
Sandy loam	2·3	2·3	2·3	2·3	—	2·1
Sandy clay loam	0·5	0·5	0·5	0·5	0·5	0·4
Clay loam A	0·1	0·2	—	0·2	0·2	0·1
Clay loam B	182	196	220	230	229	224
Potting compost	5·2	5·4	5·5	5·5	—	5·1
Seed compost	6·8	7·2	7·2	7·2	7·2	7·0

Table III

Change in chlorate concentration (p.p.m.) of fresh and dry soil, unfiltered and filtered extracts with time

Time in Days	Soil[a]		Unfiltered soil[b] extract		Filtered soil[c] extract	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
1	12·7	11·4	12·7	11·4	12·7	11·4
2	12·4	11·3	11·1	9·8	12·8	11·3
3	12·2	11·3	10·6	9·2	12·7	11·4
7	11·8	11·4	9·9	8·7	12·7	11·4
14	11·5	11·4	8·8	7·7	12·6	11·3

[a] Soil extracted and filtered each day
[b] Aliquot of the suspension filtered each day
[c] Fresh volume of the extract taken each day

Table IV

Recovery of chlorate added to 2·0-ml. aliquots of extracts of contaminated and uncontaminated soils

Chlorate (μg.) added to 2 ml. of extract	Soil Extract No.				
	1	2	3	4	5*
	Chlorate found, μg.				
0	9·0	5·6	0·5	1·1	0
1	10·1	6·7	—	—	—
2	11·0	7·8	2·2	—	—
4	13·1	9·7	4·2	4·8	1·1
6	—	11·7	6·2	—	—
8	—	13·5	8·3	8·6	4·8
12	—	—	—	12·6	8·7
16	—	—	—	—	12·6

* Extract of uncontaminated soil

extract. It may be that, in extract containing small amounts of chlorate, some reducible substances are still present in some form of equilibrium with the residual chlorate.

No attempt was made to investigate the mechanism of the reactions involved as, from an analytical viewpoint, only the amount of chlorate in the soil and plant is required. However, a measure of the precision of the method can be obtained on soil extracts of low chlorate concentration by adding a known amount of chlorate to one aliquot of the extract and double the amount to another. The difference between the two readings obtained should give a recovery of 95–105% of the extra chlorate added.

Pot experiments were carried out with seeds and seedlings of tomato, lettuce and chrysanthemum plants in compost containing varying levels of chlorate. The results of the experiment showed that:

(*a*) In all cases where the plant showed visual symptoms of toxicity, determinable amounts of chlorate were extracted from the soil.
(*b*) In some cases, e.g. tomato in the dicotyledon stage and lettuce, determinable amounts of chlorate were extracted from the soil, although the plants showed no visible symptoms.
(*c*) Soils in which no chlorate could be found in the extract had no adverse effect on seed germination nor on young tomato plants, the most sensitive of the plants tested.

(2) *Plants*

Since the leaves are the first part of any plant to show visual symptoms of chlorate toxicity, these were selected as being most suitable for chemical investigation. For this purpose young tomato plants were transplanted into pots of chlorate-contaminated soil. In 2–3 days definite toxicity symptoms developed on the leaves. When the fresh leaves were extracted with water and with 0·02M-nitric acid, no chlorate was found in the extracts. A similar result was obtained when the leaves were oven-dried and ground before extraction. However when water and acid

extracts of fresh tomato stem were analysed, chlorate was found in the acid extract but not in the water extract. These observations suggested that a more detailed examination of various parts of the plant was necessary.

Tomato plants were grown normally to a five permanent leaf stage in four pots containing J.I.P. compost. At this stage each pot was watered with a solution of sodium chlorate to give final concentration of 10, 20, 30 and 60 p.p.m. of chlorate in the compost. Care was taken to avoid losses through drainage. After 6 days the plants were removed from the pots, freed from soil by rinsing in water and separated into the following parts; roots, stem, petiole and lamina. Approximately one half of each of the parts was dried at 105° and ground, and the remainder was cut into 1–2 mm. pieces. Weighed amounts of the fresh and dried tissue were shaken with water and 0·02M-nitric acid, in the ratio of 1:10 for fresh and 1:100 for dried tissue, for 1 h. in an end-over-end shaker. The extracts were filtered and chlorate determined on the clear filtrate by the proposed method. Results are shown in Table V.

Table V

Chlorate extracted from fresh and dried plant tissue

(p.p.m. ClO_3 on dry matter)

Chlorate concn. in pots, p.p.m.		Root		Stem		Petiole		Lamina	
		Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
10	Water	0	17	0	28	0	21	0	0
	HNO_3	14	31	18	44	16	36	0	0
20	Water	0	129	0	153	14	144	0	0
	HNO_3	118	187	169	200	154	193	0	0
30	Water	10	990	25	1320	ND	1210	0	0
	HNO_3	630	1250	1060	1670	930	1560	0	0
60	Water	21	2380	35	3150	180	2200	0	0
	HNO_3	1450	2950	3050	3900	2890	3560	0	0

The results confirm earlier tests that water extracts measurable amounts of chlorate only from highly contaminated samples of fresh plant tissue. Nitric acid, however, extracts from fresh tissue (apart from lamina) between 50 and 80% of that extracted from the dry tissue. On the dry tissue both extractants extract appreciable amounts of chlorate from root, stem and petiole, nitric acid being the more effective. That no chlorate was detected in lamina extracts suggests that either the chlorate is reduced completely on entering the lamina or that on colour development, the liberated chlorine reacts more rapidly with the extracted plant constituents than with *o*-tolidine. Either of these possibilities would account for earlier tests giving no reaction for chlorate despite the obvious symptoms of toxicity.

To obtain some information on which of the above possible reactions is more likely, two samples each of petiole and lamina from a healthy young tomato plant were extracted with dilute nitric acid. One extract of petiole and of lamina were shaken for about 10 sec. with 0.25 g. of activated charcoal and all extracts were filtered. Aliquots of the extracts equivalent to 2 mg. of dry tissue were added to standard solution of chlorate, the volume adjusted to 4 ml. and the chlorate content was determined after 1 h.

The results shown in Table VI indicate that the chlorate is not reduced on entering the lamina since the recoveries from carbon-treated extracts are almost complete. It would appear, therefore, that the second possibility is more likely, namely, the reaction of chlorine with extracted plant substances. Whatever reaction does take place, shaking with carbon allows the liberated chlorine to react only with the *o*-tolidine. The recoveries from the untreated extracts show that more of the active plant substances are removed from the lamina, a not unexpected result since photosynthesis occurs in the leaf, producing compounds which may react readily with liberated chlorine.

The amount of activated charcoal used should be kept within 0·2 and 0·25 g. since larger amounts decrease the amount of chlorate determined.

It should be pointed out that plants watered with chlorinated water will produce the same symptoms in the plant as chlorate, but because of the highly active nature of free chlorine, none

Table VI

Chlorate recovered (μg.) after reaction with tissue extracts for 1 h.

ClO_3 in solution, μg	Lamina extract		Petiole extract	
	No carbon	With carbon	No carbon	With carbon
2	none	1·8	none	1·9
4	none	3·8	none	3·9
8	none	7·9	0·7	7·8
12	1·7	12·0	2·4	11·9
20	5·2	20·0	10·5	19·5
24	8·3	23·9	14·4	23·8
28	12·3	27·8	18·3	27·7

would be detectable in the plant or soil, particularly since the symptoms appear some time after the application of the water. It could arise, therefore, that visual diagnosis of chlorate toxicity would not be proved by chemical tests and information on previous plant treatment would be necessary.

Discussion

The method presented has been developed for two main purposes. First, to have an easily applied method of estimating residual chlorate in soils, and second, as a rapid diagnostic test for chlorate toxicity in plants.

The use of water for extracting chlorate from soils, apart from its convenience, would doubtless remove less interfering substances than would acid extractants. However compensation for any interfering substances that may be present can be satisfactorily made. As the chlorate concentration of fresh moist soil decreases somewhat with time and that of soil suspensions to a greater extent as shown in Table III, it is desirable to extract and filter the soil as soon as possible after receipt of the sample. The method is capable of determining as little as 0·2 p.p.m. of chlorate or less in soil, which, when combined with the observations made from the limited pot experiments, may assist in indicating when an affected area could be brought into use.

Examination of plant specimens showed that the method is suitable for the analysis of the whole plant or leaf extract. In plants, the establishment of chlorate toxicity is more important than its quantitative estimation. The test is very rapid and extremely useful for corroborating visual diagnosis.

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Received 9 March, 1965

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Added in proof: Since the preparation of this paper, the author's attention has been drawn to work by Rosenfels (*J. Ass. off. agric. Chem., Wash.*, 1938, **21**, 665) concerning the use of sulphurous acid reduction in the analysis of soil extracts. While the method has much merit, it is not as sensitive, rapid and simple in operation as the *o*-tolidine procedure outlined above.

SULPHATE LEVELS IN SOIL OF VARYING pH DURING INCUBATION WITH ORGANIC MATERIALS

By A. MASSOUMI and A. H. CORNFIELD

The water-soluble sulphate levels were followed during incubation (28° for 132 days) of soil (pH 4·7, 6·1 and 7·4) treated with 2% by weight of six different bulky organic materials.

Materials with organic carbon/total sulphur (C/S) ratio of 112 or less (farmyard manure, grass, and compost 2) usually caused either very little or only slight net immobilisation of sulphate. Materials with C/S ratio greater than 112 (compost 1, straw, and cellulose) caused net immobilisation of sulphate during the earlier part of incubation, followed by re-mobilisation of part or all of the sulphur by the end of incubation. Straw was the only material which caused net immobilisation of sulphate at all pH levels by the end of incubation. Differences in the extent of mobilisation or immobilisation due to pH were small or inconsistent except for straw, when maximum immobilisation increased with pH.

Introduction

It has been shown[1,2] that sulphate in soil is immobilised when carbonaceous material is added and allowed to decompose. Barrow[3] found that the rate of mineralisation of sulphur from fresh organic materials depended on their carbon/sulphur ratio and for mineralisation to occur within 12 weeks it was necessary that this ratio was 250 or less.

Some or all of the mineral nitrogen immobilised at the start of incubation with added material of high carbon/nitrogen ratio may be mobilised again later depending on the nature of the added material.[4]

The main purpose of the study reported here was to see whether similar effects occurred with respect to sulphur in soil.

Experimental

Samples of a cultivated alluvial sandy loam (2 mm. sieved and containing 0·14% of total nitrogen and 1·31% of organic carbon), original pH 6·7, which had been kept fallow and free of weeds for 18 months (to ensure absence of easily decomposable organic material) were adjusted to a number of different pH levels by addition of calcium carbonate or aluminium chloride at different rates. The treated samples were kept moist and subjected to intermittent leaching for 3 months, to remove soluble salts, and were then air-dried. Three samples, with pH values of 4·7, 6·1, and 7·4 were selected for study.

The organic materials used were (1) B.D.H. cellulose powder as used for column chromatography; (2) wheat straw; (3) wheat straw which had been composted for 6 months after addition of sufficient ' Nitro-chalk ' to give an initial carbon/nitrogen ratio of 40 (Compost 1); (4) wheat straw which had been composted after being mixed with sufficient young rye-grass to give an initial carbon/nitrogen ratio of 40 (Compost 2); (5) young ryegrass; and (6) farmyard manure obtained from a cattle shed and which had been allowed to rot for 9 months. All these materials were dried (at 55°) and ground, where necessary, to a fine powder in a mill. Some characteristics of the materials are shown in Table I below.

Table I

Characteristics of organic additives for soils

	Total S	Sulphate S	Organic carbon	Ratio organic C/ total S
	%	%	%	
Cellulose	0	0	43·1	—
Straw	0·155	0·098	40·2	259
Compost 1	0·300	0·066	39·7	132
Compost 2	0·345	0·069	38·6	112
Grass	0·450	0·120	39·1	87
Farmyard manure	0·525	0·068	37·2	71

The organic material (2% by weight) was mixed with 10 g. of soil and the mixture placed in a test-tube (6 in. × ¾ in.). Water containing 2 mg. of potassium nitrate was added to bring the moisture level to 50% of the maximum water-holding capacity. Preliminary tests had shown that the amount of nitrogen added was sufficient to prevent nitrogen becoming limiting to the activity of the micro-organisms decomposing the organic materials. Other major and trace elements did not limit decomposition. Control soils at each pH level did not receive any organic material. The tubes were plugged with cotton wool, weighed and incubated at 28°, moisture losses due to evaporation being made up when necessary. Sufficient tubes were prepared for each treatment to allow for duplicate analyses of water-soluble sulphate, initially and after 33, 70, 100 and 132 days of incubation.

Sulphate was determined by extraction with water as described by Massoumi & Cornfield.[5] Total sulphur in the organic materials was determined by the method of Chenery & Butters[6] and organic carbon by the method of Cornfield.[7]

Results

Results are shown graphically in Figs. 1a–c, where water-soluble sulphate-sulphur levels (p.p.m. dry soil basis) after varying incubation periods are shown for the soils examined. The values shown are for net sulphate-sulphur, due allowance being made for the original sulphate-sulphur contents of the organic materials. For each pH, the sulphate-sulphur level for the control soil is taken as zero, so that positive values represent net mobilisation of sulphur and negative values net immobilisation of sulphate-sulphur at any particular sampling date.

The differences required for significance at $P \leqslant 0.05$ were 5·1, 4·8, and 5·2 p.p.m. of sulphate-sulphur for soils of pH 4·7, 6·1 and 7·4 respectively.

Farmyard manure (FYM) was the only material which caused net mobilisation of sulphate at all stages of incubation and at all pH levels, but gains were significant only in the later stages. There were no consistent differences due to pH except that, at the highest pH, sulphur mobilised initially became immobilised by the end of incubation.

The two composts behaved similarly at pH 4·7, causing a significant gain in sulphate only after 70–100 days' incubation, with a slight decrease at the end. At pH 6·1 and 7·4, compost 1 caused significant net immobilisation of sulphate after 33–70 days' incubation, followed by re-mobilisation of this sulphur after 100–132 days. Compost 2 caused small alternate gains and losses, sometimes significant, with time. Grass also caused small alternate gains and losses of sulphate with time; some of these were significant and there were no consistent differences due to pH.

Straw and cellulose caused the greatest net immobilisation of sulphate, cellulose being the most effective in this respect at the lowest and straw at the highest pH. The extent of maximum immobilisation due to cellulose was not related to pH, but that due to straw increased significantly with pH. By the end of incubation all the sulphate immobilised initially by cellulose was again mobilised at pH 4·7 and 6·1, but only about 60% at pH 7·4. By the end of incubation, 50–70% of the sulphate immobilised initially by straw was mobilised.

Discussion

The only materials causing appreciable immobilisation of sulphate were cellulose, straw and compost 1, which had total sulphur contents of 0·3% or less and C/S ratios of 132 or more. Thus it would appear that total sulphur content in organic materials in the region of 0·3% sulphur is critical for sulphate immobilisation. This immobilisation is, however, only temporary and in time some or all of the sulphate immobilised will again be mobilised. The incomplete re-mobilisation of immobilised sulphate having the straw treatment as compared that having the cellulose treatment may be due to the presence of lignin in the straw; some of the sulphate immobilised would probably be bound as modified lignin-microbial tissue complexes of low susceptibility to attack by micro-organisms and this could account for the incomplete re-mobilisation of sulphur.

The extent to which immobilisation of soil sulphate by decomposing organic materials may reduce soil sulphate to such low levels that plants may suffer, will depend on the initial sulphate

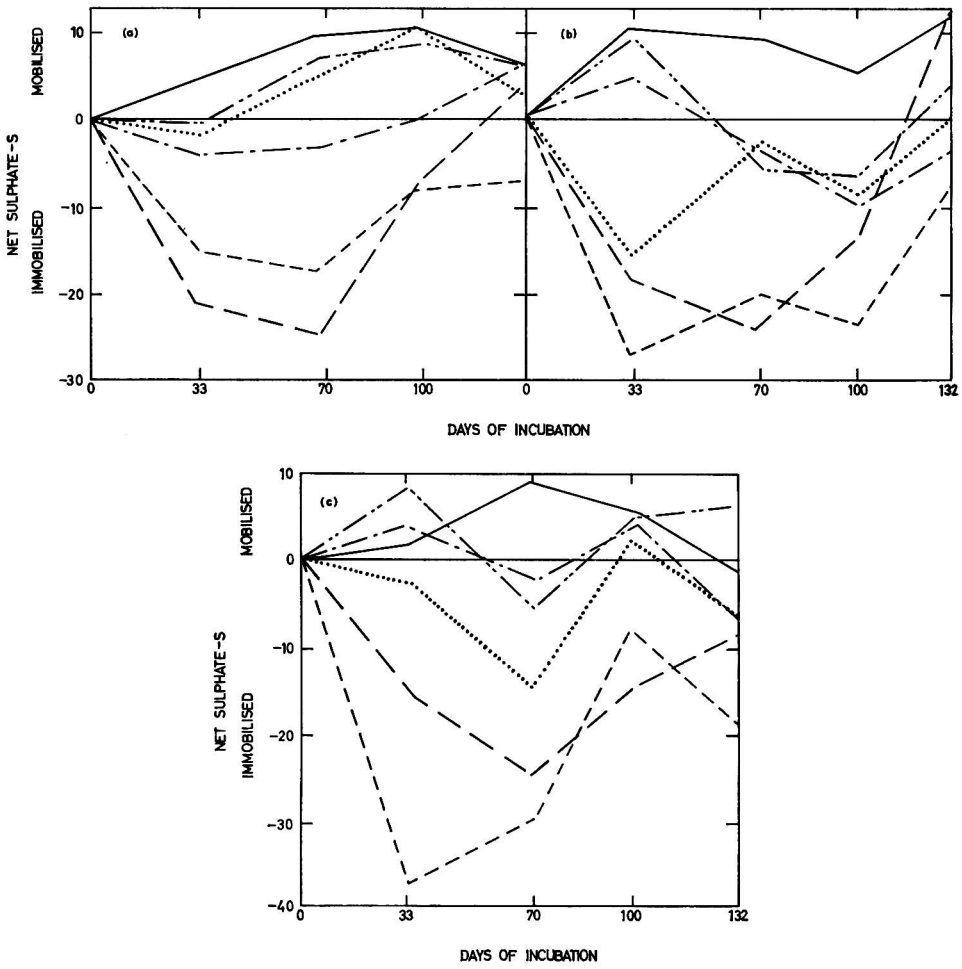

FIG. 1.—*Sulphate-sulphur levels in soils* (a) *pH* 4·7 (b) *pH* 6·1 *and* (c) *pH* 7·4, *incubated with various organic materials* (*results as p.p.m., dry basis*)

– – – cellulose · · · straw — · — grass · · · · · compost 1 — · · — compost 2 ———— farmyard manure
control: horizontal line through zero point on ordinate

content of the soil and also on its sulphur-mineralising capacity. This study has shown that even a very heavy dressing of straw (2% by weight is approximately equivalent to 20 tons per acre) in finely-ground condition, incorporated intimately with the soil and given optimum conditions of moisture and temperature for decomposition, resulted in a maximum immobilisation equivalent to about 76 lb. of sulphate-sulphur per acre, and much of this was in any case eventually re-mobilised. Under field conditions where the rate of application of straw would be lower and the extent of incorporation with soil and decomposition conditions would be much poorer, the amount of soil sulphate immobilised, even temporarily, would be very much less and probably not of significance as far as the plant is concerned, except in soils of very low initial sulphur status.

With regard to the organic materials of high total S content (compost 2, grass and farmyard manure) which caused net mobilisation of sulphur at some or all periods of sampling, at no time was this more than 9% of the total organic sulphur added, and the amounts mobilised were not

related to the organic sulphur contents of the materials. It appears that the incubation test, at least as used here, is of little value in indicating the extent of mineralisation of sulphur from organic sulphur sources within the range of sulphur contents used in this study. The lack of any consistent effect of soil pH on mobilisation of sulphur from the high-sulphur materials is rather surprising in view of reports by other workers[8,9] that increasing soil pH resulted in increasing production of sulphate in soils. However, these authors were concerned with mineralisation of native organic sulphur and the effect of pH on this would appear to be different from that on added organic sulphur, such as was used in this study.

The results obtained here for sulphate levels may be compared with those obtained in a previous study[4] for mineral nitrogen levels. The trend for both sulphate and mineral nitrogen immobilised initially to re-mobilise with further incubation was similar in both studies. The main points of difference were that grass temporarily immobilised much mineral nitrogen but not sulphate, while less nitrogen than sulphur was re-mobilised when straw was added. In addition the extent of immobilisation of nitrogen, where it occurred, was about four times greater than that of sulphate sulphur at comparable levels of the same organic materials.

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Received 9 March, 1965; amended manuscript 14 June, 1965

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EFFECT OF SOME PLANT GROWTH RETARDANTS ON THE OLEANDER APHID *APHIS NERII* (BOYER)

By A. S. TAHORI*, A. H. HALEVY and G. ZEIDLER

Oleander leaves, the stem base of which were held in solutions of Phosfon (2,4-dichlorobenzyltributyl phosphonium chloride) and other plant-growth-retarding compounds were unable to support populations of the oleander aphid. A Phosfon concentration of 10^{-3}M eliminated the aphid population.

Introduction

The dynamics of aphid populations depend among other factors on the condition of the host plant.[1] The effect of a plant-growth retarding compound, Cycocel (2-chloroethyl)trimethylammonium chloride, on the rate of increase of the cabbage aphid *Brevicoryne brassicae* (L.) has been described recently.[2] It is the purpose of this paper to present data on studies on the effect of some plant-growth retardants on an aphid population.

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Materials and methods

The following plant-growth retardants were used:

Phosfon: 2,4-dichlorobenzyltributylphosphonium chloride, used as Phosfon D, a dust containing 10% of active ingredient (obtained from Virginia-Carolina Chemical Corp. Richmond, Va.,).

Cycocel (formerly called CCC): (2-chloroethyl)trimethylammonium chloride (obtained from Cyanamide International Co.) and

B-nine: *N*-dimethylaminosuccinamic acid (obtained from U.S. Rubber Co., Naugatuck, Conn.).

The stem base of oleander (*Nerium oleander*) branches, having two to three young leaves were put into plastic vials containing 100 ml. of the solution to be tested (see Table I). Young aptera of the oleander aphid *Aphis nerii* (Boyer) were put on the leaves by means of a fine camel-hair brush, or branches containing aphids were put on top of the treated leaves for 3 days.

Table I

Effect of various growth retardants on aphid populations on oleander leaves

(Data represent average of 4 experiments)

Treatment	Concn. of solution, M	No. of aphids on leaves after days			
		3	5	7	10
A	Water	170		238	245
	Phosfon 10^{-3}	17		0	0
	Cycocel 8×10^{-3}	37		14	2
	B-nine 3×10^{-3}	107		31	4
B	Water	181	188	205	201
	Phosfon 10^{-6}	91	88	46	29
	Phosfon 10^{-4} for 48 h. then in pure water	58	70	60	48
	Phosfon 10^{-4}	32	38	34	32
C	Water	135	157	154	135
	Phosfon 10^{-6}	77	83	58	63
	Phosfon 10^{-4}	61	66	67	61
	Phosfon 10^{-3}	46	37	30	6
	Phosfon 3×10^{-3}	31	16	8	0
	Phosfon 3×10^{-2}	10	2	0	0

Treatment A: Eight leaves were immersed in pairs in four open vials containing respectively the three test solutions and water. Branches containing 300 aphids were put on top of the leaves for 3 days. The aphids could move freely to the leaves of their choice.

Treatment B: The stem base of 20 leaves were kept in Phosfon solution at the various concentrations. In each treatment 45 aphids were put on the leaves, and could move freely to the leaves of their choice.

Treatment C: The base of the stem on which there were three leaves was kept continuously in 5 Phosfon solutions or water respectively. Fifty young aptera aphids were put on the leaves by means of a camel hair brush.

Results

Results shown in Table I (treatment A) indicates that leaves held in the Phosfon solution were less infested by aphids than the untreated leaves or those held in Cycocel or B-nine solutions. Therefore further work was carried out with this compound only. Leaves, the stem base of which were held in a solution of Phosfon at 10^{-3} M for 10 days, could not support aphid populations (Table I, treatment C).

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Received 10 April, 1965

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EFFECT OF SOME PLANT GROWTH RETARDANTS ON THE FEEDING OF THE COTTON LEAF WORM

By A. S. TAHORI,* G. ZEIDLER and A. H. HALEVY

Phosfon and other plant-growth-retardants show a pronounced antifeeding effect on the cotton leaf worm *Prodenia litura* Fabricius. Foliage application was more effective than soil treatment. When larvae had either the choice between leaves held in various Phosfon solutions, or were put on treated leaves, concentrations of 3×10^{-3} M protected to a large extent leaves from being consumed.

Introduction

Insect antifeeding compounds protect an agricultural crop from insect attacks without directly killing the pest, but the insect, unable to feed, starves and dies. A number of compounds displaying antifeeding qualities have been mentioned in the literature, such as some carbamate derivatives,[1-3] an acetanilide[4] and an organotin compound.[5] It is the purpose of this paper to present data on studies on the antifeeding effects of some plant-growth-retarding compounds.

Materials and methods

In addition to the plant-growth-retardants mentioned in the preceding paper, there was used Carvadan, 3-isopropyl-4-dimethylamino-6-methylphenylpiperidine-1-carboxylate methochloride, provided as pure crystals by Dr. H. M. Cathey, Plant Industry Station, Beltsville, Md., U.S.A.

The insect used was the cotton leaf worm *Prodenia litura* Fabricius. Larvae 6 to 10 days old, all from one egg batch, were put on two cotton leaves. The leaves were either dipped for 3 min. in a solution of the material to be tested, or their petiole bases were immersed in 10 ml. of the solution. The solution was renewed every 7–10 days. All the leaves taken were of uniform size and were replaced every 3 days. In all cases where the larvae damaged the leaf–stem connection, with the leaves consequently drying up, they were replaced earlier. The leaf area consumed by the larvae was measured to the nearest 0·1 cm.^{2} by placing it on square paper and measuring the area remaining. The insects were weighed by means of a torsion balance with 1 mg. accuracy.

Results

Plant-growth-retarding compounds are either applied to the soil, or as a foliage spray (the conventional method of insecticide application.) To compare these two methods, two-week-old bean plants *Phaseolus vulgaris* var. Brittle Wax were either sprayed with 50 ml. of the candidate solution per plot, or 150 ml. of the test solution were applied to 3 kg. of soil, so that a concentration of 1·2 g. of active material per 36 kg. of soil was obtained. This is approximately the strength normally used for soil application. The following day, 25 cotton leaf worm larvae, 6 days old, were put on plants of each plot. Table I shows that Phosfon was the most efficient of the three compounds tested. Foliage application of Phosfon was more effective than soil treatment.

To determine the most effective concentration of Phosfon, the following experiments were carried out: 25 ten-day-old cotton leaf worm larvae were put on cotton leaves. Two alternative treatments were tested; either the petiole bases of the leaves were kept continuously in a solution of Phosfon, or the leaves were dipped for 3 min. in a Phosfon solution. Table II shows that there was a graded response in the amount of leaves consumed, declining with rising concentrations of Phosfon. While 10^{-2} M provided the greatest protection, this concentration sometimes produced phytotoxic symptoms. Since adults emerging from pupae weighing less than 200 mg. do not

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Table I

Effect of various growth retardants on feeding of cotton leaf worm larvae

Host: bean plants, 25 six-day-old larvae put on each plot, duration of experiment 13 days.

Treatment	Compound	No. of plants in plot	% of plants destroyed	Total leaf area, cm^2.	Leaf area consumed cm^2. per larva	% of leaf area consumed
Plants sprayed						
	Phosfon 10^{-3} M	24	12	2960	41	35
	Carvadan $1{\cdot}5 \times 10^{-3}$ M	38	8	4415	86	49
	Cycocel 10^{-3} M	38	13	4559	93	51
	Water	43	72	5048	177	92
Soil treated						
	Phosfon 1·2 g./36 kg.	25	36	3056	61	50
	Water	32	41	3772	104	67

reproduce,[6] a concentration of 10^{-3} M-Phosfon is sufficient to prevent the reproduction of this insect.

In another experiment leaves were bunched together, so that larvae could move freely from leaves held in one solution to those held in another one. Larvae had thus the choice between leaves held in various Phosfon solutions and untreated leaves. Phosfon at 10^{-2} M completely protected the cotton leaves, and at 3×10^{-3} M was still very effective (Table II).

Table II

Effect of Phosfon on weight, pupation and feeding of cotton leaf worm

Host plant: cotton leaves. 25 larvae, 10 days old, were used for each treatment.

Treatment	Phosfon concn., M	Average weight of larvae, mg., after days			No. of larvae pupating	Average weight of pupae, mg.	Average cumulative leaf area consumed (cm^2.) after days					% of leaf area consumed
		0	7	12			0	7	13	16	21	
Leaves dipped for 3 min. in solution	Water	18	173	547	21	277	0	45	133	176	202	55·8
	10^{-4}	28	269	547	18	251	0	46	126	157	172	47·5
	10^{-3}	53	252	545	10	160	0	33	95	130	133	36·7
	3×10^{-3}	47	176	337	3	182	0	21	72	90	90	24·8
	7×10^{-3}	51	218	345	3	152	0	20	65	77	77	21·2
	10^{-2}	46	104	104	0		0	11	16	21	22	6·0
Petiole bases kept continuously in solution	10^{-4}	26	235	479	16	252	0	41	119	158	173	47·8
	10^{-3}	47	187	365	8	176	0	22	75	113	132	36·5
	3×10^{-3}	25	115	254	6	187	0	15	60	83	106	29·3
	7×10^{-3}	52	122	276	2	124	0	12	44	69	84	23·2
	10^{-2}	53	124	251	4	125	0	12	44	68	70	19·3
Larvae had choice of 5 leaves, base of each petiole kept in solution	Water						0	67	130	173	197	67·6
	10^{-4}						0	52	109	142	161	51·1
	10^{-3}						0	22	56	73	75	23·8
	3×10^{-3}						0	1	11	26	26	8·2
	10^{-2}						0	1	5	6	6	1·9

Ten 10-day-old cotton leaf worm larvae were offered a choice—for 5 days—of chrysanthemum flowers the stems of which were held in solutions of Phosfon at various concentrations. Fig. 1 shows that Phosfon at concentrations of 5×10^{-4} M and 10^{-3} M protected the flowers to a large extent, while the untreated control and the flowers treated with a concentration of 10^{-6} M were destroyed.

In preliminary field trials, groundnut plants sprayed with Phosfon at concentrations of 10^{-3} M or 10^{-2} M had lower populations of cotton leaf worm larvae than unsprayed control plots. While Phosfon at concentrations of 10^{-3} M may begin to show phytotoxic effects on certain potted

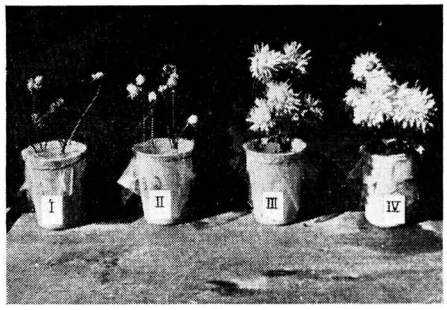

FIG. 1.—*Effect of Phosfon as antifeeding agent*

Insect: 10-day-old cotton leaf worm larvae.
Host: chrysanthemum flowers, stems of which were held in solutions of Phosfon at various concentrations.
I. water. Phosfon: II 10^{-6} M; III 5×10^{-4} M; IV 10^{-3} M

plants, no ill effects were shown by the groundnut plant. The same was true of young pepper plants, which, after being sprayed with Phosfon, were artificially infected with cotton leaf worm larvae. After 8 days the plants treated with Phosfon at a concentration of 3×10^{-2} M showed very little phytotoxic effect, while feeding damage caused by the cotton leaf worm was reduced.

Since Phosfon is highly soluble in water and possesses systemic properties, it is not necessary that the application of this compound to agricultural crops be very thorough or at frequent intervals. This quality considerably enhances its practical value by simplifying the technical problems connected with pesticide application.

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Received 10 April, 1965

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

SEPTEMBER, 1965

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Water table changes and soil moisture loss under frozen conditions. W. O. Willis, H. L. Parkinson, C. W. Carlson and H. J. Haas (*Soil Sci.*, 1964, **98**, 244—248).—The relationship between frost formation, soil moisture conditions and water table depth from Nov. to May has been studied in two soils. It was found that there was a direct relation between frost depth and depth of water table. During the winter the water table dropped as depth of freezing increased and at the same time the soil moisture in the frost zone increased.
T. G. MORRIS.

Assessing the soil factor in agricultural production. B. E. Butler (*J. Aust. Inst. agric. Sci.*, 1964, **30**, 232—240).—A technique for identifying and evaluating the soil factor for a particular locality and crop, is described. Soil factors correlate better with production than does the soil type principle. (45 references.)
E. G. BRICKELL.

Irrigation as a factor in boosting food and fibre production. H. Olivier (*Proc. Nutr. Soc.*, 1965, **24**, 8—21).—A brief, general survey which includes the Far East, Africa and South America. (15 references.)
C. V.

Modified classification procedure for rating irrigation waters. B. K. Handa (*Soil Sci.*, 1964, **98**, 264—269).—Drawbacks of the U.S. Salinity Laboratory classification system are discussed. A modification of the system is proposed based upon the suggestion that irrigation waters could be classified according to their total dissolved salts content and [Na^+] which, it is claimed, would eliminate some of the drawbacks. It is also claimed that the new classification could give an idea of the gypsum requirements of an irrigation water which the U.S. system fails to do adequately.
T. G. MORRIS.

Soil moisture content under bands of petroleum and polyethylene mulches. L. F. Lippert, F. H. Takatori and F. L. Whiting (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 541—546).—The extent of reduction of soil moisture increased with the width of band (6—24 in.) of petroleum mulch (water emulsion of petroleum resins sprayed on the soil surface) or polyethylene film. A 3-in. wide band of mulching material was ineffective.
A. H. CORNFIELD.

Moisture and strength relationships of soils as affected by 4-t-butylpyrocatechol. J. B. Hemwall and K. B. Bozer (*Soil Sci.*, 1964, **98**, 235—243).—Three soils of differing mechanical analysis were used. After drying and sieving the soils were mixed with either water alone, or water containing enough 4-t-butylpyrocatechol (TBC) to give 0·1% TBC in the soil on a dry wt. basis. After mixing, 80 g. of soil were compressed in a hydraulic apparatus to give test specimens 3 cm. dia. by 6 cm. long. The samples were then subjected to various sequential conditions of humidity or water immersion. After treatment the unconfined compressive strength of the samples was determined. After equilibrium with various R.H. the vapour absorbed by the TBC-treated soils was only slightly less than that in untreated samples, i.e. the TBC does not reduce the space available for vapour sorption. Strengths of treated samples were less than untreated by small amounts. The treated soils exhibited hysteresis during vapour-phase moisture absorption–desorption. Thus the soils have a higher moisture content during desorption than during absorption, consequently at any given moisture content the capillary potential energy of the soil is lower during desorption and strength is higher. When the soil samples were immersed in water for 24 h. the untreated samples lost all their strength but the TBC treated samples did not wet throughout their mass and the strength was consistent with the water content.
T. G. MORRIS.

Determination of quartz, feldspar and mica and studies of layer silicates in some Irish soils. P. V. Kiely (*Dissert. Abstr.*, 1964, **25**, 325).—Methods of separation and determination of the three minerals in soil based on fusion with $Na_2S_2O_7$ are described and applied to a range of soils. The distribution of quartz, feldspar and mica in the various mechanical fractions of the soils is examined. Interpretation of *X*-ray diffraction pictures of soil clays may be simplified by treatment of the clays with Na tetraphenyl boron. Separation of chlorite from talc may be effected by fusion with $Na_2S_2O_7$, whereby chlorite is dissolved.
A. G. POLLARD.

Morphological-chemical relationships of some thin A-horizon solodised soils derived from moderately fine material on a well-drained slope. E. M. White (*Soil Sci.*, 1964, **98**, 256—263).—The profiles of the soils are described together with laboratory analyses. The genesis of the soils is discussed. The amount of extractable Na in the B2 horizon could be estimated from the morphology of the soil with fair accuracy.
T. G. MORRIS.

Effect of petroleum resins mulch and polyethylene film on soil temperature and plant growth. F. H. Takatori, L. F. Lippert and F. L. Whiting (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 532—540).—Application of a petroleum resins mulch (by spraying an aqueous emulsion) or polyethylene film to the soil surface increased soil temp. during daylight. Increase in soil temp. with black polyethylene film was smaller than with either petroleum mulch or clear polyethylene film during the day, but the black film retained more heat during the night. The petroleum resin mulch hastened the emergence and maturity of eight vegetable crop species tested.
A. H. CORNFIELD.

Measuring soil moisture with high-frequency electro-magnetic waves. M. van der Westhuizen (*S. Afr. J. agric. Sci.*, 1964, **7**, 589—590).—Preliminary work on a method based on the principle that the velocity of electro-magnetic waves is lower in a medium than in a vac., is described. Gravimetric calibration is required.
E. G. BRICKELL.

Temperature distribution and performance in balloon-sheet soil steaming. J. Grainger (*Hort. Res.*, 1964, **4**, 27—41).—The technique is described. Observations on steam consumption, depth distribution of temp. and on the effects of steaming on soil fertility are presented. Pathogens which occur at considerable depths in soil (e.g., cysts of potato root eel-worm at 30 in.) are more effectively dealt with by heavy-vapour fumigants (e.g., DD).
A. G. POLLARD.

Reactions of ammonium phosphate with gibbsite and with montmorillonitic and kaolinitic soils. Y. N. Tamimi, Y. Kanehiro and G. D. Sherman (*Soil Sci.*, 1964, **98**, 249—255).—Gibbsite aggregates from a silty clay soil were washed, dried and ground to a variety of mesh sizes. The air-dry samples were then treated with water, aq. $(NH_4)_2HPO_4$ (**I**), and H_3PO_4, shaken, and the pH adjusted to different levels. After further shaking and storage the products of the reaction were isolated and examined. At all pH levels investigated **I** readily reacted with gibbsite aggregates of all sizes and at all pH levels from 1 to 5. The main reaction product was taranakite. The amount of taranakite formed depended on the size of the gibbsite aggregates and the pH. At comparable pH levels the <100 mesh gibbsite aggregates gave more than aggregates of larger sizes. With all aggregates the amount of taranakite decreased with increasing pH. The *X*-ray diffraction peaks of montmorillonite and kaolinite in Hawaiian clay soils decreased in size after the soil had been treated with **I** indicating that Al was extracted from the soils to form taranakite.
T. G. MORRIS.

Comparison of two biological methods for determination of mineral elements assimilable by plants. G. van Roey and R. Bastin (*Rev. Ferment. industr. aliment.*, 1964, **19**, 121—127, 147—156).—Compositions of nutrient media and working details are given for the use of *Aspergillus niger* and *Lemna minor* L. in the bioassay of micro-elements. Determinations of NO_3^-, Ca^{2+} and S can be made with *L. minor* but not with *A. niger*. Both methods are suitable for Mg, P, K, Fe and Zn. *A. niger* is preferable for Cu, Mn and Mo.
P. S. ARUP.

Vertical distribution of phosphorus-32-labelled ammonium phosphate after tillage operations with mouldboard plough and rotary cultivator. K. Steenberg and A. Njos (*J. agric. Engng Res.*, 1964,

9, 241—244).—Ploughing after surface application of $NH_4H_2{}^{32}PO_4$ resulted in a better turning effect than did rotary cultivation. A. H. CORNFIELD.

Reactions of iron, aluminium and calcium phosphates in six Ontario soils. A. F. MacKenzie and S. A. Amer (*Plant & Soil*, 1964, **21,** 17—25).—Phosphorus was fractionated in 6 soils 15—335 days after treatment with 200 or 2000 lb. of P_2O_5 (as PO_4^{3-}) per acre. In five of the soils Al phosphate increased with both rates of added P, Fe phosphate increased only with the high rate, whilst Ca phosphate was not affected. In a calcareous soil Ca phosphate was increased to a greater extent than were Al and Fe phosphates. A. H. CORNFIELD.

Phosphoric acid determination in soil extracts using molybdenum blue. E. Frei, K. Peyer and E. Schütz (*Schweiz. landw. Forsch.*, 1964, **3,** 318—328).—Phosphoric acid was determined in soil samples by complexing with NH_4 molybdate followed by reduction to a stable blue colour. Ascorbic acid was the best reducing agent and sulphamic acid prevents undesirable interference from N compounds extracted from heavily fertilised soils. Optimum reagent concn. were 0·4M-H_2SO_4, 0·0013M-NH_4 molybdate, 0·026M-sulphamic acid and 0·01M-ascorbic acid. (17 references.) J. B. WOOF.

Effectiveness of synthetic chelating agents as sources of zinc for calcareous soils. W. B. Anderson (*Dissert. Abstr.*, 1964, **25,** 3193—3194).—Several Zn chelates and $ZnSO_4$ were compared by application to a calcareous soil cropped with maize. In field trials the chelates produced the greater yield responses but did not eliminate Zn-deficiency symptoms entirely. In another soil a positive yield response to PO_4^{3-} overshadowed the effect of Zn. In greenhouse trials the effects of a no. of Zn chelates were inversely related to their stability constants; in general the Zn content of the maize was increased and those of Mn and Fe were diminished by the chelates. In laboratory tests the relative ability of the chelates to maintain a concn. of sol. Zn in the calcareous soil was compared. Replacement of Zn from Zn chelates by soil Fe was greatest in the case of EDDHA. A. G. POLLARD.

Anion exchange–polarographic method for the determination of zinc in plant material and in soil. E. E. Bartel and W. J. Pienaar (*S. Afr. J. agric. Sci.*, 1964, **7,** 497—508).—Wet digestion of the plant material with conc. HNO_3 and perchloric acids, separation of the Zn by anion exchange, followed by quant. determination by polarograph, is described. The same procedure may be used for soil samples by omitting the digestion step but extracting the Zn with 0·1N-HCl. Reproducibility and accuracy of the method are high and superior to those of the dithizone, Zincon and 4-chlororesorcinol methods. E. G. BRICKELL.

Factors which affect the availability of magnesium. A. D. Carr (*Proc. Nutr. Soc.*, 1965, **24,** 99—105).—A general discussion and review of the literature. (34 references.) C. V.

Cation-activity ratios in equilibrium soil solutions and the availability of magnesium. R. C. Salmon (*Soil Sci.*, 1964, **98,** 213—221).—The relationships between Mg in grass and the cation-activity ratios in the equilibrium solutions of soils with different contents of exchangeable Mg, Ca and K were examined. A clay loam and a sandy loam were limed with a fixed amount of CO_3^{2-} using different amounts of $CaCO_3$ and $MgCO_3$ to obtain four exchangeable Ca/Mg ratios from 2 to 40. After incubation for 1 month the soils were fertilised with K at four levels. Grass was then sown after fertilisation with N and P. Other soils in pots were fertilised and cropped continuously with grass. Yield of org. matter was little affected by treatment with Ca and Mg but in the clay loam addition of K markedly increased yield. The concn. of each cation increased with its content in the soil, the Ca/Mg ratio had little effect on the K concn. but increasing the exchangeable K steadily decreased the Mg and Ca concn. in the grass. Total uptake of both Mg and Ca depended upon yield. With constant K activity in the soil, the Mg concn. in grass was linearly related to $(a_{Mg}/a_{Ca+Mg})^{1/2}$ in the equilibrium soil solutions so that Mg in grass was doubled when the exchangeable Mg was quadrupled. Increasing K decreased the Mg in the grass which was then well correlated with the ratio $a_{Mg}/B.\ a_K+(a_{Ca+Mg})^{1/2}$; the proportionality factor B could be determined experimentally. With continuous cropping on soils with different exchangeable Mg, K and Ca and pH the activity of H^+ must be included. T. G. MORRIS.

Effect of phosphate application on manganese content of plants grown on neutral and alkaline soils. S. Larsen (*Plant & Soil*, 1964, **21,** 37—42).—Foliar applications of $MnSO_4$ (20—30 lb. per acre) to oats and sugar beet did not affect leaf Mn%, but soil application of triple superphosphate (200—3200 lb. of P_2O_5 per acre) increased leaf Mn% roughly in proportion to the amount of P added. The treatments did not always result in yield increases. A. H. CORNFIELD.

Rôle of *Suaeda fruticosa*, Forsk. in the reclamation of saline and alkaline soils in West Pakistan. I. I. Chaudhri, B. H. Shah, N. Naquvi and I. A. Mallick (*Plant & Soil*, 1964, **21,** 1—7).—*Suaeda fruticosa* was very effective in collecting wind-blown soil and in absorbing large quantities of salts from the topsoil. A single harvest of the aerial parts in the autumn of each year removed 2400 lb. of salts per acre. The wind-deposited sand and soil helped in reclamation by diluting the salt concn. and decreasing the salt-holding capacity of the topsoil. A. H. CORNFIELD.

Plant-available sulphur in soils. II. Availability of adsorbed and insoluble sulphates. C. H. Williams and A. Steinbergs (*Plant & Soil*, 1964, **21,** 50—62).—Addition of $CaCO_3$ to soils with pH ranging from 5·7 to 7·4 resulted in increased uptake of S by oats. The water-insol. SO_4^{2-} present in calcareous sands and associated with the $CaCO_3$ had very low availability to plants. 'Adsorbed' SO_4^{2-} (as determined by extraction with a no. of reagents) was readily available to plants. A. H. CORNFIELD.

A theoretical approach to the movement of strontium through soils. M. J. Frissel and P. Poelstra (*Soil Sci.*, 1964, **98,** 274—277).—A theory is given for the calculation of the leaching efficiency of Thornthwaite *et al.* (*Science*, 1960, **131,** 1015—1019). T. G. MORRIS.

Determination of total carbon in soils by wet combustion. W. O. Enwezor and A. H. Cornfield (*J. Sci. Fd Agric.*, 1965, **16,** 277—280).—A simplified digestion–purification train apparatus (diagram given) using H_2SO_4–H_3PO_4 and $K_2Cr_2O_7$ is described; the Zn column is eliminated, a single trap containing aq. NaOAc, AcOH and KI to fix volatile mineral acids and Cl and chromyl chloride derived from Cl^- in the soils is used. Reproducibility is good and mean results agreed well with those given by the Pregl dry combustion method. E. M. J.

Effect of varying static and changing moisture levels during incubation on the mineralisation of carbon in soil. D. M. Ekpete and A. H. Cornfield (*J. agric. Sci.*, 1965, **64,** 205—209).—With different static soil moisture levels up to 60—80% saturation, CO_2 production at 28° increased, thereafter decreasing with further increases of moisture to the water-logging point. Where moisture levels were increased to 50% from a range between air-dry and 40%, CO_2 production decreases with increasing initial moisture. Likewise a reduction in moisture content from <60% to 50% increased CO_2 production to extents increasing with initial moisture content, the effect being more marked than when the soil moisture content was brought up from lower levels to 50%. Except where the soil was air-dry, water-logging reduced CO_2 production. M. LONG.

Apparatus for measuring losses of ammonia from decomposing plant materials. P. D. Salt (*Chem. & Ind.*, 1965, 461—462).—CO_2-free air is passed through a soda lime tube and three conical flasks containing NaOH, HCl and distilled water to reach the sample. Thereafter it passes through 100 ml. of 4% w/v boric acid to absorb NH_3; Bromocresol green–Methyl red is used as indicator. Grass clippings (20 g.) were used, and after 150—200 l. of air had passed the indicator changed colour (6 days) and NH_3 continued to be evolved while a further 250—350 l. were drawn through (12—15 days). The boric acid was changed after each 70 l. of air and the absorbed NH_3 titrated with 0·1N-HCl. Two experiments were based on November grass and the third on grass collected in May. Other aspects and observations are considered. C. V.

Changes in the soil after clearing tropical forest. P. H. Nye and D. J. Greenland (*Plant & Soil*, 1964, **21,** 101—112).—About 1·5 acre of tropical forest, of known mass and chemical composition, was cleared and burned. Soil changes during clearing and two years' cropping were studied. Following burning, approx. all the K, Ca and Mg in the vegetation were accounted for by the rise in exchangeable K, Ca and Mg in the soil, and there was a marked rise in soil pH. A small but significant increase in C and N was attributed to admixture of parts of the vegetation with the soil. Following cultivation there was a rapid loss of nutrients by leaching and erosion during the first year, and a substantial loss of K and Mg, but smaller loss of Ca, in the second year. Losses of Ca were less and of K more under the local practice of shifting cultivation than under cultivation treatments involving clearing of roots followed by bare fallow or a maize–cassava rotation. Depth of cultivation had little effect on nutrient losses. Losses of org. matter were high in the first, but much lower in the second, year. A. H. CORNFIELD.

Biological clogging of sands. Y. Avnimelech and Z. Nevo (*Soil Sci.*, 1964, **98,** 222—226).—Lysimeters, 5 cm. dia., were filled with sand to a depth of 30 cm. with the outlet 10 cm. below the sand surface, the hydraulic gradient being 0·4. Sand low in org. matter was used either alone or mixed with org. materials in the top 5 cm. Water or aq. NH_4NO_3 (100 p.p.m.) was used for percolation. The rate of percolation with water was decreased by most of the org. matter used, but with aq. NH_4NO_3 the rate was not affected. In other tests using different casein/starch ratios and thus different C/N

ratios it was found that rapid clogging occurred with low C/N ratios but within a few days permeability increased almost to that of the control. When the C/N ratio was high, clogging occurred only after 5 days but it was then permanent. The degree of aggregation of the sand increased with the C/N ratio. A linear correlation was found between the concn. of polyuronides in the sand at the end of the percolation period and the extent of clogging. T. G. MORRIS.

Distribution of spore-forming bacteria in soils of different types. F. P. Vavulo and A. I. Karbanovich (*Mikrobiologiya*, 1965, **34**, 14—120).—Counts were made of *Bacillus cereus*, *B. mycoides*, *B. megatherium*, *B. mesentericus* and *B. idosus* and total bacteria at various horizons in turfy podzolic (**I**), turfy gley (**II**), peaty gley (**III**) and peaty marsh (**IV**) soils which had developed on fluvioglacial sands, loessal loams and morainic loams. In this way the rôle of parent material in the formation of soil and its effect on growth of spore-forming bacteria were determined. Four tables show total count and % of each of five organisms present at depths down to 190 cm., corresponding to known genetic horizons, in four types of soil each on three types of bed. Spore-forming bacteria were found over whole vertical soil profiles. The no. of bacteria increased steadily from **I** through **II** and **III** to **IV**. Most bacteria were found near the surface. Parent material has an important influence on bacterial development only in **I** and is insignificant in **III**. The lowest no. of bacteria were found in **I** and **II** on fluvioglacial sands. *B. cereus* predominated in all soils examined. Fertility of soil increased with total no. of spore-forming bacteria.
P. W. B. HARRISON.

Determination of survival of bacteria in soil by the thread method. M. B. Roĭzin (*Mikrobiologiya*, 1964, **38**, 1074—1077).—A method of determining the survival time of N-fixing and other bacteria in soil is described. Cotton threads, 5—6 cm. long, are impregnated with aq. suspension of organisms and buried for varying periods in 1 cm. deep layer of moist soil in Petri dishes. Threads are extracted at intervals, transferred to Ashby agar medium in 9—10 cm. Petri dish (up to 10 threads per dish) and incubated. Survival of organisms is shown by presence of growths on agar. Photographs clearly distinguish between threads kept in toxic and non-toxic soils. Comparative trials of two methods with three different podsolic soils gave generally concordant results. Longer survival times sometimes shown by the thread method may result from accumulation on the thread of bacteria, not all of which had been subjected to the toxic action of soil. Threads made from nylon-6 or other synthetic fibre may be used when cellulose-destroying organisms are present. In a field experiment threads, either bare or in Cellophane envelopes, were planted up to 25 cm. deep in soil. The thread method gave reliable results under field conditions. Cellophane slightly retarded the action of soil on organisms. P. W. B. HARRISON.

Factors influencing mechanisms of soil aggregation by micro-organisms. R. F. Harris (*Dissert. Abstr.*, 1964, **25**, 3244).—Artificial aggregates of different sizes and with controlled physical and chemical properties were prepared from a silt loam. Effects of incorporation of sucrose and of the action of various bacteria and fungi on the stability of the aggregates were examined. Stabilisation by the indigenous microflora depended largely on the ability of the organisms to synthesise binding material rather than on the no. of organisms present. Stabilisation by fungi was associated with the occurrence and development of macroscopic mycelia on the aggregates. In sucrose-treated soils the relative no. of bacteria fungi and yeasts varied with temp. The time needed to attain max. stability was similar for aggregates incubated at 35°, 25° or 15° but the subsequent decline in stability was faster at the higher temp. The period of appreciable stability of the aggregates was 4, 14 and 84 days for those incubated at 35, 25 and 15° respectively. A. G. POLLARD.

Inhibition of germination of fungus spores in soils; use of agar disk tests. D. Seidel (*Zbl. Bakt.*, 1965, **119**, II, 74—87).—Fungistasis of four soils to conidia of *Alternaria tenuis*, *Trichothecium roseum*, *Trichoderma viride*, *Helminthosporium sativum* and *Fusarium solani* was demonstrated by the agar disc method. The effect was greater in water-saturated soils than in those containing 30 or 60% of their water-holding capacity and varied with soil type. Neither the colonisation of agar disks by the organisms nor the relative no. of bacteria and fungi was a factor in the variability of the inhibitory effect. A. G. POLLARD.

Effect of vegetable tannins on nitrification in soil. J. Basaraba (*Plant & Soil*, 1964, **21**, 8—16).—Addition of 0·50—2·00% wattle and chestnut oak tannins to soil inhibited nitrification somewhat (incubation test) only during the first 1—2 weeks. Tannins at 0·125% had little effect on nitrification. The wattle tannin had a slightly stronger inhibitory effect than had chestnut oak tannin.
A. H. CORNFIELD.

***Aspergillus flavus* in relation to intermediates in nitrification.** G. E. Becker (*Dissert. Abstr.*, 1964, **25**, 2706—2707).—Two approaches were used: the detection of NO_3^- formation from various N compounds in both growing and replacement cultures and the detection of accumulated intermediates of nitrification in genetically blocked cultures. Media consisting of the salt of an org. acid and peptone supported luxuriant growth of *A. flavus* as well as vigorous nitrification. A wider spectrum of amino-acids was converted into NO_3^- with acetate as the C source. Experiments with replacement cultures of other soil hetertropes showed that more than half were capable of oxidising β-nitropropionic acid to NO_3^- but of these only *A. niger* could convert NO_2^- to NO_3^-. F. C. SUTTON.

Effect of simazine and atrazine on microflora of sandy soil. L. Yu. Klyuchnikov, A. N. Petrova and Yu. A. Polesko (*Mikrobiologiya*, 1964, **33**, 992—996).—Simazine (**I**) or atrazine (**II**) 2 kg./1000 l. of water/ha. was applied to a sandy soil. This amount is optimal for destruction of annual weeds. After 4 months bacteria and mould counts at 0—5, 5—15 and 15—25 cm. levels were compared with those in untreated controls by means of buried linen fabric plates. The total no. of organisms was somewhat reduced, especially by **I** at the 0—5 cm. depth. Inhibition of cellulose-destroying organisms by herbicides was shown by lower wt. losses of fabric buried in treated soil, **II** being more effective than **I**. Light sandy loam was treated with **II** (6 kg./1000 l. of water/ha.), sufficient to destroy perennial weeds, in two sprayings at 1-month interval. At 12 weeks after the first application, the count of micro-organisms was somewhat reduced down to the 25 cm. level and pigmentation of *Trichoderma* was retarded. A single application of **I** or **II** in these doses did not depress significantly the microfloral activity of a light soil. P. W. B. HARRISON.

Effects of organic manures on soils and crops. A. H. Bunting (*Proc. Nutr. Soc.*, 1965, **24**, 29—38).—The action of org. manures is discussed. A particular group of soils (~53% coarse sand, 28% fine sand, 10% silt, 9% clay) responded to strawy org. manures and grass, but not to sewage sludge, by improved structure (relatively stable and non-caking). The effect is entirely physical. (14 references.)
C. V.

Efficiency of superphosphate-digested compost as shown by studies with radioactive phosphorus. B. V. Subbiah and S. Mohan (*Fertiliser News*, 1965, **10**, No. 3, 29—33).—Superphosphate (**I**) was added to decomposing oat plant material in proportions of 0—32 g./100 g. During the decomposition, loss of dry matter was ~47·2—56·3% and that of N was 28·9—14·7% of the original amounts. The extent of conversion of inorg. P into the org. form was 0·059—0·104 g. with the 8, 16 and 32 g. additions of **I**. Use of **I** resulted in a more decomposed compost which was comparatively richer in N and org. P. Applications of the compost with special reference to rice, are studied in various soils. C. V.

Composting conversion of solid wastes for mushroom growing. S. S. Block (*Biotechnol. Bioengng*, 1964, **6**, 403—418).—A method of composting paper-garbage and other waste in 27 cu. ft. bins surrounded by a 6 in. layer of sawdust was developed. Temp. rises are uniformly high with no anaerobic fermentation. Indoor systems capable of handling 1 cu. ft. of compost are described and shown to give reproducible results. The use of these systems in high-yield mushroom culture is considered. J. B. WOOF.

Composting trials with pear canning wastes. J. F. Kefford (*Food Pres. Quart.*, 1964, **24**, 21—24).—Bin trials have shown that pear canning wastes can be composted successfully in approx. 2 weeks by mixing with rice hulls or wheat chaff and some lime. The compost produced was suitable for recycling as filler for subsequent waste but had no fertiliser value. S. A. BROOKS.

Agricultural value of sewage determined by pot and field trials. J. Geering (*Schweitz. landw. Forsch.*, 1964, **3**, 277—317).—Results of experiments in Switzerland on the general value of sewage were collected and evaluated both on the basis of economics and effectiveness. The agricultural values of various fertilisers (defined as the absolute cost to the farmer to produce comparable results in the field) were calculated. Specially dried sludge is expensive and normal sludges involve high transportation costs unless the plant is near to the fields. It is recommended that sewage sludge is used only for specialised viticulture applications. Similar considerations and planning are required if farmyard and liquid manure are to be used effectively as fertilisers. (75 references.) J. B. WOOF.

Production of concentrated superphosphate from Kola apatite concentrate and Moroccan crude phosphate. U. Lamm and W. Wolfrom (*Chem. Tech., Lpz.*, 1965, **17**, 90—95).—The crude phosphates were treated with a mixture of H_2SO_4 and H_3PO_4 and the time required for solidification of the products noted in laboratory and small-scale practical tests. Addition of H_3PO_4 gives a plastic intermediate stage before the final solidification. With Kola apatite, a sprayable product was obtained only if <30% of the H_2SO_4 was replaced with H_3PO_4, but with Moroccan phosphate most acid proportions were suitable. Optimum acid concn. was, for Kola apatite (small-scale test) 30—42% H_2O, and for Moroccan phosphate,

24—31% H_2O. Corresponding optimum temp. were 70—85°, and 35—80° respectively depending on the acid proportions. With increasing proportion of H_3PO_4 the P_2O_5 content of the products increased. M. GREENAWAY.

Continuous moisture determination of crude and fertilising lime by neutron bremsstrahlung. V. Netz (*Chem. Tech., Lpz.*, 1965, **17**, 95—97).—The measuring cell had a BF_3 proportional counter, a source of fast neutrons and a transistorised adaptor. The apparatus was arranged on a slide so that a continuous flow of material passed the measuring cell. The apparatus was tested with fertilising lime and the relationship between impulse rate and moisture content determined. It was possible to measure up to 25 wt.-% H_2O in fertiliser lime and 25—35% in crude lime. with variation of <1%. M. GREENAWAY.

Effect of additives of trace-elements on the chemico-physical characteristics of liquid nitro-carbonate fertiliser. S. N. Gans, R. I. Braginskaya, R. M. Danchenko and E. P. Gorbonos (*Zh. prikl. Khim.*, 1965, **38**, 263—266).—The chemico-physical characteristics of NH_4 carbonate were investigated when it contained trace element additives. The latter additives in concn. 1—2% reduced the vapour pressure of NH_3 and CO_2 over solutions and also reduced their freezing temp. NH_4 carbonate solutions with addition of 1·5—2% Cu could be applied for purifying gas from CO. Waste Cu NH_4 carbonate solutions are effective fertilisers. A. L. B.

Recovery of zinc from zinc compounds added to phosphatic fertilisers. M. S. Steyn, J. H. Rossouw and J. J. C. van Zyl (*S. Afr. J. agric. Sci.*, 1964, **7**, 411—416).—The addition of Zn compounds to a fertiliser mixture affects the water-sol. P_2O_5 content adversely only at a higher level than 1·5% Zn, but is without effect on the citric-sol. P_2O_5 content. With superphosphate the solubility of P_2O_5 in either water or citric acid is unaffected. Ammonification of a fertiliser mixture had no effect on the solubility of added Zn compounds in respect of the various extractants. E. G. BRICKELL.

Colorimetric determination of phosphorus in fertilisers. J. D. Leonard (*J. S. Afr. Chem. Inst.*, 1964, **17**, 101—113).—Use of a high concn. of molybdate in the vanadomolybdate reagent effectively prevents interference by citric acid in the colorimetric determination of P in citric acid extracts of fertilisers. The method also yields accurate and precise results in the determination of total and water-sol. P. E. G. BRICKELL.

Phosphate slag fertiliser. Hüttenwerk Salzgitter A.-G. (B.P. 953,258, 11.4.62. Ger., 16.3.62).—Liquid phosphate slag is granulated by quenching it with water and/or air, and the resulting granular material is then brought, by grinding and screening, into a range of grain sizes between 0·1 and 1 (0·2 and 0·5) mm. If the granular material does not dry out itself, it is subjected to drying before the grinding operation. J. M. JACOBS.

Fertiliser containing isobutylidenediurea. Mitsubishi Chemical Industries Ltd. (B.P. 949,408, 24.8.61. Jap., 30.8.60).—There is claimed a fertiliser containing *isobutylideneurea* (prep. described from urea and isobutanol) and optionally urea and at least 1 fertiliser selected from a water-sol. nitrogenous, phosphatic, and potassic fertiliser. F. R. BASFORD.

Fertilisers. Fisons Fertilizers Ltd. (Inventors: G. G. Brown and R. G. Wilson) (B.P. 954,423—4, 24.6.59).—[A] Phosphate rock, HNO_3 and K_2SO_4, with or without H_2SO_4, are mixed together in a multiple stirred-tank reaction system. The K_2SO_4 addition is ≮0·5 mole per mole of P_2O_5 in the rock. The reaction is carried out at 90—140°, and the rate of addition of K_2SO_4 and H_2SO_4 is adjusted so that the sol. SO_4^{2-} concn. in the liquid phase is maintained at 0·5—8% and the $CaSO_4$ is precipitated as anhydrite. The reaction product is continuously removed from the reaction system. If desired, the pptd. $CaSO_4$ is separated and the liquor is neutralised (with NH_3) dried and/or granulated. [B] The reaction is carried out at 60—90°, the sol. SO_4^{2-} concn. in the liquid phase is maintained at 0·5—4% and the $CaSO_4$ is precipitated as gypsum, $CaSO_4.2H_2O$. J. M. JACOBS.

Fertilisers. Fisons Fertilizers Ltd. (Inventors: K. S. Barclay, J. M. Crewe, and K. F. J. Thatcher) (B.P. 953,425, 27.6.59).—A process for the treatment of compound fertilisers containing phosphates and NH_4NO_3 comprises mixing the compound fertilisers or a component thereof with an amino-compound capable of reaction with HNO_2 (e.g., NH_4 sulphamate) (0·25—1·0% by wt.) and granulating the fertiliser. E. ENOS JONES.

Fermentation of waste organic material for agricultural purposes. F. Prat (B.P. 955,338, 15.4.64).—Household waste containing org. material is converted into compost by placing the material together with a minor proportion of compost from a previous fermentation in an open vat of which the side walls are such as to allow abundant natural aeration; then spraying with water containing a dissolved N-compound, to bring water content of the waste to 50—60 wt.-% and the ratio of the C/N present to 25—35:1. If desired, up to 30 wt.-% of sewage sludge may be incorporated with the waste. Apparatus is illustrated. F. R. BASFORD.

Plant Physiology, Nutrition and Biochemistry

Ultimate limits of crop production. J. N. Black (*Proc. Nutr. Soc.*, 1965, **24**, 2—8).—The complex inter-relationship of crop growth rate, leaf area index (ratio of leaf area to ground surface area) and solar energy is discussed. It is stressed that fertiliser and other experiments must be interpreted against this background and failure to do so may well lead to erroneous conclusions. (22 references.) C. V.

Formation of biochemically important compounds in prebiological stages in the evolution of the earth. A. G. Pasynskiĭ and T. E. Pavlovskaya (*Usp. Khim.*, 1964, **33**, 1198—1215).—Potential conditions of primary syntheses on the earth are discussed together with the formation of amino-acids, polypeptides, purines and pyrimidine bases, nucleosides and nucleotides, carbohydrates, aldehydes and org. acids, amines and amides, imidazole compounds and porphyrin. The final chapter contains a general review and summary of other authors' observations in this field. (104 references.) A. L. B.

Colorimetric method for measuring atmospheric carbon dioxide concentration *in situ*. R. B. Sharp (*J. agric. Engng Res.*, 1964, **9**, 87—94).—The method, which was developed for determining CO_2 in greenhouse atm., is based on measuring the change in pH of a borax–H_3BO_3–NaCl solution when shaken with a specified vol. of air. A permanent glass colour standard disk covering the range 0·02—0·40% CO_2 by vol. in air is available. A. H. CORNFIELD.

Effects of light, temperature and ionic balance on oxalate formation in spinach. J. W. Kitchen, E. E. Burns and R. Langston (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 465—470).—The % of sol. and total $C_2O_4^{2-}$ in spinach seedlings was greater when they were grown with 12-h. light periods than when grown continuously in darkness. When excised spinach leaves were treated with solutions of cations and anions and then exposed to $^{14}CO_2$ the resultant labelled $C_2O_4^{2-}$ formed was increased by the cation treatment [0·005M-$Ca(NO_3)_2$] and decreased with increasing concn. of malic acid in the treatment solution. Leaf $C_2O_4^{2-}$ values decreased with increasing temp. (10—25°). A. H. CORNFIELD.

Oxygen requirement of root crops and soils under near-field conditions. N. J. Brown, the late E. R. Fountaine and M. R. Holden (*J. agric. Sci.*, 1965, **64**, 195—203).—At normal agricultural spacings the mean daily O_2 consumption for potatoes was 2·8, for marrowstem kale 5·6, for tobacco 3·01 l./sq. m. soil area. Corresponding ranges of O_2 consumption per plant were 0·108—0·680, 0·169—0·599 and 0·175—0·986 l./day. The mean daily consumption of O_2 by an undisturbed sandy soil was 2·21 l./sq. m., range 1·8—5·3 l. and by a peat top-soil 10·81 l./sq. m., range 7·7—11·01 l. O_2 consumption increased with disturbance of a soil and with increasing amounts of org. matter. An artificial barrier against gas movement at the surface of a soil is difficult to create and capping is only likely to be a serious problem from the point of view of aeration when the surface has been waterlogged for some time. M. LONG.

Measurement of the cation-exchange capacity of plant roots. W. M. Crooke (*Plant & Soil*, 1964, **21**, 43—49).—Ground dried (80°) root material is leached free of exchangeable bases with 0·01N-HCl. The base-free material is then suspended in N-KCl and titrated to pH 7·0 with 0·01N-KOH. Cation-exchange capacity of roots of a no. of species of plants was correlated with their uronic acid content. A. H. CORNFIELD.

Growth and ion uptake by maize seedlings on solutions variable in phosphate and flow rate. S. A. Sabet, M. A. A. Salam and J. V. Lagerwerff (*Plant & Soil*, 1964, **21**, 94—100).—The effect of varying PO_4^{3-} concn. (0·1—10·0 p.p.m.) in the nutrient and varying flow rate (2—24 l. per day) of the nutrient through the roots was studied. Fresh and dry wt. of seedlings and P% of the tops increased with both nutrient P concn. and flow rate, whilst Fe% and Ca% of tops decreased and that in roots increased. The efficiency of utilisation of P by the plant (the product of P concn. in the nutrient and its flow rate in relation to P absorbed) decreased with increasing flow rate at the low and high concn. of P in the nutrient, but was not much affected at the intermediate P concn. (1 p.p.m. P). A. H. CORNFIELD.

Inorganic polyphosphates in maize roots. V. M. Vagabov and I. S. Kulaev (*Dokl. Akad. Nauk SSSR*, 1964, **158**, 218—220).—Presence of highly polymerised inorg. polyphosphates (I) in root tips of maize was proved. Seeds were grown for 4 days on filter paper moistened with solution of $KH_2^{32}PO_4$. Radioactivity was

determined in 4—5 mm. lengths cut from root tips and containing meristematic zone, and also in a further 10 mm. section. The P contents were separated chromatographically before and after hydrolysis. All unhydrolysed solutions were radioactive at the start of the chromatogram and in the orthophosphate (**II**) zone. After hydrolysis (Thilo and Wieker method) radioactivity disappeared from the start and was found in three of the four fractions in the trimetaphosphate (**III**) zone as well as in **II**. A small quantity of **I** was indicated in the last 5 mm. of root tip but not in next 10 mm. of root. Apparently highly polymerised inorg. **I** occurs in meristematic tissue of roots and on partial hydrolysis form cyclic **III**. The significance of reserves of highly polymerised inorg. **I** in rootlets is discussed.
P. W. B. HARRISON.

Compounds of manganese and iron in plants. E. A. Boĭchenko and T. M. Udel'nova (*Dokl. Akad. Nauk SSSR*, 1964, **158**, 464—466).—Mn and Fe are connected with major oxidation-reduction reactions in plants. Org. compounds of these elements, occurring in plant cells, were examined. Primula, white clover, duckweed and fresh and fallen oak leaves yielded lipids which contained >90% of the total Mn and Fe. Mn was found in lipids of linoleic (**I**) and linolenic (**II**) acid but not in those of oleic and saturated acids. Fe in leaves was mainly in non-haemic form, combined in lipoproteins (**III**) which comprised 1—6% of wt. of leaves. **III** have low oxidation-reduction potential close to 1 atm. of mol. H_2 and hence can reduce CO_2 and pyridine nucleotides; **III** also contain S and recently have been called ferredoxines (**IV**). Fe compounds were separated from proteins by extracting lipids with some solvents used to extract Mn compounds. Separation from phosphatides was achieved by alkaline hydrolysis and pptn. as Ba salts. The Fe content of **IV** was 3·8—3·9%. Chromatographic separation of **IV** from hydrolysates is described. Mn compounds with highly unsaturated acids **I** and **II** are the most powerful oxidising systems in plants and can form peroxides. The Fe compound with the unsaturated acid from **IV** is a most powerful reducing systems, actually reducing CO_2. Concn. of Mn in plant acids can increase to nearly 2% and that of Fe to nearly 4%. (21 references.)
P. W. B. HARRISON.

Inhibition of lignification in vegetable tissues by antioxidants. M. Grigoras, N. Simionescu and C. Simionescu (*Rev. roum. Chim.*, 1964, **9**, 487—489).—The inhibiting effect of quinol, gallic acid and propyl gallate on the biosynthesis of lignin in tomato plants grown in soil and in Knop solution was studied. In soil, the antioxidant was introduced into the soil in gradually increasing concn. over a 50-day growth period; for those grown in Knop solution, the plant roots were periodically treated with the antioxidant solution. Both the lignin and methoxyl values were less in the plants grown in antioxidant media although in some cases the differences from controls grown normally were slight. (In English.) J. I. M. JONES.

Methods of transforming phytin in higher plants. I. S. Kulaev, M. N. Valikhanov and A. N. Belozerskiĭ (*Dokl. Akad. Nauk SSSR*, 1964, **159**, 668—671).—Possible routes for utilisation of phytin (**I**) in growth of cotton seeds were studied. Content of P present as **I**, nucleic acids (**II**), phosphate sugars (**III**), 3-phosphoglyceric acid (**IV**), orthophosphates (**V**) in dry seeds; in seeds after 3 days on moist filter paper in daylight and in 6-day growths, are shown. After 3 days **I** had decreased significantly and **IV** increased very considerably, **II** and **V** slightly and **III** scarcely at all. Between 3 and 6 days, when green leaves formed **I** almost disappeared, **V** increased rapidly and **IV** remained constant. Similar experiments with seeds grown for 6 days in darkness showed considerable retardation of both destruction of **I** and formation of **IV**; on removal to sunlight **I** decreased and **IV** increased considerably. Formation of **IV** in the early stages of growth probably results from transformation of **I** induced by sunlight. Formation of **IV** from **I** *in vitro* was confirmed by extraction from seeds powdered at the 3-day stage, of an enzyme which catalysed decomposition of Na phytate (**VI**) at 37° and [H⁺] 5·8 yielding inositol di-, tri- and tetra-phosphates, **IV** and **V**. **IV** was not present in the unfermented extract and must have been formed from **VI**. Similar chromatograms were obtained by incubating **VI** with wheat bran extract. All data indicate direct transformation of **I** to **IV** in growth of higher plants, probably induced by sunlight. (18 references.) P. W. B. HARRISON

Identification of the flavonol myricetin in legume seeds and its toxicity to nodule bacteria. P. F. Fottrell, S. O'Connor and C. L. Masterson (*Irish J. agric. Res.*, 1964, **3**, 246—249).—Two substances (one being myricetin) having antibiotic effects on *Rhizobium leguminosarum*, were isolated from seeds of *Trifolium repens*. Addition of $NaVO_3$ to the culture medium of the bacterium largely eliminated the toxicity of the *Trifolium* extracts. $CoCl_2$ and Na_2MoO_4 had smaller protective effects. A. G. POLLARD.

Lipids of acetone-insoluble fraction from red-clover (*Trifolium pratense*) leaves. R. O. Weenink (*Biochem. J.*, 1964, **93**, 606—611).—Chromatography with diethylaminoethyl cellulose separates the constituents of the $COMe_2$-insol. fraction obtained from red clover leaves. This fraction consists approx. of waxes, 2·3; galactolipids, 25; and phospholipids, 52%. The composition (mol. proportion) of the phospholipid fraction is: phosphatidylcholine, 37; phosphatidylglycerol, 23; phosphatidylethanolamine, 15; phosphatidylinositol, 2; unidentified acidic compounds, 13; and unknown compounds, 10%. An unusual acid, probably a C_{16} unsaturated fatty acid is present preferentially in the phosphatidylglycerol fraction. (22 references.)
J. N. ASHLEY.

Content of keto-acids in plants. V. L. Kretovich and N. S. Gel'ko (*Dokl. Akad. Nauk SSSR*, 1964, **158**, 471—473).—Keto-acids content was determined in green shoots of named varieties of wheat, barley, sunflower, maize, buckwheat, peas and fodder beans. Shoots, 5—6 in. long, from plants 8—10 days old were pulverised and treated with 2,4-dinitrophenylhydrazine (**I**) in 2 N-HCl. Hydrazones were extracted with Et acetate and those of keto-acids removed by 10% aq. Na_2CO_3. The keto-acid hydrazones were separated chromatographically. Products obtained were subjected to hydrogenolysis (Pd-boride catalyst) and the amino-acids formed were identified chromatographically. Oxaloacetic, glyoxylic, pyruvic, α-ketoglutaric, oxypyruvic, phenylpyruvic, α-ketoisovaleric, α-keto-β-methyl-n-valeric acid and the half aldehyde of succinic acid (**II**) in whole plants or separate organs were determined (0—0·7 μmol. per g. of material). In all plants the content of glyoxylic acid was relatively high. Oxypyruvic acid was found in all specimens. Barley and peas contained largest quantity of **II**. The keto-acid content of roots was significantly less than in vegetative parts of young plants. (13 references.) P. W. B. HARRISON.

Further experiments on flower-bud initiation and cane dormancy in the red raspberry (var. Malling Jewel). D. L. Jennings (*Hort. Res.*, 1964, **4**, 14—21).—Exposure of fully-grown canes to 45°F and 9 h. day length for 6 weeks prevented dormancy and initiated flower bud formation; longer exposure was disadvantageous. Treatment of dormant canes with ethylene chlorhydrin vapour (1 cc. per 10 l. of air space) for 30 h. broke the dormancy. A. G. POLLARD.

Relation between leucoanthocyanins and anthocyanins in apples. M. Faust (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 85—90).—Leucoanthocyanin levels in the peel decreased through the season in red, green and yellow varieties. Anthocyanins in the peel increased through the season only in the red variety, and then only in the latter part of the season. The rates of the changes do not support the theory that leucoanthocyanins are precursors of anthocyanins. There was little change in either leucoanthocyanin or anthocyanin levels in old or young leaves through the season.
A. H. CORNFIELD.

Determination of moisture in seeds with special reference to the Marconi electrical conductance method. II. Sorghums. A. Joffe (*S. Afr. J. agric. Sci.*, 1964, **7**, 563—571).—Relationship between the Brown–Duvel distillation method and the Marconi conductance method was not constant for all sorghum classes, types or even varieties but was apparently unaffected by grade, production area and the fat, fibre, ash and protein content of the samples. The standard errors of estimate for each separate sorghum group of samples were however satisfactorily low. E. G. BRICKELL.

Plant growth-regulating substances. XIX. Stability of 2,3,6-trichlorobenzoic acid in wheat plants and in the rabbit and mouse. P. G. Balayannis, M. S. Smith, and R. L. Wain (*Ann. appl. Biol.*, 1965, **55**, 149—157).—The herbicide 2,3,6-trichlorobenzoic acid was present at harvest both in seeds and straw following its uptake by the roots of wheat or when applied as a spray at the stage of growth normally recommended for selective seed control. 17·6% of the acid fed to rabbits and 2·7% of that fed to mice was recovered in the acidified extracts of the excreta. A. H. CORNFIELD

Fruit-set studies in sweet lime. V. S. Motial (*Proc. Indian Acad. Sci.*, 1964, **60B**, 371—379).—Average fruit-set can be increased to 1·39% by bending and ringing the branches and to 2·80% by spraying with indolebutyric acid at 100 p.p.m., as against 1·09% fruit-set in the control. E. G. BRICKELL.

Site and mode of action of 1-naphthyl *N*-methylcarbamate (Sevin) in thinning apples. M. W. Williams and L. P. Batjer (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 1—10).—When ^{14}C-labelled Sevin was applied to spur leaves of apple, movement of Sevin in the vascular tissue was slow and little activity appeared in the seeds. With either leaf or fruit application of Sevin radioactivity was present mainly in the vascular tissues of the fruit. With various extractants of fruit and leaf tissue most of the recovered ^{14}C was present in the methylene chloride extract and paper chromatography showed only the presence of unchanged Sevin. Sevin interferes with the movement of vital growth factors in the vascular tissues thus preventing the growth of weak fruit and resulting in abscission, with or without seed abortion. A. H. CORNFIELD.

Control of flower formation by growth retardants and gibberellin in *Samolus parviflorus*, a long-day plant. B. Balden and A. Lang (*Amer. J. Bot.*, 1965, **52**, 408—417).—The vegetative growth of *S. parviflorus*, as measured by leaf formation was very little affected by AMO-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methyl chloride) or by CCC (2-chloroethyltrimethylammonium chloride); their inhibitory effects on flowering and on stem elongation were reversed by gibberellin, (**I**). Larger proportions of **I** were needed to reverse the action of CCC on stem elongation than of that on flower formation. The retardants act by inhibiting the endogenous formation of **I**, probably at different points in the synthetic pathways. This may explain some differences in their action on long-day and on short-day plants.
A. G. POLLARD.

Effects of gibberellic acid on the fixed oils of four plants. C. D. Ogzewalla (*J. pharm. Sci.*, 1964, **53**, 1412—1414).—Sunflower, sesame, castor bean and flax plants were sprayed, when they reached the blooming stage and at 2-week intervals until harvest, with aq. gibberellic acid (GA) (10 p.p.m.) until liquid dripped from the leaves. No morphological changes were noted in the plants; and a 5% increase in no. of flax seeds was found. GA (~ 0·5 mg. per plant), injected at similar times into the pith cavity of castor bean plants, caused internode elongation (8% increase in height) and 6% reduction in no. of seeds. No significant differences were found in the I val., unsaponifiable matter or esters in the seed oils. Sesame and sunflower oil showed significantly increased saponification values (higher proportion of short-chain fatty acids) and sesame oil a significantly reduced acid value after GA treatment. (13 references.)
A. T. CARPENTER.

Use of vegetable oils and fatty acids in biosynthesis of gibberellin by *Fusarium moniliforme*. G. S. Muromtsev and L. P. Dubovaya (*Mikrobiologiya*, 1964, **33**, 1048—1055).—Production of gibberellin (**I**), by deep cultures of *Fusarium moniliforme* No. 8, in modified Rolen–Thom media (**II**) at pH 5·5 was increased by addition to **II** of 2% sunflower or maize oil to the medium. Max **I** is formed after 8 days. Replacement of NH_4 tartrate on the original **II** by NH_4NO_3 permitted sugar or oil to be sole C source. Mould grown in the sugar medium adapted itself to fermentation in oil medium. In the latter, yields were increased by 350% above those obtained in the original **II**. Fermentation occurred in two stages: up to 4 days mycelium grew intensively with low production of **I**. By the 8th day growth had ceased, autolysis commenced and production of **I** increased rapidly. **I** is readily synthesised from C chains of tartaric acid and fatty acids from vegetable oils, but not from the C skeleton of sugar. Good yields of **I** were obtained with sunflower, cotton seed, olive and linseed (**III**) oils and Et palmitate. Max. yield with **III** was 559% of control. **I** was estimated by biological assay using dwarf peas. Various other modifications of **II** were examined. (14 references.)
P. W. B. HARRISON.

Plant growth regulant compounds and compositions containing them. U.S. Rubber Co. (B.P. 954,102, 30.11.61. U.S., 16.2.61).—There are claimed *N*-disubstituted-amino amic acids in which the substituted radical is dialkylamino, pyrrolidino, piperidino or morpolino, and the amic acid is maleamic, α-alkylmaleamic, succinamic, or α-alkyl-, α-alkenyl-, α-aryl-, α-acyloxy-, α-allcylthio-, or α-arylthio-succinamic acid, or a salt or imide or such. The products have growth-regulating properties in plants, and are prepared by reacting an appropriate disubstituted hydrazine with the anhydride of the selected carboxylic acid, then dehydrating to the imide where desired. One example is N-(*dimethylamino*)*maleamic acid*, m.p. 123—125°. It causes dwarfing and shortening of the internodes in plants (e.g., groundnut, soya-bean, pinto beans and squash).
F. R. BASFORD.

Crops and Cropping

Soil and plant nutrition in relation to crop yield. C. Bould (*Proc. Nutr. Soc.*, 1965, **24**, 21—29).—Factors controlling plant growth and yield (environment, water and nutritional needs, hormonal effects, incidence of pests and diseases) are discussed and appropriate literature is reviewed. C. V.

Effects of soil compaction on plant growth. L. E. Wittsell (*Dissert. Abstr.*, 1964, **25**, 3198).—The effects on a range of crops, of compacting a silt loam by different means and to different extents are examined. Yields of wheat grain and straw and also the kernel wt. were lowered by heavy compaction in the first year but grain yields were unaffected in two succeeding years. Other crops were affected to different extents (more severely than wheat in many cases). With increase in severity of compaction, the $[CO_2]$ in the soil atm. increased, but only under very heavy compression did it reach toxic concn. A. G. POLLARD.

Comparisons of paper and polyethylene mulching on yields of vegetable crops. J. W. Courter and N. F. Oebker (*Proc. Amer. Soc. Hort. Sci.*, 1964, **85**, 526—531).—A surface layer of black or brown paper or black polyethylene film increased early and total yields of cucumbers and summer squash to about the same extent. Soil temp. at a 4 in. depth was increased more by polyethylene than by paper. A. H. CORNFIELD.

Nitrogen supply for cereals. J. Geering (*Schweiz. landw. Forsch.*, 1964, **3**, 237—276).—Results of extensive Swiss field trials are reported in which N fertilisers were applied at various times and in different amounts. Leaching reduces the effectiveness of autumn application and it is best to apply the fertiliser in two parts in spring. The first promotes tillering (40—50 kg./ha.) and 2 weeks later a 30—40 kg./ha. application assists grain formation. Effectiveness of the second dosage depends on the type of growth occurring. Late application of N increases grain protein. (49 references.)
J. B. WOOF.

Physiology of host–parasite relations. XIV. Effect of rust infection on the nucleic acid content of wheat leaves. W. A. Quick and M. Shaw (*Canad. J. Bot.*, 1964, **11**, 1531—1540).—Increase in respiration in rust-infected leaves of Little Club wheat was followed and paralleled by an increase in RNA per g. dry wt.; there was no increase in rust-infected Khapli. Rust infection had no effect on DNA per g. dry wt. in Little Club or Khapli, but DNA per g. fresh wt. increased slightly (15%) in Little Club, indicating the synthesis of fungal DNA. Infection had little effect on the protein content of Little Club but markedly lowered that of Khapli. E. G. BRICKELL.

Effect of γ-ray-treated seed on the growth, ability to germinate, sterility and other properties of barley. A. H. Shahin (*Bull. sci. Yougosl.*, 1964, **9**, 172).—Different types of barley seeds were treated with γ-rays (1000—10,000 and 15,000r). No relationship of ray-treatment to germination could be established. Reaction appeared to depend upon the types of barley. Growth was stimulated only in some cases. Interesting mutations were observed. γ-Ray treatment influenced summer more than winter barley. A max. dose of 7000r is suggested for winter types, less than that for summer types. (In German.) I. DICKINSON.

Physiological and biochemical value of different leaves of maize in different stands of plants. T. Cupina (*Bull. sci. Yougosl.*, 1965, **10**, 16).—The hybrid of Kansas 1859 maize was planted with different spacings in dry soil to establish the upper limit of plants per plot. During growth the leaves and the yield were examined. Information about the formation of the leaf surface, the increase in wt. of groups of leaves, photosynthesis and distribution of org. matter is given. The quality of the yield decreases with the density of the crops. (In English.) I. DICKINSON.

Composition of maize plants at different stages of growth and per-acre accumulations of essential nutrients. E. J. Benne, E. Linden, J. D. Grier and K. Spike (*Quart. Bull. Mich. agric. Exp. Sta.*, 1964, **47**, 69—85).—Analyses are recorded of the various plant organs (ears, leaves, husk, silks, stalks and tassel) including individual nutrient values and mineral contents, over the period June–Oct.
A. G. POLLARD.

Shape defects of Russet Burbank potato tubers as influenced by soil moisture, temperature and fertility levels. R. H. Ruf, jun. (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 441—445).—The % of misshapen tubers was usually greater when soil moisture was maintained at a low than when at a high level and also tended to increase with soil temp. (7·2—32·2°). The level of application of NPK had no effect on the % of misshapen tubers. A. H. CORNFIELD.

Mode of action of farmyard manure. I. Influence of soil moisture conditions on the response of maincrop potatoes to farmyard manure. R. Holliday, P. M. Harris and M. R. Baba (*J. agric. Sci.*, 1965, **64**, 161—166).—The max. soil moisture deficit (**I**), calc. from meteorological data and the max. deficit, had no effect on the crop response to 10 tons of farmyard manure (FYM)/acre. Small, negative, non-significant correlations existed between **I** and the responses to 0·63 and 1·26 cwt. of N per acre. The response to 10 tons of FYM/acre in the presence of N was significantly and positively correlated with **I**. In wet years the effect of FYM was a reflection of its available N content, but in dry years FYM could not be equated to inorg. N. M. LONG.

Susceptibility of potatoes to black spot in relation to soil carbon dioxide levels. M. Yamaguchi, W. J. Flocker, F. D. Howard and H. Timm (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 446—456).—The concn. of CO_2 in the soil atm. decreased with decreasing soil moisture content. It increased to a max. with max. potato top growth and

then declined as the tubers matured. Tuber susceptibility to black spot following bruising was lower in dry than in wet soil. In the wetter soil tubers from below the 4 in. depth showed higher susceptibility to black spot than those near the surface, where atm. CO_2 concn. was lower. Susceptibility of tubers to black spot increased after postharvest exposure to 5% CO_2 for 24 h. When this treatment was followed by aeration for 24 h., susceptibility to black spot decreased to a value lower than at harvest.

A. H. CORNFIELD.

Distribution and translocation of radio-iron in the potato plant and its significance in after-cooking darkening. R. T. Wurster (*Dissert. Abstr.*, 1964, **25**, 3198—3199).—Radiographic methods showed that within potato tubers Fe accumulated mainly at the stem end just below the periderm; it was also present in various other tissues. The positions of radio-Fe accumulations corresponded closely with the darkened areas visible after cooking; the dark coloration did not appear in Fe-free areas in the tuber. The translocation of Fe in the plant was examined by a method involving grafting and the use of radioactive Fe. Translocated Fe was present in areas of active cell division, notably young leaves, axillary shoots and developing tubers. Of the total Fe in the tubers >10% was derived from the grafted scion. A. G. POLLARD.

Soil test calibration with Irish potato yields. C. A. Jaworski and W. J. Hanna. (*Soil Sci.*, 1964, **98**, 227—234).—Potatoes were grown on a loam soil with N, P and K fertilisation in factorial trial for two years. All fertilisers were applied at two or three levels. Leaf samples taken during growth were analysed for P and K and soil samples were extracted by electrodialysis and by five chemical methods. Max. yields of potatoes occurred with medium or high K levels in the soil; yields did not vary appreciably with N or P levels. The K and P contents of the leaves were affected by soil-K level but not by soil-P level. Only Bray's No. 1 and No. 2 methods for P extraction gave results that were correlated with yield variation. Soil P values obtained by electrodialysis, Olsen's aq. $NaHCO_3$ method, anion exchange resin or water extracts were not linearly related to yields. There was a close linear relationship between the K content of the leaves and the soil- and fertiliser-K values, but the leaf-P did not vary with soil or treatment. T. G. MORRIS.

Chemical composition of grasses in relation to agronomical practice. R. Waite (*Proc. Nutr. Soc.*, 1965, **24**, 38—46).—With skilful management of grass higher yields of dry matter can be accompanied by improved chemical composition and nutritive value. Cases where there has been a nutrient loss are noted and the cause is examined. C. V.

Cation–anion relationships in crop nutrition. IV. Maximum contents of cations and anions in Italian rye-grass. R. K. Cunningham and A. Karim (*J. agric. Sci.*, 1965, **64**, 229—233).—With the same level of added nutrients, summer yields of grass were about double those of winter; there was little difference between the effects of NO_3^--N+NH_4^+-N and NO_3^--N alone. The sum of cations (mequiv./100 g. dry matter) was positively correlated with %N in the dry matter and was greater in winter than in summer, and greater in glasshouse-grown than in field-grown grass. With increasing NO_3^--N the sum of cations increased steadily, but it tended to reach a max. with increasing NH_4^+-N. The sum of cations can exceed 200 mequiv./100 g. and the sum of anions 400 mequiv. The ratio, sum of cations/sum of anions is inversely proportional to %N, the relationship depending on the form of N, season and whether grass is grown in the field or under glass. M. LONG.

Lignification of grasses. I. Perennial rye-grass (S24) and cocksfoot (S37). M. J. Johnston and R. Waite (*J. agric. Sci.*, 1965, **64**, 211—219).—After head emergence, leaves contribute <10% of the dry matter of both grasses, stem 50—60%, leaf sheath 13—20% and head 20—25%. The lignification of leaf and leaf sheath tissue rises only slightly with increasing age of the grass. The thickened cells of the pericycle form the major area of lignification in the stem. After anthesis the larger cells of the tissue also become lignified. A strong inverse relationship exists between lignin % and *in vitro*, digestibility of org. matter, but this varies both between the two grasses and between their component parts. M. LONG.

Effect of varying the date of application of fertiliser nitrogen on the yield and seasonal productivity of grassland. M. E. Castle, D. Reid and R. G. Heddle (*J. agric. Sci.*, 1965, **64**, 177—184).—Application of 10 cwt. of 'Nitro-Chalk' (15·5% N) per acre led to higher dry-matter yields; the timing of the application has no significant effect. Early applications reduced the clover dry-matter yield. Crude protein in the herbage was highest where N treatment was delayed until after the second cut and where no N was applied. Delaying the treatment benefits later yields at the expense of the earlier. M. LONG.

Influence of phosphorus and potassium on forage yields in relation to fertilisation and stage of harvest of an oat companion crop. H. A. Hamilton (*J. agric. Sci.*, 1965, **64**, 157—159).—Grain and silage yields were increased by 400 lb. of 4-24-12 fertiliser per acre. Fertilisation of the companion crop produced first and second year crop increases, except where the oats were harvested at the silage stage when only first year hay yields were greater with additional P and K applications. P produced a greater effect than did K in conditions experienced in northern Ontario, and one large initial application was as beneficial as several small annual applications. M. LONG.

Grass production on blanket peat. III. Nitrogen requirements. E. J. Grennan and J. Mulqueen (*Irish J. agric. Res.*, 1964, **3**, 211—222).—Establishment of clovers on blanket peat was satisfactory when small applications of N fertilisers (≯2 cwt./acre) were given; larger dressings depressed growth. Grasses responded positively and linearly to N fertilisers up to 8 cwt./acre. A suitable balance of grasses and clovers was obtained with 1—2 cwt. applied at sowing with supplementary treatments of lime, P and K. Yields and balance of species were maintained more effectively by use of $Ca(NO_3)_2$ than by $(NH_4)_2SO_4$. A. G. POLLARD.

Contribution of white clover to a mixed upland sward. I. Effect of *Rhizobium* inoculation on the early development of white clover. D. G. Jones, J. M. M. Munro, R. Hughes and W. E. Davies (*Plant & Soil*, 1964, **21**, 63—69).—The establishment and early development of inoculated S.100 and S.184 white clover sown with grass was far superior to that of uninoculated clovers on blanket peat. Indigenous rhizobial population was low, with a high proportion of ineffective and intermediate strains. Liming encouraged the multiplication of effective strains and increased nodule size. A. H. CORNFIELD.

Effect of legumes on yield of unfertilised pastures. C. R. Horrell (*E. Afr. agric. for. J.*, 1964, **30**, 94—96).—The effect of sowing two tropical legumes, *Stylothanthes gracilis* and *Calopogonium orthocarpum*, with Rhodes and Guinea grass were studied. Over 4 years forage yields were not affected by the presence of *C. orthocarpum* with either grass. In the first year *S. gracilis* had no effect on forage yields but in the next 3 years yields were increased by 35—60%. Guinea grass gave higher yields than did Rhodes grass and the presence of *S. gracilis* increased forage yields to about the same extent with both grasses. A. H. CORNFIELD.

Response of grass-clover and pure-grass leys to irrigation and fertiliser nitrogen treatment. I. Irrigation effects. D. Reid and M. E. Castle (*J. agric. Sci.*, 1965, **64**, 185—194).—A potential irrigation requirement of 10—14 weeks was found in each of 3 years. On average 3·63 and 5·13 acre-in./year were applied respectively to provide 2 and 0·5 in. deficit. The mean response to irrigation was 168 and 156 lb. of dry herbage in the 2 and 0·5 in. deficit treatments respectively. A 5 acre-in. of irrigation produced about the same increase in herbage yield as did 40 lb. of fertiliser N/acre. Irrigation had no significant effects on clover content of a mixed herbage, yield of clover, crude protein content of a mixed herbage or the botanical composition of swards. M. LONG.

Effect of cobalt on the growth of young lucerne on a siliceous sand. J. K. Powrie (*Plant & Soil*, 1964, **21**, 81—93).—Nodulation and growth of young lucerne on a siliceous sand (pH 5·7) were greatly increased by inoculation and liming. Application of $CoSO_4$ (4 oz. per acre) in addition increased the amount of N fixed per nodulated plant (but had no effect on non-nodulated plants), nodule size, and dry matter yields of lucerne tops. A. H. CORNFIELD.

Elemental composition of apples in relation to fruit quality, soil acidity, and organic matter. W. E. Nichol and A. R. Mack (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 91—99).—Quality (flavour, texture and appearance) of McIntosh apples was positively correlated with Mn% in the flesh (dry basis) and negatively correlated with Fe% and, particularly, Cu% in the flesh. There was a significant negative correlation between Mn% in the fruit flesh and soil pH to 24 in. Cu% and Fe% in the flesh were sometimes positively correlated with org. matter content of the surface soil and pH of the subsurface soil. A. H. CORNFIELD.

Effects of *N*-dimethylaminosuccinamic acid (B-Nine) on vegetative and fruit characteristics of apples, pears and sweet cherries. L. P. Batjer, M. W. Williams and G. C. Martin (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 11—16).—Application of B-Nine sprays (500—2000 p.p.m.) after full bloom retarded shoot growth and markedly increased the amount of bloom the following spring in apples, pears and sweet cherries. Reduced growth was accompanied by shorter internodes resulting in 80—100% more leaves per linear unit. Other effects were reduction in fruit size, larger leaves, delay in blossoming, shorter fruit stems (in apple), and advanced maturity (in sweet cherry). A. H. CORNFIELD.

Effects of N-dimethylaminosuccinamic acid (B-Nine) on apple quality. M. W. Williams, L. P. Batjer and G. C. Martin (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 17—19).—Application of B-Nine (1000—2000 p.p.m.) three times at 10-day intervals, beginning 15—17 days after full bloom, largely prevented the development of scald in fruit during storage and extended the shelf life of the fruit after removal from storage. The treatment resulted in less water-sol. pectin and slightly more total pectin in the fruit after storage.
A. H. CORNFIELD.

Effect of pre-blossom defoliation on the cropping of Cox's Orange Pippin apple. V. D. Arthey and E. H. Wilkinson (*Hort. Res.*, 1964, **4**, 22—26).—Defoliation of spur leaves at the mouse-ear, green cluster, and pink bud stages showed the resulting reduction in fruit yields to be related to the time of defoliation rather than to the total leaf area removed. This effect may be related to differences in the gross amount of auxin removed in the spur leaves at the different stages of development. A. G. POLLARD.

Effects of varying levels of potassium and the leaf-roll virus upon mineral content of grape leaf tissue. D. F. Millikan, S. R. Koirtyohann and W. J. Upchurch (*Plant Dis. Reptr.*, 1965, **49**, 36—38).—The K% (dry basis) in the leaves of grape grown in sand culture was not affected by the presence of leaf roll virus when K in the nutrient was low, but decreased, in comparison with virus-free plants, with increasing level of K in the nutrient. Leaf Mg% was decreased by the presence of the virus, but only late in the season and with high K in the nutrient. Leaf Ca% was unaffected by the presence of the virus. A. H. CORNFIELD.

Growth and chemical composition of citrus seedlings as influenced by sodium additions to soils low in exchangeable K. A. L. Page and J. P. Martin (*Soil Sci.*, 1964, **98**, 270—273).—Two non-calcareous soils, both naturally low in exchangeable K were used. The exchangeable K levels in both soils were further reduced by cropping or by leaching with acid. After these treatments the soils were used to prepare 70% base-saturated soils and base-saturated soils containing a 0·25% excess of $CaCO_3$. Adjustments were also made to give known amounts of exchangeable Mg. After addition of Ca, Mn and Zn all soils received P and 0·05% vinyl acetate–maleic acid copolymer (VAMA). Sweet orange seedlings were planted and, N fertiliser was added. Increasing the levels of exchangeable Na at low levels of exchangeable K had no effect on growth at 9 months, nor did the degree of base saturation. Addition of K to both soils significantly increased growth. Na additions to K-deficient soil did not improve the growth of the plants and over the range studied; the amounts of Na in the leaves were independent of Na levels in the soil even under acute K shortage. K-deficiency symptoms were present in some plants and addition of Na had no effect on this. T. G. MORRIS.

Soil compaction effects on tomatoes. J. K. Greig, M. E. Fogleman and L. E. Wittsell (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 490—496).—Compaction of a silt loam to a bulk density >1·7 g. per cc. reduced root penetration but did not affect total root wt. by the end of the season. Compaction reduced vine growth and fruit yields but had no other apparent effects on the plant. A. H. CORNFIELD.

Radio-tracer studies of carbohydrate catabolism on tomato fruit. J. C. Ramsey (*Dissert. Abstr.*, 1964, **25**, 3230).—Glucose-1-,-2- and -6-^{14}C were incorporated into individual tomato fruits with a view to examining a possible triose recombination and the pathway of the synthesis of C_4-acids in the fruit. The distribution of active glucose confirmed the occurrence of triose recombination by the Embden–Meyerhof–Parnas (EMP) and the pentose phosphate pathways. Org. acids, separated from the fruit, afforded evidence that the C skeletons for the biosynthesis of the fruit acids are largely derived by the EMP pathway in conjunction with the tricarboxylic cycle. C_4-acids in the fruit are probably formed by a CO_2-fixation reaction of the C_3+C_1 type. A. G. POLLARD.

Effect of potassium on tomato growth and production. G. E. Wilcox (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 484—489).—Application of K to a silt loam containing 100—150 lb. of exchangeable K per acre increased tomato yields by about 30%. There was little difference in response between applications of K ranging from 200 to 1000 lb. per acre. The treatment increased internode length and decreased fruit drop. Optimum fruit yields occurred with leaf K% (dry basis) of 2·3% or greater. A. H. CORNFIELD.

Greenhouse tomato nutrition—a growth analysis study. G. M. Ward (*Plant & Soil*, 1964, **21**, 125—133).—The content of major elements in various parts of the greenhouse-grown tomato plant are reported. The total uptake of nutrients by the plant on an acre basis was N 345, P 74, K 716, Ca 295 and Mg 43 lb. Such figures are useful for indicating the fertiliser requirements of the crop.
A. H. CORNFIELD.

Phosphorus and potassium nutrition of tomato transplants. W. S. Murphy (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 478—483).—On a sandy loam P applications produced greater responses of tomato transplants than did K. K, 100 lb. per acre, tended to produce smaller plants than did 50 lb. of K. P% and K% in the stem tissue were directly related to the amounts of P and K applied. Where P and/or K was not applied NO_3^- accumulated in the stem tissue.
A. H. CORNFIELD.

Tomato fruit bronzing. J. E. E. Jenkins, D. Wiggell, and J. T. Fletcher (*Ann. appl. Biol.*, 1965, **55**, 71—81).—Two types of tomato fruit bronzing and factors affecting them are described, and a histological examination of both types is reported.
A. H. CORNFIELD.

Relation between locular seed density and the expression of blotchy ripening in tomato. J. W. Berry, jun., R. L. Carolus and R. R. Dedolph (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 497—501).—Blotchy outer wall tissue development in tomato fruit was generally associated with underlying locular areas of relatively low seed density. In these areas auxin activity was reduced; this, in turn, impared normal respiratory activity and vascular functioning. In fruit developing under conditions limiting total substrate available for lycopene pigment development, areas of low seed density may be incapable of synthesising the necessary substrates. As a result outer wall tissue exhibiting blotchy ripening is localised over areas of low seed density.
A. H. CORNFIELD.

Colour disorders of ripening tomatoes. V. Fruit colour in relation to irrigation and nutrition. M. J. Woods (*Irish J. agric. Res.*, 1964, **3**, 141—150).—In trials extending over 4 years applications of K (500—1000 lb./acre, as K_2SO_4) did not affect the occurrence of green blotch; in one year the incidence of yellow blotch and green-back was diminished. By increasing the water supply to the plants green-back was increased only in one year and yellow blotch increased in 2 years but the incidence of green blotch was scarcely affected. The proportion of uniformly coloured fruit was increased by K dressings in only one year and by lowered water supply in two years. The proportion of non-uniformly coloured fruit varied considerably between years. A. G. POLLARD.

Responses of southern pea, *Vigna sinensis*, to photoperiod and nitrogen. B. B. Brantley (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 409—413).—There were no differences in time of flowering and no. of flowers in the southern pea between day-lengths of 8 and 16 h. when grown in sand culture. Plants receiving 100—300 p.p.m. of N in the nutrient flowered one week earlier and showed greater flowering and fruit set than did those receiving 20 p.p.m. The high levels of N increased topgrowth wt. with both day-lengths, but increased height growth only with the long days. Tissue N% was highest with the short days and high N supply.
A. H. CORNFIELD.

Phosphorus and nitrogen metabolism in soya-beans as influenced by potassium. P. R. Henderlong (*Dissert. Abstr.*, 1964, **25**, 3245).—Yields of greenhouse-grown soya-beans increased exponentially with time regardless of K applications; increase in K levels raised the rate of increase in yield. Uptake of P was not associated with the K level; uptake of N was directly related to dry matter production rather than to K levels. The % of P and K in the plants were inversely related, probably as the outcome of a dilution effect of the increased crop due to K applications. Examination of changes in the nucleotide-P in leaf tissue associated with K levels and PO_4^{3-} accumulation indicated that K is concerned in the esterification of inorg. P in coupled oxidative phosphorylation. P metabolism exerts a priority over N metabolism in respect of K when the K supply is inadequate for both. A. G. POLLARD.

Influence of various factors on the longevity of potted *Chrysanthemum morifolium* flowers. B. G. Wesenberg and G. E. Beck (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 584—590).—Longevity of cut flowers was not appreciably different between a single fertiliser application at potting and weekly application of NPK fertiliser in solution. Longevity was similar between a sand–peat and a soil medium, although plant height was less and maturity 3 days later with the former medium. Soil treatment with Phosphon-D had no effect on, shading during growth reduced, whilst flower treatment with N^6-benzyladenine increased, flower longevity. A. H. CORNFIELD.

Interaction of root- and foliar-applied nitrogen on the growth of *Chrysanthemum morifolium*. M. M. Meyer, jun., and J. W. Boodley (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 564—567).—Growth of chrysanthemum was poorer with weekly foliar-applied urea sprays than with root-applied NH_4NO_3. When root-N supply was low (>75 p.p.m. in the nutrient) the response to foliar applications of N increased with level of urea (0—16 g. per l.) in the spray, but with higher levels of root-N supply there was little response to foliar urea sprays. A. H. CORNFIELD.

Effect of nutrition and light intensity on expression of leaf breaking virus in geranium, *Pelargonium hortorum*. D. E. Hartley, F. P. McWhorter and L. T. Blaney (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 594—598).—The symptoms of leaf breaking virus (LBV) in geranium plants grown in sand culture during the summer were retained when the culture solution lacked Ca, Mg or K, but were not retained when the solution lacked N, P or S or with the complete solution. With Ca and K deficiency the virus symptoms tended to increase with light intensity. A. H. CORNFIELD.

Chemical composition of foliage as an index of nutritional status in red pine, *Pinus resinosa*, Ait. H. A. I. Madgwick (*Plant & Soil*, 1964, **21**, 70—80).—The effect of K fertilisation on chemical composition (K, Na, Ca, Mg, Mn, P, N, SiO_3 and ash) of near-terminal needles and growth variables (tree height, leader length, branch length, etc.) was studied. Leader length was significantly related to foliar K by a second-order polynomial which accounted for 66% of the variation in leader length. Growth was satisfactory when leaf K (dry basis) exceeded 0·45%. A. H. CORNFIELD.

Interactions of kinetin and temperature on tobacco leaves infected with tomato aucuba mosaic virus. M. J. Daft (*Ann. appl. Biol.*, 1965, **55**, 51—56).—Application of kinetin (10 p.p.m.) stimulated the production of tomato aucuba mosaic virus in detached leaves held at 18° and 28° both in newly-infected leaves and in those infected a week previously. Kinetin counteracted the ageing effect of exposing leaves at 28°. In attached leaves kinetin either stimulated or inhibited virus synthesis, depending on the quantity applied and the age of the leaves. Kinetin increased fresh wt., sol. protein and chlorophyll contents of leaves. A. H. CORNFIELD.

Nitrogen and phosphorus content of leaf tissue in relation to sweet pepper yields. J. R. Thomas and M. D. Heilman (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 419—425).—The N% in the youngest matured leaf was sensitive to changes in the N requirements of the plant. Leaf-N% decreased as the plant approached the green mature stage. There was an inverse relation between leaf-P% and -N%. Where N was lacking leaf-P increased as the fruit matured. Fruit yields were correlated with rate of application of N up to 120 lb. per acre and also with leaf-N%. Yields were not affected by application of P (15—30 lb. per acre). A. H. CORNFIELD.

Sugar translocation in peppermint, *Mentha piperita* L. L. M. Cruz-Perez and D. Durkin (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 414—418).—The distribution of ^{14}C-labelled compounds in the peppermint plant 15—45 min. after exposure of a single leaf to labelled CO_2 was studied. Both upward and downward movement of radioactivity occurred, but radioactivity was distributed mainly basipetally. Chromatographic analysis showed that most of the radioactivity in the 80% EtOH-sol. fraction was in sugars. Verbascose, stachyose and raffinose were the main translocated sugars. A. H. CORNFIELD.

Effects of storage temperature on physiological changes in the edible corms of Chinese waterchestnut, *Eleocharis dulcis*. H. T. DeRigo and H. F. Winters (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 521—525).—Corms stored at 4·4—7·2° developed a sweet taste quicker and were higher in total and reducing sugars than did corms stored at 10—23·9°. Quality of the corms held at the lower temp. was maintained over 4 months. The time required for corms to sprout after removal from storage decreased with increasing duration and decreasing temp. of storage. A. H. CORNFIELD.

Comparison of Kjeldahl and sulphuric acid–hydrogen peroxide methods and their salicylic acid–thiosulphate modifications for determining total nitrogen in grasses. D. M. Ekpete and A. H. Cornfield (*Analyst*, 1964, **89**, 670—673).—The modified Kjeldahl, pre-treatment of samples with salicylic acid and Na thiosulphate or digestion with H_2SO_4–H_2O_2 methods gave similar results whilst the unmodified Kjeldahl method normally gave slightly lower values. Only the modified methods gave complete recovery of NO_3^--N, particularly when this was present at a high level. E. C. DOLTON.

Pest Control

Pesticides and wildlife. K. Mellanby (*Proc. R. Instn Great Britain*, 1964, **40**, 119—128).—A brief general summary of the position. C. V.

Spray drifts in apple orchards. J. B. Byass and G. K. Charlton (*J. agric. Engng Res.*, 1964, **9**, 48—59).—Measurements were made of spray deposition from high-, low-, and very low-vol. applications. It was concluded that drift hazards with most chemicals would no more limit their application in smaller vol. that in high vol. rates, nor did the use of finer, more conc. sprays increase screening or guard tree requirements in orchard spray experiments. Drift deposits contributed to an important extent to spray cover, especially on the upper surfaces of leaves and differences between types of spray in this respect may be significant when biological effects are known. A. H. CORNFIELD.

Residual effectiveness of dust and granular formulations of pentachloronitrobenzene (PCNB). J. H. Reinhart (*Plant Dis. Reptr*, 1965, **49**, 60—62).—A 10% PCNB dust formulation applied to the soil gave very good control of *Rhizoctonia solani* on cotton seedlings initially, but the effectiveness fell off rapidly with time. A 10% granular attaclay formulation gave moderate control initially, reached a max. effectiveness about 60 days after treatment and then gave somewhat lower control. Vermiculite granule formulations were usually less effective than the dust and clay granules. A. H. CORNFIELD.

3,5-Dihalogens-4-hydroxybenzonitriles and soil micro-organisms. J. E. Smith and W. W. Fletcher (*Hort. Res.*, 1964, **4**, 60—62).—The effects of the herbicides Ioxinyl (**I**) and Bromoxinyl on 50 species of fungi and 18 bacterial species in soil showed, in general, the greater toxicity of **I**. In contact with soil both compounds were rapidly deactivated. Since both are fungistatic and bacteriostatic, the soil micro-organisms may renew their growth before detoxication of the herbicides by soil is completed. A. G. POLLARD.

Synergistic effects of compounds related to 2-diethylaminoethyl 2,2-diphenyl-n-pentanoate (SKF 525 A) on the insecticidal activity of pyrethrins. A. N. Bates, P. S. Hewlett and C. J. Lloyd (*J. Sci. Fd Agric.*, 1965, **16**, 289—292).—The tests were made on lesser mealworm beetles, *Alphitobius laevigatus*, and houseflies, *Musca domestica*. Several active compounds resulted from esterification of 2-diethylaminoethanol with diphenylacetic acid and 1-substituted diphenylacetic acids. Active compounds resulted when a 2-diethylamino group was joined to a diphenylmethyl group through an ester, ketone or ether linkage; the analogous amide was inactive. None of the compounds studied approached piperonyl butoxide in synergistic activity. (16 references.) E. M. J.

Control of common bunt, *Tilletia caries*, and dwarf bunt, *Tilletia controversa*. L. H. Purdy (*Plant Dis. Reptr*, 1965, **49**, 42—46).—Almost all of the 33 formulations tested as seed treatments controlled seed-borne common bunt, but only those containing γ-BHC, pentchloronitrobenzene (PCNB), tetrachloronitroanisole (TCNA), or 4-phenyl-5-chloro-1,2-dithiol-3-one (Hercules 3944) controlled both seed- and soil-borne common bunt. Dwarf bunt was controlled by soil surface treatments with γ-BHC, PCNB or TCNA (5—10 lb. per acre). A. H. CORNFIELD.

Control of plant parasitic nematodes by water-dispersed nematicides. I. Laboratory methods with the stem nematode of narcissus, *Ditylenchus dipsaci*, Filipjev. R. E. Purnell (*Hort. Res.*, 1964, **4**, 42—48).—Effects of fumigation with chlorobromopropene, chloropicrim and methyl isothiocyanate were examined. Toxicities of all three nematicides approached 20 times that of formaldehyde and in all cases increased with rise in soil temp. A combination of nematicide treatment with the generally used hot-water-bath steep seems likely to give useful results. A. G. POLLARD.

Factors affecting control of onion bloat, due to *Ditylenchus dipsaci*, by fumigants containing 1,3-dichloropropene in organic soils. G. W. Bird and H. A. Smith (*Plant Dis. Reptr*, 1964, **49**, 33).—Application of 1,3-dichloropropene (53 gal. per acre) to org. soils gave 31—100% control of onion bloat at 22 locations. Better control was obtained when the soil was ploughed or disked to 10 in. before treatment than when no cultivation or only light disking was done. Control was better when the fumigant was applied before, than when applied after, 1st Oct. A. H. CORNFIELD.

Control of nematodes associated with blue spruce, *Picea pungens*. J. M. Ferris and A. T. Lester (*Plant Dis. Reptr*, 1965, **49**, 69—71).—A single application of 1,2-dibromo-3-chloropropane (86 lb. per acre), by injection or soil drenching, to young spruce trees held soil-inhabiting plant-parasitic nematodes at low levels for 24 months. A few trees were killed by the treatment, the loss being greater with injection than with drench application of the nematocide. A. H. CORNFIELD.

Elimination of nematodes from nursery plants by chemical bare-root dips. H. N. Miller and V. G. Perry (*Plant Dis. Reptr*, 1965, **49**, 51—53).—Root-knot nematodes were eliminated from the roots of gardenia and sansevieria by dipping them for 30 min. in *OO*-diethyl *O*-*p*-(methylsulphinyl)phenyl phosphorothioate (Bayer 25141), (600—800 p.p.m.) or *OO*-diethyl *O*-2-pyrazinyl phosphorothioate (Zinophos). Either chemical at 1000 p.p.m. eliminated root-knot nematodes from boxwood, or, at 600—800 p.p.m. the burrowing, nematode from palm plants. A. H. CORNFIELD.

Spraying arabica coffee for the control of coffee berry disease. J. A. N. Wallis and I. D. Firman (*Ann. appl. Biol.*, 1965, **55**, 139—148).—Low-vol. Cu_2O sprays were as effective as high-vol. sprays in

terms of amount of Cu deposited, reduction in inoculum potential and the no. of diseased fruit, and in increasing the yield of coffee in the first year of the trials. A. H. CORNFIELD.

Low-volume spraying to control coffee leaf rust in Kenya. I. D. Firman and J. A. N. Wallis (*Ann. appl. Biol.*, 1965, **55**, 123—137).—Arabica coffee was sprayed with a Cu_2O fungicide (50% Cu) at 0—5% w/w with a mist blower five times per year at approx. 10 gal. per acre. There was a positive linear relationship between log. spray concn. and the amount of Cu retained by the foliage. Spray concn. was negatively correlated with incidence of leaf rust, due to *Hemileia vastatrix*, and positively correlated with yield of coffee. An additional spray in May increased yields further and reduced a heavy rust attack. Leaf fall decreased with increasing spray concn. A. H. CORNFIELD.

Effect of fungicides on leaf rust, leaf retention and yield of coffee. D. A. Burdekin (*E. Afr. agric. For. J.*, 1964, **30**, 101—104).—Of a no. of materials tested sprays of Cu_2O (5 lb. per acre) were the most effective in reducing leaf rust and leaf fall. Sprays of zineb (4·5 lb.) and ziram (4·5 lb. per acre) were as effective as Cu_2O in reducing leaf rust, but not as effective in reducing leaf fall. All treatments increased coffee yields, with no significant differences between treatments. A. H. CORNFIELD.

Incidence and spread of viruses in sweet peas in relation to variety and the use of systemic insecticides. R. Hull and I. W. Selman (*Ann. appl. Biol.*, 1965, **55**, 39—5).—In two field tests common pea-mosaic virus (CPMV) and pea enation mosaic virus (PEMV) accounted for infection in most sweet pea plants. In one test there was evidence of differences in ' field resistance ' among 15 sweet pea varieties to PEMV but not to CPMV. Although soil application of the systemic insecticides disyston and menazon rendered the plants toxic to aphids, it did not significantly reduce the spread of CPMV. Incidence of CPMV infection was significantly correlated with trap catches of *Acyrthosiphon pisum*, *Myzus persicae* and *Aphis fabae* and of PEMV infection with *A. pisum* and *M. persicae*. CPMV was not transmitted mechanically by flower cutting but the rate of infection was increased when the plants were layered. A. H. CORNFIELD.

Recommended analytical methods for pesticides. XXII. Chlorfenson and its formulations. XXIII Fenson and its formulations. XXIV. Demeton-ethyl and demeton-methyl. XXV. Demeton-S-methyl. XXVI. 2,2-Dichloropropionic acid and its sodium salt. Collaborative Pesticides Analytical Committee (*Plant Prot. Bull.*, 1964, **12**, 17—21, 44—45, 70—71, 86—87, 88—91).—The methods are described. A. H. CORNFIELD.

Diquat and paraquat—new agricultural tools. W. R. Boon (*Chem. & Ind.*, 1965, 782—787).—For herbicidal activity in quaternary salts, it is essential to have a mol. in which the pyridine rings are coplanar and from mol. models it was shown that diquat (**I**) possessed a flat mol. While the mol. of trimethylene can be made to assume a flat configuration with some strain other related compounds are incapable of being forced into this configuration. Even the addition of H atoms in the dimethyl quaternary deriv. of 2,2′-bipyridyl will provide sufficient hinderance to prevent the mol. from taking up the desired configuration. Examination of quaternary salts of isomeric bipyridyls, showed that a whole range of quarternary deriv. of 4,4′-bipyridyl are active, the most potent being the dimethyl quaternary deriv., paraquat (**II**). Substitution of any one of the four C-atoms adjacent to the inter-ring bond by a methyl group, gives products which are completely inactive as herbicides. Examples are given. **I** and **II** rapidly desiccate all green tissue with which they are brought into contact but there are variations in the extent of desiccation and in the ultimate kill. **I** is more effective than **II** against broad leaved plants but **II** is the more effective against grasses and detailed examples are given; since both are effective only when applied to green tissue, it is possible to obtain selective weed control by timing the operation; the question of the necessity of ploughing is discussed. It is possible to establish cereal crops satisfactorily many weeks before the land is suitable for mechanical cultivation. C. V.

Herbicides and seed germination. R. J. Yates (*E. Afr. agric. For. J.*, 1964, **30**, 126—128).—Root elongation of cress roots in Petri dishes was stimulated by methylchlorophenoxyacetic acid (Na salt) at concn. of 0·001 p.p.m. or less, but was progressively reduced by further increasing concn. until at 10 p.p.m. there was no growth. Results are discussed in relation to the effects of residues of herbicides in soils. A. H. CORNFIELD.

Herbicidal treatment of certain hardwood trees. A. J. Watson and R. J. Mesier, jun. (*Biokemia*, 1964, No. 4, 18—21).—Foliage sprays, basal stem sprays and injection techniques of 4-amino-3,5,6-trichloropicolinic acid (**I**) are studied. **I** is considerably more effective than 2,4,5-T (**II**) when applied to cut surfaces of hardwood trees, either sprayed into a cut frill or applied with a tree injector. **I** is translocated both upward and downward from the point of injection and appears to have a distinct advantage over **II** in its downward translocation into the root systems thus preventing basal sprouting. Limited lateral translocation occurs but treatments applied to one side of a tree were relatively ineffective. C. V.

Control of *Euclea divinorum*. J. W. P. L. Parker and A. M. Parker (*E. Afr. agric. For. J.*, 1964, **30**, 89—93).—Of many materials tested for control of *Euclea divinorum*, an evergreen woody shrub, the best control was obtained by foliar application of 2,4-D esters in oil solutions. The best time of application was 3 months after the rainfall peak. A. H. CORNFIELD.

Translocation of ^{14}C-labelled compounds in cotton and oak seedlings. M. A. Clor, A. S. Crafts and S. Yamaguchi (*Weeds*, 1964, **12**, 194—200).—The direction of translocation of labelled urea, 2,4-dichlorophenoxyacetic acid (2,4-D) and amitrole (3-amino-1,2,4-triazole) applied to cotton and oak seedlings was towards regions of active growth. Translocation was slower in oak than in cotton seedlings. The rate of translocation decreased in the order urea, amitrole, 2,4-D. High humidity induced rapid and extensive uptake of the materials by oak seedlings. A. H. CORNFIELD.

Effect of herbicides on heterotropic micro-organisms of ponds. G. F. Petruk (*Mikrobiologiya*, 1964, **33**, 1018—1021).—Effects of Na salts of 2,4-D (**I**) and of TCA (**II**) on the dynamics of heterotropic organisms in pond water were studied. **I** (2 kg.) was added with sand during May to water in a fish-nursery pond, vol. 500—600 cu.m. and area 0·15 ha. The water was sampled at five positions, at intervals up to 26 days after addition. No. of organisms and coli titre were determined. The amount of micro-organisms increased from 3300 to 48,000 cells ml. in 2—3 days at position of greatest concn. of herbicide; the coli titre changed from 1·11 to 0·01. Most plentiful organisms were *Protens vulgaris*, *Escherichia coli*, *Bacillus mesentericus*, *B. subtilis and B. mycoides*. Total no. decreased after 12 days, reaching the initial value in 23—25 days. Growth of vegetation was severely retarded. In a similar experiment **II** was sprayed on to reeds in a fish-farm pond; on the second day after treatment the no. of micro-organisms increased and the coli titre declined considerably. The numbers remained unchanged until the 10th day after which they fell rapidly reaching initial values on the 14th day. Many moulds, principally *Penicillium cyclopium* and *Aspergillus versicolor* were isolated from water, the no. in some cases exceeding these of bacteria. Both materials were of value in controlling vegetation and in stimulating heterotropic organisms which increased the productivity of a reservoir. P. W. B. HARRISON.

Thin-layer chromatography of herbicides. III. Acids. H. G. Henkel (*Chimia*, 1965, **19**, 128—131).—The separation of various chlorinated phenoxy-acetic, -propionic and -butyric acids and of several benzoic acids by thin-layer chromatography is reported. The use of a no. of stationary and mobile phases and the retention times found are compiled. Limits of detection by the use of various spray reagents are reported. M. SULZBACHER.

2-Methylthio-4,6-diamino-*s*-triazine derivatives. J. R. Geigy A.-G. (B.P. 954,528, 19.11.62. Switz., 20.11.61).—An improved process for the production of the title compounds (herbicides) (in which at least 1 NH_2 group is substituted by at least 1 org-radical) comprises reacting a 2-halogeno-4,6-diamino-*s*-triazine (substituted as above) with MeSH in presence of an acid-binding agent in a *s*-alkanol solvent. Details are given for the prep. of 4,6-di(ethylamino)-2-methylthio-*s*-triazine (91·5%). F. R. BASFORD.

Triazine derivatives. Deutsche Gold- u. Silber-Scheideanstalt (Inventor: W. Schwarze) (B.P. 955,511, 28.4.61).—Compounds claimed as herbicides are *s*-triazines substituted in the 6-position with $NR^{III}\cdot CO\cdot X\cdot R^{IV}$; in the 2-position by Cl or CCl_3 and in the 4-position by NR^IR^{II} (R^I—R^{III} are H, alkyl or chloroalkyl; R^{IV} is H, alkyl or aryl; X is O, S or N between which and the R^{IV} may be an SO_2 linkage. They are made by interaction of the corresponding chlorocarbonyl analogues with $R^{IV}\times H$. One example is *4-ethylamino-2-chloro-6-N-ethylcarbamoyl-s-triazine*, m.p. 192—193°. H. S. R.

Dithiazine compounds. Deutsche Gold- u. Silber-Scheideanstalt (B.P. 953,519, 16.11.62. Ger., 29.12.61).—Dithiazine compounds (useful as pesticides) are obtained in good yield by interaction of CH_2O (or a substance yielding it) with NH_2R (R is alkyl, aryl, alkaryl, aralkyl, alicyclyl, or heterocyclyl, optionally further substituted by OH, hydroxyalkyl, CO_2H, and/or SH) and H_2S in alkaline solution (or alkali metal H sulphide). Thus, interaction of 25·8% aq. NH_2Me at room temp. with 35% aq. CH_2O and aq. NaHS gives *5-methyldihydro-1,3,5-dithiazine*, m.p. 65—66°. F. R. BASFORD.

Substituted 1-(pyrimidylmethyl)piperazines. Merck & Co. Inc. (B.P. 952,131, 23.10.61. U.S., 31.10.60).—Compounds useful in the treatment of coccidiosis in poultry comprise 4-amino-2-R-pyrimidines (and acid-addition salts thereof) substituted in the 5-position by CH_2R^I, wherein R is alkyl of 1—5C and R^I is a piperazino radical optionally containing 1-5-C-alkyl or 2-5-C-alkenylin the 4-position and 1—2 1-5-C-alkyl elsewhere. Details are given of the prep. of *4-methyl-1-(4-amino-2-methylpyrimid-5-ylmethyl)piperazine trihydrochloride*, m.p. 212—216°. F. R. BASFORD.

α-Thiocarbamoyl-benzaldoximes, their salts, and esters and compositions containing them. Shell Research Ltd. (Inventors: J. Yates and S. E. Callander) (B.P. 955,492, 27.11.61).—The title compounds are substituted in the benzene nucleus by Cl in the *p*-position or in each *o*-position. They are useful as pesticides and are prepared by reacting H_2S with the appropriate α-cyanobenzaldoxime, its salt or ester. One example is *α-thiocarbamoyl-2,6-dichlorobenzaldoxime*, m.p. 105—115°. F. R. BASFORD.

Cyanoformamide. Röhm & Haas G.m.b.H. (B.P. 955,453, 10.9.62. Ger., 16.9.61).—The title compound, $CN{\cdot}CO{\cdot}NH_2$, is an insecticide and is formed by interaction of cyanogen with an alkanol to give a cyanoformimino ether, which is then hydrolysed with HCl in anhyd. ether. H. S. R.

Insecticidal composition. Shell Internationale Research Mij. N.V. (B.P. 955,350, 26.1.62. U.S., 30.1.61).—There is claimed a solid insecticidal composition comprising an org. polymer, mol. wt. >1000 (a thermoplastic polymer or copolymer of vinyl chloride), a compound $(OR)_2PX{\cdot}OM$ (**I**) as active agent (5—75), and optionally a non-insecticidal plasticiser, viz., $R_3^{III}PO_4$ (**II**) and PhOH as stabiliser (0·1—10%), the amount of **I**+**II** forming >70 wt.-% of the composition (R^{III} is alkyl, aryl, aralkyl or alkaryl; R is alkyl; X is O or S; and M is $CR^I{:}CZ_2$, $CR^IZ{\cdot}CZ_3$, or $CR^I{:}CR^{II}{\cdot}CO_2R$; R^I is H or alkyl; Z is halogen; and R^{II} is H, alkyl, or halogen). A typical mixture is prepared from PVC 10 and dimethyl dichlorovinyl phosphate 90 pt. The compositions are stable during storage and resistant to hydrolysis. F. R. BASFORD.

Halogenophenyl thiophosphates. Boehringer Ingelheim G.m.b.H. (B.P. 956,343, 21.11.62. Ger., 30.11.61).—Pesticidal compounds of the general formula $OR^I(OR^{II}){\cdot}PS{\cdot}O{\cdot}C_6H_2Cl_2Br$-2,5,4 are claimed ($R^I$ and R^{II} are Me or Et). In an example, the prep. is described of *Me_2 4-bromo-2,5-dichlorophenyl phosphorothionate*, m.p. 51°, b.p. 140—142°/0·1 mm. Pesticidal compositions containing it are claimed. F. R. BASFORD.

Thiophosphoric acid esters, and insecticidal compositions containing same. American Cyanamid Co. (B.P. 954,234, 21.36.1. U.S., 31.5.50).—Insecticidal compounds of the general formula $OR^I(Y^IR){\cdot}PY{\cdot}S{\cdot}CH_2{\cdot}C_6H_4{\cdot}CN$ (R and R^I are alkyl of 1—4 C; Y and Y^I are O or S, Y being O when Y^I is S) are made by reacting a cyanobenzyl halide with an alkali metal salt of $OR^I(Y^IR){\cdot}PY{\cdot}SH$ at 0—100°. One compound made is *OO-Et_2 S-o-cyanobenzyl phosphorothiolothionate*, m.p. 38—39°. A solution of 0·1% of this in 65% aq. acetone is 100% lethal to aphids. F. R. BASFORD.

Phosphonodithioic acid esters. Stauffer Chemical Co. (B.P. 954,271, 2.7.62. U.S., 5.7.61).—The esters, which have pesticidal (especially insecticidal) properties, have the formula $(OR^I)R{\cdot}PS_2R^{II}$, wherein R and R^I are alkyl of 1—4 C and R^{II} is phthalimido. One example (prep. described) is *O-Et S-phthalimidomethyl ethylthiolothionophosphonate*. F. R. BASFORD.

Fluorodichloromethanesulphenic acid. Farbenfabriken Bayer A.-G. (B.P. 953,688, 2.11.62. Ger., 3.11.61).—Compounds $R{\cdot}X{\cdot}S{\cdot}CFCl_2$ useful as insecticides and acaricides are obtained by interaction of dichlorofluoromethanesulphenyl chloride with R·XH (X is O, S or NR^I; R^I is H, cycloalkyl, aryl or alkyl; or X is residue of a 5- or 6-membered heterocyclic ring; R is alkyl, chloroalkyl, alkoxyalkyl, cycloalkyl, aryl or heterocyclyl, or XR is $S{\cdot}CY{\cdot}R^{II}$; Y is O, S or NR^I; and R^{II} is NH_2 or alkoxy). Directions are given for the prep. of *2-methoxyethyl fluorodichloromethanesulphenic acid ester*, b.p. 72—76°/10 mm. At 0·2% concn. it is 100% lethal to flies. F. R. BASFORD.

Dithiocarbamates. N. V. Philips' Gloeilampenfabrieken (B.P. 952,744, 1.6.60. Neth., 4.6.59).—Compounds $NR^IR^{II}{\cdot}CS_2{\cdot}C(:NR){\cdot}NR^{III}R^{IV}$ are claimed, in which R^I and R^{II} are alkyl of 1—3 C or together comprise alkylene or alkylidene of >5 C; R^I and R^{III}—R^{IV} are H, alkyl of 1—12 C, aralkyl or aryl optionally substituted, or R and R^{III} together comprise alkylene or alkylidene of >3 C. They have bactericidal and fungicidal properties and are prepared by reacting $NR^IR^{II}{\cdot}CSCl$ with an appropriate thiourea. One example is *S-guanyl dimethyldithiocarbamate hydrochloride*, m.p. 132—133° (free *base*, m.p. 70—71° with decomp.). F. R. BASFORD.

Phosphorus-containing hydroquinone and quinone derivatives. Farbenfabriken Bayer A.-G. (Inventors: W. Gauss and O. Bayer) (B.P. 952,294, 22.6.62. Ger., 30.7. and 9.8.61).—There are claimed pesticidal compounds of the general formula $(OR)_2PS_2R^I$, wherein R is alkyl of 1—4 C and R^I is a 2,5-dihydroxyphenyl or corresponding quinone radical optionally substituted in the 3-, 4- and/or 6-position or in the 3,4-position by a fused saturated or unsaturated ring optionally containing a hetero atom, then, where the hydroquinone is formed, oxidising the latter. They can be produced by reacting a corresponding 1-halogeno-1,4-benzoquinone with $(OR)_2PS_2H$ (or a salt thereof). In one (out of 26 examples), a solution of 2,3-dimethyl-1,4-benzoquinone in EtOH is added dropwise with ice cooling to Et_2 phosphorodithionate to give *OO-Et_2 S-(2,5-dihydroxy-3,4-dimethylphenyl) phosphorothiolothionate*, m.p. 90—91°. F. R. BASFORD.

Bis(chloroethyl) disulphide. Hooker Chemical Corp. (B.P. 949,374, 18.3.60. U.S., 23.3.59).—Interaction of 1,1,2-trichloroethylene with $AlCl_3$ and S_2Cl_2 gives bis-(1,2,2,2-tetrachloroethyl) disulphide in 93% yield. The product is an active pesticide against pea aphids, southern armyworm and Mexican bean beetle. F. R. BASFORD.

Fungicidally active sulphites. Rohm & Haas Co. (B.P. 953,335, 9.5.60. U.S., 18.5.59).—The fungicidal sulphites have the formula $CN{\cdot}CR^IR^{II}{\cdot}O{\cdot}SO_2{\cdot}[CH_2]_2{\cdot}Cl$, wherein R^I and R^{II} are alkyl of 1—5 C or chlorinated Me, or R^I is H, or R^I and R^{II} together form an alkylene chain. They are made by interaction of $CN{\cdot}CR^IR^{II}{\cdot}OH$ with $Cl{\cdot}[CH_2]_2{\cdot}SO_2H$. One example (prep. detailed) is *α-cyanopropyl sulphite*, b.p. 96—97°/0·25 mm.; which is highly active against *Stemphylium sarcinaeforme* and *Monilinia fructicola*. F. R. BASFORD.

Organic phosphonic acid derivatives. VEB Farbenfabrik Wolfen (Inventor: M. Mohring and W. Faatz) (B.P. 953,109, 23.8.60).—There are claimed prep. for combating insects in which the active agents are oxonium compounds of a 2,2,2-trichloro-1-hydroxyethylphosphonic acid dialkyl (e.g., Me_2) ester with an ether (triethylene glycol). F. R. BASFORD.

Organic thiosulphonates. Stauffer Chemical Co. (B.P. 949,750, 26.9.62. U.S., 10.10.61).—Compounds claimed have the general formula $CXY{:}CZ{\cdot}CH_2{\cdot}S{\cdot}SO_2{\cdot}Me$, wherein X, Y and Z are H, Cl or Br, at least one of them being Cl or Br. They are obtained by interaction of $CXY{:}CZ{\cdot}CH_2R$ (R is halogen) with a salt of $MeSO_2{\cdot}SH$, and they active against micro-organisms (e.g., *Aspergillus niger*, *Penicillium*, *Escherichia coli*, nematodes, *Rhizoctonia*, *Fusarium* and *Pythium*). In an example, the prep. is described of *3,3-dichloroallyl methanethiosulphonate*. F. R. BASFORD.

Dithiophosphonic acid esters. Stauffer Chemical Co. (B.P. 952,698, 7.8.62. U.S., 18.8.61).—Pesticidal compounds of the general formula $(OR^I)R{\cdot}PS_2{\cdot}CH(CO_2R^{II}){\cdot}CH_2{\cdot}CO_2R^{II}$ are claimed ($R—R^{III}$ are alkyl of 1—4 C). In an example, the prep. is detailed of *O-Et S-1,2-diethoxycarbonylethyl ethylphosphonothiolothionate*. F. R. BASFORD.

Naphthalene derivatives. F. Hoffmann-La Roche & Co. A.-G. (B.P. 953,101, 26.9.62. Switz., 26.9.61).—The compounds claimed are 2H-naphtho[1,8-*cd*]isothiazole-1,1-dioxides with $SCCl_3$ group on position 2 and R^I or R^{II} on positions 3, 4 or 5 (R^I and R^{II} and H, halogen, NO_2, alkyl or acyl). They are active against *Botrytis* and *Venturia*. A representative compound is *2-trichloromethylthio-2H-naphth*[1,8-*cd*]*isothiazole-1,1-dioxides*, m.p. 167—168° (prep. described). F. R. BASFORD.

Substituted benzonitriles. Shell Research Ltd. (Inventor: J. Yates) (B.P. 951,651, 17.2.60).—Herbicidal compounds of the general formula $1,2,6\text{-}CN{\cdot}C_6H_3X{\cdot}ZR$ are claimed (X is Cl or Br; Z is O, S, SO or SO_2; R is hydrocarbon group optionally substituted by one or more halogen, CF_3, NO_2, NH_2, acylamino, OH, hydrocarbyloxy, $O{\cdot}[CH_2]{\cdot}OR^I$, CO_2H or hydrocarbyloxycarbonyl; R^I is H or alkyl of 1—4 C) and are produced by reacting a 2-chloro- or 2-bromo-6-nitrobenzonitrile with MZ^IR (M is alkali metal or NH_4; Z^I is O or S) or a mixture of RZ^IH and MOH, MH, NH_2 or aq. NH_3, at 120—250° in absence of solvent, or in fluid medium, then, where desired, oxidising SR to SOR or SO_2R. One out of 32 examples prepared is *o-phenoxy-2-chlorobenzonitrile*, m.p. 59—60°. F. R. BASFORD.

Animal Husbandry

Comparison of chromic oxide and conventional methods in digestion trials using steers fed pelleted rations. P. A. Putnam, C. J. Elam and D. Everson (*U.S. Dep. Agric. agric. Res. Serv. Tech. Bull.*, 1964, No. 1312, 13 pp.).—In trials involving steers fed pelleted rations at various planes of nutrition fewer collection days are needed when the indirect (Cr_2O_3) method is used. Incorporating Cr_2O_3 in a pelleted, complete ration and taking faecal ' grab ' samples at 9 a.m. and 3 p.m. for 5 days proved to be a satisfactory technique for estimating dry-matter digestion coeff. E. G. BRICKELL.

Rumen metabolism. IV. Effect of carbohydrate on ammonia levels in the rumen of pasture-fed cows and in rumen liquors incubated with ryegrass extracts. J. A. Robertson and J. C. Hawke (*J. Sci. Fd Agric.*, 1965, **16**, 268—276).—Lactating twin Jersey cows with rumen fistulas, stall-fed twice daily on freshly cut clover–rye-grass pasture were used to study the influence of readily fermentable carbohydrate on the utilisation of dietary protein as measured by NH_3 levels in the rumen. Increases, 7·4—14·8%, in N constituents in pasture led to higher max. concn. of NH_3 in the rumen 2—4 h. and higher min. values 6 h. after feeding. In general 900 g. of starch infused into the rumen lowered the NH_3 content to the control levels when additional N, equivalent to ~130—160 g. of protein each feeding period, was included as a pasture constituent. Incubation of rumen liquor with ryegrass extract rapidly produced NH_3. This could be prevented by adding galactose, sucrose, lactose and glucose. One of the factors that can limit the utilisation of pasture protein is the amount of sol. sugars available in the pasture. (26 references.) E. M. J.

Availability of the calcium and phosphorus of plant materials for animals. T. G. Taylor (*Proc. Nutr. Soc.*, 1965, **24**, 105—112).—Phytates influence calcification by interfering with Ca absorption by forming insol. Ca-phytate and by failing to provide inorg. PO_4^{3-} equivalent to the content of org. P. In any particular diet this may act in one, or both of these ways. Thus rickets occurring in animals fed cereal diets low in Ca is due to a deficiency of Ca while that with diets high in Ca results from deficiency of P. The P of phytic acid and phytates may be made available by the action of plant phytases (optimum pH 5) or by phytases present in the intestines of several species. A phytate-splitting enzyme, probably a non-specific alkaline phosphatase was found, but whether bacteria play a rôle in hydrolysing the phytate is not known. The rôle of Ca, Mg, Mn, K and H ions is considered. Vitamin D probably enhances the utilisation of phytate by increasing the production of intestinal phytase and by stimulating Ca absorption thus rendering the phytate more sol. The requirements of animals for vitamin D increase with the proportion of the total dietary phytate-P. (32 references.) C. V.

Use of *Penicillium chrysogenum* mycelium as animal food. S. G. Pathak and R. Seshadri (*Appl. Microbiol.*, 1965, **13**, 262—266).—The mycelial cake, when dried and specially processed, served as a source of protein in place of soya-bean meal in the diet of experimental mice and produced increased wt. as compared with the controls. Further work however would appear to be necessary so as to make the prep. more palatable and less disagreeable in odour. (16 references.) C. V.

Carotene content as a function of crude protein content of Italian rye-grass and red clover. A. Hasler and H. L. Schnetzer (*Schweiz. landw. Forsch.*, 1964, **3**, 329—337).—Crude protein and carotene were determined in the two plants grown in pots under various conditions of soil and N fertilisation. Statistical analysis of results shows positive correlation between the two sets of values and regression equations were calculated. In general the highest carotene-values were found in those plants containing the most crude protein. With the same content of crude protein, the mean content of carotene of Italian rye grass is considerably higher than that of red clover. J. B. Woof.

***In vitro* production of volatile fatty acids and dry-matter digestibility of wheat straw as affected by alkali treatment.** R. K. Wilson and J. O'Shea (*Irish J. agric. Res.*, 1964, **3**, 245—246).—Ground wheat straw was treated with NaOH (0—15 g. in 30 ml. of water per 100 g. of straw), dried at room temp., reground and stored for 26 days. *In vitro* tests showed increased production of total and individual volatile fatty acids and digestibility due to treatment with NaOH up to 9%; larger proportions had no additional effects. A. G. Pollard.

Effect of grazing management on beef production. III. Effect of stocking rate and grazing method on carcass measurements. A. Conway (*Irish J. agric. Res.*, 1964, **3**, 165—174).—Over a two-year period the effects on beef carcass characteristics of three different stocking rates on rotationally grazed (*R*) and continuously grazed (*C*) pasture are compared. The grazing system had no consistent effects on the various factors examined. Individual carcass wt. were significantly lowered by increase in stocking rates. Carcass production per acre was increased under both grazing systems by raising the stocking rate from low (1·0) to medium (1·75/acre). Further increase in stocking from 1·75 to 2·5/acre, increased production/acre under *R* but decreased it under *C*. A. G. Pollard.

Nutrition of the early-weaned calf. VIII. Effect on nitrogen retention of diets containing different levels of fish meal. T. R. Preston, F. G. Whitelaw, N. A. MacLeod and E. B. Philip (*Anim. Prod.*, 1965, **7**, 53—58).—N retention was higher on diets containing 21·7% and 19·4% of crude protein than on diets containing 16·8% and 14·8%, although as % of dietary intake it was less on the 21·7% crude protein diet. A calf of 82 kg. live-wt. gaining 909 g. daily required between 270 and 340 g. of digestible crude protein. M. Long.

Productivity and nutritive value of tropical grass/legume pastures rotationally grazed by N'Dama cattle at Ibadan, Nigeria. I. I. Okorie, D. H. Hill and R. J. McIlroy (*J. agric. Sci.*, 1965, **64**, 235—245).—Live-wt. gains in the first year at a stocking rate of 1·7 steers/acre are 207 lb. per acre and in the second 270 lb. for steers and 283 lb. for heifers at a stocking rate of 2·7 beasts per acre. *Cynodon plectostachyus* and *Centrosema pubescens*, + *Chloris gayana* and *Digitaria decumbens* + *Stylosanthes gracilis* pastures slowly revert to the dominant species of the first pasture. Crude protein content and crude fibre determinations show that the pastures are of good nutritive value. No significant differences in digestibility are found between the different pastures. M. Long.

Intensive beef production. IV. Effect of nitrogen retention of all-concentrate diets containing different levels of fish meal. H. B. Bowers, T. R. Preston, I. MacDonald, N. A. MacLeod and E. B. Philip (*Anim. Prod.*, 1965, **7**, 19—25).—At a mean live-wt. of 150 kg. the N retention increased up to a max. of 41·5 g. per day on a diet containing 22% of crude protein. At 240 kg. live-wt. the corresponding retention was 37 g. with 20% of dietary crude protein. Faecal N excretion per unit body-wt. was approx. constant at all levels of protein and at both live-wt. whilst urinary N excretion increased with protein level and was higher at the higher live-wt. M. Long.

Association between depot fat mobilisation and the presence of xanthophyll in the plasma of normal sheep. D. S. P. Patterson (*J. agric. Sci.*, 1965, **64**, 273—278).—The yellow-to-green colour of plasma of starved sheep disappears on refeeding and can be identified as xanthophyll, being mobilised from the fat depots. With non-esterified fatty acids mobilisation from depot fat is immediately inhibited by intravenous glucose injection, xanthophyll mobilisation is affected much less rapidly. M. Long.

Effects of protein intake on the storage of copper in the liver of sheep. A. MacPherson and R. G. Hemingway (*J. Sci. Fd Agric.*, 1965, **16**, 220—227).—The amount of Cu, administered orally at ~1 g. of $CuSO_4.5H_2O$ per day, required to kill a sheep (housed) ranges from 48 to 156 g. and this wide range may depend on live wt. liver size and composition of the diet. Death from Cu poisoning might occur after 3—6 months if the diet contained ~250 p.p.m. of Cu. A high protein intake may make sheep less susceptible to this order of Cu supplementation, but if only small amounts of supplementary Cu were given (10 mg./day) additional protein (dried blood meal) had no such effect. The sheep given 10 mg./day of supplementary Cu stored 3% in the liver as did those which died most rapidly when given 250 mg./day of Cu supplement. (10 references.) E. M. J.

Effect of presence or absence of rumen ciliate protozoa on some blood components, nitrogen retention and digestibility of food constituents in lambs. A. R. Abou Akkada and K. El-Shazly (*J. agric. Sci.*, 1965, **64**, 251—255).—Inoculated lambs had higher blood-haemoglobin and -protein N, and lower blood-reducing sugars, $-NH_4$-N and -urea-N than had uninoculated animals. The daily N retention was significantly higher in the inoculated lambs. M. Long.

Sustained release of chromic oxide in the rumen of the sheep from a chromic oxide–dental plaster pellet. J. E. Troelsen (*Anim. Prod.*, 1965, **7**, 127—129).—Mean values for Cr_2O_3 recovery approached 100%, but differences between individual collections were too large to justify use of the method. Increasing acidity of the ingesta did not cause an increase in disappearance of pellets from the rumen and reticulum. M. Long.

Value of feather meal as a protein supplement for growing pigs. R. S. Barber, R. Braude, A. G. Chamberlain, Z. D. Hosking and K. G. Mitchell (*Anim. Prod.*, 1965, **7**, 103—110).—On the basis of N balance trials, 5% feather meal was inferior to 7% white-fish meal when both supplements supply the same total crude protein in a barley–weatings–minerals–vitamins ration. Partial replacement of white-fish meal by feather meal gave inconclusive results. M. Long.

Effect of parenterally administered iron upon outdoor-reared piglets. E. A. Walker and J. H. Taylor (*Anim. Prod.*, 1965, **7**, 1—6).—Piglets receiving 200 mg. of Fe as a parenterally administered Fe-dextrin complex show a small positive growth response which in the case of those reared in pens, as opposed to free range, is significant. Differences in blood-haemoglobin levels, mortality and incidence of runt piglets are not significant. Total body-Fe increases during the first 3 weeks of life by a mean of 339 mg., 295 mg. of which is derived from the soil. M. Long.

Influence of various levels of dehydrated lucerne meal in growing-finishing swine rations with and without lucerne pasture. D. M. Danielson (*Dissert. Abstr.*, 1964, **25**, 3184—3185).—Pigs averaging 70 lb. live-wt. were fed one of six rations in which part of the maize of the basal ration was replaced by dehydrated lucerne meal in proportions 0—32%. Ninety test animals were fed the pelleted ration *ad lib* in confinement and others received the same ration but on lucerne pasture. The time required for animals to reach market wt. (210 lb.) was lowered by the 2, 4 and 8% replacement rations; the 14% replacement ration required the same time as the basal ration and with the 32% replacement 10 days more were needed. With pigs on pasture, increase in the lucerne % in the ration increased the no. of days to market wt., the 32% replacement ration requiring 17 days longer than did the basal ration. Feed consumption per unit gain in wt. increased with the amount of lucerne meal in the ration, there being little saving in feed consumed by the animals at pasture. Lucerne meal increased carcass length in animals in dry lot or on pasture, but lowered loin wt. although improving carcass grading. Digestibilities of dry matter, crude protein, energy and crude fibre were lowered by lucerne as pasture or as dry meal. A. G. POLLARD.

Effect of iron given orally and by intramuscular injection on live-weight gains and survival of suckling pigs. J. F. O'Grady (*Irish. J. agric. Res.*, 1964, **3**, 239—243).—Effects of oral doses of reduced Fe (1000—2000 mg.) at 3, 10 and 17 days of age were compared with those produced by injection of 200 mg. of Fe as Fe dextran. Injected Fe gave the faster early growth from 3 to 6 weeks and increased survival no. over the period of 10 to 56 days of age. G. A. POLLARD.

Unidentified growth factor in dried whey. Y. Y. Al-Ubaidi and H. R. Bird (*Poultry Sci.*, 1964, **43**, 1484—1488).—Chick growth using a purified diet (*ibid.*, 1956, **35**, 705) gave max. response with 4% dried whey added to the diet. Dried whey and fish solubles contained different growth factors, since growth response from a combination of low levels of both supplements was better than from higher levels of either supplement singly. However, there were indications that fish solubles contained a low level of the whey factor as well as its own factor. A. H. CORNFIELD.

Levels of calcium and phosphorus in the diet of young growing chickens. A. L. Mehring, jun. and H. W. Titus (*Poultry Sci.*, 1964, **43**, 1474—1484).—Chicks fed diets containing $<1{\cdot}0\%$ of Ca or $<0{\cdot}6\%$ of P showed poor growth and feed efficiency, developed rickets and had low tibia ash levels; these deleterious effects increased with decreasing level of Ca and P in the diet. Chicks fed diets containing 1·0% or more of Ca and 0·6—0·8% of P showed optimum growth and feed efficiency. Chicks receiving 1·0% Ca and 1·0—1·2% P showed reduced growth, feed efficiency and tibia ash. Growth was a more sensitive indicator of excessive levels of P in relation to the level of Ca than was bone ash. However, bone ash was a more sensitive indicator of the adequacy of the levels of Ca and P than was growth. A. H. CORNFIELD.

Pantothenic acid and unidentified requirements of young ring-necked pheasants and bobwhite quail. M. L. Scott, E. R. Holm and R. E. Reynolds (*Poultry Sci.*, 1964, **43**, 1534—1539).—The min. pantothenic acid requirement for growth and feathering of pheasants and quail to 4 weeks of age was 0·01 g. per kg. of diet. A purified diet highly enriched with all known nutrients and known to promote excellent growth and development in young domestic chickens, failed to support normal growth in pheasants and quail and caused hock enlargement and leg bowing in the pheasants. Brewers' dried yeast, liver and glandular meal contained an unknown factor(s) which prevented these troubles. A. H. CORNFIELD.

Effect of high dietary vitamin-A levels on the utilisation of carotenoids by broilers. P. N. Dua and E. J. Day (*Poultry Sci.*, 1964, **43**, 1511—1514).—In three trials carotenoid utilisation (toe-web skin carotenoid content) was depressed when 6000, 8000 and 22,000 i.u. of vitamin A or higher levels were added to the diet of broilers to 8 weeks of age. There was a high correlation between toe-web and serum-carotenoid levels, indicating that either method is suitable for measuring carotenoid utilisation. A. H. CORNFIELD.

Utilisation of vitamin A and carotene by different breeds and strains of chickens. E. M. Olsen, D. C. Hill and H. D. Branion (*Poultry Sci.*, 1964, **43**, 1488—1501).—Growth, livability and liver storage data from two strains of Columbian Rock and a strain of New Hampshire chicks indicated a min. requirement of <600 i.u. of vitamin A, but rather more of carotene, per lb. of feed. Requirements were similar between the strains and breeds, except that the New Hampshires deposited more vitamin A in their livers than did the Columbian Rocks. A. H. CORNFIELD.

Histological effects of nutrient deficiencies on chick bone development. W. O. Pollard and R. D. Creek (*Poultry Sci.*, 1964, **43**, 1415—1420).—The histopathology of bone from chicks fed diets deficient in niacin, biotin or folic acid was studied. In the epiphyseal cartilage from folic acid-deficient chicks there appeared to be interruptions of cell formation, maturation, and conversion to bone. A. H. CORNFIELD.

Effect of dietary vitamin A and temperature on performance of White Leghorn pullets. A. A. Kurnick, B. W. Heywang, B. J. Hulett, M. G. Vavich and B. L. Reid (*Poultry Sci.*, 1964, **43**, 1582—1586).—Increasing the dietary vitamin A from 500 to 3000 U.S.P. units per lb. of diet had no significant effect on wt. gains or feed efficiency to 10 or 20 weeks of age in a subtropical semi-arid climate. Liver-vitamin A level increased with level of vitamin in the diet, and liver storage of vitamin A was higher during cool than during hot weather. A. H. CORNFIELD.

Effects of cooked and raw soya-beans supplemented with niacin or a multi-enzyme preparation on the nutrition of chicks. Harbhajan Singh, P. J. Schaible, H. C. Zindel and R. K. Ringer (*Quart. Bull. Mich. agric. Exp. Sta.*, 1964, **47**, 17—23).—Food consumption by chicks was less when raw than when cooked soya-bean meal was used in the ration; difference in performance of the chicks was largely the outcome of differences in food intake. Histological differences in the pancreas (enlarged by the raw beans) are described. A. G. POLLARD.

Avian disease virus and nutrition relationships. V. Relation of vitamin K reserves to prothrombin times in chicks infected with Newcastle disease virus. R. L. Squibb (*Poultry Sci.*, 1964, **43**, 1443—1445).—Increased prothrombin times, indicating a higher requirement for vitamin K, were observed in chicks during a Newcastle disease virus infection. This phenomenon, related only to the early stages of the virus cycle, occurred in birds conditioned on diet that provided low vitamin K reserves. A commercial diet or a single dose of menadione-$NaHSO_3$ (0·00024 g. per chick) injected 24 h. prior to sampling blood the 5th day post-inoculation, provided sufficient vitamin K activity to maintain normal prothrombin times. A. H. CORNFIELD.

Influence of sodium sulphate on chlortetracycline content of the blood of chicks. T. S. Nelson, H. T. Peeler, and A. C. Walker (*Poultry Sci.*, 1964, **43**, 1546—1550).—Addition of 0·5—2·0% of Na_2SO_4 to diets containing chlortetracycline (CTC) (460 p.p.m.) and 0·65—1·40% of Ca (as $CaCO_3$ or $CaHPO_4+CaCO_3$) increased blood-CTC levels. This effect of Na_2SO_4 increased with the level of supply and was more effective in the low- than in the high-Ca diets. The omission of supplemental Ca from the diets depressed wt. gains. A. H. CORNFIELD.

High calcium diets for turkey hens. L. S. Jensen, R. K. Wagstaff, J. McGinnis and F. Parks (*Poultry Sci.*, 1964, **43**, 1577—1581).—Hatchability in Broad Breasted Bronze turkeys was not depressed by a level of 3·25% Ca as compared with 1·41% of Ca in the diet. Adding extra P or trace elements (Mn, Fe, Cu, Co, I, Zn) to the high-Ca diet did not affect the performance of the hens. Egg production, feed efficiency, and hatchability of fertile eggs did not differ significantly among hens fed isocaloric diets containing 1·75—6·25% of Ca. A. H. CORNFIELD.

Effect of copper additions to purified turkey diets. W. C. Supplee (*Poultry Sci.*, 1964, **43**, 1599—1500).—Addition of Cu^{2+} (up to 300 p.p.m.) to an isolated soya protein–glucose diet had no effect on growth of poults to 4 weeks of age, but addition of 800 p.p.m. of Cu depressed wt. gains. The extent of growth reduction due to the high Cu level decreased with increasing level of Zn (100—800 p.p.m.) in the diet. The poorer growth on the high-Cu diet may have been due to off-odours developing in the stored diet rather than to a toxic effect of Cu. A. H. CORNFIELD.

Effect of sex upon the distribution of zinc in the adult fowl. D. E. Turk (*Poultry Sci.*, 1964, **43**, 1472—1474).—The concn. of Zn in the femur, blood plasma, brain and skin of the female adult fowl was significantly greater, whilst that in the thigh muscle was significantly lower than that in the male adult fowl. There was a significant correlation between the femur-Zn content of the females and egg production of the birds during the month previous to analysis. A. H. CORNFIELD.

Calcium metabolism of pullets at the onset of egg production as influenced by dietary calcium level. S. Hurwitz (*Poultry Sci.*, 1964, **43**, 1462—1472).—A high-Ca diet (4·1% Ca) promoted a higher plasma-Ca concn. and a greater storage of Ca in the ends and cortical segments, but not in the medullary segment, of the femur than did a low-Ca diet (1·2% Ca). The high-Ca diet also increased the Ca/P ratio in all segments, but reduced the retention of ^{45}Ca in the ends and cortical segment. Production of the first five eggs resulted in a loss of stable Ca and ^{45}Ca from the end, the effect being more severe in the low-Ca birds; no such depletion occurred in the medullary segment, but the cortical segment lost some ^{45}Ca. The results indicate that medullary bone in the pullet is formed about 2 weeks before egg laying and its Ca is largely derived from structural bone. A. H. CORNFIELD.

Effects of low levels of calcium in the diet of laying chickens. A. L. Mehring, jun. and H. W. Titus (*Poultry Sci.*, 1964, **43**, 1405—1414).—Compared with the control diet (2·43% Ca) the feeding of diets containing 1·24% or 0·20% of Ca, in particular, had a marked detrimental effect on egg production, shell strength, live wt. and bone ash%. Changing the birds from a low-Ca to a normal-Ca diet or *vice versa* resulted in changes in egg shell strength in the very next egg, changes in egg production in 4—8 days, and in live wt. and egg production within 4 weeks. Some of the birds on the lowest Ca level developed a paralytic condition after 6—8 weeks, but this was completely corrected in a day by administration of 0·25 g. of $CaCO_3$ per bird. The % of ash, Ca or P in the fresh bones was a better indicator of the extent of depletion of Ca than was the % of Ca in the ash of the bone or the ratio of Ca/P in the bone.

A. H. CORNFIELD.

Effects of dewinging on chick growth and laying-house performance of experimental White Leghorns. B. A. Tower, W. A. Johnson and J. M. Dixon (*Poultry Sci.*, 1964, **43**, 1508—1511).—Dewinging, shortly after hatching, had no significant effect on growth or mortality during the first 7 days, but by 16 weeks of age body wt. was less and mortality higher in dewinged than in normal birds. In the laying house over 308 days the treatment had no effect on egg production, but the dewinged birds produced eggs which were of slightly lower wt. than those from normal birds. A. H. CORNFIELD.

Effect of thalidomide on reproduction in the hen. P. A. Kondra, J. L. Sell and J. A. McKirdy (*Poultry Sci.*, 1964, **43**, 1420—1425).—The intensity of laying was increased when thalidomide was added to the diet of hens at 0·1—0·2 g. per kg. body wt. for 6 weeks, but egg wt. was reduced at the higher level. Both levels improved fertility, but hatchability was not affected. The secretion of egg components was also affected significantly. When the level of the drug was increased to 0·6 g. per kg. body wt. per day the hens ceased laying within 12 days. All chicks hatched were normal, and the few dead embryos showed no malformations.

A. H. CORNFIELD.

Water absorption and retention by cut-up broiler parts chilled in polyphosphate solutions. M. Katz and L. E. Dawson (*Poultry Sci.*, 1964, **43**, 1541—1546).—The differences in the extent of water absorption by broiler parts during chilling in ice water was not altered by addition of mixed polyphosphates. There were slight increases in water absorption when polyphosphates were added at 8 oz. per gal., but little effect at higher rates. Loss in wt. during 3 days' storage did not exceed gains during chilling. Max. retention of water gained occurred with 8 oz. of polyphosphates per gal.

A. H. CORNFIELD.

Veterinary toxicological analysis. D. P. Braithwaite and M. L. Sapiro (*E. Afr. agric. For. J.*, 1964, **30**, 81—83).—The use of thin-layer chromatography for the detection of chlorinated hydrocarbon and org. P pesticides and of alkaloids is described.

A. H. CORNFIELD.

Search for a veterinary insecticide. I. Sulphonamides and disulphonamides active against sheep blowfly. D. Greenwood and I. R. Harrison (*J. Sci. Fd Agric.*, 1965, **16**, 293—299).—Laboratory and field tests methods are described for assessing the activity of chemicals for the control of sheep blowfly (*Lucilia sericata*, Meig). Essential properties are discussed; screening results for sulphonamides and disulphonamides (**I**) with and without *N*-substituents are presented. Of **I** none had the persistence necessary for use in a sheep dip, but the activity of **I** was quite specific to blowfly larvae. (19 references.)

E. M. J.

Resistance to BHC in the cattle tick (*Boophilus microplus*) in India. P. P. Chandhuri and R. C. Naithani (*Bull. ent. Res.*, 1964, **55**, 405—410).—Evidence is obtained of an acquired resistance of the ticks to BHC in localised areas. Ticks from a distant untreated area laid more eggs than did those from the local area.

A. G. POLLARD.

Coccidiostatic properties: experimental investigation. O. Siegmann and R. Pohl (*Biokemia*, 1964, No. 4, 6—11).—Addition of chemical prophylactics to the feedstuffs can substantially reduce the incidence of coccidiosis, and 3,5-dinitro-*o*-toluamide (**I**) is specially examined. Four different strains of *Eimeria tenella* and *E. brunetti* were studied in Leghorn chicks given 0·01% of **I** in their feed, the criterion of effectiveness being mortality, reduction in oocyst excretion, wt. development and feed conversion. Varying degrees of infection were employed and reliable degree of immunity was achieved.(10 references.) C. V.

Animal feed compositions. Monsanto Chemical Co. (B.P. 955,316, 29.12.61. U.S., 30.12.60).—There is claimed a feed for ruminant animals comprising a cellulosic roughage component (hay, straw, cottonseed hulls etc.) (≮2), an alkali metal salt of tall oil fatty acids (0·1—20 wt.-%), and optionally an antioxidant, e.g., 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (0·001—1 wt.-%).

F. R. BASFORD.

Improved animal feed compositions. Commercial Solvents Corp. (B.P. 955,642, 27.7.62. U.S., 4.8.61).—There is claimed a palatable nutrient feed composition containing 0·1—25 wt.-% of fermentation residue from the fermentative production of glutamic acid.

F. R. BASFORD.

Drink for calves. Vaasan Hoyrymylly Osakeyhtio (Inventor: K. O. Hyppola) (B.P. 955,347, 11.7.62).—A fluid food for calves (substitute for fresh milk) is produced by mixing skim-milk powder and water with up to 1 wt.-% of linolic, linolenic and/or arachidonic acid, or a deriv. thereof, or a fat or oil containing ≮30 wt.-% of the acid or its deriv. Vitamins, antibiotics and antioxidants may also be added. F. R. BASFORD.

Pyridinium compounds and compositions containing them. F. Hoffman-La Roche & Co. A.-G. (B.P. 953,875, 6.5.60. U.S., 9.11., 11.12. and 14.12.59).—Compounds claimed are 1-(4-amino-2-propylpyrimid-5-ylmethyl)pyridinium halide hydrohalides with Me substituents in the pyridine nucleus. They are useful in the control of coccidiosis in poultry and may be incorporated with the feeding stuff. One compound prepared is *2-methyl-1-(2-propyl-4-aminopyrimid-5-yl)methylpyridinium chloride hydrochloride*, m.p. 234°. H. S. R.

Hydroquinoline derivatives. Monsanto Chemicals Ltd. (Inventor: J. P. Brown) (B.P. 955,739, 10.7.61).—Compounds claimed are hydroquinolines substituted at N by SO_2R (R is org. radical) and in the same nucleus with a hydrocarbon residue. They have mycobacteriostatic, preservative and insecticidal properties. One compound prepared is *2,2,4-trimethyl-1-p-toluenesulphonyl-1,2-dihydroquinoline*, m.p. 127—129°, active against sheep tick larvae.

H. S. R.

Veterinary compositions comprising 17α-hydroxy-6α-methylprogesterone 17-acetate. Upjohn Co. (B.P. 953,107, 21.7.60. U.S., 11.8.59 and 28.6.60).—An orally active composition possessing progestational effects in ovulating mammals comprises 6α-methyl-17α-hydroxyprogesterone 17-acetate (2—200 mg. per unit dose) and a suitable carrier (e.g., a feedstuff). If desired there may also be present a diuretic and an oestrogen, and the compositions may be used to prevent ovulation, etc. in cattle, birds, dogs, etc.

F. R. BASFORD.

Nitrofurfurylidene hydrazines. Norwich Pharmacal Co. (B.P. 952,097, 16.2.61. U.S., 23.2.60).—Compounds claimed for treatment of *Eimeria tenella* in chickens and active against *Staphylococcus aurens* have the formula $RCH{:}N{\cdot}N(COX){\cdot}[CH_2]_2{\cdot}Cl$, where R is 5-nitrofur-2-yl and X is NH_2 or Me. One example (prep. described is *1-(2-chloroethyl)-2-(5-nitrofurfur-2-ylidene)-1-carbamoylhydrazine*, m.p. 194—196°. H. S. R.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Cyanogenic glucoside content of cassava and cassava products. T. Wood (*J. Sci. Fd Agric.*, 1965, **16**, 300—305).—A simple and sensitive assay method was developed. HCN was liberated from the plant tissue by autolysis followed by treatment with acid; it was distilled into Na_2CO_3 solution and later reacted with picric acid to yield orange coloured isopurpuric acid. Details are given of the assay procedure. Amounts of cyanide down to 0·02 mg./sample could be measured and in general replicate assays of extracts gave values within ±10% of their mean. Values were obtained for the HCN content of peeled cassava tuber, cassava leaves, cassava peel, konkonte flour and garri. Storage of plant tissue and tissue extracts at −20° for a few days slowed the breakdown of linamarin and was a suitable means of preservation for a short period. The peel is a rich source of the glucoside. E. M. J.

Drying of rough rice. II. Mechanism and kinetics of the drying process. A. Escardino Benlloch and F. Ruiz Beviá (*Rev. Agroquím. Technol. Aliment.*, 1964, **4**, 479—490).—The course of drying operations was studied using three varieties of rice (Stirpe 136, Balilla and Americano 1600), with initial moisture contents varying from 19·5 to 29·8% (dry basis). With air flow rates over 800 kg./h. m.^{2} (R.H. = 20%, dry bulb 50°) variations in flow rate did not affect the drying rate significantly. For recently harvested rough rice the rate of drying is limited by the rate of diffusion of water from the interior

of the grain to the surface. An integrated equation relating moisture content with drying time to within ±6% is derived. (12 references.)
E. C. APLING.

Rice quality factors. XIV. Confirmative studies on the general validity of the N quality index. Corrected N index. E. Primo, S. Barber and C. Benedito de Barber (*Rev. Agroquím. Technol. Aliment.*, 1964, **4**, 471—478).—The quality evaluation of 20 varieties of rice in order to test the validity of the N quality index (*ibid.*, 1964, **2**, 130, 135) is reported. The N index generally gave a satisfactory quality prediction but was affected by extreme differences in rice kernel size. A correction factor for these differences is proposed. (11 references.)
E. C. APLING.

Esteratic enzymes of wheat germ. C. E. Stauffer (*Dissert. Abstr.*, 1964, **25**, 1544).—The nature of the esteratic enzymes of wheat germ was investigated to clarify their rôle concerning the increase in fat acidity observed in wheat and flour during storage. Three distinct enzymes which hydrolyse ester linkages were separated from an aq. extract of wheat germ. Two are true lipases active only towards substrates emulsified in water. They require an oil–water interface. The third enzyme was an esterase, active towards esters in true aq. solution. Two of the isozymes had iso-electric points lower than pH 5·5, while the third had an iso-electric point above pH 8·6. None was particularly similar to the parent esterase.
F. C. SUTTON.

Enzymic reduction of disulphide bonds in proteins and low-molecular substances in wheat flour. N. I. Proskuryakov and E. S. Zueva (*Dokl. Akad. Nauk SSSR*, 1964, **158**, 232—234).—Content of SH-groups and S-S-linkages and activity of glutathione (**I**) and protein disulphide-reductases (**II**) were determined in 10 samples of wheat flour (70% extraction) from different varieties and locations of growth. Separate determinations of SH- and S-S- in low mol. compounds in flour were made by iodcmetric and amperometric methods. Total SH- in flour was found by rapid dispersion in 8M-urea in presence of EDTA. Total S-S- content was found similarly at 37° in presence of saturated sulphite. Disulphides, chiefly of oxidised glutathione, were present in low mol. compounds. Low mol. thiols amounted to 0·20—0·44 and disulphides to 0·7—0·99 μ-equiv./1 g. flour. In proteins SH-groups content was 0·52—1·16 and S-S-linkages 7·67—14·00. Activity of **I** varied between 1·8 and 3·52 and **II** between 2·6 and 5·2. Changes in content of SH-groups and S-S-linkages on mixing and keeping doughs made from flours with high and low reductase content were followed. Total content of S-S-linkages depends directly on ferment activity. Variation of S-H- and S-S-content in dough at 30° was plotted against storage time up to 3 h. S-H-content diminished by <62·4% in flour with lowest reductase activity and by only 28·4% in flour with highest reductase activity. S-S-linkage content was little changed. After addition of 5% yeast S-H-content of dough remained unchanged for 3—4 h. Radical difference in processes occurring in dough with and without yeast is explained by anaerobic conditions of fermentation. Besides direct effect on properties of simple proteins activity of reductases which reduce S-S-linkages shows also on thiol ferments which participate in different components of metabolism. (12 references.)
P. W. B. HARRISON.

Lipase activity in milled cereal products and its relation to relative humidity. L. Acker and H. O. Beutler (*Getreide u. Mehl*, 1965, **15**, 4—7).—Studies of the relationship between R.H. and the rate of increase of total acidity and fat acidity in wheat, rye and oat meals, and the rate of enzymic hydrolysis of triglycerides by oatmeal, are reported. The variation of lipolytic activity with R.H. for oats was found to parallel the sorption isotherm; activity was significant even at 15% R.H. At 25° and 70% R.H. oatmeal hydrolysed triolein and 1:1 mixtures of triolein and trilaurin (approx. 50% hydrolysis in 15 days) but produced only very slight hydrolysis of trilaurin alone or of tripalmitin.
E. C. APLING.

Quality of Canadian Amber Durum wheat grades and the rôle of a pentosan-rich fraction in macaroni dough quality. G. S. Bains and G. N. Irvine (*J. Sci. Fd Agric.*, 1965, **16**, 233—240).—Results outlined indicate that semolinas milled from the lower grades of Durum wheat contain relatively higher amounts of the crude residue which has a pentosan content ranging from 27·7 to 39·6%. The mixing time and stability of macaroni doughs are increased when this pentosan-rich fraction is added at levels of 2—3% to semolinas of the higher grades (**I**). **I** contained relatively lower amounts of the crude fraction than the lower grades. (35 references.)
E. M. J..

Use of uniform and practical methods for the analysis of flour in bakeries. V. Samardžić, L. Milatović and D. Žanić (*Kem. u. Industr., Zagreb*, 1965, **14**, 91—96).—Chemical and rheological methods for testing baking properties of flours were evaluated. Brands (12) of flour from Yugoslav and North American wheats and their mixtures were tested for moisture, ash and protein content, particle size, maltose value (Rumsey–Ritter test of diastase activity), and dough moisture. Also applied was the Zeleny sedimentation test, Berliner dough swelling test, Kranz-Kozmin dough test, amylogram, farinogram, extensogram and neolaborogram rheological tests designed by Brabender. A correlation of chemical and rheological methods is presented in a series of diagrams and tables. As far as baking suitability of the flours was concerned the chemical and rheological tests were in good agreement. Results of the Zeleny sedimentation test, the Berliner swelling test and the Kranz-Kozmin extensibility test coincided with data obtained by rheological instruments. Maltose value determinations are considered necessary for detailed quality control of flours, but for everyday purposes chemical methods are recommended for the use in flour mills, bakeries and cake-making. The use of one only of the above methods is not sufficient for flour control and mean results of several methods should be used. (12 references.)
A. L. GROCHOWSKI.

Preparation and properties of acid-modified cereal flours. J. C. Rankin, J. H. Samalik, M. M. Holzapfel, C. R. Russell and C. E. Rist (*Cereal Chem.*, 1964, **41**, 386—399).—A small amount of dil. aq. mineral acid is sprayed into the dry flour which is then held for a predetermined conversion time before final dry-blending with a base to neutralise the acid. In turn the product can be hydroxyethylated to broaden its potential industrial applicability. Additional improvements in flow and dispersion properties result, there is less than 3% reducing sugar present, and the products imparted high-strength increases to paper under laboratory-scale sizing operations. (17 references.)
E. G. BRICKELL.

Modified staining method for estimating the germinative capacity of wheat and barley. F. Bloch (*Cereal Chem.*, 1964, **41**, 399—401).—Wheat germs and barley kernels are exposed by partial debranning or dehulling in a hand-operated wire brush barley pearler, breakage being prevented by tempering the grain in water or 20% w/w aq. glycerol. After rinsing, the kernels are divided into 100 or 200 no. portions and soaked in 0·25% aq. 2, 3, 5-triphenyltetrazolium chloride for 1 h., strained, spread on filter paper and the no. of red-stained germ ends counted.
E. G. BRICKELL.

Influence of preparative variables on intrinsic viscosities and sedimentation values of periodate-oxidised starches. W. C. Schaefer, R. C. Burr, C. R. Russell, G. E. Babcock and C. E. Rist (*Cereal Chem.*, 1964, **41**, 406—412).—Cross-linking occurred during oxidation of granular starch and was most extensive at intermediate levels of oxidation, more taking place at 35° than at 1°. Less cross-linking occurred during oxidation of pasted starch. Drying at 105° instead of at room temp. caused little degradation but granular, 96% oxidised starch became cross-linked when treated at pH<4 and degraded at higher pH. (11 references.)
E. G. BRICKELL.

Physicochemical studies of the acid hydrolysis of maize starch. W. G. Hunt, F. T. Henzler and E. A. Sowell (*Cereal Chem.*, 1964, **41**, 375—385).—Sedimentation patterns at various solute concn. of a series of thin-boiling starches were made, using a Spinco Model E ultracentrifuge. From these patterns estimations of concn., sedimentation, heterogeneity, and concn. dependence of sedimentation were obtained for amylose and amylopectin fractions of these starches. Diffusion constants were measured at a 1% solute concn. on the A and B fractions using a Tiselius electrophoresis cell. (11 references.)
E. G. BRICKELL.

Effect of pressure on the α-amylase catalysed hydrolysis of starch. R. G. Griskey and T. Richter (*Biotechnol. Bioengng*, 1964, **6**, 469—471).—Standard starch solutions were hydrolysed with amylase in a rocking, single pedestal autoclave under pressures of 16 to 43·1 atm. After various periods of rocking (e.g., 10, 20, 30 min. etc.) residual starch was determined colorimetrically with I–KI solution. It was shown that even moderate pressures can decrease hydrolysis rate.
J. B. WOOF.

Terminology in field of starch. II. Plan for glossary, first instalment. M. Ulmann (*Ernährungsforschung*, 1964, **9**, 501—512).—In continuation of a previous communication (*ibid.*, 38), lists are given of names of starches, starch products and by-products, varieties of starches, modified starches, and decomposition products and deriv. of starches; the instalment is concluded with an alphabetical index.
P. S. ARUP.

Making yellow dextrins from starches of various origin. M. Magarašević and M. Bartoj (*Kem. u Industr., Zagreb*, 1965, **14**, 85—87).—Optimal conditions for making yellow dextrins from maize, potato and wheat starches in the laboratory were investigated. The method used was the same as for preparing white dextrins. Effects of HCl concn., temp. and duration of heating are reported in a series of diagrams. Suitable HCl concn. were from 0·5 to 1·25%. At below 0·5% HCl concn. white dextrins were obtained. A series of maize dextrins prepared at 1·25% HCl concn. by heating for 2 h. at 120, 125, 130, 150 and 160°, showed progressively higher solubility from 95 to 100%, represented as a straight line on the temp. *vs.* solubility coördinates. Similar dextrins prepared at 0·5% HCl

concn. by heating for 45 min. at 120, 130 and 135° showed solubility of approx. 90, 92·5, and 94%, respectively. A series of potato dextrins was prepared at 0·4% HCl concn. At a constant temp. of 135°, the solubility of these dextrins increased with heating time from approx. 87% on heating for 45 min. to approx. 98% on heating for 2 h. At a constant length of heating, 45 min., the solubility increased with the temp. from 80% at 130° to 97% at 145°. Yellow dextrins from wheat starch were prepared at 0·5% HCl by heating for 45 min. The solubility of the product obtained by heating at 130, 135 and 145° was 89, 92, and 94—98%, respectively. Potato starch yellow dextrins showed best adhesive properties, followed by those from maize starch, while wheat dextrins did not show adhesion at all. A. L. GROCHOWSKI.

X-ray spectrographic analysis of chlorine in bleached flour and its fractions. K. A. Gilles, E. F. Kaelble and V. L. Youngs (*Cereal Chem.*, 1964, **41**, 412—424).—Assuming an even distribution of Cl in the fat of bleached flour it was calculated that the lipid and the water-sol. fractions, which normally comprise about 5% of the total flour, contained >90% of the Cl introduced during bleaching. After these gluten was the third most important repository although the Cl found was extremely low. No appreciable Cl was retained by the starch. E. G. BRICKELL.

Influence of high flour brews on CM bread production. G. M. Trum (*Baker's Dig.*, 1965, **39**, No. 1, 46—48).—The advantages of increasing the total flour fermented in brews are discussed. They include stronger crumb body, retention of crumb resilience and increased bread vol. S. A. BROOKS.

Higher levels of non-fat dry milk in continuous dough processing. F. D. Vidal and I. Traubel (*Baker's Dig.*, 1965, **39**, No. 1, 56—64).—The effect of flour improvers on the continuous dough-mixing process was studied, with particular respect to the ability to incorporate higher amounts of non-fat dry milk solids in bread. Flour treated with azodicarbonamide yielded the best results of the various combinations of maturing and oxidising agents tested. (11 references.) S. A. BROOKS.

Qualitative factors in the evaluation of cookie flours. L. S. Brenneis (*Baker's Dig.*, 1965, **39**, No. 1, 66—69).—Some effects of the variation in the flour used in the prep. of cookies are described. S. A. BROOKS.

Hard wheat composition as related to utilisation in breadmaking. Y. Pomeranz (*Baker's Dig.*, 1965, **39**, No. 1, 70—77).—The rôle of wheat proteins in breadmaking was studied by histochemical methods. Glutamic and aspartic acids were found in wheat predominantly in the amide form. The importance of sulphydryl groups and H bonding in isolation and fractionation of wheat proteins was correlated with their effects on rheological properties of wheat dough and bread quality. S. A. BROOKS.

Improved loaf-softness tester. K. Hlynka and E. L. Von Eschen (*Cereal Sci., Today*, 1965, **10**, 84—87).—A modification of the Hill and Dalby squeeze-tester is described. It has a dial read-out with adjustable zero and a transducer element which records deformation and recovery curves. An applied weight pinches the loaf through a lever system between two bars 4 in. long. This instrument is called a Squeezometer and when compared with a Baker Compressimeter, it gives a curvilinear relationship. The tests are not destructive and the same set of loaves can be used throughout a storage period. I. DICKINSON.

Bulk handling of liquid shortenings. P. M. Koren (*Baker's Dig.*, 1965, **39**, No. 1, 82—85).—Extensive details are given of the procedure for installing bulk handling equipment for liquid shortenings. S. A. BROOKS.

Organoleptic quality of cake and bread made from cobalt-60-irradiated flour and flour from irradiated wheat. B. S. Miller, R. S. Yamahiro, H. B. Trimbo and K. W. Luke (*Cereal Sci., Today*, 1965, **10**, 80—82).—White cake and bread made from flour irradiated with 20,000 to 50,000 rads are distinguishable and less palatable than similar products baked from nonirradiated flour. Irradiating the flour and wheat at the same level gave similar results. Vanilla does not have a masking effect on the odours and flavours caused by irradiation which have been described as goaty, caprylic, ' wet-dog ', musty and mouldy. I. DICKINSON.

Quick-cooking cereal or farinaceous food products. Sister Lauras Infant & Invalid Food Co. Ltd. (Inventors: R. D. Watson, J. McLeod and H. Knock) (B.P. 955,636, 26.3.62).—A process for the prep. of a cereal or farinaceous food product containing starch comprises adjusting the product to be treated to a standard moisture content (e.g., 25—30 wt.-%, by soaking for 20—60 min. in cold or tepid water; or 2—6%, by exposing to air at ~65—140° or i.r. radiation); then causing the product to absorb water in such proportion and at a temp. above the gelatinisation temp. but below the temp. at which the starch cells would burst (e.g., at 77—93° for 15—60 min.); and without further addition of moisture continuing the heating to complete gelatinisation; then drying (at 120—138° or with i.r. radiation *in vacuo*) . The preferred cereal is rice. F. R. BASFORD.

Sugars and confectionery

Amino-acids of sugar cane. I. Amino-acids of cane juice and the effect of nitrogenous fertilisation on the levels of these substances. D. H. Parish (*J. Sci. Fd Agric.*, 1965, **16**, 240—242).—A method, according to Thompson *et al.*, for purifying extracts of plants and isolating the constituent amino-acids was used. Because of the preponderance of asparagine, detection of some of the trace constituents is difficult; 23 amino-acids were detected, pipecolic acid, methionine, tryptophan and β-alanine for the first time, the presence of a hydroxypipecolic is suggested and that of arginine is confirmed. Increase of N fertilisation (0, 30, 60 kg./acre) increases the contents of basic amino-acids; increases the neutral and acidic components at the 30 kg./acre level then decreased them slightly with higher application of N. E. M. J.

Quantitative determination of fructose in the presence of other carbohydrates in foodstuffs by enzymic analysis. H. H. Weichel (*Dtsch. LebensmittRdsch.*, 1965, **61**, 53—55).—The method is based on the spectrophotometric measurement of triphosphopyridine nucleotide (reduced form) (TPNH) (λ max. 340 mμ or 366 mμ) produced separately by glucose and fructose from TPN in triethanolamine buffer (0·3 M, pH 7·5; 0·0031 M $MgCl_2$ or $MgSO_4$) by the enzymes hexokinase (KH), phospho-glucose-isomerase (PGI), and glucose-6-phosphate-dehydrogenase (G-6-PDH). Details are given. For the determination of small amounts of fructose in the presence of glucose, glucose is first destroyed by the action of glucose-oxidase/catalase. The method can also be used for the determination of sucrose (as glucose and fructose) after inversion with invertase. Errors found in the application of the method to the determination of fructose and glucose in various diabetic foods were less than ±1%. E. C. APLING.

Treatment of natural juices extracted from sugar-containing plants. P. R. Payet (B.P. 955,799, 16.5.60. Fr., 20.7.59).—Juice extracted from sugar cane or sugar beet is buffered (e.g., with a phosphate) to the natural pH such that it remains constant at its natural value, and this pH is maintained during clarification, whereby the organoleptic nature is preserved and maintained. F. R. BASFORD.

Sucrose organo-phosphorus esters. Società per Azione Ferrania (Inventors: G. Faraone and A. Giorgetti) (B.P. 955,757, 17.4.62).—The prep. is described of *sucrose octa(diphenylphosphate)* which has outstanding properties for the gelatinising of cellulose esters. F. R. BASFORD.

Sugar product. J. L. Hulett & Sons Ltd. (B.P. 955,533, 20.7.67. S. Africa, 21.7.61).—Sugar-containing fluid is adjusted to pH ~8 (with $CaCO_3$ filter cake); evaporated to 5—15% of water; then the concentrate is kept in saturated or supersaturated state; and is finally mixed with dry particulate material (e.g., granular sugar product) (using stirrers rotating at 50—300 r.p.m.), to give a free-flowing product suitable for use as animal feedstuff. F. R. BASFORD.

Fermentation and Alcoholic Beverages

Effect of hydroxymethylfurfural on yeast. L. K. Stakhorskaya and B. I. Tokarev (*Mikrobiologiya*, 1964, **33**, 1056—1060).—Amounts varying from 0·01 to 1·0% of hydroxymethylfurfural (**I**) were added to solutions containing 2% sugar in Rider medium fermented by industrial alcohol yeasts. 0·2% of **I** halved reproduction of yeast and reduced catalase activity. Below 0·3% **I** (quantity of **I** present in wood hydrolysates) alcohol yield was not affected. Multiplication of yeast ceased with 0·4% **I**. With 1% **I** some sugar remained unfermented and alcohol yield fell to 35% of control value. Severe toxic action of **I** was noted in fermentation of 2% sugar solution by pure culture of *Schizosaccharomyces adherentes*. There was an indication of slight adaption of culture to presence of <0·4% **I** at first transfer. As little as 0·01% **I** reduces yield of fodder yeast to 81% of control value and catalase activity to 83% 0·05% **I** affected aerobic respiration of fodder yeast, measured by Tödt electrochemical method, which could serve as indicator of quality of medium. Experiments showed that growth of yeast, particularly fodder yeast considerably reduced amount of **I** which remained in the medium after fermentation. (12 references.) P. W. B. HARRISON.

Influence of ascorbic acid on the quality of bottled wines. D. Premužić (*Kem. u Industr., Zagreb*, 1964, **13**, 861—867).—The effect of additions of ascorbic acid (30—70 mg. per l.) to various bottled

wines was investigated and compared with the effect of combined addition of 25 mg./l. ascrobic acid and 17·5 mg./l. SO_2, and with the effect of SO_2 only (25 ml./l.). Wines were tested after 1, 3, 6, 12 and 24 months using three parallel samples of each type. Detailed results are reported for Solvene Riesling, Traminer, and three other wines. Additions of 50 or 70 mg./l. resulted in maintaining a low redox potential over the studied period. Organoleptic evaluation did not give conclusive results, in some cases the taste of wine containing added ascorbic acid deteriorated slightly after 1 year; in most cases after 2 years the taste of wine containing 50 mg./l. ascorbic acid and that containing the combined additive was better than that of a similar wine containing either smaller amounts of ascorbic acid or containing SO_2 only. A. L. GROCHOWSKI.

Direct fluorometric determination of malvin in solutions and its application to dilutions of red wine. J. Eisenbrand, O. Hett and G. Becker (*Dtsch. Lebensmittl Rdsch.*, 1965, **61**, 8—11).—The fluorescence of malvin in solution is greatly increased by dilution in acetone, dioxan or glacial AcOH and exhibits a max. at approx. 580 mμ. Malvin in red wine can be determined simply by comparison of the fluorescence intensity at 580 mμ of a 1:100 dilution in glacial AcOH against that of a 1:200 dilution in glacial AcOH of a 10 mg.% solution of malvin hydrochloride in methanol. A fluorescence intensity from the wine dilution greater than that of the standard is evidence of the presence of wine from hybrid grapes. Comparative evaluations of wines of known origin by this rapid method and by the more time-consuming paper chromatographic method (*Bundesgesundheitsblatt*, 1963, **6**, 125) are presented. E. C. APLING.

Thin-layer chromatographic separation and colorimetric analysis of barley or malt lipid classes and their fatty acids. D. E. Walsh, O. J. Banasik and K. A. Gilles (*J. Chromatography*, 1965, **17**, 278—287).—Lipids extracted from barley or malt with light petroleum are separated into four fractions (phospholipids, mono- and diglycerides, triglycerides, and hydrocarbons) by chromatography on a 0·25 or 1-mm. layer of Silica Gel G, and determined colorimetrically with acid dichromate solution at 440 mμ (hydrocarbons) or with Fe^{3+} perchlorate–hydroxylamine reagent at 532 mμ (phospholipids and glycerides). To determine the fatty acid components in each fraction, the lipids are transesterified to the methyl esters by the BF_3 method and the unsaturated esters are separated from the saturated esters by adduct formation with Hg^{2+} acetate and thin-layer chromatography; the individual esters are then separated by thin-layer chromatography and determined colorimetrically.

A. R. ROGERS.

Beer spoilage bacteria and their control with a phosphoric acid-ammonium persulphate wash. S. Bah and W. E. McKeen (*Canad. J. Microbiol.*, 1965, **11**, 309—318).—*Flavobacterium proteus* (**I**). *Acetobacter aceti*, *Acetobacter capsulatum* (**II**), *Pediococcus cerevisiae* (**III**) and *Lactobacillus pastorianus* were isolated from contaminated yeasts. **I** was the most prevalent in ale yeast and **II** and **III** in lager yeasts but the plant appeared to influence the exact distribution. The indicated wash proved very effective and has been used for over four years; treatment is short (2 h.) and this results in a great saving in plant equipment and labour. The fermentative ability and viability of *Saccharomyces cerevisiae* is not affected and this yeast is not killed by a solution six times more conc. than that recommended, e.g., 75 ml. concn. H_3PO_4 (Sp. Gr. 1·75 88%) added to 270 l. water at 5°, and after pH determination 0·75% by wt. of predissolved NH_4 persulphate. Full details of the results are given. (26 references.) C. V.

Analytical determination of methanol in vinegar. Mª. J. Fernández, C. Llaguno and J. Garrido (*Rev. Agroquim. Technol. Aliment.*, 1964, **4**, 491—495).—The chromotropic acid method for the determination of methanol in alcoholic beverages (*Off. Methods of Anal.*, Ass. off. agric. Chem., Wash., 1955, 142) is applicable to vinegar with slight modification of the oxidation conditions and of the solution used as standard. For vinegar, the distillate (1 ml.) is oxidised in the cold for 1 h. with 3% $KMnO_4$ solution containing 15% of H_3PO_4, and the standard solution (treated similarly) containing 0·025% of methanol in 0·6% ethanol. Decolorisation, reaction with chromotropic acid and colorimetry are carried out as in the official method. Results are reproducible to within ±20 mg. per l. (for methanol contents up to 400 mg. per l.). E. C. APLING.

Treatment of hops. J. Fromm, L. Mayer-Bass G.m.b.H. (B.P. 954,879, 8.1.63. Ger., 13.1.62).—Hops for storage and consignment are processed by grinding the green-dried material (and, if necessary, subsequently kiln-dried) to a degree of fineness approximately between chaff and powder (e.g., to 0·1—2 mm.). Grinding is preferably effected in the frozen state in presence of an inert gas, e.g., CO_2 (and a sulphurisation agent if desired), and the ground hops are stored in silos or may be packed in small amounts in air-impervious containers (plastic bags). F. R. BASFORD.

Fruits, Vegetables, etc.

Influence of controlled atmosphere storage on organic acids of apples. D. A. Kollas (*Nature, Lond.*, 1964, **204**, 758—759).—Apple extract from 'McIntosh' pulp tissue was separated into 12 acid fractions by gradient elution with formic and acetic acids from a 23-cm. column of Dowex-1 resin (200—400 mesh, acetate form). Total concn. of free acid was much higher in fruit stored in a controlled atm. (3% O_2—5% CO_2 at 38°F) than in that stored in air at 32°F, malic acid (**I**) accounting for most of the difference (7·4 mequiv./100 g. *vs.* 4·1 mequiv. in air). Other acids, including probably quinic and shikimic, also increased but H_3PO_4 and citric acid (**II**) decreased. The differences are ascribed to varying rate of depletion of acids and/or higher production of certain acids in a specific storage atm. The higher concn. of **I** in apples stored in a controlled atm. may arise from CO_2 fixation rather than from limited oxidation at low O_2 pressures, but the higher concn. of **II** in air-stored fruit is probably the result of O_2-dependence. W. J. BAKER.

Intermediary metabolism in ripening Bartlett pears. J. T. Meynhardt, E. C. Maxie and R. J. Romani (*S. Afr. J. agric. Sci.*, 1964, **7**, 485—496).—Operation of the tricarboxylic acid cycle at all stages of the climacteric was shown by the presence of labelled intermediates of the cycle after incubation with radioactive glycine, citric acid and succinic acid. Interconversion of C between the sol. and protein-N fractions occurred at an early stage of the respiration curve, but not during the climacteric max. Operation of the pyruvic oxidase enzyme system was demonstrated by incubations with pyruvate-1-^{14}C and pyruvate-2-^{14}C. (25 references.) E. G. BRICKELL.

Effects of physiological maturation and storage on physical and biochemical changes in peach (*Prunus persica*, S. and Z.) and apricot (*Prunus armeniaca*, L.) fruits. P. B. Deshpande (*Dissert. Abstr.*, 1964, **25**, 3508).—Peach and apricot fruits were harvested at three different stages of maturation and ripened at 70°F and R.H. 85%. In a second year additional fruits were refrigerated at 34°F and 95% R.H. for 9 days before ripening at 70°F. With advancing maturation and ripening loss of wt. (including decay) pH, sol. solids/acidity, volatile reducing substances, ascorbic acid, taste and flavour scores and carotenoids increased, whereas acidity, firmness and total pectins in the fruits diminished, except that sol. solids in peaches did not increase during ripening. Total reducing sugars increased in apricots and tannins decreased in peaches during maturation; during ripening sugars and tannins did not alter in either fruit. Optimal harvest maturity of peaches for fresh transport was associated with firmness <13 lb., sol. solids >9·5% and sol. solid/acidity >13. Corresponding characteristics for apricots were, firmness <13 lb., sol. solids/acidity, >6, volatile reducing substances, 500—700 mequiv./100 g., total sugars <6% and sugar/acid ratio >4. Pre-ripening refrigeration of both fruits increased storage life by 10 days without deterioration in ripening or dessert quality. A. G. POLLARD.

Leucoanthocyan material from immature peaches. C. Hsia, L. L. Claypool, J. L. Abernethy and P. Esau (*J. Fd Sci.*, 1965, **29**, 723—729).—A leucocyanidin was obtained from immature Elberta peaches by counter-current extraction. The principal leucoanthocyan is a mol. with two flavonoid units, one with a modified cyanidin structure, the other having the configuration of (2R:3S) (+)-catechin. Cyanidin is formed by cleavage of the leucyan in HCl. (25 references.) E. M. J.

Anthocyanin pigments in Bing cherries. D. Y. C. Lynn and B. S. Luh (*J. Fd Sci.*, 1965, **29**, 735—743).—The extraction, purification and identification of anthocyanin pigments in Bing cherries (*Prunus avium*, L. var. Bing are described. They were purified by paper chromatography and identified by R_F values in various solvents, absorption spectra and partial acid hydrolysis into intermediate pigments. The aglycones of the pigments were identified by alkaline degradation into products which were then identified by co-chromatography with known phenolic compounds. (22 references.) E. M. J.

Effect of *p*-chlorophenoxyacetic acid (PCPA) spray on composition and residue in boysenberries. B. S. Luh, D. L. Gutnick and R. S. Bringhurst (*J. Fd Sci.*, 1965, **29**, 744—749).—A 100 p.p.m. spray of PCPA 46 days before harvest produced berries 10—15% larger and heavier than the control sample, total yield being 9% higher for the sprayed sample. A gas–liquid chromatographic method was used to determine PCPA as a ^{14}C-labelled methyl ester on a column of 20% Dow 11 high-vac. silicone grease on Chromosorb. The plants sprayed once, as above, showed a residue of 0·09 p.p.m. in the berries; those sprayed twice at 46 and 33 days before harvest showed a residue of 0·26 p.p.m. (21 references.) E. M. J.

Isolation, characterisation and recovery of pectin from purple passion fruit waste (rind). J. S. Pruthi (*Chem. & Ind.*, 1965, 555—559).—The composition of passion fruit pectin was studied by

paper chromatography. The quality and recovery of pectin is optimum when (i) 0·75% citric acid is used, (ii) the fruit is partially purple, (iii) two 60 min. extractions with a 1·4 solid : extractant ratio are made and (iv) 2·5—5·0 polyphosphate is used. (17 references.) E. C. DOLTON.

New researches into the structure of pectinic substances. H. Neukom (*Dtsch. LebensmittRdsch.*, 1965, **61**, 35—38).—Recent studies of the properties and reactions of pectins, the fractionation of pectinases and the specificity and mode of action of the enzyme fractions are reviewed. Pectinases are classified as polymethylgalacturonases and polygalacturonases, which hydrolyse pectins and pectinic acids respectively, and pectin-transeliminases and pectinic acid transeliminases which split the glycosidic link of pectins or pectinic acids by β-elimination (liberating an anhydro-ester). Each group is subdivided into endo-enzymes, which attack glycosidic bonds indiscriminately (liquefying action, causing rapid fall in η) and exo-enzymes, the action of which is limited to the terminal glycosidic bond of the pectin mol. (saccharifying action, causing only a very gradual drop in η). Commercially available pectinase prep. generally consist of mixtures of enzymes from one or more groups in the classification. (17 references.) E. C. APLING.

Modified method for the micro determination of citric acid. R. Buschbeck (*Pharmazie*, 1965, **20**, 27—28).—The sample (10—60 μg. of citric acid) is converted into pentabromoacetone and this is determined by the red colour obtained on treatment with pyridine in alkaline solution. The average error is $\simeq \pm 4\%$. A. R. ROGERS.

Influence of 31 MeV electron-rays on potatoes. A. Berger and J. Wolff (*Atompraxis*, 1965, **11**, 214—218).—Selected seed potatoes were irradiated. Similar results were obtained to those found with *X*-ray irradiation, wt.-loss diminishing with an increasing dose of electron-rays. The limiting value required to suppress sprouting for 30 weeks' storage is at 20 kr, e.g., a greater value than that attained by irradiation with 60 keV *X*-rays. The correlation between CO_2-formation during storage and the irradiation dose as well as the resulting artificial radio activity are studied and discussed. C. V.

Stability of various vitamins in dried potatoes and dried vegetables. I. Vitamin contents in stored dried potatoes and dried vegetables. A. Schillinger and G. Zimmermann (*Dtsch. LebensmittRdsch.*, 1965, **61**, 45—52).—Determinations of vitamin contents in dried potatoes and dried vegetables stored for up to 3 years under N_2 or *in vac.* are reported. Levels of thiamine, riboflavin and nicotinic acid were unchanged throughout the storage period; no losses in ascorbic acid were observed during the first year, but after 2 years losses varied in the different vegetables from 0 to 42%. β-carotene was well retained in dried carrots and dried parsley (as was ascorbic acid); in other vegetables losses over 2 years varied from 38 to 55%. Stability of thiamine, riboflavin and nicotinic acid was unaffected by the presence of air or moisture, but losses of β-carotene were increased in the presence of air and the rate of loss of ascorbic acid was greatly increased in the presence of air and with increasing moisture content. The methods of determination used are described, and methods for the determination of ascorbic acid in the presence of other reducing substances are critically reviewed. (28 references.) E. C. APLING.

Accelerated freeze drying of green beans. Comparative study of varieties grown in the Valencia area of Spain. F. Piñaga Otamendi, B. Lafuente Ferriols and E. Primo Yúfera (*Rev. Agroquím. Technol. Aliment.*, 1964, **4**, 458—465).—Evaluations of eight varieties of green beans after experimental freeze-drying are reported. Beans were washed, cut transversely into 3 cm. portions, blanched for 6 min. in steam, frozen to −25° and freeze-dried (plate temp. 50°; condenser temp. −52 to −55°; pressure, initial 250 μ, final 50 μ). Determinations of reconstitution ratio, vitamin C, fibre, colour and organoleptic acceptance are reported. The varieties Seminole, Harvester, Tendercrop and Topcrop had the highest acceptability. (10 references.) E. C. APLING.

Varietal, physical and chemical factors affecting the quality of canned dry-line beans. M. N. Hamad (*Dissert. Abstr.*, 1964, **25**, 2729)—Unprocessed peas and beans varied in their total pectic content. Rates of water-imbibition were conversely related to pectic contents. A direct relationship exists between pectic content and the corresponding average drained wt. of the canned products. Chemical, physical and physicochemical means were used to determine the mechanism of action of Myverols in reducing cohesion in canned beans. Pectic fractions were extracted from three varieties of beans and were allowed to complex with the monoglyceride Myverol-1807. The intrinsic η values were obtained for the pectic fractions and their corresponding complexes. Techniques used are given. F. C. SUTTON.

Effect of certain chemicals and pressure on cookability of pulses. P. V. Subba Rao, T. K. Ananthachar and H. S. R. Desikachar (*Indian J. Technol.*, 1964, **2**, 417—418).—The cookability of red gram dhal is studied by determining the amount of dispersed solids. $NaHCO_3$, Na_3PO_4, $(NH_4)_2CO_3$ and pressure cooking all reduce the cooking time. $NaHCO_3$ raises the pH and dcreases the organoleptic quality and pressure cooking is preferred to chemical addition. E. C. DOLTON.

Preliminary note. Soft putrefaction of onions. E. Primo, E. Hernández and P. Cuñat (*Rev. Agroquím. Technol. Aliment.*, 1964, **4**, 496—498).—Investigations into recent spoilage troubles occurring in onions of the variety 'grano de oro' after storage from 2 to 3 months are reported. The external appearance of the spoiled onions is unchanged, but the core is softened, becoming yellow cream in colour, and darkening with time. The spoilage organism responsible was identified by morphological and cultural characteristics and biochemical tests as *Erwinia carotevora*. E. C. APLING.

Use of nisin in the processing of canned asparagus. E. Hernández, L. Durán and J. Morell (*Rev. Agroquím. Technol. Aliment.*, 1964, **4**, 466—471).—Studies aimed at reducing processing time and improving product quality are reported. Large cans (155 × 173 mm.; 3 kg.) of asparagus were inoculated with *Clostridium sporogenes* and dosed with 0, 2, 5 or 10 p.p.m. of nisin, processed for 17 min. at 115—116° and stored for 3 months at 37° and for 6 months at room temp. All cans containing 10 p.p.m. of nisin were in good condition at termination of the storage period; at lower treatments most cans were spoiled by surviving bacteria. E. C. APLING.

Nitrogenous constituents of the dehydrated mushroom, *Boletus edulis*, and their relation to flavour. J. D. Craske and F. H. Reuter (*J. Sci. Fd Agric.*, 1965, **16**, 243—250).—The true mushroom flavour was non-volatile, the residue after steam distillation of dehydrated *B. edulis* being of undiminished flavour intensity. Extracted with water about half of a sample was sol. and this extract contained the flavour components. These were concentrated into the basic fraction by an ion-exchange technique and further separated into fractions containing amino-acids of progressively increasing basicity by ion-exchange chromatographic technique. The most basic compounds tasted most characteristically of mushrooms, but all the N constituents contributed to the overall flavour profile. The flavour contribution of the highly basic components is of greater significance than simple examination of the threshold dilution figure would indicate. (35 references.) E. M. J.

Rapid paper chromatographic method for the detection of aflatoxin in groundnuts. V. Sreenivasamurthy, A. Jayaraman and H. A. B. Parpia (*Indian J. Technol.*, 1964, **2**, 415—416).—The detection and semi-quant. estimation of aflatoxin by paper chromatography using a 5 : 3 : 2 cyclohexane : benzene : AcOH solvent is described. Separation into the B and G components with good resolution is achieved in 1 h. E. C. DOLTON.

Physical and chemical characteristics of bottled fresh and dehydrated horseradish. F. E. Weber II (*Dissert. Abstr.*, 1964, **25**, 1142).—Prepared style horseradish stored 6 months at 0°F showed only a slight decrease in original allyl isothiocyanate (**I**) content and darkening in colour, but at 45°F, the rate of loss of **I** and discoloration showed the preservation effected by low temp. Horseradish powders containing the normal enzyme content or 2% of the normal were made to contain 2% and 5% moisture. After storage at 100°F for 9 months the 5% moisture samples of both enzyme levels darkened, developed a strong off-odour; the 2% samples developed off-odour to be unacceptable but no other degradation in initial qualities. Moisture content rather than enzyme level appears to be the more important factor to stability of dehydrated horseradish. F. C. SUTTON.

Physico-chemical studies on milled olive pastes. XXI. Pressing of olive pastes as a filtration process. J. M. Martínez Moreno, C. Gómez Herrera, C. Janer and J. Pereda (*Grasas y Aceites.*, 1964, **15**, 299—307).—Kinetics of olive oil pressing are studied, the operation being considered as a filtration of an emulsified org. phase in a protective aq. colloidal solution. (29 references.) L. A. O'NEILL.

Composition of fatty matter of nuts during growth and ripening of fruit of *Juglans regia* L. M. O. Mirić and A. F. Damanski (*Fruits, Paris*, 1965, **20**, 3—8).—During growth and ripening steady increases in the content of glycerides (~2—94%) and in the total fatty acids (~68—94%) in the almonds were accompanied by decreases in the acid value (47—0·1%) and in the unsaponifiable matter (25—1·4% in the fatty matter). During the growth stage the most important changes in the fatty acid composition (free or combined) were a decrease in the content of palmitic acid and an increase in the linoleic acid; during the maturing stage there was a marked increase in the oleic acid. Differences in the compositions of the oil and free acids in the seed and endosperm were also examined. P. S. ARUP.

Non-alcoholic beverages

Detection of adulteration of orange juices with additions of β-carotene and carotenoids. Preliminary note. E. Primo and D. Mallent (*Rev. Agroquím. Technol. Aliment.*, 1964, **4**, 499—500).—The additions of mixtures of β-carotene (**I**), β-apo-8′-carotinal (**II**), canthaxanthin (**III**) and β-apo-8′-carotenoic acid methyl ester (**IV**) to orange juice can be detected by thin-layer chromatography of the extracted total carotenoids on plates of Kieselgel G, using a mixture of light petroleum and isopropanol (95:5) as developing solvent. **II** and **III** (R_F 0·22 and 0·12) are recognised as reddish spots; under u.v. light the fluorescence of natural **I** is masked by the presence of synthetic **I** (R_F 0·57) and **IV** appears as a dark spot (R_F 0·35).
E. C. Apling.

Tea, coffee, cocoa

Peptidase activity in shoot tips of tea plant (*Camellia sinensis* L.). G. W. Sanderson and G. R. Roberts (*Biochem., J.* 1963, **93**, 419—423).—The progressive increase in free amino-N in tea shoot tips during withering is due to activity of an endogenous tea peptidase system. A method for the assay of this enzyme system in a $COMe_2$ extract of the tips is described. The peptidase has optimum pH near 5·0 and max. activity is shown at 52° in 1 h. incubations. The activity of the enzyme varies markedly in various parts of the shoot tips and in different cloves. (22 references.) J. N. Ashley.

Organic acids in tea plants. Non-volatile organic acids separated on silica gel. G. W. Sanderson and R. R. Selvendran (*J. Sci. Fd Agric.*, 1965, **16**, 251—258).—The study was particularly of tea shoot tips (flush) comprising the bud and first two leaves, and the included stem, these parts being harvested and used in the manufacture of tea. The major acids found were: oxalic, malic, citric; also found were isocitric, succinic and five unknown acids (>0·1 μequiv./g. fresh wt.). The acid levels varied quant. between clones and between sampling dates. In the manufacture of tea the levels of succinic and malic acids were reduced during withering, that of oxalic acid remained nearly constant. (37 references.) E. M. J.

Refractometric determinations of fat in cocoa products with the aid of tritolyl phosphate. M. Filajdić, J. Desaty and V. Vojnović (*Kem. u Industr., Zagreb*, 1965, **14**, 97—100).—A rapid and sufficiently reliable method suitable for the production control of fat content in chocolate products and in dehydrated soups was developed. It is based on the use of tritolyl phosphate as the solvent for analysed fats (cf. J. Stanley, *Industr. Engng Chem., Anal. Edn*, 1937, **9**, 132). Compared with standard extraction method taking 5—12 h., the tritolyl phosphate refractometer method takes only 15—20 min. Parallel analyses of 30 named samples of chocolate products and dehydrated soups showed that results of the new rapid method were consistently 0·44% lower than those obtained by normal extraction technique. A standard diagram for the tritolyl phosphate refractometer readings was constructed using the least square method for calculating regression equations and based on *n* tables of tritolyl phosphate containing 2·5—95·0% cocoa fat. The refractometer method gives satisfactory results for all lipid components having the refractive index at 40° equal 1·4570 ± 0·0020. For fats more widely different from cocoa fat, separate diagrams are necessary. (32 references.) A. L. Grochowski.

Milk, Dairy Products, Eggs

Temperature changes of milk stored overnight in cans under different ambient conditions. E. C. Synnott and S. F. O'Donovan (*Irish J. agric. Res.*, 1964, **3**, 201—209).—Changes in temp. of milk were substantially the same as those in water stored under the same conditions. During the cooling of water from 90°F to atm. temp. differences up to 8°F at different points in the can were recorded. With overnight temp. from 40°F to <32°F, 8¾ h. elapsed before the temp. of water in 12-gal. cans reached 60°F; in warmer atm. conditions the water did not reach 60°F at the end of the usual cooling period of 15 h. When cooled to 45°F and then stored in an atm. of 73°F water reached a temp. of 58°F in 1·5 h., but when the initial temp. was 67°F this temp. was maintained overnight in an atm. of 62°F. A. G. Pollard.

Rennet coagulation of cow and buffalo milk. R. R. Sharma and V. R. Bhalerao (*Indian J. Dairy Sci.*, 1964, **17**, 1—5).—The rennet coagulation time of cow milk was increased by dilution of the milk with distilled water, whereas that of buffalo milk was unaffected up to 100% dilution. The rennet coagulation time of buffalo milk was not affected by the addition of small amounts of NH_4 oxalate but the addition of similar amounts to cow milk significantly increased its rennet coagulation time. A description is given of a test, based on the above observations, for detecting the adulteration of cow milk with buffalo milk. (11 references.) M. O'Leary.

Complexing of calcium by hexametaphosphate, oxalate, citrate and EDTA in milk. I. Effects of complexing agents on turbidity and rennet coagulation. S. Odagiri and T. A. Nickerson (*J. Dairy Sci.*, 1964, **47**, 1306—1309).—Turbidity of milk was decreased by the addition of the Ca-complexing agents Na hexametaphosphate (HMP), K oxalate, Na citrate, and Na_2 ethylenediaminetetra-acetate (EDTA). The addition of Ca counteracted this effect and the mole ratio for HMP:Ca was 1:6 and those of citrate, EDTA, and oxalate to Ca were 1:1. Coagulation time of milk was prolonged in logarithmic proportion of complexing agent added and was restored to normal by the addition of Ca. Mole ratios of HMP, oxalate, citrate, and EDTA to Ca were, respectively, 4:5·8, 1:1, 1:1, and 1:1. As total solids in milk increased coagulation time was shortened and less affected by HMP. M. O'Leary.

The κ-casein complex. II. The isolation of a sialic acid-containing fraction by disrupting the complex at low pH in the presence of sodium chloride. R. Beeby (*J. Dairy Res.*, 1965, **32**, 57—63).—Up to 80% of the sialic acid of κ-casein was recovered from the supernatant after pptn. of the protein at pH 3. Cystine or cysteine were not detected. Two fractions were obtained from the supernatant by chromatographic analysis on DEAE cellulose, one of which contained 0·4—0·6% sialic acid and the other 4—6%. As most of the sialic acid of the latter fraction was sol. in 12% trichloroacetic acid following treatment with rennin, it is suggested that the glycopeptide released from casein by the action of the enzyme originates from that function. (13 references.) M. O'Leary.

Oxidation of casein by hydrogen peroxide. P. F. Fox (*Dissert. Abstr.*, 1964, **25**, 3240).—The effect of dil. H_2O_2 on casein with respect to the action of proteolytic enzymes and behaviour in the presence of inorg. ions, especially Ca^{2+} was studied. The products of proteolysis were investigated by electrophoretic and chromatographic techniques. H_2O_2-treated casein is more susceptible to pptn. by Ca^{2+}, max. pptn. being obtained at 30 mM-$CaCl_2$ as compared with 50 mM for the control. The effects of photo-oxidation indicate that tryptophan is probably not involved in the oxidation of casein by H_2O_2, but the oxidation of methionine (**I**) to **I** sulphoxide is the chief reaction taking place. F. C. Sutton.

Constancy of amino-acid composition of cow's milk protein under changing ration. W. R. Featherston, D. R. Frazeur, D. L. Hill, C. H. Noller, and C. E. Parmelee (*J. Dairy Sci.*, 1964, **47**, 1417—1418).—Analysis of milk from two Holstein cows showed that raising the grains:hay ratio in the ration did not significantly affect the amino-acid composition of the milk. M. O'Leary.

Physico-chemical properties of milk. XVII. Introduction of milk and some of its constituents with iodine in aqueous solution. B. R. Puri and S. Parkash (*Indian J. Dairy Sci.*, 1964, **17**, 6—9).—The amount of I_2 removed by milk from aq. I_2 solutions varied with the protein content of the milk and was unaffected by the presence of fat or lactose. The interaction of I_2 with casein was irreversible and the amount of I_2 in the resulting complex increased with increase in the ratio I_2/casein in the reaction mixture. M. O'Leary.

Physico-chemical properties of milk. XVIII. Heats of dissolution of some edible fats and oils in benzene. B. R. Puri, K. M. Bedi and S. Parkash (*Indian J. Dairy Sci.*, 1964, **17**, 13—16).—The heats of dissolution of ghee, hydrogenated vegetable fat, goat tallow, lard, and mustard, coconut, linseed and groundnut oils were determined by a calorimetric technique. The results showed that sufficient differences exist between the heats of dissolution of the various fats to permit the detection of adulteration of ghee with hydrogenated fats or goat tallow. M. O'Leary.

Effect of stage and number of lactation on the yield and composition of cow's milk. J. A. F. Rook and R. C. Campling (*J. Dairy Res.*, 1965, **32**, 45—55).—The results of experiments with Friesian heifers indicated that milk fat content was highest at the beginning and end of the lactation period and varied little throughout the middle of the lactation. There was a tendency for solids-not-fat to decrease until late in the lactation when there was a marked rise. Following a rapid rise in the early days of lactation, the lactose content remained constant for the greater part of the lactation period but declined towards the end. There was a tendency for the concn. of all major milk constituents secreted from the second to fifth months of lactation to decrease from lactation to lactation. (24 references.) M. O'Leary.

Composition of milk. V. Effect of stage of lactation. S. N. Ghosh and C. P. Anantakrishnan (*Indian J. Dairy Sci.*, 1964, **17**, 17—28).—After the colestral period, the major constituents of buffalo milk decreased for the first 7 weeks of the lactation and those of cow milk decreased for the first 4 weeks of the lactation. Thereafter, in both cases, there was a general increase during the remainder of the lactation. (42 references.) M. O'Leary.

Influence of surface active agents on some lactic streptococci. R. B. Maxey (*J. Dairy Sci.*, 1964, **47**, 1285—1290).—Studies with cultures of *Streptococcus lactis* and *S. cremoris* showed that growth of lactic streptococci was affected by the surface activity of the medium. The effects of fatty acids, nonionic and anionic surface active agents decyl alcohol, growth medium, and temp. variation growth of the organisms were directly related to surface activity and involved physical phenomena at the bacterium:mentruum interface. (12 references.) M. O'LEARY.

Effect of ultra-high-temperature heat treatment on the content of thiamine, vitamin B_6 and vitamin B_{12} of milk. M. E. Gregory and H. Burton (*J. Dairy Res.*, 1965, **32**, 13—17).—Analysis of UHT treated milks from indirectly and directly heated plants indicated that the loss of thiamine during processing at temp. up to 150° was negligible. Milk treated on indirectly heated plants lost up to 12% vitamin B_6 and up to 35% vitamin B_{12} whereas milk treated on directly heated plants lost up to 35% vitamin B_6 and up to 30% vitamin B_{12}. (10 references.) M. O'LEARY.

Development and flavour properties of methyl ketones in milk fat. J. E. Langler and E. A. Day (*J. Dairy Sci.*, 1964, **47**, 1291—1296).—The presence of water was shown to be essential for the formation of heat-induced ketones in milk fat. A study of the average flavour thresholds of individual ketones and of a ketone mixture, similar to that present in milk fat, indicated that (*a*) ketone mixtures exhibit a synergistic effect which results in the appearance of a perceptible flavour at concn. of individual components below their average flavour thresholds; and (*b*) that there is sufficient methyl ketone precursor in milk fat to give rise to perceptible flavours in beverage-type milk. (27 references.) M. O'LEARY.

Fatty acid composition of milk. II. Some differences in common dairy breeds. J. W. Stull and W. H. Brown (*J. Dairy Sci.*, 1964, **47**, 1412).—Analysis of monthly composite milk samples from Holstein, Guernsey, and Jersey cows over a 2-year period indicated that the C_{10} and C_{12} fatty acid contents of Holstein milk fat were significantly lower than those of milk fat from the other two breeds, whereas the reverse was true in the case of the C_{16}, $C_{16}{}^{1=}$, and $C_{18}{}^{1=}$ acids. M. O'LEARY.

Effect of a commercial drying process on the long chain fatty acids of milk. J. H. Moore and D. L. Williams (*J. Dairy Res.*, 1965, **32**, 19—20).—A comparative study of the fatty acid compositions of raw milk and of dried milk, produced on a double effect evaporator and twin drum rollers, indicated little difference between the two. There was no evidence that the roller-drying process resulted in any loss of linoleic (18:12) and arachidonic (20:4) acids. (13 references.) M. O'LEARY.

Application of the acetylcholinesterase inhibition method for detecting organophosphate residues and related compounds in milk. J. E. Beam and D. J. Hankinson (*J. Dairy Sci.*, 1964, **47**, 1297—1305).—A procedure for detecting cholinesterase-inhibiting pesticides in fluid milk is described. The method consists of extraction of the pesticides with acetonitrile and $CHCl_3$ followed by oxidation with H_2O_2-AcOH. *In vitro* cholinesterase assays were then carried out by a direct colorimetric method and the pesticide concn. calculated by reference to a standard curve. (24 references). M. O'LEARY.

Nature and cause of seaminess in Cheddar cheese. J. Conochie and B. J. Sutherland (*J. Dairy Res.*, 1965, **32**, 35—44).—The results of microanalytical and microscopical examinations of sections of seamy cheddar cheese indicated that the seams are due to the formation of a layer of crystals of $CaHPO_4$, $2H_2O$ as a result of dehydration of the curd surfaces by the NaCl added during manufacture. It is suggested that seaminess may be controlled by working the curd particles with aq. $CaCl_2$. (12 references.) M. O'LEARY.

Method for studying the factors in milk which influence the deposition of milk solids on a heated surface. H. Burton (*J. Dairy Res.*, 1965, **32**, 65—78).—A description is given of a method for investigating the factors which control the formation of deposits on heated surfaces, in which deposits are formed on electrically heated Pt wire under controlled heat transfer conditions. Using the technique, results were obtained which suggest that sensitivity of milk to deposit formation increases with decrease in pH and that forewarming of milk reduces deposit formation. M. O'LEARY.

Fatty acids in Irish butter-fat. T. Richardson and T. C. A. McGann (*Irish J. agric. Res.* 1964, **3**, 151—157).—Gas chromatographic examination of the Me esters of the fatty acids of the butter-fat is described. Of the 23—25 esters identified the major proportions were; myristic, 9·1—11·0; palmitic, 21·6—24·2; stearic, 12·6—14·4; oleic, 26·8—30·8; linoleic, 2·0—2·9; linolenic, 3·0—4·0; palmitoleic, 3·1—3·8; lauric, 2·3—3·4%. Results show a general pattern very similar to that reported for American butter-fats. The bearing of the results on relationships between certain aspects of the incidence of heart disease and dietary fat intake is considered. A. G. POLLARD.

Viscoelasticity of cheese. M. Fukushima, S. Taneya and T. Sone (*J. Soc. Mater. Sci., Japan*, 1964, **13**, 331—335).—Four types of rheometer are described. The instantaneous elasticity, retarded and flow η, elasticity and retardation time were measured. The η was 10^7—10^8 poises, shear rigidity 10^5—10^6 dynes/cm.^{2} and the retardation time was 200—500 sec. at room temp. The logarithmic relation between creep compliance and time could be reduced to a master creep curve by the shift along the axis of the time scale at temp. 5—45°. The yield value was 2×10^5 dynes/cm.^{2} as measured by a cone-penetrometer. Under certain conditions a logarithmic plot of the degree of sectility and of micro-penetration are straight lines. (14 references.) (From English summary.) R. J. M.

Destruction rates, lethal factors, metabolic injury, and the cause of death of microbiological cells in soft ice cream during freezing. J. Foley (*Dissert. Abstr.*, 1964, **25**, 2708).—Reductions in standard plate and coliform counts were evident during the freezing of soft serve ice cream. Agitation of soft ice cream mixes, when ice crystals were present, had a marked lethal effect on cells of *Escherichia coli* and *Saccharomyces lactis*, and a theory to explain the destruction of microbial cells during freezing, through disintegration by abrasion and collision impacts with rapidly moving ice crystals, was proposed. Electron micrographs of *E. coli* cells after different exposure times to freezing with agitation showed that they underwent progressive disintegration with increase in time of the freezing treatment. F. C. SUTTON.

Chocolate flavouring materials for ice-cream. IV. Basic and flavour bean products. J. B. Lindamood and I. A. Gould (*J. Dairy Sci.*, 1964, **47**, 1432—1435).—The relationship between flavour quality of chocolate ice-cream and differences in the type of product made from the basic cacao bean, *Accra* and the flavour bean, *La Guayra* were studied. Panel evaluations indicated that basic bean products were at least as good as flavour bean products and that there was a general inverse relationship between flavour quality and the cocoa fat content of the chocolate flavouring materials. M. O'LEARY.

Egg-yolk content of mayonnaises. E. Benk and L. Brixius (*Dtsch. LebensmittRdsch.*, 1965, **61**, 11—13).—Egg-yolk contents of commercial mayonnaises, calculated from determinations of lecithin-P_2O_5 are tabulated and their significance is discussed. Egg-yolk contents found varied from nil to nearly 10%, compared with egg-yolk contents of from 5·5 to 12·5% calculated for mayonnaises prepared according to 16 recipes recommended in standard cookery books (domestic and commercial). It is recommended that products low in egg content should be treated as adulterated under the food law unless sold under a description not including the word mayonnaise. Parallel determinations of cholesterol in several products by the method of Riffart and Keller (*Z. LebensmittUntersuch.*, 1934, **68**, 113) showed the results to be related to the sterol content of the edible oil used rather than to egg-yolk content due to interference by phytosterols. E. C. APLING.

Removing rust stains from eggs. E. Ross (*Poultry Sci.*, 1964, **43**, 1601—1602).—Rust on egg shells, derived from contact with wire-floor cages, was removed by soaking the eggs in 2% AcOH in tap water for 2—5 min. before passage through an eggwashing machine. The AcOH treatment had no effect on albumin height, shell thickness, odour or flavour of the eggs. A. H. CORNFIELD.

Edible Oils and Fats

Determination of the purity of lard by gas chromatography. D. Grieco (*Riv. ital. Sostanze grasse*, 1964, **41**, 261—266).—The presence of tallow in lard is detected gas chromatographically from the content of branched chain C_{14} and C_{16} acids, which are present in small amount in tallow, and almost absent in lard. About 5 to 10% of tallow may be detected. L. A. O'NEILL.

Adulteration of lard and refined pork fats. II. E. Pascucci and F. Paolini (*Riv. ital. Sostanze grasse*, 1964, **41**, 315—320).—The Boemer method can be used to detect the presence of $<10\%$ of tallow in lard provided the index value is raised to 74. Better indication of adulteration is given by the ratios of the fatty acids determined by gas chromatography. Thus, for lard, the ratio of $C_{14}+C_{16}+C_{18}$ saturated acids to linoleic acid should be 4 to 5 and adulteration should be suspected if this ratio is >5·5. Also, the ratio ($\times100$) of linolenic to oleic acid in lard should be 1 to 2 and values >2 should be suspect. L. A. O'NEILL.

Control of the purity of lard by gas chromatography. P. Armandola (*Riv. ital. Sostanze grasse*, 1964, **41**, 587—593).—The fatty acid composition of lard from different parts of the body of the pig was examined by gas chromatography, distinct although small differences being observed. To establish the genuineness of a sample of lard a combination of gas chromatographic methods, e.g., with calculation of indices of fatty acid ratios, with classical methods, e.g., Bömer index determination, seems necessary. (20 references.) L. A. O'NEILL.

Analysis of mixtures of animal and vegetable fats. V. Separation of sterol acetates by thin-layer chromatography in reversed-phase systems and on silica gel G–silver nitrate layers. J. W. C. Peereboom and H. W. Beekes (*J. Chromatogr.*, 1965, **17**, 99—113).—A method for the separation of mixtures of sterol acetates on kieselguhr G by the reversed-phase system undecane/acetic acid–acetonitrile (1 : 3) is described. By adding 0·5% of Br_2 to the mobile phase several critical pairs of sterols were resolved. The procedure and properties of this 'bromine system' are discussed. Sterol samples for analysis were applied to silica gel G–$AgNO_3$ plates prepared according to De Vries (*Fette Seif. Anstrichm.*, 1963, **65**, 725). Development was with a mixture of $CHCl_3$–ether–acetic acid (97·0 : 2·3 : 0·5). After development the plates were sprayed with a 0·2% ethanolic dibromofluorescein and examined under u.v. light where the sterol spots showed a bright fluorescence. The sterols and their acetates were separated according to their degree of unsaturation. C. PEARCE.

Effect of temperature on the oil content and fatty acid composition of the oils from several oil seed crops. D. T. Canvin (*Canad. J. Bot.*, 1965, **43**, 63—69).—The oil content of sunflower, safflower and castor bean was not affected by the temp. at which the plants were grown whereas the oil content of rape and flax decreased with increase in temp. Fatty acid composition of the oil from safflower and castor bean was not affected but in rape, sunflower and flax highly unsaturated fatty acids decreased and oleic acid increased with increase in temp. Saturated fatty acids were not affected. (15 references.) S. A. BROOKS.

Fatty acid distribution in lipids of marine plankton. H. Brockerhoff, M. Yurkowski, R. J. Hoyle and R. G. Ackman (*J. Fish. Res. Bd Can.*, 1964, **21**, 1379—1384).—The polyunsaturated fatty acids of the triglycerides were found accumulated in the β-position of the glycerol in the diatom, *Skeletonema costatum*, and in a zooplankton sample. This supports the theory that the typical structure of marine triglycerides originates in phytoplankton. The fatty acid composition of several other lipid fractions of plankton samples were also determined. (21 references.) S. A. BROOKS.

Detection of adulterant mineral oil in vegetable oils by thin layer chromatography. V. V. S. Mani and G. Lakshminarayana (*Indian J. Technol.*, 1964, **2**, 416).—Contamination of vegetable oils with >2% mineral oil is shown by applying a 10% $CHCl_3$ solution of the oil to a thin layer of alumina and then developing for 5 min. with 60—80° light petroleum. Detection is by spraying with 2,7,-dichlorofluorescein and viewing under u.v. light. E. C. DOLTON.

Detection of coconut oil and palmkernel oil interesterified with other fats. D. C. Leegwater and H. W. van Gend (*Fette Seif. Anstrichm.*, 1965, **67**, 1—3).—Gas chromatograms of coconut oil and palmkernel oil are shown. A chromatogram of a mixture of palmkernel oil and palm oil (3/7) was compared with a similar chromatogram on the mixed oils after they have been interesterified. The results differ; the C_{40}—C_{46} peaks were higher after interesterification. The chromatograms obtained by the gas chromatographic analysis of two margarine fats and their fatty acids were studied. One fat had considerably more C_{40}—C_{46} peaks than the other. A consideration of the ratios of the fatty acid peaks indicated that it had been interesterified with coconut oil or palmkernel oil. W. E. ALLSEBROOK.

Gas chromatography of fatty acids having triple bonds. I. Zeman (*J. Gas Chromatogr.*, 1965, **3**, 18—20).—The behaviour of two fatty acids with a triple bond (stearolic and behenolic acid) was investigated on a polar phase (20% polyethylene glycol adipate on Celite) and a non-polar phase (20% Apiezon L on Celite). The columns were operated at 170° and 220° respectively. Results confirm that the higher polarity of the triple bond causes considerable delay when passing the polar phase, this polarity being even higher than that corresponding to two double bonds. When the non-polar phase is used, acids containing a triple bond move faster than the corresponding saturated acids, but acids containing one double bond move even faster. The values of the separation factors for the CH_2-groups in the three series investigated are found to differ only very slightly. C. PEARCE.

Quantitative determination of linolenic acid by Lovelock ionisation detector. G. V. Novitskaya, A. V. Kaverina and A. G. Vereshchagin (*Dokl. Akad. Nauk SSSR*, 1964, **159**, 672—675).—Chromatographic and other methods of analysis give high results in determining linolenic acid (**I**) using Lovelock ionisation detector. Hence authors investigated whether proportionality exists between mass of **I** present and detector signal when estimating fatty acid and glyceride content of linseed oil in which **I** predominates. Triglycerides were converted to methyl esters which were analysed in Pye argon chromatograph. Detector voltage could be varied between 750 and 1500 V. Methods of improving thermal stability and efficiency of high temp. column filled with 10% polyethyleneglycol adipate on Celite 545 are described. Reference mixture of equal wt. of methyl esters of palmitic acid (**II**) and **I** gave correct peak area ratio of 1 : 1 when detector electrode potential was 750 V. When voltage was increased to 1000, 1250, 1500 V, however, ratios became 1 : 1·1, 1·23 and 1·74 respectively. Relative values of signals were determined using known mixture of methyl esters of **I**, **II**, stearic, oleic and linoleic acids with detector run at four voltages mentioned. Only **I** showed anomalous increase of signal value with increase of voltage. Gas–liquid chromatography with Lovelock argon detector is a reliable method for quant. determination of methyl esters of higher fatty acids containing high proportion of **I**, but electrode potential must not exceed 750 V. (12 references.) P. W. B. HARRISON.

Identification of fatty acids from oils by high efficiency capillary columns. M. Riva, F. Poy and P. Gagliardi (*Riv. ital. Sostanze grasse*, 1964, **41**, 267—268).—Gas chromatographic apparatus using capillary columns, with Apiezon L stationary phase and flame ionisation detector, for fatty acid analysis is described. Results with virgin olive oil confirm the absence of elaidic but show the presence of ~1·5% of petroselinic acid. In esterified oils <0·5% elaidic acid is present. L. A. O'NEILL.

Composition of fatty acids obtained in the lipids extracted from oil-seeds and fruits. D. Grieco and G. Piepoli (*Riv. ital. Sostanze grasse*, 1964, **41**, 283—287).—The fatty acid composition was determined by gas chromatography of oils and fats from 36 species of seeds and fruits of known provenance. These include groundnut, rape, linseed, sesame, soya, grapeseed, coconut and palm. (28 references.) L. A. O'NEILL.

Kreis reaction and peroxide value in Italian olive oils. A. Montefredine and C. Testo (*Riv. ital. Sostanze grasse*, 1964, **61**, 269—273).—Peroxide values have been determined and Kreis tests carried out by the Watts and Major (*J. Amer. Oil chem. Soc.*, 1946, 22) and Romani and Valentinis methods (*Boll. Lab. Chimici Prov.*, 1961, 357) on numerous samples of virgin and rectified oils. The Kreis tests can give quant. results but there is no agreement between the two methods. The samples have been classified according to their peroxide contents and Kreis tests performance. The peroxide value gives an earlier indication of the onset of rancidity than the Kreis tests. (27 references.) L. A. O'NEILL.

Use of sodium chloride, pectolytic enzyme and ascorbic acid in oleification. P. G. Garoglio and C. Stella (*Riv. ital. Sostanze grasse*, 1964, **41**, 431—439).—The use of salt in the winning of oil from various types of olives is examined. A sample of olives was treated by the classical and Baglioni processes, and the latter process with enzyme + ascorbic acid or with salt. The yields, properties of the oil and its stability were compared. L. A. O'NEILL.

Extension to all olive oils of spectrophotometric examination. A. Jaforte (*Riv. ital. Sostanze grasse*, 1964, **41**, 452—458).—The use of suitable parameters for differentiating virgin, sansa, rectified and oxidised olive oils is considered. The parameters are based on measurements at the max. and min. around the areas of diene and triene conjugation rather than at specific λ. L. A. O'NEILL.

Theory and practice of industrial neutralisation of olive and sulphur olive oils. I. Mathematical concepts on neutralisation loss. J. Espejo Gutiérrez (*Grasas y Aceites* 1964, **15**, 308—310).—Equations are developed relating fall of acidity with neutralisation loss in a two-stage process. L. A. O'NEILL.

Gas–liquid chromatography of lipids. A. G. Vereshchagin (*Usp. Khim.*, 1964, **33**, 1349—1370).—The review is divided into two main parts: gas–liquid chromatography of Me esters of aliphatic acids, and of free aliphatic acid and their deriv. In the first part, conditions of separation of the Me esters are discussed, also their synthesis for chromatographic separation; solid carriers; chromatography in polar and non-polar liquid phase; identification of aliphatic acids and their quant. determination and radioactive determination during gas-chromatographic separation. The second part includes methods of separation of free aliphatic acid, hydrocarbons, alcohols, aldehydes and amines and also glycerides. (146 references.) A. L. B.

Analysis of oils and fats by gas chromatography. George R. Jamieson and Elizabeth H. Reid (*J. Chromatography*, 1965, **17**, 230—237).—Three transesterification methods and three saponification and esterification methods for preparing the methyl esters of the fatty acids in oils were studied, and two gas chromatographic procedures were used for separating and determining the methyl esters. All six methods are satisfactory for the analysis of the major acids if precautions are taken to avoid the loss of the compounds of lower mol. wt., but there are significant differences in the results for the minor constituents. A. R. ROGERS.

Quantitative gas–liquid chromatography of volatile fatty acids. A method for the determination of C_1 to C_6 acids in biological material. G. W. Lanigan and R. B. Jackson (*J. Chromatography*, 1965, **17**, 238—244).—Ten saturated carboxylic acids containing from 1 to 6 C atoms are liberated from their Na salts (5 to 10 μmole) with 30% H_3PO_4 solution and separated on a column containing 20% of

behenic acid and 4% of H_3PO_4 on acid-washed Chromosorb W; N saturated with water is used as the carrier gas. The temp. is programmed to rise from 80 to 140° and the acids are detected and determined with a commercial recording automatic titrator. The mean recovery of eight acids from a synthetic mixture was 98·9%, the coeff. of variation for each acid (six determinations) was ±1%. A. R. ROGERS.

Edible fats. Canada Packers Ltd. (B.P. 955,788, 28.11.62. U.S., 29.11.61).—An edible coating fat, for use in making coatings for biscuits, candy bars, etc., is produced by blending a hydrogenated, non-interesterified palm kernel oil of I val. <3 (15—85%) with a hydrogenated palm kernel oil of I val. <3 and in which the fatty acid radicals have been randomly arranged by inter-esterification. The blend should have m.p. 35—46°. F. R. BASFORD.

Meat and Poultry

Determination of tin in corned beef. J. H. Shelton and J. M. T. Gill (*J. Ass. Publ. Analysts*, 1964, **2**, 98—100).—In the rapid method described, the sample is ignited overnight at 500°, tin is dissolved from the ash by boiling with 50% NaOH solution and determined colorimetrically (after neutralisation of the extract) by the toluene-3,4-dithiol procedure (De Giacomi, *Analyst*, 1940, **65**, 216). The results are comparable with, but generally slightly lower than, those obtained following wet oxidation of the sample. E. C. APLING.

Tenderising of mutton. J. R. Iyengár, S. Kuppuswamy and D. S. Bhatia (*Indian J. Technol.*, 1964, **2**, 409—411).—The cooking time of dehydrated mutton mince may be reduced by holding the raw meat at low temp. or by freezing and incorporating papain during mincing. Satisfactory tenderising is achieved by incorporating 2 mg.-% papain in the cooking brine or ~5·5 mg.-% papain on cooked deboned meat during mincing. Mutton mince containing 18·5% fat and 1·9% water packed under N_2 in sanitary cans can be stored for 6 months at 37°. (14 references.) E. C. DOLTON.

Mechanism of cell damage during freezing and thawing and its prevention. J. Farrant (*Nature, Lond.*, 1965, **205**, 1284—1287).—Two new methods have been devised for cooling cells to −79°, in the presence of dimethyl sulphoxide, in which the concn. of solutes in the liquid phase is prevented from rising above normal values. The functional activity of smooth muscle was better preserved by these new techniques than by previous methods. S. A. BROOKS.

Available lysine in meat and meat products. Z. Dvořàk and I. Vognarová (*J. Sci. Fd Agric.*, 1965, **16**, 305—312).—The dependence of the available lysine content on the composition of meat (beef and pork) including the proportion of connective tissue, the effect of spoilage, of heat processing and of reduction of water content were studied. Because of the free ε-amino-group in lysine, its availability may be decreased by processing, e.g., effect of heat in presence of sugars. Available lysine in meat is influenced by other reactions: NO_3^- with the free amino group of proteins causes the destruction of free ε-amino-group of lysine bound in proteins. This group may also be blocked by aldehydes (especially HCHO always present in smoke). (15 references.) E. M. J.

Sensory and chemical comparisons of certain flavour constituents of chicken and turkey meat. K. N. Hall (*Dissert. Abstr.*, 1964, **25**, 3508—3509).—Flavour components of the light and dark meat of chicken and turkey, as characterised by a taste panel, were water-sol. and dialysable. Changes in adenosine-5′-mono-,-di-and-triphosphate and inosine-5-monophosphate during storage for 1 or 7 days are recorded. Concn. of these substances were substantially the same in both chicken and turkey meats. A. G. POLLARD.

Cooked sausage-like products. Emhart Mfg Co. (Inventors: B. Sassen, G. D. Mylchreest and A. J. Kargl) (B.P. 954,811, 20.6.60).—There is claimed a method (and apparatus) for automatic continuous production of cooked skinless sausage-like products, e.g., frankfurters, which can be carried out more rapidly than before. F. R. BASFORD.

Fish

Protein denaturation in frozen fish. X. Changes in cod muscle in the unfrozen state, with further observations on the principles underlying the cell fragility method. R. M. Love, M. M. Aref, M. K. Elerian, J. I. M. Ironside, E. M. MacKay and M. G. Varela (*J. Sci. Fd Agric.*, 1965, **16**, 259—267).—Changes in muscle proteins that occur when cod are kept in ice were studied by protein extractability in salt solution and ' cell fragility ' values as criteria. Protein extractability falls slowly caused by an aggregation of the structural proteins (**I**) at mol. level. Some of **I** are altered in some way; in stale fish which is subsequently frozen, **I** aggregate less readily than in fresh fish. ' Cell fragility ' can change independently of protein extractability and the fragility of the myofibrils is influenced by bacterial action. Reasons for obtaining different results by the two methods are discussed; photomicrographs of homogenates of cod muscle are given. (26 references.) E. M. J.

Quality of Newfoundland cod. X. Effect of commercial freezing rates on frozen and stored quality of trap cod. W. A. MacCallum, D. A. Chalker, E. J. Laishley, W. J. Dyer and D. R. Idler (*J. Fish Res. Bd Can.*, 1965, **22**, 411—420).—Time–temp. curves are reported for points at the centre, ¼ of the depth and at the surface of blocks of dressed-cod and blocks and packages of cod fillets, frozen in commercial plate freezers. Freezing rates at the centres varied by a factor of ~4 where thickness of the pack was the only variable, the same factor applying to the fastest and slowest-frozen fish in 4½ in.-thick blocks. Dressed trap-caught cod and cod fillets were frozen (*a*) at the fast rate and (*b*) at the slow rates normally encountered at the centre of 1½- and 4½-in.-thick commercial packs, respectively (0·7 and 2·7 h. through temp. range 0 to 15°). The frozen and stored (−10°F) samples were of similar quality, up to 22 weeks, based on taste panel assessment. Little difference may be expected in the market condition of trap cod of high initial quality frozen at any of these commercial rates and package thicknesses and stored at −10°F. (26 references.) E. M. J.

Polyphosphate treatment of frozen cod. III. Taste panel evaluation, chemical assessment and thaw-drip in once-frozen Newfoundland trap-caught cod. W. A. MacCallum, D. A. Chalker and J. T. Lauder (*J. Fish. Res. Bd Can.*, 1964, **21**, 1397—1402).—Cod fillets treated with Na tripolyphosphate before packaging and freezing had significantly better texture characteristics than had untreated fillets. Thaw drip was somewhat less in treated samples while lipid hydrolysis proceeded at the same rate. There were no significant differences in the electrophoretic patterns of proteins. (13 references.) S. A. BROOKS.

Effect of polyphosphates and other salts on drip loss and oxidative rancidity of frozen fish. J. W. Boyd and B. A. Southcott (*J. Fish. Res. Bd Can.*, 1965, **22**, 53—67).—Pretreatment of fresh fillets of certain varieties of fish with Na tripolyphosphate prior to freezing was found to be effective in reducing drip loss during thawing and in increasing product yield of both frozen and thawed fish. Comparable results were obtained in some trials with NaCl and citrates at high concn. Na tripolyphosphate dip was not an effective antioxidant for storage of frozen salmon. (17 references.) S. A. BROOKS.

Effects of salting and smoking on protein quality of cod. I. C. Munro and A. B. Morrison (*J. Fish. Res. Bd Can.*, 1965, **22**, 13—16).—Salting and smoking had no effect on the biological value of cod protein, as indicated by the protein efficiency ratio, gross protein values, total lysine and methionine content, ' available lysine ' values, and plasma free lysine and methionine levels in human subjects. It was concluded that smoked and salted codfish can provide protein of high nutritional value. S. A. BROOKS.

Carbonyl compounds in salted cod. I. Thin-layer chromatography of aliphatic monocarbonyl 2,4-dinitrophenylhydrazones. M. Yurkowski. **II. Separation and identification of volatile monocarbonyl compounds from heavily salted cod.** M. Yurkowski and M. A. Bordeleau (*J. Fish. Res. Bd Can.*, 1965, **22**, 17—25, 27—32).—I. Methods are described for the separation of 2,4-dinitrophenylhydrazones of normal aliphatic monocarbonyl compounds by thin-layer chromatography. Magnesia–cellulose plates were used to separate mixtures into classes and propylene glycol- and petrolatum-impregnated silica gel to separate each class into homologues. Two-dimensional separations have also been accomplished. (14 references.)

II. Volatile monocarbonyl compounds have been isolated from heavily salted cod as the 2,4-dinitrophenylhydrazones and separated by thin layer chromatography. Formaldehyde, and to a lesser extent acetaldehyde and propanol, were the major monocarbonyls found. (23 references.) S. A. BROOKS.

Characteristics and nutritional value of various fish protein concentrates. H. E. Power (*J. Fish. Res. Bd Can.*, 1964, **21**, 1489—1504).—Fish protein concentrates have been prepared from whole cod, headed, eviscerated cod, cod trimmings, cod press cake and whole herring. Final fat contents for cod concentrates were between 0·018—0·056%; that of herring was 0·17%. The corrected protein efficiency ratios for all except cod press cake concentrate were higher than that for casein. Lysine values were between 9·1 and 15·03% and available lysine values were between 6·1 and 10·4% of the protein. (14 references.) S. A. BROOKS.

Effects of fat content on diffusion of water in fish muscle. A. C. Jason (*J. Sci. Fd Agric.*, 1965, **16**, 281—288).—The coeff. of diffusion of water D_I, D_{II} associated with states of ' high ' and ' low ' hydration respectively in fish muscle are sensitively dependent on the amount of fat present. The diffusion resistivity D^{-1} for various species

may be represented by straight lines: $D^{-1} = \alpha + \beta F$ where F is fat content and α and β are constants. Experimental evidence is generally incompatible with a muscle microstructure consisting of a dispersion of fat in a non-fatty medium but is more in accord with one consisting of relatively non-fatty tissue surrounded by fat the thickness of which increases linearly with fat content. E. M. J.

Rancidity in lean fish muscle. I. A proposed accelerated copper-catalysed method for evaluating the tendency of fish muscle to become rancid. J. MacLean and C. H. Castell (*J. Fish. Res. Bd Can.*, 1964, **21**, 1345—1359).—A method that can be carried out within 24—72 h. is suggested for the determination of the tendency of fish muscle to become rancid. It consists of adding measured, trace amounts of Cu^{2+} ion to muscle that has been blended with water (1:3) followed by storage at 0°. Rancidity is observed subjectively by noting the odours that develop and objectively by the thiobarbituric reaction. S. A. BROOKS.

Rancidity in lean fish muscle. III. Inhibiting effect of bacterial activity. C. H. Castell and J. MacLean (*J. Fish. Res. Bd Can.*, 1964, **21**, 1371—1377).—Actively growing bacteria suppressed the development of Cu-catalysed rancidity in lean fish muscle. Apart from the antioxidant effect of the bacteria themselves, it could not be shown that fish muscle undergoing microbial deterioration became any less susceptible to oxidative rancidity as spoilage progressed. S. A. BROOKS.

Atomic absorption determination of traces of lead in fish flour. A. Strasheim, E. Norval and L. R. P. Butler (*J. S. Afr. chem. Inst.*, 1964, **17**, 55—70).—The method described compensates for the scattering of radiation by Ca and P. Agreement with chemical determinations was satisfactory and a coeff. of variance of 6% obtains. E. G. BRICKELL.

Tetracycline antibiotics in shrimp preservation. B. A. Southcott and J. W. Boyd (*J. Fish. Res. Bd Can.*, 1965, **22**, 117—129).—Certain bacteria isolated from raw and cooked shrimp displayed reduced sensitivity to Tetracycline antibiotics in the presence of multivalent metallic cations or shrimp extracts; addition of a chelating agent resulted in reduction of the antagonism of such ions and extracts toward the antibacterial action of these antibiotics. A dip in the chelating agent increased the efficacy of a subsequent Tetracycline antibiotic dip for shrimp preservation. (21 references.) S. A. BROOKS.

Volatile sulphur compounds of oysters. A. P. Ronald and W. A. B. Thomson (*J. Fish. Res. Bd Can.*, 1964, **21**, 1481—1487).—The compound responsible for the characteristic odour of fresh Pacific oysters, *Crassostrea gigas* (Thunberg), was identified as dimethyl sulphide by the formation of Hg salts and by i.r. analysis and gas chromatography. The effect of bacterial action on the oysters was observed and a no. of volatile organo-S decomposition compounds, produced during room storage at 20—21°, were identified by gas chromatography and by the formation of their Pb and Hg salts. (10 references.) S. A. BROOKS.

Spices, Flavours, etc.

Photometric procedure for the micro determination of potassium ferrocyanide in common salt. E. Kroller (*Z. anal. Chem.*, 1965, **210**, 34—37).—K ferrocyanide may be added to salt, to prevent caking, in amounts below 20 p.p.m. 5 g. salt is dissolved in 45 ml. water and treated with 10 ml. 5% tartaric acid. The solution is heated to boiling to form HCN and 25 ml. distillate collected in 2 ml. 0·1N-NaOH. Cyanide in the distillate is determined by the method of Aldridge (*Analyst*, 1964, **69**, 262). T. R. ANDREW.

Volatile esters of Bartlett pear. IV. Esters of *trans*-2-*cis*-4-decadienoic acid. W. G. Jennings, R. K. Creveling and D. E. Heinz (*J. Fd Sci.*, 1965, **29**, 730—734).—Ethyl *trans*-2-*cis*:4-decadienoate was identified as a flavour component of Bartlett pears. The acid moiety was synthesised and was identical with that isolated from Bartlett pear. This acid was isolated from the seed oil of *Sapium sebiferum* and was used to synthesise esters with pear-like odour. E. M. J.

Pear aroma: relation of instrumental and sensory techniques. D. E. Heinz, R. M. Pangborn and W. G. Jennings (*J. Fd Sci.*, 1965, **29**, 756—761).—The aroma intensities of pear essences correlate well with the intensities of their absorptions at 263—267 mμ. This absorption is due to esters of 2,4-decadienoic acid which have been identified as character impact compounds of Bartlett pear aroma. Unlike essences concentrated by reflux or bubble plate columns, flash vaporisation techniques did not degrade aroma, and resulting concentrates could be rediluted without apparent change. E. M. J.

Preservatives

Substituted adenines and living plant materials preserved therewith. Shell Internationale Research Mij N.V. (B.P. 953,897, 29.3.61. U.S., 31.3.60).—The compounds claimed are adenine deriv. (6-aminopurines) with R^I and R^{II} substituents on the NH_2 group and R on positions 4, 7, 8 and 9 (R and R^{II} are H or alkyl of 1—8 C, optionally interrupted with hetero-atom, at least 1R being alkyl and at least one being on position 7 or 9). Details are given for the prep. of *N⁶-benzyl-2-methyladenine*, m.p. 285—286°. The products are claimed for use in the prevention of the deterioration of, e.g., fruits and green leafy vegetables, their action being probably inhibition of proteolysis. F. R. BASFORD.

Pesticides in Foods

Selective detection of phosphorus, sulphur and halogen compounds in the gas chromatography of drugs and pesticides. H. P. Burchfield, D. E. Johnson, J. W. Rhoades and R. J. Wheeler (*J. Gas Chromatogr.*, 1965, **3**, 28—34).—A method employing a microcoulometric gas chromatograph is described. The sample containing halogen and S is applied to the chromatographic column, swept through by N_2 and then O_2 is added and the sample combusted. The halogens are measured selectively in a titration cell with Ag and Pt electrodes and 70—85% Ag acetate as electrolyte. S is measured in the same way in a cell where all the electrodes are of noble metal and the electrolyte is 0·04—0·05% KI in 0·4% acetic acid. It is possible to detect 0·001 μg. of Cl. The application of the method to the study of the metabolism of chlorpromazine is described. Org. phosphates, after separation on the column, are converted into PH_3 by reduction with mol. H_2 at 950°. The reduction products pass through a tube containing Al_2O_3 or silica gel (to remove HCl and H_2S) and into the titration cell for estimation of P. The application of this technique to the analysis of insecticides and urine extracts is described. C. PEARCE.

Quantitative determination of polychlorocyclohexane using gas chromatography. H. Fürst and J. Lauckner (*Chem. Techn., Lpz.*, 1965, **17**, 111—113).—A model mixture of γ-hexachlorocyclohexane, and hepta- and octa-chlorocyclohexane and also the technical product of C_6H_6 chlorination were analysed. The mixture was dried under vac. and dechlorinated with Zn, in presence of a little $CuSO_4$ under reflux for 20 min. at 40—45° and then for 3 h. at 85°. The org. product obtained by micro-steam-distillation was analysed by gas chromatography, using a column of 20% tritolyl phosphate on Sterchamol at 100°, the carrier gas being H_2. Average error for individual measurements was ±0·7%. Quant. determination is possible if more than 1·5% of an individual component is present. M. GREENAWAY.

Determination of residues of *OO*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate in fruit and vegetables after previous separation of the co-extracted dyestuffs by thin-layer chromatography. J. Kovač and E. Sohler (*Z. anal. Chem.*, 1965, **208**, 201—204).—A light-petroleum extract of the plant material is shaken with acetonitrile, the acetonitrile is evaporated and the coloured co-extractives are separated by thin-layer chromatography. The insecticide is isolated and its alkaline hydrolysis product determined photometrically at 400 mμ. Results are reproducible. Lowest limit of the method is 0·1 p.p.m. B. RIPP.

Food Processing, Refrigeration

Isolation and identification of volatile fatty acids present in hickory wood sawdust smoke and their penetration into meat. H. A. Hamid (*Dissert. Abstr.*, 1964, **25**, 3509).—Acetic (**I**), propionic (**II**), iso-valeric, iso-caproic and n-caproic acids were identified in the smoke. Penetration of the acids into meat at 75 ±5° during 2 h. was in the general order **II** > **I** > higher acids in order of increasing mol. wt. A. G. POLLARD.

Iron sulphide blackening in canned protein foods. Interactions of constituents in the vapour phase. G. N. Pigott, E. J. Guardia and A. M. Dollar (*J. Fd Sci.*, 1965, **29**, 750).—The formation of volatile bases and acids during heat processing of tuna was studied, using a closed system (diagram given) swept with N_2 gas. The ratio of S to Fe responded directly to the initial vac. present in canned tuna. At high vac. the ratio was 0·575. S was absent from the black deposits when the cans were sealed without evacuation, even when high levels of cystine were added. In commercially packed shrimp the S:Fe ratio was 0·322, consistent with a deposit of mixed oxides of Fe combined with FeS. Addition of AcOH at 10 mmol./can suppressed the formation of black deposits at all closing vac. in a model system containing added cystine; addition of aq. NH_3 or no addition resulted in black deposits at 26 in. Hg of closing vac. (11 references.) E. M. J.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Some recent developments in fat nutrition. W. O. Lundberg (*Chem. & Ind.*, 1965, 572—582).—This communication divides into two parts: the first relates to the nutritional and metabolic aspects of the so-called essential fatty acids while the second is concerned with the relationship between the dietary fats and vascular disease. (24 references.) C. V.

Dietary fat and coronary heart disease. A. N. Howard and G. A. Gresham (*Chem. & Ind.*, 1965, 831—837).—The dietary fat hypothesis is reviewed together with the occurrence of spontaneous arterial disease; the production of atherosclerosis is reported but the results so far attained would appear, on one count or another, to be unsatisfactory. The response of different species to cholesterol in the diet is discussed and compared with the incidence of arterial disease with diets of low cholesterol content. As the result of experimentation it is concluded that dietary fats are important in the aetiology of vascular disease and that the characterisation of the various nutritional factors involved could have important applications to the human disease of coronary thrombosis. (57 references.) C. V.

Relation between dietetic fats and the development of bile stones in animals. H. Dam (*Riv. ital. Sostanze grasse*, 1964, **41**, 416—424).—Hamsters fed digestible carbohydrates in absence of fats have a tendency to develop bile stones with a high content of cholesterol. Inclusion of fats or rice starch into the diet greatly cuts down the formation of cholesterol bile stones. (18 references.)

L. A. O'NEILL.

Detection of polyglycerides by thin layer chromatography: supplementary report. A. Seher (*Fette Seif. Anstrichm.*, 1965, **67**, 24).—Recent biological tests on rats fed partial esters of polyglycerides are reviewed. There is no reason to believe that polyglycerides have a toxic effect. This is important since polyglycerides are used as emulsifiers in foodstuffs. W. E. ALLSEBBROOK.

Determination of vitamin A in isomeric mixtures demonstrated by example of a vitamin A acetate mother liquor. J. Proll and H. Plessing (*Ernährungsforschung*, 1964, **9**, 513—517).—A 40% solution of synthetic vitamin A acetate, 52% of which consisted of the all-*trans* isomer, was tested in rat feeding experiments, in comparison with standard prep. of vitamin A. The results of the Brüggemann liver-storage bioassay were in both cases much lower when the prep. were administered in solution in neatsfoot oil than when administered in groundnut oil. Whilst consistent results were obtained with the standard prep., the results with the liquor varied with the dosage and its dilution. P. S. ARUP.

Improved paper chromatographic method for the photometric determination of ascorbic acid. R. Pohloudek-Fabini and W. Fürtig (*Pharmazie*, 1965, **20**, 128—135).—The sample (10 μl.), containing 0·1 to 0·5% of ascorbic acid (**I**), is submitted to paper chromatography with isopropyl alcohol—2% oxalic acid solution (7:3) as the solvent in an atm. of H_2. **I** is eluted with a mixture of 2% HPO_3 (4 ml.) and 4·84% Na acetate solution (1 ml.) and determined by shaking for 15 sec. with 0·001N-2,6-dichlorophenolindophenol (1 ml.), extraction with amyl acetate (10 ml.) and measurement of the extinction of 525 mμ. The error is $\simeq \pm 5\%$. If the chromatography is carried out in an atm. of H_2S, **I** and dehydroascorbic acid are determined together. A. R. ROGERS.

Unclassified

Paper chromatographic technique for screening volatile chemicals for their reactivity with the constituents of foods. S. K. Majumder, S. Godavaribai, M. Muthu and K. Krishnamurthy (*J. Chromatography*, 1965, **17**, 373—381).—If a fumigant reacts with an amino-acid, paper chromatography may reveal a reduction in the amount of amino-acid and the presence of additional spots due to the products of the reaction. A suitable technique is described for the exposure of 10-μg. amounts of amino-acids (especially methionine) to the equivalent of 100 mg. of fumigant per l. of air for 100 h., development with butanol–AcOH–water (4:1:5) and formation of colour with ninhydrin reagent. MeBr, MeI, EtBr, vapona and β-propiolactone show high reactivity with methionine. A. R. ROGERS.

New method of adjustment of composition of medium during continuous cultivation of micro organisms. N. D. Ierusalimskiĭ, L. D. Shaforostova and V. I. Balashov (*Mikrobiologiya*, 1965, **34**, 73—78).—Construction and operation are described in detail of glass apparatus which meters very small quantities of up to four separate components of a culture medium used in continuous fermentation. Apparatus works on principle of chemostat and can be made in any laboratory. It has been worked in sterile conditions for up to 2½ months without stopping, maintaining composition of medium at desired level. Ratio of separate feed components can be varied at will at flow rates as low as 0·1 ml./cycle. Pumping cycles are controlled by an electric timer and magnetic valve which regulate flow of air from compressor. Tables show: (i) consistent flow rates in five repeat determinations with combination of four separate measuring devices with settings varied from 0·1 to 2·5 ml./cycle; (ii) results of culturing *Bacillus megatherium* in synthetic medium to which varied ratios of additional medium, NaOH solution and water were added. Pumping cycles varied from 4 to 12/min. Strict maintenance of flow rates at pre-set levels was confirmed by optical density of culture and residual content of N and sugar. $[H^+]$ remained constant with all variants for 2½ months.

P. W. B. HARRISON.

Measurement of the kinetics of biological systems at elevated temperature utilising flow techniques. D. I-C. Wang, A. E. Humphrey and L. C. Eagleton (*Biotechnol. Bioengng*, 1964, **6**, 367—379).—Thermal inactivation of *Bacillus stearothermophilus* spores in a tubular flow reactor was studied. With an efficient mixing valve very short exposure times could be studied, Water and a standard spore suspension in phosphate buffer, pH 7, were passed into the reactor from different reservoirs under N_2 pressure with flow rates controlled by needle valves. Overall flow rate was determined by collecting rates in a cooled chamber and viable counts were then made. Stepped changes in salt concn. detected by a conductivity cell at the reactor outlet permitted determination of the dwell time distribution. Theoretical predictions on the basis of a model having two equal-vol. stirred tanks were in good agreement with the experimental data for a 2·07 ml. reactor over the whole range of flow rates but for a 7·1 ml. reactor between 165 and 287 ml./min. they are inadequate. In this case the data fitted if the ratio of tank vol. was assumed to be 0·3. Account must be taken of distribution of dwell times when designing a unit, otherwise degree of destruction may be lower than expected. J. B. WOOF.

Steam sterilisable probes for dissolved oxygen measurement. M. J. Johnson, J. Borkowski and C. Engblom (*Biotechnol. Bioengng*, 1964, **6**, 457—468).—An unsealed probe is described which is readily sterilised and suitable for used in fermenters. The probe has Pb anode, Ag cathode and an acetate buffer electolyte. Teflon was used as the membrane. Details of construction are given. Output in the absence of O_2 is low and the response is rapid. Probes gave linear response when checked against a Beckman paramagnetic O_2 analyser if concn. electrolyte is used (5M-AcOH, 0·5M-NaOAc). (14 references.) J. B. WOOF.

Determination of carbonyl compounds with *N*-methylbenzothiazolone hydrazone [MBTA]. M. A. Paz, O. O. Blumenfeld, M. Rojkind, E. Henson, C. Forfine and P. M. Gallop (*Arch. Biochem. Biophys.*, 1965, **109**, 548—559).—The physical characteristics of the MBTH deriv. of 15 different carbonyl compounds are given. The azine and 'osazine' deriv. formed under defined conditions possess characteristic spectra which allow the identification of saturated and unsaturated aldehydes, ketones, keto-acids and many related compounds in a simple spectrophotometric assay. The azines of aldehydes can be further reacted with the oxidised form of MBTH to give tetra-azapentamethine cyanine dyes which are used for colorimetric procedures. The sensitivities of these methods is such that amounts of a carbonyl compound can be identified and measured rapidly and accurately. Since carbohydrates with pyranose structures are unreactive in the colorimetric and spectrophotometric methods, many aldehydes and ketones can be measured in their presence. (12 references.) C. V.

Use of 2,4-dinitrophenylhydrazine [DNP] for the estimation of micro amounts of carbonyls. R. C. Lawrence (*Nature, Lond.*, 1965, **205**, 1313—1314).—The causes of the low recoveries of methyl ketones as their 2,4-DNP hydrazones appear to be the volatility of the carbonyls and the instability of the carbonyl-reagent adduct. Under closed conditions the reaction of 2,4-DNP with carbonyls up to C_{11} was shown to be quant. and rapid. (10 references.)

S. A. BROOKS.

Soya for food. Société Industrielle des Oléagineux and M. Rambaud (B.P. 955,773, 17.7.62. Fr., 27.7.61).—Soya is improved in food quality and use of the wholemeal flour facilitated (having its natural fat content, stable, not fatty to touch, and easily dispersible) by cooking in alkaline aq. suspension (pH 8—9) at $\gtrsim 80°$, then drying. F. R. BASFORD

Beverage powder. Borden Co. (Inventor: J. F. Hole and W. B. Smith) (B.P. 955,756, 23.3.62).—Granulated sugar (100) is heated at 55—88° but below the temp. of formation of off-flavours, then molten lecithin (0·5—4) is added, and the mixture is stirred until the lecithin becomes adhered in tacky form to the sugar particles. The material is then mixed with cocoa powder and/or malted milk powder (8—60), and optionally pulverised sugar (20—80 pt.) to produce an improved cocoa powder. F. R. BASFORD.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

SEPTEMBER, 1965

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

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J. Sci. Fd Agric., 1965, Vol. 16, September

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Printed in Great Britain by Unwin Brothers, Ltd., Woking and London (B4159)