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MYCOLOGICAL PRODUCTION OF CITRIC AND OXALIC ACIDS FROM CANE MOLASSES. II.*—Effects of Some Enzyme Inhibitors

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A comparative study has been made of the behaviour of preformed mats of a strong and a weak acid-producing strain of *A. niger* when floated on sucrose, molasses and citric acid solutions containing various concentrations of sodium arsenite, sodium monoiodoacetate, or 2,4-dinitrophenol (on carbohydrate solutions only). Tracing of the α-keto-acids which accumulated in the metabolism solutions containing arsenite was carried out by paper chromatography of the 2,4-dinitrophenylhydrazones. The data obtained are discussed in relation to each other and in light of the findings of other workers.

Introduction

Dressel[1] found that both respiration and anaerobic glycolysis of yeast cells were inhibited by arsenite, but glucose could protect the cells against the effect of the poison on oxygen consumption. Arsenite in higher concentrations inhibits respiration but has no effect on alcoholic fermentation in yeast cells.[2] Krebs[3, 4] accumulated evidence that arsenite is a specific inhibitor for oxidative decarboxylation reactions of α-keto-acids, while Walker[5] studied its effects on the preformed mats of *Aspergillus niger*.

Iodoacetate is known to interfere with the metabolism of carbohydrates, possibly by inhibiting CoA in a competitive and non-competitive fashion.[6]

The first to show that 2,4-dinitrophenol (D.N.P.) in low concentrations blocks completely the synthetic reactions in certain living tissues without interfering with oxidation was Clifton.[7, 8] Hotchkiss[9] showed that D.N.P. prevented phosphate uptake by respiring yeast cells. The fact that Toply[10] found that no bound ^{32}P accumulated in washed kidney fractions when D.N.P. was added led Kaplan[11] to assume that the inhibitor may cause a breakdown of the primary energy-rich phosphate bonds formed during oxidation in the tricarboxylic acid cycle (T.A.C.). Several workers have investigated the effect of D.N.P. on phosphorylation reactions in living cells.[12, 13]

Experimental

The behaviour of 6-days-old mats of *Aspergillus niger* (strains NI and B_8) was studied. The mats were initially developed on a molasses medium containing (g./100 ml. of medium) molasses sugar 10, NH_4NO_3 0·2, the initial pH being adjusted to 5·5. The metabolism solutions of the preformed mats were decanted and replaced by equal volumes of a salt solution of the following composition (g./100 ml.): NH_4NO_3 0·2; KH_2PO_4 0·03; $MgSO_4,7H_2O$ 0·025, which is found to keep the mats physiologically active. After the mats had been floating on the salt solution for 4 h. in the incubator, the solutions were decanted and replaced by equal volumes of the final replacement solutions. These solutions contained molasses, sucrose or citric acid and various concentrations of sodium arsenite or sodium iodoacetate. The final replacement solutions which were prepared to study the effect of the nitrophenol contained either the molasses or sucrose together with the inhibitor. After 3 days' incubation the flasks were sterilised and the different estimations were carried out as described previously.[14] Quintuplicate sets of 125-ml. Erlenmeyer flasks were used and each flask was charged with 25 ml. of liquid. The results are set out in Tables I–III.

Quantitative estimation of pyruvic and α-ketoglutaric acids in the metabolism solutions containing arsenite

Six-days-old mats were developed on 50-ml. portions of the molasses medium in 250-ml. Erlenmeyer flasks and the replacement technique conducted as above. The replacement solutions were distributed in such a way that three flasks for each different case were obtained.

* Part I: *J. Sci. Fd Agric.*, 1960, **11**, 592

Table I

Behaviour of A. niger *in presence of sodium arsenite*

STRAIN B_8

Culture at time of replacement

Felt wt., g.	1·638 ± 0·0217
Change in titratable acidity	70·4
Sugar consumed, g.	4·97

STRAIN NI

1·425 ± 0·0116	
122·5	
5·67	

Initial pH of final replacement solutions, 5·5

Initial concn. of sugar in replacement solutions: {Molasses = 13·500 g. / Sucrose = 12·845 g.

Data obtained after replacement

(incubation for 3 days: all results calc. for 125 ml. of medium)

Concn. of arsenite, M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A*	B*	Concn. of arsenite, M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A*	B*
Molasses													
0·2	−0·125	0·000	0·008	1·42	—	0·60	0·2	−0·128	0·05	0·002	2·14	2·4	0·09
0·1	−0·067	0·000	0·036	1·67	—	2·2	0·1	−0·061	0·12	0·005	3·35	3·7	0·15
0·05	0·087	0·02	0·084	2·18	0·91	3·8	0·05	0·227	0·30	0·096	4·03	7·4	2·4
0·01	0·376	0·06	0·072	2·36	2·5	3·0	0·01	0·357	0·45	0·084	4·27	10·5	1·9
0·005	1·833	0·20	0·068	4·28	4·7	1·6	0·005	1·826	1·25	0·068	7·95	15·7	0·85
0·001	2·054	0·24	0·054	8·47	4·5	1·0	0·001	1·698	1·12	0·065	8·32	13·4	0·78
0·000 (Control)	1·948	0·15	0·032	7·63	3·2	0·69	0·000 (Control)	1·607	0·87	0·058	8·18	10·6	0·70
Sucrose													
0·2	−0·286	0·000	0·003	2·85	—	0·1	0·2	−0·387	—	0·011	0·86	—	1·3
0·1	−0·126	0·000	0·012	4·71	—	0·29	0·1	−0·321	—	0·057	1·73	—	3·2
0·05	0·215	0·000	0·084	4·96	—	1·6	0·05	−0·052	0·05	0·121	3·54	1·4	3·4
0·01	0·713	0·10	0·067	5·12	1·9	1·3	0·01	0·419	0·24	0·159	4·17	5·7	3·8
0·005	1·2777	0·20	0·062	5·62	3·5	1·1	0·005	0·757	0·74	0·146	6·64	11·1	2·2
0·001	1·352	0·25	0·037	6·73	3·7	0·53	0·001	0·961	0·63	0·115	7·53	8·3	1·5
0·000 (Control)	1·338	0·17	0·025	9·34	2·9	0·40	0·000 (Control)	0·733	0·55	0·092	7·05	7·7	1·3

Culture at time of replacement

Felt wt., g.	1·652 ± 0·0477
Change in titratable acidity	65·25
Sugar consumed, g.	6·17

1·447 ± 0·0250	
107·5	
5·64	

Initial pH of final replacement solutions, 1·7

Initial concn. of citric acid in replacement solutions, 7·5

Data obtained after replacement

(incubation for 3 days: results calc. on 125 ml. of medium)

Citric acid

Concn. of arsenite, M	Increase in felt wt., g.	Oxalic acid, g.	Citric acid consumed, g.	B*	Concn. of arsenite, M	Increase in felt wt., g.	Oxalic acid, g.	Citric acid consumed, g.	B*
0·20	−0·137	0·000	0·00	—	0·20	−0·009	0·000	0·00	—
0·10	−0·088	0·001	0·00	—	0·10	−0·037	0·000	0·00	—
0·05	−0·009	0·015	0·02	75·00	0·05	0·243	0·008	0·03	26·66
0·01	0·015	0·025	0·14	18·56	0·01	0·310	0·014	0·33	4·24
0·006	0·039	0·065	0·63	10·31	0·005	0·312	0·019	0·72	2·64
0·001	0·127	0·088	1·88	4·67	0·001	0·320	0·023	2·84	0·80
0·000	0·246	0·125	3·68	3·42	0·000	0·375	0·028	3·27	0·86

* In this and the following tables, $A = \frac{\text{Citric acid (g.)}}{\text{Sugar consumed (g.)}} \times 100$, $B = \frac{\text{Oxalic acid (g.)}}{\text{Carbon source consumed (g.)}} \times 100$

The carbon source may be molasses sugar, sucrose or citric acid

The flasks were re-incubated at 28° for 20 h., and the liquid contents of each set were then bulked and filtered. To each filtrate three volumes of a recently filtered solution of 2,4-dinitrophenylhydrazine (4 g./l. of 2N-HCl) were added and the mixtures were kept for 6 h. The precipitates were separately filtered through sintered glass crucibles (porosity 4) and then washed with warm N-$NaHCO_3$. The crucibles with the hydrazones were dried over calcium chloride in a vacuum desiccator. The weights of the precipitates obtained are shown in Table IV.

The precipitates were dissolved in chloroform and the solutions tested for their contents of α-keto-acid hydrazones by one-dimensional paper chromatography (Cavallini *et al.*[15]). The area and intensity of the colour of the different spots to which the precipitates were resolved were considered as a rough indication of the amounts of the different hydrazones.

The unknown precipitates showed two spots; the top one lay in line with and in between the two lateral spots of the authentic hydrazone of α-ketoglutaric acid while the second bottom spot ran slightly further than that of the hydrazone of pyruvic acid. With a mixture of the authentic hydrazones the two spots of the unknown lay at the same level and in line between the spots of the authentic hydrazones (see Fig. 1). The precipitates obtained from carbohydrate solutions were principally the dinitrophenylhydrazone of α-ketoglutaric acid at lower

Table II

Behaviour of A. niger *in presence of iodoacetate*

	STRAIN B_8	STRAIN NI
Culture at time of replacement		
Felt wt., g.	1·557 ± 0·0373	1·314 ± 0·0147
Change in titratable acidity	66·25	107·5
Sugar consumed, g.	5·32	6·18

Initial pH of final replacement solutions, 5·5
Initial concn. of sugar in replacement solutions: {Molasses = 13·125 g. / Sucrose = 13·375 g.

Data obtained after replacement

(incubation 3 days: results calc. on 125 ml. of medium)

Concn. of iodoacetate, M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A	B	Concn. of iodoacetate, M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A	B
Molasses													
0·002	1·334	0·11	0·030	3·17	3·40	1·40	0·002	2·383	1·52	0·161	5·67	26·8	2·80
0·001	1·681	0·23	0·086	3·95	5·80	2·20	0·001	2·463	1·00	0·115	5·99	17·3	1·90
0·0005	0·204	0·19	0·058	4·59	4·10	1·10	0·0005	2·589	0·95	0·063	7·60	12·5	0·83
0·0000 (Control)	2·177	0·18	0·038	4·65	3·80	0·80	0·0000 (Control)	2·578	0·84	0·055	6·62	12·7	0·82
Sucrose													
0·002	0·639	0·46	0·130	4·70	14·2	3·20	0·002	1·438	1·25	0·446	4·65	27·7	9·60
0·001	0·724	0·45	0·136	5·04	8·90	2·70	0·001	1·426	0·65	0·187	4·77	13·5	3·90
0·0005	0·972	0·25	0·047	5·68	4·30	0·83	0·0005	1·653	0·42	0·125	7·54	6·40	1·90
0·0000 (Control)	1·038	0·20	0·025	5·57	3·50	0·47	0·0000 (Control)	1·725	0·35	0·049	7·30	4·80	0·67

Culture at time of replacement		
Felt wt., g.	1·030 ± 0·0382	1·666 ± 0·0124
Change in titratable acidity	50·5	92·5
Sugar consumed, g.	6·05	6·28

Initial pH of final replacement solutions, 5·5
Initial concn. of sugar in replacement solutions: {Molasses = 13·300 g. / Sucrose = 12·863 g.

Data obtained after replacement

(incubation 3 days: results calc. on 125 ml. of medium)

Concn. of iodoacetate, M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A	B	Concn. of iodoacetate, M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A	B
Molasses													
0·01	0·815	0·00	0·009	1·63	—	0·55	0·01	1·148	0·41	0·015	4·06	10·1	1·08
0·005	1·116	0·08	0·018	3·29	2·30	0·53	0·005	1·572	0·87	0·053	5·59	18·8	1·15
0·0001	1·743	0·16	0·027	5·63	2·80	0·48	0·0001	2·248	0·56	0·036	8·58	6·50	0·42
0·0000 (Control)	1·787	0·10	0·029	3·94	2·50	0·72	0·0000 (Control)	2·076	0·55	0·032	5·43	10·9	0·60
Sucrose													
0·1	0·591	0·10	0·011	2·55	3·90	0·44	0·01	0·640	0·36	0·032	3·82	9·50	0·85
0·005	0·663	0·15	0·020	4·28	3·50	0·49	0·005	0·836	0·43	0·051	5·64	7·6	0·90
0·0001	1·065	0·22	0·036	6·66	3·30	0·54	0·0001	1·518	0·48	0·082	7·55	6·40	2·86
0·0000 (Control)	0·883	0·19	0·022	5·29	3·50	0·41	0·0000 (Control)	1·389	0·48	0·068	6·47	7·40	1·04

Culture at time of replacement		
Felt wt., g.	1·946 ± 0·0332	1·825 ± 0·0441
Change in titratable acidity	87·4	123·8
Sugar consumed	6·87	5·89

Initial pH of final replacement solutions, 1·8
Initial concn. of citric acid in replacement solutions, 7·5 g.

Data obtained after replacement

(incubation 3 days: results calc. for 125 ml. of medium)

Concn. of iodoacetate, M	Increase in felt wt., g.	Oxalic acid, g.	Citric acid consumed, g.	B	Concn. of iodoacetate, M	Increase in felt wt., g.	Oxalic acid, g.	Citric acid consumed, g.	B
0·01	0·115	0·046	1·05	4·40	0·01	0·027	0·008	0·72	1·11
0·005	0·120	0·088	2·10	4·20	0·005	0·138	0·013	1·05	1·24
0·002	0·164	0·097	2·75	3·52	0·002	0·215	0·025	2·48	1·00
0·001	0·186	0·124	3·33	3·72	0·001	0·295	0·028	2·66	1·05
0·0005	0·203	0·137	3·88	3·53	0·0005	0·325	0·035	3·34	1·04
0·0001	0·215	0·140	4·00	3·50	0·0001	0·314	0·039	3·65	1·07
0·0000 (Control)	0·227	0·138	4·10	3·36	0·0000 (Control)	0·350	0·038	3·74	1·01

concentrations of arsenite and mainly of that of pyruvic acid at higher concentrations. Pure samples of the hydrazone of α-ketoglutaric acid were isolated from the precipitates obtained from cultures containing the lowest levels of arsenite and from those separated from poisoned citric acid solutions, with ethyl acetate/ligroin mixture. M.p. and mixed m.p. of the pure yellow needles was 213·4–213·8°

Table III

Behaviour of A. niger *in presence of 2,4-dinitrophenol (D.N.P.)*

STRAIN B_8

(A) *Culture at time of replacement*

Felt wt., g.	1·942 ± 0·0430
Change in titratable acidity	58·25
Sugar consumed	4·75

Initial concn. of sugar in replacement solutions: {Molasses = 13·125 g. / Sucrose = 12·50 g.

Initial pH of replacement solutions, 5·5

Data obtained after replacement

(incubation 3 days: results calc. for 125 ml. of medium)

Concn. of D.N.P., M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A	B
Molasses						
0·005	0·214	0·00	0·001	3·26	—	0·03
0·0025	0·320	0·00	0·007	3·47	—	0·20
0·0000 (Control)	2·165	0·10	0·021	5·72	1·74	3·67
Sucrose						
0·005	−0·407	0·00	0·001	4·03	—	0·02
0·0025	−0·257	0·00	0·004	4·58	—	0·08
0·0000	1·284	0·056	0·011	6·38	0·87	0·17

STRAIN NI

Concn. of D.N.P., M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A	B
Molasses						
0·005	0·295	0·00	0·007	3·38	—	0·20
0·0025	0·297	0·25	0·009	3·43	7·28	0·26
0·0000 (Control)	2·088	1·15	0·055	7·60	15·13	0·72
Sucrose						
0·005	−0·099	0·00	0·000	3·84	—	—
0·0025	−0·052	0·00	0·001	3·67	—	0·02
0·0000	1·023	0·52	0·093	5·96	8·72	1·56

(B) *Culture at time of replacement*

Felt wt., g.	1·630 ± 0·135
Change in titratable acidity	58·5
Sugar consumed, g.	5·18

2·044 ± 0·0138
95·2
5·86

Initial concn. of sugar in replacement solutions: {Molasses = 12·84 g. / Sucrose = 12·35 g.

Initial pH of final replacement solutions, 5·5

Data obtained after replacement

(incubation 3 days: results calc. for 125 ml. of medium)

Concn. of D.N.P., M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A	B
Molasses						
0·0005	1·841	0·14	0·053	6·73	2·0	0·78
0·0000 (Control)	1·775	0·12	0·036	6·59	1·8	0·54
Sucrose						
0·0005	1·038	0·08	0·013	5·63	1·42	0·23
0·0000 (Control)	0·979	0·06	0·009	5·81	1·0	0·14

Concn. of D.N.P., M	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	A	B
Molasses						
0·0005	1·690	1·82	0·086	6·11	30·0	1·4
0·0000 (Control)	1·853	1·35	0·048	6·11	22·0	0·8
Sucrose						
0·0005	0·602	0·43	0·109	7·47	5·6	1·4
0·0000 (Control)	1·072	0·38	0·075	6·38	5·8	1·2

Table IV

Yield (g./100 ml.) of dinitrophenylhydrazones of acids obtained from media containing arsenite

Concn. of arsenite, M	Molasses Strain		Sucrose Strain		Citric acid* Strain	
	B_8	NI	B_8	NI	B_8	NI
0·20	0·12	0·08	0·05	0·01	0·00	0·00
0·10	0·17	0·14	0·08	0·03	0·00	0·00
0·05	0·22	0·20	0·11	0·06	0·01	0·01
0·01	0·19	0·16	0·13	0·08	0·03	0·04
0·005	0·14	0·13	0·10	0·04	0·04	0·03
0·001	0·11	0·10	0·06	0·04	0·02	0·02
0·000 (Control)	0·05	0·04	0·01	0·01	0·00	0·01

* Precipitates consisted mainly of the hydrazone of α-ketoglutaric acid, with traces of that of pyruvic acid

Discussion

Arsenite

The formation of felts was appreciably suppressed in sucrose and citric acid solutions poisoned with arsenite. The effect was less in molasses solutions probably because of the formation of nitrogenous cell constituents, which process would be less sensitive to the inhibitor than the one leading to the formation of non-nitrogenous cell components. The decrease in the initial weights of felts which was recorded at the highest levels of arsenite may be due to

the marked restriction imposed on the uptake of sugar or citric acid so that insufficient carbohydrate was available for the metabolic processes. This would compel the organisms to utilise their own reserve materials and thus reduce the initial weights of the felts.

The effect of arsenite on the uptake of sugar can be visualised as taking place in two stages: (1) a slight stimulatory influence recorded in most cases at lowest level and (2) a restricting effect at higher concentrations. The stimulating influence may be due to the failure of the organisms to make the full use of the T.A.C. (because of the stabilising effect of arsenite on further transformation of pyruvic and α-ketoglutaric acids). To overcome this the organisms consumed sugar at a comparatively greater rate. Higher levels of arsenite suppressed further decarboxylation of pyruvic acid resulting from sugar breakdown. The accumulated pyruvic acid would tend to retard the catabolism of sugar and thus restrict the sugar uptake. This is substantiated by the results of paper chromatography of the dinitrophenylhydrazones. Thus arsenite at lower levels disturbs the flow of metabolites from sugar to the T.A.C. at pyruvic/acetate stage while strongly restricting the α-ketoglutaric/succinic transformation. Higher levels of the inhibitor interfere strongly at the pyruvic/acetate stage so as to deprive the T.A.C. of enough acetate to maintain the normal rate, thus restricting the formation of α-ketoglutaric acid.

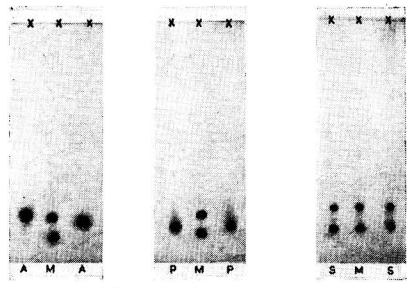


FIG. 1.—*Chromatograms showing resolution of 2,4-dinitrophenylhydrazones of pyruvic and α-ketoglutaric acids*

M = 2,4-dinitrophenylhydrazones of acids in metabolism solutions
A, P, S = Authentic 2,4-dimitrophenylhydrazones of α-ketoglutaric, pyruvic acids and a mixture of these, respectively

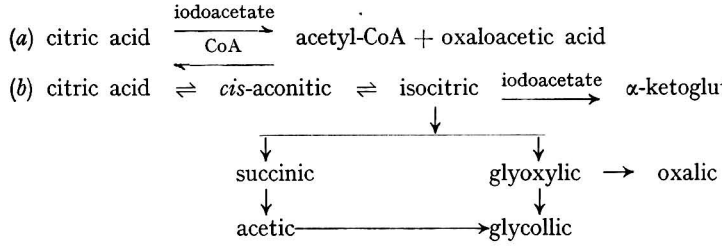
Citric acid formation was promoted by the lowest levels of arsenite. The calculated figures for citric acid per 100 g. of sugar consumed are usually higher for the cultures containing the highest levels of arsenite than those of the corresponding controls. This might be due to the high sensitivity of the citric acid uptake to the poison. The accumulation of α-ketoglutaric acid in citric acid solutions containing arsenite increased up to 0·01M. Above this level the inhibitor exerted a general non-specific toxic effect.

Yield of oxalic acid in poisoned solutions of molasses and sucrose increased with increasing concentrations up to 0·05M of arsenite, reduced yields being recorded only at the highest levels of arsenite. The inhibitor restricted the acid formation from citric acid, but the figures calculated for oxalic per 100 g. of consumed citric acid are generally higher in case of poisoned cultures than the corresponding controls. From all this evidence the sequence of events which take place from sugar to oxalic acid can be envisaged. In carbohydrate solutions arsenite would stabilise pyruvic and α-ketoglutaric acids. The accumulation of the former acid might possibly increase the fixation of CO_2 to form oxaloacetic acid which might be cleaved to oxalic and acetic acids.[16] Acetic acid may be oxidised to oxalic acid via glycollic and glyoxylic acids,[17] or may condense with oxaloacetic acid to produce citric acid. This acid might be degraded to oxalic acid either via oxaloacetic and acetic acids and/or isocitric acid when cleaved to glyoxylic and succinic acids.[18] Thus at concentrations of arsenite where it exerts its selective inhibitory influence on the oxidative decarboxylation reactions of α-keto-acids, the increase in the yields of oxalic acid would be anticipated.

Iodoacetate

The effects of iodoacetate are expected on basis of its presumed effect suggested by Shimi.[6] Its influence on the sugar uptake in sucrose and molasses solutions showed that in addition to the main glycolytic route there is another route by which sugar is degraded. In carbohydrate cultures poisoned with iodoacetate, CoA is presumably inhibited and thus the formation of acetyl-CoA would be restricted. As a result less oxaloacetic acid would be involved in the formation of citric acid and the rest of the former acid might be cleaved to oxalic acid. Thus increased yields of oxalic acid would be expected in most of the cultures poisoned with iodoacetate. The suppressing influence of iodoacetate on the formation of oxalic acid from citric

acid may be attributed to the interference of the inhibitor in the two main pathways of the T.A.C. through which the latter acid would be catabolised, viz.,



Route (*b*) would not be highly sensitive to iodoacetate as it affects the isocitric dehydrogenase. Since the yields of oxalic acid which were formed from citric acid were markedly reduced by iodoacetate it would be reasonable to exclude this route as a major channel through which citric acid is degraded to oxalic acid under the present experimental conditions. This implies that the acetic/oxalic route is the one actually followed.

Dinitrophenol

The behaviour of the organisms floated on either molasses or sucrose solutions poisoned with the nitrophenol was similar in both cases. The accumulation of citric and oxalic acids and the up-take of sugar in both types of cultures were markedly restricted. This indicates that these metabolic processes require high-energy phosphate bonds. The yields of citric and oxalic acids produced per 100 g. of sugar consumed were markedly reduced in poisoned cultures. This is probably due to the interference of the nitrophenol in some metabolic changes leading from sugar to the two acids.

Conclusions

(1) In molasses and sucrose solutions sugar is degraded mainly via the well-known glycolytic route and also a minor channel which is insensitive to iodoacetate.

(2) Molasses sugars are mainly catabolised through the T.A.C. and the citric acid produced is a metabolic product of this cycle.

(3) The formation of mycelial cell constituents is promoted in molasses probably because of the presence of nitrogenous compounds available for such a metabolic process.

(4) Oxalic acid is formed in molasses and sucrose solutions via at least more than one pathway and its formation through the cleavage of isocitric acid is of minor importance.

(5) The hydrazones isolated from poisoned molasses solutions were larger in amount than those from poisoned sucrose solutions: this is considered as an indication that the rate of circulation of the T.A.C. is higher in the former case.

(6) The processes involved in the production of citric and oxalic acids from sucrose or cane molasses require energy-rich phosphate bonds.

(7) The ability of strain B_8 to consume citric acid is greater than that of strain NI and is the reason for the greater yields of citric acid in cultures of the latter strain.

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MYCOLOGICAL PRODUCTION OF CITRIC AND OXALIC ACIDS FROM CANE MOLASSES. III.*—Effects of some Mixed Substrates

By I. R. SHIMI and M. S. NOUR EL DEIN

The effects on the *A. niger* fermentation of sucrose and molasses exerted by various concentrations of ethanol, acetate and pyruvate are examined in presence of the optimum concentrations of ferrocyanide and phosphate. Such mixtures gave appreciable increases in the yields of citric acid with also traces of oxalic acid and the time of incubation could be reduced. The behaviour of two potent citric-acid-producing strains of *A. niger* on molasses medium supplemented with methanol was also studied. The results are discussed in the light of well-known metabolic pathways.

Introduction

Kormberg & Madson[1] found that with *Pseudomonas KBI* acetate enters the cycle at two sites, to form citrate at one and a compound metabolically close to malate at the other. Strauss[2] has obtained data indicating that acetate is oxidised via the tricarboxylic acid cycle (T.A.C.) in *Neurospora*. Kormberg & Quayle[3] showed that, when *Pseudomonas KBI* was grown on ^{14}C-acetate as sole source of carbon, intermediates were obtained from the T.A.C. and were utilised to provide the carbon skeleton of the amino-acids synthesised. Shimi[4] obtained appreciable increase in the yields of citric acid in submerged cultures of *Penicillium spinulosum* when acetate was added to the metabolism solutions, and discussed the energy relations of the metabolic processes involved in the formation of citric acid and fat when some of the presumed intermediate is present.

Moyer[5] described a process for the production of citric acid in which molasses is supplemented by different alcohols. The addition of 1–3% of methanol to the medium greatly increased the yields of citric acid and the tolerance of the organism to iron, zinc and manganese.

Experiments are described here on the effects of the presence of acetate, pyruvate and ethanol on the fermentation of molasses and sucrose by *Aspergillus niger*.

Experimental

The replacement technique was adopted as described before,[6] the only difference being that the final replacement solutions contained acetate, pyruvate or ethanol with either sucrose or molasses. The quantities of these compounds added were those calculated to give the same amounts of total carbon as are present in equal volumes of the corresponding controls. The organisms used were *Aspergillus niger* strains NI and B_8. As the results for the B_8 strain are in general similar to those for the other strain, only the data concerning the latter are set out in this paper, although the yields of citric in cultures of strain NI were higher than those for strain B_8.

* Part II: preceding paper

The static surface culture technique with strains NI and C_{IIe} was also adopted without the replacement procedure. These experiments were carried out to study the effects exerted by the optimum amounts of ferrocyanide and phosphate and suitable concentrations of each of the compounds mentioned above on the formation of citric and oxalic acids.

Fourteen triplicate sets of 1-l. Erlenmeyer flasks were used, seven sets being devoted for the growth of each organism with different media. The media contained 15·41% w/v of molasses sugar; 0·2% w/v of ammonium nitrate; and had initial pH 5·5. The amounts of ferrocyanide and phosphate and the technique of their supplementation to molasses were as described in a previous communication.[7] After incubation for 8 days 10-ml. portions were withdrawn from each flask at 2-day intervals. The three portions of each particular set at any given incubation period were mixed together and the different estimations were carried out.

The experiment carried out to study the effect of methanol was performed as described above for ferrocyanide, except that methanol was the sole supplement added to the molasses medium.

Results

These are shown in Tables I–VI.

Behaviour of A. niger (*strain NI*) *in presence of* (**Table I**) *sodium acetate.* (**Table II**) *sodium pyruvate*

Table I

Table II

Culture at time of replacement

Felt wt., g.	0·889 ± 0·0445	1·257 ± 0·0744
Change in titratable acidity	70·5	75·2
Sugar consumed, g.	6·25	6·18

Initial pH of replacement solutions, 5·5

Data obtained after replacement

(incubation after replacement for 3 days: results calc. for 125 ml. of medium)

Carbon source, g.	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	Oxalic/citric	A*	B*	Carbon source, g.	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	Oxalic/citric	A*	B*
(m) 11·25 + (a) 1·79	2·700	2·50	0·337	8·49	0·13	29·4	3·95	(m) 11·25 + (p) 1·607	2·452	1·41	0·173	6·70	0·12	21·05	2·58
(m) 11·50 + (a) 1·49	2·61	1·56	0·133	7·42	0·085	21·0	1·79	(m) 11·50 + (p) 1·286	2·309	1·35	0·109	7·32	0·08	18·44	0·15
(m) 13·62	2·593	1·56	0·054	6·35	0·34	24·6	0·85	(m) 13·80	2·292	1·00	0·059	6·34	0·06	15·77	0·93
(s) 11·25 + (a) 1·79	1·734	0·38	0·184	7·49	0·48	5·07	2·46	(s) 11·25 + (p) 1·607	1·848	0·32	0·173	5·65	0·54	5·67	3·06
(s) 11·50 + (a) 1·49	1·768	0·25	0·079	7·02	0·32	3·56	11·3	(s) 11·50 + (p) 1·286	1·8334	0·25	0·123	5·36	0·49	4·66	2·39
(s) 13·13	1·673	0·025	0·040	5·36	1·60	0·47	0·75	(s) 13·80	1·88	0·23	0·048	5·39	0·21	4·26	0·89

(m) molasses sugar (a) sodium acetate (s) sucrose (p) sodium puruvate * see preceding paper

Discussion

Acetate slightly increased the weight of felt, possibly due to the formation of some polysaccharides in the mycelial cells.[8] The increase shown in the yields of citric acid on fermentation in presence of acetate has been frequently reported by other authors. The presence of the added acetate may promote the formation of acetyl-CoA which may subsequently condense with oxaloacetate to form citric acid. Stern & Ochoa[9] found that such a process is exergonic in nature and 7720 cal. are liberated. The direct condensation of acetate with oxaloacetate to yield citric acid without the involvement of CoA[10] has also been reported. In a previous communication[6] the so-called route b was excluded as a major pathway for the biosynthesis of oxalic acid from citric acid by the organisms employed. The cleavage of oxaloacetic acid to oxalic and acetic acids was considered as a pathway through which appreciable amounts of the oxalic acid is biosynthesised. If oxalic acid is formed only by this process it would be expected that in presence of added acetate the yields of citric acid would increase at the expense of oxalic acid, which was not the case. Thus the repeated oxidation of acetate to oxalate is a process leading to appreciable yields of oxalic acid. The present results are in accord with this view.

Behaviour of A. niger *in presence of* (**Table III**) *ethanol or* (**Table IV**) *methanol*

Table III

Strain NI

Cultures at time of replacement

Felt wt., g. 1·344 ± 0·0222
Change in titratable acidity 76·35
Sugar consumed, g. 6·18

Initial pH of final replacement solutions, 5·5

Data obtained after replacement

(incubation after replacement 3 days: results calc. for 125 ml. of medium)

Carbon source, g.	Increase in felt wt., g.	Citric acid, g.	Oxalic acid, g.	Sugar consumed, g.	Oxalic/citric	A*	B*
(m) 11·25 + (e) 1·0	2·319	2·04	0·489	8·93	0·24	22·85	5·48
(m) 11·88 + (e) 0·5	2·318	1·64	0·312	7·86	0·19	20·86	3·97
(m) 12·5	3·112	1·33	0·085	7·62	0·06	17·45	1·12
(s) 11·25 + (e) 1·0	1·551	0·92	1·578	5·83	1·72	15·78	27·06
(s) 11·88 + (e) 0·5	1·603	0·77	1·234	5·41	1·61	14·24	22·80
s) 12·5	1·375	0·32	0·047	5·08	0·15	6·29	0·92

(m) = molasses sugar (e) = ethanol (s) = sucrose * see preceding paper

Table IV

Strains NI and C_{IIe}

Initial pH of media, 5·5
Final pH of media, 2·0–6·0
Initial concn. of molasses sugar: 14·5 g./100 ml. of medium
Results calc. on 100 ml. of media

Methanol concn., (g.) %	Citric acid, g.			Oxalic acid, g.			Sugar consumed, g.		
	Incubation period in days								
	9	12	15	9	12	15	9	12	15
Strain C_{IIe}									
0·00	2·18	3·25	2·80	0·082	0·218	0·635	6·37	8·47	11·62
0·50	0·92	1·54	1·87	0·114	0·236	0·576	5·63	7·18	9·88
1·00	1·61	2·33	2·88	0·156	0·248	0·714	6·94	8·54	11·73
2·00	3·76	6·85	5·37	0·052	0·086	0·127	7·79	9·17	12·54
3·00	0·00	1·25	0·47	0·000	0·002	0·004	3·17	4·68	6·55
Strain N1									
0·00	3·76	4·02	3·26	0·585	0·713	0·954	9·29	12·66	14·08
0·50	1·71	2·55	2·61	0·203	0·287	0·352	5·07	8·55	11·45
1·00	1·12	1·84	1·53	0·162	0·205	0·266	7·38	11·16	13·86
2·00	0·58	0·90	0·78	0·133	0·156	0·187	5·96	9·38	12·44
3·00	0·00	0·00	0·00	0·000	0·000	0·000	1·85	2·69	4·35

Behaviour of A. niger *on mixed substrates*

Table V

Strain NI

Initial pH of media, 5·5: final pH 1·5 4·0
Results calc. on 100 ml. of media

Medium	Citric acid, g.			Oxalic acid, g.			Sugar consumed, g.		
	Incubation period in days								
	8	10	12	8	10	12	8	10	12
A	5·98	8·87	9·93	0·024	0·031	0·048	9·37	11·87	13·82
B	2·89	3·62	5·31	0·042	0·056	0·081	8·62	10·34	12·76
C	3·02	3·86	4·97	0·051	0·060	0·082	9·01	10·47	12·81
D	2·92	3·78	5·02	0·046	0·059	0·078	9·12	10·66	13·11
E	6·81	9·09	10·66	0·023	0·047	0·077	11·21	14·61	15·14
F	6·76	9·12	9·47	0·024	0·051	0·083	10·97	14·38	15·14
G	7·11	9·89	9·28	0·031	0·046	0·061	11·16	14·22	15·41
H	2·98	3·37	3·98	0·024	0·035	0·054	8·34	10·74	12·35

Table VI

Strain C_{IIe}

Medium	Citric acid, g.			Oxalic acid, g.			Sugar consumed, g.		
	Incubation period in days								
	8	10	12	8	10	12	8	10	12
J	6·10	9·12	10·72	0·021	0·036	0·039	10·31	12·47	14·12
K	3·33	4·84	6·68	0·045	0·078	0·126	11·07	13·64	15·23
L	2·98	3·81	5·31	0·051	0·083	0·184	11·13	13·72	15·41
M	3·16	4·10	5·67	0·047	0·124	0·177	11·41	13·61	15·41
N	7·93	11·82	10·84	0·044	0·052	0·081	12·13	14·79	15·41
O	6·08	9·17	10·19	0·045	0·059	0·088	11·91	14·98	15·41
P	6·86	10·96	10·12	0·041	0·047	0·078	11·97	14·87	15·41
Q	1·97	2·21	3·14	0·039	0·065	0·114	6·23	8·16	11·64

Composition of the molasses media (g./100 ml.)

A Ferrocyanide, 0·16 + phosphate, 0·01
B Sodium acetate, 1·79
C Sodium pyruvate, 1·607
D Ethanol, 1·0
E Ferrocyanide, 0·16 + phosphate, 0·01 + sodium acetate, 1·79
F Ferrocyanide, 0·16 + phosphate, 0·01 + sodium pyruvate, 1·607
G Ferrocyanide, 0·16 + phosphate, 0·01 + ethanol, 1·0
H Molasses control (15·41 molasses sugar + 0·2 ammonium nitrate)

J Ferrocyanide, 0·14 + phosphate, 0·01
K Sodium acetate, 1·79
L Sodium pyruvate, 1·607
M Ethanol, 1·0
N Ferrocyanide, 0·14 + phosphate, 0·01 + sodium acetate, 1·79
O Ferrocyanide, 0·14 + phosphate, 0·01 + sodium pyruvate, 1·607
P Ferrocyanide, 0·14 + phosphate, 0·01 + ethanol, 1·0
Q Molasses control (15·41, molasses sugar + 0·2, ammonium nitrate)

An assumption that the main bulk of oxalate is formed by oxidation of acetate would not agree with the data obtained during the work with enzyme inhibitors. Thus the repeated oxidation of acetate, the cleavage of oxaloacetate and the so-called route b all participate to different extents in the formation of oxalic acid. Since the results in the ethanol and pyruvate experiments are in general similar to those with acetate it would be reasonable to assume that they are transformed to acetate before being involved in the metabolic activities leading to the formation of citric and oxalic acids. Pyruvate could also yield oxalate subsequent to its transformation to oxaloacetate.

It is obvious that in the presence of acetate, pyruvate and ethanol the yields of citric acid are higher in the molasses solutions than in those containing sucrose. The formation of oxalic acid was influenced in an opposite manner, i.e., yields of the acid were higher in sucrose than in molasses.

Then the effects of different combinations of ferrocyanide/phosphate and each of the above compounds were examined. The most suitable concentrations of the three organic supplements for citric acid formation were added to the ferrocyanide/phosphate/molasses solutions. Yields of citric acid in cultures of strain C_{IIe} accounted for 76·63% of the initially available molasses sugar. This crop of acid was obtained after 10 days' incubation, which is a comparatively short period for such a surface fermentation process. The yields of oxalate were sufficiently low to cause little difficulty during the purification of the citric acid. In cultures of strain NI the yields of citric acid were equivalent to 69·18% of the initially available sugar, and oxalic acid was again produced in small amounts. In general, the presence of acetate, ethanol and pyruvate and the optimum concentrations of ferrocyanide/phosphate stimulated the accumulation of citric acid and uptake of sugar, acetate apparently being the most suitable. These findings might have some industrial significance.

Methanol influenced the metabolic activities of the two strains of *A. niger* in a different manner. In cultures of strain NI the concentrations of methanol used were all toxic to the organism. In cultures of strain C_{IIe} the maximum yields of citric acid, obtained with 0·2% methanol, were equivalent to 74·7% of the sugar consumed and 47·24% of the initially available sugar.

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THE NITRATE NITROGEN CONTENT OF HERBAGE. I.—Observations on Some Herbage Species

By G. ap GRIFFITH and T. D. JOHNSTON

Nitrate accumulation in grass which occurs when heavy dressings of nitrogen are applied varies in amount with species and strain of grass. With rape, nitrate accumulation occurred with only moderate dressings of nitrogen and persisted for up to 8 weeks.

Introduction

One of the principal criteria for the evaluation of herbage has always been the so-called crude protein content. This is an arbitrary figure arrived at by multiplying total N% by the factor 6·25 on the assumption that (*a*) the nitrogen content is all in the form of protein, and (*b*) the protein has a N content of 16%. Both these are only approximately true. In earlier pasture work the crude protein content provided a useful rough-and-ready means of

comparison of species and varieties. As the need for more accurate assessment has developed, greater attention has been given to the nature of the compounds comprising the nitrogenous part of the herbage in a search for differences in quality, on the one hand, or for the cause of digestive upsets, on the other.

In keeping with this trend, the non-protein nitrogen has become one of the main subjects of investigation at this Station, and following from the observation of a somewhat high nitrate content in a composite reference sample of herbage used in the checking of routine analyses, interest was centred on one constituent only, namely, nitrate-N. The composite sample consisted of a mixture of some hundreds of herbages, and the inference was that if it had such an unexpectedly high nitrate content then some of the herbage samples which contributed to it might be expected to be still more abnormal in this respect.

Experimental and results

An experiment in which eight grasses had been grown in swards and as spaced plants, seemed to offer likely material to start the investigation.

Nitrogen had been applied as Nitro-chalk, all plots receiving a total of 12 cwt. per acre applied in three dressings of 4 cwt. per acre each ; the herbage was sampled at successive dates throughout the growing season. Table I summarises the results of nitrate determinations on this material.

Table I

Nitrate-N % in dry matter of herbage at successive stages of growth (primary growth) at Cae Glanrafon, Gogerddan, 1957 (elevation 80 ft.)

(4 cwt. of N per acre applied on 18 March, 13 May and 12 June)

	Date of sampling				
	9 May	25 May	7 June	20 June	3 July
Swards					
S.23 perennial ryegrass	0·003	0·035	0·022	0·182	0·054
S.24 ,, ,,	nil	0·096	0·054	0·243	0·112
S.170 tall fescue	nil	0·051	0·054	0·157	0·077
S.59 red fescue	0·016	0·042	0·032	0·269	0·128
S.143 cocksfoot	0·006	0·074	0·038	0·077	0·048
S.48 timothy	0·003	0·051	0·045	0·090	0·080
S.50 ,,	nil	0·064	0·035	0·122	0·048
Br 616 bent	0·006	0·048	0·016	0·160	0·125
Spaced plants					
S.23		0·365	0·138	0·253	0·099
S.24	Too	0·182	0·083	0·121	0·051
S.170	little	0·109	0·055	0·099	0·042
S.59	growth	0·109	0·061	0·137	0·042
S.143	for	0·343	0·163	0·250	0·138
S.48	cutting	0·214	0·045	0·128	0·035
S.50		0·195	0·086	0·147	0·054
Br. 616		0·141	0·042	0·106	0·035

On the first two dates the spaced plants in most cases had more nitrate than had plants in the swards, the quantities often being very high. This results from the fact that fewer plants are competing for the nitrogen ; crude protein was also generally much higher in the spaced plants. The nitrate remaining is the excess over what the plants have been able to utilise. In the June cut, when the plants are approaching maturity, the swards show this excess to the same degree as the spaced plants, the effect persisting until the next sampling date without a further application of nitrogen.

In the spaced plants, S.23 perennial ryegrass and S.143 cocksfoot without exception have the highest nitrate at each date, but it is of interest to note that these two grasses do not show this distinction in the swards. The implications of this call for investigation. Spaced plants of S.23 always have a higher nitrate content than S.24. This, again without exception, is reversed in the swards.

A similar experiment with identical layout and treatments was carried out in the same season at Tynpynfarch, at an altitude of 700 ft. The results are summarised in Table II.

Table II

Nitrate-N% in dry matter of herbage at successive stages of growth (primary growth) at Tynpynfarch, 1957 (elevation 700 ft.)

(4 cwt. of N per acre applied on 18 March, 17 May and 12 June)

	Date of sampling			
	10 May	22 May	6 June	21 June
Swards				
S.23	0·010	0·070	0·013	0·080
S.24	0·006	0·067	0·035	0·106
S.170	0·013	0·077	0·045	0·086
S.59	0·006	0·054	0·029	0·080
S.143	0·006	0·166	0·054	0·102
S.48	0·010	0·090	0·032	0·109
S.50	0·003	0·083	0·026	0·054
Br.616	0·006	0·096	0·026	0·090
Spaced plants				
S.23			0·224	0·317
S.24			0·090	0·102
S.170	Insufficient		0·083	0·093
S.59	growth		0·054	0·125
S.143	for		0·234	0·346
S.48	sampling		0·118	0·102
S.50			0·102	0·118
Br.616			No sample	0·083

The higher nitrate in the spaced plants of S.23 and S.143 confirms the observation made on the plots in Cae Glanrafon (80 ft.). At the higher elevation there is generally more nitrate in mid-May herbage and less in June herbage. This may be a reflection of the later season at the higher altitude when less use is made of nitrate at the earlier date, and more at the later date when growth at the lower elevation is approaching maturity. There is a somewhat shorter interval, however, between N application and sampling on the May herbage at Tynpynfarch. S.23 is much higher in nitrate than S.24 in the spaced plants but on the swards S.24 tends to be higher than S.23.

Material was also examined from a third experiment with a similar layout, but instead of sampling the herbage at successive stages of growth as in the first two experiments the grass was cut at each date indicated, and allowed to recover for re-cutting at the next date. The nitrate results from this material are summarised in Table III. The equivalent of 16 cwt. of Nitro-chalk was given in four dressings.

Table III

Nitrate-N % in dry matter of herbage cut at repeated intervals (recovery growth)

(4 cwt. of N per acre applied on 18 March, 6 May, 12 June and 1 August)

	Date of cutting			
	26 April	28 May	16 July	5 September
Swards				
S.23	0·016	0·013	0·013	0·160
S.24	0·016	0·035	0·029	0·350
S.170	0·010	0·035	0·029	0·045
S.59	0·006	0·022	0·013	0·384
S.143	0·026	0·048	0·029	0·096
S.48	0·010	0·038	0·022	0·093
S.50	0·019	0·029	0·016	0·384
Br.616	0·029	0·026	0·016	0·051
Spaced plants				
S.23	0·304	0·330	0·048	0·326
S.24	0·256	0·179	0·080	0·330
S.170	0·195	0·106	0·090	0·297
S.59	0·125	0·125	0·085	0·259
S.143	0·317	0·330	0·163	0·362
S.48	0·211	0·214	0·109	0·300
S.50	0·144	0·160	0·058	0·253
Br.616	0·176	0·125	0·048	0·205

The higher nitrate content of S.23 and S.143 in the spaced plants, although less marked, is well in evidence, and again this does not apply to the swards. S.23 is only higher in nitrate than S.24 in the first two cuts of the spaced plants. In the swards, S.24, as in the previous experiments, has the higher nitrate. There is no tendency to nitrate accumulation in the swards during mid-season. This may be because of the much longer interval between N application and cutting, and also that at each cut the bulk of the N applied up to that date is removed, and the recovery growth has to start its uptake of nitrogen afresh; accumulation occurs in September when growth is slowing down.

The results show unmistakably that nitrate accumulation can vary both with species and variety. Only S.23 and S.24 perennial ryegrass and S.143 cocksfoot have been mentioned in the above discussion, but there are other, though less definite, indications of specific differences. For example, the high nitrate figures for S.59 red fescue in June and July of the first experiment are paralleled in the third experiment by an abnormally high figure for this grass in September.

Further work is needed, and is now being planned, to investigate the factors which may control the accumulation. There has been a tendency to regard nitrate accumulation as an abnormality due to ill-timed application of N. There is no doubt that this is often the cause, and Tables I and II are sufficient evidence of this, especially where the intervals between manuring and cutting are exceptionally short. A long interval, however, is not always a safeguard, as is shown by the results for the swards cut in September in the third experiment, where the interval is 5 weeks. Further evidence of this is presented in Part II.

Nitrate content of rape

Preliminary observations confirm that rape occasionally shows nitrate accumulation. During 1958 a replicated trial was carried out on rape varying the time of N application and date of cutting, and comparing Nitro-chalk with sulphate of ammonia each at two levels. In order to approach farming practice as closely as possible the levels of N applied were not high. The variation in dates of application and of cutting gave a series of intervals between application and cutting with a corresponding variation in nitrate content.

The nitrate results are summarised in Table IV.

Table IV

Nitrate-N % in dry matter of giant rape, 1958

	Cut on							
	7 October				12 November			
	Nitro-chalk		Sulphate of ammonia		Nitro-chalk		Sulphate of ammonia	
	Low	High	Low	High	Low	High	Low	High
Date of application of N								
August 15	0·031	0·028	0·029	0·030	0·011	0·026	0·019	0·023
August 29	0·046	0·100	0·063	0·086	0·022	0·029	—	0·023
September 12	0·118	0·420	0·088	0·366	0·030	0·074	0·042	0·079

Low = 30 lb. of N per acre High = 60 lb. of N per acre

These results show that the earliest date of application of N gives no nitrate accumulation at either level of N or either date of cutting, the interval between application and cutting here being approximately 8 and 13 weeks respectively. For the second date of application the first cutting date (6 weeks interval) shows nitrate accumulation at both levels of application, which has gone by the second cutting date (about 11 weeks interval). For the third date of application nitrate accumulation at the first date of cutting (over 3 weeks interval) is marked at the 2-cwt. level and high at the 4-cwt. level. For the second cutting (8 weeks interval), there is still a suggestion of some nitrate remaining at the 2-cwt. level, and quite a considerable quantity at the 4-cwt. level. There does not appear to be any difference between Nitro-chalk and sulphate of ammonia in respect of nitrate accumulation—in other words, to apply the N as ammonia is no safeguard against nitrate accumulation. Under the conditions of this experiment the conversion of ammonia into nitrate in the soil has taken place quickly enough to result in much of the nitrogen being taken up by the plant as nitrate.

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The material for investigation of the grass species and varieties was kindly supplied by Mr. Ll. Iorwerth Jones.

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THE NITRATE NITROGEN CONTENT OF HERBAGE. II.*—Effect of Different Levels of Application of Sulphate of Ammonia on the Nitrate Content of Herbage

By G. ap GRIFFITH

In a field experiment where sulphate of ammonia was applied in April at 0, 2, 4, 6, 8 and 12 cwt. per acre to three grass mixtures which were cut for silage in early June, the nitrate-N content of the herbage was detectable at the 4-cwt. level and considerable at the 12-cwt. level. A close relation was demonstrated between nitrate-N content and the crude protein content of the herbage.

Introduction

Nitrogen in the form of mineral nitrate in herbage is known to be toxic to farm animals. In the U.S.A. it has been stated that a 500-lb. animal eating only 5·5 lb. of hay containing 5% of potassium nitrate could be fatally poisoned, whilst the lower limit of toxicity has been placed at 1·5% potassium nitrate, equivalent to 0·22% of N as nitrate.[1] New Zealand results suggest that 1% potassium nitrate equivalent may be associated with weight losses in hoggets.[2] Muhrer *et al.*[3] reported that animals fed with sub-lethal amounts of nitrate equivalent to 0·5–1·5% potassium nitrate have reproduction and lactation difficulties.

It is desirable that more should be known of the circumstances in which an accumulation of nitrate nitrogen may be expected. Heavy N-manuring has been the major cause of such accumulations as have been reported in this country, although in New Zealand high nitrates have been found occasionally on unmanured grass.[2]

Results are here presented of determinations of nitrate-N on material from a field experiment where various levels of N were applied to different grass mixtures. The experiment carried out by the National Agricultural Advisory Service[4] at Trawscoed, near Aberystwyth, was to investigate the effect on yields of dry matter and crude protein of varying dressings of sulphate of ammonia. For the purpose of the present study of nitrate content it is to be noted that the dates of both N application and of cutting were normal for the district, and that the N was applied as sulphate of ammonia, which might reasonably be expected not to exaggerate any tendency to nitrate accumulation.

Experimental

Material and methods

Four different grass mixtures were seeded down directly from permanent pasture in June 1949. The material for the present enquiry was provided by the 1951 cut from three of these mixtures, namely:

* Part I: preceding paper

	lb. per acre
Mixture A	
Perennial ryegrass S.23	10
,, ,, S.101	10
White clover S.100	1
Wild white clover	½
Mixture C	
Cocksfoot S.37	13
,, S.143	5
White clover S.100	1
Wild white clover	¼

	lb. per acre
Mixture B	
Italian ryegrass	6
Perennial ryegrass S.101	4
Timothy S.48	3
Cocksfoot S.26	3
,, S.143	3
,, S.37	3
,, Danish	3
Alsike	2
Red clover S.123	3
White clover S.100	1
,, ,, S.184	½

The experiment as laid down consisted of three randomised blocks of nine treatments on each mixture with a plot size of approximately 40 sq. yd., but only six of these treatments are relevant to the present enquiry, namely, six levels of sulphate of ammonia at 0, 2, 4, 6, 8 and 12 cwt. per acre and for the purpose of this paper the experiment has been regarded as three randomised blocks of six treatments each.

Nitrogen was applied in 1950 and 1951 with a basal dressing of 3 cwt. of superphosphate and 2 cwt. of muriate of potash per acre each year, and in 1951 sulphate of ammonia was given at the end of April. Mixtures A, B and C were cut on 5 June, 31 May and 4 June, respectively.

Nitrate was determined on the air-dry ground samples of herbage by the following method: 100 ml. of distilled water were added to 1 g. of the sample and the whole brought to the boil, set aside for 10 min., stirred occasionally, filtered, and washed three times with distilled water. Celite filter-aid was added to speed up filtration and nitrate was determined without delay on the filtrate (delay in carrying out the determination causes nitrate losses which in a matter of hours can be considerable). The filtrate was boiled for 30 min. to drive off ammonia, Devarda's alloy added and boiling continued for 1 h. with distillation into 0·01N acid. Determinations were made in duplicate.

The results for these mixtures are summarised in Table I, and shown graphically for general-purpose Mixture B (Fig. 1). Graphs for Mixtures A and C are not included as they are essentially similar.

Table I

Nitrate-N % on air-dry material

(three replicates for each treatment)

	Cwt. per acre of sulphate of ammonia					
	0	2	4	6	8	12
Ryegrass Mixture A						
	0·012	0·008	0·012	0·024	0·101	0·146
	0·017	0·006	0·052	0·039	0·027	0·144
	0·007	0·012	0·011	0·032	0·042	0·172
Average	0·012	0·009	0·025	0·032	0·057	0·154
Sig. diff. between averages	0·040 at 5% level;		0·059 at 1% level;		0·082 at 0·1% level	
General-purpose Mixture B						
	0·011	0·008	0·022	0·048	0·181	0·293
	0·004	0·010	0·035	0·087	0·193	0·262
	0·013	0·020	0·057	0·157	0·069	0·281
Average	0·009	0·013	0·038	0·097	0·148	0·279
Sig. diff. between averages	0·074 at 5% level;		0·106 at 1% level;		0·153 at 0·1% level	
Cocksfoot Mixture C						
	0·017	0·011	0·017	0·035	0·111	0·140
	0·006	0·008	0·010	0·013	0·169	0·228
	0·011	0·015	0·006	0·028	0·067	0·168
Average	0·011	0·011	0·011	0·025	0·116	0·179
Sig. diff. between averages	0·061 at 5% level;		0·087 at 1% level;		0·126 at 0·1% level	

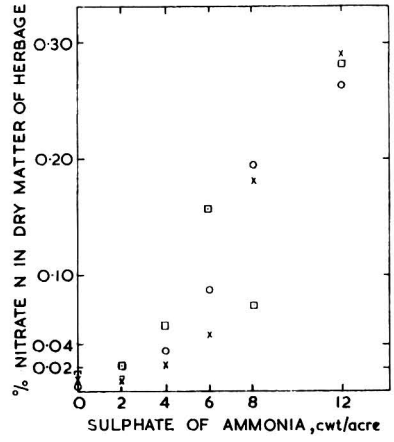

FIG. 1.—*Relation between nitrate-N and applied N on a general-purpose seeds mixture*
⊙ × ⊡ represent three replicates of each treatment

Variation between replicates

The differences between replicates of the same treatment are such as greatly to reduce the level of significance of differences between treatments. In more than one instance (e.g., Mixture B, 6 cwt.) the difference is over 200%.

This variation between replicates appears to be a characteristic of the nitrate-N. It was encountered to the same degree in another field trial which was studied earlier and was eventually in fact abandoned because of it. Butler[2] found nitrate to vary widely in New Zealand pastures. Sampling every 10 ft., he found within 50 ft. nitrate contents in the herbage varying from 0·1% to over 2% nitrate expressed as potassium nitrate (about 0·015 to 0·3% expressed as N). Some of the highest figures he attributes to urine patches (which he had marked) but even excluding these, the remaining variation was still large. He mentions botanical composition and height, and maturity of herbage, as factors probably contributing to the variation.

In planning future experiments the possibility of such variation between replicates will have to be taken into account and its causes investigated.

Relation between nitrate-N and crude protein

In Fig. 2, nitrate-N is plotted against crude protein for the three grass mixtures together, the data from the individual replicates of each experiment having been averaged. There is a close connexion between the crude protein and the nitrate content and there is a tendency for the nitrate to become evident when the crude protein value is higher than 18% or 19%. Since a nitrate-N content of as low as 0·07% (equivalent to 0·5% potassium nitrate) has been reported as toxic in effect, in this experiment one would treat with caution any material having more than 21% crude protein.

This close relationship between nitrate-N and crude protein only holds when the herbage material to be compared has been cut at the same time and has had the same interval between cutting and the time of application of nitrogen. In an experiment briefly reported previously[5] results for a number of cutting dates and with different intervals between cutting and nitrogen application showed that, although there was a general relationship between nitrate-N content and crude protein at the same cutting date and interval, this relationship did not extend over all the results of the experiment, and high nitrates were often found when the crude protein was low. In other words, although it has been a general experience that a high crude protein content is associated with a high nitrate content the fact that the crude protein value is low is not in all circumstances a guarantee that there will also be a low nitrate-N value.

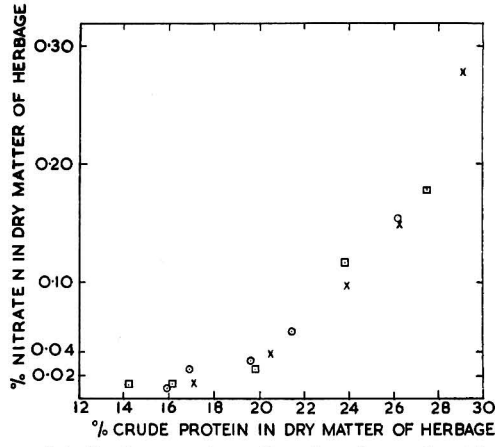

FIG. 2.—*Relation between nitrate-N and crude protein of herbage*
⊙ ryegrass mixture ⊡ cocksfoot mixture
× general-purpose mixture

A further interesting point is that, whereas the range of crude protein at the six levels of N varies with the three mixtures, their range of nitrate content shows a similar variation, so that the points for the three fall quite strikingly on the same curve.

Acknowledgments

The material for this study was provided by Dr. Rice Williams and Mr. J. R. Lloyd of the N.A.A.S. who also supplied the crude protein data. The statistical analysis of the results was carried out by Miss Margaret Ford of this Station.

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DRYING OF SEAWEEDS AND OTHER PLANTS. III.*—Through-circulation Drying of *Zostera marina*†

By J. H. MERRITT

Tests on the through-circulation drying of *Zostera marina* on a semi-commercial scale have been conducted with a batch dryer to determine the optimum conditions. There was a maximum feasible loading for grasswrack (eel-grass) of approximately 3·8 lb./sq. ft. in each layer. The effects of variations in the rate of air flow, of amount of air recirculated, of air-flow reversal and of temperature (160–220° F) have been studied with particular reference to output and efficiency.

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Introduction

In previous investigations the optimum conditions for the drying of a rockweed[1] and a kelp[2] were determined on a semi-commercial scale in a batch dryer. The present paper describes the desirable conditions for the drying of grasswrack (eel-grass), *Zostera marina*, a common seaweed used commercially as a heat insulator. No previous observations on the drying of this plant have been published. If dried by exposure to the sun outdoors when the humidity is high, considerable decomposition and deterioration occur.

Grasswrack (*Z. marina*) is a spermatophyte, a member of the pondweed family (*Najaceae*), but not botanically a true grass. It grows wholly under water, from a creeping rootstock, and forms very long grass-like ribbon-shaped leaves.

Experimental

The dryer was the same as that previously described.[1] A supply of *Z. marina* was obtained locally as cast weed. Tests were conducted from July to November, 1958, on plants which had been harvested less than two days before drying.

A weighed amount of wet grass was distributed evenly in layers on trays of expanded aluminium. The initial moisture content was approximately 86% and the final was 5%.* Otherwise the procedure was identical to that used previously for rockweed.[1]

Results

Independent variables

As in previous tests,[1] loading and air mass flow were found to be largely independent of other variables.

The maximum feasible loading was 0·53 lb. B.D.S. (bone-dry solids)/sq. ft./layer beyond which compression of the grass, due to its own weight, resulted in non-uniform drying. With a loading of 0·53 lb. B.D.S./sq. ft./layer in four layers, output was measured for various air velocities at zero recirculation (Fig. 1).

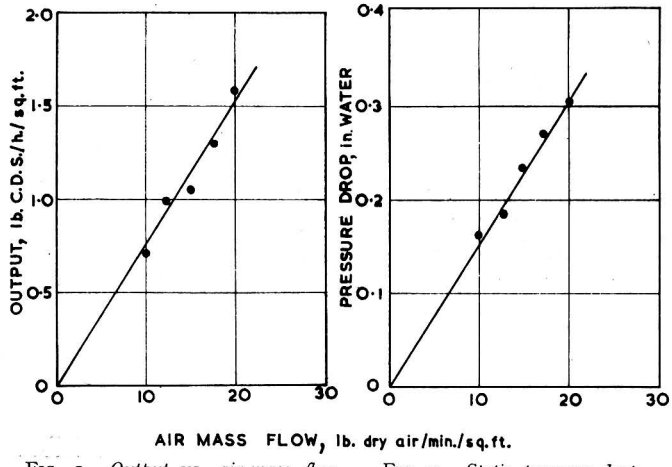

FIG. 1.—*Output* vs. *air-mass flow*
Loading 0·53 lb. B.D.S./sq. ft./layer in four layers
Recirculation zero
Control temperature 200° F

FIG. 2.—*Static pressure drop*
Loading 0·53 lb. B.D.S./sq. ft./layer in one layer
Control temperature 200° F

The reversal of air flow was found to promote even drying but did not improve efficiency and output. Good results were obtained with one reversal about half-way through the run.

After drying to a moisture content of 5% grasswrack becomes dark brown in colour.

Rate of drying

In all tests, the time of drying at a constant rate was short relative to the total drying time. Efficiencies for the constant-rate period were 35–40 %.

* C.D.S. = commercial dry solid, 5% water

Static pressure drop

The static pressure drop was measured for a loading of 0·53 lb. B.D.S./sq. ft./layer in one layer. The result is shown in Fig. 2. These values are for a dry batch of grasswrack and are approximately 13% lower than for the corresponding wet batch.

Temperature and recirculation

With a loading of 0·53 lb. B.D.S./sq. ft./layer in four layers and an air mass flow of 20 lb. dry air/min./sq. ft., tests were made at various control temperatures and recirculation values. The results are shown in Fig. 3.

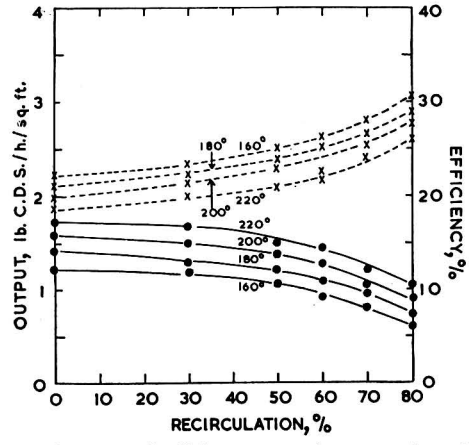

FIG. 3.—*Output and efficiency at various control temperatures*
Air mass flow 20 lb. dry air/min./sq. ft.
Loading 0·53 lb. B.D.S./sq. ft./layer in four layers
Make-up air temperature 70° F
Output —●— efficiency - - - - × - - - -

With the above conditions, and with the ideal dryer chart as a guide, runs of varying recirculation[1] were made. The results are shown in Table I for runs with ideal efficiency at 40–50% based on the definition of this term as previously postulated.[1]

Table I

Output and efficiency for runs of varying recirculation

Control temperature, ° F	Output, lb. C.D.S./h./sq. ft.	Efficiency, %
160	0·93	28·0
180	1·10	27·0
200	1·21	28·5
220	1·35	24·0

Discussion

In the operation of a commercial dryer of the through-circulation type, it should be possible to have an efficiency and output higher than those given in Table I by the continuous or intermittent introduction of fresh layers. Thus, in a well-designed system with a sufficient number of layers, it should be feasible to maintain efficiency near the values given for the constant-rate period.

Conclusions

Pilot-plant tests on through-circulation drying of *Z. marina* have been conducted with a batch dryer. Results show that there is a maximum feasible bed depth and that multiple layers should be employed to make full use of drying air.

Efficiency can be improved by recirculating air towards the end of a run. It has been found that, for even drying, a reversal of air flow about half-way through the run is desirable.

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RELEASE OF IRON OXIDE IN RED-BROWN SOIL FORMATION FROM THE WEATHERING OF LIMESTONE. II.*—Rôle of Grass Extract

By D. H. KHAN†

An aqueous grass extract was allowed to react with powdered limestone rock under controlled laboratory conditions at room temperature. The solution mobilised iron from limestone, in amount about 100 times that released by carbonic acid leaching. It is suggested that the action of the aqueous extract of grass coupled with that of carbonic acid liberated by the ample amount of rainfall accounts for the release and mobilisation of iron oxide which results in the formation of red-brown soil on limestone.

Introduction

Reifenberg[1] reported that colloidal silica sol peptises ignited iron oxide, but in an earlier communication[2] it was shown that no peptisation occurs in presence of Ca ions as these cause flocculation of the silica. The investigation was continued by a study of the carbonic acid leaching of limestone rock under controlled laboratory conditions, when a gradual release of Fe (and Si, Al, Ti, etc.) in the leachate was found,[3] although the amount of iron so mobilised was not appreciable in the light of the findings[4] which suggested that 70–79% of the total iron oxide was lost from the seat of weathering, and this is the major loss of non-calcic constituents.

An examination has now been undertaken of the effect of an aqueous grass extract in releasing iron from limestone rock. Such an extract was selected as grass is the vegetative cover commonly and widely distributed over the limestone areas of England and Wales.

A search of the literature brings forth some interesting results. Bloomfield,[5] from an exhaustive study on the solution effect of different leaf and grass leachates on solid iron oxide, has established that formation of a ferrous–organic complex takes place in such processes, so initiating the movement of iron. He further made the important observation that solution of iron and aluminium may take place at pH 8·0, provided there is a complex formation with organic compounds dissolved from grass, tree leaves and bark, etc., by rain water.

* Part I: *J. Sci. Fd Agric.*, 1959, **10**, 483

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Experimental

Materials

A Jurassic limestone from Somerset, England (So 140/3), was used, the physical, chemical and mineralogical properties being shown in the Appendix. The overlying soil formation, according to Avery,[6] belonged to the 'Red and Brown Calcareous Soils'. Khan,[4] however, found these soils to be closely related to the *terra rossas* and *terra fuscas* of the Mediterranean regions. The morphological descriptions of the soil profile overlying the Jurassic limestone (So 140/3) under study are also given in the Appendix.

The limestone rock, cleaned of weathered residue from the surface with a wire brush, was ground in tungsten-steel and agate mortars, avoiding contamination. An aqueous extract of grass (cocksfoot, *Dactylis glomerata*) was obtained by soaking 5 g. of oven-dried powdered grass in 400 ml. of distilled water overnight, and then filtering. A synthetic $CaCO_3$–Fe_2O_3 mixture was prepared with a cold-precipitated iron oxide, which was dark brown in colour, and was presumably limonitic material. The $CaCO_3/Fe_2O_3$ ratio in the synthetic mixture was kept at 100 : 1 in order to maintain a ratio identical with that in the limestone (So 140/3) : the iron content was 1·65% on the ignited basis.

Procedure

Ten g. of powdered limestone, and (10 + 0·1) g. of the synthetic $CaCO_3$–Fe_2O_3 mixture, were allowed to react with 400 ml. of the grass extract in filter flasks, fitted with delivery tubes through which the samples could be removed. Any suspended particles were retained by cotton-wool plugs fitted to the lower end of the tubes. The flasks were swept out periodically with nitrogen introduced through the delivery tubes, which were provided with glass stop-cocks. Anaerobic conditions were maintained, on the assumption that the reacting medium at the seat of limestone weathering is saturated with carbonated water, resulting in an anaerobic condition.

The reaction was allowed to proceed at room temperature. Fermentation appeared to commence after 4 days, and the first sampling was made at the end of 1 week, bubbles of gas being observed in the outlet U-tube filled with water. Five samples were taken at weekly intervals, and each was analysed for iron with $\alpha\alpha'$-dipyridyl,[7] after centrifuging for a prolonged time in polythene tubes at high speed, and destruction of organic matter with aqua regia plus H_2SO_4–$HClO_4$ mixture. The pH of the grass extract was determined electrometrically using glass-electrodes.

Results

Results are presented in Table I which is self-explanatory.

Table I

Release of iron oxide from limestone by grass extract

Time (in days) of action of the grass extract	Fe, mg./l.		pH	
	Limestone So 140/3 + extract	$CaCO_3$–Fe_2O_3 + extract	Initial extract	At the end of 5 weeks' fermentation
7	92	101	5·3	7·2 ± 0·2
14	91	160		
21	68	234		
28	51	212		
35	54	164		

Discussion

Table I shows an appreciable release of iron from limestone and also from the synthetic $CaCO_3$–Fe_2O_3 mixture, but the amount of iron extracted from limestone is less than that from the synthetic mixture throughout the period of fermentation. This is possibly due to the attack on the iron oxides of the limestone only, rather than on iron-bearing silicate minerals. The mechanism of reaction involved in the solution process of iron oxide is not understood at

this stage of work, but in the light of Bloomfield's findings,[8] it may be that the constituents of lower molecular weight in the grass extract play an important rôle in solubilising iron from limestone rock.

A plot of the results shows a maximum followed by a gradual fall. This shows that there is an optimum point of reaction when the reducing capacity of the extract is exhausted, after which the iron in solution starts reprecipitating. This might be due to (i) partial oxidation during sampling, or (ii) re-sorption of ferrous iron on ferric oxide and calcite particles. Bloomfield[9] found similar results with leaf leachates and experimentally demonstrated the sorption of ferrous organic reaction products on undissolved ferric oxide.

The absolute values obtained for the solution of iron from limestone are much less than found by Bloomfield[5] for leaf leachates on solid iron oxide. According to Bloomfield, the solution of iron is related to the reducing capacity which is always greatly in excess of the amount of iron dissolved. The relatively low values for solution of iron from limestone, however, indicate that the solvent action of the grass extract has been effective on some compounds, presumably calcium carbonate, as well as on iron oxide. In view of the fact that there is about 100 times more $CaCO_3$ than iron oxide in the system, addition of fresh extract would possibly have effected further solution of iron from the same material. Under the natural conditions in limestone countries, the ample rain-water leaching of grass vegetation would result in fresh supply of dissolved organic compounds to effect progressive solution of iron from limestones.

In a previous communication[3] it was reported that one l. of carbonic acid solution in four portions extracted 0·097 mg. of Fe_2O_3 per g. material of limestone rock. Here 400 ml. aqueous grass extract extracted 52·64 mg. of Fe_2O_3 per 10 g. of limestone rock after 1 week. The initial iron content of the grass extract would not be significant (0·68 mg. of Fe_2O_3 per 400 ml. of extract). This indicates that aqueous grass extract has a much greater solubility effect than carbonic acid on the release of iron from limestone rock.

Conclusions

From the findings of this study and those of the previous communication[3] it is suggested that the release of iron oxide at the seat of limestone weathering resulting in red-brown soil formation is probably a function of aqueous extracts of grass and other plant materials with rain water. To this may be added the rôle of simple carbonic acid leaching due to the appreciable rainfall over limestone districts.

It is also suggested that the process of reprecipitation of iron which comprises the other part of the reversible and continuous reaction from solution to solid phase, is perhaps operative in the limestone environment where there are localised changes in ionic concentration. The red and brown effect of soil colour of *terra rossa* and allied soils may be due to the redistribution, following reprecipitation, of iron oxide over mineral particles occupying a larger surface area and affecting an appreciable scattering of light.

Acknowledgments

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Appendix

I. *Physical, chemical and mineralogical properties of the limestone rock (So 140/3) used*

Physical properties	Location	Somerset, England
	Period of formation	Jurassic, Inferior Oolite
	Nature of composition	Calcitic
	Colour	Grey
	Impurities—natdre	Dominantly clay-sized Greyish brown
	Impurities—amount	3·0%
	Crystallinity	Coarsely crystalline
	Hardness	Hard
Chemical composition	MgO 2·50; SiO_2 1·91; Al_2O_3 1·19; Fe_2O_3 1·65; TiO_2 0·05%	
Mineralogical properties	Light minerals in sand sized residue	Angular quartz, dominant with moderate chert; some felspars with little collophane
	Heavy minerals in sand sized residue	Flood of opaques; some garnet and zircon, with little tourmaline, apatite, and muscovite
	Minerals in clay sized residue	Dominant mica with little kaolin and vermiculite; trace of quartz and geothite

II. *Morphological description of the red-brown soil profile (So 140) overlying the Jurassic limestone rock*

Profile No. So 140

Locality: Quarry on Creech Hill, Somerset

Series: Sherborne

Type: Clay loam

Genetic group: Red-brown soil

Topography: Level plateau

Vegetation: Old pasture, fine-leaved fescues probably dominant with cocksfoot, etc.

Underlying rock: Coarse-grained Jurassic limestone (oolitic)

Drainage: Free

Horizon	*Depth* in.	*Description*
A	0–5	Reddish brown (5 YR 4/3) clay loam; soft granular structure; moist; some angular fragments of light yellowish-brown limestone; friable and mellow; moderate intimate organic matter; worms active; abundant grass roots; slightly calcareous.
(B)	5–10	Yellowish-red clay containing abundant red-brown stained angular limestone fragments; moist; granular; friable.
C	10+	Broken coarse-textured oolitic limestone, mainly horizontally bedded, but irregular and rubbly. (Limestone sampled at 24–30 in.)

DETERMINATION OF THE COMPONENTS OF PLANT CUTICLES*

By J. T. MARTIN

Quantitative methods are described for the determination of waxy substances and cutin in plant cuticles. Surface waxy materials are obtained by immersing leaves or fruits in chloroform at room temperature; waxy substances embedded within the cuticles of fruits are recovered by further extraction of the skin after surface washing. Acidic materials are removed from the extract, and true wax and 'oil' fractions are obtained by partition between n-heptane and methanol. The cuticular membranes are then separated by treatment with ammonium oxalate-oxalic acid solution, and the cutin in the membranes is determined by saponification. Work by other investigators on the nature and determination of components of plant cuticles is reviewed.

Introduction

The cuticle of the plant is the protective layer of non-living material which lies over and gradually merges into the outer walls of the epidermal cells. Because of its close association with these cells, a strict morphological definition of the cuticle is not easy, and indeed, the term has been used in different senses in the botanical literature. Work on the structure and properties of the leaf cuticle has recently been reviewed by Van Overbeek;[1] it consists essentially of waxy substances embedded within and extruding from the surface of a spongy framework of cutin. Some leaves possess hardly discernible cuticles but in others they are well defined. The waxy surface of the plant repels water droplets and so affects the deposition of spray chemicals, while the cuticle forms the chief barrier to the penetration of toxicants. The cuticle may also contribute to a natural defensive mechanism of the plant against invasion by parasitic organisms. The investigation of these problems has led us to an examination of plant cuticles and especially to the quantitative determination of their chief components.

* Read at meeting of Agriculture Group, 20–21 April, 1960

The components of plant cuticles

Various workers have shown by staining reactions or examination with polarised light,[2] by electron microscope studies[3] or by dissolving away the cutin,[4] that in the leaf, a zone of pectin occurs in the region where the cutin layer merges into the outer cellulosic walls of the epidermal cells. Cellulose may also be present within the cutin. Miss M. F. Roberts has shown (unpublished work) that in the cuticle of the *Euonymus* leaf, pectin and cellulose occur within the cutin layer nearest to the epidermal cells. The pectin and cellulose resist oxalate and cuprammonium extraction of the intact cutin and react to stains only after the cutin has been partially dissolved away. The amount of pectin present is not known but the cellulose content does not exceed 2%. The major components of leaf cuticles are clearly waxy materials and cutin. Huelin[5] has obtained fractions containing carbohydrate and protein from apple fruit cutin.

Our knowledge of the constitution of the waxy substances is due largely to the researches some 30 years ago of Chibnall & Piper and their co-workers. These authors in work reviewed elsewhere[6] showed that the waxes of the cuticles of fruits and leaves consisted of mixtures of long-chain paraffins, primary and secondary alcohols and ketones. n-Nonacosane, n-heptacosane, *d*-10-nonacosanol, n-hexacosanol, n-octacosanol and n-triacontanol were obtained, for example, from the wax of the apple fruit.[7] Chibnall obtained his waxy materials by precipitating them with acetone from concentrated plant extracts. Other workers have examined the ' hard ' or true wax thrown out by acetone and the ' soft ' wax or oil fraction remaining in solution in the solvent. Mackie & Misra,[8] for example, identified palmitone and primary alcohols in the acetone-insoluble wax, and palmitone, unsaturated acids and sesquiterpene oils in the soft wax fraction of the cuticle of leaves of *Anona senegalensis*. Gane[9] fractionated the waxy covering of the apple fruit into wax, ursolic acid and oil. These components were also investigated by Markley & Sando[10] and Huelin & Gallop.[11] Apple cuticle oil was recently found by Davenport[12] to contain saturated acids, chiefly stearic and arachidic.

It has long been known that cutin is saponifiable to yield a mixture of long-chain acids. Early work on hand-stripped *Agave americana* leaf cuticle, leading to the isolation of cutic and cutinic acids, has been described elsewhere.[13] This cuticle has recently been re-examined by Matic[14] who obtained from its cutin a mixture of hydroxylated octa- and hexa-decanoic acids. Of these, phloionolic acid predominated, comprising about 25% of the cutin. Huelin[5] found that the apple fruit cutin was readily saponified with 0·5N-ethanolic potash at 25° to yield about 80% of ether-soluble acids. The basic component of cutin is therefore a polyester closely related chemically to suberin of cork.

Quantitative determination of components

Waxy substances

So far little work has been done on the quantitative determination of the waxiness of leaves. When the waxy covering has been obtained, there remains the further problem of its quantitative subdivision into its constituent groups of compounds. Kurtz[15] refluxed air-dried leaves with light petroleum and separated the wax and non-wax components by acetone precipitation. Schieferstein & Loomis[4] washed the wax from measured leaf surfaces with successive portions of light petroleum and removed the non-wax fraction from the extract with acidified alcohol. The materials obtained were dried to constant weight at 45° and volatile substances then determined by further heating at 100°. Subsurface waxy materials were obtained by Soxhlet (light petroleum) extraction of stripped epidermises from which the surface waxes had been removed.

Quantitative analyses have been made of the waxy components of the apple fruit cuticle. Markley & Sando[10] determined the total ether extract, the oily fraction by extraction with light petroleum and the ursolic acid by titration. Huelin & Gallop[11] extracted whole apples with boiling light petroleum to remove the surface waxy materials, which were then resolved into wax and oil components by differential solubility in acetone. Subsequent extraction of the skin with ether gave the ursolic acid fraction.

Cutin

Methods for the quantitative determination of cutin were described by König in 1906–14 (see Paech & Tracey[16]). König removed the soluble constituents of plant tissues with dilute

acid and treated the crude fibre remaining with ammoniacal hydrogen peroxide to remove lignin and with cuprammonium solution to remove cellulose, the residue being cutin. König also used 72% H_2SO_4 to eliminate the cellulose, but this procedure was found by Markley & Sando[10] to lead to partial decomposition of the cutin. Skoss[17] extracted waxy materials from separated leaf cuticles with alcohol and weighed the residue to obtain a value for the cutin.

The ready saponification of cutin with alcoholic potash provides the most dependable basis for an analytical method. Paech & Tracey[16] give a method of direct saponification of plant material followed by the recovery of the ether-soluble acids. Markley & Sando[10] extracted isolated apple fruit cuticles with dilute acid and alkali and measured cutin by loss in weight on saponification. Huelin & Gallop[11] in similar work preferred the recovery of the ether-soluble acids, liberated on saponification, as a measure of cutin content.

Examination of leaves and fruits

We obtain the waxy materials by dipping whole leaves or fruits in a suitable solvent (see below) at room temperature. While this procedure may be relied upon to remove surface waxes, there is no assurance in the examination of leaves that it recovers waxy substances located within the cutin layer. More thorough extraction brings the complication that fatty substances may be extracted from within the leaf tissues. The position with fruits is less difficult. After the removal of surface waxes the skins may be separated from the flesh and extracted to recover the embedded waxes.

In work already reported[6, 13, 18] on the fractionation of leaf and fruit waxy materials the procedure of Chibnall and later workers of precipitating the hard or true wax with acetone was followed. Extraction of the residual material, in ether solution, with dilute alkali removed an acidic fraction, leaving the oil in solution in the ether. Small amounts of steam-volatile material were also estimated. Recent work has shown that the acetone precipitation method of determining the true wax is not completely reliable, and that a more rapid and efficient separation of wax and non-wax components may be obtained by partitioning the waxy materials between non-polar and polar solvents. The selection of suitable solvents was not easy since a low degree of miscibility and relatively low boiling point to facilitate the removal of solvent without the loss of wax were required. Partition between cyclohexane and methanol was satisfactory, but on the suggestion of Dr. G. S. Hartley, n-heptane and methanol were found to give better solvent separation and were adopted.

After the removal of the surface waxy substances, disks of leaves or fruit skins were taken for the determination of cutin. The leaf cuticular membranes were first isolated in order to eliminate the cellular tissue. Other workers have removed the cuticles from leaves or fruits by digestion with enzyme preparations[4, 5, 17, 19] or by chemical treatment.[11, 20] On refluxing the disks with ammonium oxalate–oxalic acid solution, the membranes separated readily; those from the upper and lower surfaces of leaves were obtained at different times permitting the assay of cutin in each surface. The membranes obtained from leaves consisted of layers of cutin with attached cellulosic remnants of epidermal cells, similar to those obtained from apple fruits by Huelin & Gallop.[11] Treatment with cuprammonium solution for a few minutes removed the attached cellulose to give thin translucent sheets consisting almost entirely of cutin. In the determination of cutin we have used reagents suggested by Markley & Sando[10] and Huelin & Gallop[11] and acknowledge our indebtedness to these workers.

The expression of results for cuticle components as percentages of leaves or fruits means little because of the widely differing surface-weight relationships. Measurements of the surface areas of tissues examined have given more realistic values as μg./cm.^{2}

The solvent for the extraction of surface waxy materials

Chloroform is now used for removing waxy substances from leaves or fruits in preference to ether employed in earlier work. In comparative tests at room temperature with chloroform, ether and n-hexane in which apple leaves were immersed individually for 10 sec. for each immersion in four successive portions of each solvent, chloroform was clearly the most efficient solvent, removing over 60% of the total waxy material in the first immersion. The waxy deposits obtained were: chloroform 35; ether 32 and n-hexane 7 μg./cm.^{2} Similar extraction

patterns resulted in other tests with, at times, lower recoveries with ether. Only 2 μg./cm.^{2} resulted from the fourth immersion of the leaves in chloroform. On Soxhlet extraction of the leaves after immersion, considerable further quantities of fatty substances were obtained. In view of this large reserve within the tissues, the small amount of material extracted by the fourth immersion in chloroform indicated that the solvent was not leaching fats from within the leaves. The relatively low amount of waxy deposit obtained with n-hexane consisted chiefly of substances precipitated by acetone. While chloroform satisfactorily removes the surface waxy material from leaves, it has not so far been possible to assess how much remains embedded within the cutin.

Fractionation of the waxy materials

Cox apple fruits from storage were immersed individually, with rotation, in four successive portions of chloroform, the extracts combined and made up to volume. Aliquots were taken for comparisons of the acetone precipitation and solvent partition methods of fractionation.

Direct partition between cyclohexane and methanol gave a mean value of 53·8% of wax (taken out by the cyclohexane) in the waxy mixture. In other tests, the acidic substances were first removed by extraction, from ether, with dilute alkali, and the residue was partitioned between the solvents. When the proportions of waxy material to solvent mixture were varied, the results were in good agreement and a mean value of 52·1% of wax was obtained. The recoveries by the summation of the fractions were 96–102% of the material taken. The initial removal of acidic substances resulted in cleaner separations of the cyclohexane and alcohol layers. The material taken out by the cyclohexane was waxy in appearance, readily precipitated on cooling a hot acetone solution to room temperature and melted close to 60°. The fraction remaining in the methanol was not precipitated from acetone at 0° and melted at 190–200°. The acidic substances melted in the region of 250°. Similar effects were obtained in parallel experiments using partition between n-heptane and methanol.

When the waxy material from the fruits was dissolved in hot acetone and kept at 0° overnight, wax separated to the extent of 34·5% of the mixture. The precipitated material melted in the region of 54–56°. The substances remaining in solution in the cold acetone were then partitioned between cyclohexane and methanol and a further 20·5% of wax was recovered. In other tests, different values were obtained. Acetone precipitation of the wax is empirical and dependent upon conditions, and the solvent partition method is much to be preferred.

Tests were made in which the dry waxy material from the fruits was extracted with successive portions of hot cyclohexane; 60% dissolved and this on partition yielded 50·7% of wax in the original mixture. The procedure, however, was unsatisfactory for quantitative work. The method adopted consists of removing the acidic substances from the waxy materials, followed by solvent partition of the residue into wax and oil. The term 'oil', although retained, is strictly a misnomer since after the separation of alkali-soluble materials and wax the residue is a powder.

Analytical method for waxy materials and cutin

Following these experiences, a routine method has been devised for the analysis of leaves and fruits in which operations have been reduced to the minimum.

Leaves

Waxy materials.—The surface areas of leaves are measured from their outlines on paper. The leaves are washed with four successive portions (30 ml. each) of chloroform at room temperature, as described above, the extracts are combined with straining through cotton wool to remove dust, and the solvent is removed. Immediately after immersion, the leaves are placed in 25% methanol to maintain turgidity.

The residue from the chloroform extraction is dissolved in 40 ml. of ether and extracted with 20, 10 and 10 ml. of 0·1% aqueous KOH solution. The alkaline extracts are combined, washed with ether (which is added to the main layer), acidified with dilute HCl and extracted twice with ether. The acidic substances are obtained from the ether solution, after washing and drying, by removal of the solvent to constant weight.

The main ether layer is washed free from alkali, dried, the solvent removed and the residue, consisting of wax and oil, dried to constant weight. n-Heptane (b.p. 98–99°, 1 vol.) and methanol (absolute redistilled, 0·65 vol.) are equilibrated by vigorous shaking and the layers separated with efficient drainage. The wax–oil mixture is dissolved in 10 ml. of the methanol and 5 ml. of the heptane layer and washed into a small separating funnel with a further 5 ml. of the heptane layer. After vigorous shaking for 1 min. and setting aside the methanol solution is run into a second separating funnel, allowing efficient drainage before tapping off. The heptane solution is washed twice with vigorous shaking with 5-ml. portions of the equilibrated methanol, and the methanol solution with two 5-ml. portions of the equilibrated heptane. After standing the methanol layers are combined, allowing complete drainage from the heptane, and the solvent is removed to constant weight to give the oil. The wax is determined by difference. The dry weights of the original material and wax from the heptane layer may be taken to check recovery.

Cutin.—Disks are punched from the leaves, avoiding the midrib, and extracted with methanol in a Soxhlet until colourless. They are then refluxed with ammonium oxalate 1·6%–oxalic acid 0·4% solution until the cuticles loosen or float away. The cuticles are removed (upper and lower separately if required), refluxed with 1·25% w/v H_2SO_4 acid for 30 min. and then with 0·2% KOH, with renewal of the KOH after intervals of 1 h., until no further coloration of the solution is seen. The solid material is filtered off, washed with water and ethanol and refluxed with 0·5N-ethanolic KOH for 3 h. The solution, after filtration, is almost neutralised with 2·5N-HCl, and the solvent removed at 35° in a rotary evaporator. The residue is taken up in water, acidified with 2·5N-HCl and the liberated cutin acids are extracted with ether, recovered and dried to constant weight.

A small proportion of the cutin acids remains in the aqueous layer, but may be recovered by extracting its dry residue with dry acetone.[6] The ether-soluble acids, however, give a satisfactory measure of cutin content.[13] Replicate analyses show good concordance provided the extraction with aqueous KOH is carried to completion.

Some leaves, e.g., cabbage and tomato, show poorly-developed cuticles whose isolation without fracture is difficult. Analysis is then carried out on the disks, without attempting to obtain the cuticles, up to the completion of the extraction with aqueous potash. The disks then consist of the cutin layers held together by the cellulosic remnants of the mesophyll tissue. Treatment with cuprammonium dissolves away the cellulose and liberates the cutin, which is then saponified. The results of analyses on disks taken through the method without the removal of cellulose agreed well with those made on the isolated cuticles.[13]

When the attached cellulose is removed from the isolated cuticular membranes before saponification, the residue finally obtained gives a measure of the cellulose occurring within the cutin layer.

Fruits.—The surface areas of fruits are assessed from mean diameters. After the removal of waxy substances by the immersion of the fruits in chloroform, the skins are removed with as little attached flesh as possible, and disks of known area are taken. Some are air-dried and extracted with ether in a Soxhlet to recover the waxy materials not removed by surface washing; others are subjected to the method for cutin.

Notes on the method

Sufficient leaves or fruits are taken to give about 50 mg. of waxy materials for each fractionation. For cutin in leaves, 100 disks of 1 or 3 cm.^{2} area are used according to cuticle thickness. For cutin in large fruits whose skins may be separated in sheets, 100 1-cm.^{2} disks are used; small fruits such as blackcurrant are halved and the flesh removed. In the cutin method 200-ml. quantities of oxalate solution, dilute acid, dilute KOH and alcoholic KOH are used. Ether solutions are dried over anhydrous sodium sulphate, and organic solvent is removed by drawing a current of dry air over the solution at 40–50°. Drying to constant weight is carried out at 40°. Alternatively cutin may be determined by loss in weight on saponification.[13]

The degree of waxiness and of cutin formation in leaves and fruits

Detailed results will be published elsewhere, but general conclusions from past work may be given. Considerable variations have been found in the amounts and relative proportions of surface waxy materials and cutin.

The leaves of tomato, banana and red beet show low levels of waxiness and cutin (<50 μg./cm.²). Cauliflower and cabbage leaves show little cutin, but are considerably waxy. The leaves of different apple varieties carry 30–70 μg./cm.² of surface waxy substances and 70–120 μg./cm.² of cutin. Rhododendron and laurel leaves show higher degrees of waxiness and cutin deposition of about 250 and 450 μg./cm.² respectively. The ornamental *Euonymus japonicus* leaf is exceptional in having little surface wax (20 μg./cm.²) and 600 μg./cm.² of cutin. More cutin has invariably been found in the upper than in the lower leaf surface.

The blackcurrant fruit cuticle carries about 150 μg./cm.² of waxy materials and 150 μg./cm.² of cutin. Apple fruits of different varieties differ considerably in skin thickness; surface waxy deposits vary from 600 to 1200 μg./cm.² and cutin is present to the extent of 750–1000 μg./cm.² As the tomato fruit develops, outer layers of cells become progressively impregnated with cutin and in this way substantial skins, containing about 1000 μg./cm.² of cutin, are formed.

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STUDIES ON CASEIN. III.*—Preparation of a Carbohydrate-rich Fraction and a Calcium-sensitive Fraction from α-Casein†

By M. E. Q. PILSON, G. O. HENNEBERRY and B. E. BAKER

α-Casein was fractionated by the addition of calcium chloride to an α-casein solution (pH 7) which had previously been kept for 45 min. at pH 12. One fraction (Fraction A) accounted for 13·4% and another (α-casein — Fraction A) for 56·7% of the original α-casein.

The sugar contents (anthrone) of Fraction A, and α-casein — Fraction A, were 3·94 and 0·68 mg./g., respectively, and the hexosamine contents were 4·58 and 0·68 mg./g., respectively.

The product α-casein — Fraction A was precipitated from aqueous solution (pH 7) by the addition of calcium ion whereas Fraction A was not precipitated under the same conditions. No precipitate was formed on addition of 0·1M-calcium chloride solution to a solution containing 3 parts of α-casein — Fraction A to one part of Fraction A but a precipitate was formed if Fraction A was pretreated with rennin.

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Introduction

In 1939 Mellander[1] applied the classical Tiselius electrophoretic technique[2, 3] to the separation of the components of the casein complex. He obtained three distinct electrophoretic components designated α-, β- and γ-casein in order of decreasing mobility. Warner[4] described a method for the isolation of α- and β-casein which is based on the higher solubility of the latter in water at pH 4·4 and at a temperature of 2°.

Hipp *et al.*[5] described two methods for the separation of the three components of casein in quantity, viz., (1) based on differences in their solubility in 50% alcohol in the presence of salts, and (2) based on the solubility in aqueous urea solution at the isoelectric point of casein.

Von Hippel & Waugh[6] described a procedure for the preparation at constant pH and at reduced temperature, of a mixture of α- and β-casein. The method involved the addition of calcium chloride to skim milk, the isolation of the micelles by high-speed centrifugation and the removal of calcium from the micelle by precipitation with potassium oxalate followed by dialysis. The casein was recycled through the same process and the resultant second-cycle casein was a mixture of α- and β-casein in approximately the same proportions in which they occur in skim milk.

MacRae & Baker[7, 8] separated the α-, β- and γ-components of the casein complex by filter-paper electrophoresis. The papers were stained for carbohydrates according to the method of Koiw & Gronwall[9] and staining was observed only at the position of the α-casein band. In a subsequent paper, Reynolds *et al.*[10] reported that the total sugar contents of α-, β- and γ-casein were 1·5, 0·8 and 0·9 mg./g. respectively. Individual sugars were determined by filter-paper chromatography and it was observed that α-casein contained mannose and galactose and β- and γ-casein contained only galactose. These sugars accounted for about half the sugar content of the three casein fractions as determined by the anthrone method.

The present paper deals with the isolation of a fraction from α-casein (Warner), which is relatively high in sugar content and possesses some of the properties of the κ-casein prepared by Waugh & Von Hippel.[11]

Experimental

Materials

Whole casein.—This was prepared from a composite sample of milk (Macdonald College herd) by the method of Warner.[4]

α-Casein.—This was prepared from whole casein by the method of Warner.[4]

Fraction A.—α-Casein (33·5 g.) was suspended in distilled water (1250 ml.). Aqueous NaOH (1N) was added slowly to the suspension until pH 12 was reached. The resultant solution was kept at 25° for 45 min. and then HCl (3N) was added slowly until the pH was 7·0. The total volume of the casein solution at this point was approximately 2000 ml. Sufficient calcium chloride solution was added to the casein solution to adjust the solution to 0·25M with respect to calcium ion. The mixture was set aside for 14 h. at 4° and the precipitate (P_1) then recovered by centrifugation. The supernatant was dialysed for 5 h. against five changes each of 2000 ml. of distilled water at 4°. HCl (0·1N) was added to the dialysed solution until the pH was 4·5. The product, which was recovered by centrifugation, was washed with water and then with alcohol and with acetone. The weight of air-dried material (P_2) was 6·0 g.

The product (P_2) was suspended in distilled water (400 ml.) and NaOH (1N) added until the pH was 12. The resultant solution was kept at 25° for 45 min. and then HCl (3N) was added slowly until the pH was 7·0. Sufficient calcium chloride was then added to adjust the solution at 0·25M with respect to calcium ion. The resultant solution, which was opalescent, was filtered and then dialysed for 5 h. against four changes of distilled water (each 12,000 ml.) and then for 5 h. against 2000 ml. of 0·5% Versene solution (Bersworth Chemicals Co., Framingham, Mass.) (pH 8·5, 4°) and against two changes of distilled water each of 12,000 ml. at 4°. HCl (0·1N) was added to the dialysed solution to give pH 4·5. The precipitate was recovered by centrifugation and was washed with water and then with alcohol and with acetone. The weight of air-dried material (Fraction A) was 4·5 g. (13·9% N).

α-Casein — Fraction A.—The precipitate P_1, which was obtained in the preparation of

Fraction A, was suspended in distilled water (1000 ml.). Versene (Bersworth Chemicals Co., Framingham, Mass.) (10 g.) was added and then NaOH (0·2N) to give pH 8·5. The resultant solution was dialysed for 5 h. against four portions of distilled water (12,000 ml. each, 4°) which had been adjusted to pH 8·5 with NaOH, and against two changes (12,000 ml. each, 4°) of distilled water. The dialysed solution had pH 8·0 and this was adjusted to pH 4·5 by addition of 0·1N-HCl. The precipitated casein was recovered by centrifugation and then washed with water, alcohol and acetone. The weight of the air-dried product (P_3) was 22·0 g.

The precipitate (P_3) was subjected to the same treatment as had been used in the preparation of Fraction A and the resultant product was further purified by the method used for precipitate P_1. The weight of air-dried material (α-casein — Fraction A) was 19·0 g. (13·9% N).

Results and discussion

Carbohydrate and phosphorus contents of the casein fractions

Total sugars, galactose, mannose and glucose, and hexosamine, were determined by the methods described by Reynolds *et al.*,[10] and phosphorus by the method of Martin & Doty.[12] Table I gives the results of the analyses.

Table I

Carbohydrate and phosphorus contents of α-casein fractions[a]

Sample	Phosphorus	Total sugar (anthrone)	Hexosamine	Galactose	Mannose	Glucose
	%	mg./g.	mg./g.	mg./g.	mg./g.	mg./g.
Fraction A	0·92	3·94	4·58	2·75	0·51	0·00
α-Casein — Fraction A	1·38	0·68	0·68	0·00	0·00	0·00
α-Casein	1·22	1·51	2·11	0·75	0·48	0·00

[a] Based on 15% N

It will be observed that the total sugar figure for Fraction A was about six times that for α-casein — Fraction A as determined by the anthrone method, and the hexosamine content of Fraction A was nearly seven times that of α-casein — Fraction A. The fact that no glucose or mannose was detetected in α-casein — Fraction A by filter-paper chromatography, whereas the anthrone method revealed the presence of 0·68 mg./g. of sugar, would suggest that α-casein — Fraction A contained sugars other than galactose or mannose.[13]

Paper electrophoresis of the casein fractions

Samples of α-casein, Fraction A and α-casein — Fraction A were subjected to filter-paper electrophoresis.[7] Single bands of identical mobilities were obtained with the separate fractions, and with a mixture of Fraction A and α-casein — Fraction A (1 part Fraction A to 3 parts α-casein — Fraction A).

Behaviour of casein fractions towards calcium ions

1% solutions of Fraction A and α-casein — Fraction A were prepared by the slow addition of 0·2N-NaOH to aqueous suspensions of the protein until pH 7·0 was reached. The solutions were then diluted with distilled water to the appropriate volumes. The solutions were then examined in order to ascertain the behaviour of the casein fractions toward calcium ions.

When equal volumes of Fraction A solution and 0·1M- or 0·5M-calcium chloride solutions were mixed, an opalescent solution was formed but there was no precipitate. α-Casein — Fraction A solution gave an immediate, heavy, white, stringy precipitate on addition of calcium chloride. The addition of trichloroacetic acid to the supernatant gave no further precipitation.

A mixture of 3 parts of α-casein — Fraction A solution and 1 part of Fraction A solution turned milky when mixed with an equal volume of 0·1M-calcium chloride solution, but with 0·50M-calcium chloride a precipitate formed and the supernatant was opalescent. A mixture of α-casein — Fraction A solution and Fraction A solution (4 parts α-casein — Fraction A to 1 part Fraction A) gave a milky solution and slight precipitation when mixed with an equal volume of 0·1M-calcium chloride solution.

Waugh & Von Hippel[11] showed that the addition of calcium ion to second-cycle casein, at concentrations markedly lower than those found in skim milk, led to formation of a coarse heavy precipitate. It will be noted that α-casein — Fraction A behaved in a similar manner. Also it may be noted that the fraction designated in the present paper as Fraction A yielded an interaction product with α-casein — Fraction A which formed stable micelles on the addition of calcium ion as did κ-casein.[11]

Behaviour of casein fractions towards rennin

These experiments were performed with 1% protein solution at pH 6·0 and commercial rennin extract (Canada Packers Ltd.). The rennin extract was added to the protein solutions in amounts to give a dilution of 1 part in 5000.

Equal volumes of Fraction A solution and 0·1M-calcium chloride solution were mixed and then rennin was added. No change was observed for the first 10 min. and then a white precipitate slowly separated in the reaction mixture. No change was observed when rennin was added to the α-casein — Fraction A solution in the absence of calcium chloride.

A solution containing 3 parts of α-casein — Fraction A to 1 part of Fraction A was mixed with an equal volume of 0·1M-calcium chloride solution. Rennin was then added to the resultant milky solution. In 2–5 min. a precipitate began to form and after 10 min. most of the protein had precipitated. The experiment was repeated except that the calcium chloride solution was added to the solution of α-casein — Fraction A and Fraction A which had been set aside for 10 min. after the addition of rennin. A precipitate appeared immediately on the addition of the calcium chloride.

A mixture of α-casein — Fraction A solution and rennin was kept for 10 min. A sufficient volume of Fraction A solution was then added to give a ratio of α-casein — Fraction A to Fraction A of 3 : 1, followed by an equal volume of 0·1M-calcium chloride solution. A precipitate began to form after 3–5 min. This experiment was repeated, except that rennin was allowed to act on Fraction A for 10 min. before the addition of the α-casein — Fraction A and the calcium chloride solution. A large precipitate formed immediately on the addition of the α-casein — Fraction A and the calcium chloride to Fraction A which had been treated with rennin.

These experiments show that the stable micelle formed when calcium ion is added to a mixture of α-casein — Fraction A and Fraction A will precipitate in the usual way on the addition of rennin. If Fraction A is first treated with rennin and then added to α-casein — Fraction A in an amount which would normally prevent the precipitation of α-casein — Fraction A by 0·1M-calcium chloride, it will be found that Fraction A has lost its stabilising effect.

It is of interest to note that the fractions designated in the present paper as Fraction A and α-casein — Fraction A are similar in their behaviour to Fraction S and second-cycle casein described by Waugh & Von Hippel.[11]

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PHOSPHOLIPID HYDROLYSIS IN COD FLESH STORED AT VARIOUS TEMPERATURES

By JUNE OLLEY and J. A. LOVERN

Cod were stored at 20, 0, −14, −22 and −29°, samples being withdrawn at intervals, the lipids extracted and analysed for free fatty acids (FFA) and phosphorus. Chloroform or toluene vapour ultimately inhibited enzymic liberation of FFA, hence studies at 0 and 20° were with small pieces of flesh obtained aseptically and stored without preservative. Tests with cooked fish showed that non-enzymic hydrolysis is negligible. Comparison of sterile fish at 0° with non-sterile iced fish showed that all phospholipid breakdown in iced cod can be attributed to autolysis. At 0°, but not at the other temperatures, there is a marked initial lag before rapid hydrolysis begins. Only rough comparison is possible from the data available, but the rate of hydrolysis at −14° is about 10 times that at −22°. At 0° hydrolysis proceeds little faster than at −14°, while the rate at 20° is about 3 times that at 0°. Data at −29° are too few even for rough comparison. At all temperatures studied the only products accumulating from phospholipid degradation are FFA and water-soluble phosphorus derivatives. The similar course of phospholipid hydrolysis and of protein denaturation in frozen cod was confirmed and its possible significance is discussed.

Introduction

During prolonged storage of cod in crushed ice, phospholipids in the flesh are degraded to free fatty acids and water-soluble fragments.[1] Under such conditions hydrolysis might be due to enzymes of either bacterial or fish tissue origin, with perhaps some non-enzymic hydrolysis[2, 3] also occurring.

The work reported here was designed partly to provide more information on the cause of phospholipid breakdown in iced cod, and partly to amplify published findings[4] on lipid hydrolysis in frozen cod. Data were also obtained on the course of phospholipid hydrolysis in cod flesh at a relatively high temperature (20°).

Experimental

Bacterial sterility is commonly maintained in enzyme studies by means of chloroform or toluene, but the incubation period seldom exceeds 24 hours or so. It is unlikely that small concentrations of these substances would inhibit non-enzymic hydrolysis, but chloroform is known to produce slow inhibition of phospholipases A[5] and C.[6] Gradual loss of activity in a mixed preparation of phospholipases A and B in the presence of a chloroform–toluene mixture has also been noted,[7] although not attributed to the preservative.

Sterile storage at 0° and 20°

Strict comparison with previous work on iced fish demands periodical withdrawal of three whole fish at a time. It is impossible to set up a storage experiment on this scale in which sterility is obtained and maintained solely by aseptic preparation and precautions. Accordingly chloroform–toluene was used. Small cod, of about 1 kg. when gutted, headed and tailed, were placed three at a time in a series of 12 large cans with well-fitting but not air-tight lids. Six cans were placed completely inside steam baths for 75 min. To each can were added 100 ml.

of chloroform and 50 ml. of toluene. As a check on the individual preservatives, an additional can of raw fish was treated with 150 ml. of chloroform alone and another with 150 ml. of toluene alone. All cans were then stored at 0°.

The fish used were in *rigor* or pre-*rigor* condition, hence bacterial contamination would be confined to the surface and sterilisation by the preservative should have been fairly rapid. In fact, throughout the whole period of storage, no signs of bacterial attack were observed. After 56 days the raw fish had appearance indistinguishable from fresh. Cans of both raw and cooked fish were withdrawn after 2, 3, 4, 5, 6 and 7 weeks, the fish filleted and skinned, the flesh minced and well mixed, and a 1-kg. sample extracted with acetone and chloroform–methanol as described previously.[1] The extract was purified by the washing procedure of Folch *et al.*[8] and aliquots used for the determination of total lipids, free fatty acids (FFA) and phosphorus. The methods were as described previously,[1] except for FFA. This was determined by titration with 0·1N ethanolic KOH and phenolphthalein, after removal of part of the phospholipids by precipitation with acetone at 0° (see Discussion).

With the assistance of Mr. W. Hodgkiss, a small-scale experiment was conducted with fish muscle obtained aseptically, no preservative being used during storage. Pieces of flesh of about 20–30 g. were dissected, with suitable precautions, from freshly-killed fish and placed individually in small, sterilised bottles with screw stoppers. Some bottles were stored at 0°, others at 20°. When a sample was withdrawn for analysis, it was first swabbed for bacteriological examination and then placed immediately in methanol (20 ml. per 10 g. of fish). Bacteriological testing involved 48-h. incubation in nutrient broth at 20°. If the result was negative, the sample was used—otherwise it was discarded. About half of the samples had to be rejected, despite all precautions during dissection.

The acceptable samples, muscle plus methanol, were homogenised for 2 min. (M.S.E. 'Nelco' homogeniser). The homogenate was mixed with chloroform (chloroform–methanol ratio 2 : 1), set aside for 30 min. and filtered. The residue was re-extracted twice with chloroform–methanol (2 : 1), each time with 10 ml. of solvent per 10 g. of original tissue. The three filtrates were combined and sufficient methanol added to give a single phase. This solution was then washed by the procedure of Folch *et al.*,[8] using water at 16 times the total volume of methanol present.[9]

Total lipid, FFA and phosphorus were determined on suitable aliquots of the purified extract. FFA was determined as total titratable acidity, in view of the small amounts available (see Discussion).

Frozen storage

Advantage was taken of some experiments by Connell[10] and Love,[11] involving storage of cod at −14 and −22° respectively. From the residual portions of their samples, each involving three and four fish respectively, 250-g. or 500-g. batches of minced flesh were taken. A few batches of cod stored at −29° also became available. When drip formed on thawing it was included in the minced sample. In addition, experiments of our own were set up at −14°, in which paired skinless fillets were stored raw and also after cooking. Fillets (raw and cooked) were individually wrapped in aluminium foil before freezing in an air-blast freezer running at −29°.

Preliminary tests with fillets steamed for 30 min. in a casserole showed that, although they appeared to be adequately cooked, lipid hydrolysis proceeded in them during cold storage at virtually the same rate as in the paired raw fillets. A steaming period of 90 min. was therefore adopted. It was confirmed that the cooking process itself did not cause any lipid hydrolysis.

Lipids were extracted with acetone and chloroform–methanol and washed as described above. Since the production of FFA was the main feature under study, particular attention was paid to the method for its determination. Dyer and colleagues[4, 12] titrated the mixed lipids to phenolphthalein in ethanol–benzene–chloroform (1 : 1 : 1). We find that identical values are obtained in the more usual ethanol alone. We have, however, commented in previous work[1] that direct titration gives misleading results when phospholipids are present since certain of these titrate as acids. Accordingly, phospholipids were removed chromatographically and the opportunity taken to determine their separate contribution to the total titratable acidity.

The column contained 10 g. of silicic acid (Mallinckrodt, analytical reagent), heated overnight at 120° and packed as a slurry in ethyl ether. The lipid (~500 mg.) was added dissolved in 50 ml. of light petroleum (b.p. 60–80°) and the column washed through with 100 ml. of ether, to remove all FFA in a phosphorus-free fraction. The eluate was taken to dryness in a rotary vacuum evaporator, the lipid dissolved in 25 ml. of ethanol and titrated with 0·1N ethanolic KOH. As a precaution, a further 100 ml. of ether were passed through the column but this never eluted more than traces of acidic material. All remaining lipids were eluted with two portions of 100 ml. of chloroform–methanol (1 : 4). These extracts were similarly evaporated, transferred to ethanol and titrated.

In the procedure finally adopted the aliquot for chromatography was only transferred to light petroleum immediately before being placed on the column. Storage trials at 0° with cod lipids dissolved in light petroleum (b.p. 60–80°), chloroform or chloroform–methanol (4 : 1), respectively, showed progressive darkening and loss of solubility in the light petroleum. This occurred more rapidly with lipids from fish which had been stored for lengthy periods. In chloroform alone the lipids merely darkened extremely slowly, without any precipitation or loss of subsequent solubility in light petroleum. In chloroform–methanol there were no obvious changes in 20 weeks. Although a temperature of −30° is used for lengthy storage of lipid preparations, it is often more convenient to keep them in the laboratory refrigerator at about 0° during preparative and analytical work. The unwashed lipid extracts are in chloroform–methanol. After the washing it is useful to add methanol, e.g., in making up to a definite volume.

The first frozen cod to be examined were those stored at −22°. The lipids from them had been transferred to light petroleum some considerable time before chromatography and the elution pattern was abnormal. Total recovery was often low, e.g., 60%. In this series, therefore, total titratable acidity was determined on the unchromatographed lipids. With the series run at −14 and −29°, the washed lipids were held in chloroform solution until the day before chromatography. An aliquot was then taken to dryness in a rotary vacuum evaporator at about 35°, dried overnight in an exsiccator over P_2O_5 and dissolved in 50 ml. of light petroleum. Since either incompletely dried or partially degraded phospholipids are not entirely soluble in light petroleum, the use of this solvent is a check that a normal elution pattern may be expected. In the final series with paired fillets, delay of any kind between extraction and chromatography was avoided. These three later series gave normal elution and satisfactory total recoveries.

Phosphorus and total titratable acidity were determined in all series on the unchromatographed extracts. An aliquot was evaporated to determine total lipid content.

Results

In the previous paper[1] results were related to the wet tissue, rather than to the total lipids. When cooked fish are involved, loss of water invalidates comparisons on a tissue basis. Hence all results below, except those for total lipids, are expressed on a lipid basis. This has the incidental advantage of facilitating comparison with the data of Dyer *et al.*[4, 12] A mean equivalent of 300 has been assumed for the FFA.

Sterile storage at 0° and 20°

The results of storage in chloroform–toluene vapour at 0°, which do not lend themselves to graphical presentation, are given in Table I. The development of FFA in sterile pieces of flesh stored without preservative at 0° and 20° respectively is shown in Fig. 1. A deduction of 8 units has been made from each analytical value to allow for the contribution of acidic phospholipids (see below). For comparison with storage in ice, the FFA values obtained in the previous work[1] have been recalculated on a lipid basis and are shown in Fig. 1. Values after 28 days in the second series have not been included (see Discussion).

Frozen storage

Development of FFA in raw cod stored at −14, −22 and −29° respectively is shown in Fig. 2. The sum of true FFA plus the acidity due to phospholipids, determined separately on chromatographic fractions, always showed reasonable agreement with the total titratable

Table I

Development of free fatty acids (FFA) in the flesh lipids of cod preserved in chloroform–toluene vapour at 0°

(FFA as g./100 g. lipid, calc. at mean equivalent 300)

Storage time, weeks	Raw fish	Cooked fish
2	10·7	5·1
3	22·6	4·5
4	28·5	12·8
5	16·2	4·7
6	13·0	6·7
6*	32·9	—
6†	28·5	—
7	20·6	7·7

* Preserved with chloroform alone
† Preserved with toluene alone

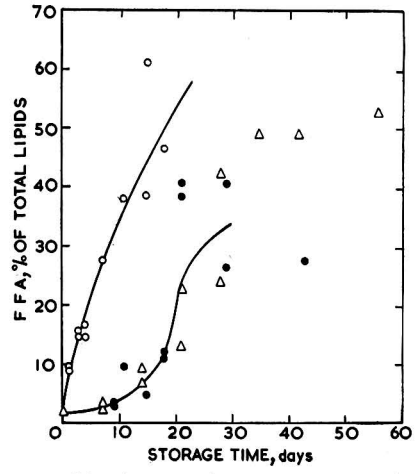

FIG. 1.—*Development of free fatty acids (FFA) in the lipids of unfrozen cod flesh*
● sterile storage at 0° ○ sterile storage at 20°
△ iced fish

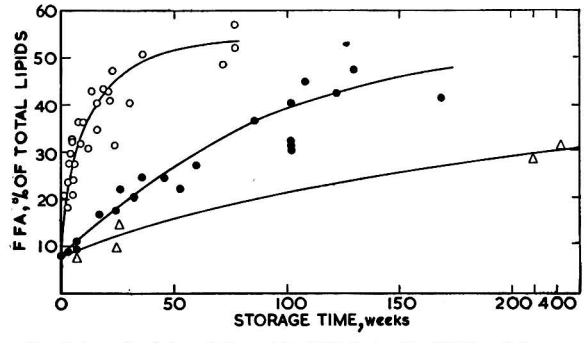

FIG. 2.—*Development of free fatty acids (FFA) in the lipids of frozen cod flesh*
○ at −14° ● at −22° △ at −29°

acidity of the unchromatographed lipid (e.g., see Table II). For 42 samples the phospholipid contribution ranged from 4·7 to 10·4% (calculated as FFA of mean equivalent 300), with a mean of 8·0%. There was no trend with increasing duration of storage. Only one value (14·7) has

been encountered outside this range and 28 samples fell within the range 7·0 to 9·0%. In the series run at −22°, where only the total titratable acidity was measured, an allowance of 8 units has been made for the phospholipid contribution.

Comparison of raw and cooked paired fillets gave the data shown in Table II. The control samples (zero storage time) were frozen like the rest.

Table II

Lipid hydrolysis in raw and cooked paired fillets of cod stored at −14°

[Total lipids (TL) as g./100 g. flesh. Free fatty acids (FFA) and phosphorus (P) as g./100 g. total lipids. Total titratable acidity (TTA) and phospholipid titratable acidity (PTA) as equivalent FFA in g./100 g. total lipids. Ro, R3, R4, etc. raw fillets stored 0, 3, 4, etc. weeks. Co, C3, C4, etc. paired cooked fillets]

Sample	TL	FFA	TTA	PTA	P
Ro	0·66	9·4	17·9	6·2	2·9
Co	0·87	5·0	14·1	8·0	3·0
Ro	0·58	6·9	15·3	8·1	3·1
Co	0·82	3·9	12·8	9·2	3·1
R3	0·57	18·0	28·2	8·6	2·5
C3	0·74	4·0	13·3	8·9	3·0
R4	0·54	22·1	29·8	6·8	2·6
C4	0·85	3·8	12·6	9·0	2·9
R5	—	25·5	32·2	7·4	2·1
C5	0·79	4·1	12·4	9·3	3·1

In the previous paper[1] it was noted that there was no accumulation of lysophosphatides during storage of cod in ice. The present data can be used to test whether in frozen fish also there is a more or less simultaneous release of both fatty acids, to give only water-soluble phosphorus derivatives. For this purpose FFA content is plotted against phosphorus content in Fig. 3. The data used relate to fish stored at −14°, including the raw paired fillets, and −29°. Data obtained in the other series are well distributed about the same straight line but show rather more scatter, attributable to inferior technique for FFA determination and, for the sterile pieces of fish, increased variability in the raw material.

Discussion

Consideration of post-mortem changes necessitates acceptable data on freshly-killed fish. This in turn raises the questions of methods and of natural variation.

Extraction

We have confirmed that our procedure[1] gives complete extraction of the total lipids. The residual tissue, after hydrolysis with HCl, does not yield any further lipid to organic solvents.

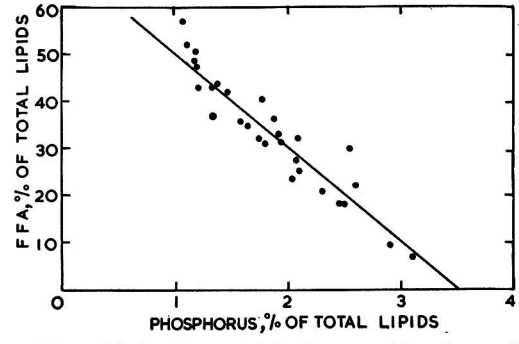

FIG. 3.—*Fall in lipid phosphorus with development of free fatty acids (FFA)*
Data from fish stored at −14 and −29°

With care in the separation of the chloroform phase from the washing procedure,[8] replicate samples of minced cod flesh give identical yields of total lipids. However, we discard the initial aqueous acetone extract since it has been repeatedly confirmed with fresh raw fish, and with freshly-frozen fish, that this extract does not contain significant amounts of lipid. Typical values are 0·2–0·4% of the total lipids.

Re-examination of laboratory records of our studies with iced cod[1] has shown that at some point between 28 and 35 days' storage some of the lipid becomes soluble in the aqueous acetone. From 35 days onward this material amounts to 10–20% of the total lipids. Titration shows that it is virtually all FFA. One sample tested showed a particularly high degree of unsaturation (iodine value 288) and was considerably oxidised (peroxide value 89). In the previous paper[1] an apparent fall in total lipids, and a parallel apparent fall in FFA, was noted in the second storage experiment from 35 days onward. It was then attributed to bacterial destruction of fatty acids, but it can now be accounted for as loss in the discarded acetone. In the first storage experiment, with a different extraction series and no solvent discarded, there was no such loss after 35 days. The fall in iodine value after 28 days may have a similar explanation.

Unfortunately this re-appraisal was made after most of the work now described had been completed. A sample of fish stored at −14° for 78 weeks yielded 0·50% of lipid containing 55% FFA by the extraction procedure used in the present work. In this case the discarded acetone proved to contain lipid, entirely FFA, amounting to 0·028% of the tissue. Thus the true values would be 0·53% of lipid containing 57% FFA. An error of this magnitude cannot affect the general picture revealed by the data.

There remains a need for a general extraction procedure suitable not only for fresh fish but for fish after long storage when hydrolysis and oxidation may be considerable. Bligh & Dyer[13] have developed a rapid method of extraction and purification of fish flesh lipids. We have tested this and agree with the authors that, applied to fresh fish, the losses of lipid during purification are negligible. However, we find that with fish which has been cold-stored for a long time, or become very stale in ice, the upper phase during lipid purification is never clear, even when centrifuged. Under these conditions lipid losses occur in the discarded upper phase comparable to those in the first acetone extract of our original procedure. Thus, with a sample of cod stored at −14° for 88 weeks, 9% of the total lipid was found in the upper phase. With cod stored in ice for 54 days the loss was about 40% of the total. In both these cases the lipid entering the upper phase contained some 50% FFA, i.e., it was probably almost a random sample of the total lipids.

The method adopted with the sterile pieces of flesh has proved to give quantitative extraction and negligible loss during purification, even with the most difficult raw material, e.g., 1·6% of the total lipids from fish stored at −14° for 90 weeks and 5% from fish stored in ice for 66 days. Unlike Bligh & Dyer's procedure, the method does not require modification according to the water-content of the original tissue.

FFA in fresh cod flesh

Dyer & Fraser[4] quote a value of 15% FFA, calculated as oleic acid, in the lipids of fresh cod flesh. The extraction procedure then used was admittedly incomplete and almost certainly selective. Moreover, FFA was determined by simple titration and would include an indeterminate phospholipid contribution. Garcia *et al.*[14] reported a value of 5·5%, isolated and weighed, but this material would have contained impurities amounting to about 43%.[15] The corrected figure is thus 3·1%. Work with iced cod[1] gave values of 2·0 and 2·1%, isolated and weighed after two different chromatographic techniques. Present data on cooked cod after freezing (Table II) give values ranging from 3·8 to 5·0%, by titration after chromatography, calculated at a mean equivalent of 300. Freshly frozen raw fish showed higher values than paired cooked fillets (comparison of Ro and Co in Table II). These fillets were wrapped in aluminium foil and freezing would be slow. It is possible that slow freezing has led to some lipid hydrolysis, perhaps by enzyme activation (see below). Fillets frozen rapidly in solid CO_2 and minced frozen directly into acetone at −14° gave a lipid with only 1·4 and 1·6% FFA, in two experiments.

It appears that a normal range of about 1·5–5·0% FFA can be expected in the lipids of freshly-killed cod flesh.

Natural variation in other lipids

In all experiments except those with small sterile pieces of flesh (Fig. 1), whole fillets have been used. Apart from the paired fillet studies (Table II), samples were from the pooled flesh of three or four fish. The range in total lipid content which we have observed for North Sea cod, with 50 samples covering every month of the year, is 0·43–0·78%. The mean value for each month varied from 0·56 to 0·71%, with no definite seasonal trend. This relative constancy in amount is reflected, so far as our data go, in approximate constancy of composition, e.g., as shown by the phosphorus content. Hence data on lipid hydrolysis probably have comparable significance whether expressed on a tissue basis or a lipid basis.

When only a small portion of flesh is used, as in the series at 0 and 20° without preservative, variation may be greater. The only data on variation within a single fish are from successive layers inward from just below the skin to the backbone, on each side of one specimen. A total of 16 such layers, 8 from each side, had lipid contents ranging from 0·23 to 0·71%. There was no regular progression with depth, apart from highest values in the layers immediately below the skin. The lipid from these subcutaneous layers contained less phosphorus (1·5 and 1·9%) and more total cholesterol (10·4 and 11·0%) than that from other layers (2·3–3·0% and 8·0–10·3% respectively). Excluding the subcutaneous layers, variations in phosphorus and cholesterol content did not show any regular correlation with depth. Total lipid content in the sterile pieces of fish ranged from 0·38 to 0·90%.

Non-enzymic hydrolysis

The data on cooked fish show that non-enzymic hydrolysis, such as has been reported to occur in aqueous emulsions of phospholipids at room temperature or above,[2, 3] does not occur under chloroform–toluene at 0° (Table I) or in a product frozen at −14° (Table II). Moreover, it does not occur during 90 min. cooking with steam. The anomalous value of 12·8% FFA in sample C4 (Table I) may be due to incomplete heat inactivation of enzymes. As stated above, 30-min. steaming had no effect on the rate of development of FFA at −14°, although the fish was cooked by culinary standards. A high degree of thermal stability has been reported for the phospholipase A of snake venom, which requires prolonged boiling for its complete inactivation.[16, 17] The heat stability of phospholipase A seems to vary with its source, but is relatively high at favourable pH values.[18, 19] Phospholipase B of bacterial origin is also relatively heat stable,[18] but not pancreatic phospholipase B.[19] We have no information on the relative rôles of phospholipases A and B in the present studies.

Enzymic hydrolysis at 0°

It is clear from comparison of Table I and Fig. 1, that both chloroform and toluene ultimately inhibit the tissue phospholipases. With these preservatives some initial hydrolysis occurs, but the analytical data are erratic and hydrolysis ceases after about 2–3 weeks.

The FFA values for small pieces of fish, shown in Fig. 1, may understandably show more scatter than would be expected in experiments involving several whole fish at a time. The scatter is just as great if the results are expressed on a tissue basis rather than a lipid basis, i.e., it is not due to variations in total lipid content.

It is evident that, at least in the initial period, say up to about 30 days' storage, hydrolysis at 0° follows a similar course in sterile and non-sterile fish. There is an initial lag period of about 10 days, a period of rapid hydrolysis, and finally a progressively falling rate. Data are inadequate after 30 days to decide whether hydrolysis eventually ceases at the same level in sterile as in non-sterile fish.

Certain preparations of phospholipase A can exhibit an initial lag phase in their attack on lecithin.[5, 20] The terminal fall-off in reaction rate is typical of enzyme systems and may be attributed to inactivation or inhibition. Phospholipid hydrolysis in iced cod, even when this has become putrid, can be attributed to the action of tissue enzymes and there is no evidence that bacterial enzymes play a significant rôle, at least up to about 4 weeks after death.

Enzymic hydrolysis at 20°

As would be expected, hydrolysis at 20° proceeds much faster than at 0°. In addition, no lag period can be detected. If it exists, it is less than 24 h. There are no data on the later

stages of hydrolysis at 20°. The different shapes of the earlier part of the curves prevent adequate comparison of reaction rates at the two temperatures, but a rise from 10 to 25% FFA, i.e. subsequent to the lag phase at 0°, takes about 3 days at 20° against 10 days at 0°.

Enzymic hydrolysis in frozen fish

At the temperatures employed, only tissue enzymes can be involved in lipid hydrolysis in frozen fish. We have confirmed Dyer & Fraser's observation[4] that development of FFA in frozen cod is unaffected by preliminary storage of the fish in ice for up to 8 days. The series run at −22° included fish kept in ice for periods ranging from less than 1 day to 6 days before freezing. There were no significant differences in the scatter of the resultant FFA values in Fig. 2.

The general course of hydrolysis at −14 and −22°, shown in Fig. 2, is similar to that reported by Dyer & Fraser[4] for the same temperatures, although their actual values are higher for reasons discussed above. There are insufficient data at −29° to do more than illustrate the marked retardation of hydrolysis. There is no evidence of an initial lag in any of the curves. If it exists, it is less than 2 weeks at −14°, by which time the FFA may have already reached 20%.

Comparison of the reaction rates at −14 and −22° can only be approximate. Uncertainty about the final equilibrium level prevents proper calculation of reaction rates. Further, the rate of enzyme inactivation or inhibition is different at −14 and −22°, so that comparison of the curves at various points gives varying ratios. Thus, a level of 25% FFA is reached after about 4 weeks at −14° and after 40 weeks at −22°. This 10-fold ratio becomes one of 9·2 at the 30% FFA level, 7·7 at 35% FFA or 6·3 at 40% FFA. It seems desirable, since no lag phase complicates the situation, to compare rates at an early stage, before inactivation has become appreciable. A 10-fold increase in the rate of lipid hydrolysis in frozen cod per 8° temperature rise is in line with data obtained for other reactions in frozen fruit and vegetables.[21, 22]

Dyer & Fraser[4] state that the reaction rates at −14 and 0° are almost the same, but their experiments at 0° only extended to 3 weeks. The existence of a marked lag at 0°, whether the fish is sterile or not, seems to preclude any simple comparison of reaction rates over a short period. In Fig. 1 a level of 25% FFA is reached at 0° after about 3 weeks and a level of about 32% FFA in 4 weeks; i.e., despite the lag it is somewhat quicker at 0°. The steepest part of the curve at 0° shows a hydrolysis rate undoubtedly greater than that of a comparable part of the curve at −14°. Thus a rise in FFA from 10 to 25% FFA at 0° requires about 10 days against nearly 28 days at −14°. Nevertheless, the 10-fold increase per 8° rise clearly does not extend from −14 to 0°. Assuming that it extends to the temperature at which the fish begins to freeze (about −2°), there must have been a marked increase in hydrolysis rate due to freezing. Dyer & Fraser's statement implies this even more strongly. Various enzyme systems are known to show a sharp break in the temperature/reaction rate relationship at or just below 0°.[22–24] Curiously, actual freezing may not be necessary, e.g., the same break occurs with pancreatic lipase when freezing is prevented by addition of glycerol, and a similar break may occur in some systems at other temperatures, e.g., −12°.[25] It should also be noted that freezing a tissue may activate an enzyme system. Thus, phospholipase A could only be demonstrated with certainty in undried ox pancreas after a period of frozen storage.[19] Synthesis of phosphatidic acid in suspensions of guinea pig brain microsomes was markedly promoted by preliminary freezing.[26]

The marked similarity of the course of lipid hydrolysis and of protein denaturation in frozen fish has been emphasised by Dyer & Fraser,[4] who suggest that the two phenomena are related. This similarity is again apparent by comparison of our curves in Fig. 2 with the data of Love[11] for protein denaturation. However, Love, who takes special precautions to avoid biological variations,[27] does not find the initial lag in protein denaturation which Dyer & Fraser noted and associated with the development of a 'critical' level of lipid hydrolysis (see also Fraser & Dyer[28]). Similar reaction rates at various temperatures are not, in themselves, proof that one effect is conditioning or governing the other. Temperature may rather be affecting some common factor, such as the proportion of water frozen, diffusion rate, etc. It has been mentioned above that various apparently unrelated reactions in frozen tissues show closely similar

temperature/rate effects.[21, 22] Protein denaturation, as measured by loss of solubility in 5% NaCl,[11] is another reaction with a similar temperature coefficient, namely an 8-fold acceleration between −22 and −14°. Work in progress, involving comparison of several species of fish in which the rate of protein denaturation at any particular temperature varies considerably, suggests caution in deducing any simple connexion between lipid hydrolysis and protein denaturation.

Hydrolytic route

Finally, it should be noted that in frozen fish, as in iced fish[1] or sterile fish at 0 and 20°, the enzyme system promoting phospholipid hydrolysis liberates both fatty acids more or less simultaneously. Production of about 40% FFA, by liberation of both fatty acids, would produce a fall in lipid phosphorus from 3 to 1%. This calculation agrees with the observed initial and final values in Fig. 3 and the straight-line regression shows that the situation does not alter at any stage of hydrolysis.

Conclusions

Phospholipid hydrolysis in cod flesh stored at temperatures ranging from +20 to −29° is promoted entirely by tissue enzymes, with non-enzymic reactions and the bacteria present on iced cod playing negligible rôles. Freezing appears to activate the system, eliminating the initial lag at 0° and permitting nearly as rapid hydrolysis at −14 as at 0°. The temperature coefficient is about 8 times greater in frozen than in unfrozen fish, over the ranges −22 to −14° and 0 to 20° respectively. In all cases there must be virtually simultaneous loss of both fatty acids from the phospholipid molecule. Phospholipid degradation and protein denaturation follow a similar course in frozen fish, but this is not evidence of a causal relationship.

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ESTIMATION AND DISTRIBUTION OF SOME PHOSPHORUS FRACTIONS IN LEAVES OF PLANTS*

By E. J. HEWITT and B. A. NOTTON

A procedure for fractionation of phosphorus compounds in leaves has been studied. Serial extractions with 85% ethanol, 0·2N-HCl, 1N-$HClO_4$ at 4°, 0·5N-$HClO_4$ at 70° and 2N-NaOH removes 99·8% of the total phosphorus from tomato or cauliflower leaves. A method, involving preliminary treatment of fractions with isobutanol, was designed to eliminate interfering effects of unknown compounds on the determination of inorganic and acid-labile phosphorus by the phosphomolybdic acid method used.

Several extractions with ethanol and 0·2N-HCl are required for exhaustive removal of phosphorus compounds by each of these reagents. Single extractions by these reagents remove about 75% of the respective fractions. A major part of the organic phosphorus remaining after exhaustive ethanol extraction is readily removed by 0·2N-HCl. By using ^{32}P-labelled inorganic phosphorus it was shown that whereas ribonucleic acids (RNA) are not decomposed during extraction in 1N-$HClO_4$ at 4° for 18 h. there is appreciable decomposition of nucleic acids including deoxyribose nucleic acid (DNA) during subsequent extraction by 0·5N-$HClO_4$ at 70° for 20 min. RNA is estimated as organic phosphorus in the 1N-$HClO_4$ extract and DNA as total phosphorus in the 0·5N-$HClO_4$ extract. Light extinction values at 260 mμ tend to be higher than expected in relation to phosphorus contents, especially for DNA.

The distribution of organic phosphorus by single consecutive extractions into the fractions described above was determined for seven different species or varieties of plants grown in sand culture.

Introduction

The work described here was originally undertaken in relation to investigations on the possible relationships between molybdenum and phosphorus metabolism which have been reviewed recently.[1, 2] Some observations relevant to the extraction of some phosphorus fractions from leaves and the proportions in which they occur are reported here.

Experimental

Plant material

Plants were grown in a greenhouse in sand or water culture during spring and late summer and included different species and varieties. Leaf lamina from which major vascular tissues had been removed was used. The samples were sub-sampled to give weights between 1 and 5 g. and were extracted immediately after removal from the plants.

Estimation of phosphorus

Phosphorus was determined as total (TP), inorganic (IP) and acid labile (7′P) (7-min. hydrolysis at 100° in 1N-HCl). Organic phosphorus (OP) was estimated by difference between total and inorganic P. TP was determined as described by Eggleston & Hems.[3] IP was determined as described by Weil-Malherbe & Green[4] by extraction in isobutanol using the same method but without prior destruction of organic material with the ashing fluid. 7′P was estimated by difference in IP before and after hydrolysis. All isobutanol extracts were washed with acid before analysis as prescribed.[3]

Certain extracts, especially those in ethanol or 0·2N-HCl, contained compounds that interfered with the measurement of light absorption of the blue molybdenum compounds obtained by reduction of the phosphomolybdic acid in isobutanol. Similar interferences have been experienced particularly in the estimation of 7′P by G. Ducet (private communication). It was found that the interfering compounds were removed by extraction with isobutanol, in the presence of the sulphuric–perchloric acid mixture specified by Eggleston & Hems,[3] but without the addition of the molybdate reagent. IP and 7′P were then estimated as described above without trouble on adding the molybdate and extracting again in isobutanol.

In experiments using IP labelled with ^{32}P as Na_2HPO_4 it was shown that losses due to the modification described were less than 0·5% of the IP present in model systems. In the

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presence of plant extracts, losses were less than 2·5%. The slightly increased losses were probably due to slight emulsification of the isobutanol and aqueous phases in the presence of plant extracts.

Extraction of phosphorus fractions

Extraction of phosphorus fractions from plants is complicated by the presence of metal ions which may form sparingly soluble salts, by enzyme action during the prolonged time required to disrupt cells with tough cellulose walls, and by the possibility of occlusion by large cell residues. To arrest enzymic action rapidly, fresh material was immersed in boiling 85% ethanol for 2 min. as recommended by Benson[5] and Markham[6] in preference to grinding in cold 0·2N-HCl as described by Loughman & Martin.[7]

After this pretreatment, five similar sub-samples of tomato leaf were extracted by macerating for 5 min. in a blendor in 85% ethanol, filtered and the precipitate washed with 85% ethanol. The residues were extracted separately with water, 5% or 10% trichloroacetic acid, 0·2N-HCl or 1N-$HClO_4$ by macerating again at room temperature and filtering and washing the residue. The ethanol fraction was diluted with water (2 vol.) and extracted with ether to obtain the lipoid phosphorus (LP) fraction.

The distribution of phosphorus obtained in these fractions is shown in Table I as the percentage of TP in the tissues. About 60% of the ethanol-soluble phosphorus in tomato was lipoid in nature, and this fraction comprised about 13% of TP. Water extracted less and N-$HClO_4$ extracted more phosphorus than 0·2N-HCl or trichloroacetic acid solutions, which yielded similar amounts. The variations in the proportion of lipoid P present in the alcohol-soluble fraction occurred because inorganic phosphorus was extracted in varying amounts at this stage.

On the basis of these results the sequence described by Loughman & Martin[7] was used to follow the preliminary extraction in ethanol. Results obtained with tomato leaf are given in Table II.

Table I

Extraction of phosphorus fractions from similar sub-samples of tomato foliage using different reagents

(Values as % of total P in sample)

Sub-sample no.	First extraction in alcohol	Second extraction		P in residue	Ether-soluble P	
		Reagent	P		% of alcohol-sol. P	% of total P
1	20·3	Water	53·6	26·0	68·8	14·0
2	21·6	5% TCA*	59·8	18·6	60·5	13·1
3	23·0	10% TCA*	58·4	18·6	56·6	13·1
4	22·0	0·2N-HCl	61·2	16·8	56·8	11·6
5	22·5	N-$HClO_4$	67·0	10·5	55·6	12·9
Mean	21·9		59·8	18·1	59·5	12·95

* TCA = trichloroacetic acid

Table II

Distribution of phosphorus in tomato leaves as μg./g. fresh wt. or as % of total phosphorus in leaf in successive fractions

Lipoid (LP) and non-lipoid organic phosphorus (NLOP), other abbreviations as in text

Solvent	μg./g. fresh wt.					% of total P in plant				
	LP	NLOP	OP	IP	TP	LP	NLOP	OP	IP	TP
85% EtOH	99·4	55·9	155·3	10·3	165·6	14·9	8·4	23·3	1·6	24·8
0·2N-HCl			55·3	324·7	380·0			8·3	48·7	57·0
1N-$HClO_4$			37·9	15·2	53·1			5·7	2·3	8·0
0·5N-$HClO_4$			52·3	7·0	59·3			7·8	1·0	8·9
2N-NaOH			3·9	3·7	7·6			0·6	0·5	1·1
Residue			1·3		1·3			0·2		0·2
Totals			307·3	359·6	666·9			46·0	54·0	100

The composition of successive fractions differed considerably. A substantial quantity of OP was extracted by 0·2-HCl which also removed about 90% of the IP content of the tissues. The ethanol fraction and the $HClO_4$ and NaOH fractions also contained appreciable quantities of IP. Analysis of the residue showed that 99·8% of all the phosphorus of the leaves had been extracted.

The extracts in 1N- and 0·5N-$HClO_4$ were clear and colourless. Ultra-violet absorption spectra were characteristic of those of ribose- and deoxyribose-nucleic acids (RNA and DNA). The relationships between light extinction at 260 mμ and phosphorus content per ml. are shown in Fig. 1. These fractions are referred to as RNA and DNA for convenience. The lower and upper lines in Fig. 1 represent the theoretical values[6] for native and fully hydrolysed nucleic acids respectively (ε_P = 8000–12,000). Most of the values for RNA fall within these limits indicating partial degradation, whilst many of those for DNA appeared to be high with respect to light extinction. This feature is considered again later.

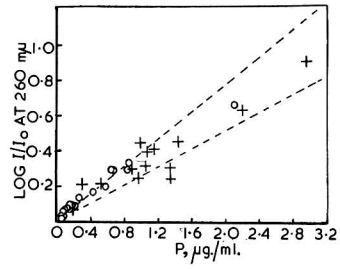

FIG. 1.—*Relationship between light extinction at 260 mμ and content of phosphorus (μg./ml.) as OP in* 1N-$HClO_4$ (+) *or as TP in* 0·5N-$HClO_4$ (o) *extracts from plants of different species, varieties and treatments*

Broken lines indicate values for ε_P = 8000 and 12,000 $M^{-1}cm.^2$

The persistence of IP in successive extracts in Table II was investigated in more detail, by repeating the ethanol and then the 0·2N-HCl treatments several times before extraction with 1N-$HClO_4$. This was done with cauliflower leaves for which results are shown in Table III, for extraction up to the RNA stage. Five extractions with ethanol were required to remove all traces of pigment from the residue, and a sixth was given for completeness. Although there was a progressive decrease to a negligible value in OP extracted, the IP values appeared to reach an equilibrium level. These levels probably saturated the ethanol extracts with IP salts. Subsequent extraction by 0·2N-HCl, however, released not only the bulk of the IP but a substantial quantity of OP as well, which probably comprised calcium or other salts of sugar phosphates and nucleotides which were not soluble in ethanol. It is apparent that at least four extractions by 0·2N-HCl were required to reduce the OP and IP to comparatively negligible values. Subsequent extraction of RNA in 1N-$HClO_4$ removed very little IP.

Table III

Effects of repeated extractions on removal of phosphorus from cauliflower leaf

(P as μg./g. fresh wt.)

Solvent		OP	IP	TP	Solvent		OP	IP	TP
Alcohol	1	77·2	7·3	84·5	0·2N-HCl	1	29·5	77·8	107·3
	2	21·2	2·0	23·2		2	10·7	14·3	25·0
	3	4·2	1·4	5·6		3	3·9	3·4	7·3
	4	3·1	0·4	3·5		4	2·6	1·1	3·7
	5	2·2	0·7	2·9	Totals		46·7	96·6	143·3
	6	0·5	1·8	2·3					
Totals		108·4	13·6	122·0	1N-$HClO_4$		41·7	0·6	42·3
					Totals		196·8	110·8	307·6

In order to identify the origin of the IP found in the 0·5N-$HClO_4$ fraction obtained from tomato (Table II), another experiment was carried out with the aid of ^{32}P. ^{32}P as Na_2HPO_4 with carrier was added to the macerate (50 ml.) at the first ethanol extraction stage before separation from the residue and was allowed to equilibrate with insoluble IP for 2 h., during which interval no exchange between ^{32}P and ^{31}P in nucleic acids would be expected in the absence of enzyme activity. Serial extractions and estimations of TP and IP were carried out as described above and radioactivity counts were made in triplicate. It was hoped to find from ratios of $^{31}P/^{32}P$ in IP isolated in isobutanol as phosphomolybdate whether decomposition of nucleic acid fractions occurred during extraction or whether IP found in earlier experiments had been carried over from a previous fraction.

The results of the experiment are given in Table IV. There was a fairly constant ratio $^{31}P/^{32}P$ in ethanol 2–5 stages of 3·61 ± 0·15 and in 0·2N-HCl of 5·03 ± 0·4 but the initial decrease in the ethanol series was probably real. There was no change in ratio at the 1N-$HClO_4$ stage (18 h. at 4°) in spite of the low levels involved. No decomposition of RNA is therefore likely at this stage. Further extraction with 0·5N-$HClO_4$ at 70° for 20 min. (Ogur & Rosen[8]), however, released IP which was entirely unlabelled, and about 15% decomposition of residual nucleic acids had evidently occurred.

In the light of these results it was decided to use OP values for RNA-P extracted in 1N-$HClO_4$ and TP values for DNA-P and residual RNA extracted in 0·5N-$HClO_4$. The partial breakdown of nucleic acids during extraction in 0·5N-$HClO_4$ at 70° probably accounts for the tendency for light extinction values of the DNA-fraction in Fig. 1 to be high, since decomposition raises extinction per mole of P but does not alter total P content. The use of OP values would only accentuate the increase on a phosphorus basis.

The reason for the decrease in the ratio $^{31}P/^{32}P$ between the first and second ethanol stages is not clear. The rise in the ratio between the fifth stage ethanol and 0·2N-HCl might indicate incomplete penetration of ^{32}P during equilibration. The existence of the discrepancy does not, however, invalidate the conclusions relating to nucleic acid breakdown which the experiment was designed to investigate.

The data for the OP fraction from tomato in Table IV confirm the results in Table III of the earlier experiment with cauliflower, that 0·2N-HCl extracts contain a substantial proportion of OP fractions not readily extracted in ethanol, which removes both lipoid and non-lipoid OP fractions.

Table IV

Repeated extraction of phosphorus fractions from tomato leaf after addition of ^{32}P as Na_2HPO_4

P as μg./g. fresh wt.; ^{32}P as counts per sec. per g. fresh wt.

Solvent	TP	IP	^{32}P	OP	Ratio IP/^{32}P
Ethanol 1	1155·0	1034·3	174·3	120·7	5·93
2	69·3	40·1	10·6	29·2	3·78
3	27·9	22·6	6·4	5·3	3·53
4	25·5	21·5	5·9	4·0	3·65
5	23·2	17·9	4·8	5·3	3·73
Total	1300·9	1136·4		164·5	
0·2N-HCl 1	878·5	798·5	147·3	80·0	5·42
2	175·5	154·3	33·0	21·2	4·67
3	39·6	37·3	7·4	2·3	5·06
4	10·4	8·4	1·7	2·0	4·95
Total	1104·0	998·6		105·5	
1N-$HClO_4$	48·0	1·5	0·3	46·5	5·00
0·5N-$HClO_4$	32·0	4·5	0	27·5	>300
2N-NaOH*	11·8			(11·8)*	∞
Total	2496·7	2152·7		365·8	

* Phosphoprotein is estimated as IP but included in total OP.
Standard deviation difference between mean sample and mean background = ±0·0093 c.p.s.

Distribution of phosphorus in different fractions from leaves of different plants

The procedure outlined at the beginning of this paper was used, and results are shown in Table V. IP comprised between 12 and 60% of the total. Ethanol-soluble OP always slightly or greatly exceeded 0·2N-HCl-soluble OP. More acid-labile phosphorus (7′P) was obtained in 0·2N-HCl than in ethanol extracts, and 7′P often comprised 8–12% or more of OP in 0·2N-HCl. Combined nucleic acid P comprised 30–50% of the OP. Phospho-protein (in 2N-NaOH) comprised generally between 1 and 3% of OP. Cauliflower varieties showed a remarkable range in apparent DNA-P. This was confirmed by analyses (not given here) of plants from four nutritional treatments for each variety.

Table V

Distribution of phosphorus in separate fractions from leaves of different plants

Values as μg. P/g. fresh wt.

Plant	Total P (TP)	Inorganic P (IP)	Organic P (OP) extracted and estimated as under: Ethanol OP	Ethanol 7′P	0·2N-HCl OP	0·2N-HCl 7′P	N-$HClO_4$ OP	0·5N-$HClO_4$ TP	2N-NaOH TP	Total OP
Tomato March	774	216	178	4	165	12	166	35	14	558
Tomato September	785	190	333	4	37	4	74	107	44	595
Cauliflower (Tremendous) March	706	193	243	4	77	5	164	17	12	513
Cauliflower (Majestic) September	1579	319	441	7	281	73	204	318	16	1260
Mustard September	566	181	258	12	30	21	23	72	2	385
Sunflower September	513	65	150	4	36	12	175	71	16	448
Vegetable marrow April	1161	627	166	6	152	12	182	29	5	534

Conclusions

Phosphorus fractions in plants can be removed completely by successive extraction in 85% ethanol, 0·2N-HCl, 1N-$HClO_4$ at 4°, 0·5N-$HClO_4$ at 70° and 2N-NaOH. Two extractions with ethanol and 0·2N-HCl remove over 90% of the organic phosphorus compounds soluble in these reagents. RNA is stable in 1N-$HClO_4$ at 4° for 18 h. but residual nucleic acid is decomposed significantly by 0·5N-$HClO_4$ at 70° in 20 min. Acid-labile phosphorus is more abundant in 0·2N-HCl than in ethanol extracts, which contain both lipoid and non-lipoid organic phosphorus. Acid-labile phosphorus comprises usually only 2–4% of ethanol-soluble and 8–12%, and occasionally much more, of 0·2N-HCl-soluble organic phosphorus. The amounts and proportions of organic phosphorus fractions in plants appear to vary widely between different species or varieties and between the same species on different occasions.

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STUDIES ON THE LIPIDS OF FLOUR. I.—Effect of Chlorine Dioxide Treatment on the Essential Fatty Acids

By N. W. R. DANIELS, P. W. RUSSELL EGGITT and J. B. M. COPPOCK

The essential fatty acid (E.F.A.) contents of white flour treated with twice and with twenty times the present usage rate of chlorine dioxide were examined by gas–liquid chromatography and by ultra-violet spectrophotometry of the isomerised oil. Although little change occurred at both levels immediately after treatment, the heavily overtreated flour suffered severe loss of E.F.A. on storage for 12 days in air. When this flour was stored under nitrogen the loss of E.F.A. was considerably reduced, suggesting that heavy treatment of flour, in destroying the protective tocols, exposes the E.F.A. to rapid oxidation in the presence of air. At the lower level of treatment the presence of chlorine in the chlorine dioxide gas did not have any significant effect on the E.F.A.

Introduction

In the commercial production of white bread flour certain chemical oxidising agents are used[1] to improve the baking quality and the consistency of performance of the flour.[2] Suggestions[3] have been made that the use of such oxidative improvers results in a serious loss of the nutritionally important essential fatty acids present in flour. Following these suggestions workers both in this country and in America have examined the lipids of flour with the object of evaluating quantitatively any changes which may occur in the E.F.A. content as a result of chemical treatment.

Fisher *et al.*[4] examined flour which had been treated with chlorine dioxide at the rate of 3·5 g. and 35·0 g. per sack of 280 lb. (These are referred to as 'normal' ×1 and ×10 treatments respectively, and because many of the nutritive studies cited have used flours treated at these levels, this terminology has been retained. In all cases, however, these levels are twice and twenty times the levels used at the present day. Thus, for chlorine dioxide, current treatment levels of 70% extraction white flour are approx. 1·8 g. per sack.) Analysis of the E.F.A. content of these flours showed little significant change after treatment even when the ×10 treatment was used. Subsequent work by Gilles *et al.*[5] confirmed these results and provided additional confirmatory evidence by the use of infra-red spectrophotometry.

The effect of chlorine dioxide, potassium bromate, ascorbic acid, ammonium persulphate and benzoyl peroxide on E.F.A. content of flour, and the effect of dough making and bread baking has also been examined[6] and in no instance, even when ×10 treatments were used, was there appreciable loss of E.F.A. as a result of treatment.

In this published work the method of analysis of E.F.A. content of the extracted flour oil was that described in the A.O.C.S. Tentative method Cd7-48 using alkaline isomerisation of the lipid material followed by ultra-violet spectrophotometry of the conjugated unsaturated fatty acids. Although used extensively for the analysis of polyunsaturated fatty acids, it is known nevertheless to be subject to errors arising from artifacts produced in the absorption spectrum of the oil during the isomerisation procedure.[7] An instance of this type of interference was found to be caused by the presence of autoxidised fatty acids in the lipid sample to be

analysed. In such samples erroneous results indicating for example the presence of arachidonic acid have been obtained.[8]

Consequently it was thought desirable to investigate the effect of oxidative improvers on the E.F.A. of flour lipids with the alternative and more direct method of gas–liquid chromatography (G.L.C.). In the work reported here, chlorine dioxide was used as before at the ×1 and ×10 levels of treatment, and a comparison was made of the results obtained by G.L.C. analysis with those obtained using the alkaline isomerisation method. The effect of ageing of the treated flours on their E.F.A. content was studied, and also the effect of using chlorine dioxide containing up to 30% of chlorine in the flour treatment. A preliminary publication of the results obtained has been made.[9]

Experimental

Flour

Throughout this work the flour used was an untreated unbleached sample of a commercial bread flour (approximately 70% extraction) free from Creta Preparata and all other flour additives. On a 14%-moisture basis the flour had 11·3% of protein (N × 5·7) and 0·43% of ash.

Treatment

Samples of flour (not less than 2000 g. of each sample) were treated with known amounts of chlorine dioxide while being continuously stirred in a sealed container fitted with rotating paddles. Chlorine dioxide was generated by the method of Daniels & Whitehead,[10] the ratio of hypochlorite to chlorate being controlled so that either pure chlorine dioxide or chlorine dioxide containing a pre-determined percentage of chlorine was generated. After treatment the flour was stored in sealed tins for at least 48 h. to equilibrate before the first samples were taken for analysis.

Treatment at the ×1 level caused little visible change in the flour, and the pH of a 10% suspension was unchanged at 6·0. When ×10 treatment was used the flour acquired a pink colour, as observed by other workers,[11] and the pH of a 10% suspension dropped from 6·0 to 5·8.

Extraction and saponification

Flour samples were extracted by mixing with redistilled carbon tetrachloride under nitrogen (approximately 4 ml. per 3 g. of flour) and shaking for 2 h. in the dark, on a 'Microid' flask shaker. The suspension was filtered on a Buchner funnel, the flour resuspended in fresh solvent, and the extraction repeated for a further 2 h. The two lipid extracts were combined, filtered if necessary to remove all traces of flour, and concentrated under vacuum in a rotary evaporator. By this procedure 1% by weight of the flour was extracted as lipid material, irrespective of the treatment received.

The flour lipids were saponified under nitrogen (oxygen free) by refluxing for 2 h. with 0·5N methanolic KOH. Excess methanol was distilled off, and after dilution with water, the solution was acidified with dilute H_2SO_4. The liberated free fatty acids were extracted with ether, which in turn was extracted with 0·7N aqueous KOH. The solution of potassium soaps was separated from ether-soluble unsaponifiable material (centrifuging if necessary) and re-acidified, when the free fatty acids were extracted once more with ether.

The ether extract was dried over magnesium sulphate, the excess ether was distilled off and the residue of free fatty acids methylated by adding excess diazomethane in ether. Diazomethane was prepared by the method of De Boer & Backer,[12] and was distilled directly on to the fatty acids at each methylation. Ether and excess diazomethane were driven off in a stream of nitrogen at 40° leaving the methyl esters of the fatty acids ready for analysis by G.L.C.

The samples of methyl esters were stored in glass vials, loosely stoppered with cellulose wadding, in a container filled with solid carbon dioxide. In this way, the low temperature, together with the heavy inert atmosphere, ensured that oxidation of the unsaturated acids in the samples did not occur during storage.

Gas liquid chromatography

Quantitative analysis of the methyl esters was carried out with the Pye Argon Chromatograph, which uses argon as the carrier gas and incorporates an ionisation detector system as

described by Lovelock.[13] The system combines simplicity of construction with extreme sensitivity, being capable of detecting as little as $2{\cdot}10 \times 10^{-12}$ moles of most organic compounds. Because of the very low loads consequently imposed on the chromatographic column, high column efficiencies may be attained and closely similar chemical compounds separated.

Advantage was also taken of the new synthetic polar polymers introduced as stationary phases in G.L.C. by Orr & Callen.[14] These allow better separation of fatty acids, differing only in their degree of unsaturation, than has previously been possible with the relatively non-polar greases and silicone oils of earlier workers.

With a polymer of ethylene glycol and adipic acid, polyethyleneglycol adipate (P.E.G. adipate), it was possible to separate oleic, linoleic and linolenic acids, although satisfactory separation of oleic from stearic acid was not obtained.

Two difficulties were encountered in the application of G.L.C. analysis to the detection of differences in E.F.A. content of treated and untreated flour. Firstly it was found that in many of the flour treatments examined the difference in E.F.A. content between the test and control flours was very small and was of the same order as errors involved in injecting the very small samples required (0·025 μl.) on to the chromatographic column. Secondly, samples of fatty acids from heavily treated flour contained material which was not eluted during the G.L.C. experiment, and which was therefore not recorded on the chromatograph chart obtained.

To overcome these difficulties, and to eliminate small variations in the sensitivity of the detector during the experiments, multiple consecutive runs were made in which an external standard of fatty acids from the untreated flour control was followed by the same volume of test sample. A series of up to ten consecutive pairs of chromatograms was made for each analysis reported.

In the calculation of the results the total peak area (peak area taken as height of peak multiplied by the width at half the height) found for the external standard was taken as the expected 100% area for the subsequent test chromatogram. The calculations were then examined statistically, when the mean values and standard deviations from the mean were obtained. In this way the degree of significance of differences between analyses could be assessed.

To complete the large number of chromatograms required for this statistical treatment it was necessary to reduce to a minimum the time for each chromatogram, while still retaining adequate separation between the major fatty acids of flour including the E.F.A. This was achieved by preparing a high-molecular-weight polymer of P.E.G. adipate and using a very light coating (2·3% w/w) as stationary phase on 60–100 mesh Celite (May & Baker, ' Embacel '). This column (120 cm. × 0·4 cm.), when used at 200° with a flow of 20 ml. of argon per minute, gave the required separation between the E.F.A., the chromatogram being completed within 6 min. The retention volumes of the different fatty acids are given in Table I.

This method of analysis provided a rapid and satisfactory method for measuring the major fatty acids of the flour lipid, although it was realised that minor peaks on the chromatogram, particularly those preceding palmitic acid, may not have been detected. With this proviso, the method was capable of analysing the E.F.A. content of lipids giving results within known limits of variation and significance.

Alkaline isomerisation

The procedure described in the A.O.C.S. Official method Cd7-58 was followed. All isomerisation experiments were performed on duplicate samples of flour oil and isomerisation was carried out under a stream of oxygen-free nitrogen.

Table I

Retention volumes of the major acids of flour oil

Acid	Time of emergence, sec.	Retention volume, ml.	Relative retention volume (linoleic acid = 1·00)
Palmitic	98	31	0·45
Oleic	187	59	0·86
Linoleic	218	69	1·00
Linolenic	272	86	1·25

Following recommendations given in this recent official method, calculations were made only when a peak could be detected at the characteristic wavelength. Arachidonic acid was not found in the G.L.C. analyses, and the small amounts which could be calculated from the isomerisation data (~0·02%) were ascribed to artifacts and are not reported.

Spectroscopic methanol was prepared from AnalaR methanol by refluxing for 3 h. with KOH and zinc dust. The methanol distilled from this mixture conformed to the required absorbence specifications. Measurement of the unisomerised oil was made in spectroscopic iso-octane, except when heavily oxidised oils were examined. These oils were partially insoluble in this solvent, and were dissolved in spectroscopic cyclohexane.

Results and discussion

Analysis by G.L.C. of the fatty acid methyl esters from flour freshly treated with chlorine dioxide at the rate of 3·5 g. per sack showed no significant change in its E.F.A. content as compared with untreated flour. The results of this analysis are shown in Table II, together with the analysis of the same treated flour 39 days after treatment. On storage there was a slight fall in linoleic acid content from 63·4% to 56·4% (significant at the 0·1% level) which was accompanied by a rise of 5·0% in palmitic acid (significant at the 0·1% level).

Table II

G.L.C. analysis of lipids of flour treated at ×1 *level*

Treatment	Untreated flour		3·5 g. of ClO_2 per sack			
Age of flour after treatment	—		5 days		39 days	
Number of chromatograms	6		7		10	
Fatty acid (as % of mixed methyl esters)	%	Standard deviation	%	Standard deviation	%	Standard deviation
Palmitic acid	15·4	0·9	16·3	1·7	21·3	0·7
Oleic acid	16·2	1·0	16·5	1·4	17·1	0·8
Linoleic acid	63·2	1·1	63·4	1·8	56·4	1·6
Linolenic acid	5·3	0·4	3·8	0·6	3·7	0·2
Undetected	0·0		0·0		1·5	

In Table III the effect is shown of a similar storage time on the fatty acids of flour heavily treated with chlorine dioxide at the ×10 level. Analysis of the freshly treated flour, 5 days old, showed no significant loss of linoleic acid (not significant at 1·0% level) compared with the untreated flour (Table II), although a fall in linolenic acid from 5·3% to 2·6% was found (significant at 0·1% level). After storage for 39 days, however, severe loss of all unsaturated acids was found, the E.F.A. content falling from 68·5% to 14·5%, giving 58·2% of undetectable fatty acid material.

This severe destruction of E.F.A. in heavily treated flour on storage has not been reported previously, although it has been shown that such treatment completely destroys vitamin E in flour,[15] and that as a result of such treatment rancid flavours are produced.[16]

The results of E.F.A. analysis by the isomerisation technique are given in Table IV and show no loss of E.F.A. after ×1 treatment, either at 5 days or at 80 days after treatment. The apparent rise in linoleic acid content from 42·0% to 46·8% is similar to that observed by Fisher,[4] but is not in agreement with the G.L.C. results of Table II.

Table III

G.L.C. analysis of lipids of flour treated at ×10 *level*

Treatment	35·0 g. of ClO_2 per sack			
Age of flour after treatment	5 days		39 days	
Number of chromatograms	6		5	
Fatty acid (as % of mixed methyl esters)	%	Standard deviation	%	Standard deviation
Palmitic acid	20·3	1·8	19·8	3·6
Oleic acid	16·8	0·9	7·5	0·6
Linoleic acid	60·1	2·1	14·5	1·5
Linolenic acid	2·6	0·5	nil	—
Undetected	0·2		58·2	

Table IV

Isomerisation analysis of ×1 and ×10 ClO_2-treated flour

Treatment	Untreated flour	×1 treatment 3·5 g. of ClO_2 per sack		×10 treatment 35·0 g. of ClO_2 per sack	
Age of flour after treatment	—	5 days	80 days	5 days	80 days
Fatty acid (as % of extracted oil)	%	%	%	%	%
Linoleic acid	42·0	46·8	48·2	38·7	15·9
Linolenic acid	2·4	2·3	2·6	0·6	nil
Conjugated diene	1·04	1·41	1·24	7·20	8·80
Conjugated triene	0·014	0·009	0·004	0·013	0·016

At the ×10 level of treatment, little change was observed after 5 days, but on storage for 80 days a marked fall in E.F.A. content occurred. Comparison of these figures with the results in Tables II and III shows close agreement in the trends of E.F.A. content with the various conditions examined. However, in general the G.L.C. results are higher than the figures obtained with the isomerisation method. This is most likely due to the presence of non-fatty acid material (lipoprotein, sugars, glycerol and unsaponifiable matter) in the flour oil used in the isomerisation analysis which is removed during the preparation of the fatty acid methyl esters used in the G.L.C. analysis. This was investigated and confirmed by preparing a sample of free fatty acids from untreated flour oil, and analysing the composition by both methods. Table V shows that when dealing with such a purified sample, the results obtained by the two methods are more comparable.

Flour treatment with chlorine dioxide/chlorine mixtures

Previous investigations of E.F.A. destruction in treated flour might be criticised on the grounds that substantially pure chlorine dioxide has been used in treating flour for such studies whereas in the commercial treatment the gas used may contain up to 20% of chlorine. Consequently treatment of flour with chlorine dioxide containing 5%, 15% and 30% of chlorine was examined. G.L.C. analysis of the fatty acids of flour oil from these treated flours 39 days after treatment is shown in Table VI. In each case the flours were treated at the ×1 level.

The presence of chlorine in the chlorine dioxide used for these treatments was found to have no effect whatever on the E.F.A. of the flour lipids at the ×1 level of treatment, even after storage for 39 days after treatment.

Effect of air on storage of heavily treated flour

In order to assess the importance of air oxidation in the loss of E.F.A. from heavily treated flour, a sample of chlorine-dioxide-treated flour (35·0 g. per sack, ×10 treatment) was divided

Table V

Comparison of G.L.C. and isomerisation method on sample of fatty acids

Method	% composition of mixed fatty acids				
	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Conjugated diene
G.L.C.	17·3	16·0	61·5	5·2	—
Isomerisation	—	—	55·4	3·0	1·2

Table VI

G.L.C. analysis of fatty acids from flour treated with Cl_2/ClO_2 mixtures

Percentage Cl_2 in ClO_2	(Pure ClO_2)		5%		15%		30%	
Number of chromatograms	10		5		10		8	
Fatty acid (as % of mixed methyl esters)	%	Standard deviation	%	Standard deviation	%	Standard deviation	%	Standard deviation
Palmitic acid	21·3	0·7	20·8	3·2	20·9	1·3	20·8	1·8
Oleic acid	17·1	0·8	16·5	2·3	17·6	1·5	17·2	1·4
Linoleic acid	56·4	1·6	58·3	3·3	57·2	1·6	57·8	1·9
Linolenic acid	3·7	0·2	3·6	0·7	4·2	0·6	4·4	0·5
Undetected	1·5		0·8		0·1		0·8	

into two samples after equilibrating for 5 days after treatment. One sample was stored in a closed tin in the presence of air; the other was stored in a sealed vacuum desiccator in which the air had been replaced with oxygen-free nitrogen by repeated evacuation and filling. Samples were taken from each flour after 12 days and 30 days and the E.F.A. content of the flour lipid analysed by G.L.C.

Fig. 1 shows the E.F.A. content of the flour fatty acids plotted against storage time for the two conditions of storage. Exclusion of air resulted in a marked reduction in the rate of destruction of E.F.A. compared with the rapid destruction in the air-stored flour. Thus, whereas with air storage the E.F.A. content fell by 70·6% between the 5th and 12th day after treatment, the flour stored under nitrogen showed only a 16·1% fall for the same period of time. During the period between the 12th and 30th day of storage, the air-stored flour lost 49·0% of its remaining E.F.A. while the nitrogen-stored flour again lost only 16·9%. A possible explanation of this small, consistent fall in the E.F.A. of flour stored in nitrogen may be that at each sampling a small amount of air was retained by the flour in spite of the evacuating technique and caused the loss of E.F.A. observed.

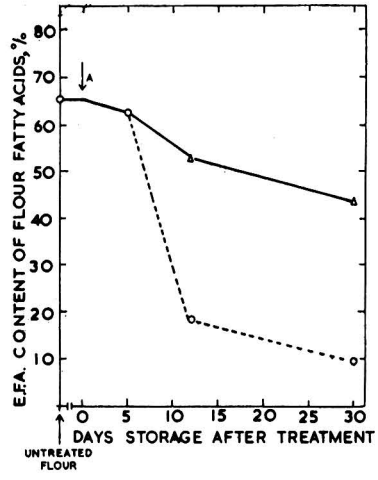

FIG. 1.—*E.F.A. content (G.L.C. analysis) of air and nitrogen stored flour*

At A flour treated with 35·0 g. of ClO_2 per sack
△ flour stored in nitrogen
○ ,, ,, ,, air

The rapid and extensive loss of E.F.A. in the air-stored sample would thus seem to be due to air-oxidation of the lipids rather than their destruction by the direct action of chlorine dioxide used in the flour treatment. That this air oxidation should occur in the heavily treated flour is in agreement with the work of Moran[17] who suggested a loss of tocopherols following treatment, and with the findings of Frazer *et al.*[15] who showed complete destruction of the vitamin E content of flour at the ×10 level of treatment with chlorine dioxide. Flour thus denuded of its antioxidants would be expected to display such rapid autoxidation of its unsaturated lipids as was found in these experiments. The findings of Frazer[15] are also in agreement with the observed stability of E.F.A. in ×1 treated flour, since this was shown to have retained 14% of the normal amount of vitamin E after treatment. At a lower level of treatment with 2·1 g. of chlorine dioxide per sack and a 72% extraction Australian flour, 46·5% of the original protective tocols have been shown to remain following treatment.[18] Thus with the current treatment of 70% extraction flour at 1·8 g. of chlorine dioxide per sack there would appear to be little danger of loss of E.F.A. caused by air oxidation during storage.

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CHANGES IN THE LIPIDS OF FLOUR INDUCED BY TREATMENT WITH CHLORINE DIOXIDE OR CHLORINE, AND ON STORAGE

By D. G. H. DANIELS

Fatty acid composition of wheat flour oils was measured by gas chromatography, and results were presented either as fractions of the total C_{16} and C_{18} acids or, by application of an internal standard, as the percentage of each acid in the original oils.

Flour treated with chlorine dioxide (up to 120 p.p.m.) and stored for periods up to 15 weeks showed no changes in the fatty acid composition of the mixed oil extracted successively by light petroleum and acetone. Heavier treatment resulted in significant oxidative changes. After 27 weeks' storage, however, both untreated and lightly treated flour (up to 33 p.p.m. of ClO_2) showed changes in the acetone—but not in the light petroleum—extracts. These findings were correlated with measurements of the induction periods of whole flours when heated in oxygen at 100°, both treatment and storage diminishing the induction periods.

Chlorine treatment of the flour caused decreases in linoleic acid contents of the extracted mixed oils at all levels of treatment. The effect on induction period was complex, but in general chlorine treatment caused long induction periods, which diminished during storage less than those of untreated flour. The significance of these results is discussed.

Introduction

In studies of the effect of treatment of flour with chlorine dioxide on the linoleic and linolenic acid content of flour oils, Fisher *et al.*[1, 2] found no significant changes at the ' normal ' level of treatment (30 p.p.m.), and only minor changes at the ×10 level (300 p.p.m.). These measurements were made immediately after treatment. It has however been recognised by workers in these laboratories and elsewhere[3, 4, 5] that one of the immediate results of the application of gaseous improvers is a reduction in the effectiveness of the antioxidants present in the flour, notably the tocopherols, the content of which has been found by Moore *et al.*[5] to fall from 1·57 to 0·19 mg./100 g. on treatment with 30 p.p.m. of chlorine dioxide. If all the protective factors undergo losses of this magnitude, large reductions in unsaturated acid contents might be expected to occur when the treated flour is stored.

The present work was undertaken to test this possibility. The C_{16} and C_{18} fatty acids in flour lipids have been measured by a gas chromatographic method, and changes in effectiveness of the natural antioxidants have been assessed by measuring the induction periods of intact flours when subjected to an accelerated oxidation test.

Since this work was completed, Coppock *et al.*[6] have published measurements of the fatty acid composition of lipids from flours treated with chlorine dioxide, chlorine and mixtures of the two, before and after storage. Their results are in substantial agreement with those described here. (See also preceding paper.—Editor.)

Experimental

Materials

Flours were prepared on a Bühler laboratory mill. Manitoba wheat (No. 2 grade) was milled to about 67% extraction rate, a single sample being used for Expts. 2 and 3, and another one for Expt. 1.

Light petroleum (b.p. below 40° and b.p. 60–80°) was purified by shaking with conc. H_2SO_4, washing, drying and redistilling.

Acetone, ethanol and methanol were redistilled before use.

Glyceryl trimargarate (GTM) was prepared from margaric acid and glycerol by Garner's method[7] and recrystallised before use.

Flour treatment

Chlorine dioxide, chlorine dioxide–chlorine mixtures, and chlorine were prepared and assayed as described by Daniels & Whitehead.[8] The quantities of sodium hypochlorite and sodium chlorite were adjusted to give ' pure chlorine dioxide ' with less than 0·7% w/w of chlorine, or ' chlorine dioxide–chlorine ' with the gases in the proportions of 4 : 1 w/w.

A 5-litre flask containing 200 g. of flour was evacuated, and then connected to a gas-washing bottle holding the acidified reagents. By suitable adjustment of the connecting stop-cock, a steady stream of air was drawn through, carrying the chlorine dioxide with it. When the flow of air had ceased, the flask was disconnected, shaken vigorously, and re-evacuated, the process being repeated two or three times, until all the chlorine dioxide had been swept over.

Flours were stored in screw-capped glass bottles in the dark at room temperature (about 20°). The bottles were opened periodically to observe any abnormal odours developed on storage.

Extraction of lipids

Flour (20 g.) was extracted by percolation, firstly with light petroleum (b.p. <40°, 100 ml.) and secondly with acetone (100 ml.). The oils were recovered by distilling off the solvents under reduced pressure, and dried overnight *in vacuo* over phosphoric oxide.

Saponification and esterification

The flour oil (0·25 g. or less) was rapidly saponified with ethanolic KOH containing pyrogallol.[9] After extraction of non-saponifiable materials, the aqueous layer was acidified, and extracted three times with 25 ml. of light petroleum (b.p. 60–80°). The fatty acids were esterified with diazomethane.

Gas chromatography

The methyl esters of fatty acids were chromatographed at 197° in nitrogen, using as stationary phase 20% diethylene glycol polyadipate[10] (LAC-1-R296, Cambridge Industries Co. Inc., Cambridge 42, Mass., U.S.A.) on silicone-treated Celite, 80–100 mesh.[11] A gas density balance was used as detector (Abbotsbury Laboratories, Elstree, Herts). Peak areas were measured as the product of peak height and width at half-height.

Each sample of methyl esters was chromatographed four times to obtain mean values of fatty acid compositions. In some experiments, a known weight of glyceryl triheptadecanoate (glyceryl trimargarate, GTM) was added to the flour oil before saponification: the methyl heptadecanoate produced from the GTM served as an internal standard.

Oxygen uptake of flours at 100°

The recording manometric apparatus employed was a modification of that of Sylvester *et al.*,[12] designed primarily for measuring the induction periods of oils and fats when heated in oxygen at 100° and atmospheric pressure. The modifications enabled samples of 0·1–0·2 g. of oil to be examined. It was found that intact flour (10 g. sample) could also be examined in the apparatus, provided that all moisture were first removed by drying overnight over phosphoric oxide at 0·02 mm. Hg.

A full description of the apparatus will be published elsewhere.[13]

Results

General

The main peaks in gas chromatograms of methyl esters from flour oils corresponded to the esters of the C_{16} and C_{18} acids: palmitic, stearic, oleic, linoleic and linolenic. Minor peaks corresponding in retention volumes to C_9, C_{11} and C_{12} esters were usually present, but could not be estimated quantitatively, as it was difficult to avoid losses of these more volatile components when putting such a mixture on to a column at 197°.

There was some evidence of the presence of higher fatty acids in minor amounts. In some samples, a shallow peak appeared, with a retention volume corresponding to a C_{20} monounsaturated acid. One sample which showed this peak was hydrogenated, and then had about 1% of C_{20} saturated ester in addition to the expected palmitate and stearate peaks.

The margaric acid used as internal standard was 98% pure, the impurities being traces of C_{15}, C_{16}, C_{18} and C_{19} acids, which were too small in amount to make a significant contribution to these acids in chromatograms derived from flour oil mixed with glyceryl trimargarate.

Experiment 1

Flour was treated, immediately after being milled, with 'pure' chlorine dioxide, with chlorine dioxide–chlorine (4 : 1) or with chlorine, each at three different dosages. Those of chlorine dioxide–chlorine (4 : 1) were respectively equivalent in total oxidising power (towards acidified potassium iodide) to the corresponding levels of pure chlorine dioxide. The two upper levels of chlorine treatment were similarly equivalent to the corresponding chlorine dioxide ones, but the lowest was equivalent to a dosage of 60 p.p.m. of chlorine dioxide. After storage, the light petroleum- and acetone-soluble oils were extracted. In most cases, the two extracts from each sample were mixed before conversion into esters.

The flours which had had the highest dosages of chlorine dioxide and chlorine dioxide–chlorine became rancid after one week's storage. Those with the intermediate dosages became rancid in about 8 weeks. Those with the lowest dosages remained odourless, the 'fresh' odour of untreated flour having been immediately destroyed by the chlorine dioxide. The flours treated with chlorine at all levels immediately acquired an abnormal 'sweetish' odour which apparently changed very little on storage.

Table I records the results of fatty acid determinations: the proportions of the five principal acids are expressed as percentages of their total (col. A), and also, where determined, as mean percentages of these five C_{16} and C_{18} acids in the original oils (col. B). Examination of col. A showed that the two heavier dosages of chlorine dioxide or chlorine dioxide–chlorine, and all three dosages of chlorine, caused the linoleic acid contents to be significantly lower than the control mean (a difference greater than 1·1% was significant at the 5% level). No peak corresponding to dichlorostearic acid[6] was observed in the chromatogram.

Experiment 2

Flour was treated, about 2 weeks after being milled, with chlorine dioxide–chlorine (4 : 1) at dosages of 33 and 330 p.p.m. of chlorine dioxide. The oils were extracted 1 week (control only), 6 weeks and 27 weeks after treatment, and the light petroleum and acetone extracts examined separately. The flour given the heaviest dosage developed a rancid odour after about 1 week's storage, but the untreated and lightly treated flours were unchanged in odour for the whole period.

Table I

Changes in fatty acid composition of oils extracted from flour variously treated and stored

Dosage ClO_2, p.p.m.	Dosage Cl_2, p.p.m.	Storage time, weeks	Extraction solvent	Yield, %	Additions of GTM	Principal acids: Palmitic A	Palmitic B	Stearic A	Stearic B	Oleic A	Oleic B	Linoleic A	Linoleic B	Linolenic A	Linolenic B	Total $C_{16} + C_{18}$ fatty acids in oil, %
Control flour																
0	0	11	P	0·79	+	18·6	14·6	1·3	1·0	13·6	10·7	63·2	49·6	3·3	2·6	78·5
0	0	11	A	0·27	+	16·5	9·1	1·5	0·8	9·0	5·0	69·3	38·3	3·8	2·1	55·3
0	0	11	P + A	1·06	+	18·2	13·2	1·3	0·9	12·7	9·2	64·4	46·8	3·4	2·5	72·6
0	0	10	P + A	1·04	+	17·9	12·7	1·4	1·0	12·6	8·9	64·9	46·0	3·3	2·3	70·9
0	0	8	P + A	1·05	−	18·1		1·1		13·4		64·2		3·2		
Treated flour (pure chlorine dioxide)																
30	0	10	P + A	1·08	+	19·2	12·9	1·2	0·8	12·5	8·4	63·9	42·8	3·2	2·2	67·1
150	0	10	P + A	1·02	+	20·3	12·9	1·6	1·0	14·1	9·0	61·0	38·7	3·0	1·9	63·6
300	0	10	P + A	0·85	+	30·1	13·8	2·7	1·2	19·4	8·9	46·4	21·2	1·5	0·7	45·8
Treated flour (chlorine dioxide–chlorine)																
27	7	11	P + A	1·06	+	18·7	13·6	1·2	0·9	12·4	9·0	64·2	46·6	3·6	2·6	72·6
137	34	11	P + A	1·00	+	20·6	13·7	1·6	1·0	14·2	9·4	60·5	40·1	3·1	2·1	66·3
274	68	11	P + A	0·87	+	29·5	14·2	2·1	1·0	18·4	8·9	48·5	23·4	1·6	0·8	48·4
Treated flour (chlorine)																
0	155	8	P + A	1·04	−	19·5		1·0		13·3		63·1		3·1		
0	395	8	P + A	1·06	−	21·1		1·5		13·5		61·0		3·0		
0	790	8	P + A	1·08	−	23·3		1·4		12·6		59·6		3·2		

P = light petroleum (b.p. below 40°) A = acetone

Columns A acids as % of $C_{16} + C_{18}$ acids

,, B ,, as % of total extracted oil

Table II records the results of fatty acid determinations, the proportions of the five principal acids being expressed as percentages of their total. Examination of these results showed that the slight changes in composition of light petroleum extracts of both control and lightly treated flours were without statistical significance ($P > 5\%$), both with respect to storage time and to the effect of treatment, but that the corresponding changes in heav treated flour were highly significant ($p < 1\%$). However, with acetone extracts of control and lightly treated flours, the fall in linoleic acid and rise in oleic acid which occurred on storage appeared to be real (p about

Table II

Changes in fatty acid composition of oils extracted from flour treated with several dosages of chlorine dioxide–chlorine (4 : 1) and stored

Dosage, p.p.m. ClO_2	Dosage, p.p.m. Cl_2	Storage time, weeks	Yield %	Principal acids (as % of $C_{16} + C_{18}$ acids): Palmitic	Stearic	Oleic	Linoleic	Linolenic
(1) *Light petroleum extracts*								
Control flour								
0	0	1	0·75	19·5	1·1	14·5	61·8	3·2
0	0	6	0·74	19·4	0·9	14·9	61·7	3·1
0	0	27	0·73	18·5	1·2	14·8	62·2	3·3
Treated flour, low dosage								
33	8	6	0·76	19·4	1·0	14·7	61·8	3·2
33	8	27	0·72	19·3	0·9	15·4	60·9	3·5
Treated flour, high dosage								
330	80	6	0·54	34·5	1·2	22·8	40·6	0·9
330	80	27	0·30	43·6	2·2	26·8	26·1	0·6
(2) *Acetone extracts*								
Control flour								
0	0	1	0·31	14·5	0·9	8·6	71·5	4·5
0	0	6	0·32	15·6	0·7	9·0	70·7	4·1
0	0	27	0·28	16·8	1·0	10·5	68·1	3·7
Treated flour, low dosage								
33	8	6	0·30	15·7	0·6	9·2	71·0	3·6
33	8	27	0·28	16·7	1·1	9·6	69·4	3·4
Treated flour, high dosage								
330	80	6	0·37	27·8	0·9	15·6	53·7	2·1
330	80	27	0·37	39·5	2·0	21·6	36·2	0·9

1%), with no significant difference between the two flours in these respects. The differences in composition between the heavily treated flour and the other two were again highly significant; moreover there was a significant difference in composition between the oils extracted by acetone from the heavily treated flour at 6 and 27 weeks.

Experiment 3

Flour was treated with 'pure' chlorine dioxide on the same day as it was milled, seven levels of treatment being applied (15–300 p.p.m.). The oils were extracted after 6 weeks' and 15 weeks' storage, and the light petroleum and acetone extracts were mixed before converting into esters.

The most heavily treated flour became rancid less than 2 weeks after treatment. The flours which had received 90, 120 and 150 p.p.m. of chlorine dioxide developed rancid odours more slowly, but were appreciably rancid at the end of 15 weeks. The three most lightly treated flours and the control flour remained non-rancid after 15 weeks' storage.

The results obtained from this series of treated flours are recorded in Table III. An analysis of their variance showed that the differences between the control flour and the first five treated flours were non-significant in respect to palmitic, oleic and linoleic acid contents (the sets of results for the other two acids were not analysed as the acids were present in minor amounts and differences were irregular). However, as in Expt. 2, the heavily treated flour (300 p.p.m.) showed appreciable effects at 6 weeks' storage; these increased still more in the next 9 weeks. By contrast, neither storage nor level of treatment resulted in significant effects on the fatty acid composition of the control and first five treated flours.

Oxygen uptake of flour at 100°

The flour samples of Expt. 3 were heated in oxygen at 100° in the recording manometric apparatus, 4 days, 6 weeks and 15 weeks after treatment. As the apparatus accommodated only four samples at a time, odd- and even-numbered samples, in order of dosage, were grouped together. The results are plotted in Fig. 1. No attempt has been made to draw smooth curves.

Table III

Changes in fatty acid composition of oils extracted from flours treated with several dosages of pure chlorine dioxide and stored

ClO_2 treatment, p.p.m.	Storage time, weeks	Extraction solvent	Yield, %	Principal acids (as % of $C_{16} + C_{18}$ acids)				
				Palmitic	Stearic	Oleic	Linoleic	Linolenic
Control flour								
0	6	P + A	1·17	18·4	0·8	15·0	62·8	3·4
0	15	P + A	1·04	18·3	1·0	15·2	62·3	3·2
Treated flour								
15	6	P + A	1·16	18·0	1·1	14·4	63·3	3·2
15	15	P + A	1·02	18·1	1·0	15·1	62·7	3·2
30	6	P + A	1·14	17·6	1·3	15·1	63·0	2·9
30	15	P + A	1·05	18·6	1·0	14·9	62·6	3·1
58	6	P + A	1·20	17·8	0·9	14·0	63·6	3·6
58	15	P + A	1·05	18·2	1·2	14·2	63·2	3·4
88	6	P + A	1·15	18·5	1·0	13·3	64·2	3·0
88	15	P + A	1·15	19·0	1·2	15·5	61·5	2·8
120	6	P + A	1·15	18·3	1·1	14·5	63·0	3·1
120	15	P + A	1·13	18·4	1·1	15·2	62·2	3·2
—	—	—	—	—	—	—	—	—
150	15	P + A	1·09	19·4	1·1	15·4	61·2	3·1
300	6	P + A	0·94	22·6	1·4	17·2	56·9	2·0
300	15	P + A	0·88	32·4	1·6	22·4	42·5	1·2

Standard errors for means:
Palmitic acid ±0·43% Oleic acid ±0·57% Linoleic acid ±0·53%

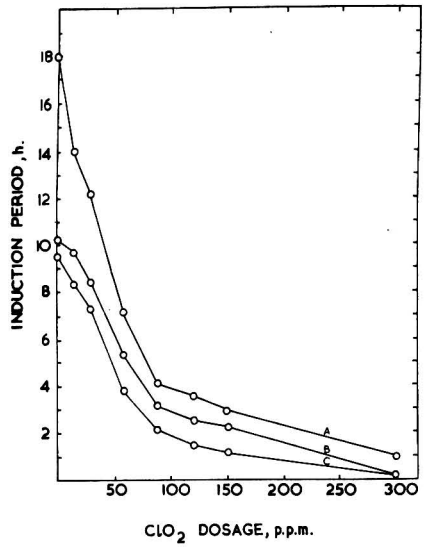

FIG. 1.—*Relationship between induction period and dosage in flours heated in oxygen at* 100°, *at various storage times after treatment with chlorine dioxide*

A 4 days' storage B 7 weeks' storage
C 15 weeks' storage

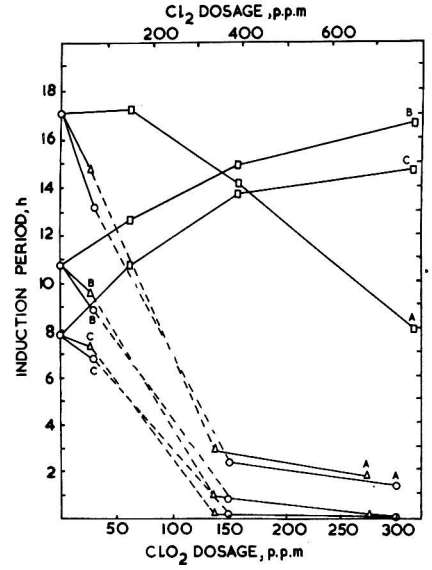

FIG. 2.—*Relationship between induction period and dosage in flours heated in oxygen at* 100°, *at various storage times after treatment with chlorine dioxide or chlorine*

○ Pure chlorine dioxide □ Pure chlorine
△ Chlorine dioxide–chlorine (4 : 1)
A 2 days' storage B 7 weeks' storage
C 17 weeks' storage

Fig. 2 shows the result of similar measurements with the flours of Expt. 1. There is a noteworthy difference between the behaviour of flours treated with chlorine and those treated with chlorine dioxide, whether or not additional chlorine was present in the latter.

Discussion

The present experiments in general confirm the findings of Fisher *et al.*[1, 2] and Coppock *et al.*[6] that treatment at normal commercial levels (30 p.p.m. or less of chlorine dioxide) has no measurable effect on the content of the major fatty acids of flour, at any rate in the more readily extractable lipid fractions. Minor differences from their results are probably caused by natural variations in the flours and differences in extraction procedure.

The stability of the unsaturated acids persists up to quite high levels of treatment (about 120 p.p.m. of chlorine dioxide in Expt. 3). There is thus no support for the simple hypothesis, put forward in the introduction, that the complete antioxidant system of flour has the same order of stability as the tocopherols, which would be completely destroyed at such a heavy dosage as this. The accelerated oxidation test throws considerable light on this question, if one may take the induction period in this test as a measure of total antioxidant content in the flour. Treatment with 30 p.p.m. of chlorine dioxide reduces it by only 30% in contrast with the fall of 88% in tocopherol content produced by a similar dosage.[5] Indeed, from Fig. 1, mere storage for 6 weeks (the average period from milling to baking in commerce) lowers the antioxidant level of the untreated flour rather more.

The three curves of Fig. 1 show marked inflexions at the 90 p.p.m. dosage. A possible explanation is that two antioxidant systems exist in flour, both affected by chlorine dioxide and atmospheric oxygen. While any of the first, more potent one, remains, it fully protects the remainder of the system, including the second antioxidant. At a dosage of about 90 p.p.m. all the first has been destroyed and the unsaturated lipids are then protected by the second, but it would seem that it is a less effective protective agent, as deterioration of the fatty acids sets in even when there is enough antioxidant present to give one hour's induction period in the accelerated test.

Turning to Tables I and II, it is found that the fatty acid compositions of light petroleum and acetone extracts are dissimilar, and that these lipids differ in their susceptibility to oxidation when the intact flour is stored. This is probably a reflexion of their different contents of polar lipids. It has been shown[14] that the acetone extract contains relatively large amounts (up to 70%) of galactosyl glycerides, while unpublished chromatographic studies of mixed acetone and light petroleum extracts in these laboratories suggest that in the latter only 10–15% are galactosyl glycerides. These compounds may well be more susceptible to oxidation than normal fats.

The few results on chlorine-treated flours indicate that a different reaction mechanism must be involved. The falls in contents of linoleic acid, even at small dosages of chlorine, suggest that the predominant feature is addition at double bonds in the fatty acids themselves rather than oxidation. Moreover, measurements of induction periods show a complex state of affairs, the full explanation of which must await further experimental work. A tentative explanation of the shape of the curves at 2 days' and 7 weeks' storage may be that the effect of chlorine on the antioxidant system is to form intermediate substances, themselves poor antioxidants, which change on storage to more powerful antioxidants. The curve at 17 weeks shows the kind of general lowering that might be expected from further aerial oxidation over a period of 10 weeks.

In conclusion, one may emphasise that while moderate doses of these bleaching and 'improving' agents have little or no effect on the contents of 'essential' and other fatty acids in the flour, they appear to have marked effects on the natural antioxidant substances therein, and further investigation of this aspect is under way.

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APPLICATION OF THE KARL FISCHER METHOD TO THE DETERMINATION OF WATER IN SUGAR CONFECTIONERY MATERIALS

By D. SANDELL

The techniques are described and the suitability discussed of the Karl Fischer method for the determination of water content of sugar confectionery and sugar confectionery raw materials. The method is shown to have some considerable advantages over the conventional, oven-drying methods used for most control and investigational purposes. It is rapid, tests being carried out in 7–40 min.; it has a precision of ±1% of the water content; and it is productive of results in quantity. Comparable results obtained by the Karl Fischer and oven-drying methods are presented, showing good agreement in many cases, but some discrepancies in others.

Introduction

The difficulties associated with the determination of water in foodstuffs are well known, and are reflected in the large number of techniques described in the literature. The most widely used method, oven drying, is slow and is in general neither quantitative nor specific for water.[1, 2] While many modifications of oven drying are satisfactory in a limited application, a general method, reasonably specific for water, accurate and convenient, has been lacking. It is suggested that the Karl Fischer method can be adopted to fill this gap.

The use of this method for foodstuffs is by no means new. Since the method was first proposed by Fischer[3] in 1935 (for the determination of water in sulphur dioxide) many workers have indicated its obvious usefulness in the testing of foodstuffs. Among others, Fosnet & Haman[4] describe application to cereals and cereal products; dairy products were tested by Heinemann,[5] dried vegetables by Johnson,[6] sugars by Zimmermann,[7] and some industrial materials by Almy *et al.*[8] Mitchell & Smith, in their excellent book dealing with the Karl Fischer method,[9] give a valuable bibliography.

The Karl Fischer reagent is a solution of iodine, sulphur dioxide and pyridine in methanol. It reacts quantitatively with water but deteriorates due to side reactions and thus requires standardisation at intervals. The rate of deterioration is at a maximum when the reagent is freshly prepared and falls to a low steady value after a few days; with the commercial reagent, daily standardisation has been found to be quite adequate. For a fuller description of the reagent, the reader is referred to the volume by Mitchell & Smith.[9]

Experimental

The experimental work which has been carried out can be readily divided into two parts:

(1) Development of a suitable titration apparatus.
(2) Development of a suitable technique for extracting water from the material under test, so that it may be completely titrated.

In the following text, abbreviations have been used as follows:

KF = Karl Fischer reagent, water equivalent 5 mg./ml. when freshly prepared.
SWS = Standard solution of water in methanol, water equivalent 2·5 mg./ml.
MA = Methanol, dry, of analytical reagent quality.

(1) *Development of titration apparatus*

The requirements were: (*a*) the reagents should be fully protected against atmospheric moisture; (*b*) an electrical method of indicating the titration end-point, since coloured materials mask the colour change of excess reagent (brown) to spent reagent (yellow); (*c*) a standard and inexpensive titration vessel with rapid interchangeability, in which both the extraction and titration could be carried out.

The apparatus as developed is centred on a standard Bakelite, screw-capped 2 fl. oz. jar.

The cap is drilled out to take a polythene plug carrying the KF and SWS burette tips and the electrode assembly. A second jar is fitted to carry the MA burette tip. The burettes are, respectively: left-hand KF burette, capacity 25 ml.; right-hand SWS burette, capacity 25 ml.; and a standard 50-ml. burette for the MA. Each burette is fitted with two-way tap, guard bulb, and a B7 cone on the inlet, fitting directly into a B7 socket on the reservoir head.

The three reservoirs are identical, being made up from Pyrex 2-litre conical flasks fitted with B40 cone and socket; each cone carries a delivery tube to a burette, fitted with a B7 socket; a connexion to a guard tube; and a refill tube fitted with B10 socket and stopper.

Three guard tubes are required, filled with silica gel.

The titration vessel is supported over a suitable magnetic stirrer, which can be swivelled about a retaining bolt so that the jar is easily unscrewed and removed.

Burette filling is carried out by the vacuum side of a Dymax Mk. II compressor, and the pressure side of the same compressor provides an air stream which, after being dried, is used to mix the liquids in the reservoirs after the burettes have been rinsed.

The electrode system is of the 'dead stop' type, and employs a Siemens 1·5-V B5 cell, a Clarostat type 58 100-kΩ wire-wound linear potentiometer, a Sifam type M35 0–25 micro-ammeter, and two electrodes made up from 0·5 mm. diameter platinum wire.

The whole apparatus (Fig. 1) is set up on a Perspex case, which has removable panels to allow easy access to the reservoirs for filling.

Method of titration and the standardisation of the reagents

The burettes are filled and drained two or three times, and a small quantity of air is blown through the reservoirs to ensure homogeneity of the reagents. The burettes are then filled and brought to the zero mark.

The first balance required is that between the KF and SWS. Ten ml. of KF are run into the jar, which contains a magnetic float; the speed of the float is adjusted, and SWS added until the electrodes are immersed. The potentiometer is then adjusted until a deflection of 25 μA is observed, a considerable excess of KF being present, as shown by the dark brown colour. SWS is then added until the colour turns to a light brown, and finally the additions are made in increments of 0·1 ml. with an interval of 10 sec. between additions to ensure completeness of reaction. As the end-point is approached, the meter reading falls slightly, and at the end-point, a marked deflection is observed from approximately 23 to 12 μA. The titration is repeated, and the two values should not differ by more than 0·1 ml.

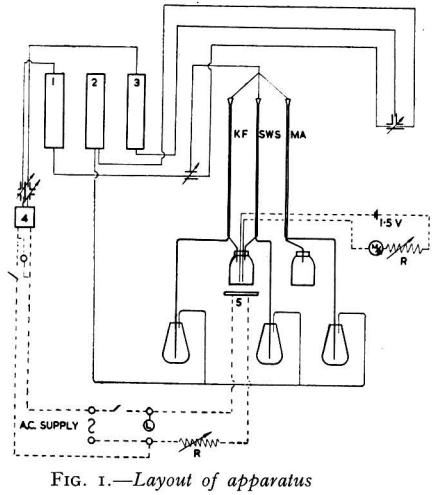

FIG. 1.—*Layout of apparatus*

1 Vacuum drying tube
2 Atmospheric pressure drying tube
3 Positive pressure drying tube
4 Compressor
5 Magnetic stirrer

The second balance required is that between KF and water. One drop of distilled water is weighed into a jar whose lid is fitted with a rubber washer, and excess KF is run in. The KF is back-titrated as described above, and a duplicate titration then carried out.

The third balance is that between MA and KF. Exactly 10 ml. of MA are treated with excess KF and back-titrated, in duplicate.

In an actual titration to determine water content, the water-containing extract is treated with excess KF and back-titrated with SWS as above. The water equivalent of the KF, and the KF equivalent of the SWS and MA are known from the standardisation ; the volume of KF actually used in titrating the water in the extract is therefore

total volume of KF added — (volume used in reacting with water in methanol and volume used in reacting with SWS on back-titration).

Since the water equivalent of the KF is known, the quantity of water in the extract, and hence the water content of the material under test, can be calculated.

(2) *Extraction techniques*

Initially, attempts were made to titrate the water in some materials directly. In most cases, low values were obtained, and in all cases results were erratic. Some form of extraction technique was, therefore, essential, and two methods were devised.

Method A

The material was heated with methanol in the standard jar, which was covered with a loosely fitting Bakelite cap. It was found that the mixture of methanol plus material could be safely heated until rapid boiling commenced, no appreciable amount of water being gained from or lost to the atmosphere. When rapid boiling occurred, some water was lost. A hotplate was employed for heating, set so that the mixture was just coming to the boil after heating for 5 min. ; this 5-min. period gave complete extraction of water from a considerable range of materials, especially those of a finely divided nature. Table I shows the effect of heating methanol/water mixtures (plus a little dry sand to promote boiling at the earliest possible moment) for varying times by method A. The quantity of water present was that which would ideally be present in a normal determination, i.e., equivalent to about 20 ml. of KF ; the quantity of methanol was 10 ml., the standard extraction quantity.

Method B

As in method A, the material was heated with methanol in the standard jar, but the jar was fitted with an air condenser. Here it was shown that extraction times of up to 30 min. would be quite safe. The results are detailed in Table II.

With either method of extraction, it is obvious that risk of loss of water depends on the rate of boiling. Vigorous boiling, even with an air condenser fitted, carries a risk of loss of water. The hotplate used was therefore modified by insulating half the heating surface with a hardboard sheet ; with a thermostat setting to give a hotplate temperature of 130°, the temperature on the insulated surface is 70°, i.e., not much higher than the boiling point of methanol (65°). The mixtures are brought to the boil on the hot surface, and then maintained at a gentle simmer for the rest of the extraction period on the insulated surface.

A useful guide as to the efficiency of the air condensers is that the loss of alcohol on heating for 60 min. should not exceed 0·25 g., i.e., 2·5% of the weight of alcohol.

Table I

Recoveries of water in methanol/water mixtures heated by Method A

Time of heating, min.	% Recovery of water			Mean recovery %
	Series 1	Series 2	Series 3	
0	100·0	99·6	99·7	99·7
2	100·0	100·7	100·3	100·3
4	100·0	100·7	100·3	100·3
6	101·0	101·0	100·8	100·9
8	101·0	99·6	99·4	100·0
10	101·0	99·6	100·3	100·3
15	98·2	99·0	98·6	98·6

Table II

Recoveries of water in methanol/water mixtures heated by Method B

Time of heating, min.	% Recovery of water Series						Mean recovery %
	1	2	3	4	5	6	
0	98·8	98·8	98·7	100·1	99·9	100·7	99·5
10	98·7	98·7	98·8	99·7	99·1	99·7	99·1
20	98·7	98·8	98·6	100·0	100·1	100·2	99·4
30	99·1	99·0	98·3	100·0	100·3	99·7	99·4
40	100·2	100·0	101·3	+	+	+	100·5
50	100·0	100·0	101·0	+	+	+	100·3
60	100·2	100·4	100·2	+	+	+	100·3

+ no observation made

Extraction equipment

(*a*) Hotplate: Townson and Mercer model X103 hotplate operating at 130 ± 2°, fitted with a hardboard sheet across half the surface, with a surface temperature on the sheet of 70–75°.

(*b*) Extraction vessels: standard 2 fl. oz. jars, transferred direct to the titration apparatus after extraction.

(*c*) Air condensers: specification given in Fig. 2.

(*d*) Glass sheets: microscope slides cut to 2·5 cm. × 1·9 cm.

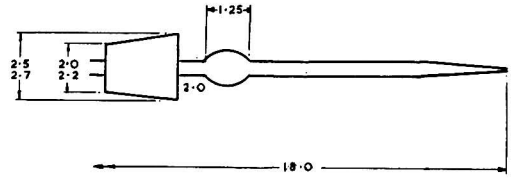

FIG. 2.—*Air condenser for extraction by method B*
Made up in 6 mm. o/d soda glass; dimensions in cm.

Application to confectionery materials

The first step was to devise methods of treating the materials for extraction so as to give a suitably large surface area. Suitable solids were ground in the usual way, in a mill, to a given mesh size; other, flexible solids (e.g., liquorice in sheet form) were extruded through metal rollers to give a thin sheet of constant weight per unit area. Pastes and viscous liquids were spread thinly on a glass sheet. Some materials, such as thin syrups, finely divided materials (e.g., flours) and materials soluble in methanol (e.g., fats) did not need any preliminary treatment.

Extraction curves for each material were then obtained by extracting samples for varying periods and plotting the apparent water content as ordinate against the time of extraction. The value at which the curve flattened out was taken as the water content. An extraction time was then set for the particular material by taking the time required for the curve to flatten, plus approximately 100% as a safety factor, subject to the proviso that extraction times by method A should not exceed 6 min., and by method B, 30 min.

A further check was applied in the case of 'difficult' materials such as cooked starch paste, gelatin etc. The extract was titrated immediately on completion of the extraction period, and then stirred for 3 min. with excess KF before retitrating. Since KF is much more hygroscopic than MA, two values of water content were thus obtained, and two curves plotted, which theoretically should have joined at the point of flattening out. In point of fact, with some materials, the two curves did not actually meet, the 'immediate titration' values remaining 0·1–0·2% below the other '3-min. stirring' values. For materials which hold water tenaciously, therefore, a 3-min. stir period with excess KF is specified before back-titration. It was established that this 3-min. stir period was sufficient to complete removal of water from the material.

Fig. 3 illustrates typical curves obtained by method B.

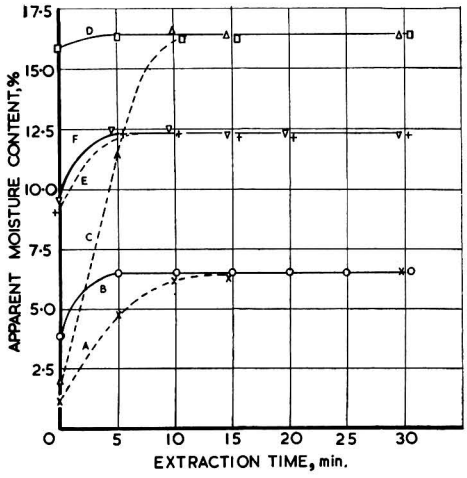

FIG. 3.—*Extraction curves by method B for some materials*

A Fudge, immediate titration
B Fudge, titration after 3-min. stir with excess KF
C Gelatin/sugar syrup, immediate titration
D Gelatin/sugar syrup, titration after 3-min. stir with excess KF
E Pectin, 60/80 mesh, immediate titration
F Pectin, 60/80 mesh, titration after 3-min stir with excess KF

Results

Tables III and IV give the experimental procedures for a range of raw materials, intermediate products and confectionery, based on extraction curves obtained by the methods detailed in the preceding paragraph.

Table V gives an indication of the significance of results obtained by the Karl Fischer method, with confidence limits for single and triplicate tests at 95% probability. The sample number in all cases was 10.

Table III

Methods for confectionery raw materials

Material	Preparation	Extraction method	Time, min.	3 min. stir KF	Approx. sample weight, g.
Sugar, granulated	None	A	5	No	5·0
,, raw	,,	A	5	,,	5·0
,, icing	,,	A	5	,,	5·0
Maltose	,,	A	5	,,	2·0
Dextrose	,,	A	5	,,	2·0
Thin sugar syrups	,,	A	5	,,	0·5
Glucose syrup	Thin film[a]	B	30	Yes	0·5
Caramel	,, ,, [a]	B	15	No	0·5
Treacles	,, ,, [a]	B	15	,,	0·5
Gelatin	+30 −40[c]	B	30	Yes	0·5
Gum arabic	+60 −80[b]	B	30	,,	0·5
Liquorice extract	+30 −40[b]	B	30	,,	0·5
Agar	+30 −40[c]	B	15	No	0·5
Pectin	+60 −80[c]	B	15	,,	0·5
Wheat flour	None	A	5	,,	0·5
Starch maize	,,	A	5	,,	0·5
Starch, acid treated	,,	A	5	,,	0·5
,, moulding	,,	A	5	,,	0·5
,, farina	,,	A	5	,,	0·5
Cocoa powder	,,	B	15	,,	2·0
Coconut, medium	,,	B	20	,,	2·0
,, fine	,,	B	10	,,	2·0
,, flour	,,	B	10	,,	2·0
Condensed milk	Thin film[a]	B	15	,,	0·5
Citric acid	None	A	5	,,	1·0
Fats soluble in methanol	,,	A	5	,,	2·0

[a] liquid spread as thin film on glass sheet
[b] ground by mill to give mesh size
[c] sample graded to give correct mesh size

Table IV

Methods for confectionery in process and finished confectionery

Material	Preparation	Extraction method	Time, min.	3 min. stir KF	Approx. sample weight, g.
Liquorice paste (undried)	Thin film[a]	B	20	Yes	0·3
Liquorice (dried)	Extruded into thin sheet	B	30	,,	0·5
Cream paste	Small pieces[b]	A	5	No	1·0
Fondant	,, ,, [b]	B	15	Yes (until dissolved)	1·0
Gelatin/sucrose syrup	Thin film[a]	B	15	Yes	0·5
Gelatin-based sweets before depositing	,, ,, [a]	B	15	Yes	0·5
Starch-based sweets before depositing	,, ,, [a]	B	15	,,	0·5
Gum arabic-based sweets before depositing	Thin film[a]	B	30	Yes	0·5
Boiled sweets	Broken into small pieces[b]	None	Dissolved in excess KF before back-titration		2·0
Fudge	Small pieces	B	30	Yes	1·0
Chocolate	Shavings by razor blade	A	5	,,	2·0
Fondants	Chopped by razor blade	B	15	Stirred until dissolved	1·0
Marshmallows	Chopped by razor blade	B	15	No	0·5
Pontefract cakes	Extruded into thin sheet	B	30	Yes	0·5
Lozenges	Ground by mill, +30 −40	B	10	No	2·0

[a] liquid spread as thin film on glass sheet
[b] broken into small pieces by hand

Table V

Significance of results (n = 10 in all cases)

Material	Average % water	Range	Std. deviation	Limits at 95% probability	
				Single test	Mean of triplicates
Sugar, raw	2·12	0·09	0·035	±0·07	±0·04
,, syrup	26·0	0·5	0·35	±0·7	±0·4
Treacle	18·5	0·3	0·09	±0·2	±0·1
Glucose syrup	18·4	0·6	0·23	±0·45	±0·26
Gelatin	16·7	0·4	0·13	±0·27	±0·16
Pectin	12·2	0·4	0·16	±0·32	±0·19
Wheat flour	14·4	0·25	0·07	±0·14	±0·08
Starch	9·3	0·6	0·16	±0·33	±0·19
Coconut	3·2	0·16	0·05	±0·10	±0·06
Cream paste	4·9	0·15	0·04	±0·03	±0·02
Lozenges	1·85	0·03	0·02	±0·05	±0·03
Boiled sweets	3·49	0·18	0·05	±0·10	±0·06
Chocolate	1·35	0·08	0·03	±0·06	±0·04
Marshmallows	18·5	0·4	0·22	±0·44	±0·25

Table VI shows the relationship between values obtained by the Karl Fischer method and by oven drying; the oven drying procedure in section 1 of the table was direct drying for 5 h. at 100°; in section 2, the material was dispersed on sand before drying for 16 h. at 100°. All values quoted are means of duplicate determinations.

Discussion and conclusions

The obvious advantages of the method are:

(*a*) Rapidity of test. Tests can be carried out on all the materials listed in this paper in 40 min. or less. Many materials take only 7 or 8 min.

(*b*) Reproducibility of results. The degree of precision indicated in Table V is adequate

for most purposes; greater sensitivity could be obtained (at the expense of some of the convenience of the method) if required. Generally speaking, a precision of ±1% of the water content, based on the mean of triplicate determinations, is quite possible.

(*c*) Specific nature. The method is not absolutely specific for water, but interfering substances (e.g., bases) are not common constituents of foodstuffs. There is a special advantage over oven drying when heat-sensitive materials, or materials with volatile components, are tested.

Table VI

Relationship between Karl Fischer and oven drying values of water content

Section 1			Section 2		
Material	Karl Fischer %	Oven drying %	Material	Karl Fischer %	Oven drying %
Raw sugar	2·1	2·1	Condensed milk	27·3	27·2
Coconut, fine	2·8	2·7	Fondants*	9·2	9·1
,, medium	3·0	3·0	Marshmallows*	18·5	16·4
,, flour	3·2	3·0	Boiled sweets*	4·0	3·0
Cocoa powder	4·6	4·3	Chocolate*	1·4	1·4
Starch (i)	6·5	6·0	Lozenges	1·9	4·9†
,, (ii)	9·7	8·7	Liquorice Allsorts	9·9	9·9
,, (iii)	10·6	9·6	Treacles (mean of 26)	20·9	22·7
Flour (i)	11·8	11·0	Cream paste (mean of 12)	6·0	5·7
,, (ii)	16·8	14·4			
Agar	18·2	16·4			

* Samples purchased from confectionery retailers
† This high value is due to the presence of volatile matter other than water

The question of whether the results obtained with the reagent are absolute is a difficult one, because of the lack of a general reference method; however, the evidence indicates that the results are at least as genuine as those obtained with oven drying. In this connexion, an examination of the results in Table VI will reveal the difference between comparative values by Karl Fischer and oven-drying methods. These fall into two sections:

(1) Where the oven drying was for 5 h. at 100°, a method applied to finely divided solids.
(2) Oven drying at 100° for 16 h., after dispersion of the material on sand.

In section (1) results for both methods are in good agreement for materials of low water content, but an increasing discrepancy is noted for materials of higher water content. As a matter of interest, materials dried in this manner can be titrated with the reagent, and an apparent residual water content, equal to the discrepancy, found. This suggests that the discrepancy is due to incomplete driving off of water in the oven.

In section (2) there does not appear to be any pattern in the discrepancies; good agreement is obtained with some materials but substantial differences with others. In the case of lozenges, the large difference is due to the presence of volatile material other than water, i.e., chloroform. Values for treacle by oven drying are much higher than Karl Fischer values, which suggests that some decomposition occurs on heating; and with the marshmallows, where the water is tenaciously held by the colloid components, oven drying appears to give low values.

Finally, some indication of possible sources of error in the light of experience, might be fitting. Possibly the greatest weakness of the method lies in its sensitivity, which means that only small sample weights can be tested of materials with a high water content. Precautions must be taken to prevent loss of water, especially when the material is to be spread as a thin film; these include the use of rubber-sealed jars and the weighing of material when cold or only just warm. Care in extraction is also necessary; the material must be prepared to the correct specification for surface area, and rapid boiling must be avoided. The reagents used are all hygroscopic, and must not be exposed to air for longer than is necessary. All equipment in contact with methanol must be dried by heating before re-use.

If any doubt exists about completeness of extraction, a useful check is to leave the extract for a few minutes in contact with excess KF after titration, and then retitrate.

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TREATMENT OF MEATS WITH IONISING RADIATIONS. IV.—Comparison of the Deterioration in Quality during Storage of Eviscerated Chicken Carcasses Treated with Chlortetracycline or Radiation*

By B. COLEBY, M. INGRAM, H. J. SHEPHERD and M. J. THORNLEY

Eviscerated chicken carcasses were treated with chlortetracycline (CTC) or irradiated with 0·3 or 0·6 Mrad, and then stored at 0°. Changes in quality during storage up to 26 days were studied, and microbial counts made on the carcasses. 0·6 Mrad caused a fairly rapid decline in quality, but with 0·3 Mrad or CTC deterioration of quality was only noticed when the carcasses had been stored at 0° for 19 days or longer. This loss of quality was not due to the growth of micro-organisms.

Introduction

In earlier papers it was shown that pasteurising doses of ionising radiation (up to 1 Mrad) considerably delay the microbial spoilage of eviscerated chicken carcasses stored at chill temperatures[1] but that deterioration of eating quality is apparent before that type of spoilage occurs.[2] The present experiments were made to determine if this deterioration is due to slow growth of the micro-organisms present, or is a consequence of irradiation, or whether it is a natural occurrence during storage of unfrozen chicken carcasses.

Eviscerated chicken carcasses can be stored at chill temperatures for only a short period (usually not more than about 12 days at 0°) before putrid smells develop, indicating microbial spoilage. Treatment with tetracycline antibiotics delays this spoilage, and enables quality

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changes to be studied over longer periods. In meats so treated and stored unwrapped in crushed ice, a progressive and eventually marked decline in flavour, which may be ascribed to leaching,[3] has been reported during storage for 18–19 days.[3–5] When the carcasses are wrapped in plastic films, however, and stored at similar temperatures, deterioration in flavour is slight or undetectable: Jacobson *et al.*[6] reported no deterioration by the fourteenth day of storage, and Baker[4] none by the nineteenth day, while Carlin *et al.*[7] reported only a slight decline during 10–11 days of storage. Both Baker[4] and Jacobson *et al.*[6] reported some deterioration in odour, appearance and 'feel' of the carcasses during storage. Several other papers (e.g. [8, 9]) have described the delay in the growth of micro-organisms on chicken carcasses treated with antibiotics. Most report agreement between bacterial counts and 'off' odour as criteria of spoilage, 'off' smells developing with counts between 10^7 and 10^8/cm.[2] On balance, it appears that only slight deterioration in quality might be expected during storage at chill temperatures for about 18–19 days in the absence of microbial spoilage, but published observations do not cover the storage period (15–40 days), which is of interest for the spoilage of irradiated chicken carcasses.[2]

In the present experiments, a comparison has been made between the progressive decline in quality of carcasses treated with chlortetracycline (CTC) or irradiated with 0·3 and 0·6 Mrad when stored for up to 26 days at 0°. Microbial counts have been made at intervals during this period.

Experimental

Chicken carcasses, of about $2\frac{1}{4}$ lb. eviscerated weight, were cooled in ice-slush, or in ice-slush containing 20 p.p.m. of CTC, and packed in sealed polyethylene bags, as described earlier.[2] Carcasses were transported in crushed ice; similar ones for controls were transported frozen at −75°, and stored subsequently at −20°.

Irradiation with ^{60}Co γ-rays took place at 1° ± 1° within 10–30 h. of slaughter of the chickens. The dose rate was about 86,000 rads/h., and the dose distribution throughout the carcasses was within 10% of the stated dose.

Quality assessment by an experienced laboratory panel was made in a manner similar to that of the earlier experiments.[2] Flavour assessments of the white and dark meat from roasted carcasses were made separately by 10 panel members, but as similar results were obtained in the two cases, the data have been combined for presentation in the tables. Except where indicated otherwise, all carcasses were frozen at −20° for a short period before assessment, to render them comparable in this respect with controls which had been stored frozen.

Microbiological examinations

These were made only on control and treated carcasses which had not been frozen at any stage in the process.

Samples were removed by Method 2 of Barnes & Shrimpton.[10] Weighed quantities of skin from under the wing and surface portions of tissue from around the vent and inside the visceral cavity were combined to form a 5-g. sample. This represented an area of approximately 50 cm.[2] of the original surface. The sample was homogenised with 45 ml. of 0·1% peptone water,[11] and this homogenate used as the first dilution. Serial dilutions were made in the same fluid. Volumes of 0·03 ml. of appropriate dilutions were spread on quarters of agar plates, while for very low counts, 0·5 ml. of the first dilution was spread over the whole surface of specially dried plates. Heart infusion agar (Difco) was used for counts at 20° and 0°. The medium developed by King *et al.*[12] to promote maximum fluorescein production was used at 0° only. Duplicate plates were used for each count, and incubation times were 3 days for the 20° count and 14 days for the 0° count. Plates of the medium of King *et al.*[12] were examined under ultra-violet light, and the fluorescent colonies counted. Previous experience had shown these to be pigmented strains of *Pseudomonas*.[1]

Results and discussion

The numbers of organisms counted after different periods of storage are shown graphically in Fig. 1.

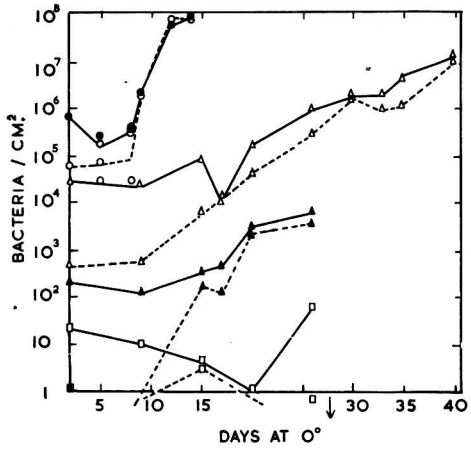

FIG. 1.—*Number of micro-organisms present on eviscerated chicken carcasses during storage at 0° after treatment with CTC or γ-radiation*

In the control samples the initial counts (2 days after slaughter) were 6×10^5/cm.² at 20° and 5×10^4/cm.² at 0°. The 0° count showed psychrophiles capable of multiplying on the carcasses at 0°, while the 20° count included these organisms, but also many more mesophiles originating from the gut and other sources. After storage of the carcasses at 0°, the 20° count decreased slightly, as expected, and after 8 days the number of psychrophiles increased rapidly, both counts then being the same. Putrid smells were noticed on the carcasses sampled after 12 and 14 days, with counts of 6×10^7 and 9×10^7/cm.² Pigmented *Pseudomonas* were present as a significant proportion of the flora in all but two of the control samples (Table I).

With CTC treatment, change in numbers was much slower, and after storage for 8 days, both 20° and 0° counts were nearly the same as the initial counts; in later samples a gradual increase in numbers took place. This is similar to other observations in the literature (e.g., Ayres *et al.*[13]). With two exceptions (carcasses stored for 17 and 30 days) the counts at 20° were appreciably higher than those at 0°. This was expected in the early stages, as in the control samples. In later samples, where multiplication had taken place, the difference was probably due largely to yeasts, which were included in the 20° count but grew too slowly at 0° to appear on the plates after 14 days. A small proportion of pigmented *Pseudomonas* was present in the later stages of storage, confirming the observations of Barnes & Shrimpton.[10] Definite ' off ' odours were observed after 30 days or more, but these differed from those of the controls in being sweeter and less offensive, and in developing more gradually.

After irradiation with 0·3 Mrad, the 20° count was 3×10^2/cm.²—only 0·04% of the control count—while the 0° count was less than 1/cm.²—less than 0·002% of the control. An increase in numbers was shown after 15 days, and continued till 26 days when storage was terminated. Counts were then 6×10^3/cm.² at 20° and 4×10^3 at 0°, and no ' off ' odour was observed.

Table I

Pigmented Pseudomonas (%) *in the colony count at 0°, for control and CTC-treated carcasses*

Control		CTC-treated	
Storage time at 0°, days	Pigmented *Pseudomonas* as % of 0° count	Storage time at 0°, days	Pigmented *Pseudomonas* as % of 0° count
2	3	2	—
5	39	9	—
5	+	15	—
8	—	17	—
8	—	20	—
9	53	26	—
12	21	30	1
14	9	33	6
		35	—
		40	1

+ = present but not counted — = absent

The higher dose of 0·6 Mrad gave an initial 20° count of 20/cm.^{2} and this declined during storage for 20 days, while the 0° plates indicated less than 1/cm.^{2} except for the 15-day sample with 3/cm.^{2}. Some multiplication had taken place after 26 days, for 75/cm.^{2} was indicated by the 20° count, although the 0° plates still showed less than 1/cm.^{2} Examination of the colonies showed these organisms to be largely yeasts. No ' off ' odours were present after 26 days.

Pigmented *Pseudomonas* were absent from all of the irradiated samples, during the storage period studied. This agrees with reports in the literature of their low radiation resistance.[14] The fact that far more of the psychrophilic (0°) than of the mesophilic (20°) organisms are destroyed explains why radiation produces a much greater proportionate increase in storage life if the carcasses are stored at low temperatures.[2]

For the present investigations, however, the important microbiological question was whether deterioration during storage was, or was not, caused by micro-organisms. In the two sets of irradiated carcasses, the microbial counts were below 10^4/cm.^{2} after 26 days at 0° and no ' off ' smells had been observed. It is thus unlikely that any flavour changes taking place during this period could have been due to microbial action (cf. Carlin[7]).

With CTC-treated carcasses, the 0° and 20° counts were $4{\cdot}2 \times 10^4$/cm.^{2} and $1{\cdot}7 \times 10^5$/cm.^{2}, respectively, after 20 days, and $3{\cdot}0 \times 10^5$/cm.^{2} and $9{\cdot}5 \times 10^5$/cm.^{2}, respectively, after 26 days. These are below the usually quoted level of 10^7–10^8/cm.^{2} for microbial spoilage, but since large numbers of yeasts are often observed on carcasses treated with CTC,[10, 13] and since yeasts have a metabolic activity per cell some 10–100 times greater than bacteria, some slight spoilage cannot be completely ruled out with counts in the region of 10^5/cm.^{2} It is worth noting that ' off ' odours were noticed after 30 days, when counts were only 2×10^6/cm.^{2}

The effect of freezing on quality

Fresh untreated chicken carcasses for comparison could not be stored in an unfrozen condition for the duration of this experiment (26 days), hence frozen carcasses were used as controls. To minimise any differences in quality due only to freezing (e.g., in texture) all treated carcasses were frozen at −20° for a short period before assessment for quality. Because of the possibility that assessment of frozen carcasses might differ from assessment of carcasses which had never been frozen, some direct comparisons were made by a laboratory taste panel of 10 members between frozen and unfrozen carcasses. Typical data are shown in Table II. These tests failed to reveal any significant differences due to the freezing of the carcasses (this relates strictly to the criterion here used, viz., the eating quality of a small portion of the cooked meat).

Appearance of chicken carcasses

Before being cooked, carcasses were ranked in order of preference for appearance by a small panel of experienced observers. The results are shown in Table III. Although the agreement

Table II

Effect of freezing on quality of chicken carcasses

Samples were ranked for preference and awarded a hedonic score[2]
R = average rank, S = average hedonic score, both for 20 judgments

Sample	R	S	Significance of ranking*
Carcass held at 0° for 5 days	1·45	7·4	n.s.†
Carcass stored at −20°	1·55	7·4	
Carcass held at 0° for 9 days	1·4	7·6	n.s.
Carcass stored at −20°	1·6	7·4	
Carcass held at 0° for 5 days after irradiation with 0·6 Mrad	1·3	7·3	
Carcass held at −20° for 5 days after irradiation with 0·6 Mrad	1·7	7·0	n.s.

* The statistical significance of ranking data in this and other tables was evaluated by the procedure of Kendall.[15]

† n.s. = not significant

Table III

Comparison of the appearance of irradiated or CTC-treated raw chicken carcasses after storage at 0°

Panel members ranked the thawed carcasses in order of preference

Storage time at 0° (days)	Average rank for					No. on panel	Significance of ranking
	Control	0·3 Mrad	0·6 Mrad	CTC	Stored control		
2	1·8	2·5	1·7	4·0	—	6	1%
9	1·6	2·85	3·85	1·7	—	7	1%
15	1·4	3·0	3·9	1·7	—	7	1%
16	2·0	3·3	2·2	2·5	—	6	n.s.
17	1·6	2·9	4·0	1·6	—	7	1%
18	1·9	2·9	1·7	3·5	—	7	5%
19	1·6	3·2	2·8	2·4	—	5	n.s.
20	2·2	3·2	4·5	1·6	3·5[a]	6	1%
26	1·2	4·0	5·0	2·0	2·8[b]	5	0·1%
Average rank for period 2–19 days	1·7	2·9	2·9	2·5	—		0·1%

[a] Stored at 0° for 5 days, then held at −20° for comparison
[b] Stored at 0° for 8 days, then held at −20° for comparison

between panel members was significant on most occasions, there was no consistent trend in the rankings after different periods of storage except that the frozen control was usually preferred. If the rankings over the period 2–19 days are averaged, a distinct trend is apparent: the frozen control was preferred, followed by the CTC treated, and then the irradiated carcasses, in that order. The carcasses irradiated with 0·3 and 0·6 Mrad were ranked almost equally. The slight pinkness in colour brought about by irradiation appears, therefore, to be a disadvantage, as was suggested earlier.[2]

Odour of raw chicken carcasses

A small laboratory panel ranked the carcasses in order of preference for odour before they were roasted, and the results are shown in Table IV. As with appearance, there is scarcely any trend in the rankings unless the results are averaged over the period 2–19 days, when the irradiated carcasses are clearly shown to be least preferred, though the panel members individually had not recorded the slight irradiation odour as sufficiently pronounced to be objectionable.

Flavour of roasted chicken carcasses

The carcasses were roasted as described earlier[2] and assessed by a laboratory panel. The results of assessment after storage for various periods at 0°, in Table V, show a deterioration in

Table IV

Comparison of odour of irradiated or CTC-treated raw chicken carcasses after storage at 0°

Panel members ranked the carcasses in order of preference

Storage time at 0°, days	Average rank for					No. on panel	Significance of ranking
	Control	0·3 Mrad	0·6 Mrad	CTC	Stored control		
2	1·7	2·7	3·0	2·6	—	7	n.s.
9	1·4	2·7	3·9	2·0	—	7	1%
15	1·3	3·1	3·9	1·7	—	7	1%
16	2·0	2·5	2·8	2·3	—	6	n.s.
17	1·4	3·3	3·4	1·9	—	7	1%
18	2·0	2·5	3·0	2·5	—	6	n.s.
19	2·0	3·0	2·6	2·4	—	5	n.s.
20	2·2	2·7	4·2	2·2	3·8[a]	6	5%
26	1·0	3·6	4·6	2·8	3·0[b]	5	1%
Average rank for period 2–19 days	1·7	2·8	3·2	2·2	—		0·1%

[a] Stored at 0° for 5 days, then held at −20° for comparison
[b] Stored at 0° for 8 days, then held at −20° for comparison

quality during storage. The deterioration in flavour of the irradiated carcasses confirms previous findings[2] and the data on CTC-treated carcasses extend the range of storage beyond that of previously published observations. The decline in average hedonic scores awarded during storage is shown in Fig. 2. The carcasses irradiated with 0·6 Mrad deteriorated most rapidly,

Table V

Effect of storage at 0° on flavour of irradiated or CTC-treated chicken carcasses after roasting

Samples were ranked for preference and awarded a hedonic score[2]
R = average rank, S = average hedonic score, both for 20 judgments

Storage time at 0°, days	Sample: Control		0·3 Mrad		0·6 Mrad		CTC		Stored control		Significance of ranking
	R	S	R	S	R	S	R	S	R	S	
2	2·3	6·9	2·7	6·6	2·4	6·9	2·6	6·6	—	—	n.s.
9	2·5	6·7	2·4	6·8	3·2	5·8*	1·9	6·8	—	—	5%
15	1·8	7·1	2·5	6·7	3·2	5·9**	2·5	6·5	—	—	1%
16	1·9	7·0	2·3	6·7	3·5	5·9**	2·3	6·9	—	—	1%
17	1·9	7·1	2·4	6·6	3·5	5·6**	2·2	6·8	—	—	1%
18	2·2	6·9	2·6	6·5	3·2	5·7**	2·0	6·8	—	—	5%
19	1·7	7·2	2·7	6·4*	3·4	5·7**	2·2	6·8	—	—	1%
20	1·6	7·1	3·5	6·0**	4·6	4·7***	2·7	6·1**	2·6[a]	6·5	1%
26	1·8	6·7	3·3	5·2**	4·7	3·8***	2·6	5·8*	2·6[b]	6·1	1%

* Difference in S, compared with control, significant at 5% level
** " " " " " " " " 1% "
*** " " " " " " " " 0·1% "
} evaluated using Student's t-distribution

[a] Stored at 0° for 5 days, then held at −20° for comparison
[b] Stored at 0° for 8 days, then held at −20° for comparison

being distinctly inferior after storage for only 9 days, and much worse after 26 days. Carcasses treated with 0·3 Mrad or CTC remained of high quality for longer periods, but by the 20th and 26th day of storage these, too, were significantly inferior to frozen control carcasses. That this deterioration was not due to microbiological spoilage in the case of the irradiated carcasses is clearly indicated by the microbial counts illustrated in Fig. 1. This conclusion was further strengthened by the inclusion in the quality assessment, after 20 and 26 days of storage, of untreated carcasses which had been stored at 0° for 5 and 8 days, respectively, before being held at −20°. The bacterial counts on such carcasses (before freezing) would exceed those of any of the irradiated carcasses, and yet these carcasses were awarded higher hedonic scores than any of the treated carcasses (Table V). Some possible microbiological spoilage cannot

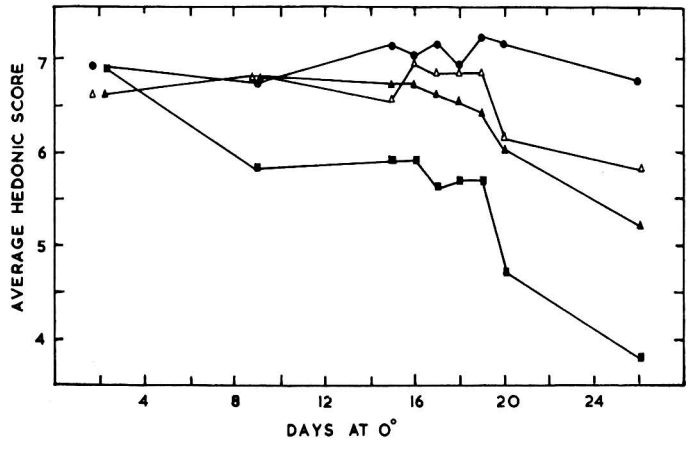

FIG. 2.—*Decline in the average hedonic score awarded to chicken carcasses which had been treated with CTC or radiation, and stored at 0° before roasting* (20 tastes were made on each carcass)

● Control (frozen) ▲ 0·3 Mrad △ CTC ■ 0·6 Mrad

be entirely discounted in the case of the CTC-treated carcasses after storage for 20 and 26 days, though the similarity in extent of the decline of quality of the CTC-treated carcasses and those irradiated with 0·3 Mrad suggests that the deterioration was non-microbial in origin.

Conclusions

Although irradiation with pasteurising doses (up to 1 Mrad) can considerably delay microbial spoilage of eviscerated chicken carcasses stored under chilled conditions,[1, 2] this is only achieved at the expense of accelerating the deterioration of the quality of the chicken during storage. With 0·3 Mrad, which will delay microbiological spoilage at 0° for about 30 days,[2] deterioration is scarcely accelerated, but nevertheless after storage for 19 days, decline in quality is apparent. This deterioration is probably an inherent characteristic of the carcasses, for those treated with CTC also lose quality after a similar period, and it is unlikely that treatment with antibiotics will predispose a carcass towards chemical deterioration in the same way as irradiation. As has been shown earlier,[2] the deterioration cannot be ascribed to rancidity. Therefore, it appears that carcasses cannot be held in an unfrozen state for more than about 18–19 days without losing quality, i.e., about half as long again as the period required for microbial spoilage of an untreated carcass. Storage for periods longer than this would require the halting of chemical or enzymic deterioration by freezing or other methods.

It is, perhaps, worth remarking that the slight, but statistically insignificant, preference which the taste panel showed for frozen carcasses, as against those treated with CTC or 0·3 Mrad, during the storage period 15–19 days (Fig. 2), might operate as a bias against irradiated chickens, in terms of large-scale consumer preference, even though the changes caused by irradiation were so slight as to be not obviously apparent. Similarly, the detectable changes in odour and colour caused by irradiation, though not objectionable, might produce adverse consumer discriminations.

Acknowledgments

Grateful acknowledgment is made to the Wantage Radiation Laboratories of the U.K. Atomic Energy Authority for providing γ-radiation facilities, and to the Buxted Chicken Co. for their co-operation.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

NOVEMBER, 1960

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

INDEX OF AUTHORS' NAMES

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

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NOVEMBER, 1960

I.—AGRICULTURE AND HORTICULTURE

General : Soils and Fertilisers

Morphology and genesis of the Caribou catena in New Brunswick, Canada. R. E. Wicklund and E. P. Whiteside (*Canad. J. Soil Sci.*, 1959, **39**, 222—234).—Field data and detailed chemical and physical data are recorded for four soil profiles representative of the sequence of the catena. The influence of drainage properties on soil characteristics is discussed. A. G. POLLARD.

Mineralogy of three soils of the Mississippi river alluvial plain. L. E. DeMumbrum and R. R. Bruce (*Soil Sci.*, 1960, **89**, 333—337).—The mineralogical composition of the silt and clay fractions of the soils is reported. The main materials found were montmorillonite, micas, kaolinite and quartz. T. G. MORRIS.

Rendzinas and red-brown soils on limestone : genetic inter-relationship. D. H. Khan (*J. Sci. Fd Agric.*, 1960, **11**, 477—484).—The chemistry of the soils, exchange status and composition, and the insol. silicate residue of the underlying limestone rocks were studied. The findings that the rendzinas have a high base status with no decalcification, and the red-brown soils are relatively more decalcified but maintain a high base status, are not in agreement with those of Robinson. (15 references.) E. M. J.

Mineralogical study of core samples from the Bearpaw formation. S. A. Forman and H. M. Rice (*Canad. J. Soil Sci.*, 1959, **39**, 178—184).—Details of deep borings (150 ft.) in this shale formation are recorded. A. G. POLLARD.

Occurrence of chlorite and vermiculite in the clay fraction of three British Columbia soils. A. A. Theisen, G. R. Webster and M. E. Harward (*Canad. J. Soil Sci.*, 1959, **39**, 244—251).—*X*-Ray diffraction methods showed chlorite to be the predominant mineral in these soils. Discrete units of vermiculite also occurred. The presence of kaolinite could not be confirmed as criteria commonly used for its detection in presence of chlorite proved unsatisfactory. A. G. POLLARD.

Profiles from major soil zones in Saskatchewan and Alberta. H. M. Rice, S. A. Forman and L. M. Patry (*Canad. J. Soil Sci.*, 1959, **39**, 165—177).—Chemical and mineralogical analyses of these soils are recorded. The dominant minerals were montmorillonite and illite with small proportions of kaolinite. A. G. POLLARD.

Mineralogical composition of sand in southern Ontario. C. I. Dell (*Canad. J. Soil Sci.*, 1959, **39**, 185—196).—An examination of the sand fraction of unweathered tills is described. A. G. POLLARD.

Stratification of soil constituents in a mountainous high moor. H. Daiber (*Z. PflErnähr. Düng.*, 1960, **89**, 55—61).—Marked sesquioxide, humus and phosphate stratification in newly laid drainage tiles is considered due to chelation by humus followed by subsequent destruction in the precipitation zone. M. LONG.

Clay minerals in a glei soil. I. Chemical analysis, differential thermal analysis and *X*-ray diffraction. II. Dehydration and rehydration. N. Uchiyama and Y. Onikura (*Tohoku J. agric. Res.*, 1959, **9**, 199—212, 213—236).—I. Extensive analytical data are recorded. The existence of mixed-layer clay minerals of the 2 : 1-lattice type is postulated.

II. Evidence for the occurrence of a mixed-layer type of hydrated halloysite is presented. Other mixed-layer minerals are also discussed. A. G. POLLARD.

Clay minerals in Carpathian flish soils. L. Pavel and S. Uziak (*Ann. Univ. Mariae Curie-Sklodowska*, 1958, **13, E**, 49—70).—In most Carpathian soils illite is the predominant mineral; it is accompanied by some montmorillonitic material. In brown acid soils mica replaces illite and montmorillonite. Kaolinite may occur in considerable amounts in mountain basins. (52 references.) A. G. POLLARD.

Classification and description of soil pores. W. M. Johnson, J. E. McClelland, S. B. McCaleb, R. Ulrich, W. G. Harper and T. B. Hutchings (*Soil Sci.*, 1960, **89**, 319—321).—A tentative classification of soil pores is presented. A descriptive nomenclature suggested relates to abundance, diameter, length or continuity, vertical orientation, location and shape. T. G. MORRIS.

Effect of tillage traffic on certain physical properties and crop yield on a Brookston clay soil. E. F. Bolton and J. W. Aylesworth (*Canad. J. Soil Sci.*, 1959, **39**, 98—102).—The influence of crop sequences and of different intensities of tillage operations on the % pore space of the soil was examined. No consistent deterioration of the physical condition of the soil or appreciable loss of crop yield could be ascribed to tillage practices. Yields were more affected by the nature of the preceding crop. A. G. POLLARD.

Effects of soil management practices on infiltration rates. L. H. Stolzy, T. E. Szuszkiewicz, M. J. Garber and R. B. Harding (*Soil Sci.*, 1960, **89**, 338—341).—Mature citrus orchard soils were treated by various combinations of irrigation methods, fumigation and mulching with sawdust or shavings. In practically all cases bulk density was highest in the 5—8 or 9—12 in. depths, probably due to compaction by cultivation. Infiltration was greater in untilled plots; mulching and fumigation had little effect on infiltration. T. G. MORRIS.

Energy dissipation during absorption and infiltration. II. J. R. Philip (*Soil Sci.*, 1960, **89**, 353—358).—A theoretical discussion following an earlier paper (*ibid.*, 1960, **89**, 132). T. G. MORRIS.

Rate of soil drainage following an irrigation. I. Nature of soil drainage curves. J. C. Wilcox (*Canad. J. Soil Sci.*, 1959, **39**, 107—119).—Mathematical relationships are established between time after irrigation and rate of fall of moisture content. A. G. POLLARD.

Apparatus for testing soil permeability. S. Zawadzki (*Ann. Univ. Mariae Curie-Sklodowska*, 1958, **13, E**, 71—78).—Soil samples, e.g. from a profile, are packed in their natural order in sections of a cylinder which can be united to reproduce the original profile. The upper section is fitted with a constant water level device, and the bottom section is a funnel for collecting the percolate. A. G. POLLARD.

Aggregate-size distribution and aggregate stability in Haldimand clay treated with four soil additives. L. R. Webber (*Canad. J. Soil Sci.*, 1959, **39**, 252—253).—Comparative data showing the effects of two synthetic polymers and two commercial waste products from the paper industry are recorded. The action of the polymers (HPAN, VAMA) diminished in the year following application and disappeared completely in six years. The other prep. showed more variable effects. A. G. POLLARD.

Effect of frost action on structure of Haldimand clay. D. E. Logsdail and L. R. Webber (*Canad. J. Soil Sci.*, 1959, **39**, 103—106).—Samples of a poorly aggregated soil cropped with maize for three successive seasons and another from a well-aggregated 10-year blue-grass sod were subjected to repeated freezing and thawing. The latter showed extensive breakdown which increased with the moisture content. The former soil was very little affected. A. G. POLLARD.

Porosity of soil aggregates. I. Determination of soil particle and pore volumes of soil aggregates. R. Sunkel (*Z. PflErnähr. Düng.*, 1960, **89**, 17—27).—By a method described using Hg, the vol. of aggregates and apparent sp. gr. are found by the dry wt./difference in vol. of Hg filling a vessel empty and that after the soil is placed therein. Temp. corrections are applied. Density of the soil particle is determined by the method of Zunker. M. LONG.

Effect of cropping systems and fertilisers on mean weight-diameter of aggregates of Breton plot soils. J. A. Toogood and D. L. Lynch (*Canad. J. Soil Sci.*, 1959, **39**, 151—156).—The mean wt.-diameter (*MWD*) of these soils under a five-year rotation of cereals and grass-legume mixtures was nearly double that in plots under a wheat-fallow rotation. Differences due to fertilisers were small. *MWD* was related to the polysaccharide content of the soils. A. G. POLLARD.

Influence of erosion on some properties of cretaceous rendzinas in the Lublin district. I. R. Turski (*Ann. Univ. Mariae Curie-Sklodowska*, 1958, **13, E**, 1—47).—In these soils loss of org. matter and of plant nutrients on sloping areas are greatest with types of sheet-erosion coupled with the destructive effects of cultural machinery. In sedimentation areas diminution in soil permeability is associated with decrease in no. of micro-organisms and changes in the distribution of species. A. G. POLLARD.

Water erosion of the cretaceous rendzinas on the anti-erosion field, Nowosiolki. B. Dobrzanski (*Ann. Univ. Mariae Curie-Sklodowska*, 1958, **13, E**, 79—113).—Physical and chemical data for profiles in the area are recorded. Water erosion is an important factor in the development of brown soil types; cretaceous rendzinas have been formed from the original podsolic soils. A. G. POLLARD.

Soils of the Agricultural Experimental Station, Elizowka : influence of water erosion. B. Dobrzanski, J. Borowiec and J. Gawlik (*Ann. Univ. Mariae Curie-Sklodowska*, 1958, **13, E,** 115—144).—A study, physical and chemical, of eroded loess soils. A. G. POLLARD.

Washing out of clay from the top layer of agricultural soils. J. Kohnlein (*Z. PflErnähr. Düng.*, 1960, **89,** 49—55).—Lysimeter trials confirm that there is a connection between carbonate levels and the washing out of clay. M. LONG.

Change of moisture tension with temperature and air pressure : theoretical. A. J. Peck (*Soil Sci.*, 1960, **89,** 303—310).—A theoretical discussion with particular reference to the bubbles of air trapped in the soil moisture of unsaturated soils and the effects on the bubble vol. of external air pressure, and of the change in surface tension of water with temp. The analysis derived allows the prediction of changes in moisture tension with external air pressure and of the vol. of air trapped in an unsaturated soil. T. G. MORRIS.

Concentrations of radon in subsoils of certain regions of the Lublin district. E. Trembaczowski (*Ann. Univ. Mariae Curie-Sklodowska*, 1958, **13, E,** 195—207).—Radon concn. were highest in clay and loess formations ($7–19 \times 10^{-10}$ curie/l.). Sandy and turf-covered areas contained smaller amounts. A. G. POLLARD.

Exchangeable and water-soluble potassium in soils and degree of saturation in relation to tomato yields. R. L. Halstead and H. B. Heeney (*Canad. J. Soil Sci.*, 1959, **39,** 129—135).—In various soil types examined exchangeable K (*E*), % saturation with K (*S*) and water-sol. K (*W*) were significantly correlated with yield response to K fertilisers in sandy loams but not in loams or clay-loams. In the modified Mitscherlich equation the "*c*" value for K based on *S* or *W* exceeded that based on *E*. Values of *E* and non-exchangeable K (sol. in N-HNO_3) increased and *W* decreased with rise in the clay content of the soil. *W* and *S* were significantly correlated in sandy loam and loam soils. A. G. POLLARD.

Effects of potassium chloride and dolomitic limestone on growth and ion uptake of sweet potato. W. A. Jackson and G. W. Thomas (*Soil Sci.*, 1960, **89,** 347—352).—Sweet potatoes were grown in a loamy sand with a cation-exchange capacity of 2 mequiv. per 100 g. K as KCl and dolomitic limestone were broadcast at rates of up to 468 and 2000 lb. per acre respectively. P and N were applied as row treatments. Leaching of K during the season was proportional to the rate of application, but was greatest early in the season. With the heaviest KCl treatment after 56 days the bulk of the K remained in the top 12 in. but most of the Cl was between 12 and 24 in. down. After 117 days the distribution of K was more uniform but Cl had moved down. After 199 days essentially all the Cl was below 30 in. Some exchangeable K still remained above this. Changes in Ca and Mg content were less marked than that of K. Mg was influenced more than Ca by the different rates of application of dolomite. Uptake of K by plant tops was increased by K treatment. Yields were increased by both K and lime; the yield of roots was much more dependent on K levels than was that of the tops. High rates of K decreased the Ca and Mg content of plants and in some cases Mg deficiency appeared. T. G. MORRIS.

Magnitudes and forms of phosphates retained in acid soils. J. C. Laverty (*Dissert. Abstr.*, 1960, **20,** 3491—3492).—The % fixation of P_2O_5 applied to 80 different soils was determined by comparing amounts of available P extracted from the treated and untreated soils by the Bray acid F method. Chang and Jackson's method for fractionating soil phosphates was shown statistically to be reasonably accurate and reproducible. Fe and Al phosphates in about equal amounts accounted for more than 80% of P applied to one soil. Amounts of Ca phosphate formed did not vary with soil pH in the range 5·0—7·4. Changes in Ca phosphate in soils were mainly due to unchanged fertiliser phosphates. The nature of the fertiliser phosphate had little effect on the amounts of Fe and Al phosphates formed, but did affect "saloid-bound" phosphate. Uptake of P by oat seedlings was the same from soils receiving different P fertilisers containing the same amount of citrate-sol. P. Increasing fertiliser content of NH_4 phosphate decreased (or in one case did not affect) the uptake of P. M. D. ANDERSON.

Effects of heavy applications of phosphate and lime on nutrient uptake, growth, freeze injury, and root distribution of grapefruit trees. W. F. Spencer (*Soil Sci.*, 1960, **89,** 311—318).—Grapefruit trees received N, K, Mg, Mn, Cu and Zn with differing amounts of P and limestone. After 5 years trees receiving P alone or with limestone were significantly smaller than those receiving lime only. Different rates of application of P or Ca had no effect. Frost injury was more severe in trees receiving P than in those without. Applications of P markedly reduced the concn. of feeder roots in the surface foot of soil, this being unaffected by Ca. High rates of P were more effective than low in reducing root concn. Leaf analyses indicated that superphosphate increased the uptake of P, Ca and Mn but decreased that of K, Mg, Fe, Ca, B and Al. Limestone increased the uptake of Ca and Fe and of P in unphosphated plots but decreased that of K, N, Mn, Mg, Cu, Zn and Al. Leaf-P and -Ca were greatly increased by the higher rates of applied superphosphate, -Ca being increased as much by superphosphate as by lime. Treatments which increased leaf -Ca decreased -Mg and -K. The effect of the treatments was the same on roots as on leaves. T. G. MORRIS.

Ammonium sulphate usage and the availability of soil phosphorus to citrus. D. Bouma (*Aust. J. agric. Res.*, 1960, **11,** 292—303).—Growth and P uptake of lemon cuttings and orange seedlings were better in soil without $(NH_4)_2SO_4$ and these plants reacted favourably to the application of phosphate. Superphosphate had very little effect if applied to soil from the high-$(NH_4)_2SO_4$ plots. A better response was obtained if applied to limed plots, or with a mixture of Ca and Mg silicate, or by using crushed phosphate. Soil analysis indicated that in the acid, high-$(NH_4)_2SO_4$ soil a greater proportion of soil P was present as Al phosphate. (11 references.) E. G. BRICKELL.

Effect of lime on availability of phosphorus on several Jordan plot soils. A. B. Awan (*Dissert. Abstr.*, 1960, **20,** 3459).—Of the four plots of different histories examined, the highest total P was in an acid soil from which little P had been removed by the low crop yields. Soil limed to pH 8·0 had less total P because of higher crop yields; org. P was increased. Liming soil of pH 5·1 increased the yield of tall fescue, but not root growth; liming soil of pH 4·3 increased both yield and root growth. The P and Ca contents of the grass were increased by liming. Of methods for determining available P the Na acetate method gave results correlated with yield and P uptake of fescue; the Truog and continuous leaching methods showed some correlation (not statistically significant), and both Bray methods gave negative correlation. M. D. ANDERSON.

Factors affecting the solubility of phosphates during the microbial decomposition of plant material. S. M. Bromfield (*Aust. J. agric. Res.*, 1960, **11,** 304—316).—Ferric phosphate and di- and tricalcium phosphates became more sol. when *Trifolium subterraneum*, L. was submerged and incubated under air or under N_2 but Nauru rock phosphate and $AlPO_4$ did not. $FePO_4$ was not dissolved during incubation under well-aerated, moist conditions but became more sol. as the degree of aeration decreased and as the amount of clover, $FePO_4$ or water in the system increased and in the presence of Fe and Al oxides. The pH of submerged clover incubated under N_2 decreased from about 6·0 to between 5·0 and 4·5 and remained at this level; under air the pH rapidly increased to values as high as 8·5. During decomposition under well-aerated, moist conditions the pH steadily increased from 6·0 to 8·5. (24 references.) E. G. BRICKELL.

Capillary movement of nitrate towards tropical soil surfaces. R. Wetselaar (*Nature, Lond.*, 1960, **186,** 572—573).—Increasing concn. of NO_3^- (from 38 to 230 p.p.m.), and also of Cl^- (950 to 3000 p.p.m.), in the top in. of soil (red loam) during the dry season following rains in N. Australia is ascribed to capillary movement. The max. concn. is determined by the base of the soil crust (0·5—0·75 in.) rather than by soil depth. W. J. BAKER.

Bacterial count, cellulose decomposition and the action of nitrogen on the decomposition process in moor soils and sand mixtures at different liming levels. W. Frercks, D. Puffe and H. Findeisen (*Z. PflErnähr. Düng.*, 1960, **89,** 27—42).—Bacterial counts increased with heavier liming; fungi decreased correspondingly. Nutrient solutions containing N greatly increased cellulose decomposition. Higher liming levels increased the intensity of decomposition of slightly decomposed high moor peats when peat, peat + cellulose, or peat + N additions were made. When peat, cellulose and N were added together, CO_2 production greatly increased, although higher N additions produced no further increase. The optimum N addition was in the range of normal practice. M. LONG.

Effect of trace elements on nitrogen fixation by *Azotobacter*. V. Iswaran and W. V. B. Sundara Rao (*Proc. Indian Acad. Sci.*, 1960, **51,** 103—115).—Mo, Co, Ni and Fe^{3+} stimulate N fixation when present in traces in a medium allowing growth of *Azotobacter* but increasing concn. of Pb brought about a gradual and regular decrease in activity. Combination of Co, Mo, Ni or Fe^{3+} gave lower fixations of N than those obtained by Mo alone, the latter giving a max. at 0·02 p.p.m. in Fred and Waksman's medium. There was definite evidence of absorption of Mo and Co in *Azotobacter* cells. (20 references.) E. G. BRICKELL.

Effects of DDT and malathion on forest soil microarthropods. R. C. Hartenstein (*J. econ. Ent.*, 1960, **53,** 357—362).—Application of DDT (1), or malathion (2 lb./acre) with Sovicide as a solvent did not affect the population-levels of beneficial soil mites. In a mixed

hardwood there was a temporary increase in the populations 15 weeks after treatment with DDT (10 lb. or 50 lb./acre). In a pine plantation DDT at 50 lb./acre gave reduced populations for the whole period. (19 references.) C. M. HARDWICK.

Effects of thiourea, ethyl urethane and some dithiocarbamate fungicides on nitrification in Fox sandy loam. R. P. Jaques, J. B. Robinson and F. E. Chase (*Canad. J. Soil Sci.*, 1959, **39**, 235—243).—Nitrification of $(NH_4)_2SO_4$ in this soil was inhibited by thiourea ($1{\cdot}6 \times 10^{-3}$), ethyl urethane ($1{\cdot}6 \times 10^{-2}$), manzate and zineb ($2{\cdot}1 \times 10^{-4}$) and by ferbam ($3{\cdot}5 \times 10^{-4}$ moles per kg. of soil) for varying periods up to 28 days. Inhibition of nitrification of NH_4 carbonate by the thiocarbamates was less severe than that of $(NH_4)_2SO_4$. Higher concn. of thiocarbamates ($2{\cdot}1 \times 10^{-3}$ moles per kg.) delayed nitrification for 150 days. Ferbam probably inhibits NO_2^--oxidising as well as NH_3-oxidising micro-organisms. A. G. POLLARD.

Stimulated decomposition of soil organic matter during the decomposition of added organic materials. N. J. Barrow (*Aust. J. agric. Res.*, 1960, **11**, 331—338).—Accumulation of NH_4^+ caused high pH which in turn increased the production of CO_2, SO_4^{2-} and mineral N from soil org. matter. (17 references.) E. G. BRICKELL.

Clay–humus complexes in a chernozemic and a podsol soil. J. E. Brydon and F. J. Sowden (*Canad. J. Soil Sci.*, 1959, **39**, 136—143).—Fractionation (Tyulin method) of the clay–humus complexes of a dark brown chernozem and a podsol is recorded. In all fractions montmorillonite, illite and small proportions of kaolinite were the principal minerals. Podsol colloids contained more "free" Fe_2O_3 and Al_2O_3 than did the chernozem colloids; in both soils the humate fraction contained more sesquioxides than did the KCl-floc fraction. Amino-acids were probably concerned in linking mineral colloids with the org. matter. A. G. POLLARD.

Effects of varying the nitrogen, sulphur and phosphorus content of organic matter on its decomposition. N. J. Barrow (*Aust. J. agric. Res.*, 1960, **11**, 317—330).—Mineralisation of N does not occur until respiration lowers the C/N ratio to ~5 and that of S does not occur until the C/S ratio is ~50. Mineralisation of P occurs before the C/P ratio is reduced to any consistent figure due possibly to a sub-optimal supply of N. (19 references.) E. G. BRICKELL.

Mulch influence on soil temperature and maize growth. W. C. Burrows (*Dissert. Abstr.*, 1960, **20**, 3451—3452).—In field experiments with maize, the average soil temp., at depths of 0·25, 2 and 4 in., was highest for bare soil, and decreased with each added increment of mulching material (0 to 8 tons per acre). Plant height, dry matter content, uptake of N, and rate of growth, decreased with increasing amounts of mulch. Heating the soil hastened emergence and attainment of maturity; plant height and dry matter content increased with average soil temp. N uptake was not affected by heating. Heating only during the early part of the season was as effective as heating all the season. Yield of grain was not related to soil temp. The effect of mulch in retaining soil moisture late in the season appears to counterbalance its effect in lowering soil temp. early in the season. M. D. ANDERSON.

Soil sulphate changes in the presence and absence of growing plants. J. R. Freney and K. Spencer (*Aust. J. agric. Res.*, 1960, **11**, 339—345).—Pot experiments with *Phalaris tuberosa*, L. are described. Mobilisation of the org. S occurred at the nil, 4, 12 and 36 p.p.m. levels of SO_4^{2-} S addition except with a lateritic krasnozem when only at nil and 4 p.p.m. was there any net release of SO_4^{2-}. The modifying effect of growing plants is of great significance in the cycling of S in the soil and is probably due to the activities of rhizosphere micro-organisms. (16 references.) E. G. BRICKELL.

Uptake of copper by summer cereals from copper-deficient soils. K. Scharrer and E. Schaumlöffel (*Z. PflErnähr. Düng.*, 1960, **89**, 1—17).—Higher uptake of Cu and higher crop yields result from applications of Cu powder (**I**) than of $CuSO_4$ to Cu-deficient soils. **I** has a residual effect. Cu availability is dependent on the sorption of the soil which, in turn, is related to the org. matter content, but fixation by humus does not occur. An application of Cu equivalent to 100 kg./ha. is adequate for slightly acid Cu-deficient soils. Cu availability is min. at pH 6. M. LONG.

Current New Jersey research in chemical soil testing. W. J. Hanna and R. L. Flannery (*J. agric. Fd Chem.*, 1960, **8**, 92—94).—A summary of problems under investigation at Rutgers University. (16 references.) M. D. ANDERSON.

Interpretation of soil tests and application as charted by current research. E. J. Kamprath and J. W. Fitts (*J. agric. Fd Chem.*, 1960, **8**, 94—96).—Problems encountered in interpreting the results of soil analyses are discussed. M. D. ANDERSON.

Chemical methods for determining available phosphorus and potassium in soils. J. R. Miller (*J. agric. Fd Chem.*, 1960, **8**, 87—91).—Determinations of P by six methods in 17 Maryland soils receiving superphosphate often showed little difference between methods in correlation with crop response of lucerne in pot culture. The method of Miller and Axley, employing 0·03N-H_2SO_4 and 0·03N-NH_4F as extractant, gave the closest correlation. The amounts of 20% superphosphate required to establish similar levels of chemically available P in different soils varied widely. Type of soil largely determined the correlation between chemically available P and crop yield, soils giving a poor correlation being usually high in extractable P, and showing little or no response to added P. When the soils did not receive superphosphate, the modified Truog method for determining P gave the closest correlation with crop response. On soils treated with rock phosphate, the Truog and other methods using acid extractants removed more P than was readily available to the crop in field experiments; maize, clover and wheat all gave higher yields with 75 lb. per acre of P_2O_5 when given as superphosphate than when given as rock phosphate. Yields of tobacco were closely correlated with soil P and K, extractable by the N. Carolina methods. The thermal method proposed by Kolterman and Truog showed promise for determining the relative capacity of soils to supply non-exchangeable K to plants. (20 references.) M. D. ANDERSON.

Relation of soil test values to fertiliser response by the potato. I. Nitrate production and crop yield. D. C. MacKay, C. R. MacEachern and R. F. Bishop (*Canad. J. Soil Sci.*, 1959, **39**, 144—150).—Production of NO_3^- in soil samples leached to remove existing NO_3^- was determined by incubation at 30° for 14 days. Values thus obtained were related to potato yields using Bray's modification of the Mitscherlich equation. Differences in "*c*" values between locations and varieties were significant. A. G. POLLARD.

Assessing nitrogen requirements of some Alberta soils. K. N. Synghal, J. A. Toogood and F. C. Bentley (*Canad. J. Soil Sci.*, 1959, **39**, 120—128).—Determinations of total N (Kjeldahl), available N (Truog) and "N value" (Munson and Stanford, *Proc. Amer. Soil Sci. Soc.*, 1955, **19**, 464) were of little value in predicting the N requirement of these soils. Crop response to added N was more closely correlated with the amount of NO_3^- accumulating in unleached soils incubated for 14 days at 28°. Preliminary leaching of the soil or prolongation of the incubation resulted in poorer correlation. A. G. POLLARD.

Ammoniacal liquor as a fertiliser. W. G. Foyer (*J. Inst. Sew. Purif.*, 1960, 92—96).—A review. O. M. WHITTON.

Isolation of urea–formaldehyde compounds and their decomposition in soil. M. I. E. Long and G. W. Winsor (*J. Sci. Fd Agric.*, 1960, **11**, 441—445).—The difficulties in preparing satisfactory slow-acting fertilisers consisting of mixtures of the various methylene-ureas condensed under acid conditions are discussed. Methylene-diurea is too rapidly decomposed in the soil and compounds containing more than four urea units in the chain are highly resistant to decomposition. In limed soil, a mixture of di- and tri-methylene-ureas with methylene-diurea or urea possesses some small reserve of slowly available N by virtue of the lag phase preceding their decomposition. Methylene-ureas prepared by condensation of urea with formaldehyde under acid conditions seem unlikely to possess the properties of a slow-acting fertiliser especially at pH values of horticultural soils. (17 references.) E. M. J.

Comparison of rock phosphate and superphosphate as sources of phosphorus on a black earth. K. Spencer (*J. Aust. Inst. agric. Sci.*, 1960, **26**, 63—67).—In three-year field experiments the effects of finely ground rock phosphate (4, 8 and 16 cwt./acre) and superphosphate (2, 4 and 8 cwt./acre) on subterranean clover were compared. On the basis of dry matter yields and P availability, the commercial use of rock phosphate was uneconomical. P. M. KINGSTON.

Penetration of radioactive superphosphate into a podsol soil. D. C. MacKay and J. B. Eaton (*Canad. J. Soil Sci.*, 1959, **39**, 215—221).—Superphosphate labelled with ^{32}P was incorporated in the surface 1 in. of soil. After 2 weeks only 5·2% of the P was located in the 2—6 in. layer of soil. Rainfall or irrigation >1 in., addition of limestone or of org. matter had little influence on the movement of P. A. G. POLLARD.

Waste sulphite liquor. I. Effect on crop yields and on soil properties. A. A. MacLean and J. J. Doyle. **II. Waste sulphite liquor as a soil aggregating agent.** J. J. Doyle and A. A. MacLean (*Canad. J. Soil Sci.*, 1959, **39**, 87—91, 92—97).—I. An NH_4^+-base sulphite applied at the rate of 2 tons/acre (providing N 120 lb./acre) increased yields and N contents of oats and Italian rye-grass to extents comparable with those obtained with 60 lb. of N/acre as $(NH_4)_2SO_4$. The liquor also increased the org. matter content, base-exchange

capacity, exchangeable H^+ and % of water-stable aggregates in the soil without altering the moisture equiv.

II. Aggregation of soils after treatment with the liquor (up to 1·6% of the soil on dry basis) increased to a max. in about 3 weeks, then declined somewhat but increased again to a second max. at 6 weeks. These variations were paralleled by changes in microbial activity as measured by loss of org. C from the soil. The aggregating action of the liquor was $\frac{1}{8}$ and $\frac{1}{36}$, respectively, of that of HPAN and VAMA. A. G. POLLARD.

Quantitative paper chromatography of inorganic ions in soils and plants. C. B. Coulson, R. I. Davies and C. Luna (*Analyst*, 1960, **85**, 203—207).—From an oxidised extract of the soil Cu, Co and Zn are extracted with dithizone in $CHCl_3$, and after addition of dimethylglyoxime the Ni complex is extracted with $CHCl_3$. The evaporation residue of the combined extracts is evaporated repeatedly with HCl, dissolved in HCl and from an aliquot the metals are separated by ascending paper chromatography with acetone–ethyl acetate–HCl as solvent. A rubeanic acid reagent is used to identify Cu, Ni and Co and 1-nitroso-2-naphthol to identify low concn. of Co. To determined Cu, Co and Ni a double-beam reflectance densitometer is used. Zn is determined colorimetrically. With slight modification the method is applied to plant material oxidised with H_2SO_4, HNO_3 and $HClO_4$. (10 references.)
A. O. JONES.

Determination of exchangeable cations in soils with the Beckman model B flame spectrophotometer. P. F. Pratt and G. R. Bradford (*Soil Sci.*, 1960, **89**, 342—346).—Methods are described for the elimination of interference in the flame photometric determination of cations in soils where wide ranges of anions and cations are found. Ion-exchange methods were used to remove anions other than Cl^- from the sample solutions. In determinations of Ca interference from Mg up to 400 p.p.m. was severe, but above 1500 p.p.m. it was small. 1700 p.p.m. of Mg was the most suitable level for Mg in both standards and unknown. With this addition of Mg the interference of either Na or K (up to 400 p.p.m.) in the determination of Ca was small. Interference by Ca in Mg determinations could not be eliminated and Ca was complexed with mannitol, the Mg precipitated with NaOH, collected and subsequently determined. Agreement with the oxalate method for Ca and the 8-hydroxyquinoline method for Mg was good. T. G. MORRIS.

An *in-situ* technique for the quantitative determination of fertiliser phosphorus in growing plants. M. H. Miller and C. H. E. Werkhoven (*Canad. J. Soil Sci.*, 1959, **39**, 205—214).—The plants are grown between a Plexi-glass sheet and a fibre-glass screen and supplied with ^{32}P. Two thin-walled Geiger-Müller tubes are coupled together and placed horizontally at a level about half the final height of the plants and close to the screen. The measured activity is corrected for the height of growth by use of regression equations determined separately. A. G. POLLARD.

Use of lanthanum chloride to prevent interference in the flame photometric determination of exchangeable calcium in soils. C. H. Williams (*Anal. chim. Acta*, 1960, **22**, 163—171).—Interference by Al^{3+}, PO_4^{3-}, SO_4^{2+} or SiO_3^{2-} has been prevented by the addition of La to the solution. E. G. CUMMINS.

Manometric determination of calcite and dolomite in soils and limestones. S. I. M. Skinner, R. L. Halstead and J. E. Brydon (*Canad. J. Soil Sci.*, 1959, **39**, 197—204).—The mixed calcite and dolomite is treated with excess of 4N-HCl at 25° with shaking and the CO_2 liberated is measured at intervals. The log of amount of CO_2 not liberated is plotted against time. Rapid decomposition of calcite is followed by slower release of CO_2 from dolomite. The graph approaches a straight line extrapolation of which to zero shows the amount of dolomite originally present: calcite is determined by difference from the total CO_2 obtained.
A. G. POLLARD.

Fertilisers from calcium nitrate and urea. Office National Industriel de l'Azote (B.P. 816,573, 27.6.55. Fr., 26.6.54).—$Ca(NO_3)_2$ impregnated with HNO_3 and containing ≮0·4 wt.-% of H_3PO_4 calculated as P_2O_5, which has been separated in the course of the production of complex fertilisers by the HNO_3 stabilisation of natural phosphates and the elimination of part of the Ca content as $Ca(NO_3)_2$, is treated with urea so as to form urea nitrate and urea phosphate. J. M. JACOBS.

White calcium cyanamide. Süddeutsche Kalkstickstoff-Werke A.-G. (Inventors: F. Kaess, H. Kronacher, H. Hoegner and W. Dichtl) (B.P. 814,534, 21.12.55).—In an improved method of making Ca cyanamide (white Nitrochalk), the initial chalk or lime in a state of fine division is reacted (in a vertical reactor) in presence of air with dry HCl or SO_2 at 600—900 (720—850)° until its $CaCl_2$ or $CaSO_4$ content reaches 0·8—1·5% by wt., and thereafter a (pre-heated) gas mixture containing HCN or CO and NH_3 is passed through the product in manner to keep the product in the fluidised state. Gas leaving the reactor during the conversion may be stripped of its CO_2 content before recirculation to the reactor by passing it through a suspension of lime. H. L. WHITEHEAD.

Plant Physiology, Nutrition and Biochemistry

Variations in respiratory responses in *Fusarium*-infected tomato plants. R. P. Scheffer (*Phytopathology*, 1960, **50**, 192—195).—Infection by *Fusarium oxysporum* f. *lycopersici* in tomato plants grown with low N supplies was followed by increased O_2 uptake by the leaves; the extent of the change was influenced by environmental conditions. No such effects occurred in leaves of plants given high levels of N. A. G. POLLARD.

Impairment of respiration, ion accumulation, and ion retention in root tissue treated with ribonuclease and EDTA. J. B. Hanson (*Plant Physiol.*, 1960, **35**, 372—379).—Treatment of soya-bean or maize root tips with RNA or EDTA or high concn. of KCl impaired respiration after 1—3 hr. Concurrently, cold-acid-sol. nucleotides were lost; polynucleotides began to degrade. RNA in membranes is implicated in ion accumulation, solute retention and oxidative phosphorylation but divalent ions normally protect it from degradation. Substitution by monovalent ions leads to the degradation of membranous RNA and the loss of physiological and biochemical functions. E. G. BRICKELL.

Germination of wheat at various levels of soil moisture as affected by applications of ammonium nitrate and muriate of potash. J. S. Chapin and F. W. Smith (*Soil Sci.*, 1960, **89**, 322—327).—Wheat seeds were germinated at 26° in soil treated with five rates of NH_4NO_3 or KCl (20—100 lb./acre) and maintained at different moisture levels (30—10%). At 30% moisture KCl had little retarding effect on germination; NH_4NO_3 delayed it but by the end of the germination period the difference was not significant. With 25% soil moisture KCl at the 60-, 80- and 100-lb. rates significantly reduced germination as also did the two heaviest rates of N. With decreasing soil moisture germination was delayed increasingly by the lower rates of application of N and K until at 10% moisture level all rates of K and the 20-lb. rate of N reduced germination significantly and all other N rates prevented it entirely.
T. G. MORRIS.

Effect of fertilisers and osmotic pressure on germination. S. Dubetz, R. L. Smith and G. C. Russell (*Canad. J. Soil Sci.*, 1959, **39**, 157—164).—Germination of various crop seeds was tested in iso-osmotic concn. of mannitol and aq. NH_4NO_3 in sand and also in soil to which various fertilisers were added. Germination was not significantly affected by moisture levels alone but N fertilisers reduced germination to extents which increased with falling moisture content and which were greater than those produced by iso-osmotic concn. of mannitol. Of seeds examined maize was the most and field beans the least susceptible to germination injury by fertilisers. Germination of sugar-beet diminished in solutions of osmotic pressure >4 atm.; toxicity of NO_3^- is indicated. A. G. POLLARD.

Factors affecting subsequent germination of cereal seeds sown in soils of sub-germination moisture content. H. A. H. Wallace (*Canad. J. Bot.*, 1960, **38**, 287—306).—Wheat seeds sown in soils too dry for germination sometimes decay and die, max. loss occurring at a moisture content about halfway between that in air-dry soil and that adequate for germination. Extent of loss is greater with wheat and rye than with oats and barley and varies with samples but is not affected by sterilising the "dry" soil; it is correlated with the extent of injury to seed coats by threshing, absence of hulls, sprouting, growth cracks and frost; damaged seeds become infected by spp. of *Penicillium*, *Aspergillus*, *Rhizopus* and *Mucor*; which prevent germination. Treatment of seeds with mercurials does not increase germination in moist soil, but triples it in "dry" soil; soaking the seeds in cold water for 4 hr. is as effective. No treatment completely prevents fungal infection of damaged seed sown in "dry" soil. A sound seed coat is the best protection against infection. (22 references.) M. D. ANDERSON.

Uptake of ions by plants growing in soil. T. W. Walker (*Soil Sci.*, 1960, **89**, 328—332).—A review and discussion. T. G. MORRIS.

Uptake of cations by storage tissues. R. M. Chasson (*Dissert. Abstr.*, 1960, **20**, 3483—3484).—Slices of plant storage tissues were slung from rods and alternately dipped into solutions containing radioisotopes, and lifted free, to ensure aeration and mixing. After uptake of radioisotope, slices were removed to solutions of stable isotope, to remove exchangeable radioisotope; they were then frozen, and sap was expressed. Slices which had lost all exchangeable ^{45}Ca to a stable Ca solution yielded large amounts of ^{45}Ca after the tissues had been killed and sap expressed. DNP stimulated

uptake of Ca by potato slices, and inhibited uptake of Rb; in carrot slices, DNP inhibited uptake of both Ca and Rb. In certain conditions, DNP diminished Ca in cell sap of potato, while increasing uptake into tissue. Evidence was obtained for the participation of mitochondria in the absorption of ions.
M. D. ANDERSON.

Differential absorption of metal chelate components by plant roots. L. O. Tiffin, J. C. Brown and R. W. Krauss (*Plant Physiol.*, 1960, **35,** 362—367).—Zinnia, sunflower and soya-bean plants were studied. Increase in chelating capacity was due to an increase in Fe-free ethylenediaminedi-(*o*-hydroxyphenylacetic acid) concomitant with Fe uptake by the roots. Probably these plants selectively absorb Fe, the EDDHA remaining, for the most part, in the nutrient medium. E. G. BRICKELL.

Effect of soil temperature and moisture on the uptake of phosphorus by oats. K. Simpson (*J. Sci. Fd Agric.*, 1960, **11,** 449—456).—In pot tests the effects of two levels of soil moisture, two temp., five rates of application of superphosphate to two soils, of high and low available P, were studied. An increase in temp. of ~5° increased uptake of soil P especially in soil of low-available P, increased uptake of fertiliser P from high-P soil and of total P from both soils. Lowering soil moisture tension to field capacity increased the uptake of fertiliser P from both soils. Yield was unaffected by superphosphate treatments of high-P soil despite high levels of uptake of fertiliser P. (10 references.) E. M. J.

Rôle of calcium in absorption of monovalent cations. L. Jacobson, D. P. Moore and R. J. Hannapel (*Plant Physiol.*, 1960, **35,** 352—358).—The effect of Ca on absorption is related to pH as well as to specific monovalent cations. Absorption of K, Rb and Cs is enhanced at low pH; Na only slightly. Li absorption is repressed almost completely by Ca at all pH values. The stimulating effect of Ca is considered to be essentially a blocking of interfering ions.
E. G. BRICKELL.

Physiological rôle of molybdenum in plants. E. I. Minina (*Dokl. Akad. Nauk SSSR*, 1960, **130,** 461—464).—The effect of Mo on lettuce growth was investigated on a heavy acid clay (pH 4·2, mobile Al content 4·3 mg. per 100 g.). Mo had a marked effect in the presence of NO_3^-, serving as an activator for oxidation–reduction processes and increasing sugar content in the carbohydrate-albumin exchange as a result of increased photosynthesis. (24 references.) E. SEMERE.

Temperature and ionising radiation effects on solute translocation in plants. R. H. Hodgson (*Dissert. Abstr.*, 1960, **20,** 3485).—Labelled CO_2 assimilated by bean leaves was translocated at a rate independent of petiole temp. Labelled sucrose supplied by "flap injection" was less mobile, and rate of translocation was more sensitive to petiole temp. The translocation of ^{32}P was more sensitive to petiole temp. when supplied by "flap injection" than by application of a spot to the leaf surface. Massive doses of ionising radiation to petiole and hypocotyl tissue did not affect the rate of translocation of ^{32}P, confirming the major part played by enucleate sieve cells in translocation. Doses to terminal buds much lessened the translocation of ^{32}P to them and to adjacent tissue.
M. D. ANDERSON.

Products of orthophosphate absorption by barley roots. P. C. Jackson and C. E. Hagen (*Plant Physiol.*, 1960, **35,** 326—332).—Orthophosphate was incorporated into five major compounds containing 80 to 90% of the total P absorbed in periods from 5 sec. to 2 hr. They were uridine diphosphate glucose, glucose-1-phosphate, inorg. orthophosphate and two unknowns, the first three being the earliest formed. E. G. BRICKELL.

Cold hardening and cold hardiness of young winter rye seedlings as affected by stage of development and temperature. J. E. Andrews (*Canad. J. Bot.*, 1960, **38,** 353—363).—Winter rye seedlings respond to cold hardening during sprouting in much the same way as that previously observed in winter wheat. Max. of cold hardiness in rye seedlings at 1·5° occurred (i) just after germination, when coleoptiles were 1 mm. long and roots 5 to 10 mm., and (ii) after 5 or 6 weeks of growth, when coleoptiles were 15 to 30 mm. long. Min. of cold hardiness occurred after about 1 week of growth. Genetic differences are best determined at the second max. of cold hardiness, exposing the seedlings to −14° for 16 hr. as a freezing test. Exposure to −4° much increased the cold hardiness of very young seedlings, whether or not they had been pre-hardened at 1·5°, but had little effect on seedlings showing max. hardiness after 5 weeks at 1·5°. M. D. ANDERSON.

Chromatographic analyses of the free amino-acids, organic acids and sugars in wheat plant extracts. B. S. Miller and T. Swain (*J. Sci. Fd Agric.*, 1960, **11,** 344—348).—Qual. and proximate quant. differences in the 80% ethanol-sol. amino-acid, org. acid, and sugar composition of three hard red winter wheat varieties differing in susceptibility to attack by hessian fly (*Mayetiola destructor*, Say) were examined at the fourth leaf stage. Eleven amino-acids, five org. acids and two inorg. acids were identified; seven sugars were found but two were unidentified. Allulose was found in extracts of the highly susceptible (Tenmarq) and semi-resistant (Ponca) but not in the resistant variety (C.I. 12855). Sorbitol was found in the Tenmarq extract but not in those of the other two varieties. No differences were observed in the amino-acid or org. acid composition of the three varieties. (33 references.)
E. M. J.

Tannins of *Acacia arabica*. I. Fractional extraction and paper-chromatographic examination of the fruit. H. Endres, H. El Sissi and M. Hilal (*Egypt. J. Chem.*, 1959, **2,** 375—384).—Anaerobic extraction of the fruits with ether, ethanol, ethyl acetate and water followed by chromatography showed the presence of condensed tannins containing mixtures of 30—35 different phenolic compounds. A. G. POLLARD.

(2-Chloroethyl)trimethylammonium chloride and related compounds as plant growth substances. II. Effect on growth of wheat. N. E. Tolbert (*Plant Physiol.*, 1960, **35,** 380—385).—Compounds of the structure $(CH_3)_3N^+CH_2{\cdot}CH_2X$ were active as plant growth substances when X was Cl, Br or $=CH_2$. The most characteristic growth alterations were shorter and thicker stems, broader and greener leaves, earlier and stronger tillering, and more uniform growth. They occurred without a change in wet or dry wt. and were similar to those produced by high light intensity and the opposite from those caused by gibberellin. E. G. BRICKELL.

Differential uptake of 2,4-D acid and its octyl ester by seedling maize roots and coleoptile sections. D. J. Morre and B. J. Rogers (*Plant Physiol.*, 1960, **35,** 324—325).—Probably the inherent toxicity of 2,4-D is not changed by esterification but a large difference was found for the concn. necessary for 50% inhibition of root growth (for 2,4-D, 4·5 and for the octyl ester 9·7 mg./l.).
E. G. BRICKELL.

Physiological effects of gibberellic acid. I. On carbohydrate metabolism and amylase activity of barley endosperm. L. G. Paleg (*Plant Physiol.*, 1960, **35,** 293—299).—Concn. of gibberellic acid (**I**) from 2×10^{-4} μg. to 2×10^{2} μg. per 3 ml. of solution were active in producing increasing amounts of both maltose and glucose from the endosperm. At pH 5·5, 0·01M-acetate and -citrate buffers inhibit the effect. Production of reducing sugars in an amylase assay was greatly increased when endosperm was pretreated with **I**, providing evidence that **I** increases its water-sol., amylolytic activity.
E. G. BRICKELL.

Effect of gibberellic acid and photoperiod on indoleacetic acid oxidase in *Lupinus albus*, L. R. Watanabe and R. E. Stutz (*Plant Physiol.*, 1960, **35,** 359—361).—Application of 50 μg. of gibberellic acid to terminal buds of lupin plants in 5 μg. aliquots decreased the IAA oxidase activity in bud and stem tissues but had no significant effect in leaves and hypocotyls. Treatment increased the length of the main axis and the no. of visible nodes. Increased photoperiod reduces IAA oxidase activity in stem tissues but had the opposite effect in bud tissue. Activity in leaves and hypocotyls was unaffected. E. G. BRICKELL.

Net assimilation rate and growth behaviour of beans as affected by gibberellic acid, urea and sugar sprays. P. de T. Alvim (*Plant Physiol.*, 1960, **35,** 285—288).—Gibberellic acid increased net assimilation rate, relative growth rate, stem dry wt., leaf area and plant height; root dry wt. was reduced and leaf dry wt. not significantly altered. The reduction in root dry wt. induced by gibberellic acid could be effectively controlled by 10% sucrose and stem elongation was increased by urea. Both sugar and gibberellic acid were effective in protecting against injury by 2% urea spray.
E. G. BRICKELL.

Gibberellic acid. XI. Growth-promoting activities of functional derivatives of gibberellic acid. J. S. Moffatt and M. Radley (*J. Sci. Fd Agric.*, 1960, **11,** 386—390).—In general salts (11) and acyl deriv. (4) showed activities similar to that of gibberellic acid when applied to the leaves or roots of dwarf pea seedlings. Esters (5) and acyl deriv. of esters (9) had no significant activity when applied to the leaves in ethanol, but showed moderate to high activity when applied in aq. solution to the roots. E. M. J.

Promotion of lettuce seed germination by gibberellin. A. Kahn (*Plant Physiol.*, 1960, **35,** 333—339).—Gibberellin is capable of replacing red light in every situation examined in which light promotes germination and brief irradiances with far-red light do not negate this promotion although they may lead to delay.
E. G. BRICKELL.

Shoot histogenesis: sub-apical meristematic activity in a caulescent plant and the action of gibberellic acid and Amo-1618. R. M. Sachs, A. Lang, C. F. Bretz and J. Roach (*Amer. J. Bot.*, 1960, **47,** 260—266).—The action of gibberellins (**I**) on shoot development in

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Chrysanthemum morifolium results from its ability to regulate sub-apical meristematic activity. Amo-1618 [(4-hydroxy-5-isopropyl-2-methylphenyl)trimethylammonium chloride 1-piperidine carboxylate] is antagonistic to **I** in respect of meristematic activity and exerts a dwarfing effect on the plant. A. G. POLLARD.

Recovery of gibberellins. C. Pfizer & Co. Inc. (B.P. 819,110, 20.9.57. U.S., 2.7.57).—Gibberellins are recovered from filtered fermentation broth containing them by extracting (at pH 2—3·5) with a water-immiscible org. solvent, viz., EtOAc, COMeEt or COMeBu^t, preferably in a countercurrent process, then concentrating the extract, filtering off pptd. impurities, and either crystallising gibberellins from the concentrate or precipitating them with Ph acetate. F. R. BASFORD.

Crops and Cropping

Quality of plant foods. II. Influence of variety and environment on the riboflavin content of hulled rice with special reference to its effective factors. K. Sone (*Tohoku J. agric Res.*, 1959, **9**, 179—197).—Environmental conditions affected the riboflavin content of hulled rice more on a peaty soil than on a clay loam. Varietal differences in this respect were considerable as also were the effects of fungicides; fertiliser treatment was a less influential factor. A. G. POLLARD.

Paddy soils and rice production. H. Greene (*Nature, Lond.*, 1960, **186**, 511—513).—Mainly a review of literature. Wet paddy soils in Japan occur on low-lying recent alluvium near the permanent water table and are characterised by a leached and denitrified, reduced gley horizon with a Mn–Fe pan at the base. They include fertile well-drained soils and infertile (degraded) types poor in Fe or with excessive Al. Soil chemistry (including the redox balance at different seasons and different parts of the profile) and use of fertilisers to attain optimum conditions for rice growth are summarised. (38 references.) W. J. BAKER.

Effect of nitrogen, phosphorus and potassium concentrations on the development of *Gibberella* stalk- and root-rot of maize. P. Thayer and L. E. Williams (*Phytopathology*, 1960, **50**, 212—214).—Development of stalk-and root-rots in maize was diminished by increase in P concn. of the nutrient. Variations in N and K supply had no effect on root-rot but produced positive or negative responses in stalk-rot which were dependent on the relative levels of supply of the two nutrients. A. G. POLLARD.

Yield and size distribution of potatoes as influenced by seed rate. A. J. Reestman and C. T. De Wit (*Netherlands J. agric. Sci.*, 1959, **7**, 257—268).—In two-year field experiments smaller size seed gave a heavier crop owing to a greater relative skin surface area. With larger seed, a greater no. of stems per potato resulted in a lowering of yield. A theoretical relationship between yield and plant spacing is postulated. P. M. KINGSTON.

Production and use of grass. Symp. Dublin and Dist. Sect. Agric. Group, V sessions. (*Soc. chem. Ind. Monogr.*, 1960, No. 9).—The symposium comprised 16 papers: (i) Use of fertilisers on grassland. G. W. Cooke, pp. 5—18 (14 references); Manuring and conservation with intensive grassland. J. A. Collier, pp. 19–34; Conditions in which nitrogen fertilisers may be used in pastures without affecting health of animals. A. Voisin, pp. 35—43. (ii) Utilisation of ground rock phosphate by different species of pasture plants. M. Neenan, pp. 47—54; Effects of fertiliser treatments and cutting managements on yield and quality of grass and clover swards. M. E. Castle and D. Reid, pp. 55—68 (14 references); Effect of organic and inorganic nitrogenous compounds on yield, chemical composition and botanical constitution of grass swards. S. McConaghy, J. S. V. McAllister, J. Lowe and P. A. Linehan, pp. 69—88; Legumes and herbage production. T. W. Walker, pp. 89—101 (22 references). (iii) Response of grass–clover mixtures to lime and sulphate of ammonia. P. Bergin, pp. 105—116; Chemistry of silage making. W. S. Ferguson, pp. 117—124; Effect of growth-regulating weed-killers on the botanical composition and productivity of permanent grassland. R. S. L. Jeater, pp. 125—132; Chemical composition of peat supporting *Schoenus nigricans* in north Mayo. P. J. O'Hare and G. A. Fleming, pp. 133—145; Mineral composition of pasture in relation to animal health and production. I. Hypomagnesaemia in cattle and sheep. II. Delayed breeding in cows and heifers. L. B. O'Moore, pp. 146—164. (iv) Measurement of quantities of herbage consumed by grazing animals. J. L. Corbett and J. F. D. Greenhalgh, pp. 167—180 (50 references); Herbage composition and nutritive value. W. F. Raymond, J. M. A. Tilley, R. E. Deriaz and D. J. Minson, pp. 181—190 (42 references); Grass, nitrogen and metabolism of the dairy cow. M. J. Head, pp. 191—201; Physical and chemical aspects of digestion of grass and grassland products by the ruminant. A. M. Frens, pp. 202—217. (v) General discussion, pp. 221—228. E. M. J.

Effects of nitrogen and potassium fertilisers on the mineral status of perennial ryegrass (*Lolium perenne*). I. Mineral content. II. Anion–cation relationships. H. Rahman, P. McDonald and K. Simpson (*J. Sci. Fd Agric.*, 1960, **11**, 422—428, 429—432).—I. The effects of NH_4NO_3 (**I**) applied at two levels and K_2SO_4 (**II**) at one level on the N, Ca, Mg, Na, K, P, S and Cl contents of *L. perenne* grown pure and with clover were studied, three cuts over two seasons being made. **I** increased the % of N (chiefly non-protein N), Na, P, K, at all cuts, Mg at the third cut, and decreased % of Cl. Without **I** N content was higher in *L. perenne* grown with clover. **II** increased K, Cl contents and decreased Ca, Na at all cuts, decreased non-protein N generally and Mg at the first cut. The presence of clover increased K and decreased S contents. (15 references.)

II. Alkali alkalinity (AA), total alkalinity (TA), base excess (VA) and the K/(Ca + Mg) ratio were increased by N and K fertilisers. Many negative alkaline earth alkalinity (EA) values were obtained. In samples of herbage taken from "tetany" farms none of the K/(Ca + Mg) ratios approached 2·2, but most were characterised by high AA values. The (K + Ca)/Mg ratios gave a better indication of tetany-inducing herbage. E. M. J.

Effect of salt and other fertilisers on yield and mineral composition of forage crops. I. Turnips. II. Kale. R. G. Hemingway (*J. Sci. Fd Agric.*, 1960, **11**, 349—355, 355—362).—I. The literature on the influence of Na on P utilisation and replacing of K by Na in the nutrition of Brassica crops is reviewed. NaCl increased the yield of turnips by greater amounts than did KCl. Uptake of Na was decreased by KCl and increased (~50%) by NaCl. NaCl improved P uptake and utilisation by turnips on soils of low-P status in absence of superphosphate. (31 references.)

II. NaCl increased the yield of kale on soils not unduly deficient in K. Negative K–NaCl interactions may be indicative of greater responses from NaCl on soils of lower K status. NaCl and KCl depress the uptake of Ca (by 0·1%) and of Mg (by 0·01%), not materially affecting the food value of kale for animals. NaCl did not increase P utilisation although the soils were P-deficient. NaCl and $(NH_4)_2SO_4$ increased Na uptake, KCl reduced it. The Na content of crops varies within wide limits relative to those of K, P, Ca and Mg. E. M. J.

Relationships between chlorosis in fruit trees and soil-pH. M. J. Liwerant (*C. R. Acad. Agric. Fr.*, 1960, **46**, 352—358).—Observations in several orchards show that peach and pear trees can resist chlorosis in spite of low active soil-Ca (<1—3%) provided that the soil-pH is >7·7—7·9 and 8·1, respectively. P. S. ARUP.

Magnesium nutrition of *Nicotiana tabacum* in relation to multiplication of tobacco mosaic virus. R. J. Shepherd and G. S. Pound (*Phytopathology*, 1960, **50**, 195—198).—The concn. of virus in infected tobacco plants was lower in Mg-deficiency than with normal Mg nutrition. A. G. POLLARD.

Effect of modified atmospheres and various temperatures during storage on the respiration rates, colour indices and keeping quality of Better Times roses. T. E. Pope (*Dissert. Abstr.*, 1960, **20**, 3463—3464).—The most satisfactory storage conditions for roses after cutting were: holding the flowers with stems in water at 50°F for 12 hr.; packing in sealed Cellothene bags in normal air; and storing at 32°F for not more than 12 days. Modified atm. did not increase storage life, and had undesirable effects. Quality was better assessed by visual observation than by measuring changes in flower colour. M. D. ANDERSON.

Pest Control

Apparatus for insecticide assay. C. C. Hassett, R. Kirk and G. B. Craig (*J. econ. Ent.*, 1960, **53**, 483).—Modifications of the apparatus used by Burchfield *et al.* (*Contr. Boyce Thompson Inst.*, 1952, **17**, 57) based on a negative photomigration test are described and illustrated. C. M. HARDWICK.

Use of an animal membrane in the evaluation of chemical repellants against the stable fly. P. Granett (*J. econ. Ent.*, 1960, **53**, 432—435).—Repellancy was determined by the no. of caged flies that did not feed through a membrane of which the untreated side was in contact with warm beef blood. A fluorescent dye added to the blood aided recognition of those flies which had fed. The surface to which the chemical was applied affected the results; membranes gave results closer to those on cattle than did cheesecloth. C. M. HARDWICK.

***O*-Ethyl *S*-2-(ethylthio)ethyl alkylphosphonothioates as systemic insecticides.** R. L. Metcalf and T. R. Fukuto (*J. econ. Ent.*, 1960, **53**, 127—130).—The importance of optical activity in the indicated

compounds is stressed; the L-isomer is six to ten times as toxic to the insects as the D-. Contact toxicity and cholinesterase inhibition were examined. With *Musca domestica* the toxicity decreased with increase of the alkylphosphonate group, methyl > ethyl > propyl > isopentyl, and similar findings are recorded for *O*-ethyl *O*-*p*-nitrophenyl alkylphosphonate. With the phosphonothionate esters, the LD_{50} values are not informative. Systemic activity is evaluated on the cotton plant by topical application to the stem or as a 50% charcoal powder to the seeds, *Tetranychus telarius*, *Heliothrips haemorrhoidalis* and *Buccalatrix thurberella* being used as test organisms. With the ethyl phosphonates the thiol isomer was markedly superior to the thiono while the thiono-thiol was specially active in seed treatment. Compounds resulting in the highest anticholinesterase activity in leaves are the most active systemic insecticides. (13 references.) C. V.

Persistence of Dimethoate and metabolites following foliar application to plants. W. C. Dauterman, G. B. Viado, J. E. Casida and R. D. O'Brien (*J. agric. Fd Chem.*, 1960, **8**, 115—119).—The systemic insecticide Dimethoate (**I**), labelled with ^{32}P, was applied to the leaves of maize, cotton, pea and potato plants; it was rapidly absorbed, and was decomposed both on the surface and inside the tissues by oxidation and hydrolysis. Only trace amounts of **I** and its oxygen analogue were found 32 days after application. Of the five hydrolysis products identified, the predominant one from peas was phosphoric acid, and from the other plants *OO*-dimethyl *S*-carboxymethyl phosphorothiolate on the surface, and *O*-methyl *O*-hydrogen *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate within the leaf tissue. (14 references.) M. D. ANDERSON.

Determination of dieldrin. E. A. Baker and E. J. Skerrett (*Analyst*, 1960, **85**, 184—187).—Benzene washings of the sprayed surfaces are mixed with lime and acetic anhydride and passed through an Al_2O_3 column which is eluted with benzene. The evaporated eluate is heated at 100° with acetic anhydride containing H_2SO_4. The product is shaken with benzene and water, the residue from evaporation of the washed benzene layer is dissolved in ethanol and heated with 2,4-dinitrophenylhydrazine in H_2SO_4. To the washed solution of this product ethanol and a drop of tetraethylammonium hydroxide solution are added and the extinction is measured at 440 mμ and referred to a calibration graph. Recovery from spray target papers is good. From mangold leaves recovery is reasonably good provided a high blank value can be tolerated.
A. O. JONES.

Determination of *OO*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate in technical Rogor and its formulations. L. F. Dupée, K. Gardner and P. Newton (*Analyst*, 1960, **85**, 177—184).—The solution of *OO*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate (Dimethoate) in diethyl ether is passed through a column of Hyflo Super-Cel which is eluted with a diisopropyl ether–light petroleum mixture. Separate fractions of the eluate, after removal of the solvent, are heated to fuming with $HClO_4$ and HNO_3, diluted with water and boiled. NH_4^+ vanadate is added and the extinction is measured at 470 mμ and referred to a calibration graph prepared with KH_2PO_4. Alternatively, with samples free from interfering substances, the aq. extract of the combined fractions of the eluate is treated with a $KBrO_3$–KBr reagent and HCl, KI is added and the liberated I_2 is titrated.
A. O. JONES.

Systemic disease control by 2-pyridinethiol-1-oxide and its derivatives. S. I. Cohen, J. H. Reinhart and S. S. Ristich (*Phytopathology*, 1960, **50**, 239).—Promising results are reported in the control of leaf and root diseases in cucumber and tomato by use of 2-pyridinethiol-1-oxide derivatives (notably Zn salt and disulphide) as a systemic fungicide applied as a spray (especially to the undersides of leaves) or as a soil drench. A. G. POLLARD.

Toxicity of cellulose acetate sheeting to leguminous plants. R. W. Kieckhefer and J. T. Medler (*J. econ. Ent.*, 1960, **53**, 484).—The toxic constituent of cellulose acetate sheet which killed goldfish within 3 days was a phthalate. None was present in vinyl sheeting and this had no toxic action. C. M. HARDWICK.

Measuring the surface area of apples for expression of pesticide residues. R. J. Daum and J. E. Dewey (*J. econ. Ent.*, 1960, **53**, 462—467).—Projection of the surface of an apple on a plan surface and its measurement or weighing gave errors of <0·5%. The error of the vol. displacement method differed with variety, as these deviated from a sphere but was likely to be <1%. The variation in sp. gr. due to fruit development, and that between seasons caused error in surface area determinations by wt. Values by the displacement and wt. methods may differ by 4% from the planimeter value. Diameter measurements should be the max. from calyx to end of stem. Sample size and other causes of variation are discussed. (16 references.) C. M. HARDWICK.

Parathion and EPN residue studies on Concord grapes. E. F. Taschenberg and A. W. Avens (*J. econ. Ent.*, 1960, **53**, 441—445).—The reduction of residues for 3 weeks after spraying was determined. Interference with determination of residues was related to the amount of methyl anthranilate present; this was dependent on variety and state of maturity. Two washings with 10% HCl removed up to 86% of this. EPN residues decreased more slowly than those of parathion. The presence of $Ca(OH)_2$ did not increase residue losses. (21 references.) C. M. HARDWICK.

Residues of Sevin and 1-naphthol on forage from aerial applications of Sevin in oil. E. W. Huddleston and G. G. Gyrisco (*J. econ. Ent.*, 1960, **53**, 484).—Residues of Sevin were below the sensitivity level of 0·2 p.p.m. 21 days after application. No 1-naphthol was detected. C. M. HARDWICK.

Sevin residues on soya-beans following its use for army worm control. L. J. Hardin, W. W. Stanley, D. E. Gonzalez and S. E. Bennett (*J. econ. Ent.*, 1960, **53**, 481—482).—Application of Sevin wettable powder at 1·5 lb./acre gave 90% control of *Pseudaletia unipuncta* in 4 days with residues of <4 p.p.m. At 3 lb./acre an almost complete kill is obtained but residues of 30 p.p.m. were present. Residue analysis involved hydrolysis by methanolic KOH and colour developed in glacial acetic acid by means of the fluoroborate reagent. The amount of Sevin is then determined by comparison of absorbance with that of a standard curve. C. M. HARDWICK.

Rapid combustion and determination of residues of chlorinated pesticides using a modified Schöniger method. D. J. Lisk (*J. agric. Fd Chem.*, 1960, **8**, 119—121).—Residues of chlorinated pesticides on plants are determined by evaporating a benzene extract in a cone of cellulose acetate, which is then burned in an O_2-filled flask, the HCl formed being absorbed in dil. NaOH. Complete combustion is obtained by means of a specially designed Pt holder for the folded cone, and a balloon is attached to the flask to ensure safety. Recoveries of DDT, Thiodan and lindane from lucerne were 63 to 109%. M. D. ANDERSON.

Malathion residues on and in the leaves of *Phaseolus vulgaris*. F. Matsumura (*J. econ. Ent.*, 1960, **53**, 452—454).—^{32}P-labelled malathion was applied to a bean leaf; its dissipation was very rapid for 3 days due to evaporation and the rate of entry into the tissue satisfied Langmuir's adsorption equation. (11 references.)
C. M. HARDWICK.

Extraction and determination of ethylene dibromide in soils. J. J. Jurinak, A. L. Brown and P. E. Martin (*J. agric. Fd Chem.*, 1960, **8**, 113—115).—Ethylene dibromide (**I**) in soils is determined by vac. distillation of a sample mixed with glycerol, collecting the separated product in NaOH and H_2O_2, converting it into inorg. Br by catalytic oxidation (using electrically heated Pt wire) and determining Br by $AgNO_3$. Recovery over the range 164 to 0·4 mg. of **I** per 100 g. of soil fell from about 98 to about 75%, and was somewhat decreased when the soil was moist instead of air-dried, when the soil had a high content of org. matter, and when glycerol was omitted.
M. D. ANDERSON.

Development of granary weevils and storage fungi in columns of wheat. II. C. M. Christensen and A. C. Hodson (*J. econ. Ent.*, 1960, **53**, 375—380).—Wheat with a moisture content of 13—14% was stored in cylinders at 25° and 65—70% R.H. Unconfined weevils were concentrated near the bottom after 2 months and nearer the middle after 4 months. Moisture contents increased to 20% in the upper part of the grain and *Aspergillus repens* and *A. restrictus* increased greatly. When insects were confined at the bottom there was increased moisture content above them associated with increased fungal growth. Fumigation with CCl_4 + ethylene dichloride and ethylene dibromide killed all the weevils but after 2 months there was no detectable effect on the fungi. C. M. HARDWICK.

Influence of grain moisture and storage temperature on the effectiveness of malathion as a grain protectant. R. G. Strong and D. E. Sbur (*J. econ. Ent.*, 1960, **53**, 341—349).—Mortality of *Sitophilus granarius*, *Sitophilus oryza* and *Tribolium confusum* was used as the criterion of the effectiveness of malathion (**I**) (10 p.p.m.). To avoid dangerous residual concn. of **I**, the max. safe level of moisture in grain stored at 60°F was 12%. The length of effective life of malathion decreased with rising temp. (60—120°F). (11 references.)
C. M. HARDWICK.

Effect of Ronnel upon the adult rice weevil, *Sitophilus oryza*. P. K. Harein (*J. econ. Ent.*, 1960, **53**, 372—375).—Ronnel solution applied to wheat at 26 p.p.m. gave 80% mortality in 14 days. Although dosages of 1 p.p.m. produced only 7% mortality reproduction was reduced by 94%. The wheat had no odour at dosages of 5 p.p.m., but at 10 p.p.m. some was detectable and at 25 p.p.m. it persisted after airing for 3 months. C. M. HARDWICK.

Laboratory studies of three-lined potato beetle and control with various insecticides. T. W. Kerr and C. E. Olney (*J. econ. Ent.*,

1960, **53,** 480—481).—Field-collected *Lema trilineata* were exposed to deposits on glass jars. High residues of dieldrin failed to kill 1% of overwintering or first-generation beetles, thus explaining the recent increased populations. DDT deposits were effective against overwintering adults but more was required later in the season. Thiodan was effective at lower dosages and endrin gave erratic results. C. M. HARDWICK.

History of aphid control on potatoes in Southern Florida, 1946—59. D. O. Wolfenbarger (*J. econ. Ent.*, 1960, **53,** 403—405).—Percentage control of *Myzus persicae* is given for each material tested, each year. In general, org. P compounds were more effective than chlorinated hydrocarbons. At first DDT was the most effective compound but this was superseded by parathion for 10 years and then by Thiodan. C. M. HARDWICK.

Further studies on control of the clover root borer in Virginia. A. M. Woodside (*J. econ. Ent.*, 1960, **53,** 449—450).—Heptachlor 0·6, BHC 1, chlordane 2·5 and aldrin 0·7 lb./acre gave similar control of *Hylastinus obscurus*. Sprays were more effective than granules. The season of application was unimportant. Heptachlor at the 1-lb. level was effective for two seasons and at 2 lb. for three seasons. C. M. HARDWICK.

Laboratory studies on the toxicity of various insecticides to different stages of the oriental fruit moth. M. H. Brunson (*J. econ. Ent.*, 1960, **53,** 468—471).—Methods are described for testing the toxicity of numerous insecticides to eggs, adults and larvae of *Grapholitha molesta*. Of 54 compounds tested Guthion, parathion, methyl parathion, chlorthion and EPN and Sevin were the most effective. C. M. HARDWICK.

Soil treatments in lieu of spraying for plum curculio control in peach orchards. O. I. Snapp (*J. econ. Ent.*, 1960, **53,** 439—441).—In soil boxes, aldrin, dieldrin and heptachlor dusts prevented the emergence of adult *Conotrachelus nenuphar* placed in the boxes as larvae. Chlordane, isodrin, endrin and Thiodan were less effective. 2%-aldrin, -dieldrin or -heptachlor granules gave effective control for at least three seasons in an orchard and added larvae were nearly all killed. C. M. HARDWICK.

Plum curculio control experiments in 1955—58. O. I. Snapp (*J. econ. Ent.*, 1960, **53,** 335—337).—The knockdown and residual effectiveness of Guthion, diazinon, Trithion and Sevin in cage tests against *Conotrachelus nenuphar* on peaches is recorded. In field experiments with various chlorinated hydrocarbons and org. P compounds three applications of dieldrin were most effective. Residues of Guthion, Sevin and diazinon were below tolerance level; dieldrin should not be used later than 2 weeks after shuck-off. C. M. HARDWICK.

Control of grape mealybug on apricots. H. F. Madsen and L. B. McNelly (*J. econ. Ent.*, 1960, **53,** 354—357).—All insecticides tested were more effective if applied as petal fall sprays (i.e. spring emergence of crawlers) rather than pink bud or summer sprays. Diazinon and parathion were the most satisfactory. C. M. HARDWICK.

Systemic insecticides for control of scales, leaf miners and lace bugs. J. C. Schread (*J. econ. Ent.*, 1960, **53,** 406—408).—Soil drenches of Phorate (**I**) and Phosdrin controlled *Asterolecanium* sp. on holly but Disyston and Systam did not. The heaviest application of **I** granules gave about 44% control of *Phenacaspis pinifoliae*. **I** and Dimethoate (**II**) granules were effective against *Fenusa pusilla* but Phosphamidon (**III**) gave poor results. **II** was more toxic to *Phytomyza ilicis* and *Monoarthropalpus buxi* than were **III** granules. Complete control of various *Stephanitis* spp. was obtained with **III** as a soil drench or granules or **II** as granules. C. M. HARDWICK.

Effect of copper compounds on control of citrus rust mite with zineb. R. B. Johnson (*J. econ. Ent.*, 1960, **53,** 395—397).—Prevention of fruit russet is dependent on good control of *Phyllocoptruta oleivora*. Zineb was the most effective treatment. Addition of any of six Cu compounds reduced its effectiveness in summer sprays but not in post-bloom sprays; this was overcome by the addition of an emulsified oil but not by parathion. Where summer populations were moderate they increased after treatment. C. M. HARDWICK.

Citrus red mites resistant to demeton and Ovex and their response to Tedion and Kelthane. F. Munger, J. E. Gilmore and A. W. Cressman (*J. econ. Ent.*, 1960, **53,** 384—388).—The adults of a strain of *Panonychus citri* collected from demeton-treated groves were 163× as resistant as an untreated strain when tested on dipped lemons. Ovex-resistance of 131× was measured by the no. of adults maturing from eggs laid on treated lemons. There was no cross-tolerance to Tedion or Kelthane. C. M. HARDWICK.

Field evaluation of Sevin as an insecticide for pests of vegetables in New Jersey. P. Granett and J. P. Reed (*J. econ. Ent.*, 1960, **53,** 388—395).—The value of the spray (0·25—1 lb./acre/100 gal.) on 11 crops over a period of 4 years was determined. There was no increase in yields. Spray residues varied with the crop; all decreased to low levels within a week. (11 references.) C. M. HARDWICK.

Field tests with various insecticides for control of Lygus bugs and the maize earworm on lima beans. M. W. Stone, F. B. Foley and R. E. Campbell (*J. econ. Ent.*, 1960, **53,** 397—403).—In experiments over 7 years, the best control of *Lygus hesperus* was given by dieldrin, followed by toxaphene, endrin, DDT–toxaphene and DDT. The highest yield of beans was associated with DDT–toxaphene and these had least pitting. C. M. HARDWICK.

Effect of seed potato dip treatments on the incidence of sweet potato sprout decay caused by *Diaporthe batatatis*. R. H. Daines, E. Brennan and I. A. Leone (*Phytopathology*, 1960, **50,** 186—187).—The incidence of the disease was greatest when the temp. of the plant bed was 85°. Of seed dips tested, org. Hg prep. gave best control followed, in diminishing order of efficiency, by Tersan DM (tetramethylthiuram disulphide), Dyrene [2,4-dichloro-6-(*o*-chloroanilino)triazine] and captan. A. G. POLLARD.

Insecticides for control of the Nantucket pine moth, *Rhyacionia frustrana*, and the European pine shoot moth, *R. buoliana*. D. E. Donley (*J. econ. Ent.*, 1960, **53,** 365—367).—Based on counts of infested pine shoots, sprays of 45 gal./acre of Guthion, Delnav, malathion, Trithion and methyl parathion were as effective as or better than DDT. C. M. HARDWICK.

Influence of timing and insect biology on the effectiveness of insecticides applied for control of European pine shoot moth, *Rhyacionia buoliana*. J. W. Butcher and D. L. Haynes (*J. econ. Ent.*, 1960, **53,** 349—354).—Spring sprays of Guthion, Bayer 22408 (*OO*-diethyl *O*-naphthalimido phosphorothioate), Phosdrin, Dimethoate and DDT in two oil formulations gave good control of overwintering larvae. Applications of Sevin, DDT and Dimethoate made during the summer indicated that treatment is most effective when 50% of the eggs have hatched. Malathion and BHC did not reduce populations. C. M. HARDWICK.

Suitability of tobaccos for the growth of the cigarette beetle, *Lasioderma serricorne*. R. T. Yamamoto and G. Fraenkel (*J. econ. Ent.*, 1960, **53,** 381—384).—Comparison of the growth of *L. serricorne* on four different types of tobacco was favoured by adequacy of nutrients and stimulated by addition of yeast and B-vitamins. Only flue-cured tobacco seems susceptible to beetle attack. (13 references.) C. M. HARDWICK.

Soil treatments with insecticides for control of the eye gnats, *Hippelates collusor* and *H. hermsi*. M. S. Mulla, M. M. Barnes and M. J. Garber (*J. econ. Ent.*, 1960, **53,** 362—365).—Use of aldrin or heptachlor as granules or sprays reduced the no. of gnats initially but had no residual effect at 9 months. Dieldrin, toxaphene and Thiodan were also unsatisfactory. DDT granules (13 lb./acre) lowered the no. of gnats by 95%, initially. C. M. HARDWICK.

Chlorinated hydrocarbon insecticides as soil treatments against the eye gnat *Hippelates collusor* (Townsend) in the laboratory. M. S. Mulla (*J. econ. Ent.*, 1960, **53,** 367—372).—Shell SD-4402 (1,3,4,5,6,-7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan) at 2·5 lb./acre reduced the emergence of gnats by 98%. DDT (18 lb./acre) gave an 80% reduction and endrin (5 lb./acre) a 95% reduction. The effectiveness of DDT granules increased with 3 months' ageing but was still less than that of sprays. In soil, the activity of aldrin and dieldrin decreased for 3 months, that of DDT and endrin remained the same and that of toxaphene increased. C. M. HARDWICK.

Effect of lime applications to soil on Japanese beetle larval population. J. B. Polivka (*J. econ. Ent.*, 1960, **53,** 476—478).—Additions of lime which increased soil pH in all cases depressed the no. of *Popillia japonica*. C. M. HARDWICK.

Pickleworm control and residue studies with malathion and Phosdrin. J. E. Roberts and L. E. Anderson (*J. econ. Ent.*, 1960, **53,** 482—483).—Application of 4% malathion (**I**) or 2% Phosdrin (**II**) dust at 5—7 day intervals significantly reduced damage by *Diaphania nitidalis*. Residues of **II** were below tolerance levels within 2½ hr. and none were found 2½ hr. after **I** application. C. M. HARDWICK.

Susceptibility of *Lucilia cuprina*, Wied. to some organic phosphorus compounds. R. J. Hart (*J. Aust. Inst. agric. Sci.*, 1960, **26,** 70—71).—Dosage-mortality tests were made using diazinon, Trolene, parathion, malathion and Rogor insecticides. P. M. KINGSTON.

Absorption of herbicides by roots. A. S. Crafts and S. Yamaguchi (*Amer. J. Bot.*, 1960, **47,** 248—255).—Radioactively-labelled herbicides were added to culture media for barley, beans, cotton and *Zebrina*. Movement of 2,4-D from roots into the tops of barley, *Zebrina* or bean was small but in cotton was considerable. Barley roots readily absorbed 2,4-D, MH, ATA, urea, monuron, dalapon,

Simazin and IAA ; movement into tops was least with 2,4-D followed by urea and much greater with the other materials. ATA, MH, IAA and dalapon moved readily in phloem ; monuron and Simazin were translocated only in xylem. A. G. POLLARD.

Factors regulating herbicidal effectiveness of 3-(*p*-chlorophenyl)-1,1-dimethylurea (monuron) on beets. L. W. Feddema (*Dissert. Abstr.*, 1960, **20**, 3460—3461).—Variability in the effectiveness of monuron in killing *Chenopodium album* in beet was not related to the amount of irrigation water, but the nature of the soil was important. Inactivation of monuron was best correlated with the amount of an org. soil fraction readily oxidised by H_2O_2. The substance which combines with and inactivates monuron is perhaps a product of decomposition of org. matter ; raw org. matter was without effect. Electrochemical adsorption of monuron by soils was found to be of minor importance. M. D. ANDERSON.

Influence of 2,4-D on pathways of glucose utilisation in bean stem tissues. S. C. Fang, F. Teeny and J. S. Butts (*Plant Physiol.*, 1960, **35**, 405—408).—Absorption and metabolism of glucose were increased three-fold in seven days by 2,4-D, there being a relative increase in the amount of glucose catabolised via the glycolytic sequences. Synthetic pathways for cellular constituents in the stem tissue seem unaffected. E. G. BRICKELL.

Phytocidal action of 3-amino-1,2,4-triazole. M. C. Carter (*Dissert. Abstr.*, 1960, **20**, 3483).—The metabolism of the phytocide 3-amino-1,2,4-triazole (ATA) labelled with ^{14}C, in honeysuckle (*Lonicera japonica*) and bean (*Phaseolus vulgaris*) was examined. Translocation of labelled ATA was rapid, but unaltered ATA was not found in stem tips or roots ; 13 deriv. were detected, of which " compound I " preponderated. ATA had little or no effect on respiration, dark fixation, or metabolism of glucose and succinate, but it modified the metabolism of glycine and serine ; in presence of unlabelled ATA, ^{14}C from either of these amino-acids appeared in the " compound I." On acid hydrolysis of compound I, ATA was recovered, but glycine and serine were not. A common deriv C_1 of these amino-acids is probably involved in the reaction with ATA ; shortage of C_1 fragments for syntheses may be the cause of the phytotoxicity of ATA. M. D. ANDERSON.

Synthesis of carbon-14-labelled dalapon and trial applications to soya-bean and maize plants. F. A. Blanchard, W. W. Muelder and G. N. Smith (*J. agric. Fd Chem.*, 1960, **8**, 124—128).—Radioactive Na dalapon (2,2-dichloropropionate) was prepared by direct chlorination of 2-^{14}C-propionic acid, and separated from other chlorinated acids by gradient elution from a phosphate-buffered silica gel column. Dalapon-^{14}C supplied to the roots of maize and soya-bean plants was distributed throughout the plants. When it was applied to a leaf, over 95% of the activity was recovered from the treated leaf, but all parts of the plant showed some activity. Active material was identified as dalapon by paper chromatography ; no breakdown products were found. (15 references.)
M. D. ANDERSON.

Synthesis and preliminary evaluation of amino-acid derivatives of 2-(2,4,5-trichlorophenoxy)propionic acid. C. F. Krewson, J. F. Carmichael, T. F. Drake, J. W. Mitchell and B. C. Smale (*J. agric Fd Chem.*, 1960, **8**, 104—106).—The *N*-[DL-2-(2,4,5-trichlorophenoxy)propionyl] deriv. of a series of D-, L- and DL-amino-acids were prepared, and 22 of 33 compounds were evaluated as growth regulators on pinto bean, sunflower and cucumber seedlings by the lanolin assay method. Responses differed widely. Deriv. of the L- and DL-amino-acids usually showed marked activity, except the deriv. of L-tryptophan, which was inactive. Deriv. of D-amino-acids, except those of D-alanine and D-tryptophan, were usually inactive. Activities were usually less than for the corresponding halogenated phenoxyacetyl compounds, or for the parent-propionic acid. M. D. ANDERSON.

New compounds containing phosphorus and sulphur. CIBA Ltd. (B.P. 819,066, 17.11.55. Switz., 17.11.54).—Compounds $OR(OR^I)\cdot PO\cdot CCl_2\cdot CO_2\cdot R^{III}\cdot S\cdot R^{II}$, useful as systemic insecticides, are obtained by interaction of $OR(OR^I)\cdot P\cdot OR^{IV}$ with $CCl_3\cdot CO_2\cdot R^{III}\cdot S\cdot R^{II}$ (R—R^{II} are alkyl or alkenyl, optionally substituted by OH, CNS, CN, esterified CO_2H or halogen ; R^{III} is alkylene ; R^{IV} is low-mol. alkyl). Et_3PO_3 and methylmercaptoethyl trichloroacetate give a brown oil comprising *Et$_2$ dichloro[carbo-2-(methylthio)ethoxy]methylphosphonate.* F. R. BASFORD.

Derivatives of dialkyldithiophosphoric acids with a double amide function in the molecule and disinfesting compositions prepared therefrom. Montecatini Società generale per l'Industria Mineraria & Chimica (B.P. 819,742, 10.7.56. It., 14.7.55).—Compounds $(OR)_2PS\cdot S\cdot CH_2\cdot CO\cdot NR'\cdot R''\cdot NR'''\cdot CO\cdot CH_2\cdot S\cdot PS(OR)_2$, useful as pesticides, are obtained by interaction of $CH_2X\cdot CO\cdot NR'\cdot R''\cdot NR'''\cdot CO\cdot CH_2X$ with $(OR)_2PS_2Y$ (in a solvent) (R is Me or Et ; R′ and R‴ are H when R″ is divalent aliphatic, aromatic or aliphatic-aromatic residue, or together with R″ and both N form a heterocyclic radical ; X is halogen ; Y is alkali metal or NH_4). Thus, a solution of $(OEt)_2PS_2H$ (40) in acetone 200 c.c. is neutralised with Na_2CO_3 (12), then *NN′*-bis-chloroacetylethylenediamine (21) is slowly added. After 2 hr. stirring the mixture is kept (at room temp.) overnight, then boiled for 3 hr., and subsequently freed from solvent. The residue is stirred with water during 30 min., then filtered, to give a solid product (34 g.) comprising *SS′-ethylene di-*[1,2-*biscarbamoylmethyl*]*bis-*(*OO-diethyl phosphorothiolothionate*) $[CH_2\cdot NH\cdot CO\cdot CH_2\cdot S\cdot PS(OEt)_2]_2$, m.p. 103—105° (from 70% aq. MeOH). F. R. BASFORD.

Quaternary ammonium salts of dialkylaminoalkyl thiophosphate esters. Campbell Pharmaceuticals Inc. (B.P. 819,735, 9.5.56. U.S., 12.5 and 9.9.55).—Quaternary halides and sulphates of esters $(OR)_{3-n}PX(X\cdot CHR'\cdot CH_2\cdot NR''R''')_n$ are claimed as inhibitors of cholinesterase and as insecticides [R,R″ and R‴ are alkyl, R′ is H or alkyl or substituted (dialkylamino) alkyl ; X is O or S, one X is S ; *n* is 1—3]. A typical example is *2-dimethylaminoethyl Et$_2$ phosphorothionate methosulphate*, m.p. 92·5—93·5°.
F. R. BASFORD.

New phosphoric esters and compositions containing same. Société des Usines Chimiques Rhône-Poulenc (B.P. 819,850, 26.11.57. Fr., 3.12.56 and 27.9.57).—Compounds $(OR)_2PX\cdot X\cdot CH_2R'$ are claimed (R is alkyl of 1—4 C ; X is O or S but at least one X is S ; R′ is benzthiazol-2-yl or benzoxazol-2-yl). They are useful as pesticides, being especially effective against flies (e.g., *Ceritilis capitata*), mites (e.g., *Paratetranychus pilosus* and *Tetranychus telarius*), aphids (e.g., *Aphis fabae* and *A. pomi*) and weevils (e.g., *Centhorrhynchus assimilis*), and compositions containing them (0·005—5%) for such use are also claimed. *OO-Dimethyl S-benzthiazol-2-ylmethyl phosphorothiolothionate*, an oil, is prepared. F. R. BASFORD.

Chlorinated and brominated polycyclic hydrocarbons and insecticidal compositions containing them. Shell Research Ltd. (Inventors : W. C. Webber and P. A. Harthoorn) (B.P. 819,240, 20.5.58).—Adamantane (tricyclo[3,3,1,1^{3,7}]decane) is treated with a chlorinating agent (Cl_2,SO_2Cl_2,PCl_5,$SbCl_5$) or a brominating agent (Br_2, etc.) at high temp. in presence of a catalyst (actinic light) in a solvent (halogenohydrocarbon), to give a chloro- or bromo-derivative. The compounds are useful as intermediates or (when <2 halogen atoms are present) as insecticides. In a typical example, a solution of the hydrocarbon (13·6 g.) in CCl_4 (100 c.c.) is treated with Cl_2 during 1·5 hr. in presence of actinic light, to give a 75·5% yield of *dodecachloroadamantane*, m.p. 290°.
F. R. BASFORD.

Dithiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 819,672, 4.10.56. Ger., 4.10.55).—Compounds $(OR)_2PS\cdot S\cdot C_6H_2R'R''\cdot NO_2$ are claimed (R is alkyl of 1—5 C or Ph ; R′ and R″ are H, alkyl of 1—4 C or halogen); they are obtained by condensing $SX\cdot C_6H_2R'R''\cdot NO_2$ with $(OR)_2PS\cdot Y$, in presence of acid binding agent if desired (X is H, NH_4 or metal ; Y is halogen). The products are valuable insecticides (especially contact insecticides) and are effective (in 0·0001—1% concn. with a liquid or solid carrier) against flies, aphids and mites. *OO-Diethyl S-p-nitrophenyl phosphorothiolothionate*, m.p. 49°, is prepared.
F. R. BASFORD.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 819,689, 12.3.57. Ger., 22.3.56).—Esters of the formula $R'\cdot S(O)_n\cdot Ar\cdot O\cdot PS(OR)_2$, where R is an alkyl radical containing 1—4 C-atoms, R′ an alkyl radical or an aryl radical which may be unsubstituted or substituted, Ar a phenylene radical which may be unsubstituted or substituted and *n* is 0 or 1, are obtained when a substituted phenol is reacted with an *OO*-dialkyl phosphorochloridothionate. An example given is the prep. of *OO-Et$_2$ O-p-methylmercaptophenyl phosphorothionate*. The new compounds are especially useful as pesticides or as intermediates for the production of other pesticides. E. ENOS JONES.

Methylmercuric pentachlorophenoxide and fungicidal compositions containing same. N.V. Aagrunol, Chemische Fabriek (B.P. 819,130, 16.5.58. Neth., 17.6.57).—An aq. solution of $HgMeNO_2$ is treated with aq. Na pentachlorophenoxide, and the pptd. solid is filtered off, washed with water and recrystallised from EtOH, to give *methylmercuric pentachlorophenoxide* (e.g., in 76·9% yield). The compound possesses insecticidal and fungicidal properties, and compositions containing it for these purposes are claimed. F. R. BASFORD.

Dithiocarbamate derivatives. Rohm & Haas Co. (B.P. 819,087, 11.1.57. U.S., 19.1.56).—*Isopropoxycarbonyl dimethyldithiocarbamate* (m.p. 61—62°), useful on account of its fungicidal properties, is made by treating $ClCO_2Pr^i$ with a (Na) salt of dimethyldithiocarbamic acid ($NMe_2\cdot CS_2H$) in solution at $<20°$. The product is highly active against *Stemphylium sarcinaeforme* and *Monilinia fructicola*, and is compounded in the usual way for the purpose.
F. R. BASFORD.

Sulphonyl ethylene fungicides. Pittsburgh Coke & Chemical Co. (B.P. 819,069, 5.12.55. U.S., 6.12.54).—Compounds useful as fungicides (and seed protectants) comprise di(alkylsulphonyl)ethylenes, viz., $(:CH\cdot SO_2R)_2$, especially the *trans*-isomers (R is alkyl of 3—12 C). They may be obtained by oxidation of $(:CH\cdot SR)_2$ with, e.g., H_2O_2. A typical example describes the prep. of cis- and trans-1,2-*di*-(*propylsulphonyl*)*ethylene*. Both isomers are active against *Phytophthera infestans, Alternaria oleracea* and *Sclerotinia fructicola*. F. R. BASFORD.

Fungicidal substance. U.S. Rubber Co. (B.P. 819,779, 23.12.57. U.S., 28.1.57).—Na_2 ethylenebis-dithiocarbamate (nabam) is treated with phosgene at −10° to %20° during 1—20 hr. in aq. or org. solvent medium to give a good yield of a polyethylene-bisthiuram sulphide, highly active as a fungicide. F. R. BASFORD.

New triazine derivatives and herbicidal compositions containing them. E. I. du Pont de Nemours & Co. (B.P. 819,520, 18.3.58. U.S., 18.3.57).—Compounds useful as herbicides comprise 2-chloro-*s*-triazines substituted in the 4- and 6-positions by $NH\cdot[CH_2]_n\cdot O\cdot[CH_2]_m\cdot H$ (*m* is 1—8; *n* is 2—8; $m + n$ is ⩽4). They may be obtained by condensing cyanuric chloride with 2 mol. of $NH_2\cdot[CH_2]_n\cdot O\cdot[CH_2]_m\cdot H$ at 0—5° in aq. dioxan. The prep. of 2-*chloro*-4,6-*di*-(3-*methoxypropylamino*)-*s*-*triazine*, m.p. 159—163°, in 81% yield is detailed. This, pulverised to <50 μ and compounded (75) with Na alkylnaphthalenesulphonate (1·5), low-η methylcellulose (0·25), and synthetic fine SiO_2 (23·25%) affords a wettable powder which, may be diluted with water to provide an aq. spray (80 lb. of active ingredient per 300 gal. of water) capable of giving excellent control of grass and broad-leaf weeds. F. R. BASFORD.

Herbicidal amine salts of chlorobenzoic acids. Amchem Products Inc. (Inventor: A. W. Schneider) (B.P. 819,127, 9.4.58).—Compounds useful as herbicidal agents, comprise NHRR′R″ salts of $CO_2H\cdot C_6H_{5-n}Cl_n$ (*n* is 1—5; R—R″ are C_{1-2}-alkyl optionally substituted by NH_2, or hydroxyalkyl of 2—4 C; or R—R′ are H). As an example of prep., an isomeric polychlorobenzoic acid mixture (Hooker X-33) is admixed with water, then an aq. solution of triethanolamine is added during 30 min., while keeping at 10—27°. There is thus produced a herbicidal concentrate, d^{20} 1·181, containing the amine salt. F. R. BASFORD.

Substituted urea herbicides. Farbenfabriken Bayer A.-G. (B.P. 819,853, 17.12.57. Ger., 21.12.56).—A herbicidal composition, especially effective against dicotyledonous plants (cress, mustard, charlock), comprises a substituted urea, viz., NHR·CO·NY·COX (R is Ph optionally carrying a substituent; Y is H or aryl; X is OR′ or NR″R‴; R′ is saturated or unsaturated alkyl of 1—6 C; R″ and R‴ are H or as R′, or together with N comprise a heterocyclic radical), a solid or liquid carrier (i.e., talc, or flour prepared from wheat or cottonseed, cyclohexanol, acetone, cottonseed oil, etc.), a conditioning or an emulsifying agent, and optionally a surface-active agent (5—30 wt.-%), e.g., Na oleate, sulphonated petroleum oil, Na lauryl sulphate or polyethylene oxide. The preferred urea compound is selected from 1-phenyl-, 1,3-diphenyl-, 1-*p*-tolyl-, 1-*p*-chlorophenyl-, 1-*p*-ethoxyphenyl- or 1-(4′-chloro-3′-nitrophenyl)-5,5-dimethylbiuret, or from allyl, methyl, ethy., isopropyl or isobutyl phenylallophanate. F. R. BASFORD.

Animal Husbandry

Use in animal nutrition of fermentation residues containing vitamin B_{12} from streptomycin production. E. Kraak (*Ernährungsforschung*, 1960, **5**, 124—130).—A review covering the results of feeding experiments in which the dried mycelia have been used as supplements. (28 references.) P. S. ARUP.

Digestibility of *Medicago tribuloides* (barrel medic) pods. J. E. Vercoe and G. R. Pearce (*J. Aust. Inst. agric. Sci.*, 1960, **26**, 67—70).—Results of controlled feeding tests on caged sheep are presented and discussed in relation to reported nutrition studies by other workers. P. M. KINGSTON.

Lupins in catch-crop mixtures for silage. F. Schmidt, S. Tabin and J. Woznica (*Ann. Univ. Mariae Curie-Sklodowska*, 1958, **13, E**, 363—383).—Yields of green matter were greater when sunflower than when other crops (millet, buckwheat, maize) were grown in association with white lupins or when the lupins were grown alone. Food constituents (except protein) in the green matter of the sunflower–lupin mixture generally exceeded those in the pure lupin crop. Silage prepared from the mixture (up to 60% of either component) was of good quality (pH 4·0—4·3). Larger proportions of lupin resulted in inferior silage (pH 5·5, NH_3-N 37% of total N). A. G. POLLARD.

Chicory as a continuous fodder crop. S. Tabin (*Ann. Univ. Mariae Curie-Sklodowska*, 1958, **13, E**, 209—246).—Effects on the yield and composition of roots and tops of chicory of frequency of cutting, no. of years of cultivation, protection from frost and soil conditions, are recorded. A. G. POLLARD.

Water-soluble forage constituents and their influence on metabolic activity of the rumen. A. Bondi and H. Tagari (*Bull. Res. Counc. Israel*, 1960, **9A**, 143—148).—The cellulose-digesting capacity (*in vitro*) of the rumen liquor of sheep is generally positively related to the content in the feed of sol. carbohydrates or of sol. carbohydrates + sol. non-protein N. Ryegrass (fresh or dried), however, gives rise to a liquor of comparatively high cellulolytic capacity in relation to its content of sol. carbohydrates; this may be due to its (exceptional) content of fructosans. The de-aminating capacity of liquor from sheep fed on hay (in two trials) exceeds that of sheep fed on fresh clover or ryegrass (in three trials). No correlation is found between cellulolytic and de-aminating capacity. (13 references.) P. S. ARUP.

Stepwise hydrolysis of grass holocellulose. T. G. Phillips, D. G. Routley and J. T. Sullivan (*J. agric. Fd Chem.*, 1960, **8**, 153—155).—Holocelluloses from 2 spp. of grass were heated with 0·01N-H_2SO_4 in a boiling-water bath for 1 hr., and the simple sugars, uronides, oligosaccharides and hemicelluloses extracted were determined. The process was repeated with the holocellulose residues, which were heated with acid for 2 hr., and subsequent residues for 4 and 16 hr. All the arabinose, galactose and rhamnose, practically all the uronides, and 75% of the xylose were finally extracted. Glucans accompanied the other hemicelluloses, but glucose does not appear to be a constituent of the polyuronide hemicelluloses. The oligosaccharides found indicate that considerable branching occurs in the structure of the polyuronide hemicelluloses. (10 references.) M. D. ANDERSON.

Water-soluble hemicelluloses of grass holocellulose. J. T. Sullivan, T. G. Phillips and D. G. Routley (*J. agric. Fd Chem.*, 1960, **8**, 152—153).—Hemicelluloses were extracted by hot water from the holocelluloses of 5 spp. of forage grasses. Analyses for component sugars showed xylose to be the most abundant (56—73%), followed by glucose (11—22%), arabinose (9—13%), galactose (5—7%) and rhamnose (1—2%). Some uronic acid was present. Analyses of orchard grass at different stages of growth showed that glucose and rhamnose decreased, and xylose increased, with increasing maturity. M. D. ANDERSON.

Investigations of fodder using the formic acid method for the determination of cellulose, starch and protein. E. Lehmann and H. Birsgal (*Z. PflErnähr. Düng.*, 1960, **89**, 42—49).—The formic acid technique may be used to assess homogeneous and heterogeneous fodders, but in the latter minerals and keratin may be present. The minerals occur in the cellulose residue if insoluble and in the low-molecular fraction if soluble. Keratin is found in the cellulose fraction from which is can be removed by boiling for ½ hr. in dilute NaOH solution and subsequently determined. M. LONG.

Properties and classification of lactobacilli isolated from grass and silage. R. M. Keddie (*J. appl. Bact.*, 1960, **22**, 403—416).—The characteristics of 61 representative strains are described. (34 references.) C. V.

Drought feeding of cattle. I. Comparison of hay-grain and all-grain rations with observations on vitamin status. II. Comparison of daily and weekly feeding of all-grain rations with observations on vitamin A status. W. H. Southcott and G. L. McClymont (*Aust. J. agric. Res.*, 1960, **11**, 439—444, 445—456).—I. All-grain rations are satisfactory. Serum-vitamin A levels declined to subnormal levels during the experiment with yearling Hereford steers, but clinical signs of deficiency were not evident. (10 references.)

II. The practicability of weekly feeding is confirmed but the practice does incur a greater risk of digestive disturbance and losses were heavier than with daily feeding. Cattle on carotene-deficient drought rations for 38 weeks showed no patent night blindness or other evidence of vitamin A deficiency, although individual serum values fell as low as 2 μg. per 100 ml. (10 references.) E. G. BRICKELL.

Experimental bloat in ruminants. V. Effects on rumen motility of volatile fatty acids introduced into the rumen. Y. Tsuchiya and M. Kayama (*Tohoku J. agric. Res.*, 1959, **9**, 237—250).—Addition of volatile fatty acids by fistula to the rumen of goats inhibited the motility of the rumen to extents varying in the order butyric > propionic > acetic acid. Complete inhibition by sufficient concn. of these acids is associated with increased total fatty acids in the blood serum and marked symptoms of acidosis. A. G. POLLARD.

Influence of the sense of taste on feed and fluid consumption. M. R. Kare and H. L. Pick, jun. (*Poultry Sci.*, 1960, **39**, 697—706).—Data on many trials on the effects of flavouring materials on the intake of feed and water and on wt. gains of chicks are presented. A. H. CORNFIELD.

Oxidative metabolism of the chick embryo. I. Factors influencing oxygen uptake. II. Oxidation of Krebs cycle intermediates and the effect of dinitrophenol on it. G. L. Feldman and J. R. Couch (*Poultry Sci.*, 1960, **39**, 517—520, 521—523).—I. Oxygen consumption of the 10-day-old chick embryo was stimulated by addition of adenosine triphosphate (**I**) and $MgSO_4$ (**II**). F^- inhibited O_2 uptake in the presence of **I** but the inhibition was overcome by adding more **I**. Inhibition of O_2 consumption due to addition of Ca^{2+} was overcome by adding more **I**.

II. Cell-free extracts from homogenates of 10-day-old chick embryos oxidised Krebs cycle intermediates. Dinitrophenol (**III**) stimulated the oxidation of α-ketoglutarate and succinate by sucrose prep. when the system contained Mg^{2+} and **I**. Only α-ketoglutarate oxidation was stimulated by the drug in the absence of Mg^{2+} and **I**. The oxidation of malate, α-ketoglutarate and isocitrate by cell-free extracts was stimulated by **III** in the absence of additional pyridine nucleotides required by the systems. When the pyridine nucleotides were supplied, the oxidation of these substrates was inhibited. A. H. CORNFIELD.

Metabolisable energy of feed ingredients for the growing chick. L. M. Potter and L. D. Matterson (*Poultry Sci.*, 1960, **39**, 781—782).—The metabolisable energy of 61 samples of feed ingredients representing 27 poultry feedstuffs is presented. A. H. CORNFIELD.

Metabolisable energy of grain and grain products for chickens. F. W. Hill, D. L. Anderson, R. Renner and L. B. Carew, jun. (*Poultry Sci.*, 1960, **39**, 573—579).—The N-corrected metabolisable energy values of 21 grains and grain products are presented. A. H. CORNFIELD.

Metabolisable energy of soya-bean oil-meals, soya-bean mill feeds and soya-bean hulls for the growing chick. F. W. Hill and R. Renner (*Poultry Sci.*, 1960, **39**, 579—583).—Average metabolisable energy values, corrected for N retention, on air-dry basis computed to 10% moisture were for 50%-protein dehulled soya-bean oil-meal 1150 kg.-cal. per lb., for 44%-protein soya-bean oil meal 1020 kg.-cal., for high- and low-protein soya-bean mill feeds respectively 570 and 350 kg.-cal. and for soya-bean hulls 5 kg.-cal per lb. A. H. CORNFIELD.

Factors affecting the metabolisable energy content of poultry feeds. I. R. Sibbald, J. D. Summers and S. J. Slinger (*Poultry Sci.*, 1960, **39**, 544—556).—Metabolisable energy values for maize, but not for wheat, showed significant variations when combined with different basal diets. The metabolisable energy of maize showed no variation due to age of bird (2 weeks to 16 months). The metabolisable energy of wheat was used more efficiently for carcass energy deposition than was that of maize, although maize appeared to have a slight advantage during the ration acclimatisation period. Alphacel, which is considered to have no nutritive value, actually showed a positive metabolisable energy value indicating an interaction which allowed better utilisation of the basal diet when diluted. A. H. CORNFIELD.

Determination of digestible carbohydrates in poultry foods. W. Bolton (*Analyst*, 1960, **85**, 189—192).—The sample is boiled to gelatinise starches and the liquid is incubated with takadiastase. The filtered liquid is clarified, acidified and heated, and its sugar content is determined by titration with Fehling's solution. Statistical comparison of results with those of digestibility trials showed that available carbohydrate + 4·9 is a reasonable measure of the digestible carbohydrate in foods for adult and growing poultry. For young chicks 4·9 should be replaced by 2·5. The compositions of some compounded diets and foods are given. (12 references.) A. O. JONES.

Blood meal as a source of protein in turkey starting diets. W. C. Lockhart, R. L. Bryant and D. W. Bolin (*Poultry Sci.*, 1960, **39**, 720—728).—Replacing 3—6% of the soya-bean oil-meal protein of a turkey diet with freeze- or vat-dried blood-meal protein resulted in reduced wt. gains and feed efficiency. Freeze-dried blood meal was less deleterious than was vat-dried blood meal. Addition of fish meal, methionine, isoleucine and lysine to diets containing blood-meal protein did not improve their growth-promoting effects. A. H. CORNFIELD.

Defatted starfish meal as source of nutrients in poultry rations. E. Levin, N. T. Rand, J. D. Mosser, D. S. Varner and V. K. Collins (*Poultry Sci.*, 1960, **39**, 646—654).—Tests with growing chicks showed that defatted starfish meal was as satisfactory nutritionally and economically as was fish meal as a source of protein, Ca and unidentified growth factors. A. H. CORNFIELD.

Growth and production performance of birds fed all-plant proteins supplemented with chicken manure from day-old through the laying stage. R. B. Gapuz (*Araneta J. Agric.*, 1959, **6**, 65—110).—A ration containing all-plant protein (*P*) supplemented with chicken manure (with or without addition of Co) was compared with a normal commercial ration which included animal protein (*A*). From 1 day to 12 weeks of age the *P* ration produced the slower growth, the Co supplement having some beneficial effect. From 12 to 24 weeks the growth response was similar with both rations. Egg production reached the max. rate within the first month of laying, with the *A* ration, but within 2—3 months with the *P* ration. Egg yields over the whole 9 months' laying period were substantially the same with both rations but hatchability was greater with the *A* ration. Supplementary Co improved hatchability. (72 references.) A. G. POLLARD.

Methyl acceptors for the biosynthesis of choline. P. Vohra, B. W. Langer, jun. and F. H. Kratzer (*Poultry Sci.*, 1960, **39**, 626—630).—Aminoethanol, HCHO-reacted aminoethanol, and hydroxymethylserine failed to improve the perotic condition of poults fed choline-deficient diets. Monomethylaminoethanol replaced choline in preventing perosis but was less growth-promoting than choline or dimethylaminoethanol, both of which have some antiperotic and growth-promoting activity. A. H. CORNFIELD.

Effect of dietary methionine on the methionine and cystine content of poultry meat. J. L. Fry and W. J. Stadelman (*Poultry Sci.*, 1960, **39**, 614—617).—The methionine and cystine contents of turkey meat were little affected by level of supplemental methionine (0% to 0·5%) in the diet. Muscle-cystine, but not -methionine, increased with age from 6 to 12 weeks. A. H. CORNFIELD.

Tolerance of growing chickens for dietary copper. A. L. Mehring, jun., J. H. Brumbaugh, A. J. Sutherland and H. W. Titus (*Poultry Sci.*, 1960, **39**, 713—719).—Chick growth to 10 weeks of age was reduced when the dietary Cu content exceeded 500 p.p.m. When the added excess Cu was left out of the diet after 10 weeks of age, subsequent growth was much improved. Addition of Cu to the feed increased the storage of Cu, Mo and SO_4^{2-} in the liver and spleen. Feed efficiency was improved by addition of Cu up to the toxic level. A. H. CORNFIELD.

Interrelationships between phosphorus, fluoride and fat in chick diets. J. D. Summers, S. J. Slinger, I. Motzok and G. C. Ashton (*Poultry Sci.*, 1960, **39**, 664—671).—Addition of F^- (150—385 p.p.m. as NaF) to chick diets depressed wt. gains when 6% of fat was added but had no consistent effect where no fat was added. Growth depression was due to reduction in feed intake. F depressed bone ash (%) at low, and increased it at adequate, P levels. Addition of fat depressed bone ash (%) with a P-deficient diet, but had no effect with a normal diet. F in soft phosphate was relatively unavailable to chicks. A. H. CORNFIELD.

Comparison of methods for estimating thyroid secretion rate in chickens. W. J. Mellen and B. C. Wentworth (*Poultry Sci.*, 1960, **39**, 678—686).—The goitre-prevention test gave thyroid secretion rates averaging 65% of those derived from the radioiodine method. A radioiodine method based on measuring the effect of increasing thyroxine doses on individual birds gave values of 3·5—4·0 μg. L-thyroxine per 100 g. per day. A. H. CORNFIELD.

Effects of unidentified factors on the body and spleen weight of chicks. R. L. Atkinson and F. H. Kratzer (*Poultry Sci.*, 1960, **39**, 631—637).—Liver L and splenic tissue contained factor(s) which increased body and spleen wt. when fed to chicks. The growth factor was stable to autoclaving at 15 lb. pressure at pH 1—9 and occurred in both free and bound form. The bound form was released by autoclaving at pH 7—9. The factor affecting the wt. of spleen was stable to autoclaving and was rendered partially sol. in $CHCl_3$ after autoclaving at pH 9. A. H. CORNFIELD.

Effect of diethylstilboestrol implantation in chickens on growth and body composition. G. A. Donovan and W. C. Sherman (*Poultry Sci.*, 1960, **39**, 757—765).—Wt. gains of 9—10-week-old birds implanted with diethylstilboestrol (0·012 g. pellet) were accelerated, compared with controls, during 14 days following treatment. Growth rate then declined to that of the controls but later levelled off, while that of the controls continued decreasing. Male birds showed greater effects than did females. No significant changes in carcass composition occurred in the first week following treatment, but deposition of carcass fat increased in the following 3 weeks. Wt. gains to 15 weeks were improved more by implantation at 10 than at 13 weeks of age and were similar to those produced by implantation at both 10 and 13 weeks of age. Two 0·015 g. diethylstilboestrol injections, 3 weeks apart, were necessary to equal the efficacy of one pellet implantation. A. H. CORNFIELD.

Influence of cortisone, liver L and dienoestrol diacetate on the body and organ weight of male chicks. R. L. Atkinson and F. H. Kratzer (*Poultry Sci.*, 1960, **39**, 638—645).—Cortisone (0·0075 g. per week) reduced wt. gains, increased the wt. of comb and testes and slightly reduced spleen wt. Liver L (50 g. per kg. of feed) increased body, spleen and testes wt. When supplied in combination with cortisone there was a considerable increase in comb wt. Dienoestrol diacetate

(0·01 g. per kg. of feed) had little effect on body or spleen wt., but decreased comb and testes wt. A. H. CORNFIELD.

Chemical and electrophoretic analysis of young chickens' serum following sex hormone administration. I. Proteins and protein distribution. K. Perk, M. Perek, K. Loebl and D. Allalouf (*Poultry Sci.*, 1960, **39,** 775—780).—Treatment of young chickens with diethylstilboestrol resulted in an increase in total serum proteins, with globulin increasing to the greatest extent, especially the α-2 and β-3 fractions. Treatment of the birds with testosterone propionate had no significant effect on serum proteins. A. H. CORNFIELD.

Formation of 16-epi-oestriol from oestradiol-17-β in the laying hen. H. F. MacRae and R. H. Common (*Poultry Sci.*, 1960, **39,** 707—712).—Radioactive 16-epi-oestriol (**I**), oestriol (**II**), oestradiol and oestrone were found in the intestinal tract and excreta following injection of a laying hen with radioactive oestradiol-17-β. All compounds except **I** were also found in the bile. The ratio **II** : **I** in the excreta of the laying hen was considerably lower than that in human pregnancy urine. A. H. CORNFIELD.

I. Supplemental Terramycin does not change cholesterol content of tissues in chicks. W. Joussellin, P. Gantes and J. Ladrat. **II. Effect of antibiotic supplements on chick-rearing under antiseptic conditions.** J. Ladrat and W. Joussellin (*C. R. Acad. Agric. Fr.*, 1960, **46,** 303—308, 309—311).—I. Terramycin (10 or 200 mg. per kg. of the ration) given to cockerels, reared in the open, caused no differences up to 9 weeks of age in the rate of growth or levels of blood-, liver- or fat-cholesterol, and no structural abnormalities in the blood-vessels.

II. In experiments similar to those described in the previous abstract, neither oleandomycin (1, 2 or 4 mg. per kg.) nor Terramycin supplements had any effect on the growth or food consumption of cockerels whether reared in the open or in a battery. P. S. ARUP.

Dietary antibiotics for turkey poults. P. E. Waibel, E. L. Johnson and J. W. Hassing (*Poultry Sci.*, 1960, **39,** 611—613).—Poult growth to 4 weeks of age was improved by addition of erythromycin-thiocyanate (10—50 g./ton of feed), or streptomycin + procaine penicillin + sulphaquinoxaline (100 g.) to the diet. Chlortetracycline (10 g.), procaine penicillin G (10 g.) and oleandomycin (10 g.) were less effective. Male birds usually made greater responses than did female birds. A. H. CORNFIELD.

Effect of feeding various antibiotics on the haemorrhagic condition in chickens. R. J. Dempsey and P. E. Sanford (*Poultry Sci.*, 1960, **39,** 691—696).—Addition of four different antibiotics (10—200 g. per ton of feed) usually increased the incidence of haemorrhages in chickens. Incidence was greater when antibiotics were added to a maize–soya-bean than when added to a purified diet. Crude feeding grades of antibiotics caused higher incidences of haemorrhages than did equiv. amounts of cryst. forms. Crude feeding grade sources of oxytetracycline and Zn bacitracin caused the highest incidence, whilst penicillin and chlortetracycline were sporadic in causing haemorrhages. A. H. CORNFIELD.

Effect of feeding *Escherichia coli* to turkey poults and chicks in the presence of antibiotics. W. K. Warden and P. J. Schaible (*Poultry Sci.*, 1960, **39,** 728—734).—Crop inoculation of turkey poults and broiler chicks with pure cultures of *E. coli* did not affect the growth-stimulating action of Zn bacitracin or oxytetracycline. A. H. CORNFIELD.

Influence of orally and parenterally administered salts of terephthalic acid on oxytetracycline serum levels in chickens. K. E. Price, Z. Zolli, jun. and G. A. Donovan (*Poultry Sci.*, 1960, **39,** 617—628).—Administration of terephthalic acid (**I**) or its Na or ethanolamine salts increased serum oxytetracycline levels over those of controls receiving the antibiotic only. When oxytetracycline was given orally the effect of **I** was greater with the higher level of antibiotic (500 g. per ton of feed or 0·275 g. per l. of drinking water) than with the lower level (200 g. per ton of feed or 0·11 g. per l. of water). The most effective levels of **I** were 0·05—0·15% in water and 2—4 times this quantity in the feed. The optimum effect of **I** when administered subcutaneously occurred with 0·025—0·150 g. per 1·5 lb. bird. A. H. CORNFIELD.

Effect of trifluoperazine and reserpine on reproductive efficiency in chickens. J. C. Gilbreath, Q. B. Welch, R. E. Waggoner and R. D. Morrison (*Poultry Sci.*, 1960, **39,** 735—739).—Addition of reserpine (0·0015—0·0025 g. per kg. of feed) to the diet of laying hens had no effect on egg production, feed efficiency with respect to egg production, feed consumption or egg quality factors. Addition of trifluoperazine (0·154—0·307 g. per kg. of feed) increased feed efficiency with respect to egg production, but reduced slightly egg wt. and shell thickness. A. H. CORNFIELD.

Effect of feeding 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline to chickens. F. X. Gassner, E. G. Buss, M. L. Hopwood and C. R. Thompson (*Poultry Sci.*, 1960, **39,** 524—533).—The LD_{50} of the drug for chicks was 10 g. per kg. body wt. The presence of even 0·075% of the drug in the feed had no effect on growth, feed consumption, mortality, egg production and fertility and hatchability of eggs. The treatments had no effect on the progeny of the birds or on various organs and tissues except that testes were enlarged and thyroid size was reduced with the higher levels of the drug. A. H. CORNFIELD.

Pigmentation-enhancing effect of 2-methyl-1,4-naphthaquinone in growing chickens. P. Griminger and H. Fisher (*Poultry Sci.*, 1960, **39,** 706—707).—Addition of 2-methyl-1,4-naphthaquinone (menadione, 5 g. per kg. of feed) to a maize meal–soya-bean oil-meal type diet considerably enhanced pigmentation in chicks to 3 weeks of age, but decreased wt. gains. Addition of an equiv. amount of menadione–$NaHSO_3$ complex enhanced pigmentation to a smaller extent but reduced wt. gains to a smaller extent. Addition of the antioxidant Santoquin (0·5 g. per kg. of feed) enhanced pigmentation only slightly, but did not reduce wt. gains. A. H. CORNFIELD.

Oral toxicity to poultry of a commercial octylamine. S. P. Clark and R. T. DuBose (*J. agric. Fd Chem.*, 1960, **8,** 147—151).—Broiler-type chickens were fed rations containing 0·036—0·36% of commercial primary n-octylamine, under investigation as a solvent for removing gossypol from cottonseed meals. After 9 weeks no definite toxic symptoms were observed. Birds to which amine was administered by tube, at levels above those acceptable in the food, showed typical injury and many died. The lethal dose was similar to that reported for other amines (LD_{50} 400 to 700 mg. per kg.). Toxicity was much less marked when the amine was given in a mixture with food than when it was given alone. M. D. ANDERSON.

Temperature and pH changes in poultry breast muscles at slaughter. J. W. Dodge and F. E. Peters (*Poultry Sci.*, 1960, **39,** 765—768).—Both pH and temp. of the breast muscles of chickens, geese and turkeys increased to a max. a few min. after slaughter and then declined. The effects occurred to a greater extent with males than with females of each species. A. H. CORNFIELD.

Influence of periodic removal of calcium sources from chicken rations on growth and egg production. G. A. Donovan, W. C. Sherman, K. E. Price and J. H. Hare (*Poultry Sci.*, 1960, **39,** 750—756).—Chick growth on low-Ca diets (0·138—0·702%) was significantly slower to 2 weeks of age than that on high-Ca diets in some, but not all, trials. Growth retardation up to 4 weeks of age due to low Ca occurred in even fewer cases. Low-Ca rations had little or no effect on feed efficiency. Wt. gains with a ration containing only 0·2% Ca from 0—4 days or during 4—8-day periods from 4 weeks to 8 weeks of age were not less than those with a high-Ca diet. During the later stages of growth 0·6% Ca caused no growth depression when supplied for periods of up to 8 days. Egg production was reduced by about 10% by a ration containing 0·119% of Ca for a 5-day period, but returned to normal when high Ca was supplied. A. H. CORNFIELD.

Effect of 3% added animal fat on laying hen performance. W. E. Donaldson and C. D. Gordon (*Poultry Sci.*, 1960, **39,** 583—587).—Addition of 3% stabilised animal fat, replacing 3% maize, to a 19% protein laying ration fed over 350 days had no significant effect on hen-day egg production. Egg production on a hen-housed basis was depressed by the treatment, but only with the heavy breeds. The treatment reduced feed efficiency and increased mortality and incidence of internal haemorrhage and obesity in heavy breeds, but had no effect on egg quality. A. H. CORNFIELD.

Ether extracts of yolks of eggs from hens on feed containing different fats. J. G. Ostrander, R. Jordan, W. J. Stadelman, J. C. Rogler and G. E. Vail (*Poultry Sci.*, 1960, **39,** 746—750).—Addition of maize oil or beef tallow (10%) to the hen's diet had no effect on % of ether extract in egg yolks. Maize oil increased, whilst tallow decreased, the I_2 absorption value of the ether extract of the yolk. A. H. CORNFIELD.

Factors affecting components of eggs from adult hens. K. N. May and W. J. Stadelman (*Poultry Sci.*, 1960, **39,** 560—565).—There were significant differences due to strain of hen in the % of moisture in the egg and in the % of protein in fresh and dried eggs. Age of hen and the season significantly influenced egg contents (wt.), albumin height, Haugh units, g. protein per egg, and in one case, the % of moisture and of protein in dry egg. There were significant differences for every variable studied between individual birds in a given strain. Correlations between the different variables are presented. A. H. CORNFIELD.

Storage of hatching eggs and the post-hatching body weights of chickens. W. A. Becker (*Poultry Sci.*, 1960, **39,** 588—590).—In four tests using meat-type chickens the body wt. at 8—9 weeks of birds hatched from eggs stored for 2 weeks before incubation were

lower than the body wt. of birds from eggs stored for a shorter period. A. H. CORNFIELD.

Effect of vitamin E and condensed fish solubles on hatchability of chicken eggs. F. T. Basilio (*Araneta J. Agric.*, 1959, **6**, 111—136).—Supplementing the hens' diet with condensed fish solubles slightly improved the hatchability of the eggs; vitamin E produced a far greater improvement. A. G. POLLARD.

Acute toxic effects upon livestock and meat and milk residues of dieldrin. R. D. Radeleff, W. J. Nickerson and R. W. Wells (*J. econ. Ent.*, 1960, **53**, 425—429).—Externally applied dieldrin was fatal to or badly affected over half the baby calves, most lambs, angora goats and Jersey steers. Pigs were unaffected. Oral administration of a 5% emulsion killed most calves but only temporarily affected pigs, sheep and horses. Residues in omental fat at different times up to 3 weeks after spraying were determined by infra-red spectroscopy. Symptoms of poisoning are described. C. M. HARDWICK.

Excretion of dieldrin, DDT and heptachlor epoxide in milk of dairy cows fed on pastures treated with dieldrin, DDT and heptachlor. N. Gannon and G. C. Decker (*J. econ. Ent.*, 1960, **53**, 411—415).—Sprays were applied at twice the normal level. Dieldrin appeared in the milk within 12 hr. and rose to 4 p.p.m. by the third day, then declined abruptly as pasture residues declined. The sharp peak was not found if cows were not pastured until the 14th or 21st day after spraying. The peak was proportional to the magnitude of the residues as also was the amount accumulating in the fat. Similar changes occurred with DDT but the residues were more persistent. Heptachlor was present in milk at much lower levels but variations showed a similar pattern. (13 references.) C. M. HARDWICK.

Face fly control [on cattle]. W. N. Bruce, S. Moore, III, and G. C. Decker (*J. econ. Ent.*, 1960, **53**, 450—451).—Baits of maize syrup (75) and water containing DDVP or Dimethoate (25%) applied to the foreheads of cattle in the morning attracted 60% of *Musca autumnalis* feeding on them. DDVP killed flies in 40 sec., but Dimethoate required 20 min. DDVP was effective for 1 day only whereas Dimethoate lasted 2—5 days. Regular treatment progressively reduced flies over a 3-week period. The amount of toxicant was too small to affect the cattle. C. M. HARDWICK.

Residual effectiveness of certain insecticides in horn fly control. W. T. Johnson and G. S. Langford (*J. econ. Ent.*, 1960, **53**, 477—478).—The treatment of cows with malathion, Sevin, Korlan, Dimethoate or methoxychlor dusts or sprays gave up to 2 weeks protection against *Siphona irritans*. Rain reduced this. In general dusts were better than sprays. C. M. HARDWICK.

Feed lot tests with Ronnel for control of cattle grubs. H. E. Thurber and G. D. Peterson, jun. (*J. econ. Ent.*, 1960, **53**, 339—341).—A single bolus of Ronnel (100 mg./kg. of body wt.) was most effective against *Hypoderma lineatum* and *H. bovis* when given before grubs reached the backs of the cattle. Grub control did not increase wt. gains. C. M. HARDWICK.

Relationship between coccidiosis and vitamin A nutrition in chickens. J. Erasmus, M. L. Scott and P. P. Levine (*Poultry Sci.*, 1960, **39**, 565—572).—After infection with coccidiosis chicks receiving 8000 i.u. of vitamin A per lb. of diet regained their appetites and grew faster than did those receiving 800 i.u. per lb. of diet. Infection resulted in lower liver storage of vitamin A. Liver storage of vitamin A of both infected and uninfected chicks was much lower where β-carotene than where an equiv. amount of stabilised vitamin A was supplied. Ataxia occurred only in chicks showing liver storage levels below 0·9 i.u. per g. of liver. A. H. CORNFIELD.

Anti-coccidial drugs against experimental infections with *Eimeria tenella* and *necatrix*. E. H. Peterson (*Poultry Sci.*, 1960, **39**, 739—745).—Tests with seven commercial anti-coccidial prep. are recorded. A. H. CORNFIELD.

Stages in the life cycle of *Eimeria tenella* affected by nicarbazin. D. K. McLoughlin and E. E. Wehr (*Poultry Sci.*, 1960, **39**, 534—538).—Tests in which medication with nicarbazin was begun at varying periods prior to and following experimental infection of birds with *E. tenella* indicated that the drug exerted its greatest activity on the second generation schizont, with some inhibitory effect on the preceding stages. The drug also had a definite suppressive action on the life cycle of *E. tenella*. A. H. CORNFIELD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking.

Grain drying rates and zone depths at the steady state. N. C. Ives (*Dissert. Abstr.*, 1960, **20**, 3670—3671).—In a specially designed counterflow dryer it was observed that the true drying time, or the travel time through the drying zone, of a kernel of wheat was dependent only on the moisture contents of the wheat entering and leaving the counterflow column, and the temp. and humidity of the entering air. Steady state drying conditions were obtained on a laboratory scale. The results were expressed mathematically and a general prediction equation was developed. A. M. SPRATT.

Chromatographic method for fractionating globulins of maize. T. A. McGuire, E. M. Craine and R. J. Dimler (*Cereal Chem.*, 1960, **37**, 324—333).—The method, which uses ion-exchange chromatography on carboxymethylcellulose, is described. (12 references.) S. G. AYERST.

Five proposed methods for determining smut content in wheat. R. M. Johnson (*Cereal Chem.*, 1960, **37**, 289—308).—Five simple, uniform and accurate methods for quant. determination of the total smut contamination in wheat were developed. They are (i) light transmittance, (ii) sedimentation, (iii) catalase activity, (iv) light reflectance and (v) light absorption. The results are compared with a microscope method. Method (i) is the cheapest and requires about 5 min. per sample, but method (v) can be used directly on bulk wheat and 500 samples can be tested in 8 hr. by one operator. (10 references.) S. G. AYERST.

Absorption of liquid water by the wheat kernel. H. A. Becker (*Cereal Chem.*, 1960, **37**, 309—323).—The pattern of liquid water absorption was studied and is described. A possible explanation of the observations is proposed. (17 references.) S. G. AYERST.

Weight and volume changes in wheat during sorption and desorption of moisture. W. Bushuk and I. Hlynka (*Cereal Chem.*, 1960, **37**, 390—398).—Wt. and vol. isotherms were obtained under identical conditions for the sorption and desorption of water vapour by wheat The variations in density of the wheat used, with moisture content, during sorption and desorption, which can be derived from the wt.-vol. data, are reported and discussed. (13 references.) S. G. AYERST.

Improved tempering and modified milling techniques for small samples of wheat. L. D. Sibbit, D. H. Classon and R. H. Harris (*Cereal Chem.*, 1960, **37**, 398—404).—A rotary shaker for tempering wheat, and a micro mill used for a short milling procedure, are described. Some miscellaneous data, obtained from flours milled by this method, are presented. S. G. AYERST.

Lipids of wheat: fractionation on silicic acid. N. Fisher and (in part) M. E. Broughton (*Chem. & Ind.*, 1960, 869—870).—The fatty acid distribution, determined by vapour-phase chromatography (argon/polyethylene glycol adipate), and contents of N and P are reported for three crude lipid fractions (petrol-sol.; petrol-insol.; petrol-sol–acetone-insol.) obtained from wholemeal wheat flour. Results of fractionation of the petrol-sol. material on silicic acid, eluting with increasing concn. of diethyl ether in light petroleum followed by increasing concn. of methanol in diethyl ether, are briefly reported. E. C. APLING.

Modern flour storage in baking factories. W. Hachmann (*Brot u. Gebäck*, 1960, **14**, 96—98).—Bulk storage flour plant for feeding mixing machines is described and discussed. J. V. RUSSO.

Effect of extraction rate of flour and of supplementation with soya meal on the nutritive value of bread proteins. K. Guggenheim and N. Friedmann (*Food Technol.*, 1960, **14**, 298—300).—In rat tests (*a*) all dietary protein was derived from bread, (*b*) bread proteins provided half and casein the other half and (*c*) bread diet was gradually supplemented with lysine and methionine. When bread only provided the dietary protein, the net protein ratio (NPR) rose as the % of extraction and of soya added increased. When bread provided half of the dietary protein, neither extraction rate nor soya improved the nutritional value of the protein. Lysine, the limiting amino-acid in bread proteins and supplied by increasing the % of extraction or of soya added, is not limiting in bread–casein diets. (17 references.) E. M. J.

Loss of vitamin A during preparation of bread made from enriched flour. H. Iwao, Y. Takai and A. Kenmoku (*Annu. Rep. nat. Inst. Nutr., Tokyo*, 1959, p. 76).—Bread made with flour enriched with vitamin A (600 i.u. %) retained 75 to 86% of the vitamin. M. D. ANDERSON.

Critical studies of titrimetric, colorimetric and polarimetric methods of starch determinations. H. Hadorn and F. Douvelaar (*Mitt. Lebensm. Hyg. Bern*, 1960, **51**, 1—68).—The titrimetric method of von Fellenberg which depends on the dissolution of starch in aq. $CaCl_2$, followed by the formation of a starch iodide complex and oxidation with $K_2Cr_2O_7$ and conc. H_2SO_4 is applied with modifications to a variety of precooked and native starches. A spectrophotometric method based on measuring the blue colour intensity of the starch iodide complex under various conditions is discussed;

and the polarimetric method of Baumann and Grossfeld is studied. Results obtained by the three methods on various different foodstuffs are compared and contrasted. (35 references.)
J. V. Russo.

Damaged starch. Quantitative determination in flour. R. M. Sandstedt and P. J. Mattern (*Cereal Chem.*, 1960, **37**, 379—390).—A method is proposed, based on the facts that damaged starch is rapidly digested by the amylases, whereas undamaged starch is more resistant. (16 references.) S. G. Ayerst.

Photomicrographic study of mechanically damaged wheat starch. R. M. Sandstedt and H. Schroeder (*Food Technol.*, 1960, **14**, 257—265).—Types of injury are discussed and photographed. The degree of injury varies widely and correspondingly, granule swelling, solubility and digestibility (by α- or β-amylase) also vary. (19 references.) E. M. J.

Wet-milling high-amylose maize containing 49 and 57 per cent amylose starch. R. A. Anderson, C. Vojnovich and E. L. Griffin, jun. (*Cereal Chem.*, 1960, **37**, 334—342).—In high-amylose maize, difficulties were encountered in the starch-gluten separation. Modifications to the usual method of separation are described. The wet-milling characteristics of high-amylose maize are discussed and compared with an ordinary dent maize. (11 references.)
S. G. Ayerst.

Effect of malting procedure and wheat storage conditions on alpha-amylase and protease activities. J. R. Fleming, J. A. Johnson and B. S. Miller (*Cereal Chem.*, 1960, **37**, 363—370).—Wheat was malted on a laboratory scale under a wide range of steep moisture levels, germination times and temp., kilning temp. and wheat storage conditions. The effects of these variations in processing on the enzyme production are recorded and discussed. Optimum malting conditions were ~42% steep moisture level and 60°F germination temp. Kilning temp. >110°F caused loss of both enzymes. Enzyme activities reached max. after storage of the wheat for 2 months at 40°F. (14 references.) S. G. Ayerst.

Effect of environment, variety and class of wheat on alpha-amylase and protease activities of malted wheat. J. R. Fleming, J. A. Johnson and B. S. Miller (*Cereal Chem.*, 1960, **37**, 371—379).—Several varieties of soft white winter, soft red winter, durum, hard red winter and hard red spring wheat were evaluated for enzyme activity of their malts. The soft white wheats were superior to all others and hard red spring showed least enzyme activity. (15 references.) S. G. Ayerst.

Improved denaturation test for gluten based on solubility in acetic acid. W. C. Schaeffer, C. A. Wilham, R. W. Jones, R. J. Dimler and F. R. Senti (*Cereal Chem.*, 1960, **37**, 411—412).—Modifications of the solubility test described by Blish (*Adv. Prot. Chem.*, 1945, **2**, 337) are presented. The speed, simplicity and accuracy of the test are increased. S. G. Ayerst.

Radioactive tracers. V. The reduction of ^{35}S-labelled persulphate to sulphate in flour and dough systems. C. C. Lee and D. G. Small (*Cereal Chem.*, 1960, **37**, 280—288).—The methods of determining ^{35}S recovery and sulphate content are described and the results discussed. The behaviour of persulphate (**I**) is quite different from that of bromate; the flour lipids do not appear to exert any appreciable effect on the rapid decomposition of **I** by the dough. (12 references.) S. G. Ayerst.

Bromate reaction in dough. II. Inhibition and activation studies. W. Bushuk and I. Hlynka (*Cereal Chem.*, 1960, **37**, 343—351).—The amperometric titration method was used to study effects of 17 different chemicals on the bromate reaction in dough. Iodate and bromate react with the same group(s) in the flour, but with greater speed in case of the iodate; sulphydryl is the main group involved. (11 references.) S. G. Ayerst.

Effect of remixing on the structural relaxation of unleavened dough. A. H. Bloksma and I. Hlynka (*Cereal Chem.*, 1960, **37**, 352—362).—The change in rheological properties of unleavened doughs caused by remixing was studied, with particular attention to recovery, and the effects of remixing of doughs which contained flour improvers such as bromate (**I**), iodate (**II**) and *N*-ethylmaleimide (**III**). The addition of **III** or **II** results in doughs with little sensitivity and good recovery; **I** and O_2 are associated with high sensitivity and poor recovery. Differences between flour improvers that change rheological properties of dough are more important possibly than differences between oxidising and thiol-blocking reagents. (16 references.) S. G. Ayerst.

Conjugated fatty acids, tocopherols and keeping quality of Swedish crispbread. T. Halden and D. Karp (*Brot u. Gebäck*, 1960, **14**, 104—108).—Nutritional aspects of the lipid constituents of rye and crispbread are discussed and the effect of storage on them is considered. (10 references.) J. V. Russo.

Behaviour of active dry yeast [ADY] in breadmaking. J. G. Ponte, jun., R. L. Glass and W. F. Geddes (*Cereal Chem.*, 1960, **37**, 263—279).—Active dry yeast and compressed yeast were compared using baking tests, gassing power, and farinograph and extensograph studies. At 40° rehydration temp. ADY gave optimum baking performance, comparable with that of compressed yeast. Lower rehydration temp. caused leaching of various substances from the cells of ADY and considerably affected dough properties and baking performance. Compressed yeast was unaffected by temp. up to 40°. (12 references.) S. G. Ayerst.

Effect of the relative quantity of flour fractions on cake quality. D. H. Donelson and J. T. Wilson (*Cereal Chem.*, 1960, **37**, 241—262).—A cake flour was fractionated into water-solubles, gluten, starch tailings and prime starch. Flour blends using various proportions of the fractions were prepared, and cakes baked from them. By means of a central composite statistical design, a multiple regression equation was derived relating cake vol. to the relative proportions of the fractions making up the flours. The balance of flour components conditioned the contribution to cake structure of each component, and had a significant effect on the quality of the cake. (13 references.) S. G. Ayerst.

Leavening acids, their effect on shelf-life of cake mixes and cake grain. J. F. Conn and T. P. Kichline (*Cereal Sci.*, 1960, **5**, 143—147).—Cake mixes leavened with blends of monocalcium phosphate monohydrate and a slow-reacting sodium acid pyrophosphate (SAPP-RD) have a longer shelf-life than mixes leavened with blends of anhydrous monocalcium phosphate (AMCP) and fast-reacting sodium acid pyrophosphate (SAPP-40). Loss of soda on storage is mainly due to reaction with acids in the flour and milk; the difference in shelf-life between blends is due to loss of the leavener delay property of the AMCP on storage. The two Ca phosphates have similar effects in imparting a finer grain structure to the baked goods. E. C. Apling.

Kilning of cereal grains. Research Association of British Flour-Millers, J. B. Hutchinson and J. Thomlinson (B.P. 819,241, 17.8.54).—The grains are subjected to a no. of heating stages in each of which they are exposed to an upward moving stream of air heated to ~150° for ~5 min., the grains being withdrawn from the influence of the heated air between the stages. The grain is passed down a tower having perforated walls and an air chest at each end, and a succession of perforated floors. Means are provided within the tower for agitating the descending grain both vertically and laterally.
J. M. Jacobs.

Sugars and confectionery

Effects of ionising radiations on carbohydrates and related substances. L. J. McCabe (*Dissert. Abstr.*, 1960, **20**, 3957).—Solid carbohydrates underwent colour, organoleptic and other changes when irradiated. Sucrose in powder form or in 50% solution was hydrolysed to an extent increasing with dosage of radiation; about 10% was transformed to non-reducing substances. Yield of invert sugar was higher at lower temp. Decomposition of sucrose by soft *X*-rays was less than by cathode and γ-rays. Methyl α-D-glucopyranoside was hydrolysed to D-glucose by cathode rays. Sorbitol irradiated in aq. solution yielded glucose, gulose, arabinose and xylose. D-Mannitol yielded mannose and arabinose, and at high dosages some mannuronic acid. D-Fructose yielded three unidentified sugars, probably ketoses. Starch was extensively degraded, yielding dextrins and sugars. Browning in sugar–amino-acid systems increased with increasing dosage of radiation; pre-irradiation, especially of the sugar, enhanced browning.
M. D. Anderson.

Microbiological assay of glucose as applied to starch hydrolysates. M. D. Smith, M. W. Radomski and J. J. Kagan (*Analyt. Chem.*, 1960, **32**, 678—680).—The method is based on the ability of *Lactobacillus casei* to ferment glucose selectively in a mixture of sugars found in starch hydrolysates. The response is determined by titration with standard alkali and the dose–response curve is linear up to 4 mg. of glucose. A heavy-inoculum technique shortens the incubation time to 2 hr. and is sensitive to small quantities of glucose. Recoveries averaged 100·1% at the 1 mg. level.
G. P. Cook.

Removal of interfering ions in determination of betaine in sugar-beet juices and plant material. A. Carruthers, J. F. T. Oldfield and H. J. Teague (*Analyst*, 1960, **85**, 272—275).—The betaine solution is applied to a column of a mixture of De-Acidite FF (OH^-) and Amberlite IRC-50 (H^+). The effluent is assayed conventionally for betaine, e.g., by pptn. with ammonium reineckate, the removal of reineckate as Ag salt and titration of betaine with aq. NaOH. The method is applicable to aq. extracts of chopped plant material.
A. O. Jones.

Maple syrup. XIII. Sterilising effect of sunlight on maple sap in transparent tubes. H. A. Frank and C. O. Willits (*Appl. Microbiol.*, 1960, **8**, 141—145).—Germicidal effect is related to transparency of tubes to u.v. radiation; environmental temp. is also a factor. (27 references.) C. V.

Composition of honey. V. Separation and identification of organic acids. E. E. Stimson, M. H. Subers, J. Petty and J. W. White, jun. (*Arch. Biochem. Biophys.*, 1960, **89**, 6—12).—Acids from clover honey were isolated by ion-exchange adsorption and separated by silicic acid partition chromatography and ion-exchange chromatography with six solvent systems. I.r. spectra of the Na-salts were examined. Those definitely identified were, acetic, formic, citric (**I**), gluconic (**II**), lactic, malic, pyroglutamic acids. Oxalic acid was tentatively identified. The principle acid was **II**, not **I** as previously proposed. (28 references.) C. V.

Fermentation and Alcoholic Beverages

Kinetics of the continuous alcoholic fermentation of blackstrap molasses. W. Borzani, M. Falcone and M. L. R. Vairo (*Appl. Microbiol.*, 1960, **8**, 136—140).—It is now shown that the kinetic order of the process is ~0 to 0·5; this indicates that the previous value of $n = -1$ is erroneous. (22 references.) C. V.

Chromatography of the colouring matter of hybrid red wines. Jaulmes and Ney (*Ann. Falsif., Paris*, 1960, **53**, 180—183).—A paper chromatographic method for the analysis of the colouring matter in red wines is described, using Schleiches and Schüll paper (2043) and a mixture of H_3PO_4, AcOH, H_3BO_3 and water as the developing solvent. Development takes ~2 hr. and the spots are examined under u.v. light. A scale of reference was prepared using the synthetic colour pinacyanol. J. V. Russo.

Brewing water. P. Kolbach (*Mschr. Brauerei*, 1960, **13**, 77—86).—The effects of the salts present in brewing waters on the quality of the beer are reviewed in the light of present brewing technique: covering (i) the influence on acidity of wort and beer; (ii) effects independent of acidity. The relations between pH-raising and pH-lowering ions as expressed by residual alkalinity, and their effects on the pH of the beer, are considered; and the effects of salts and their ions on the beer flavour are described. About half the salts in the beer are derived from the malt. (40 references.) C. L. Hinton.

Significance in brewing technology of reactions between water salts and malt phosphates. H. Waller and G. Krauss (*Brauwissenschaft*, 1960, **13**, 171—177).—In experimental brewings with demineralised water containing (separately) each of the six salts causing permanent or temporary hardness, approx. 70% of the $CaCO_3$ or $MgCO_3$, and 28% of the $CaCl_2$ or $CaSO_4$ are precipitated as phosphates; comparatively little of the $MgCl_2$ or $MgSO_4$ is precipitated. The effects of the salts on buffering at various pH, and of the residual alkalinity on brewing results are examined. P. S. Arup.

Determination of tannin in the course of brewing. G. Krauss and H. Egner (*Brauwissenschaft*, 1960, **13**, 178—180).—The methods of Owades *et al.* and of Harris and Ricketts (described) are suitable for the determination of tannins and leucoanthocyanins, respectively. An account is given of the changes in content of these substances observed during two brewings. P. S. Arup.

Spectrophotometric measurement of colour of beer. G. Krauss, H. Egner and A. Rebmann (*Brauwissenschaft*, 1960, **13**, 181—183).—Results obtained in collaborative tests for 28 pale beers by the EBC method are compared with those obtained by measurement at 430 mμ in the Hellige comparator, with corrections (if necessary) for turbidity measured at 700 mμ. The latter method is found to give satisfactory results. A formula is given for conversion of the spectrophotometric results into EBC units. P. S. Arup.

Detection of stabilisation of beer. K. Silbereisen (*Mschr. Brauerei*, 1960, **13**, 73—77).—Methods of detection of proteolytic enzymes, protein-precipitants, reducing agents and preservatives added as stabilisers to beer are discussed, with particular attention to the difficulties of drawing conclusions as to the presence or absence of these additions. (26 references.) C. L. Hinton.

Ultrasonic experiments on the hop double extraction process. E. Schild and H. Weyh (*Brauwissenschaft*, 1960, **13**, 206—210).—Experiments aimed at economising on hops without affecting the quality of the resultant beer are described. Double extraction processes with and without ultrasonic influences are compared and contrasted and the beers subjected to flavour and analytical tests. 33% of the hops can be saved without affecting the quality of the beer if the ultrasonic process is used. J. V. Russo.

Behaviour of strontium-89, caesium-137 and fission product contamination during brewing of Pilsner beer. H. Bergh, H. Kringstad and B. Ottar (*Bryggmesteren*, 1959, **16**, 233—241; *Brauwissenschaft*, 1960, **13**, 194—195).—Known amounts of the radioactive materials were added to the mashes in two experimental brewings. The average % of the added matter found in the beer were, for ^{89}Sr 10, for ^{137}Cs 70, and for the fission product contamination 12·5. The bulk of the ^{89}Sr and of the fission products was removed with the spent grains. P. S. Arup.

Fruits, Vegetables, etc.

Formation of pyrrolidonecarboxylic acid in processed fruit and vegetable products and its effect on flavour. A. A. Mahdi (*Dissert. Abstr.*, 1960, **20**, 4072—4073).—Determinations of glutamic acid, glutamine and pyrrolidonecarboxylic acid (**I**), in canned foods before and after sterilisation, and during storage, showed that content of glutamic acid remained stable throughout. **I** was absent from products having no glutamine, and was formed from glutamine at a rate increasing with temp. In foods of low and medium acidity processed at 240° to 260°F, all free glutamine was converted to **I** during sterilisation. In foods of high acidity processed below 212°F, glutamine was only partly converted into **I** during sterilisation, and the reaction continued during storage, at a rate depending on temp. **I** caused off flavour in sweet maize and tomato juice at concn. >0·05%, and in peas and beans at a higher concentration; it had little effect on taste panel judgements of beetroot flavour. The content of **I** in most canned fruits and vegetables was low. M. D. Anderson.

Residual effects of insecticides on fruit and vegetables. S. Dormal (*Span*, 1960, **3**, 77—80).—A review. (20 references.) E. G. Brickell.

Kinetics of colour, ascorbic acid and total acid diffusion in frozen syrup-packed raspberries. D. G. Guadagni, S. H. Kelly and L. L. Ingraham (*Food Res.*, 1960, **25**, 464—470).—A kinetic theory for distribution of colour and acid between raspberries and syrup is postulated. Diffusion of these constituents follows a first-order process with rate constants which appear to obey the Arrhenins equation. An equation which fits experimental data obtained from commercial samples of frozen berries is derived. Various parameters in the equation are discussed with respect to their significance in the estimation of temp. history in frozen raspberries. E. M. J.

Growth of food spoilage bacteria in banana puree. E. N. Bilenker and C. G. Dunn (*Food Res.*, 1960, **25**, 309—320).—Five types of spoilage bacteria were tested by dispersal in samples of sterile banana puree. The puree supported vigorous growth of *Bacillus coagulans*, but inhibited that of Thermophilic anaerobe No. 3814, Putrefactive anaerobe No. 3679 and *Clostridium botulinum* No. 62A and No. 213B. The banana puree was neutralised prior to inoculation with the two strains of *C. botulinum*, but the bacteriostatic effect persisted. (14 references.) E. M. J.

Sub-tropical fruits in Israel. II. Citrus fruits. A. Comelli (*Fruits d'outre mer*, 1960, **15**, 129—138).—Exports of citrus fruits from Israel were: 1938/9, 15 million cases; 1948/9, 4 million; 1958/9, 10 million. Methods of cultivation and irrigation are outlined. Infestations with scale insects have been very successfully controlled by importation from Hong Kong of the small wasp *Aphytis lignanensis*. The fruit fly *Ceratitis capitata* is controlled by dusting with malathion mixed with hydrolysed protein. Packing is well organised and highly mechanised. The fruit is washed in a solution of borax or Na phenylphenate, containing also ethylene dibromide to destroy *Ceratitis*; it is then washed with soap, rinsed, waxed, dried and polished. M. D. Anderson.

Toxicological study of diphenyl: citrus fungistat. A. M. Ambrose, A. N. Booth, F. DeEds and A. J. Cox, jun. (*Food Res.*, 1960, **25**, 328—336).—Inhibition of growth of male and female rats on dietary levels of 0·5 and 1·0% of diphenyl is due to decreased food consumption, possibly because of decreased palatability, rather than direct toxic action. Haemoglobin values on 1·0% level decreased at 300 days (in males) and 400 days (in females) and on 0·5% at 500 and 600 days respectively. Kidney lesions occurred. E. M. J.

Mycelial efflorescences on fruits. C. and M. Moreau (*Fruits d'outre mer*, 1960, **15**, 239—241).—The formation of mycelial growths on two types of fruit and methods of inhibiting their formation by reduction of temp. and humidity during storage are discussed. J. V. Russo.

Constituents of crystalline deposits on dried fruit. M. W. Miller and C. O. Chichester (*Food Res.*, 1960, **25**, 424—428).—These deposits were determined by paper chromatographic techniques: on prunes and figs they contained glucose and fructose, traces of citric and malic acids and of lysine, asparagine and aspartic acid;

on apricot and peach, in addition, sucrose and large amounts of asparagine and aspartic acid; on raisins deposits were similar to those on figs but with large amounts of tartaric acid in addition. No yeasts were found. E. M. J.

Pilot-plant studies on the preparation of crude papain from raw papaya. G. V. Krishnamurthy, B. S. Bhatia, Girdhari Lal and V. Subrahmanyan (*J. Sci. Fd Agric.*, 1960, **11**, 433—436).—The yields and total solids contents of the latex of six separate tappings of 20 batches of papaya fruit and the yields and activity of the crude papain obtained from the sun- or vac.-dried latex are presented. The effects of chemical treatment of the latex (addition of K metabisulphite, 0·5%, alone, or + thymol, 0·2%) on the activity of the crude papain are described. E. M. J.

Nitrogen compounds of cabbage. I. Relation of non-protein to total nitrogen with special reference to essential amino-acids. E. G. Kelley, R. M. Zacharius, S. Krulick and R. B. Greenspun. **II. Chromatographic analysis of non-protein nitrogen.** R. M. Zacharius, E. G. Kelley and J. J. McGuire (*Food Res.*, 1960, **25**, 401—413, 414—418).—I. The distribution of total N and Van Slyke amino-N in protein and non-protein fractions of cabbage was studied and the amounts of "ten" essential amino-acids in whole cabbage and in both fractions after separation by electrodialysis and other methods were determined. Wisconsin and Danish strains of Copenhagen Market cabbage were used. Total solids and total N values of the two strains differed by 30% and 16% respectively, but the extractable solids and total N differed by only 2%. After electrodialysis nine amino-acids were recovered quant. (±10%) in cathode and residue fractions, lysine recovery was somewhat variable, tryptophan was partially destroyed. (46 references.)

II. The major amino-N compounds of non-protein N fraction of cabbage are: (i) glutamine, (ii) *s*-methylcysteine sulphoxide and (iii) arginine. These three compounds make up 43% of the Van Slyke N of the fraction. Individual components (23) assayed by chromatographic method account for 76% or the Van Slyke N-value and 60% of the Kjeldahl value. (16 references.) E. M. J.

Quality of cabbage dehydrated after chemical or steam inactivation of enzymes. R. U. Makower and M. M. Boggs (*Food Technol.*, 1960, **14**, 295—297).—In cabbage infiltrated with acid-ethanol–surfactant mixture, enzymes were as effectively inactivated as in samples blanched in steam, and residual colour, flavour and ascorbic acid were as effectively stabilised during storage for 6 months at a raised temp. Rehydration resulted in large losses of ascorbic acid in all samples. E. M. J.

Application of orthogonal polynomials to data on palatability. J. N. Eisen, A. B. Parks and E. H. Dawson (*Food Technol.*, 1960, **14**, 237—239).—Panel mean scores for texture of stems of fresh broccoli cooked electronically for four equally spaced times were evaluated. E. M. J.

Changes of vitamin C in vegetables kept in cold storage. K. Morimoto, H. Matsumuro, M. Shimizu and H. Ikarimoto (*Annu. Rep. nat. Inst. Nutr., Tokyo*, 1959, pp. 92–93).—Retention of vitamin C in green vegetables kept at 0° was, at 3 and 7 days respectively: cucumber 75 and 65%; Chinese cabbage 94 and 86%; cabbage 93 and 91%; Japanese radish 88 and 85%; broccoli 100 and 96%. Retention in head lettuce at 3 days was 48%. M. D. Anderson.

Loss of vitamin C by home cooking. V. K. Morimoto, H. Matsumuro, M. Shimizu and Y. Matsumoto (*Annu. Rep. nat. Inst. Nutr., Tokyo*, 1959, pp. 94—95).—Retention of vitamin C in green peppers after cooking in various ways was: 86 and 97% for boiling in fresh and salted water respectively, and 70 and 1% when afterwards fried; 44% for boiling after frying; 92% for frying in deep oil; and 59% for frying in batter (of which 9% had moved into the batter). M. D. Anderson.

Influence of blanching conditions on sloughing, splitting and firmness of canned snap beans. J. P. Van Buren, J. C. Moyer, D. E. Wilson, W. B. Robinson and D. B. Hand (*Food Technol.*, 1960, **14**, 233—236).—Beans blanched at temp. between 150—210°F showed tendency to slough and split with rise in temp. Below 180°F increased blanching time led to less sloughing. General firming effect of moderate temp. blanching continued in the interval between blanching and retorting. Tendency to slough was increased by soaking in solutions of oxalate or Calgon prior to blanching; decreased by aq. $CaCl_2$. E. M. J.

Factors affecting the blanching of green beans. J. O. Mundt and I. E. McCarty (*Food Technol.*, 1960, **14**, 309—311).—Of several factors studied the variety of bean is the most influential in determining bleaching time. At 205°F a difference of 22 sec. exists in time of inactivation between the most easily blanched and the most resistant varieties. Very small residuals of peroxidase (10—15 units as measured by the Klett-Summerson colorimeter) will result in the effects of underblanching when the beans are in prolonged storage. (11 references.) E. M. J.

Isolation and partial characterisation of a volatile essential oil fraction from bitter canned carrots. R. S. Shallenberger, J. D. Atkin and J. C. Moyer (*Food Res.*, 1960, **25**, 419—423).—By distilling bitter carrots (122 kg.) and extracting with ether, a highly refractive dense, pale yellow oil with a harsh, hot flavour was obtained. On the basis of observations the oil was considered to be a sesquiterpene or mixture of sesquiterpenes. (14 references.) E. M. J.

Red pigment of the root of the beet (*Beta vulgaris*) as a pyrrole compound. R. G. Peterson and M. A. Joslyn (*Food Res.*, 1960, **25**, 429—441).—The literature on betanin is reviewed. Betanin is not a nitrogenous anthocyanin as previously considered; a proposed explanation that it is a pyrrole compound is supported by experimental data, including spectral evidence, type reactions and breakdown products. A yellow N-free pigment changeable into a N-free red pigment may be a furan analogue of the pyrrole pigments. The variety of *B. vulgaris*, Green Top Bunching, yielded pure pigment easily. (19 references.) E. M. J.

Carotene distribution in the sweet potato. R. M. Ampey (*Dissert. Abstr.*, 1960, **20**, 3482).—The concn. of carotene in sweet potato varieties was in the order: Queen Mary > Porto Rico Unit I > Porto Rico. Within the root, the concn. was in the order: stem end > centre > root end. The core usually contained more carotene than the peelings, but concn. in the latter was considerable. Synthesis of carotene continued after harvest, and it became more evenly distributed. M. D. Anderson.

Egyptian foods. Calcium, phosphorus and iron content of popular vegetables. S. R. Morcos and M. M. Amin (*Egypt. J. Chem.*, 1959, **2**, 367—373).—Analytical data for *Allium kurrat, Daucus carota, Eruca sativa, Mentha spicata, Petroselinium crispum* and *Raphanus sativus* show these, in general, to be good sources of Ca. A. G. Pollard.

Semi-micro determination of calcium and of magnesium in vegetable samples. J. Dumes and C. Egoumenides (*Fruits d'outre mer*, 1960, **15**, 233—238).—A complexometric method for the determination of Mg after separation of Ca by oxalate pptn., using Na_2 ethylenediaminetetra-acetate as the chelating agent is described. The effect of interfering ions: heavy metals (Fe, Mn and Al) is studied. Results are compared with those obtained by the classical NH_4 Mg phosphate method and with Beley and Cuemji's method in which, after pptn. of Ca, heavy metals are separated by diethyldithiocarbamate and the Mg titrated by oxine diazotisation. The classical method tends to give high results, whereas the other two methods give results in good agreement. J. V. Russo.

Non-alcoholic beverages

Effect of variety and maturity of fruit on acetylmethylcarbinol and diacetyl content of fresh citrus juices. E. C. Hill, F. W. Wenzel and R. L. Huggart (*Food Technol.*, 1960, **14**, 268—270).—Variety and maturity had an effect on diacetyl value of fresh citrus juices. Pineapple and Valencia orange and Marsh grapefruit juices showed consistent increases in diacetyl values as the fruit matured; Dancy tangarine, Hamlin orange and Duncan grapefruit showed an upward trend. The diacetyl values were due most probably to acetylmethylcarbinol. (12 references.) E. M. J.

Detection of β-carotene in orange juice. Anon. (*Fruchtsaft-Industr.*, 1960, **5**, 181—182).—The method whereby the ratio of β-carotene to xanthophylls is determined, requires overnight saponification. A simple method is described in which the artificial emulsion of the added pigment is broken by centrifuging. The orange juice is diluted if necessary to 12° Brix., 50 ml. are centrifuged at 3000 r.p.m. for 15 min., 4 ml. of light petroleum (b.p. 65—110°) are added in such a way that only the drops floating on the surface are dissolved. If a yellow solution is obtained the absorption spectrum between 400 and 500 mμ is determined and compared with that of β-carotene in light petroleum. I. Dickinson.

Tea, coffee, cocoa

Carbohydrates of the coffee bean: isolation of a mannan. M. L. Laver (*Dissert. Abstr.*, 1960, **20**, 3955—3956).—A holocellulose insol. in 10% KOH, isolated from green coffee, was hydrolysed by anhydrous H_2SO_4, and yielded L-arabinose, D-glucose, D-galactose and D-mannose, in the proportions 1·0/2·2/1·8/6·2. This holocellulose was acetylated, and yielded $CHCl_3$-sol. and $CHCl_3$-insol. acetates; on hydrolysis both yielded arabinose, glucose, galactose and mannose. A fraction dissolved from the holocellulose by 18% NaOH yielded a mannan containing 95% of mannose. M. D. Anderson.

Analysis of coffee volatiles by gas chromatography. A. Zlatkis and M. Sivetz (*Food Res.*, 1960, **25**, 395—398).—Collection, separation by gas–liquid chromatography and identification by mass

spectrometry are discussed. The coffee samples considered were the coffee aroma essence fraction composed largely of aldehydes and sulphides and representing ~200 p.p.m. of the roasted coffee bean (**I**); and the dry vac. aroma fraction (ether extracted) which represents 0·1% of **I**. More than 30 volatile compounds contributing to aroma and flavour were isolated and identified, comprising mercaptans, aldehydes, ketones, esters, acids and heterocyclics.
E. M. J.

Rheology of cocoa butter. I. Effect of contained fat crystals on flow properties. C. Sterling and J. J. Wuhrmann (*Food Res.*, 1960, **25,** 460—463).—Purified cocoa butter is characterised by a slight structural η, even in absence of suspended particles. As fat crystals form this η effect is enhanced. Sub-microscopical aggregation of fat mol. causes increase of the η coeff., this effect being apparent before the formation of microscopically visible fat crystals. (16 references.) E. M. J.

Extracts of coffee, malt coffee, chicory or mixtures thereof. W. K. Titkah and A. C. H. G. N. Voet (B.P. 819,611, 5.7.57. Neth., 6.7.56).—In the prep. of an extract of coffee, malt coffee, chicory or mixtures thereof, batches of the product to be extracted are periodically traversed in countercurrent flow by (cold) water, such that the extract is successively left in contact for a predetermined time with progressively more-extracted batches. The final extract may be thickened (e.g., with gelatin) to form a paste product.
F. R. Basford.

Milk, Dairy Products, Eggs

Investigating β-lactoglobulin and κ-casein solutions and their interactions by means of fluorescence polarisation. C. V. Morr (*Dissert. Abstr.*, 1960, **20,** 3943—3944).—The fluorescent dye 1-dimethylaminonaphthalene-5-sulphonyl chloride was conjugated with (i) β-lactoglobulin, (ii) κ-casein prepared by a urea fractionation method and by a constant pH method, and (iii) mixtures of β-lactoglobulin and κ-casein, either unheated, or heated at 65° for 1 hour. The properties of the proteins were not much altered by conjugation with the dye. Using the polarisation of fluorescence method, the apparent mol. vol. of β-lactoglobulin was calculated as 64,580 c.c./mole in 0·02 ionic strength phosphate buffer, and 98,310 c.c./mole in 0·10 ionic strength phosphate buffer. No change occurred when the concn. of lactoglobulin was varied. Addition of sucrose caused decrease of apparent mol. vol., and increase in relaxation time. The properties of κ-casein were also profoundly changed by addition of sucrose. The effects on κ-casein of the addition of other substances were investigated. Observations on solutions containing both lactoglobulin and casein suggest that heat-induced complexes between them have a central core of casein and an outer layer of lactoglobulin, stabilising them against further increases in size on additional heating. M. D. Anderson.

Biochemical alteration of milk proteins by γ- and ultra-violet irradiation. H. F. Kraybill, M. S. Read, R. S. Harding and T. E. Friedemann (*Food Res.*, 1960, **25,** 373—381).—The proteins of raw skim milk were studied. When the treated milk is injected into milk-sensitised guinea-pigs a decrease in anaphylactic response occurs; γ-irradiation causes the greater changes, increasing η and in sulphydryl and disulphide contents, but no formation of Me mercaptan. Electrophoresis of the casein and whey fractions shows that below 5·58 megarads, the component proteins are denatured or destroyed; at 5·58 megarads and above an immobile component appears in both; at 9·30 megarads, solution was very difficult. (26 references.) E. M. J.

Gas chromatography as a means of detecting odours in milk. J. D. Wynn, J. R. Brunner and G. M. Trout (*Food Technol.*, 1960, **14,** 248—250).—The degree of effectiveness of odour removal, the nature of the odoriferous substances removed and sensitivity of gas chromatography as a tool were studied. Approx. 85% of the measurable flavour components of milk were removed by vac. pasteurisation. Characteristic gas chromatograms were obtained for volatile substances in milk from cows fed lucerne silage, onion tops or beet tops and gas chromatography offered a valuable means of measuring the effectiveness of vac. pasteurisation in removing volatile flavour components of milk. E. M. J.

Vitamin B_6 activity of heat-sterilised milk. F. W. Bernhart, E. D'Amato and R. M. Tomarelli (*Arch. Biochem. Biophys.*, 1960, **88,** 267—269).—An S-containing compound with varying amounts of vitamin-B_6 activity was formed by the reaction between pyridoxal and cysteine. Its presence in evaporated heat-sterilised milk was identified by paper chromatography. (14 references.) C. V.

Structure of a sulphur-containing compound with vitamin B_6 activity [in milk]. G. Wendt and F. W. Bernhart (*Arch. Biochem. Biophys.*, 1960, **88,** 270—272).—The compound formed during heat sterilisation of milk is a di-4-pyridoxyl disulphide. (11 references.)
C. V.

Industrial refrigeration and alterations of milk and milk products. I. J. Moreno Calvo (*Rev. Cienc. apl.*, 1960, **14,** 107—120).—This review surveys the composition and physico-chemical structure of milk, the bacterial contamination and stability of milk in relation to refrigeration, and fundamentals of the industrial treatment of milk.
H. Friedmann.

Keeping quality of vacuum-dried whole milk. C. D. Degener (*Dissert. Abstr.*, 1960, **20,** 4072).—The flavour stability of vac.-dried whole milk in storage at 22° improved as fat content was decreased from 28 to 16%, and at 8% satisfactory flavour was retained throughout 180 days. Packaging with a glucose-oxidase-catalase O-scavenging system gave satisfactory flavour throughout 150 days at −14° and 12°, and up to 90 days at 22°. The metal-chelating agent salicylaldoxime, added to the conc. milk before drying, also improved flavour stability. Modified dried products made from pure butter oil and skim milk, with low phospholipid contents, developed a stale rather than an oxidised flavour during storage at 22°, and showed increased peroxide values. The deterioration of vac.-dried whole milk involves both fat and phospholipid.
M. D. Anderson.

Infant food from buffalo milk. VI. Large-scale production of roller dried infant food. VII. Shelf-life of roller dried infant food. M. R. Chandrasekhara, M. Narayana Rao, M. Swaminathan, D. S. Bhatia and V. Subrahmanyan. **VIII. Infant feeding trials with roller dried food.** M. R. Chandrasekhara, T. R. Doraiswamy, M. Narayana Rao, A. N. Sankaran, M. Swaminathan and V. Subrahmanyan (*Food Sci., Mysore*, 1960, **9,** 1—3, 3—5, 6—7).—VI. Roller drying is more economical in areas where only 10—20 thousand lb. of milk are available. The conditions of roller drying have been standardised. The buffalo milk was analysed within 2—3 hr. of milking for its fat content, non-fatty solids and methylene blue reduction time. The pasteurisation temp. was 85°. The fat content was adjusted to 2·5%, the milk was transferred to a stainless steel tank where a temp. of 60° was maintained. The water-sol. vitamins thiamine, riboflavin, niacinamide and pyridoxine were dissolved and added. The fat-sol. vitamins A, D and E were premixed with hydrogenated fat and redissolved in cream before adding to the milk. Na_2HPO_4 and Na citrate in solution and cane sugar (1 : 3 pt. of total milk solids dissolved and boiled for 5 min.) were added. The milk was then homogenised under pressure of 1000 lb./sq. in., preheated to 60° and fed on to roller dryers which were heated internally with steam at a pressure of 50 lb./sq. in. The dried milk was powdered and packed in 1-lb. tins.

VII. Samples of roller dried infant food with and without added Fe were prepared from the same batch of milk. Ferric citrate was added until the dried food contained 6 mg. per 100 g. of added Fe. The samples were packed in 8 oz. seamed unlacquered tins in air with min. head space and stored at 25—29° and at 37°. The water content, solubility, fat acidity, thiobarbituric acid value and thiamine content were determined and the samples were tested organoleptically. Both samples kept at room temp. had an expected shelf-life of 20 months and those kept at a higher temp., of 10 months. The thiobarbituric acid, which was a better guide than the peroxide value, increased steadily during storage and was in fair agreement with the organoleptic acceptability. Thiamine was lost to the extent of 15 and 25% respectively when stored at room temp. and at 37°.

VIII. At the beginning of the trials (on 17 infants under medical supervision) the wt. was plotted against age. The food contained 22% protein, 14% fat and was fortified with vitamins A and D and B-complex. All infants digested the food readily after consuming it with relish. No cases of vomiting were reported. The average rate of growth was satisfactory and was similar to that observed earlier on infants fed on spray-dried infant food.
I. Dickinson.

Caesium-137 in spray-dried Danish milk. P. G. Jensen (*Nature, Lond.*, 1960, **188,** 562—563).—Concn. of ^{137}Cs (measured by γ-scintillation spectrometry) increased slowly to ~20 μμc/g. of K in milks produced between 1949 and December 1958, and then rapidly to a max. of ~50 μμc during 1959. Low concn. of ^{137}Cs may be partly due to high concn. (~3·2g./sq. m. per annum) of fertiliser used in Denmark. Average rainfall was 60—75 cm.
W. J. Baker.

Dairy research in south Asia, 1953–9. C. P. Anantakrishnan and K. K. Iya (*Dairy Sci. Abstr. Rev. Art.* No. 89, 1960, **22,** 377—384, 427—435).—A review. (About 450 references.)
A. G. Pollard.

Factors in egg white which control growth of bacteria. J. A. Garibaldi (*Food Res.*, 1960, **25,** 337—344).—The rôle of conalbumin in controlling microbial (Gram negative) spoilage bacteria in egg white is discussed. When the conalbumin was saturated with

respect to Fe, all bacteria tested developed to dense populations in egg white ; the high pH of egg white shortly after the egg is laid was not inhibitory. In unsupplemented egg white the stability of the Fe conalbumin co-ordination compound increases as pH increases and Fe becomes less available to micro-organisms. Ovomucoid has no effect on rate or extent of growth of Gram negative bacteria. (19 references.) E. M. J.

Edible Oils and Fats

Vegetable oil based on polyunsaturated fatty acids : oil from grape pips, analytical characteristics and dietary qualities. P. Morand and J. Silvestre (*Ann. Falsif., Paris*, 1960, **53**, 193—203).—Oil from dried grape pips contains fatty acids, chiefly linoleic (>50%), oleic, palmitic and stearic, and unsaponifiable matter (chiefly tocopherols). Data on physical, chemical and stability tests show that the oil compares well with other vegetable oils used in cooking. (19 references.) J. V. Russo.

Determination of gallates in edible fats. W. Cassidy and A. J. Fisher (*Analyst*, 1960, **85**, 295—297).—The liquid sample (or solid sample with liquid paraffin) is shaken with MeOH and then warmed in water at 40°—45°. The upper layer is separated and the MeOH extraction is repeated. The combined extracts are shaken with $CaCO_3$ and filtered. An aliquot of the filtrate is shaken with acetone and powdered ferrous ammonium sulphate and extinction is measured at 580 mμ. This value multiplied by 0·622, 0·785 or 0·952 gives content of Pr^n, *n*-octyl or n-dodecyl gallate. A. O. Jones.

Meat and Poultry

Environmental factors affecting the quality of frozen meat. J. L. McBee, jun. (*Dissert. Abstr.*, 1960, **20**, 3685—3686).—Changes in amounts of coagulable N and amino-N in frozen ground beef, indicating degree of proteolysis and denaturation in the lean tissue, followed a consistent pattern, but were small ; changes in flavour were associated, not with protein degradation, but with changes in the fat. Organoleptic ratings for rancidity and desirability were closely correlated with 2-thiobarbituric acid values. Freezing ground beef or steaks did not increase tenderness, but sometimes slightly decreased it. Temp. of freezing did not affect tenderness. Warner–Bratzler shear determinations on cooked meat gave the best agreement of any mechanical method with organoleptic scores. Tenderness press evaluations of cooked meat also showed correlation. Warner–Bratzler shear, and tenderness-press determinations on raw meat, and micro-tenderness-press determinations on raw and cooked meat, were less useful. M. D. Anderson.

Methods of evaluating freeze-dried beef. L. J. N. Cole and W. R. Smithies (*Food Res.*, 1960, **25**, 363—371).—The criteria studied were : salt solubility, ATPase activity and electrophoretic mobility, and observations are reported on the sedimentation behaviour of the fraction extracted by strong salt solution. There are only small differences between frozen and freeze-dried beef. Exposure to temp. of 37° or above causes appreciable but not consistent differences in % of extractable actomyosin N. There was no marked decrease in the level of specific ATPase activity after drying. In general all of the freeze-drying techniques are sufficiently mild to avoid gross damage to the proteins. (13 references.) E. M. J.

Effect of various environmental conditions during ageing on shrinkage and organoleptic characteristics of beef and the efficacy of oxytetracycline for ageing beef. R. B. Sleeth (*Dissert. Abstr.*, 1960, **20**, 3686—3687).—An attempt was made to find conditions for ageing beef that would minimise shrinkage and microbial growth, while allowing rapid development of tenderness, flavour and aroma. Ageing under u.v. radiation, at 80°F or above, encouraged microbial growth on the surface of the meat, and deep spoilage. Injection of oxytetracycline intramuscularly at 0·5 to 6 h. *ante mortem*, and spraying the carcasses with oxytetracycline and nystatin, controlled bacterial growth during high-temp. ageing. Ageing temp. did not affect the rate of disappearance of antibiotic from muscle. Antibiotic in raw steak diminished considerably when the meat was cooked " medium rare," and disappeared when it was " well done." When meat containing small amounts of antibiotic was fed to rats for 5 weeks, there were no detectable residues in the rat tissues. M. D. Anderson.

Tenderness of beef as related to tissue components, age, stress and post-mortem biochemical changes. N. B. Webb (*Dissert. Abstr.*, 1960, **20**, 3458).—Increased tenderness during ageing of beef carcasses was associated with changes in pH, water-holding capacity and water-extractable N and minerals. Tenderness was not related directly to water-extractable Na, K, Mg, Ca, P or N. Tenderness decreased with degree of physiological stress before slaughter, and with age of cattle. In determinations of water-holding capacity, the only constituent of the expelled juice that appeared to be related to tenderness was Mg. The content of hydroxyproline in fresh beef muscle was correlated with tenderness (evaluated organoleptically). M. D. Anderson.

Beef quality. VIII. Observations on the nature of drip. A. Howard, R. A. Lawrie and C. A. Lee (*Commonw. sci. industr. Res. Org., Aust., Div. Fd Pres. Transp.*, 1960, tech. Paper No. **15**, 29 pp.).—Data relating to the effect of various treatments on the quality of frozen beef and the composition of drip fluids are presented. Quarter (**I**) and butchers' drip (**II**) (especially **I**) have high ratios of ash to total solids ; chloride to ash ; albumin to total protein ; and high contents of haemoglobin. Cube and laboratory drip (**III**) approximate in composition to muscle press juice. The solutes in **I** originate chiefly from extracellular spaces whereas **III** contains solutes originating from extracellular and intracellular spaces in the proportions as *in vivo*. **II** contains less than **I** and cube drip contains more than **III** of solutes of extracellular origin. Present in unhydrolysed muscle press juice and muscle extract are carnosine/anserine and α-alanine ; and in the hydrolysed fluids, histidine/lysine and β-alanine. In **I** and **II** the carnosine/anserine proportion is lowered, suggesting dilution by interstitial fluid which does not contain these peptides. Patterns obtained by paper electrophoresis and ultracentrifuge show small differences for **III**, muscle press juice and weep from fresh muscle, but **I** shows a different pattern, due to presence of albumin from extracellular fluid. (36 references.) E. M. J.

Analysis of myoglobin fractions on the surfaces of beef cuts. R. W. Dean and C. O. Ball (*Food Technol.*, 1960, **14**, 271—286).—Various transmittancy or " absorbancy ratio " and reflectance methods are discussed and results are described from the application of the reflectance ratio method to the surface of beef cuts packaged under various conditions in film materials, cans and jars. The results are detailed. E.g., samples vac. packaged in films of extremely low permeability or in cans or jars are characterised by the presence of less metmyoglobin and more oxymyoglobin than are samples in Cellophane. Presence of O_2 in excess in prepackaged beef after the first day of storage seems to cause formation of metmyoglobin and of small quantities of O_2 in vac. packaging to produce oxymyoglobin. Reduced myoglobin apparently present in large amounts as reservoir can be converted easily into either of the other pigments. It is these small amounts of metmyoglobin or oxymyoglobin that drastically affect colour of the meat. (18 references.) E. M. J.

Bone-taint in beef. II. Bacteria in ischiatic lymph nodes. P. M. Nottingham (*J. Sci. Fd Agric.*, 1960, **11**, 436—441 ; cf. J.S.F.A. Abstr., 1956, ii, 201).—A detailed study of the bacteria in ischiatic lymph nodes of freshly-killed cattle (162) was made ; only two outbreaks of bone-taint (involving four and two sides of tainted beef) were reported and tainted meat samples from both showed Gram-positive rods. Of 81 isolations, 47 were aerobes and 34 anaerobic spore-forming bacteria (clostridia). Coliforms, micrococci, achromobacter and pseudomonads were isolated from tainted meat and lymph nodes. The bacterial load in each lymph node was less after a period of high rainfall for the two months prior to slaughter ; evidence was that the micro-organisms gained entry into the animal before death. Spoilage can be prevented by chilling the carcass quickly enough to inhibit multiplication of the spoilage bacteria. (12 references.) E. M. J.

Production and prevention of irradiated odour in beef. P. A. Hedin, G. W. Kurtz and R. B. Koch (*Food Res.*, 1960, **25**, 382—388).—The odour arises from a water-sol., non-dialysable fraction which is a mixture of at least two electrophoretically separable proteins. The odour is associated with sulphydryl compounds ; cysteine and methionine could not be detected in the protein after irradiation, but Fe and P were found. The irradiated odour was released when water was added to the protein irradiated in the dry state. As chromatographically shown, 1-anthraquinonesulphonic acid, $AgNO_3$–bromophenol blue, 1-fluoro-2,4-dinitrobenzene, 3,5-dinitrobenzoyl chloride and PtI_4 formed deriv. with compounds present in the irradiated protein, but not with the original sample. (18 references.) E. M. J.

Influence of fat, moisture and cooking liquor on dried mutton mince. A. Howard and A. R. Prater (*Commonw. sci. industr. Res. Org. Aust., Div. Fd Pres. Transp.*, 1960, tech. Paper No. 18, 19 pp.). —Addition of cooking liquor, concentrated to ~24% solids, to the mince prior to drying, improves the meat flavour, tenderness and juiciness and reduces the rate of production of stale flavour. As an additive mutton liquor extract is superior to beef concentrate or commercial meat extract. Fat when present as a component of the tissue reduces the score for woolliness and particle size and increases juiciness and tenderness. E. M. J.

Effect of cooking and carcass part on the methionine and cystine content of chicken meat. J. L. Fry and W. J. Stadelman (*Food Res.*, 1960, **25**, 442—447).—Methionine and cystine were stable to the

effects of baking in a covered container; significant increases in both amino-acids occurred, as very small amounts were lost from the meat into the drip. Light meat contained most methionine, 2·97 g. per 16·0 g. of N. Liver contained most cystine, 2·12 g. per 16·0 g. of N. Skin contained least of both amino-acids. (13 references.) E. M. J.

Flavour differences in broths prepared from hormonised and non-hormonised turkeys. G. Bennett (*Food Technol.*, 1960, **14,** 231—232).—Judges were able to distinguish between 3% levels of fat homogenised into the broth in which it was cooked and between samples of the broth alone. The difference between broth prep. of control and hormonised turkeys is not caused by texture or flavour differences in the fat but by differences in the broth itself. Turkey flavour of broths was rated: hormonised having least amount of turkey flavour, control, and hormonised with added fat having most flavour. (11 references.) E. M. J.

Fish

Change of the vitamin A concentration in enriched fish sausage during storage in summer. H. Iwao, Y. Takai and A. Kenmoku (*Annu. Rep. nat. Inst. Nutr., Tokyo,* 1959, p. 77).—Fish sausage enriched with vitamin A by addition of conc. fish liver oil lost no vitamin A during storage for 72 days in the refrigerator, nor during 24 days at room temp.; there was a considerable loss after 72 days at room temp. Boiling did not affect the vitamin A content. M. D. ANDERSON.

Spices, Flavours, etc.

Effect of electron beam irradiation on the microbial content of spices and teas. P. A. Lerke and L. Ferber (*Food Technol.*, 1960, **14,** 266—267).—Data are given on the effect of exposure to 2 megarep from a resonant transformer electron beam generator on the microbial content of a variety of spices, flavouring herbs and several tea samples. Bacteria, yeasts, moulds were completely eliminated or reduced to insignificant levels. Black pepper lost most of the aromatic component; cinnamon lost the pleasing aromatic component but retained a more medicinal type of odour; cloves had a weaker characteristic odour. E. M. J.

Estimation of residual solvents in spice oleoresins. P. H. Todd, jun. (*Food Technol.*, 1960, **14,** 301—305).—A technique is reported for evaluating residual solvents at levels below 50 p.p.m. with accuracy of within ±12 p.p.m. Each solvent (e.g., methylene chloride, ethylene dichloride, trichloroethylene, hexane, methanol, isopropanol and acetone) was studied individually and in combination with other solvents. E. M. J.

Examination of lemon oil by gas–liquid chromatography. II. Hydrocarbon fraction. J. R. Clark and R. A. Bernhard (*Food Res.*, 1960, **25,** 389—394).—Examination of the terpene fractions from several cold-pressed California lemon oils revealed that there are between 10 and 12 distinct peaks in the gas–liquid chromatograms when separated on a LAC-2-R466 column. By determining the corrected retention vol. of known terpenes and comparing these with those of unknown peaks, a large no. of the terpenes present were tentatively identified. In the hydrocarbon fraction were: α-pinene, camphene, β-pinene, myrcene, D-limonene, terpinolene, γ-terpinene and *p*-cymene. A proximate analysis is given of the terpene fraction of a typical California lemon oil. (10 references.) E. M. J.

Analysis and composition of oil of lemon by gas–liquid chromatography. R. A. Bernhard (*J. Chromatography,* 1960, **3,** 471—476).—Conditions are given for the analysis of cold-pressed California lemon oil from which 22 components have been identified. Another 10 peaks were bunched together close to the start and have not been identified. G. BURGER.

Colouring matters

Colour of capsicum spices. W. D. Pohle and R. L. Gregory (*Food Technol.*, 1960, **14,** 245—247).—It is suggested that the colouring matter may be evaluated more accurately if the different instruments used are calibrated with, e.g., β-carotene, and the colour in the samples is expressed as equiv. to μg. of β-carotene per g. of sample. E. M. J.

Structure of δ-carotene. T. E. Kargl and F. W. Quackenbusch (*Arch. Biochem. Biophys.*, 1960, **88,** 59—63).—δ-Carotene (**I**) isolated as a crystalline, yellow-orange compound from tomatoes, possessed a monocyclic isoprene structure. The perhydro deriv. was identical with perhydro-γ-carotene. Alkaline isomerisation of **I** yielded γ-carotene and treatment with *N*-bromosuccinimide gave rise to dehydro-**I**. It is suggested that **I** is the α-ionone analogue of γ-carotene. (11 references.) C. V.

Food additives and their mutation-promoting effects. VI. Examination of permitted and originally suggested dyes in West Germany for the mutation effects on *Escherichia* [*Bacterium*] *coli.* H. Lück and E. Rickerl (*Z. LebensmitUntersuch.*, 1960, **112,** 157—174).—Strains of *B. coli* which can grow only in presence of streptomycin or lysine were grown in a suitable medium with and without added dyes. The no. of bacteria which underwent mutation, i.e. those which formed colonies in absence of streptomycin or lysine, were counted in a medium free of these substances. The method of prep. of samples is given. The concn. of the dyes (solubility permitting) was usually 0·5 g./100 ml. Results from 21 dyes are given. The "mutation fraction" which is the ratio of the no. of mutated bacteria to the whole no. of bacteria present, was calculated. Only Erythrosin had a mutation fraction which was 3—6 times higher than normal. Acridine Orange which is known to cause mutation in *Drosophila* (Clark, *Amer. Naturalist,* 1953 **87,** 295) was used as comparison and showed this no. at a concentration of only 0·0002%. Of the dyes studied only Chrysoin S and Erythrosin were found to inhibit the growth of *B. coli.* All dyes except Quinoline yellow, Chrysoin S, chlorophyllin and Erythrosin lost their colour when added in concentration of 1 mg./100 ml. to a growing culture of *B. coli.* (27 references.) I. DICKINSON.

Paper chromatography of water-soluble colours. J. C. Riemersma and F. J. M. Heslinga (*Mitt. Lebensm. Hyg., Bern,* 1960, **51,** 94—104).—Experiments aimed at finding a generally applicable method for the paper chromatographic determination of water-sol. colouring matter in foods show that the most easily reproducible results are obtained by using t-butyl alcohol : propionic acid : water (50 : 12 : 38) as the mobile phase. (12 references.) J. V. RUSSO.

Preservatives

Effect of different length of chill periods with chlortetracycline [CTC] and different holding conditions on the shelf life of dressed [poultry] fryers. E. O. Essary, L. E. Dawson and W. L. Mallmann (*Food Technol.*, 1960, **14,** 286—289).—Total bacterial counts were highest for fryers chilled 24 hr. Total count on controls (chilled 2 hr.) was lower than on the 24 hr. chill group but higher than on groups chilled in CTC for 2 and 12 hr. In most cases, 2 hr. chill periods, with or without CTC, gave lower total counts and higher raw odour scores than did 12- and 24-hr. chill periods. Total bacterial counts were lower and raw odour scores were higher for a longer storage period for CTC-treated birds, chilled for 2, 12 and 24 hr. and held in tray packages than for similarly treated birds held in flaked ice for similar periods of time. (10 references.) E. M. J.

Food Processing, Refrigeration

Canning processes. III. Cooling phase of processes for products heating by conduction. P. W. Board, N. D. Cowell and E. W. Hicks (*Food Res.*, 1960, **25,** 449—459).—The contribution of the cooling phase to the lethal value of a process (F_c) varied widely with the nature of the pack and the processing conditions. This phase was evaluated by (*a*) measurements in the laboratory retort, (*b*) in industrial retorts and (*c*) practical considerations. In cans which cooled predominantly by conduction, F_c calculated by Gillespy's method agreed with F_c calculated graphically; calculated by Ball's method it was less than F_c calculated graphically. In industrial retorts the value of F_c calculated by the general method (Bigelow *et al.*) varied with position in the retort. The quality of some sensitive products could be improved by control of cooling conditions to ensure a high F value and a corresponding reduction of the length of the heating phase. (13 references.) E. M. J.

Preservation of boiled rice. S. Nagai and A. Sato (*Annu. Rep. nat. Inst. Nutr., Toyko,* 1959, pp. 88—89).—Cooked rice is stored in a large wooden tub, or, increasingly, in a vac. jar. Records were made of temp. and bacterial counts of the rice in both containers. In cold weather, rice in the wooden tub cooled rapidly, and bacterial multiplication was checked; in the vac. jar, temp. fell more slowly, bacterial multiplication did not begin quite so soon, but continued much more actively for much longer. In warm weather, the start of bacterial multiplication in the jar was delayed, but the no. of bacteria in the tub rose more rapidly. M. D. ANDERSON.

Evaluating tomato varieties for processing. P. W. Board (*Commonw. sci. industr. Res. Org., Aust., Div. Fd Pres. Transp.*, 1960, tech. Paper No. 17, 11 pp.).—Field design and sampling procedures are described for use in variety trials for evaluation of tomatoes for processing (from the viewpoint of the comminuted product) in terms of yield, chemical composition and organoleptic qualities. The results obtained by application of these procedures to four crops including nine varieties are reported. The variety Urbana was superior to all others in yield and solids content. (10 references.) E. M. J.

Heat resistance and growth characteristics of micro-organisms isolated from semi-perishable canned hams. W. L. Brown, C. A. Vinton and C. E. Gross (*Food Res.*, 1960, **25,** 345—350).—Most *Bacilli* spores are destroyed before a process of F_0 0·6 is reached. Small no. of anaerobic spores are able to withstand a process of F_0 1·0 but are destroyed before the heating level has reached F_0 3·0. A cocci culture isolated from semi-perishable ham is able to withstand a process of 40 min. at 150°F. The same culture when grown in meat can survive a process of 400 min. at 150°F. The relationship between types of organisms present after processing in semi-perishable hams is discussed. (F_0 = min. at 250°F.) E. M. J.

Thermal and non-thermal degradation of Acronize chlortetracycline in fish and some shellfish. E. F. Kline, A. Abbey, M. C. Firman and P. F. Hopper (*Food Technol.*, 1960, **14,** 305—308).—For average cooking conditions (e.g., frying of flounder fillets in deep fat for 10 min. completely degraded the antibiotic) and normal canning, pickling and smoking procedures no chlortetracycline is found if the antibiotic is used at recommended levels. (13 references.) E. M. J.

Radiation sterilisation of food. I. Procedures for evaluation of the radiation resistance of spores of *Clostridium botulinum* in food products. C. F. Schmidt and W. K. Nank (*Food Res.*, 1960, **25,** 321—327).—Experimental details and data are presented. The occurrence of non-toxic spoilage yielding either toxic or questionably toxic cultures was noted. Although a mixture of strains, 3 Type A and 2 Type B was used as inoculum, all survivors produced Type A toxin. Approx. the same resistance of the inoculum was found in chicken pieces, steak and whole kernel maize; survival at 2·8 megarads, no survival at 3·0 megarads, radiation D value (calculated from partial spoilage data) 0·33—0·35 megarad. In pork loin resistance was slightly lower but on the borderline of significance. On the basis of comparison of D values, resistance in green beans of the suspension appeared significantly lower by 40—45%. E. M. J.

Preservation of fish with ionising radiation: bacterial studies. D. P. MacLean and C. Welander (*Food Technol.*, 1960, **14,** 251—254).—The effects of ionising radiations at levels varying from 0·27 to 2·0 megarads on Pacific cod (*Gadus macrocephalus*) during storage in melting ice were studied. In unirradiated control samples typical spoilage micro-organisms: *Micrococcus, Achromobacter, Flavobacterium* and *Corynebacterium* predominated; *Sarcina, Pseudomonas, Alcaligenes, Mycoplana, Protaminobacter, Bacillus,* yeast (*Torulopsis*), *Aerobacter* and *Streptococcus* were in small no. In irradiated samples *Micrococcus, Sarcina, Achromobacter, Flavobacterium* and *Corynebacterium* were predominant; *Alcaligenes, Bacillus,* yeast (*Torulopsis*) and *Mycoplasma* were less numerous. *Micrococcus* were resistant under conditions studied but did not appear to be typical food poisoning organisms. (14 references.) E. M. J.

Time-temperature tolerance of frozen foods. XXII. Relationship of bacterial population to temperature. H. D. Michener, P. A. Thompson and W. C. Dietrich (*Food Technol.*, 1960, **14,** 290—295).—Bacterial counts on vegetables (peas, beans, cauliflowers and spinach) were followed after exposure to various temp. E.g., at initial temp. −20°F the count ranged from $<10^5$ to $>10^7$ per g.; at temp. from −10° to 20°F, bacterial growth never occurred but large no. survived (~50%). Flavour deterioration occurred. Bacterial growth took place at temp. of 25°F and above. Population doubled in 8 weeks at 25 and 30°F and in less than a week at 40°F. Off flavour was detected before the count had increased significantly. (29 references.) E. M. J.

Ice crystal formation in biological materials during rapid freezing. J. L. Stephenson (*Ann. N.Y. Acad. Sci.*, 1960, **85,** 535—540).—A mathematical discussion. (12 references.) C. V.

Freezing injury of plant tissue. J. Levitt (*Ann. N.Y. Acad. Sci.*, 1960, **85,** 570—575).—Stresses are caused by direct pressure of ice formation, by ice formed within the cell and by collapse and subsequent expansion of the cells due to dehydration. The circumstances and results of these changes are discussed. C. V.

Physical factors implicated in the death of micro-organisms at sub-zero temperature. P. Mazur (*Ann. N.Y. Acad. Sci.*, 1960, **85,** 610—629).—A review. (44 references.) C. V.

Principles of freeze drying. H. T. Merryman (*Ann. N.Y. Acad. Sci.*, 1960, **85,** 630—640).—A review. (10 references.) C. V.

Theory and practice of freeze drying. T. W. G. Rowe (*Ann. N.Y. Acad. Sci.*, 1960, **85,** 641—681).—A very detailed discussion. (27 references.) C. V.

Freezing, freeze-drying and freeze-concentration of foodstuffs. R. Gane (*Research*, 1960, **13,** 207—211).—Applications are described. (23 references.) O. M. Whitton.

Packaging

Effect of in-package desiccation on the keeping quality of air-dried mutton mince. A. R. Prater and G. G. Coote (*Commonw. sci. industr. Res. Org., Aust. Div. Fd Pres. Transp.*, 1960, tech, Paper No. 16, 12 pp.).—The study was made in presence and absence of O_2 at storage temp. of 77 and 99°F over a storage period of 2 years. Reduction of the moisture content of the air-dried precooked mutton mince to <2% by in-package desiccation (CaO) improved the keeping quality at 77 and 99°F in air and N_2 packs, but it is doubtful whether the additional cost involved for the small improvement would justify commercial adoption of the process. E. M. J.

Rubber hydrochloride film for packaging prunes and the like. Goodyear Tire & Rubber Co. (B.P. 818,940, 27.5.57. U.S., 11.10.56).—The film, at least one surface of which includes a polyethylene glycol alkyl thio-ether which is a non-ionic surface-active agent with a mol. wt. of 422—462, contains 100 parts by wt. of rubber hydrochloride, 7·5 parts of butyl stearate, 5 parts of di-isobutyl adipate and 2·5 parts of polyethylene glycol t-dodecyl thio-ether. E. Enos Jones.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Nutritional value of defatted fish flour. A. B. Morrison and J. A. Campbell (*Canad. J. Biochem. Physiol.*, 1960, **38,** 467—473).—Weanling rats were fed diets (otherwise adequate) containing various protein levels supplied by fish flour or casein, or they were fed whole wheat flour or white bread, with fish flour added. The effects of these diets on liver, kidney and adrenal wt., wt. gain, liver lipids and protein efficiency ratios (P.E.R.) are discussed. The P.E.R. values for bread and flour diets were found to be a direct function of the lysine content of the protein. Defatted fish flour (of high quality protein) is of particular value in supplementing diets deficient in lysine. (20 references.) S. G. Ayerst.

Nutritional study on bread-yeast protein. H. Iwao, Y. Takai and T. Kuga (*Annu. Rep. nat. Inst. Nutr., Tokyo*, 1959, pp. 72—73).—Rats received, as source of protein in an otherwise complete diet, 1·6% of N in yeast dried (i) in cool air, (ii) in hot air, (iii) after autolysis, (iv) after hydrolysis. Rats receiving (i) and (iv) lost wt. Gains of wt. were small on (ii), and on (iii) were about half those of rats receiving an equiv. amount of casein. Figures are given for the contents of moisture, fat and protein in the carcasses of the rats. M. D. Anderson.

Supplementary value of composite protein foods containing a blend of coconut meal, groundnut flour and Bengal gram to poor rice diet. P. K. Tasker, K. Krishnamurthy, R. Rajagopalan, M. Swaminathan and V. Subrahmanyan (*Food Sci., Mysore*, 1960, **9,** 84—86).—Compositions (2) of protein food were prepared by mixing low-fat groundnut flour, coconut meal and Bengal gram flour in different proportions. They were fortified with vitamins A, D, thiamine, riboflavin and Ca phosphate. Results of analysis and growth tests on rats fed (*a*) a poor rice diet, (*b*) poor rice diet of which 12·5% is substituted with coconut meal, (*c*) 12·5% substituted with protein food I, and (*d*) protein food II are given. The average weekly increase in wt. (g.) was: (*a*) 4·74, (*b*) 7·30, (*c*) 14·32, (*d*) 13.59. The composite protein foods studied form a good supplement to poor diets of people in regions where coconut meal is available in large amounts. I. Dickinson.

Nutritive value of composite protein foods based on blends of groundnut, soya-bean and sesame flours. K. Krishnamurthy, T. N. Ramakrishnan, R. Rajagopalan, M. Swaminathan and V. Subrahmanyan (*Food Sci., Mysore*, 1960, **9,** 86—88).—Compositions (2) of the above protein foods were prepared and fortified with vitamins A, D and B. The protein efficiency ratios of the proteins as compared with that of skim milk powder proteins were determined by the rat growth method. Average weekly gain in body wt. was for (*a*) rats fed protein food I, 13·7 g., (*b*) II, 16·7 g., and (*c*) skim milk powder, 15·8 g. These results show that diets containing protein from the prepared protein foods are as effective as that containing skim milk powder. I. Dickinson.

Supplementary effects of amino-acids to the protein of Japanese diet. I. II. III. E. Tamura, H. Baba and A. Tamura (*Annu. Rep. nat. Inst. Nutr., Tokyo*, 1959, pp. 35—36, 37—39, 40—41).—I. Rats were fed diets resembling the average diet of the Japanese people, which is low in tryptophan and S-containing amino-acids, according to the provisional recommendations of FAO. Supplements of tryptophan increased the growth rate of the rats, also protein efficiency, and activity of xanthine oxidase in the liver. The increases were more marked when methionine was given as well as tryptophan. The amount of N retained was not increased.

II. Supplements of lysine and threonine increased growth rate, protein efficiency, and liver xanthine oxidase. The two amino-acids together had a more marked effect than either singly; their joint effect was about the same as that produced by tryptophan + methionine. All four amino-acids together were not more effective than either pair.

III. Supplements of methionine had little effect on growth rate or protein efficiency, but increased liver xanthine oxidase. Addition of decolorised *Scenedesmus* (4 or 8%) increased wt. gain but decreased protein efficiency. M. D. ANDERSON.

Determination of amino-acids. II. Effect of hydrolysis time of protein on the determination of amino-acids. N. Matsuno, A. Nishihara and S. Isobe. **III. Hydrolysis time and effect of carbohydrate on tryptophan determination.** S. Isobe (*Annu. Rep. nat. Inst. Nutr., Tokyo*, 1959, pp. 42—44, 45—46).—II. Figures are given for the amounts of amino-acids obtained from casein, egg albumin, gelatin, zein, fish meal and decolorised *Chlorella*, after hydrolysis with 4N-HCl for 2, 4, 6, 8, 10 and 12 hr. Methionine, threonine, isoleucine, lysine. leucine, phenylalanine, valine gave max. values at 6 hr., except lysine from fish meal, phenylalanine from casein and egg albumin and valine from gelatin, zein and fish meal for which values were highest at 8 hr. and valine and lysine for *Chlorella* at 10 hr. Addition of starch diminished the yields of amino-acids.

III. Casein and albumin were hydrolysed with 4N-NaOH at 121° alone, with cysteine, under CO_2, with cysteine and under CO_2 with glucose, and with starch, and tryptophan was determined in the hydrolysates at 10, 12, 14 and 16 hr. Yields of tryptophan from albumin were somewhat increased in the presence of cysteine, and decreased by carbohydrate; effects on yields from casein were slight. M. D. ANDERSON.

Effect of a dietary lysine deficiency on the concentration of amino-acids in deproteinised blood plasma of chicks. J. A. Gray, E. M. Olsen, D. C. Hill and H. D. Branion (*Canad. J. Biochem. Physiol.*, 1960, **38**, 435—441).—A lysine-deficient basal diet, with and without supplementary lysine, was fed to chicks for 4 weeks. Twelve amino-acids were then determined microbiologically in the deproteinised blood plasma. The amino-acid patterns and the effects of acid hydrolysis of the deproteinised plasma on the amino-acid concn. are reported. (17 references.) S. G. AYERST.

Available lysine in food protein. I. Examination of method of determination. H. Baba, T. Izawa, and E. Tamura. **II. Determination of available lysine in animal and vegetable protein.** H. Baba, Y. Kobatake, T. Izawa and E. Tamura (*Annu. Rep. nat. Inst. Nutr., Tokyo*, 1959, pp. 51—52, 53).—I. Carpenter's method for determination of free ε-amino-groups in lysine by treatment with fluoro-2,4-dinitrobenzene in ethanol, hydrolysing with HCl, removing excess reagent, and measuring the DNP compound spectrophotometrically, was applied to casein and ovalbumin. Figures for "available lysine" thus obtained were close to those for total lysine. An adaptation of the method for use on vegetable protein was investigated.

II. Available lysine in animal proteins ranged from 60 to 95% of total lysine. In vegetable proteins, available lysine, determined by an adaptation of Carpenter's method, was 47 to 83% of total lysine. M. D. ANDERSON.

Effect of cooking on edible fat and oil. V. On heated lard and old lard. T. Hashimoto, K. Muramoto, A. Nakamura and H. Matsumuro (*Annu. Rep. nat. Inst. Nutr., Tokyo*, 1959, pp. 90—91).—Rats received complete diets, based on dextrin and casein, with 15% of fresh or heated lard. Male rats receiving lard that had been heated to a high temp. for a short time grew almost as well as controls; those receiving lard that had been repeatedly used for frying ate less, and showed poorer growth. In females, the differences were not significant. M. D. ANDERSON.

Modified fat. R. D. Coleman (*Dissert. Abstr.*, 1960, **20**, 3940—3941).—Rats fed acetostearin as sole fat at relatively high levels showed retarded growth and high mortality; supplements of linoleate caused some improvement, and of cottonseed oil marked improvement. Rate of absorption of acetostearin was slow; increasing when the m.p. of the product was lower, and when cottonseed oil was fed simultaneously. Retention of Ca was not affected by acetostearin. Plasma cholesterol was decreased, and liver and adrenal cholesterol were increased, in rats fed acetostearin as sole fat. These and other pathological effects are similar to those caused by deficiency of essential fatty acids and low kg.-cal. consumption. The abnormalities disappeared when cottonseed oil or linoleate was given. M. D. ANDERSON.

Biosynthesis of riboflavin by *Eremothecium ashbii*. I. A synthetic medium suitable for growth and vitamin B_2 production. H. G. Osman and M. Y. H. Kamel (*Egypt. J. Chem.*, 1959, **2**, 385—395).—The medium contained *m*-inositol (**I**), thiamine hydrochloride (**II**) and biotin (**III**) with $NH_4H_2PO_4$ as sole source of N and glucose as dominant C source. **I** had a growth-promoting but not a flavenogenic effect whereas **II** and, to a smaller extent, **III** were flavenogenic but not growth-promoting. A. G. POLLARD.

Decomposition of vitamin C and inhibition of its decomposition in food industry. I. Rôle of oxygen in changes in the content of vitamin C of foods during storage; processes reducing this effect. F. Balla (*Acta chim. hung.*, 1959, **21**, 363—373).—Dissolved O_2 is shown to be a primary factor in the oxidation of ascorbic acid to furfural in foods. The decomposition rate is lower the higher the acid concn. Sugars protect by virtue of the lower solubility of O_2 in their solutions. Some protection is afforded by removal of the dissolved O_2 in a vac., but decomposition will have already commenced. (12 references.) (In Russian; from summaries.) J. L. PROSSER.

Unclassified

Viscometry of non-Newtonian food materials. S. Charm (*Food Res.*, 1960, **25**, 351—362).—A method is described for establishing the flow constants using an empirical generalised relationship between shear stress and rate of shear. E. M. J.

Effect of γ-rays on food micro-organisms. I—III. γ-ray resistivity of *Escherichia coli*. 1.—3. IV. Influence of amino-acids and proteinaceous materials on survival of *E. coli* by γ-ray irradiation. V. Survival of *E. coli* irradiated with γ-rays. W. Watanabe (*Bull. agric. chem. Soc. Japan*, 1958, **22**, 68—77, 255—261; 1959, **23**, 73—77; 1960, **24**, 75—83, 84—92).—I. A simplified method was studied for the measurement of γ-ray resistance of *E. coli*. Comparison was made of each survival curve for 11 spp. of bacteria. The effects of addition of sucrose, NaCl or thiourea were also studied.

II. This report deals with the influence of the heating procedure applied to irradiated samples after irradiation, the γ-ray resistance of strains kept for long periods, and the influence of the addition of heat-sterilised cells of the same strain and chemicals (tartaric, citric or succinic acid) on media.

III. This report deals with the survival of the strain in various types of media under γ-ray irradiation, the influence of the preservation procedure at room temperature prior to γ-ray irradiation, and the minimum dose-rate of γ-ray for regular survival of the strain under a weak γ-ray field.

IV. The addition of amino-acids to a medium resulted in the protection of *E. coli* irradiated with γ-rays from ^{60}Co. The protective effects were different for different amino-acids. Proline was most effective, phenylalanine the least and arginine was ineffective in this protection. The addition of proteinaceous materials (horse serum, yeast extract or lactalbumin) also resulted in the protection of *E. coli* irradiated with γ-rays.

V. The survival ratios of *E. coli* irradiated with an equal γ-ray total dose were almost constant at a dose-rate exceeding a certain limit which differed according to conditions. For a nutrient medium containing 10% horse serum, the limit was 4000 rep/hr., for a minimum medium 2000, and for a non-nutrient medium 1700. When the dose-rate was below a certain limit, the lower was the dose-rate, the more bacteria survived. When *E. coli* was kept in an ice-box for 6 hr. in nutrient agar or on a pure agar before irradiation, no change was observed in either case in the survival of the strain on irradiation. When *E. coli* suspended in saline was kept in an incubator for 6 hr. before preparation of the samples, survival of the strain was higher than that of the control. The higher survival is probably due to the formation of resting cells of the bacteria. When dead cells sterilised with γ-rays were added to the living cells of the same strain, no influence on the survival curve of the living cells was observed. The survival of *E. coli* irradiated and incubated at 18° was slightly higher than that of *E. coli* incubated at 37°. The survival of samples irradiated in vacuum was higher than for those irradiated in air. The protective effects of Pereston-N (containing 6% polyvinylpyrrolidon regarded as an artificial serum) were shown during irradiation of *E. coli* with γ-rays. S. KAWAMURA.

Survival of *Salmonella typhimurium*, *Staphylococcus aureus* and *Streptococcus faecalis* in simplified food substrates. M. J. Woodburn and D. H. Strong (*Appl. Microbiol.*, 1960, **8**, 109—113).—Survival over a 10-week period at −11, −21 and −30° was studied; rice flour, maize syrup or egg white was added. In all cases the no. of viable cells increased, differences being due to the organisms used, the time and the temp. Recovery rate was most marked in the case of Na alginate. Except in one case, the greatest no. of cells surviving in each period were those subjected to the lowest temp. (−30°) and least in the highest. C. V.

Zinc-65 in foods. G. K. Murthy, A. S. Goldin and J. E. Campbell (*Science*, 1959, **130**, 1255—1256).—^{65}Zn was determined in a variety of food samples purchased in the open market. The isotope was found in all foods tested but the highest levels occurred in shellfish;

oysters from Chesapeake Bay contained 124—178 $\mu\mu$c/kg. Local use of the isotope in this area does not account for these levels and it is assumed that high level fall-out is responsible. No health hazard exists at this level. T. G. MORRIS.

Chemistry of natural substances. A. Stoll (*Experientia*, 1960, **16**, 85—100.—A review on carotenoids, vitamins A, E and K, curare alkaloids, ergot alkaloids and their deriv., cardiac glycosides of squill, digitalis and others, as well as recent results of research on chlorophyll—the crystallisation of natural chlorophyll *a* and *b*. M. LAPIDOT.

Comprehensive review of leucoanthocyanins and leucoanthocyanin-tannins and their importance in foods. K. Hermann (*Z. Lebensmitt-Untersuch.*, 1960, **112**, 105—118).—The literature on the chemistry, natural occurrence, extraction, detection and estimation of the leucoanthocyanins is reviewed, and the phenomena and problems associated with the occurrence of these products and the leucoanthocyanin-tannins in foods are discussed. Medical applications are briefly mentioned. (>100 references.) C. L. HINTON.

Industrial production of enzymes. I. D. Manuel Rosell Pérez (*Afinidad*, 1960, **37**, 16—18).—Review. A. J. B.

Determination of aldehydes and ketones in foodstuffs. I. Microtitration after preliminary treatment with 2,4-dinitrophenylhydrazine. V. Hamann and A. Herrmann (*Dtsch. LebensmittRdsch.*, 1960, **56**, 95—99, 133—138).—The micro-method described is based on reaction of the carbonyl compounds with a standard solution of 2,4-dinitrophenylhydrazine, followed by iodometric titration of the excess of reagent. Reaction conditions and results obtained are tabulated for a large number of carbonyl compounds. (23 references.) E. C. APLING.

Submerged fermentation process. R. F. Beers, jun. (B.P. 819,336 1.11.57. U.S., 21.11.56).—Cells of *Micrococcus lysodeikticus* (rich in catalase) are obtained in large quantity by inoculating aq. nutrient medium with *M. lysodeikticus*, and aerating the medium at pH 7—9·5 with stirring. F. R. BASFORD.

3.—SANITATION

Relative cleanability of stainless steel finishes after soiling with inoculated milk solids. O. W. Kaufmann, T. I. Hedrick, I. J. Pflug, C. G. Pheil and R. A. Keppeler (*J. Dairy Sci.*, 1960, **43**, 28—41).—Bright cold-rolled, grit-finished and grit + buff-finished stainless steel showed no significant differences in bacterial cleanability using a semi-automatic spray-washing device. Bacterial counts decreased in the order: rinsing, rinsing + alkaline detergent wash, rinsing + washing + sanitising (100 p.p.m. Cl_2 solution). A. H. CORNFIELD.

Comparative tests of machine and manual washing of milk containers as measured by their bacterial content and bacterial growth during storage. B. Goldinger (*Mitt. Lebensm. Hyg. Bern*, 1960, **51**, 75—93).—Experiments aimed at establishing bacterial standards of purity for 40-l. milk containers are described and discussed. Bacterial counts are made by rinsing and swabbing methods. (14 references.) J. V. RUSSO.

Selection of pyrethrum-resistant strain of grain weevil *Calandra granaria*, L. E. A. Parkin and C. J. Lloyd (*J. Sci. Fd Agric.*, 1960, **11**, 471—447).—A farm granary strain of *C. granaria* initially twice as resistant to pyrethrins in oil solution as a laboratory standard strain was exposed to selection pressure with pyrethrins in 17 out of 22 generations during 5 years when resistance increased to 18 times that of the standard. Simultaneous increase in resistance to pyrethrins synergised with piperonyl butoxide was only 2 times. The granary strain adults were slightly smaller and of a darker colour than the standard strain adults. (18 references.) E. M. J.

Assessment of insect infestation and acceptability of market samples of food grains. I. Wheat flour. S. Venkatrao, K. Krishnamurthy, K. S. Narasimhan, V. A. Daniel, S. K. Majumder and M. Swaminathan (*Food Sci., Mysore*, 1960, **9**, 8—10).—Samples of flour (60) purchased over the counter were examined to establish whether the amount of uric acid present would give an indication of the degree of insect infestation. The following were determined: (i) insect fragment count, (ii) uric acid and (iii) the organoleptic qualities from dough and unleavened bread made from the samples. The uric acid content indicated the degree of acceptability more uniformly than the insect fragment count. On the basis of organoleptic quality, samples containing up to 15 mg. of uric acid/100 g. sample, were acceptable. I. DICKINSON.

Grain storage studies. XXX. Chitin content of wheat as an index of mould contamination and wheat deterioration. M. Golubchuk, L. S. Cuendet and W. F. Geddes (*Cereal Chem.*, 1960, **37**, 405—411).—Chitin content of wheat samples was determined by two methods and related to the mould count, fat acidity and viability of the samples. (15 references.) S. G. AYERST.

"Grey mould" of oranges. C. Moreau (*Fruits d'outre Mer*, 1960, **15**, 69—71).—Algerian oranges in 1959 showed an abnormal no. of infections with *Botrytis cinerea* (grey mould); infections with *Penicillium digitatum* (green mould) and *P. italicum* (blue mould) were less noticeable than usual. Primary infections with *B. cinerea* usually begin in the region of the stalk, and are favoured by damp conditions following exposure of the fruit to cold. Infection in stored fruit is controlled by good ventilation, low humidity and regular disinfection. M. D. ANDERSON.

Effect of insect infestation on stored field bean (*Dolichos lablab*) and black gram (*Phaseolus mungo*). S. Venkatrao, R. N. Nuggehalli, S. V. Pingale, M. Swaminathan and V. Subrahmanyan (*Food Sci., Mysore*, 1960, **9**, 79—82).—Infestation of pulse samples (7 lb.) was induced with eggs laid by *Bruchus chinensis* (120 eggs on 100 grains of each sample) two days earlier. The infested bags were kept in separate earthenware pots in insect-proof rooms at a temp. of 85°F and a R.H. of 70—75%. The physical and chemical changes were studied at intervals. Results showed considerable loss in viability and wt. as the infestation progressed. The uric content due to the presence of insect excreta increased steadily. An increase in fat acidity and non-protein N and a decrease in thiamine content were observed. Pulses infested for a period >2 months were organoleptically unacceptable. I. DICKINSON.

Retention of acrylonitrile and carbon tetrachloride by shelled walnuts fumigated with Acrylon. B. Berck (*J. agric. Fd Chem.*, 1960, **8**, 128—131).—Insect infestation of shelled walnuts was successfully controlled by packing them in 55-lb. batches in polyethylene bags, and injecting each bag with 3 or 6 ml. of Acrylon (acrylonitrile/CCl_4, 34/66 v/v). After exposure to the fumigant at 70—75°F, the walnuts were aerated, air-cleaned, packed in Cellophane bags in ½-lb. lots, and stored at room temp. Determinations of acrylonitrile by polarography, and of CCl_4 by spectrophotometry, showed that residues were increased by larger dose, and longer exposure, and decreased by aeration. After storage for 38 days, residues ranged from 0·0 to 8·5 p.p.m. of acrylonitrile, and 5·7 to 34·5 p.p.m. of CCl_4. Fumigation under reduced pressure gave lower residues. (13 references.) M. D. ANDERSON.

Comparison of four pyrethrum synergists. G. D. Glynne Jones and P. R. Chadwick (*Pyrethrum Post*, 1960, **5**, No. 3, 22—30).—A series of laboratory tests to assess the values of four commercially available pyrethrum synergists is reported. With piperonyl butoxide as a standard and using houseflies and a measured drop technique, the average order of potency relative to piperonyl butoxide was: piperonyl butoxide = 1, sulphoxide = 1·1, Bucarpolate = 0·7, S.421 = 0·4. As a space spray against houseflies, the effects of three were similar; and for S.421 the effect was reduced. With grain weevils *Calandra oryzae* and *C. granaria*, sulphoxide and piperonyl butoxide were equal in potency; the other two synergists were appreciably less potent. E. M. J.

Control of insects with pyrethrum sprays in wheat stored in ships' holds. G. L. Phillips (*Pyrethrum Post*, 1960, **5**, No. 3, 31—32, repr. *J. econ. Ent.*, 1959, **52**, 557—559; cf. J.S.F.A. Abstr., 1960, i, 46). E. M. J.

Pyrethrum for control of red mite in poultry houses. P. R. Chadwick (*Pyrethrum Post*, 1960, **5**, No. 3, 3—4, 34).—In laboratory tests piperonyl butoxide (**I**) 0·065 mg./sq. cm. on filter paper killed 23% of the mites in 24 hr., pyrethrin (**II**) deposit 0·0013 mg./sq. cm. killed 70%, a mixture of **II** and **I** (1 : 2·5 by wt.) killed 87%. For field tests in poultry houses a water-miscible formulation [pyrethrin extract, xylene, Ethylan B.C.P. (an arylalkylpolyethylene glycol)] containing pyrethrins concn. of 6·25% wt./wt. giving on dilution at 24 c.c./gal. a 0·03% solution, was prepared. Two treatments of spray concn. 0·025% pyrethrins at a 7-day interval gave satisfactory control, perches being sprayed to run-off, nest boxes and walls less heavily. A monthly treatment of the houses prevented the mite population developing. E. M. J.

Sulphoxide: a pyrethrum synergist. R. W. Price (*Pyrethrum Post*, 1960, **5**, No. 3, 5—11, 30).—The development of pyrethrum synergists and the prep. of sulphoxide (**I**) from isosafrole and octyl mercaptan are reviewed. Data are presented (*a*) for several concn. of pyrethrins, the amount of **I** required for an LD_{90} for houseflies and (*b*) for several concn. of **I**, the amount of pyrethrins required for an LD_{90}. (47 references.) E. M. J.

Susceptibility to pyrethrins of three species of moth infesting stored products. C. J. Lloyd and P. S. Hewlett (*Pyrethrum Post*, 1960, **5**, No. 3, 12—13).—*Anagasta kühniella* (Zell.), *Ephestia elutella* (Hb.) and *E. cautella* (Wlk.) were exposed to residues of pyrethrins in oil and were dosed topically. Female *A. kühniella* were more susceptible to pyrethrins than the male, but the reverse was true for *E. cautella*; male and female *E. elutella* were about equally susceptible. E. M. J.

Relative toxicities of barthrin and pyrethrum. P. R. Chadwick and D. G. Glynne Jones (*Pyrethrum Post*, 1960, **5**, No. 3, 14—16).—Barthrin (6-chlorophenyl ester of DL-*cis*-*trans*-chrysanthemic acid) is less toxic than pyrethrum to grain weevils (LD_{50} values being 10·6 and 6·6 p.p.m., respectively), houseflies, cockroaches and flour beetles; the knockdown action is slow and it is less suitable for use in domestic sprays. Piperonyl butoxide was not an effective synergist for barthrin when tested against houseflies. E. M. J.

Screening tests with synthetic compounds as synergists for house-fly sprays. P. G. Piquett, W. A. Gersdorff and B. H. Alexander (*J. econ. Ent.*, 1960, 299—301).—Synthetic compounds (56) were tested by the turntable method as synergists for 0·5 mg./ml. of pyrethrins or 0·25 mg./ml. of allethrin. The most effective were the butyl 2-methyl-3-(3,4-methylenedioxyphenyl)propyl acetal of acetaldehyde and its isobutyl-analogue. C. M. HARDWICK.

Relative toxicity, knockdown effectiveness, and intensity of synergism of mixtures of piperonyl butoxide with barthrin and some of its isomers. W. A. Gersdorff and P. G. Piquett (*J. econ. Ent.* 1959, **52**, 1168—1171).—Barthrin and its D-*trans*, DL-*trans* and DL-*cis* isomers were used in 10 : 1 space sprays with pyrethroids against houseflies. The mortality and knockdown were compared with allethrin. There was a 3-fold synergism at the 50% mortality level for all the esters. The knockdown level was only $\frac{1}{4}$ that of the mortality level relative to allethrin. C. M. HARDWICK.

Resistance of houseflies to γ-benzene hexachloride and dieldrin. R. G. Bridges and J. T. Cox (*Nature, Lond.*, 1959, **184**, 1740—1741).—Addition of graded amounts of dieldrin to the larval medium of *S*-flies (originally resistant to dieldrin) causes rapid rise of resistance, and after six generations, when 150 p.p.m. of dieldrin are present, the resulting *R*-flies are very resistant to dieldrin and γ-benzene hexachloride. When eggs from *R*-flies are bred through a dieldrin-free larval medium, the adults (*RND*-flies) have a decreased resistance to both insecticides. This effect is reversible. Presence of dieldrin in larval food or adult tissue causes greater resistance to γ-benzene hexachloride of the adult fly which receives dietary milk fat or other associated fraction. This property is not transmitted genetically in absence of dieldrin pressure. *R*-flies metabolise γ-benzene hexachloride at the same rate whether they are fed on glucose alone or on glucose and milk, and rather more rapidly than do *RND*-flies. *S*-flies metabolise the insecticide at an intermediate rate. The results are discussed. J. N. ASHLEY.

Mechanisms of insect resistance to chlorohydrocarbon insecticides. F. R. Bradbury and H. Standen (*J. Sci. Fd Agric.*, 1960, **11**, 92—100).—The literature is reviewed especially in consideration of apparent contradictions in the present knowledge of the resistance mechanism. Penetration and metabolism of DDT and BHC in susceptible and resistant houseflies and mosquitoes are discussed. (38 references.) E. M. J.

Supposed correlation between the ratio X_5 and DDT resistance in houseflies. P. R. Sokal and T. Hiroyoshi (*J. econ. Ent.*, 1959, **52**, 1077—1080).—No correlation was found between the level of DDT-resistance and four sets of measurements in eight strains. The correlation found by F. O. Morrison (*J. econ. Ent.*, 1957, **50**, 554) is disputed. (17 references.) C. M. HARDWICK.

Effect of DDT resistance on the development of malathion resistance in houseflies. G. C. LaBrecque and H. G. Wilson (*J. econ. Ent.*, 1960, **53**, 320—321).—Flies, highly resistant to DDT but with only a slight resistance to malathion, increased this tendency in successive generations at a much more rapid rate than did a strain susceptible to all insecticides. C. M. HARDWICK.

Metabolism of malathion in houseflies and cows' liver. D. E. Weidhaas (*J. Ass. off. agric. Chem., Wash.*, 1959, **42**, 445—446).—Tests with homegenates of cow's liver (cf. Cook *et al.*, *ibid.*, 1957, **40**, 665; 1958, **41**, 399, 407) demonstrated the hydrolysis of malathion, but Cook's mechanism was not demonstrated in susceptible or malathion-resistant houseflies. A. A. ELDRIDGE.

Toxicity to house-fly larvae of droppings from chickens fed insecticide-treated rations. M. Sherman and E. Ross (*J. econ. Ent.*, 1960, **53**, 429—432).—A 50%-mortality was found in house flies feeding on faeces from chicken receiving insecticides in food at rates of Co-Ral 22, Dipterex 28, Dow ET-15 38, diazinon 47, Ronnel 48, malathion >1102 or phenothiazine 6000—11,023 p.p.m. C. M. HARDWICK.

Alkylphosphonic acid esters as insecticides. T. R. Fukuto, R. L. Metcalf and M. Winton (*J. econ. Ent.*, 1959, **52**, 1121—1127).—The prep. of many alkyl *p*-nitrophenyl alkylphosphonates and their S analogues is described. Many were effective against houseflies although the alkyl groups varied widely. (19 references.) C. M. HARDWICK.

Field studies of house-fly resistance to organophosphorus insecticides. E. J. Hansens (*J. econ. Ent.*, 1960, **53**, 313—317).—Resistance to diazinon, Ronnel, lindane and DDT was found in flies collected from barns sprayed with diazinon or Ronnel for 5 years, and from surrounding barns. The no. of flies found was related to the level of resistance. In field experiments Dimethoate and malathion were unsatisfactory where considerable resistance to diazinon and Ronnel was found. C. M. HARDWICK.

Relationships between structure and insecticidal activity of some organotin compounds. M. S. Blum and J. J. Pratt, jun. (*J. econ. Ent.*, 1960, **53**, 445—448).—The toxicity of 42 org. Sn compounds applied topically to *Musca domestica* is given. LD_{50} values were determined on a molar basis. Max. toxicity was obtained with tri-substituted compounds. Di- and tetra-substituted compounds were of similar toxicity and mono-substituted compounds were the least toxic. (14 references.) C. M. HARDWICK.

Effects of a diet containing gibberellic acid on growth and food consumption of *Periplaneta americana* L. A. N. Siakotos and J. E. Dewey (*J. econ. Ent.*, 1959, **52**, 1214—1215).—The cockroaches were divided into three groups based on weight and fed for 35 days on diets containing gibberellic acid (0·01, 10 and 100 p.p.m.). The intermediate wt. group consumed the greatest quantity of food but the heaviest group showed greater wt. increases. Mortality was variable, being highest in the youngest group of roaches. Egg production was not affected. C. M. HARDWICK.

Relationship between metabolism and differential toxicity of malathion in insects and mice. H. R. Krueger and R. D. O'Brien (*J. econ. Ent.*, 1959, **52**, 1063—1067).—Column and ion-exchange chromatography were used to analyse the metabolites, of which 11 were found. Topical applications rapidly disappeared from both cockroaches and the housefly. The percentage metabolised was the same for high and low doses. A comparison of LD_{50} following injection of malathion showed that the difference between *Periplaneta americana* and *Blattella germanica* was in the amount absorbed. The level of Malaoxon was higher and remained constant longer in the mouse than in *P. americana*. (12 references.) C. M. HARDWICK.

Control of the tropical rat mite. W. Ebeling (*J. econ. Ent.*, 1960, **53**, 475—476).—A SiO_2 aerogel containing 4·7% of NH_4 fluosilicate at 1 lb./1000 sq. ft. eliminated severe infestations of *Ornithonyssus bacoti* for up to a year. Its action is based on dehydration due to lipin removal. Mites from birds' nests have been eliminated by similar dusts. C. M. HARDWICK.

Food contamination by rodents and birds. W. W. Dykstra (*Cereal Sci.*, 1959, **4**, 303—304).—The points at which food is most likely to be contaminated by rodent and bird filth during its journey from the harvest field to the processing plant are discussed. Mice are considered to be a more important source of contamination than rats. S. G. AYERST.

Insecticides for control of Drosophila breeding. H. C. Mason, T. J. Henneberry and R. Lehr (*J. econ. Ent.*, 1959, **52**, 1136—1138).—Field experiments with boxes of tomatoes and strawberries, and piles of cull tomatoes, showed that sprays of malathion, Dipterex, heptachlor, aldrin, dieldrin, Dicapthon, methoxychlor and Ethion gave almost 100% control of Drosophila breeding for at least one month. In a commercial experiment 1·5% malathion gave satisfactory control for a month when sprayed on tomato skins. C. M. HARDWICK.

Effect of repellants on evolution of carbon dioxide and moisture from human arms. H. K. Gouck and M. C. Bowman (*J. econ. Ent.*, 1959, **52**, 1157—1159).—A method of estimating CO_2 output and moisture from human arms is described. The reduction in the amount of CO_2 evolved, following application of dimethyl phthalate, diethyl toluamide and ethyl hexanediol, is insufficient for repellancy (to mosquitoes) to be based on this. Natural attractiveness is based on high CO_2 production and low moisture evolution. (12 references.) C. M. HARDWICK.

Estimating residual insecticides on wall surfaces. A. V. de Courcy (*Span*, 1960, **3**, No. 1, 30—32).—The methods of application are discussed with special reference to the persistence of deposits and the limitations of bioassay. The necessary precautions in sampling are indicated. C. V.

Compatibility of dehydrated army rations with chlorinated and iodine-treated surface-waters. M. R. Rogers, A. M. Kaptan and E. Pillion (*Food Technol.*, 1960, **14**, 240—245).—Foods rehydrated with swamp water were less acceptable than those rehydrated with ground and river waters, and control samples were generally more acceptable than halogen-treated samples, but none of the average scores fell into the hedonic "dislike" category or below. E. M. J.

Evaluation of factors contributing to the rapid death of bacteria in sea water. A. F. Carlucci (*Dissert. Abstr.*, 1960, **20**, 3469).—Cells of *Bacterium coli* died more rapidly in deionised water than in 25% sea water, but survival decreased with increasing concn. of sea water above 25%. Survival in sea water and NaCl solutions varied inversely with salt concn.; a pH of 5·0 was the most favourable. More cells survived in sea water than in NaCl solutions of the same salinity and pH. Addition of nutrients aided survival; 0·1 p.p.m. of cysteine or thioglycollate was especially effective. No antibiotic active against *B. coli* was obtained from over 200 strains of bacteria isolated from sea water. Chlortetracycline and penicillin were rapidly inactivated in sea water; some other antibiotics persisted for 30 days. Bacteriophages were readily isolated from sea water, but did not affect survival of *B. coli* except in water supplemented with nutrient. Coliphages were rapidly inactivated in natural sea water, but persisted in sterilised sea water. Survival of *B. coli* was increased in four out of six cases by filter-sterilising sea water. Autoclaved sea water was more favourable to survival than filter-sterilised water in four out of six cases. *B. coli* survived longer in autoclaved than in untreated samples of artificial sea water. M. D. ANDERSON.

Control of cleaning wastes in the food industries. A. J. Steffen (*Chem. Engng Progr.*, 1959, **55**, no. 11, 79—81).—Careless cleaning produces large quantities of dilute warm waste liquors. Re-use of cleaning solutions—generally hot water with detergent—can effect considerable economy. Settling tanks and screens should be used as for process solutions. F. RUMFORD.

Engineering study of effluent-disposal problems of Louisiana raw sugar industry. J. E. Wheeler, jun. (*Dissert. Abstr.*, 1960, **20**, 2729).—Causes of pollution are outlined. Some improvements have been made by revising entrainment removal devices on the evaporators, and by reducing the cane wash effluent vol. in a multi-stage washing process. The effects of aeration, temp., and nutrient addition on the bio-stabilisation of the cane wash effluent have been studied in batch reactors. A. M. SPRATT.

Pollution control in fermentation industry. C. S. Boruff (*Chem. Engng Progr.*, 1959, **55**, no. 11, 82—86).—The fermentation industry must recover large quantities of org. residue. It is essential to treat stillage by secondary fermentation, while all solids should be recovered in, e.g., brewers' grains. Antibiotics, newest of fermentation industries, produce waste liquor of high B.O.D., but a proper waste recovery can cut this by over 80%. (24 references.) F. RUMFORD.

Disposal and utilisation of organic wastes. J. S. Hardy (*J. Inst. Sew. Purif.*, 1959, 186—189).—Disposal and utilisation of org. wastes are discussed, particularly prep. of org. manures; i.e., processed org. manures, commercially sterilised, rich in humus and in suitable condition for spreading; uses other than for farming projects; and comparative costs. O. M. WHITTON.

Bacteria used in industrial purification installations for removal of phenol in waste water. N. T. Putilina (*Mikrobiologiya*, 1959, **28**, 757—762).—Cultures of phenol-destructive bacteria were introduced into the aeration tanks of the coke and chemical works at Kadiev, USSR, in 1951; results of ten years' study were reported. Very simple bacteria and also actinomyces and moulds are not sufficiently effective in oxidation of phenol. Ten non-pathogenic strains originally isolated from urban soil were selected. Important changes in biochemical and morphological properties were observed in the 10—12 years' cultivation. Gram-positive bacilli became Gram-negative, the mobile sporogenous types immobile and non-sporogenous. Transfer of the effective strains of soil origin from a phenol medium to one containing glucose was found to lower their phenol oxidative capacity from >92 to 17%. It is possible to obtain phenol-destructive strains from pure cultures of *Bacillus subtilis*, *Bac. megatherium* and *Bacterium coli*, previously not known to oxidise phenol. A. GROCHOWSKI.

Laboratory determination of sludge-filtration characteristics. P. K. Eastwood (*J. Inst. Sew. Purif.*, 1959, 198—202).—Application of the concept of sp. resistance in measuring filterability has been examined experimentally. For comparing filterabilities the "cracking" test appears preferable. O. M. WHITTON.

Oligomycin. Wisconsin Alumni Research Foundation (B.P. 816,070, 9.7.56. U.S., 15.7.55).—Oligomycin is obtained by growing an oligomycin-producing organism, e.g., *Streptomyces diastatochromogenes* in aq. nutrient medium containing whole or part of the assimilable C in the form of a fatty oil (e.g., lard oil) in excess of the organism's requirements, then adding $CaCO_3$ after 10—30 hr. (when pH has fallen below 6), separating the mycelium, and recovering oligomycin therefrom by solvent extraction. The product is inactive against bacteria but is active against fungi, insects, ants, beetles and nematodes. F. R. BASFORD.

4.—APPARATUS AND UNCLASSIFIED

Flame-photometric determination of calcium in plants. J. C. Brogan (*J. Sci. Fd Agric.*, 1960, **11**, 446—449).—The interfering effects of PO_4^{3-}, SO_4^{2-} and Al^{3+} in flame-photometric determinations of Ca were studied. In presence of large excess of PO_4^{3-} or SO_4^{2-}, Ca emission is independent of small variations in both. A simple flame-photometric procedure is suggested in which the Ca present is determined in solution containing a large excess of SO_4^{2-} and results are free from errors caused by variations of P and S contents of the plant. E. M. J.

Propagation of flames in mixtures of eucalyptus-oil vapour and air. A. C. McLaren (*Aust. J. appl. Sci.*, 1959, **10**, 321—328).—For oils with constituents having b.p. within a narrow range, flame propagation in air–vapour mixtures begins at ~60°, attains a max. velocity (~50 m./sec.) at ~70° and falls to zero at ~90°. Concn. of oil vapour at initiation, max. and end of propagation are ~2, 3 and 7%. Oil from *E. dives* (cineol ~60, piperitone ~40%) has a wider range of b.p., and flame propagation extends over 60—120° with min. velocity at ~100°. Clouds of electrostatically dispersed NaCl or $NaHCO_3$ (0·2—0·4 g./l.) will stop flame propagation. Application to eucalypt-forest fires is discussed. W. J. BAKER.

Detection and differentiation of E605 [parathion], methyl-E 605 [methyl parathion] and Chlorthion [*OO*-dimethyl-*O*-3-chloro-4-nitrophenyl phosphorothioate]. H. Sperlich (*Dtsch. ApothZtg*, 1960, **100**, 774—777).—The title compounds, e.g. in insecticide prep. or biological tissue, are extracted and then separated by chromatography on oil-impregnated paper with 70% methanol as solvent, and detected by reduction with aq. $TiCl_3$, diazotisation with $EtNO_2$ or amyl nitrite vapour and coupling with *N*-(naphth-1-yl)ethylenediamine hydrochloride. Chlorthion is further identified by the colour change on treating the resultant azo dye with H_2O_2. (17 references.) A. G. COOPER.

Complexometric titration of calcium and magnesium in the presence of phosphate in milk and blood plasma. T. H. Kamal (*J. agric. Fd Chem.*, 1960, **8**, 156—158).—Ca and Mg in biological materials, especially in milk and blood plasma, are determined by adding excess of the disodium salt of (ethylenedinitrilo)tetra-acetic acid to a neutral system, and back-titrating the excess with standard Ca and Mg solutions, using calcein indicator for Ca, and Eriochrome Black T indicator for Mg. This method avoids the necessity for removing P and protein, and is rapid and sensitive. M. D. ANDERSON.

Strontium-90 in human bone in the U.K. 1956—8. N. T. J. Bailey, F. J. Bryant and J. F. Loutit (*A.E.R.E.*, 1960, R 3299, 40 pp.).—The rôle of Ca and Sr in the body and the nature of the hazard are briefly reviewed, together with the method of sampling and assay of ^{90}Sr and a statistical appraisal of the results of the determinations. There is strong evidence of an increase in the values of the $^{90}Sr/Ca$ ratio over the period, but the data are insufficient to indicate whether there was any rise in the rate of increase towards the end of 1958. J. M. JACOBS.

Rapid radiochemical determination of caesium-137. N. Yamagata and T. Yamagata (*Analyst*, 1960, **85**, 282—285).—Sample (food or biological material) containing ~0·1 g. of K is ashed. To an acid solution of the ash a CsCl carrier and $FeCl_3$ are added and the liquid is scavenged by pptn. of $Fe(OH)_3$. The filtrate is treated with Na picrylaminate; the K and Cs salts pptd. are weighed. The solution of the ppt. in $COBu^tMe$ is extracted with aq. HCl; to the aq. solution of residue from evaporation of the extract chloroplatinic acid is added and the ppt. of Cs_2PtCl_6 is collected, weighed and its activity counted in a low-background β-counter and corrected for self-absorption and scatter. A. O. JONES.

Drying of grain and like agricultural products.—Jessop & Co. Ltd. (Inventor: A. Jardine) (B.P. 814,536, 9.2.56).—An installation for the drying of grain, etc., is figured and claimed. F. R. BASFORD.

Journal of Applied Chemistry

The following papers are appearing in the November, 1960, issue

Adhesion in bitumen macadam
By R. I. Hughes, D. R. Lamb and O. Pordes

Stabiliser degradation in picrite propellants
By Paul E. Gagnon, R. MacDonald, C. Haggart and J. L. Myers

Measurement of thickness and porosity of oxide films on iron and aluminium
By K. F. Lorking

The system barium oxide, zinc oxide and water at 90° and 70° C.
By H. Monk and A. V. W. Mortifee

A comparison of the corrosiveness of indoor atmospheres
By J. F. Stanners

The setting of road tar in surface dressing
By J. R. Dewhurst

Green grow the olives

Perhaps an outwards-bound Roman legionnaire or a home-going proconsul once plucked an olive as he passed through Apulia, along the famous Appian Way. For this ancient province (forming the "heel" of Italy) was, and still is, noted for its excellent olives. Its people, whose forbears have known Roman, Greek, Saracen, Norman and Spanish overlords, today strive to overthrow yet another "foreigner" on their soil . . . *Rhynchites cribripennis*, the olive weevil.

This time they have an effective weapon. It is dieldrin, and it is proving highly successful. The trees and the surrounding soil are given two sprayings when the young fruits first appear. These prevent the female weevil burrowing into the olives and laying her eggs within. Thus, the year's harvest gets off to a sturdy start. Nor does dieldrin's good work finish there. The two sprayings of this persistent Shell insecticide usually control the first and second generations of the olive fly as well. Other pests, such as the olive thrip and the olive moth, are also held in check by the treatment. Between them, aldrin, dieldrin, endrin, Phosdrin, D-D and Nemagon can control virtually every major world pest.

dieldrin

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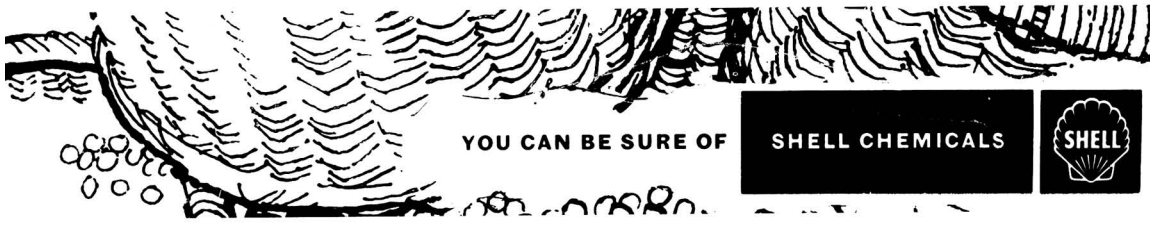

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