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

Published by the Society of Chemical Industry

Volume 14 No. 8 August, 1963

SOCIETY OF CHEMICAL INDUSTRY

FOUNDED IN 1881 INCORPORATED BY ROYAL CHARTER 1907

President:
MONROE E. SPAGHT, A.M., Ph.D., Sc.D.

Hon. Treasurer:
J. S. GOURLAY, B.Sc., PH.D.

Hon. Foreign Secretary:
E. L. STREATFIELD, PH.D., B.Sc., F.R.I.C., M.I.CHEM.E.

Hon. Secretary for Home Affairs:
H. K. CAMERON, PH.D., B.Sc., F.R.I.C., M.I.CHEM.E., M.I.E.E.

Hon. Publications Secretary:
PROF. W. G. OVEREND, D.Sc., PH.D., F.R.I.C.

General Secretary and Editor-in-Chief:
FRANCIS J. GRIFFIN, O.B.E., F.C.C.S., A.L.A.

Editor:
H. S. ROOKE, M.Sc., F.R.I.C.

Advertisement Manager:
P. R. WATSON

Publications Committee:

W. G. Overend (*Chairman*), H. Egan (*Chairman, The Journals and Chemistry & Industry*), G. Brearley (*Chairman, Annual Reports and Monographs*), G. L. Baldit, H. J. Bunker, L. C. Dutton, D. V. N. Hardy, S. R. Tailby, W. Wilson, and the Officers

Journals Sub-Committee:

H. Egan (*Chairman*), A. L. Bacharach, H. J. Bunker, D. H. F. Clayson, G. A. Collie, L. C. Dutton, J. Elks, J. Grant, J. L. Hewson, J. H. Nicholas, J. E. Page, A. G. Pollard, W. H. Read, M. K. Schwitzer, (Miss) R. M. Scott, V. W Slater, S. R. Tailby, K. A. Williams, F. Wormwell, and the Officers

Abstracts Advisory Sub-Committee:

(Miss) D. M. Brasher, H. J. Bunker, C. B. Casson, M. B. Donald, D. Gall, J. E. Garside, A. G. Pollard, and the Officers

Offices of the Society: 14 Belgrave Square, S.W.1
Telephone: BELgravia 3681/5

Annual Subscription to the *Journal of the Science of Food and Agriculture*, £15 post free, single copies £1 17s. 6d. post free

OXIDATION BY MOLECULAR OXYGEN OF THIOL GROUPS IN UNLEAVENED DOUGHS FROM NORMAL AND DEFATTED WHEAT FLOURS

By A. H. BLOKSMA

Thiol groups in doughs from wheat flours that have been defatted by extraction with light petroleum, carbon tetrachloride, n-butanol–water (6 : 1, v/v) or acetone–water (4 : 1, v/v) mixtures are more slowly oxidised by molecular oxygen than thiol groups in doughs from normal flours. The oxidation essentially occurs during mixing; in a resting dough the reaction is established with difficulty in spite of the occlusion of an ample amount of oxygen. Fat extractions with light petroleum or carbon tetrachloride increase the rate of gas occlusion in the dough on mixing; with the other solvents this phenomenon was not observed.

Introduction

Atmospheric oxygen[1] and small amounts of oxidising flour improvers, such as potassium bromate or iodate (see, for example, [2]), increase the resistances of wheat flour doughs against deformation and decrease their extensibilities. Similar effects on the rheological properties are caused by the addition of compounds that react specifically with thiol groups.[3] These observations have led to the hypothesis that thiol groups of the flour proteins are important for the rheological properties of dough, presumably by the way of thiol–disulphide interchange reactions.

The flour lipids in dough affect its rheological properties by protecting the protein fraction from oxidation by atmospheric oxygen. This protective effect was discovered by rheological measurements with the Brabender Extensograph and only demonstrated originally with lower grade or clear flours with high fat and enzyme contents.[4, 5] Later it was also found with a normal flour.[6] This protection can be explained by a competition for the available oxygen between the highly unsaturated flour lipids and the protein thiol groups. The observation that blocking the thiol groups increases the extent of oxidation of flour lipids during mixing of dough in an atmosphere of oxygen, was recently explained in similar terms.[7]

The present author observed that, on prolonged mixing in air, the gluten structure broke down more rapidly after extraction of the flour with an acetone–water mixture (4 : 1, v/v) as compared with an unextracted flour.[8] The two theories above were combined to explain the phenomenon. Mixing of dough in air causes oxidation of thiol groups, which leads to an increase in resistance. If the thiol–disulphide interchange no longer provides for a mechanism for reversible breakage of bonds, the dough in the mixer may comply with the imposed deformations by irreversible breakage of chemical bonds. The latter process can explain a decrease in gluten yield upon washing the dough. Extraction of fat with acetone–water mixture would increase the oxidation of thiol groups and consequently the breakdown of the gluten.

After a GRL-mixer[9] had become available, in which a dough can be mixed in a controlled gas atmosphere, it was confirmed that a breakdown of the gluten structure occurs in oxygen, but not in nitrogen. However, the experiments described in this paper showed the second part of the hypothesis to be false; the oxidation of thiol groups in dough by oxygen is not enhanced by extraction of fat from the flour.

Experimental

Flours

Two commercially milled, unbleached bread flours, free from improvers and Creta preparata, were used. Further details of the flours are given in Table I. In this paper all results of analytical determinations and the composition of the doughs are expressed on the basis of flour with 14% moisture.

Flour I was extracted by shaking it for 4 h. with one of the following freshly distilled solvents or mixtures of solvents: light petroleum (LP), boiling range 40–60°; carbon tetrachloride (CT); n-butanol–water 6 : 1 v/v (BW), and acetone–water 4 : 1 v/v (AW). The ratio solvent/flour was 3 ml. per g. The suspension was filtered by suction and the flour on the filter washed

Table I

Analysis of the flours used

(LP = light petroleum, CT = carbon tetrachloride, BW = n-butanol–water, AW = acetone–water)

	Flour I					Flour II	
	Unextracted	After extraction with				Unextracted	After
	U	LP	CT	BW	AW	U	extraction with LP
Kjeldahl N × 5·7, %	10·2					10·0	9·9
Kjeldahl N, mmoles/g.	1·28					1·25	1·24
Thiol groups, μmoles/g.	0·56	0·55	0·55	0·43	0·48	0·61	0·60
Total fat after acid hydrolysis, %	1·42	0·62	0·62	0·39	0·57	1·23	0·61
Fatty acids after acid hydrolysis, %	0·89	0·49	0·51	0·32	0·39	0·77	0·48
Ash, %	0·40	0·41	0·40	0·39	0·37	0·43	0·43
Farinograph water absorption,[10] %	54·0					55·8	
Dry material in extract, % of flour	—	1·11	1·11	1·23	1·38	—	0·97
Non-lipid material in extract, % of flour	—	0·31	0·31	0·20	0·53	—	0·35

first with 1 ml./g. of the solvent used and then with 1 ml./g. of diethyl ether, and finally dried in vacuum at 35°. If necessary, flour lumps were broken up in a percussion grinder.

Flour II was extracted similarly but with light petroleum only. The extraction was performed in two stages; after sedimentation, the supernatant was decanted and replaced by 2·4 ml./g. of fresh light petroleum. The suspension was shaken again, filtered, etc. For washing the flour on the filter, no diethyl ether was used, but only 0·6 ml./g. of light petroleum.

Solvents and suspensions were purged with nitrogen. Contact with atmospheric oxygen could not be avoided completely, however, during filtration and drying of the extracted flour.

To check the removal of lipids from the flours by these procedures, 20 g. of flour were hydrolysed by boiling with 25 ml. 1·4N-hydrochloric acid for 1 h. The lipids set free were isolated by extraction of the water phase with a mixture of 40 ml. of 96% ethanol and 50 ml. of light petroleum (boiling range 60–80°), and dried at 105°. The lipids were weighed and the fatty acids in them were titrated with potassium hydroxide in alcoholic solution. For the calculation of the weight of the fatty acids, a mean molecular weight of 280 (linoleic acid) was assumed. The weights of total fat and of fatty acids are reported in Table I. From the reduction in the total fat content of the flour by extraction, and the total amount of dry material in an aliquot part of the combined extracts and wash liquids, the amounts of non-lipid material in the extracts were calculated. They are also reported in Table I.

Doughs

Doughs were prepared from 100·0 g. of flour, 1·0 g. of chemically pure sodium chloride (dissolved in the dough water), and 55·2 (flour I) or 54·5 (flour II) ml. of distilled water. They were mixed for various times in a GRL-mixer[9] at a speed of 66·5 r.p.m. in an atmosphere of either nitrogen or oxygen. When nitrogen was used, the flour was previously stored overnight under nitrogen, and the dough water was also purged with nitrogen for 5 min. before addition to the flour. Doughs were prepared at 25° and, if necessary, stored at the same temperature and high humidity for 45 min.

Thiol groups

For the determination of thiol groups in flour, about 3·2 g. of sample were dispersed in 82 ml. of an ammonia buffer solution of pH 8·4, also containing ethylenediaminotetra-acetic acid (EDTA). Then 24 g. of urea were added, bringing the volume of the solvent up to 100 ml. and the final concentrations to 4M-urea, 1M-ammonium nitrate, 0·05M-ammonia, and 0·001 M-EDTA. The solvent and the dispersion were purged with nitrogen. The dispersion was titrated under nitrogen at 20° with 0·01M-silver nitrate from a micro syringe in a vessel described

earlier.[11] Every minute a 40-μl. portion was added. The end point was detected amperometrically; the potential of the platinum electrode was −0·4 V in respect of a saturated calomel electrode. The electrode was cleaned with hot nitric acid after about 20 titrations. After cleaning, the electrode was covered with a fresh layer of silver by means of a slow, blank titration (0·02 μmole of silver nitrate in 15 min.). Results of blank titrations were usually in the order of 2×10^{-7} moles per litre of solvent, or 0·006 μmole per g. of flour, and can therefore be neglected. All reagents were analytical reagent-grade except urea (Merck puriss. crist.). Water was distilled twice, the second time in a glass still.

For a determination of thiol groups in dough, about 5·0 g. of dough were triturated with a pestle in a mortar with part of the buffer solution under a stream of nitrogen. The slurry was transferred quantitatively to the titration vessel with the remainder of the solvent. Then urea was added and the procedure for flour then followed. The agreement between determinations in doughs after short mixing times in nitrogen and in flour makes it probable that no more thiol groups were lost during the dispersion of doughs than in the case of flour. Evidently the procedure adopted is at least as adequate as the more complicated one, in which doughs are frozen and lyophilised.[12]

The titration conditions described above were chosen in spite of the possibility of obtaining higher results with a lower ammonia concentration and at a lower temperature.[13] The higher results at a lower pH may be due to binding of more than one silver atom per thiol group.[14] Because of the results of Sullivan *et al.*[15] results of titrations of flour and of doughs are not comparable if the dry flour is dispersed and titrated in a cold buffer solution, whereas the doughs are made and stored at 25°.

Gas occlusion

The gas occlusion was measured immediately after mixing by means of the apparent density of the dough according to the method of Baker & Mize.[16] Samples of dough were dropped into a series of cylinders containing calcium chloride solutions with densities in successive cylinders differing by 0·008 g./cm.^{3}. For a determination of the true density d_0, some doughs that had been mixed for only a few minutes at atmospheric pressure were placed in the mixer again, and were mixed in vacuum 2 or 3 times for 2-min. intervals until their apparent density remained constant. The average true densities, g./cm.^{3}, thus found were:

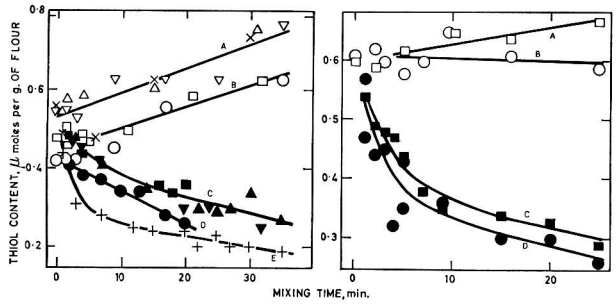
Flour	Average	Standard deviation	Number of determinations
I	1·247	0·011	21
II	1·253	0·005	8

The gas occlusion a in cm.^{3} of gas per cm.^{3} of dough plus gas is calculated from the apparent density d with the equation $a = 1 - d/d_0$. The reliability of the determinations of a is estimated to be about 0·01 or 1% of gas.

Results

Determinations of thiol groups in doughs immediately after mixing (b) and 45 minutes later (c) gave essentially identical results. The average value of the ratio c/b was 0·98 (98 experiments, standard deviation 0·07) with no significant difference between mixing in nitrogen and in oxygen. Although this average value differs significantly from unity, the two titrations of the same dough were treated as duplicates, the mean value of which will be reported below.

Figs. 1 and 2 show that, when doughs are mixed in oxygen for 25 min., the thiol contents are reduced to roughly one half of their original values, the remainder of the thiol groups reacting very slowly. In nitrogen the thiol content is constant or increases slowly. This slow increase is occasionally found with both normal and defatted flour. Its nature has not yet been clarified. In Fig. 1 the points obtained after mixing in nitrogen fall into two groups: those from the flours that did not lose thiol groups during fat extraction and from the unextracted flour (U, LP, CT) being higher than those from the flours that lost some thiol groups during extraction (AW, BW).



Thiol contents of doughs from flours I (FIG. 1, left) *and II* (FIG. 2, right), *and from the same flours after fat extraction, all after varying mixing times in nitrogen or in oxygen*

U = unextracted flour. Extracted flours are indicated by the solvent used: LP = light petroleum, CT = carbon tetrachloride, AW = acetone-water, and BW = n-butanol-water. Points at zero mixing times represent the averages of determinations in the flours. The curves for mixing in oxygen obey equation (1) with the values of the parameters given in Table II.

curve A U, LP and CT in nitrogen
" B AW, BW " "
" C LP, CT, AW " oxygen
" D BW " "
" E U " "
Mixed in nitrogen U × LP △ CT ▽ AW □ BW ○
" " oxygen U + LP ▲ CT ▼ AW ■ BW ●

curve A □ LP in nitrogen
" B ○ U " "
" C ■ LP " oxygen
" D ● U " "

Sokol *et al.*[12b] described the results of similar experiments with mixing in air as from a simple first-order reaction. It is not possible to do so with the present experiments in oxygen. Their results can be described reasonably well with the assumption that part of the thiol groups react according to a first-order reaction, and that the remainder follow a zero-order reaction. In fact, the oxygen curves in Figs. 1 and 2 obey the equation

$$C = A.\exp(-k_A t) + B.(1 - k_B t) \quad . \quad . \quad . \quad . \quad . \quad (1)$$

with the values for A, B, k_A, and k_B given in Table II; A and B have been chosen in such a way that their sum is identical with the thiol contents of the flours, given in Table I. That the rate constants k reported by Sokol *et al.*[12b] are 10–50 times lower than the k_A values in Table II is primarily due to the fact that the equation used by them did not include the last term of equation (1). Their measurements can be described very well by means of equation (1) with values for k_A and k_B comparable to those in Table II. Some examples are given in Table III. It is not possible to detect an effect of the difference in conditions (Farinograph mixing bowl and GRL-mixer, 29 and 25°, air and oxygen as atmosphere, respectively) between the experiments of Sokol *et al.* and the present ones.

Figs. 1 and 2 show that the thiol groups in doughs from defatted flour are oxidised more slowly than in doughs from normal flour. The magnitude of the effect is much larger for flour I than for flour II. The behaviour in oxygen of the flour extracted with wet n-butanol is slightly different from that of the other three defatted flours from the same original flour. In spite of these variations in magnitude, the direction of the effect is always the same.

The apparent densities and gas occlusions in Fig. 3 confirm the observation of Cookson & Coppock[17] that after extraction of flour with carbon tetrachloride, gas occlusion in the dough proceeds more rapidly than in doughs from unextracted flour. The behaviour of dough after extraction with light petroleum is apparently similar (see also Fig. 4). Fig. 3 shows, however, that increased gas occlusion is not a general property of doughs from defatted flours, independent of the solvent used. The dashed line in Fig. 3 is not very reliable since it is only based on three observations on doughs the gluten structure of which had been broken down considerably.

Discussion

The results show that the author's hypothesis that flour lipids protect thiol groups from oxidation by oxygen[8] is false. This conclusion is supported by results of Smith *et al.*[5] The present results are more conclusive than theirs since they are based on measurements of both

Table II

Parameters for the description by means of equation (1) *of the oxidation of thiol groups in dough on mixing in oxygen*

	Flour I			Flour II	
	Unextracted U	After extraction with LP, CT or AW	BW	Unextracted U	After extraction with LP
A, μmoles/g.	0·28	0·11	0·01	0·24	0·21
B, μmoles/g.	0·28	0·41	0·42	0·37	0·39
A + B, μmoles/g.	0·56	0·52[a]	0·43	0·61	0·60
$k_A \times 10^3$, sec.$^{-1}$	7·7	3·1	—[b]	6·2	4·9
$k_B \times 10^4$, sec.$^{-1}$	1·58	1·66	3·18	1·82	1·52

[a] Average value of the data in Table I for the three defatted flours
[b] A is too small to permit a reliable estimation of k_A

Table III

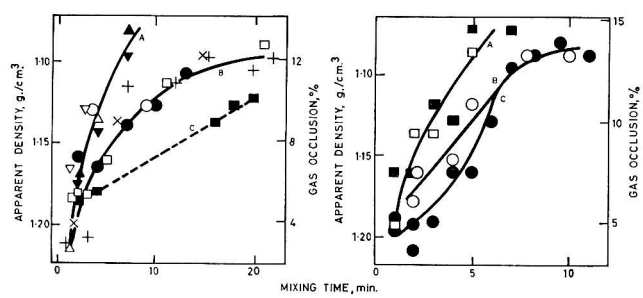
Parameters for the description by means of equation (1) *of the oxidation of thiol groups in dough on mixing in air in experiments by Sokol* et al.[12b]

	Flour			
	Elgin	Idaed	UN No. 1	Durum No. 2
A, μmoles/g.	0·29	0·03	0·03	0·27
B, μmoles/g.	0·49	0·62	0·59	0·78
A + B,[a] μmoles/g.	0·78	0·65	0·62	1·05
Thiol content of flour, μmoles/g.	0·76	0·82	0·69	0·95
$k_A \times 10^3$, sec.$^{-1}$	5·8	2·4	2·4	9·0
$k_B \times 10^4$, sec.$^{-1}$	0·68	1·9	3·1	3·4
$k \times 10^4$ as reported by Sokol *et al.*, sec.$^{-1}$	1·7	2·5	4·2	5·0

[a] Since Sokol *et al.* assume a loss of thiol groups during the processing of the doughs, the present author did not feel justified to choose A + B identical with the thiol content of the flours as has been done in Table II. Instead, A, B, k_A, and k_B were calculated so as to give a perfect fit of the experimental points after 2, 5, 10 and 20 min. of mixing. Apparently Sokol *et al.* did not use the thiol contents of the flours for the estimation of their rate constants k either.

the soluble and insoluble thiol groups in dough and not only the soluble ones ; moreover, silver nitrate is a more specific reagent than the *o*-iodosobenzoic acid used by Smith *et al.*

From the results of Tsen & Hlynka[7] it can be estimated that blocking of the thiol groups results in an increase of the amount of lipid peroxides by 0·4 μequiv. per g. of flour. This is approximately the same amount as the decrease of the thiol content on mixing in oxygen for 30 min.—0·36 μmole/g. for both flours according to equation (1) and Table II of the present paper. If oxidation proceeds to the disulphide stage, the same number of equivalents of oxygen



Apparent densities of and gas occlusions in doughs from flours I (FIG. 3, left) *and II* (FIG. 4, right), *and from the same flours after various fat extractions, all after varying mixing times in nitrogen or in oxygen*

(Abbreviations and symbols as in Figs. 1 and 2, respectively)

curve A LP and CT
" B U, AW in nitrogen, BW
" C AW in oxygen

curve A LP
" B U in nitrogen
" C U in oxygen

are required. So far, the observations can be explained by a competition for oxygen between thiol groups and flour lipids.

However, for an explanation of the slower oxidation of thiol groups as a result of fat extraction, another assumption is necessary, for instance that the oxidation of thiol groups by lipid peroxides (reaction II of Tsen & Hlynka) is a fast reaction. The occurrence of this reaction is made probable by the observations that fatty acid peroxides or their esters react with thiol groups of cysteine and ovalbumin,[18] and inactivate enzymes the activity of which is dependent on the presence of thiol groups in their molecules.[19] If this assumption is correct, the higher amount of lipid peroxides found after blockage of the thiol groups can better be explained by the failure of this reaction than by the competition for molecular oxygen. This is supported by considering the amounts of oxygen involved in the various reactions.

As stated above, 0·36 μequiv./g. of oxygen is necessary for the oxidation of the disappearing thiol groups to disulphides. If the oxidation proceeds to more highly oxidised products, a larger amount is consumed. It is improbable that on the average one thiol group consumes more than 3 equivalents.[20] Therefore 1 μequiv./g. is considered as a safe upper limit for the amount of oxygen consumed by the thiol groups. The amount of oxygen consumed by the lipid fraction is more difficult to estimate. The peroxide oxygen, reported by Tsen & Hlynka,[7] being of the order of 1 μequiv./g., can be considered as a minimum amount. Smith & Andrews reported actual oxygen uptakes of 26 μequiv./g. for a patent and 58 μequiv./g. for a first clear flour, which are largely due to the lipid fraction.[21] The amount of oxygen available during mixing is dependent upon the rate of gas occlusion. The curves in Figs. 3 and 4 represent the excess of occlusion over consumption. Therefore, the amount of oxygen available during mixing is larger than the amount occluded at the end of the mixing time. The latter varies from 3 to 14%, corresponding with 6 to 33 μequiv./g., dependent upon mixing time; more oxygen has been available during mixing. In comparing these figures one must conclude that the amount of oxygen available is usually amply sufficient for an oxidation of the thiol groups; it may or may not restrict the oxidation of the lipid fraction. Although the unsaturated lipids may consume so much oxygen that the oxidation of thiol groups is retarded, the reverse can hardly be true. It is not excluded that thiol groups retard the oxidation of lipids by other mechanisms, for instance breaking of reaction chains.

The rate of oxidation of thiol groups is decreased by fat extraction by all solvents in spite of the fact that after some extractions the rate of occlusion of oxygen is increased. This is in agreement with the conclusion that it is not the amount of available oxygen that restricts this oxidation. Its rate is more probably determined by the size distribution of the gas cells and their total surface area, or by the reactivity of the thiol groups in the dough phase.

During mixing in oxygen, the rate of oxidation of thiol groups decreases from the order of 1×10^{-3} to 7×10^{-5} μmoles/g. sec., but in a resting dough it is only of the order of 3×10^{-6} μmoles/g. sec. in spite of the fact that an ample amount of oxygen is usually available (see above). In a resting dough the oxidation of thiol groups is possibly limited by the diffusion of oxygen from the gas cells to reactive groups. These figures demonstrate the importance of the mixing process for the rates of chemical reactions in dough.

Institute for Cereals, Flour and Bread, T.N.O.
Wageningen
The Netherlands

Received 10 January, 1963

References

[1] Smith, D. E., & Andrews, J. S., *Cereal Chem.*, 1952, **29,** 1; Dempster, C. J., Hlynka, I., & Anderson, J. A., *Cereal Chem.*, 1954, **31,** 240

[2] Dempster, C. J., Cunningham, D. K., Fisher, M. H., Hlynka, I., & Anderson, J. A., *Cereal Chem.*, 1956, **33,** 221

[3] Sullivan, B., *J. agric. Fd Chem.*, 1954, **2,** 1231; Goldstein, S., *Mitt. Lebensm. (Bern)*, 1957, **48,** 87; Mecham, D. K., *Cereal Chem.*, 1959, **36,** 134; Bloksma, A. H., & Hlynka, I., *ibid.*, 1960, **37,** 352; Sokol, H. A., & Mecham, D. K., *Baker's Digest*, 1960, **34,** (6), 24, 74; Frater, R., Hird, F. J. R., Moss, H. J., & Yates, J. R., *Nature, Lond.*, 1960, **186,** 451; *J. Sci. Fd Agric.*, 1961, **12,** 269; Bushuk, W., *Cereal Chem.*, 1962, **39,** 189

References (*cont.*)

[4] Bungenberg de Jong, H. L., *Proc. Nord. Cereal-kemistförenings, 8th Kongr.* (Stockholm), 1946, p. 240; *idem, ibid., 14th Kongr.* (Oslo), 1960, p. 148; *idem, Rev. Ferment. Ind. aliment.*, 1956, **11,** 261
[5] Smith, D. E., Van Buren, J. P., & Andrews, J. S., *Cereal Chem.*, 1957, **34,** 337
[6] Narayanan, K. M., & Hlynka, I., *Cereal Chem.*, 1962, **39,** 351
[7] Tsen, C. C., & Hlynka, I., *Cereal Chem.*, 1962, **39,** 209
[8] Bloksma, A. H., *Chem. & Ind.*, 1959, p. 253
[9] Hlynka, I., & Anderson, J. A., *Cereal Chem.*, 1955, **32,** 83
[10] Swanson, E. C., 'Cereal Laboratory Methods', 6th edn, 1957, pp. 132 ff. (Amer. Ass. Cereal Chemists, St. Paul)
[11] Bloksma, A. H., *Getreide u. Mehl*, 1958, **9,** 65
[12] Sokol, H. A., Mecham, D. K., & Pence, J. W., *Cereal Chem.*, 1960, **37,** (*a*) p. 151; (*b*) p. 739
[13] Sokol, H. A., Mecham, D. K., & Pence, J. W., *Cereal Chem.*, 1959, **36,** 127; Bloksma, A. H., *ibid.*, p. 357
[14] Allison, A. C., & Cecil, R., *Biochem. J.*, 1958, **69,** 27; Cecil, R., & McPhee, J. R., *Adv. Protein Chem.*, 1959, **14,** 255
[15] Sullivan, B., Dahle, L., & Larson, E., *Cereal Chem.*, 1961, **38,** 272
[16] Baker, J. C., & Mize, M. D., *Cereal Chem.*, 1946, **23,** 39
[17] Cookson, M. A., & Coppock, J. B. M., *J. Sci. Fd Agric.*, 1956, **7,** 72
[18] Dubouloz, P., & Fondarai, J., *Bull. Soc. Chim. biol.*, 1953, **35,** 819
[19] Wills, E. D., *Biochem. Pharmacol.*, 1961, **7,** 7
[20] Hird, F. J. R., & Yates, J. R., *Biochem. J.*, 1961, **80,** 612; Bloksma, A. H., in preparation
[21] Smith, D. E., & Andrews, J. S., *Cereal Chem.*, 1957, **34,** 323

A COMPARISON OF FREEZE-DRIED BEEF MUSCLES OF HIGH OR LOW ULTIMATE pH

By I. F. PENNY, C. A. VOYLE and R. A. LAWRIE

Some of the deleterious effects of freeze-drying on the texture of beef have been obviated by using muscles with a high ultimate pH. Pre-slaughter injection of adrenaline raised the ultimate pH to 6·7. After freeze-drying the treated beef was more tender, more juicy and less woody than controls at pH 5·6. A resultant increase in water-holding capacity and rehydratability was demonstrated by myofibrillar swelling and increased fibre diameter. Dehydrated beef with a high ultimate pH showed less deterioration during storage at 37° in the attributes texture, flavour and colour.

Introduction

Considerable technological advances in the freeze-drying of foodstuffs have been made in recent years. As a result, the process of Accelerated Freeze-Drying (AFD)[1] has become commercially possible. Nevertheless the process causes changes in some products which adversely affect their quality. Raw meat subjected to the AFD process is usually tougher and drier than the original meat and in addition has a characteristic 'woody' texture. These undesirable features are paralleled by a reduction in the water-holding capacity of the proteins after drying.[2]

The protein/water-holding relationship of muscle is affected to a considerable degree by the extent of pH fall during *post mortem* glycolysis. Thus a high ultimate pH is associated with an increase in water-holding capacity.[3, 4] An ultimate pH greater than 6·5 can be induced experimentally in musculature by pre-slaughter injection of animals with adrenaline.[5] Preliminary work[6] on rabbit had shown that after being freeze-dried, muscle with an ultimate pH of 6·7 had a higher water-holding capacity, was more tender and less woody than muscle with a normal pH of 5·9. These experiments have now been extended to beef. In addition to examining the effect of high ultimate pH on the texture of the beef after freeze-drying the effect on subsequent storage has also been studied.

Experimental

Injection of steers and sample preparation

Identical twin steers (of Aberdeen Angus–Jersey breed) were employed in the hope of lessening differences due to inter-animal variation. One of these was injected subcutaneously

with adrenaline (100 μg./kg.) at 24 h. and again 4 h. pre-slaughter; the other twin, which was not injected, served as a control. The left hand side of each carcass was dissected into its constituent anatomical muscles. *Semimembranosus, biceps femoris, psoas major, longissimus dorsi* (lumbar), *longissimus dorsi* (thoracic) and deep pectoral muscles were held at +1° for 7 days before being frozen at −20°. The values for the ultimate pH of the fresh muscle are compared in Table I, from which the effect of adrenaline in depleting the glycogen reserves, and thereby in raising the ultimate pH, will be apparent. The pH increment in the *psoas major* was slightly greater, and that in deep pectoral slightly less, than those obtained with the other four muscles.

To prepare the meat for dehydration, slices 12 mm. thick were cut from the whole frozen muscle. Each set of six slices taken consecutively along the muscle comprised one unit. From each unit, two slices wrapped in polythene pouches were held frozen at −20° and the remainder were freeze-dried by the AFD process. The adrenaline treatment appeared to have little effect on the length of the drying run since the average time for the control was 5½ h. and the treated 5¾ h.

After being dried each unit was separately sealed in a can and gas packed under nitrogen. Samples were held at room temperature, at 0 and 37°.

For convenience the frozen beef from the adrenaline injected steer will be referred to as 'treated' and the beef from the other twin as 'control'.

Cooking and tasting

Dehydrated whole steaks were reconstituted by immersion in water for 20 min. During this time corresponding frozen steaks were thawed out in water. The samples were then casseroled (in the immersion water) in an oven at 185° for 1½ h. To assess texture, a taste panel (8 members) was asked to place the samples in order of increasing toughness and dryness, and also to indicate 'woodiness' when it was detected. Each taster received treated and control samples, both frozen and dehydrated, but the order in which they were tasted was random. Only one muscle was assessed at each sitting. For estimation of stored samples, an arbitrary scale was used (0 very tough; 6 tender).

Tenderometer measurements

Rehydrated and thawed steaks, wrapped tightly in aluminium foil, were cooked by steaming for 1 h. After cooling, pieces 1 cm. wide and 0·5 cm. thick were cut; the length of the piece depended on the original thickness of the steak. The pieces were cut so that the direction of the fibres was always at right angles to the shearing edge of the tenderometer. The instrument used was that described by Grünewald.[7] The toughness of a sample was determined by the work done by the tenderometer (under a constant load of 15 kg.) in shearing the pieces of meat. (Results were calculated for pieces of meat 1 cm. thick.)

Reconstitution

The influence of ultimate pH on the reconstitution of the dehydrated beef was examined by the following procedure. The dehydrated samples were first ground by passing them through a mincer with a plate having 4-mm. holes, and then washed several times with cold diethyl ether to remove all the fat. The ether was evaporated by suction in a Buchner funnel and the dried powder passed through a No. 40 mesh sieve, which removed large pieces of connective tissue and fibre clumps (approximately one-third of the whole dried powder). Of the powder

Table I

Ultimate pH of fresh muscles from adrenaline-treated and control identical twin steers

Muscle	Treated	Control
Semimembranosus	6·80	5·68
Biceps femoris	6·70	5·50
Psoas major	7·01	5·52
Longissimus dorsi (lumbar)	6·75	5·60
,, ,, (thoracic)	6·80	5·60
Deep pectoral	6·52	5·60

which passed through the sieve, 2 g. were weighed accurately in a centrifuge tube and mixed with 20 ml. of water. After being kept for 20 min. the tube was centrifuged at 2500 *g* for 5 min. The supernatant was discarded and the residue drained for 10 min. and reweighed. The reconstitution ratio was determined as the weight of water absorbed per g. of dry meat powder.

Preparation of myofibrils and measurement of water binding

Myofibrils were prepared by homogenising 30 g. of meat in ice-cold 0·1M-potassium chloride in a high-speed blendor for 2 min. From control dehydrated material, however, low yields were obtained and further periods of homogenising were necessary to prepare myofibrils in sufficient quantities for determining pH/water-binding relationships. The myofibrils were washed with 0·1M-potassium chloride until free of soluble sarcoplasmic proteins. A suspension with a concentration of 5–6 mg. fibrillar protein/ml. was prepared in 0·1M-potassium chloride. Measurement of the effect of pH on the water binding of the myofibrils was carried out on 10-ml. portions by adjusting the pH of the suspension with 0·1N-hydrochloric acid or -sodium hydroxide. After being kept for 15 min. to allow the myofibrils to equilibrate to the required pH, the suspensions were centrifuged at 1200 *g* for 5 min. The myofibril layer was weighed after decanting and draining the supernatant. Protein concentration was usually determined on a solution of myofibrils in 0·01N-hydrochloric acid either by measuring the absorption at 220 mμ or by Lowry's modification[8] of the method of Folin. In both cases standardisation was first carried out by the usual Kjeldahl procedure.

Histological studies

A series of slices from each muscle were selected for histology. Samples were obtained after being frozen at −20° and after dehydration. The samples were removed with a No. 8 cork borer and, in order to provide valid comparisons, were obtained from the same relative position in each slice.

Measurements of fibre diameter were carried out on teased preparations of the frozen samples. The cylinders of frozen tissue removed with a cork borer were allowed to thaw at room temperature before a portion was removed for teasing. The method described by Hiner and his co-workers[9] was employed. By means of an eyepiece micrometer scale three readings for each of twelve fibre fragments were obtained. The readings were taken at points approximately 100 μ apart. The ends of fragments and those showing excessive damage due to teasing were avoided. (Measurements of fibre diameter of unfrozen muscle were not materially different from those obtained with frozen tissue.)

Similar measurements were also made on reconstituted tissue. Samples were removed from the dehydrated slices in the same way as from the frozen material. The tissue cylinders were cut into slices approximately 2–3 mm. in thickness and reconstituted in distilled water. Reconstitution was carried out at room temperature in a vacuum of 600 mm. Hg. After 30 min. the samples were removed from the vacuum chamber and placed on filter paper for 5 min. to remove excess water. Fibre diameters were determined on teased preparations as above.

Moisture content

This was determined by drying minced dehydrated samples in a vacuum oven at 70° and less than 3 mm. pressure for 5 h.

Glucose estimation

Glucose was extracted by blending 0·5 g. of dehydrated beef with 3 ml. of 2N-perchloric acid and 3 ml. of water. After removal of the precipitated protein by centrifuging, 2 ml. of the supernatant were neutralised to pH 6 with 2N-potassium hydroxide and made up to 15 ml. The solution was then chilled to precipitate the potassium perchlorate which was removed by filtration and the filtrate was further diluted to 3 times its volume. Glucose was determined on 1-ml. portions by a modification[10] of the glucose oxidase method of Saifer & Gerstenfeld[11] which is specific for glucose.

Degree of browning

Washed homogenates were treated with acetone to remove the water, and the acetone was evaporated by suction at a vacuum pump. The reflectance of the acetone-dried powder was measured at 400 mμ on a Unicam SP 500 with a reflectance attachment.

Results

Tasting

From the results given in Table II the effectiveness of high ultimate pH in improving the texture of beef is clearly demonstrated. The treated (frozen) sample was placed first ($p = 0.05$) in tenderness and juiciness. This improvement was maintained after drying, the treated dehydrated sample being placed second ($p = 0.05$). The control (frozen) sample, which was third, was only slightly different from the control dehydrated meat except in the incidence of ' woodiness ' which was greatest in the latter.

Table II

Taste panel results

The sum of the rankings given by eight tasters for treated and control (both frozen and dehydrated) for tenderness and juiciness

Muscle	Treated		Treated dehydrated		Control		Control dehydrated	
	Tenderness	Juiciness	Tenderness	Juiciness	Tenderness	Juiciness	Tenderness	Juiciness
Semimembranosus	12	13	16	19	23	24	24	23
Biceps femoris	11	15	15	22	26	20	25	22
Psoas major	12	12	14	16	23	25	25	25
Longissimus dorsi (lumbar)	19	11	17	14	19	26	21	24
,, ,, (thoracic)	11	10	12	20	26	22	25	25
Deep pectoral	10	10	16	19	25	25	27	24
Total	75	71	90	110	142	142	147	143
No. of times ' woodiness ' was indicated	2		9		10		16	

Tenderometer measurements

Objective measurement of toughness by tenderometer confirmed the findings of the taste panel (Table III). As before, the treated frozen meat was the most tender. The treated dehydrated muscles were in most cases more tender than the corresponding control (frozen) muscles; and, in all cases, more tender than corresponding control dehydrated muscles. The results also show a slight, but consistent, tendency to greater toughness in the control after dehydration. It is interesting to note that the effect of the treatment varied for the different muscles. The increase in tenderness of the *biceps femoris* was slight, whereas the *semimembranosus* (which was the toughest muscle in the control animal) was very tender in the treated animal and comparable to the *psoas*.

Reconstitution

An increase in the amount of water absorbed during reconstitution was found in all the treated dehydrated muscles in comparison with corresponding control dehydrated material. The method used, because of the removal of fat and the uniform particle size, gave consistent

Table III

Measurement of work done (ergs $\times 10^6$*/cm.) by a tenderometer in shearing meat*

Muscle	Treated	Treated dehydrated	Control	Control dehydrated
Semimembranosus	9·8	13·4	24·5	25·6
Biceps femoris	12·1	13·6	12·6	15·2
Psoas major	9·2	9·5	12·9	12·4
Longissimus dorsi (lumbar)	11·5	11·8	15·2	17·7
,, ,, (thoracic)	6·9	9·1	12·9	17·2
Deep pectoral	14·8	18·3	18·6	19·4

Table IV

Reconstitution ratios (g. H_2O/g. dry wt.) of dehydrated muscle from adrenaline-treated and control steers measured after 20 min. reconstitution and centrifuging at 2500 g for 5 min.

Muscle	At ultimate pH of muscle Treated dehydrated	At ultimate pH of muscle Control dehydrated
Semimembranosus	3·10	1·99
Biceps femoris	3·12	1·96
Psoas major	3·58	2·08
Longissimus dorsi (lumbar)	3·22	1·98
,, ,, (thoracic)	3·34	2·02
Deep pectoral	3·65	1·95

results for the various muscles from the control animal as shown in Table IV. The fact that the muscles from the treated animal absorbed markedly different amounts of water can be taken as an indication that the muscles responded differently to the adrenaline treatment.

Myofibrillar swelling

The results in Fig. 1 show the pH/swelling curves, averaged for all muscles, and the curves for individual muscles in Fig. 2. It can be seen from both these figures that the myofibrils from the treated muscle absorbed more water than those from the control, this being true throughout the range of environmental pH studied. It was found, however, by microscopical examination with phase contrast illumination at a magnification ×675, that, although the myofibrils prepared from the control muscles remained separated throughout the pH range used, the treated myofibrils clumped together when the pH was lowered to less than 6·5 by the addition of 0·1N-hydrochloric acid. This altered their sedimentation behaviour during centrifuging. Accordingly, comparison between the myofibrils of the treated and control muscles could be made only when they were in a similar separated state. The effect of dehydration on the water holding of the myofibrils is also shown in the curves. The myofibrils from both treated and control dehydrated material absorbed less water than their non-dehydrated counterparts. But the treated dehydrated absorbed more water than the control dehydrated at pH 6·5 and above thus indicating the improved water-holding capacity of dehydrated meat with a high ultimate pH.

The results in Fig. 2 illustrate that the different muscles responded in varying degrees to a high ultimate pH. The pH/swelling curve for the *longissimus dorsi* (lumbar) was practically the same for the treated (frozen), treated (dehydrated) and control (frozen) samples. However, all the other muscles from the treated meat absorbed more water at pH 6·5 than corresponding controls; the greatest increase was found with the *psoas* and the *biceps femoris*. With the exception of *longissimus dorsi* (lumbar) all the treated muscles have a lower water-holding capacity after dehydration, the drop being approximately the same for each muscle. Similarly the water-holding capacity of all the control muscles was lower after dehydration. (In this particular figure the curves for three muscles only have been shown in order to avoid confusion: the other three muscles give a similar picture.)

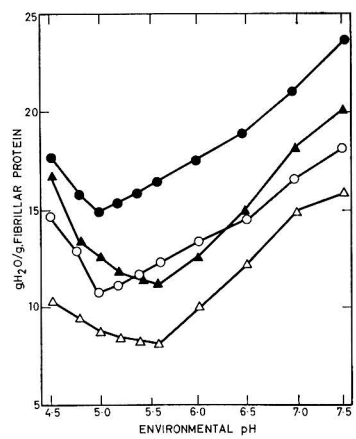


FIG. 1.—*Effect of environmental pH on the water-holding capacity of myofibrils prepared from muscles with high or normal ultimate pH*

(Average results of all muscles)

—●— Treated: ultimate pH 6·7
—○— Treated, dehydrated: ultimate pH 6·7
—▲— Control: ultimate pH 5·6
—△— Control, dehydrated: ultimate pH 5·6

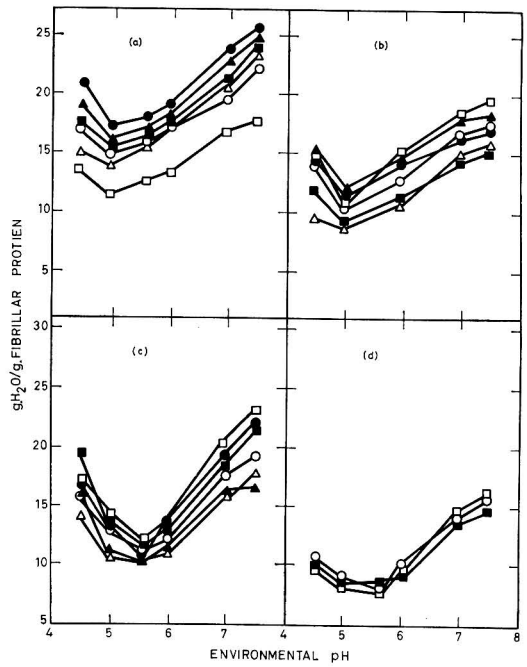


FIG. 2.—*Effect of environmental pH on water-holding capacity of myofibrils prepared from individual muscles with high or normal ultimate pH*

(a) Treated frozen, (b) treated dehydrated, (c) control frozen, (d) control dehydrated

—●— *Biceps femoris* —○— *Longissimus cervicus*
—▲— *Psoas major* —△— Deep pectoral muscle
—■— *Semimembranosus* —□— *Longissimus dorsi*

In addition to the increase in the water-holding capacity of the rehydrated muscles of the treated animal, an increase in the ease of breakdown of fibres to fibrils was observed. To estimate this difference, a weighed quantity of the fat-free powder used in reconstitution ratio measurements was homogenised with 0·1M-potassium chloride in a small blendor for 5 min. The resulting homogenate was centrifuged and the myofibril layer, which was removed as accurately as possible, was washed three times in 0·1M-potassium chloride by centrifuging and resuspension. Protein determinations were carried out on the myofibrils and the amount of fibrillar protein prepared from 1 g. of dried meat is shown in Table V. Clearly the control rehydrated meat resisted breakdown to myofibrils to a greater extent than the rehydrated meat from the treated animal.

The effect of the pH of the homogenising medium on the swelling of the myofibrils has also been studied. Control and treated frozen meat was homogenised with 0·1M-potassium chloride in 0·03M-sodium glycerophosphate buffer at pH 5·6 or 6·7. After homogenisation, sodium hydroxide or hydrochloric acid was added to correct the pH to 5·6 or 6·7 and the homogenates were left overnight to equilibrate. The myofibrils were then washed and centrifuged in the appropriate buffer. The pH/swelling curves were obtained by the method previously described. The results in Fig. 3 show that, when meat of normal ultimate pH 5·6 was homogenised at pH 6·7, no increase in swelling at pH 7 was found, but when meat with high ultimate pH was homogenised at pH 5·6, there was a considerable reduction in water holding.

Table V

Yield of fibrillar protein (mg.) from 1 g. of fat-free dehydrated meat

	Control	Treated
Longissimus dorsi	152	210
Biceps femoris	127	224
Semimembranosus	149	234

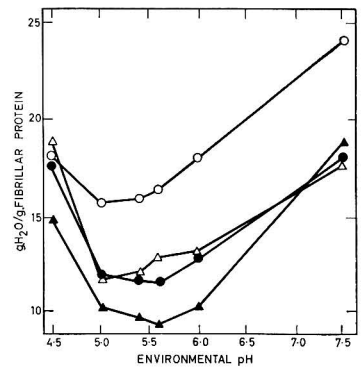


FIG. 3.—*Effect of adjusting the pH of homogenates of* biceps femoris *of high or normal ultimate pH, on the water-holding capacity of the myofibrils*

—○— Treated at pH 6·7
—●— Treated, adjusted pH 5·6
—△— Control, adjusted to pH 6·7
—▲— Control at pH 5·6

Histological studies

The mean fibre diameters of the muscles of the control and treated animals from the frozen and rehydrated states are shown in Table VI. The standard errors indicate a greater degree of scatter than was obtained with rabbit *longissimus dorsi*.

In studying rabbit *longissimus dorsi*[6] it had appeared that the mean fibre diameter increased as the ultimate pH of the muscle increased. In the work on beef muscles the greater variability between fibres may account partially for failure to observe a similar relationship. Nevertheless the induction of a high ultimate pH clearly increases rehydratability of muscle dried by the AFD process ; and this is reflected in the relatively greater fibre diameters of rehydrated muscle of high pH in comparison with corresponding muscle for the control steer.

Storage

The samples stored at −20° and 37° were examined after 4 months : the results for the *longissimus dorsi, biceps femoris* and *semimembranosus* are shown in Table VII.

Organoleptic assessment indicated that tenderness, juiciness and flavour of the meat with high ultimate pH were little affected by storage at 37° in comparison with corresponding material at −20°. The control samples of low ultimate pH, on the other hand, were considerably tougher and drier when stored at 37° than the corresponding material at −20° ; and the meat of low ultimate pH, whether held at −20° or 37°, was markedly less tender and juicy than the meat of high ultimate pH at either temperature. Measurement by tenderometer confirmed these findings on toughness, although the treated *semimembranosus* stored at 37° was found considerably tougher than the taste panel suggested.

It had been found previously that the dehydrated meat could readily be broken down to myofibrils by homogenising at high speed in 0·1M-potassium chloride. However, in these stored samples, even those held at −20°, the yield of myofibrils was very low. Accordingly the water-holding capacity was determined on fibres. A reduction in the water held by the control stored at 37° was found, but there was little diminution in the meat of high ultimate pH.

Table VI

Mean fibre diameter (μ) of muscle with normal and high ultimate pH following freezing and rehydration

Muscle	Frozen				Rehydrated				% Rehydration	
	Control	(SE)	Treated	(SE)	Control	(SE)	Treated	(SE)	Control	Treated
Semimembranosus	98·6	(23·4)	94·5	(17·5)	90·7	(27·2)	96·9	(15·1)	91·5	102·5
Biceps femoris	90·3	(17·2)	81·8	(12·5)	75·7	(14·3)	119·3	(13·5)	83·5	143·8
Longissimus dorsi	82·2	(18·2)	83·5	(22·0)	82·6	(17·0)	129·9	(15·3)	100·5	152·5
Psoas major	53·2	(11·9)	55·5	(12·0)	47·2	(11·2)	51·2	(6·8)	88·7	92·0

SE = standard error

Table VII

Effect of storage on dehydrated meat with ultimate pH 6·7 and 5·6

Muscle	Ultimate pH 6·7						Ultimate pH 5·6					
	Biceps femoris		*Semimembranosus*		*Longissimus dorsi*		*Biceps femoris*		*Semimembranosus*		*Longissimus dorsi*	
Storage temp., °C	−20	+37	−20	+37	−20	+37	−20	+37	−20	+37	−20	+37
Moisture, %	2·0	—	2·3	—	1·8	—	2·6	—	1·8	—	1·6	—
Glucose, mg./g.	0·06	0	0·10	0	0·07	0	2·9	0	3·4	0	2·6	0
Tenderness	—	4·5	3·6	3·1	4·3	4·1	3·9	2·8	—	2·0	3·4	2·0
Juiciness	—	3·2	3·9	3·4	4·4	4·4	2·8	2·8	—	3·9	4·3	3·1
Flavour	—	3·9	4·6	4·7	4·7	4·3	4·9	4·0	—	4·4	4·0	4·4
Tenderometer, ergs × 10^6	—	8·4	12·6	23·3	11·5	11·5	11·5	15·7	—	27·2	12·7	18·4
Reflectance at 400 mμ	—	0·375	0·240	0·390	0·127	0·351	0·249	0·435	—	0·565	0·152	0·497
Water holding at pH 6·5, g. H_2O/g. protein	—	6·7	8·9	9·0	10·8	10·3	8·3	6·2	—	6·6	9·8	8·2

Although the glucose content of the treated sample was low compared with that of the control, both browned on storage. The degree of browning was greater in the latter case and the general appearance was inferior.

Discussion

The main intention of the experiment with beef was to assess whether or not the beneficial effects of a high ultimate pH in reducing the ' woodiness ' and toughness of AFD-treated rabbit *longissimus dorsi* muscle would obtain when applied to a large commercial species of animal. The taste panel and tenderometer results amply confirmed that there were such benefits. Since ' woodiness ' was noted in non-dehydrated samples of beef from the control steers (i.e., of normal, low ultimate pH), however, it is possible that this adverse characteristic of AFD meat may be one of degree, rather than a specific effect of the process, and there are some preliminary indications that ' woodiness ' may become apparent even in meat of high ultimate pH on storage, although tenderness and colour may be retained at satisfactory levels.

An interesting aspect of the tenderometer results is the differential effect of high ultimate pH, and of the AFD process, between the different anatomical regions. Thus, the *semimembranosus*, which was toughest of the six muscles studied in the control steers, was of tenderness comparable to that of the *psoas* muscle in the treated steer. On the other hand, there was virtually no increase in tenderness in the *biceps femoris* muscle of the treated steer in comparison with that of the corresponding muscle of the control animal ; yet the yield of fibrillar protein from this muscle, and its ease of breakdown on homogenisation, were relatively greater after treatment than with the case of *semimembranosus*. The AFD process caused a relatively greater increase in toughness (as measured in tenderometer) in the *semimembranosus*, deep pectoral and *longissimus dorsi* (thoracic) muscles of the treated steers ; but in the control steer only the last-named muscle increased in toughness to a comparable degree. In view of the more marked responses of the taste panel to differences caused by the AFD process in both control and treated muscle, it must be assumed that the character measured by the tenderometer does not correspond exactly to the toughness or ' woodiness ' detected by the former. Another aspect of the differential response of the six muscle areas studied is exemplified by the reconstitution ratios. Although the various dehydrated muscles from the control steer absorb similar quantities of water on reconstitution, dehydrated *psoas major* and deep pectoral muscle from the treated steer appear to have improved their absorbing capacity to a relatively greater extent than did the other four muscles.

The results obtained for myofibrillar swelling with the various beef muscles also confirmed the view that the ultimate pH of the muscle is more important than the pH of the environment in which measurements are made. It has been found[12] that a protein fraction, which is soluble in 0·1M-potassium chloride when derived from a given muscle at an ultimate pH of 6·7, and hence sarcoplasmic in origin, was insoluble if the ultimate pH were 5·6, and was also denatured, since it

no longer dissolved when the pH was raised again to 6·7. The lowered water-holding capacity of the myofibrils derived from meat of low ultimate pH may be due to some intrinsic change in the properties of the myofibrillar protein and/or the precipitation of this sarcoplasmic protein on to the myofibrillar surface, as in pork muscle—when the rate of the *post mortem* glycolysis is very high.[13] Since the fraction concerned is denatured when the pH falls to 5·6, it is obvious why the subjection to a subsequent high environmental pH is of less significance with respect to water-binding than a high ultimate pH in the muscle itself.

The degree of response to high ultimate pH again differed between the muscles. The *longissimus dorsi* (lumbar) responded least and the *biceps femoris* responded most; in this respect they reflected their relative response to breakdown to the level of myofibrils, referred to above. It is noteworthy that, despite failure to detect an increased fibre diameter in the muscles of the treated steer before dehydration—indeed a marked decrease was found in *biceps femoris*—the fibre diameters *after* dehydration and reconstitution were consistently greater in the muscles of high ultimate pH (thus aligning with the data obtained with rabbit *longissimus dorsi*).

Since the glucose content of the meat with high ultimate pH was low, less deterioration in various attributes of eating quality during storage at 37° was expected. This was found with the treated meat which differed little from the corresponding samples stored at −20°. The control sample, on the other hand, markedly deteriorated. It is of interest, however, that browning had occurred in the treated samples, which suggests that even small quantities of glucose are sufficient to initiate the Maillard reaction.

The fact that the fibres of the treated and the control muscles could not be broken down easily after storage indicated that changes had occurred during this period leading to a denaturation of the protein in both. Yet, whatever the change, it had not appreciably affected the texture of the meat with a high ultimate pH.

Acknowledgments

This work was financed by the U.S. Department of Agriculture (Grant No. FG-UK-101-59). The pre-slaughter injection of the beef steer was carried out with the kind co-operation of the staff of the A.R.C. Institute of Animal Physiology, Babraham Hall, Babraham, and the anatomical dissection performed by Dr. R. W. Pomeroy and his staff. Some of the tenderometer data were supplied by Mr. J. H. Linton. The authors should also like to thank Mr. B. F. Lovey for technical assistance.

Low Temperature Research Station
Downing Street
Cambridge

Received 4 December, 1962; amended manuscript 3 April, 1963

References

[1] Hanson, S. W. F., 'The Accelerated Freeze-Drying Method of Food Preservation', 1961 (London: H.M.S.O.)

[2] Hamm, R., & Deatherage, F. E., *Food Res.*, 1960, **25,** 573

[3] Empey, W. A., *J. Soc. chem. Ind., Lond.*, 1933, **52,** 270

[4] Howard, A., & Lawrie, R. A., *C.S.I.R.O. Div. Food Pres. Tech. Pap.*, 1956, No. 2, Part III, p. 52

[5] Cori, C. F., & Cori, G. T., *J. biol. Chem.*, 1928, **79,** 309

[6] Penny, I. F., Voyle, C. A., & Lawrie, R. A., *Proc. 1st Int. Congr. Fd Sci. Tech.*, 1962, in press

[7] Grünewald, T., *Z. LebensmittUntersuch.*, 1957, **105,** 1

[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J., *J. biol. Chem.*, 1951, **193,** 265

[9] Hiner, R. L., Hankins, O. G., Sloane, H. S., Fellers, C. R., & Anderson, E. E., *Food Res.*, 1953, **18,** 364

[10] Matheson, N. A., *J. Sci. Fd Agric.*, 1962, **13,** 248

[11] Saifer, A., & Gerstenfeld, S., *J. Lab. clin. Med.*, 1958, **3,** 448

[12] Scopes, R. K., 1963, in preparation

[13] Bendall, J. R., & Wismer-Pedersen, J., *J. Fd Sci.*, 1962, **27,** 146

THE NUTRITIONALLY AVAILABLE LYSINE AND METHIONINE OF HEATED CASEIN–GLUCOSE MIXTURES

By M. NARAYANA RAO, H. SREENIVAS, M. SWAMINATHAN, K. J. CARPENTER and CLARE B. MORGAN

A mixture of buffalo casein and glucose of high moisture content was given different heat treatments which greatly reduced the value of the materials as a protein source for young rats. Their value as a source of lysine fell much more than their value as a source of methionine or 'methionine + cystine'. These feeding results confirmed the results of two laboratory prediction tests—reactivity with fluorodinitrobenzene for 'available' lysine, and a microbiological assay for 'available' methionine with *Strep. zymogenes* NCDO 592 on mild papain digests.

Introduction

It is now well known that when casein is in contact with glucose and water it is extremely sensitive to heat damage, which reduces its nutritive value.[1, 2] The hypothesis used to explain this has been that the carbonyl groups of the sugar form indigestible linkages with the free amino-groups of the protein,[3] and it has been confirmed that linkages are formed even under mild conditions.[4, 5] Nutritional damage to dried skim milk (where casein is again in contact with sugars containing free carbonyl groups), as a result of either scorching or storage under adverse conditions, is largely corrected by supplementation with lysine,[6, 7] an amino-acid which still has a second reactive amino-group even when its α-amino-group is linked into the normal peptide chain.

In more recent work, it has been demonstrated that when meat or fish is given severe heat-treatment under different conditions, the value of these materials as sources of the sulphur-containing amino-acids is reduced at least as much as their value as sources of lysine.[8, 9] It has also been found, in studies with a proteolytic strain of *Streptococcus zymogenes*, that the availability of methionine and even of isoleucine in some proteins falls to a greater extent than does the number of the reactive lysine amino-groups.[10–12]

It is the object of the present work to go back to the casein–glucose system on which much earlier work was done to examine specifically whether or not different types of heat-treatment would cause severe reduction in the availability of the sulphur amino-acids either for the rat or for *Strep. zymogenes*. With flesh materials, results with *Strep. zymogenes* have proved a good indicator of damage for the rat and the chick.[10, 12]

Experimental

Test materials

No. 1.—Control batches of 800 g. of 'casein' (prepared commercially from Indian buffalo milk by precipitation at pH 4·8; containing N 14·3%, dry matter 96·9%) were suspended in 3800 ml. of water and the slurry adjusted to pH 6·3 by addition of 192 ml. of 2·5N-sodium hydroxide and then well stirred. Glucose (commercial grade) (316 g.) was then added and the whole homogenised thoroughly in a Hobart mixer. The material was then freeze-dried in a tray dryer, with the heating plates at 45°, ground in a hammer mill to pass a 40-mesh screen and stored at −20° in sealed containers. It contained 4·5% of moisture.

No. 2.—Portions of the No. 1 material were spread on trays and a fine spray of water played over them until it was estimated that they contained 13·5–16·0 g. of water/100 g. of solids. They were then well mixed, held in sealed cans at 4° for 3 days to allow equilibration of the moisture, re-mixed and found to contain 15·0 g. of water/100 g. of solids. The mixture was then re-canned in small A.1 size tins and one-half of them were immediately transferred to a hot-air oven for 40 min. The temperature of the air in the oven was 85° but, as will be shown in Table II, it has been concluded that the temperature attained by the mixture was considerably less. The containers were cooled and the contents freeze-dried and stored at −20° as material 2.

No. 3.—The remainder of the above A.1 containers was held for 10 days in an oven kept at 37°, then the contents were freeze-dried and stored at −20°.

No. 4.—Batches of 500 g. of casein (as used for material No. 1) were mixed with 500 g. of glucose (commercial grade) and 250 ml. of water. The mixture was then autoclaved at a pressure of 15 p.s.i. for 30 min., freeze-dried to 4% moisture content and stored as before in sealed containers.

Methods of analysis

(1) *Fluorodinitrobenzene (FDNB)-available lysine* was measured by the usual procedure,[13] with a correction for losses of dinitrophenyl (DNP)-lysine during the acid hydrolysis stage based on the recovery of standard DNP-lysine added to the digests in replicate flasks just before the hydrolysis, as previously explained.[14]

(2) *'Available' methionine* was determined with *Strep. zymogenes* NCDO 592 after a mild predigestion with 0·36% of papain (B.D.H. non-crystalline grade); the papain used had an activity of $1{\cdot}3 \times 10^{-6}$ units/μg. when assayed by Anson's procedure.[15] In the original methionine assay Ford specified 0·048% of papain[10] but we found his papain to have an activity of $5{\cdot}7 \times 10^{-6}$ units/μg. Thus, under our conditions the actual enzyme activity of the digest was 1·7 times that originally recommended.

(3) *'Total methionine'* was determined by assaying acid digests (2N-hydrochloric acid at 115° for 5 h.) of the test materials with the same organism.[10]

(4) The *'protein efficiency ratios'* of rats receiving the test materials, with and without supplementary amino-acids, were determined in a feeding experiment of the standard type with each diet containing 10% crude protein from one or other of the test materials.[16, 17] The details of the supplements in each diet are set out in Table I. The levels of supplementary lysine were based on the results of the 'available lysine' determinations on the test materials and designed to bring the 'available' lysine in the diets containing test materials Nos. 2–4 approximately to the level present in the diet containing material No. 1. 'Available methionine' values were not then known, and the additions were made on the basis that they had fallen in materials Nos. 2–4 in the same ratio as those of available lysine. Finally, to test whether methionine was limiting in the control material No. 1, a supplement was added to diet B, that was estimated to increase the methionine present by approximately 25%.

The diets were stored at 0° immediately after they were mixed; each morning a weighed quantity of fresh diet was moistened to a paste with water and offered to each rat, and on the

Table I

Growth, protein efficiency ratios (P.E.R.) and net protein ratios (N.P.R.) of rats receiving different dietary treatments for 4 weeks (level of protein: 10%)

Diet	Test material No.	Supplementary amino-acids, as % of diet		Mean wt. gain, g.	Mean protein intake, g.	Mean P.E.R.*	Estimated N.P.R.
		L-lysine hydrochloride	DL-methionine				
A	1	—	—	71·7	27·1	2·64	3·68
B	1	—	0·08	76·0	25·9	2·94	4·02
C	2	—	—	61·3	27·9	2·20	3·20
D	2	0·15	—	70·0	26·3	2·66	3·73
E	2	0·15	0·08	77·3	29·5	2·62	3·57
F	3	—	—	19·0	17·7	1·07	2·66
G	3	0·375	—	36·2	21·8	1·67	2·95
H	3	0·375	0·19	40·3	23·4	1·72	2·92
I	4	—	—	11·0	15·0	0·70	2·60
J	4	0·413	—	45·7	22·1	2·06	3·34
K	4	0·413	0·21	59·7	26·5	2·24	3·31

* Standard deviation ±0·09 (50 d.f.) and least significant differences between P.E.R. values selected for comparison:

	One-tailed test	Two-tailed test
($P < 5\%$)	0·21	0·25
($P < 1\%$)	0·29	0·33
($P < 0{\cdot}1\%$)	0·40	0·43

แผนกหอสมุด กรมวิทยาศาสตร์
กระทรวงอุตสาหกรรม

following morning the residue was removed and dried so that actual food consumption could be calculated for each animal.

At the beginning of the experiment, six weanling male rats, each in an individual cage, were allotted to each of eleven treatments, by division of 66 rats into 6 groups according to their body weight and then randomisation of the 11 rats in each group amongst the treatments. The experiment continued for 4 weeks.

' Protein efficiency ratio ' (P.E.R.) was calculated for each rat as ' weight gain/weight of crude protein eaten '. This measure has been criticised as being dependent on food intake (and thus on the palatability of diets), since the first rôle of the food eaten, that of providing for maintenance, is not considered. Bender & Doell[18] have suggested that this may be overcome by the function ' (weight gain + weight loss on protein-free diet)/weight of crude protein eaten ' which they have termed ' net protein ratio ' (N.P.R.). It is not possible to keep rats on a protein-free diet for 4 weeks, and in any case they would no longer be of comparable size to those on the other treatments. As an approximation, we have calculated N.P.R. estimates from group means, with an assumed loss of 1 g./day for a protein-free diet, this being the value found over a 10-day period.

Results

Amino-acid analyses and assays

Internal standards run with materials Nos. 1–4 in the FDNB-available lysine analysis gave recoveries of 89–92%. As these were not significantly different, a common mean recovery of 91% was assumed for each and the duplicate determined values corrected by the reciprocal of this factor, with the results set out in Table II. Statistical analysis of an extended series of replicates with this procedure suggests that a difference of 5% between two such values could be considered significant.[13]

Total and ' available ' methionine assays were run in duplicate ; they gave good agreement and the mean results are included in Table II. From an earlier series of results subjected to statistical analysis[12] it would seem that a difference of approximately 10% could be considered significant.

It is seen that for both available lysine and available methionine the lowest values were obtained with material No. 4 : 39% and 77% respectively of the corresponding values for the unheated material No. 1. Materials Nos. 2 and 3 gave intermediate values, and again in each case with a greater proportional fall in ' available ' lysine than in ' available ' methionine. Heat-treatment had certainly caused no fall in ' total ' methionine ; there is even an apparent slight increase in the value obtained for material No. 4.

Feeding experiment

For each of the eleven treatments the mean initial weight of the rats was within 45 ± 0·3 g.

Table II

Effect of heat-treatment on the nutritive value of casein–glucose mixtures

Test material	Amino-acids, g./16 g. N			Protein efficiency ratios			
	Total methionine	' Available ' methionine	' Available ' lysine	Alone	+ Methionine	+ Lysine	+ Lysine + methionine
No. 1 (control)	4·1	3·1	5·4	2·6	2·9	—	—
No. 2 (hot oven* for 40 min.)	4·2	2·8	4·2	2·2	—	2·7	2·6
No. 3 (37° for 10 days)	4·1	2·7	2·4	1·1	—	1·7	1·7
No. 4 (autoclaving of different mix)	4·4	2·4	2·1	0·7	—	2·1	2·2

* The temperature of the oven was 85°, but it was concluded that the material did not reach this temperature ; the inner portions in each can were paler than the outer and the lysine value of the re-mixed sample was higher than the value of 3·8 g./16 g. N obtained after only 30 min. heating in a preliminary trial with a smaller quantity.

All the rats appeared to remain healthy over the experimental period. The mean results, together with the statistical analysis of the results for P.E.R., are set out in Table I.

With each of the heated samples (Nos. 2–4) the P.E.R. was significantly poorer than with the control material (No. 1). The control material was itself improved slightly by supplementation with methionine. The heated materials were improved, and to a greater extent, by the addition of lysine ; the further addition of methionine gave no significant improvement above that obtained with lysine alone. Only with material No. 2 did supplementation bring the P.E.R. to the value obtained for the control material.

The estimates of N.P.R. for the different diets show a close relationship to the P.E.R. values when plotted as a correlation diagram, so that there is no reason to fear that the conclusions from P.E.R. values are vitiated by differences in appetite for the diets. As expected, the ratio of the highest to the lowest value for N.P.R. was less (1·55 : 1) than the corresponding ratio for the P.E.R. values (4·2 : 1).

Discussion

Changes in nutritional value for rats

The results of the feeding experiment have confirmed, with casein from buffalo's milk instead of cow's milk, the earlier observation that in the presence of glucose and 13–16% moisture at pH 6·3, storage at 37° resulted in severe nutritional damage within a few days.[2, 5] The fall in P.E.R. found in the present experiment as a result of 10 days' storage, is intermediate between that found with cow's casein as the result of 5 and 30 days' storage respectively.[2]

For the test material No. 4, prepared by a short-time, high-temperature treatment, the pH was deliberately not adjusted to the most sensitive portion of the range[19] so as to avoid the extreme damage to all amino-acids that might be expected to result from even the minimal practicable period of autoclaving. Even for treatment at a desired intermediate temperature of 85°, the period of 40 min. considered likely to cause the desired degree of damage at pH 6·3 was too short for uniform heating of the material under the conditions that we used.

The results of amino-acid supplementation were in general agreement with those reported for skim-milk powders ;[6, 7] i.e., the response to unheated material was limited by the sulphur-containing amino-acids, whereas for the inferior, heated samples lysine was considerably more limiting than any other amino-acid. Some of the published results are summarised in Table III.

In general the present results and those of Henry & Kon[6] suggest that mild damage can be almost completely corrected by lysine addition, whereas with more severe damage there is a significant depression of response even with added lysine. There is some evidence that the further limiting factors are the other dibasic amino-acids arginine and histidine ;[6] the latter may be more sensitive to heat-damage in the presence of air.[20] Certainly with our materials some amino-acids other than methionine were limiting the performance of those heated materials that did not come up to the control value with supplementary lysine alone. On the other hand, Mauron & Mottu[7] found complete restoration of a severely damaged milk powder with lysine and methionine.

Application of ' prediction ' tests

Both the laboratory tests used for ' available ' lysine and methionine appeared to predict correctly the changes in the value of the heated materials as sources of the two amino-acids for rats, i.e., very considerable changes in ' available lysine ' and smaller changes in available methionine.

Despite the presence of glucose in the test materials, the recovery of DNP-lysine in the ' available lysine ' procedure[13] was high. Handwerck *et al.*[21] have drawn attention to the effect of fluorodinitrobenzene (FDNB) in protecting DNP-lysine from destruction in the presence of reducing sugars during acid hydrolysis. We have confirmed this, and find that with foods rich in carbohydrate analysed by modified procedures[22, 23] that remove excess FDNB but not carbohydrates before acid hydrolysis, there can be considerable losses of DNP-lysine.[12]

The value of 5·4 g./16 g. N for the FDNB-available lysine of the control casein–glucose mixture is very much lower than that of 8·3 g./16 g. N reported for specially prepared cow's

casein used by Lea & Hannan.[4] Apart from a possible effect of species difference (since a microbiological ' total ' value of 5·9% has been reported for ash- and moisture-free buffalo casein[24]), the initial casein used was of commercial grade that may already have received some damage, and further binding may have occurred during the preparation of the casein–glucose mixture in high ' laboratory ' temperatures.

For the control material, the ' available ' methionine value was approximately 75% of the total value. However, the total value of 4·1 g./16 g. N was curiously high, as compared with Lea & Hannan's value of 2·7[5] and a mean value of 3·05 (range 2·6–3·6) g./16 g. N for twelve other published analyses[25] for cow's casein. Ford also reports a high value of 3·7 g./16 g. N for skim-milk powder,[10] as compared with a mean value of 2·4 g./16 g. N for 10 published results from the use of different procedures.[25] The conditions of acid digestion[10] are considerably milder than those used in the other procedures ; certainly some peptides will remain, and certain peptides are known to stimulate the growth of other organisms.[26] The more drastic methods used to obtain complete hydrolysis may also be causing some destruction of the methionine. The discrepancies remain to be resolved. Both Ford[10] and ourselves have also recorded a small, but consistent, rise in apparent total methionine content with autoclaving of skim milk and casein–glucose respectively using his procedure, which is also unexplained.

With unheated casein or milk, the limiting amino-acid is either cystine or methionine. There is approximately seven times as much methionine as cystine in casein,[25] so that even large proportional changes in ' available ' cystine can have relatively little effect on the overall value of the material as a source of the two sulphur amino-acids.

Comparison of the present laboratory tests with others

An attempt is made in Table IV to summarise the results of other workers who have determined ' available ' lysine and methionine, by various procedures, in either milk powder or casein–glucose mixtures, and also in fish or meat products. Despite the wide range of procedures used, it has always been found that with the first class of materials there is a considerably greater fall in lysine than in methionine, whereas with the second class this is not the case.

The simplest explanation of these differences would be that the first materials are rich in reducing sugars which will bind even under mild conditions with free amino-groups, primarily those of lysine, to produce severe nutritional damage ; the other materials contain very low levels of reducing sugars and have to be exposed to severe conditions for nutritional damage to occur, which may be the result of internal ester or amide formation.[30]

Whether this hypothesis will prove generally true must await further investigation. Earlier studies have been made of the damage resulting from heating casein alone, and it has been the general conclusion that the availability of lysine is reduced to a very much greater extent than that of the other amino-acids.[31–33] However, these were made with ' commercial ' or ' edible ' grades which are likely to have contained appreciable amounts of lactose.

Table III

*Summary of some published results of the effect of heat-treatment on the nutritive value of skim-milk powders with and without amino-acid supplementation**

Authors	Test materials	Protein efficiency ratios			
		Alone	+ Methionine	+ Lysine	+ Methionine and lysine
Mauron & Mottu[7]	(i) Spray-dried powder (VI)	3·2	3·4	—	—
	(ii) Scorched roller-dried powder (VI)	0·6	0·7	2·8	3·2
Henry & Kon[6]	(i) Spray-dried powder M	2·7	—	2·7	—
	(ii) M, adjusted to 7% moisture content and stored in N_2 at 37° for 60 days	1·8	—	2·5	—
	(iii) M, adjusted to 7% moisture content and stored in air at 37° for 60 days	1·5	—	1·9	—

* The levels of L-lysine addition to the test diets were 0·54%[7] and 0·29%[6] respectively, and of DL-methionine was 0·4%[7]

Table IV

'Available' amino-acids in heated materials as % of corresponding values of control samples, as determined by various procedures

Authors	Control material	Heat-treatment	Lysine	Methionine
A. Materials rich in reducing sugars				
Lea & Hannan[5]	Casein + glucose	37° for 5 days	36[a]	76[b]
		37° for 30 days	9	43
Present authors	Casein + glucose	Treatment 2	78[a]	90[c]
		,, 3	44	87
		,, 4	39	77
Mauron *et al.*[27]	Liquid milk	Deliberately drastic roller drying, A	70[d]	90[d]
		,, ,, B	29	82
Ford[10]	Freeze-dried skim milk	120° for 30 min.	16[a]	69[e]
B. Materials low in reducing sugars				
Beuk *et al.*[28]	Fresh pork	Autoclaving at 120° for 24 h.	47[e]	48[e]
Bissett & Tarr[29]	Fresh herring & press-cake	Drying and heating at different temperatures (mean of Nos. 7–10 of their samples)	59[e]	55[e]
Carpenter *et al.*[11]	Freeze-dried herring meal	130° for 27 h. at 4–55% moisture content	55[a]	40[c]

The procedures used are described in detail by each author, but in summary they are:

[a] reaction with fluorodinitrobenzene,
[b] colour reaction after papain digestion,
[c] microbiological assay with proteolytic organism, after mild papain digestion,
[d] dialysis of enzymic digest, followed by enzymic or chemical procedure,
[e] microbiological assay on undialysed enzymic digest.

For the moment there is no serious discrepancy between the findings of different workers for the same type of starting material, and for all animal products so far investigated application of the 'available lysine' procedure, under conditions where only a single quality control test is practicable, should give satisfactory evidence as to whether or not heat-damage to the protein of the food has in fact occurred.

Acknowledgment

One of the authors (K. J. C.) is indebted to the Royal Society and the Nuffield Foundation for a grant, and to the Director, C.F.T.R.I., for facilities to work at Mysore. The work of another author (C. B. M.) was assisted by a grant from the U.S. Dept. of Agriculture. The authors are grateful to Miss K. Indiramma for the statistical analysis of the results.

Central Food Technological Research Institute
Mysore-2
India
and
School of Agriculture
University of Cambridge

Received 5 February, 1963

References

[1] McInroy, E. E., Murer, H. K., & Thiessen, R., *Arch. Biochem.*, 1949, **20,** 256
[2] Henry, K. M., & Kon, S. K., *Biochim. biophys. Acta*, 1950, **5,** 455
[3] Liener, I. E., 'Processed Plant Protein Foodstuffs', (A. M. Altschul ed.), 1958, p. 79 (New York: Academic Press)
[4] Lea, C. H., & Hannan, R. S., *Biochim. biophys. Acta*, 1950, **4,** 518
[5] Lea, C. H., & Hannan, R. S., *Biochim. biophys. Acta*, 1950, **5,** 433
[6] Henry, K. M., & Kon, S. K., *J. Dairy Res.*, 1947–1948, **15,** 341
[7] Mauron, J., & Mottu, F., *Arch. Biochem. Biophys.*, 1958, **77,** 312
[8] Miller, D. S., *J. Sci. Fd Agric.*, 1956, **7,** 337
[9] Donoso, G., Lewis, O. A. M., Miller, D. S., & Payne, P. R., *J. Sci. Fd Agric.*, 1962, **13,** 192
[10] Ford, J. E., *Brit. J. Nutr.*, 1962, **16,** 409
[11] Carpenter, K. J., Morgan, C. B., Lea, C. H., & Parr, L. J., *Brit. J. Nutr.*, 1962, **16,** 451
[12] Morgan, C. B., Miller, E. L., Milner, C. K., & Carpenter, K. J., unpublished results
[13] Carpenter, K. J., *Biochem. J.*, 1960, **77,** 604
[14] Carpenter, K. J., & March, B. E., *Brit. J. Nutr.*, 1961, **15,** 403

References (*cont.*)

[15] Anson, M. L., *J. gen. Physiol.*, 1938, **22,** 79
[16] Osborne, T. B., Mendel, L. B., & Ferry, E. L., *J. biol. Chem.*, 1919, **37,** 223
[17] Joseph, K., Narayana Rao, M., Swaminathan, M., Sankaran, A. M., Jayaraj, A. P., & Subrahmanyan, V., *Brit. J. Nutr.*, 1961, **16,** 49
[18] Bender, A. E., & Doell, B. H., *Brit. J. Nutr.*, 1957, **11,** 140
[19] Lea, C. H., & Hannan, R. S., *Biochim. biophys. Acta*, 1949, **3,** 313
[20] Henry, K. M., & Kon, S. K., *J. Dairy Res.*, 1952, **19,** 216
[21] Handwerck, V., Bujard, E., & Mauron, J., *Biochem. J.*, 1960, **76,** 54P
[22] Conkerton, E. J., & Frampton, V. L., *Arch. Biochem. Biophys.*, 1959, **81,** 130
[23] Baliga, B. P., Bayliss, M. E., & Lyman, C. M., *Arch. Biochem. Biophys.*, 1959, **84,** 1
[24] Dastur, N. N., *Dairy Sci. Abstr.*, 1956, **18,** 967
[25] Harvey, D., *Commonw. Bureau Anim. Nutr., Tech. Commun.*, 1956, No. 19 (Aberdeen: The Bureau)
[26] Hearfield, D. A. H., & Phillips, A. W., *Nature, Lond.*, 1961, **190,** 266
[27] Mauron, J., Mottu, F., Bujard, E., & Egli, R. H., *Arch. Biochem. Biophys.*, 1955, **59,** 433
[28] Beuk, J. F., Chornock, F. W., & Rice, E. E., *J. biol. Chem.*, 1948, **175,** 291
[29] Bissett, H. M., & Tarr, H. L. A., *Poult. Sci.*, 1954, **33,** 250
[30] Mecham, D. K., & Olcott, M. S., *Industr. Engng Chem.*, 1947, **39,** 1023
[31] Greaves, E. O., Morgan, A. F., & Loveen, M. K., *J. Nutr.*, 1938, **16,** 115
[32] Eldred, N. E., & Rodney, G., *J. biol. Chem.*, 1946, **162,** 261
[33] Rice, E. E., & Beuk, J. F., *Adv. Food Res.*, 1953, **4,** 233

INFLUENCE OF NITROGEN AND POTASSIUM FERTILISERS ON PECTIC ENZYME ACTIVITY IN TOMATO FRUIT

By G. E. HOBSON

The influence of nitrogen and, especially, potassium fertilisers on the quality of tomato fruit has raised the question of the mechanisms by which they have their effect. As part of a large-scale factorial trial, plants were grown in two soil levels of nitrogen combined with two levels of potassium. Good quality fruit from each treatment was analysed for pectinesterase and polygalacturonase activity, since the pectic enzymes have long been associated with ripening. Results show that an increase in the soil level of nitrogenous fertilisers was always reflected in higher total pectic enzyme activity, but potassic fertilisers gave no consistent trends. More detailed studies on one variety showed that additional nitrogen had the effect of increasing extractable protein in the fruit; potassium stimulated the specific activity of pectinesterase. The probability that the pectic enzymes are connected with the process of ripening is discussed, and the possible pathways by which potassium exerts its strong effect upon fruit quality are examined.

Introduction

The interactions and main effects of four macronutrients and lime on the yield, quality and composition of tomato fruit are being studied at this Institute.[1–4] Preliminary results of this long-term trial have indicated that the proportion of fruit showing a physiological disorder known as 'blotchy' ripening may be markedly altered by soil levels of potassium and to a lesser extent of nitrogen. The pectic enzyme complex has for many years been thought to be closely associated with the mechanisms of ripening,[5] and it has previously been demonstrated that fruit affected by 'blotchy' ripening show diminished activities of pectinesterase[6] and polygalacturonase[7] in the abnormal areas. In view of differences in the proportion of fruit affected by 'blotchy' ripening according to manurial treatment, comparison of enzyme activities in this investigation was confined to uniformly coloured fruit. If within the uniformly ripening proportion of the crop the activities of the pectic enzymes bore no relation to the manurial treatment the plants had received, it would be unlikely that these enzymes were connected with the ripening mechanism. A positive relation, however, would strengthen the association of the pectic enzymes with ripening.

Experimental

The fruit used in this investigation was obtained from a factorial nutritional trial in a

block of heated glasshouses. The lowest and highest levels of nitrogen, N1 and N3 (0·75 and 2·75 oz. per sq. yd. as N, respectively), were combined with the lowest and highest levels of potassium, K1 and K3 (3 and 12 oz. per sq. yd. as potassium sulphate, respectively), in the four selected plots; in addition, adequate and uniform amounts of phosphate, magnesium and lime were present in all cases. Samples of fruit uniform in colour and free from obvious ripening defects were obtained from these four groups of plants each week during mid-season. The varieties grown were 'Potentate' in 1961 and 'J.168' (an F_1 hybrid produced at this Institute which shows freedom from a ripening disorder known as 'greenback') in 1962.

The methods previously reported[6] were used for the extraction and assay of pectinesterase. A unit of enzyme activity is regarded as the quantity that would produce 1 mmole of free carboxyl groups from excess pectin in 1 min. at 27° in 0·05M-sodium acetate solution at pH 7·5.

The extraction and determination of polygalacturonase were carried out by a modified method of Foda.[8, 9] The unit of activity is taken as the increase in reducing power per hour measured in g. of α-D-galacturonic acid monohydrate, at pH 4·25.

The protein in 10 ml. of the enzyme solution was precipitated overnight by the addition of 1 ml. of a solution containing 1·1 g. of trichloroacetic acid. The protein complex was centrifuged down, washed with 5% trichloroacetic acid solution, dissolved in 0·2 ml. of 0·5N-sodium hydroxide and the protein determined by the method of Lowry *et al.*[10] Crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, U.S.A.) was used for the construction of a calibration curve. Solutions from 75 extractions analysed both by this colorimetric method and by the micro-Kjeldahl procedure showed that there was no significant difference between the two sets of results. Dry matter was determined by heating 100-g. samples of fresh tomato tissue in a forced-draught oven at 80° for 48 h.

Results

During 1961, the effect of varying the levels of nitrogen and potassium in the soil on the activity of pectinesterase in fruit of the variety 'Potentate' was investigated and the results are summarised in Table I. Statistical analysis shows that there was a significant interaction between nitrogen and potassium in producing higher esterase total activities with increasing nutrient levels. The higher level of nitrogen increased the pectinesterase total activity significantly at both levels of potassium, the increase being greater at the K3 than at the K1 level. The higher level of potassium increased the enzyme activity significantly at the N3 but not at the N1 level of nitrogen. The content of dry matter increased somewhat with the larger amounts of applied fertiliser, but the differences were not statistically significant.

The mean protein contents of the enzyme extracts are given in Table II. The larger amounts of nitrogen in the soil significantly increased the soluble protein, but the high potassium regime tended to produce the opposite effect. Thus the specific activity, as units per mg. of protein in the enzyme solution, was little altered by the different levels of nitrogen but significantly increased by additional potassium in the soil.

Table I

Effect of nitrogen and potassium fertilisers on pectinesterase activity and the dry matter content of ripe tomato fruit (variety 'Potentate')

(the figures within the body of each part of the Table are the means of 7 determinations)

	Units of activity per 100 g. of fresh tissue			Dry matter, g. per 100 g. of fresh tissue	
Treatment	Low nitrogen (N1)	High nitrogen (N3)	Mean value	Low nitrogen (N1)	High nitrogen (N3)
Low potassium (K1)	3·57	5·58	4·57	6·64	6·75
High potassium (K3)	4·02	7·85	5·94	6·66	7·39
Mean value	3·80	6·71			

L.S.D. between the activities for NK* interaction (at P = 0·05) = 1·25
L.S.D. between the mean values for N*** and K** (at P = 0·05) = 0·88

In this and subsquent tables
* indicates a significant difference between means at P = 0·05 in the F-test,
** indicates a significant difference between means at P = 0·01, and
*** indicates a significant difference between means at P = 0·001.

Table II

Effect of nitrogen and potassium fertilisers on the amount of protein and the specific activity of pectinesterase in solutions extracted from ripe tomato fruit (variety 'Potentate')

(the figures within the body of each part of the Table are the means of 6 determinations)

Treatment	Protein extracted, mg. per 100 g. of fresh tissue			Specific activity (units of activity per mg. of protein in the extract)		
	Low nitrogen (N_1)	High nitrogen (N_3)	Mean value	Low nitrogen (N_1)	High nitrogen (N_3)	Mean value
Low potassium (K_1)	119·3	176·4	147·8	0·028	0·031	0·030
High potassium (K_3)	103·9	165·8	138·4	0·041	0·050	0·045
Mean value	111·6	171·1		0·035	0·040	

L.S.D. between the protein means for low and high N*** (at P = 0·05) = 18·5
L.S.D. between the specific activity means for low and high K** (at P = 0·05) = 0·010

In 1962, the variety of tomato grown in the trial was changed to 'J.168', but the nitrogen and potassium treatments were unaltered. The effect of these two elements on the activity of pectinesterase was also investigated with this second variety (Table III). The higher level of nitrogen again increased the total activity of this enzyme, but the effect of potassium was not significant.

The project was then extended to embrace the effects of nitrogenous and potassic fertilisers on polygalacturonase activity in the variety 'J.168'. It had previously been established[11] that, in fruit of the variety 'Potentate', the activity of this enzyme rose exponentially with time up to the orange colour stage, with a further significant increase up to the red stage. Hence the selection of comparably ripe fruit from each of the experimental plots had to be carried out with care so that the effects of the various treatments were not obscured.

Mean values for polygalacturonase activity in fruit from each of the treatments are given in Table IV. At both the K_1 and K_3 levels additional nitrogen produced greatly increased total activity, leading to highly significant differences between the mean activities at N_1 and N_3. The effect of potassium was not significant, and it was again apparent that nitrogen was quantitatively more effective than potassium in raising the activity of the enzyme. The highest activity was reached when abundant supplies of both nitrogen and potassium coincided (N_3K_3), but the NK interaction was not statistically significant.

Discussion

Little information of the relation between mineral nutrition and the pectic enzymes has been published. Holden & Tracey[12] showed that pectinesterase in tobacco leaves was significantly increased by additional soil nitrogen but reduced by phosphate; potassium appeared to have little effect, probably because the base level in the growing medium was not a limiting factor.

The influence of nitrogen and potassium on tomatoes is reflected not only in the growth

Table III

Effect of nitrogen and potassium fertilisers on pectinesterase activity and the dry matter content of ripe tomato fruit (variety 'J.168')

(the figures within the body of each part of the Table are the means of 5 determinations)

Treatment	Units of activity per 100 g. of fresh tissue			Dry matter, g. per 100 g. of fresh tissue	
	Low nitrogen (N_1)	High nitrogen (N_3)	Mean value	Low nitrogen (N_1)	High nitrogen (N_3)
Low potassium (K_1)	4·41	7·08	5·75	6·18	6·26
High potassium (K_3)	5·64	6·74	6·19	6·53	6·07
Mean value	5·02	6·91			

L.S.D. between the mean values for N* (at P = 0·05) = 1·51

Table IV

Effect of nitrogen and potassium fertilisers on polygalacturonase activity and the dry matter content of ripe tomato fruit (variety ' J.168 ')

(the figures within the body of each part of the Table are the means of 8 determinations)

Treatment	Units of activity per 100 g. of fresh tissue			Dry matter, g. per 100 g. of fresh tissue	
	Low nitrogen (N1)	High nitrogen (N3)	Mean value	Low nitrogen (N1)	High nitrogen (N3)
Low potassium (K1)	2·95	3·93	3·44	6·17	6·46
High potassium (K3)	2·77	4·36	3·56	6·68	6·17
Mean value	2·86	4·14			

L.S.D. between the mean values for N** (at P = 0·05) = 0·61

and appearance of the plants but also in the yield, quality and composition of the fruit.[2, 13] Studies at this Institute[14] have shown that ' J.168 ' plants produced considerably better quality fruit from a particular treatment than did ' Potentate ' plants, hence the percentage of fruit in each of the grades differed widely from one variety to the other. Nevertheless, a consideration of the relative proportions of fruit in each grade from the four plots involved in this investigation show that ' J.168 ' gave 12 times and ' Potentate ' 7 times as much severely ' blotchy ' fruit in the low-potassium (K1) plots as in the high-potassium (K3) ones. This reduction in the potash level must predispose fruit to ripen irregularly so that, with the influence of further factors, areas of the fruit become ' blotchy ' in character. On the other hand, nitrogen appeared to have no clear-cut effect upon the proportions of fruit of the two varieties showing severe ' blotch ', but in general, increased nitrogen reduced the percentage of top quality fruit whether the potassium was high or low.

The interpretation of the results of the treatments on the activities of pectinesterase and polygalacturonase must depend upon the significance attached to total and specific bases of activity. Higher total activities can be held to indicate greater extraction of enzyme unless protein figures are available to show how the treatments altered them. Metabolic processes may be related to changes of the specific activity rather than of the total activity of an enzyme. Thus in the instance in this study in which figures for both types of activity are available, for total activity there was a significant difference between both nitrogen and potassium means, but for specific activity the difference was significant only between the potassium means. Hence it is possible that, although only nitrogen produces a significant difference for the total activity of pectinesterase in variety ' J.168 ', this might revert to a potassium effect if the specific activities had been considered.

Pectinesterase is a carboxylic ester whose desorption from water-insoluble cellular constituents is only efficiently achieved by moderately strong salt solutions and its activity is markedly influenced by the cationic content of the substrate.[15] The potassium content of the expressed sap from evenly ripening fruit from each of the four experimental plots used in this investigation was measured for both varieties of tomato. The average for the plots receiving potassium at the K3 level was over 85% higher for ' Potentate ' and nearly 70% higher for ' J.168 ' than the average at the K1 level.[14] These differences tend to be rather less sharp when the sodium contents are added to the potassium figures,* but nevertheless the diminished concentrations must have some effect upon the desorption and activation of pectinesterase in the fruit.

The results of tests by Jansen & MacDonnell[16] demonstrated that the velocity of the hydrolysis brought about by polygalacturonase increased largely in proportion to the decrease in the extent of esterification of the substrate. Thus the action of pectinesterase might appear

* In this connexion, the sodium/potassium ratios in the K1 and K3 plots were 0·83 and 0·28 for the ' Potentate ' variety and 0·71 and 0·25 for ' J.168 ' variety. The additional potassium in the K3 plots increased the actual potassium contents of the fruit by 86 and 68% and the sodium + potassium contents by 22 and 23% respectively, for the two varieties mentioned. Results on this and other research centres suggest that sodium has a variable and minor effect on fruit quality, but fruit on low-potassium plots tend to adsorb more sodium than that on high-potassium plots.

to influence greatly the subsequent action of polygalacturonase and the effect of potassium may thus extend by an indirect mechanism to this latter pectic enzyme as well.

Polygalacturonase activity in the green side of ' blotchy ' ripened fruit has been found to be barely 15% of the lowest activity found in evenly ripening red fruit.[7] There is an increasing amount of evidence to suggest that the ripening of fruit is a process dependent upon the synthesis of proteins and other substances.[17] Hence it might be expected that nitrogen would affect normal ripening through an influence on the synthesis of protein during this period. But even at the low nitrogen (N1) level, the grading figures show that in the presence of sufficient potassium, most of the fruit are of good quality. Thus the failure in the synthesis of polygalacturonase in 'blotchy ' tissue is unlikely to be due purely to a poor supply of nitrogen.

There are grounds for believing that potassium not only affects the activities of the pectic enzymes but is also associated with increased respiration and alteration in the nitrogen metabolism of higher plants (see review by Pirson[18]). In addition to the neutralisation of the organic acids in the fruit, potassium probably becomes concentrated in the mitochondria,[19] thought to be intimately involved in ripening and the seat of citric acid oxidations, electron transport and oxidative phosphorylations. A situation may thus occur in which the abundant supplies of nitrogen are not utilised because the synthetic mechanism is impaired through potassium deficiency.

The results reported here substantiate the widely held view[5] that the pectic enzymes are intimately associated with ripening. In normal fruit the activities of both the enzymes investigated varied in a generally regular manner with the fertiliser treatments. At the levels used, nitrogen appeared to have a greater influence than potassium on the total activities of the enzymes, but the opposite effect has been found for the occurrence of ' blotchy ' ripening.[6, 7] At present it is possible only to speculate on the precise mechanism by which the effect of potassium on fruit quality is brought about, but it seems not unlikely that the pectic enzymes are involved.

It is to be hoped that further work, especially at the cellular level, will reveal the point at which tomato tissue becomes so disorganised that normal ripening pathways are abandoned, so that the selection of varieties free from ' blotchy ' ripening may be made on a more rational basis.

Acknowledgments

The author would like to thank Drs. G. W. Winsor and J. N. Davies of this Institute for permission to use certain unpublished results on the grading and potassium content of tomatoes, also for supplies of fruit from the large-scale trial they are conducting.

Glasshouse Crops Research Institute
Worthing Road
Littlehampton
Sussex

Received 4 January, 1963; amended manuscript 14 March, 1963

References

[1] Winsor, G. W., Long, M. I. E., & Davies, J. N., *Rep. Glasshouse Crops Res. Inst.*, 1958, p. 29
[2] Winsor, G. W., Long, M. I. E., & Davies, J. N., *Rep. Glasshouse Crops Res. Inst.*, 1959, p. 30
[3] Winsor, G. W., & Davies, J. N., *Rep. Glasshouse Crops Res. Inst.*, 1960, p. 56
[4] Winsor, G. W., & Long, M. I. E., *Rep. Glasshouse Crops Res. Inst.*, 1961, p. 50
[5] Woodmansee, C. W., McClendon, J. H., & Somers, G. F., *Food Res.*, 1959, **24,** 503
[6] Hobson, G. E., *Biochem. J.*, 1963, **86,** 358
[7] Hobson, G. E., *Proc. 1st int. Congr. Fd Sci. Tech.*, 1962 (in press)
[8] Foda, Y. H., Ph.D. Thesis, 1957, Univ. of Illinois
[9] Hobson, G. E., *Nature, Lond.*, 1962, **195,** 804
[10] Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J., *J. biol. Chem.*, 1951, **193,** 265
[11] Hobson, G. E., *Rep. Glasshouse Crops Res. Inst.*, 1961, p. 60
[12] Holden, M., & Tracey, M. V., *Biochem. J.*, 1948, **43,** 147
[13] Winsor, G. W., Davies, J. N., & Long, M. I. E., *J. hort. Sci.*, 1961, **36,** 254
[14] Winsor, G. W., & Davies, J. N., 1962, unpublished results
[15] Kertesz, Z. I., 'The Pectic Substances', 1951, pp. 366, 371 (London: Interscience)
[16] Jansen, E. F., & MacDonnell, L. R., *Arch. Biochem.*, 1945, **8,** 97
[17] Freeman, J. A., & Woodbridge, G. C., *Proc. Amer. Soc. hort. Sci.*, 1960, **76,** 515
[18] Pirson, A., *Annu. Rev. Pl. Physiol.*, 1955, **6,** 71
[19] Amoore, J. E., *Biochem. J.*, 1958, **76,** 438

EVALUATIONS OF SOIL PHOSPHATE STATUS BY POT EXPERIMENTS, CONVENTIONAL EXTRACTION METHODS, AND LABILE PHOSPHATE VALUES ESTIMATED WITH THE AID OF PHOSPHORUS-32*

By E. G. WILLIAMS and A. H. KNIGHT

The implications of the quantity and intensity aspects of soil P status have been studied over a range of 40 contrasting acid soils by examining (*a*) interrelationships and (*b*) correlations with the yield and P uptake of oats from pot cultures receiving no added P, for (1) the so-called *L* value, (2) readily-soluble P (P_x) extracted by six conventional methods, and (3) the total (solid + liquid phase) P (P_q) involved in these extractions. The *L* value, representing the quantity of isotopically exchangeable P sampled by the plants in the pot cultures, and the P_q value were estimated with the aid of added ^{32}P.

The results emphasise the importance of the intensity factor, especially in relation to yield, and show that the success of conventional readily-soluble P values as criteria of P status depends largely on avoidance of attack on unavailable P, coupled with reflection of intensity through variation from soil to soil in the proportion of P_x to P_q. The highest crop correlations, especially with yield, are therefore given by mild extractions at intermediate pH, with fairly short extraction periods, relatively inactive anions, and a fairly narrow extractant/soil ratio, such as in the lactate and bicarbonate methods.

Introduction

Definition of soil P status entails not only a quantity factor referring to the amount of available P, but also an intensity factor reflecting the ease, or difficulty, of withdrawal.[1–4] In addition, rate factors[1, 2, 4, 5] may be involved, covering replenishment of the soil solution, transport to the root and, over long periods, release from slowly available reserves. These factors are interdependent and not completely separable, but as already discussed elsewhere,[3, 4] they provide a rational basis for assessing the availability of various categories of soil P, the implications of readily-soluble P values determined by conventional extraction methods, and the significance of crop characteristics.

Provided the essential experimental requirements are satisfied and certain disturbing effects are absent, the quantity factor can be equated with the pool of labile, isotopically exchangeable, inorganic P measured by the so-called *L* value[4, 6–9] obtained from plant growth experiments with ^{32}P. In its simplest interpretation the intensity factor can be regarded as the P concentration in the soil solution.

Many procedures have been devised to extract readily-soluble soil P as an index of P status.[3, 4] The main extractant properties and experimental factors which can vary are: pH, nature and concentration of anions and cations, temperature, period of extraction, and extractant/soil ratio. As discussed elsewhere[3, 4] these determine the strength of the extraction and the extent to which the P values can be regarded as intensity or quantity measurements. Weak reagents, such as water and neutral salts, reflect the intensity of the soil P, very little being actually extracted. Other reagents, such as strongly acid or alkaline solutions, extract large amounts of P and give predominantly quantity measurements. Between these extremes there is a range of conventional methods of varying extracting power which may give a composite index of quantity and intensity. The P in solution in such suspensions tends to be in equilibrium with P on the soil surfaces. Depending on the extraction method and the soil, the readily-soluble P in the extract may account for only a minor proportion of the total P taking part in the extraction. For a given method, therefore, variation from one soil to another in the proportional distribution of P between the solid and liquid phases indicates differences in the strength of P retention. In so far as such differences correspond to variations in the intensity of P supply to plants, the readily-soluble P as normally determined in the extract should therefore reflect the intensity factor. This should enhance its usefulness as a criterion of P status and make it superior in this respect to the total P involved in the extraction. The latter value is predominantly a quantity measurement comparable with the *L* value, and this comparison gives

* Read at Agriculture Group Symposium, 3–5 April, 1963

a valuable basis for deciding whether the extractant used is attacking P sources which are not available to plants.[10] The pattern of the relationships with crop performance, however, may vary depending on whether the soil P status is defined in terms of crop yield or P-uptake.[4] Quantity measurements, such as the L value and the total P involved in an extraction, should tend to correlate better with P-uptake than with crop yield, whereas readily-soluble P values which reflect intensity should tend to show the opposite behaviour.

The present study was crried out to examine the above aspects of the behaviour and inter-relationships of the L value and readily-soluble P values by six conventional methods over a range of acid soils. The experimental work comprised characterisation of P status and determination of L values from plant-growth experients in pots, and laboratory extractions of readily-soluble P, with supplementary estimations of solid-phase P with the aid of ^{32}P.

Experimental

Soils

The investigation covered 40 relatively freely-drained acid soils, pH 5·0–6·4, sampled to normal plough depth from agricultural land in north-east Scotland. Analytical values (%) range as follows: C 2·0–7·1; loss on ignition 5·4–15·5; total N 0·16–0·44; total P 0·06–0·31; total organic P 0·03–0·13; clay 8·6–22·5; silt 5·9–25·8; fine sand 20·7–39·1; coarse sand 13·1–49·7; acid-oxalate soluble Al_2O_3[11] 0·35–2·50; acid-oxalate soluble Fe_2O_3[11] 0·43–2·20, and P_2O_5 retention capacity[11] 0·56–3·01. There were 10 soils from each of four parent glacial drift materials (basic igneous, slate, Old Red Sandstone and granite), and the P contents and P sorption characteristics have already been described.[11–13] For the present purposes the 40 soils are treated as one group to provide the maximum range of properties, particularly with regard to the nature of the soil complex, the P retention capacity, and the degree of P saturation. The intensity factor is likely to be more critical, and quantity values correspondingly less adequate as criteria of P status, when the latter properties vary, because they are important in relation to the strength of P retention and the quantitative significance of the labile P for plant growth.[4, 12, 13]

Pot evaluation of phosphate status

The P status of each soil was characterised by determining the dry-matter yield and total P-uptake, both in g. per pot, of oat grain + straw from cultures receiving no P but adequate dressings of other nutrients. Except that the soils were not diluted with sand, the standard Mitscherlich[14] pot equipment, cage and technique were employed, 35 plants per pot being grown to maturity. Each treatment was in duplicate and moisture was regulated by returning drainage and periodic watering to saturation at intervals depending on the weather and stage of growth. A constant volume of soil $<\frac{1}{4}$ in. containing 20–25% of moisture was used, sufficient to fill the pots to the normal level and amounting to about 12 lb. per pot, depending on the volume-weight. Uncertainty about the volume of soil being explored by roots, which can modify the quantity factor and upset relationships in the field, can therefore be disregarded.

L *value*

This represents the pool of labile phosphate[6–9] sampled by the plants in the pot cultures and was obtained from additional treatments receiving 0·3 and 1·2 g. of P_2O_5 per pot as 150 ml. of potassium dihydrogen phosphate solution labelled with ^{32}P. The initial specific activity was arranged so that the value at sowing was of the order of 400 μc per g. of P, and well below the safety limit of radiation damage to plants. The final specific activities of the plant and fertiliser P were determined by precipitation as $MgNH_4PO_4,6H_2O$ and counting under an end-window Geiger–Müller counter. The L value was calculated from $L = (S_f/S_p - 1)X$, where S_f and S_p denote the specific activities of the fertiliser and plant P, respectively, and X is the amount of added fertiliser P.

To promote isotopic dilution, the soil and potassium dihydrogen phosphate solution were divided into three portions which were successively added to a basin and thoroughly mixed by hand. After addition of the basal nutrients, the soil was again thoroughly mixed, filled

into the pot, and set aside for about 1 week before being seeded. This procedure gave reproducible *L* values, virtually the same for the two rates of added P, for the mature straw and grain, and for samples taken 8–10 weeks after sowing. Replicate pots agreed even in the few instances where the yields happened to differ.

The average *L* value for the two P rates based on the mature grain has been adopted throughout. It has been expressed both in mg. of P_2O_5 per 100 g. of soil <2 mm. for strict uniformity with the readily-soluble P values, and in g. of P_2O_5 per pot for correlation with the yield and P-uptake of the crop.

Readily-soluble phosphorus

Extractions were carried out by the six contrasting conventional methods[15–20] detailed in Table I. In addition to the normal colorimetric estimation of the readily-soluble inorganic P in the extracts, the corresponding solid-phase P was estimated from the partition of added carrier-free ^{32}P. Sufficient of this, as orthophosphate, was added to each extracting agent to provide an analytically suitable activity of approximately 1 μc per ml. The extraction was then carried out in the normal way and the radioactivity in the extract compared with that of the extractant by counting equal volumes in a Geiger–Müller solution counter. The solid-phase P corresponding to the readily-soluble P in the extract was calculated from

$$\frac{^{32}P \text{ extractant} - {}^{32}P \text{ extract}}{^{32}P \text{ extract}} \times {}^{31}P \text{ extract.}$$

This solid-phase P + the readily-soluble P in the extract gave the required estimate of the total P taking part in the extraction during the standard extracting period. Essentially the same end could be achieved by determining the recovery of added ^{31}P, but the estimation with ^{32}P has the advantage that the P addition is negligibly small and cannot disturb the normal balance.

The conventional readily-soluble P as normally determined in the extract will be denoted by P_x, and the total (solid + liquid phase) P apparently involved in the extraction by P_q. Air-dry soil <2 mm. was used throughout and both P_x and P_q are expressed in mg. of P_2O_5 per 100 g. of soil.

Results

L *value*

The crop correlations in Table III are fully compatible with the premise that except in so far as availability to plants implies a certain range, or minimum level, of intensity, the *L* value is a quantity measurement representing the amount of P available to the crop under the particular growth conditions. Thus the correlation is appreciably better with P-uptake than crop yield, but the general level is rather poor, due to lack of reflection of the intensity factor, and possibly also some related rate factors. Since a constant volume of soil <$\frac{1}{4}$ in. was taken for the pots and the *L* value is a quantity measurement, it is better expressed in g. of P_2O_5 per pot rather than mg. P_2O_5 per 100 g. of soil <2 mm. Table III shows that the crop correlations do tend to be slightly better on the former basis. The differences, however, are very small because there was very little variation in the volume-weights of the soils and the proportion of soil <2 mm. to soil <$\frac{1}{4}$ in.

As already mentioned, the relationship of the *L* value, in mg. per 100 g. of soil, with P_q

Table I

Extraction methods

Method	Extractant	Time of shaking	pH	ml. of extractant/g. of soil
Neutral NH_4F[15]	0·5N-NH_4F	1 h.	7·0	50
Acid NH_4F[16]	0·03N-NH_4F in 0·1N-HCl	40 sec.	—	50
Acetic acid[17]	2·5% v/v, acetic acid	2 h.	2·6	40
Truog[18]	0·002N-H_2SO_4 + 3 g. $(NH_4)_2SO_4$ per litre	30 min.	3·0	200
Lactate[19]	0·02N-Ca lactate in 0·01N-HCl	2 h.	3·8	50
Bicarbonate[20]	0·5M-$NaHCO_3$	30 min.	8·5	20

should tell whether the latter includes unavailable P.[10] Because of the markedly different time factors and conditions of estimation, however, the comparisons may entail uncertainties. Even so, where P_q is much larger than the *L* value it is highly probable that it includes unavailable P, and if in addition the correlation between the two values is poor this is virtually certain. Where P_q is similar to or lower than the *L* value, attack on unavailable P is unlikely to be of any importance if the correlation is high, but cannot be ruled out if the correlation is poor. Largely irrespective of the level of correlation, however, a better relationship with *L* for P_q than P_x is strong evidence that the latter is reflecting intensity.

The *L* value can also be compared with P_q and P_x on the basis of crop relationships. Better crop correlations for *L* compared with P_q indicate that the latter includes unavailable P. Even so, if P_x reflects intensity it may still give as good or better relationships than the *L* value, especially with crop yield. The most that can be expected of P_q, however, even where there is no attack on unavailable P, is that its crop relationships should equal those of the *L* value, because both are quantity measurements and the *L* value is the reference standard in this respect. Accordingly, Table III confirms that the best P_q crop correlations are practically identical with those of the *L* value, and in no case were appreciably superior. Otherwise, the various interrelationships of the *L* value, P_q and P_x, included in Tables II and III, are noted below for the individual extraction methods.

Table II

L value, and readily-soluble P values for extraction methods, covering 40 soils

Method	P_2O_5, mg. per 100 g. of air-dried soil <2 mm.			100 P_x/P_q	
	P_x Mean content in extract	P_q* Total involved in extraction			
		Mean	Range	Mean	Range
Neutral NH_4F	33·6	54·8	10·8–130	56	28–83
Acid NH_4F	16·8	23·2	10·1–48·2	71	53–83
Acetic acid	5·7	28·5	14·0–61·4	17	5–64
Truog	8·2	11·2	4·7–28·0	71	56–83
Lactate	2·7	9·8	4·1–15·4	23	10–53
Bicarbonate	5·4	11·0	3·1–22·1	48	33–71
L value		29·9	11·0–53·4	—	

* P_q = P_x + corresponding solid-phase P estimated from partition of ^{32}P added to the soil suspension

Table III

Correlation coefficients covering 40 soils

Method	Correlations of P extraction values† and *L* values with the yield and P-uptake of oat grain + straw				Correlations of P extraction values with the *L* value	
	P_x		P_q		P_x	P_q
	Yield	P-uptake	Yield	P-uptake		
Neutral NH_4F	0·28	0·48**	0·23	0·44**	0·66***	0·76***
Acid NH_4F	0·46**	0·62***	0·39*	0·60***	0·73***	0·80***
Acetic acid	0·65***	0·53***	0·31	0·46**	0·50**	0·63***
Truog	0·63***	0·60***	0·54***	0·55***	0·78***	0·82***
Lactate	0·73***	0·64***	0·56***	0·61***	0·65***	0·88***
Bicarbonate	0·69***	0·75***	0·55***	0·65***	0·80***	0·87***
L value in g. per pot			0·58***	0·64***	—	
mg. per 100 g. soil			0·55***	0·61***		

† P_x = P in extract; P_q = total P involved in extraction
*, **, and *** denote significance at the 5, 1 and 0·1% levels, respectively

Neutral ammonium fluoride

Strong complex formation between fluorine and aluminium makes this reagent a powerful extractant of aluminium-bound P. It has relatively little effect on iron- and calcium-bound P, and on the inorganic P in sand fractions.[12, 21] Much of the inorganic P in the present soils

is aluminium-bound[11, 12] and the neutral ammonium fluoride method accordingly gives much the highest values for both P_x and P_q (Table II). P_x is widely used as an estimate of total aluminium-bound inorganic P, but its significance for these soils requires further study because it apparently accounts for only 28–83% of P_q. This three-fold variation, however, offers appreciable scope for P_x to reflect the intensity factor. Accordingly it gives an appreciably poorer correlation than P_q with the *L* value, but a slightly better relationship with crop yield, and also P-uptake (Table III). The latter differences, however, are very small, and P_x does not correlate better with yield compared with P-uptake. On the contrary, both P_x and P_q correlate significantly with P-uptake, but not with yield (Table III), indicating that both values are predominantly quantity measurements. The prime reason for the generally very poor crop relationships, however, is almost certainly not inadequate reflection of the intensity factor, but attack on relatively unavailable P by the powerful ammonium fluoride treatment. Thus the mean P_q approaches double the mean *L* value (Table II) and the correlation of 0·76 between the two values (Table III) is only moderately good and substantially poorer than for the much milder lactate and bicarbonate methods. Similarly, all the crop relationships are much better for the *L* value than for the neutral ammonium fluoride values.

Acid ammonium fluoride

The active fluoride ion coupled with the strong acidity makes this reagent capable of extensive attack on all forms of inorganic P in soil. The very short time and low fluoride concentration (Table I), however, are designed to extract only the more rapidly soluble and available categories. Several features of the results, including the generally better crop relationships (Table III), indicate that although there is probably some attack on relatively unavailable P, this is less serious than for the previous method. The mean P_q (Table II) is less than one-half the value for the neutral ammonium fluoride method and smaller than the mean *L* value. P_q determined by the acid ammonium fluoride method is also somewhat better correlated with the *L* value (Table III) and the two values give practically the same correlation with P-uptake. The P_x/P_q proportion for the acid fluoride method, however, is high, ranging from 53 to 83% (Table II). Accordingly, P_x is higher than for all the methods except neutral ammonium fluoride, and there is rather little scope for it to reflect the intensity factor. Thus the correlation for P_x compared with P_q, is only a little better with crop yield, and only a little poorer with the *L* value (Table III). The yield relationship for both P_x and P_q, however, is poor. Both values correlate considerably better with P-uptake than with crop yield, indicating that they are predominantly quantity measurements. Inadequate reflection of intensity, rather than major influence of unavailable P, would appear to be the main factor limiting the crop relationships of the conventional P_x value.

Acetic acid method

The levels of P_q (Table II) are closely similar to those for the *L* value. The correlation between the two values, however, is rather poor, and considerably lower than for the other methods (Table III). Further, the crop relationships for the acetic P_q are much poorer than those of the *L* value. The results therefore support the conclusion of Mattingly & Pinkerton[10] that the acetic reagent attacks P sources not available to plants. This is doubtless the main reason for the generally poor acetic crop correlations (Table III). Probably the major factor in these acid soils is that the fairly low pH and long period of extraction (Table I) cause substantial attack on the largely unavailable acid-soluble P in the sand fractions.[22] Any P coming into solution from such sources, however, is partially sorbed during the extraction.[11, 22]. The P concentration in the extract is controlled by the adsorbed, aluminium- and iron-bound P and varies inversely with the P retention capacity of the soil.[11, 12, 22] The full effect of any unavailable P involved should therefore be reflected in the relationships of P_q, but, depending on the P retention capacity of the soil, the effect on P_x may be much smaller. This, however, is only one consequence of the widely varying partition of P between the solid and liquid phases (Table II). On the average P_x accounts for only 17% of P_q, and the proportion varies nearly thirteen-fold over the range 5–64%. The situation is therefore most favourable for P_x to reflect variations in the intensity factor, and the crop relationships in Table III provide striking

evidence of this. The level of correlation is generally better for P_x than for P_q, and P_x correlates clearly better with crop yield than with P-uptake, whereas the reverse is true for the quantity measurement, P_q. Consequently the superiority of P_x over P_q is very marked on the basis of crop yield, but relatively small in the case of P-uptake. Finally, although the correlation of P_q with the *L* value (Table III) is rather poor, it is better than for P_x.

Truog method

The somewhat higher pH and much shorter extraction period of the Truog method (Table I) make it less liable than the acetic treatment to attack unavailable forms of acid-soluble P. In particular, the Truog method is much less effective in dissolving inorganic P from sand separates.[22] Accordingly, the Truog P_q (Table II) is much lower than the acetic acid value, and much better correlated with the *L* value (Table III). The latter correlation, however, is still somewhat lower than for the lactate and bicarbonate methods, and the upper limit of P_q is considerably higher for the Truog method (Table II). Finally, Table III shows the important point that the correlations with P-uptake follow the sequence *L* value > Truog P_q > acetic P_q. Thus although attack on relatively unavailable P is undoubtedly smaller than in the acetic method, the Truog crop relationships are probably limited to some extent by this factor.[10, 22]. Unlike with the acetic method, any such influence is likely to be largely reflected in the relationships of P_x as well as P_q. The very wide extractant/soil ratio of the Truog method (Table I) makes it highly effective in extracting the adsorbed P of the clay,[22] so that most of the P involved in the extraction appears in the extract (Table II). P_x accounts for 56–83% of P_q, and there is correspondingly less scope for it to reflect variations in the intensity factor. Accordingly the Truog crop correlation coefficients in Table III all fall in the narrow range of 0·54–0·63. Even so, there are indications that P_x does give some additional reflection of intensity. It correlates appreciably better than P_q with both crop yield and P-uptake, especially the former, and possible correlates very slightly better with yield than with P-uptake. For these soils, however, the conventional Truog P_x value would appear to be mainly a quantity measurement. In so far as inadequate reflection of intensity and some influence of relatively unavailable P are both factors limiting the crop relationships, improvement might result from a narrowing of the extractant/soil ratio, coupled perhaps with some shortening of the extraction period.

Lactate method

This has the same extraction period as the acetic method and a very similar extractant/soil ratio (Table I), but the pH is more than one unit higher. Although the lactate anion is probably more effective than acetate in displacing phosphate, the net result is that the lactate method has a much lower extracting power. The mean lactate P_q value is only about one-third of the corresponding acetic value, and bears the same relationship to the *L* value (Table II). Other results[22] have shown that lactate extraction does not appreciably attack sand separates and is controlled by the adsorbed P of the clay. Further, Table III shows that the lactate P_q gives the very high correlation of 0·88 with the *L* value and has practically identical crop relationships. There is little doubt, therefore, that a favourable factor enhancing the lactate crop relationships is that it involves very largely, if not entirely, sources of P that are also available to plants. In addition, the lactate P_x has very considerable scope for reflecting the intensity factor because the P_x/P_q ratio shows a five-fold variation over the range 10–53%, with a mean value of only 23% (Table II). The effects of this are evident in the crop correlations in Table III. P_x correlates clearly better with crop yield than with P-uptake, while P_q shows the reverse trend. Accordingly, the correlation with yield is markedly better for P_x than for P_q and the *L* value. The varying partition of P_q between the solid and liquid phases undoubtedly reflects corresponding variations in the intensity of the P supply to crops, and the lactate P_x value provides a very useful composite index of quantity and intensity. The emphasis on the latter factor is further demonstrated by the fact that P_q and the *L* value, both quantity measurements, correlate very much better than do P_x and the *L* value (Table III). Finally, the fact that the lactate P_x value correlates better than the P_q value not only with crop yield but also with P-uptake (Table III) underlines the importance of the intensity factor in determining yield, and of yield as a component of total P-uptake.

Bicarbonate method

For these acid soils, the mildly alkaline bicarbonate method with its fairly short period of extraction (Table I) would be expected to reflect largely the labile pool of exchangeable, aluminium- and iron-bound P available to plants. The relatively good crop relationships (Table III) and other features of the results confirm this view. Like the lactate value which it closely resembles (Table II) the bicarbonate P_q value is much smaller than the *L* value but is highly correlated with it, and the two values give practically the same crop correlations (Table III). The bicarbonate method, however, has the narrowest extractant/soil ratio (Table I). This is conducive to low values for P_x and P_x/P_q, but it is counteracted by displacement of phosphate by the hydroxyl and bicarbonate anions. The net result is that the mean P_x and proportion of P_x to P_q (Table II) are intermediate between the Truog and lactate values ; the bicarbonate proportion varies by a factor of 2, compared with 1·5 for the Truog method and 5 for the lactate. So the scope for the bicarbonate P_x to reflect the intensity factor is somewhat greater than for the Truog value but substantially less than for the lactate. Accordingly, the pattern of the bicarbonate correlations is also intermediate. Indications that the bicarbonate P_x value reflects intensity are given by the sequence $P_x > P_q$ for the correlation with crop yield (Table III) and the reverse sequence $P_q > P_x$ for the correlation with *L* value. The differences, however, are smaller than for the lactate method, and the bicarbonate P_x value does not correlate better with crop yield compared with P-uptake ; on the contrary, it tends to behave like a quantity measurement in that it shows the opposite trend, though not so clearly as P_q. It seems that the bicarbonate P_x value does reflect intensity, but not to the same extent as the lactate value. Accordingly the latter gives a somewhat higher correlation with crop yield (Table III) but a lower correlation with P-uptake.

Discussion

The practical usefulness of methods for evaluating soil P status is determined by their ability to predict crop yields and responses to P under field conditions. The ultimate calibration must therefore be against crop performance in field experiments, and the requirements in this respect have been noted elsewhere.[3, 4] Relatively simple laboratory extractions of readily-soluble P, however, cannot be expected to integrate the numerous interrelated factors which affect the delivery and uptake of P and regulate plant growth in the field.[3, 4] These include soil physical properties and profile characteristics, the nature of the crop, pH, temperature, moisture, aeration, biological activity, nutrient levels and interactions, cultural practices, and pests and diseases. Variations in these factors can severely upset correlations between soil P values and crop performance. In pot experiments such variations are either minimised or largely eliminated. For example, the quantity factory in the field depends on the volume of soil exploited by roots, and can vary with soil physical properties, profile features, cultivations, and the amounts of positional distribution of other nutrients and water. As already explained, this uncertainty should not materially affect pot results, and soil-P values are known to correlate better with pot than with field data.[23] Even so, the highest crop correlations in Table III, for the lactate and bicarbonate methods, do not exceed 0·70–0·75. Considering the major variations in the properties of the 40 soils, this level is quite good, but it still leaves substantial room for improvement and, in line with earlier experience,[17] it is not highly promising from the field point of view.

There are clearly two main reasons for the relatively good performance of the lactate and bicarbonate methods. It is virtually certain that, unlike the other methods, they extract very largely, if not entirely, the pool of labile P available to plants. Secondly, the varying partition of P between the solid and liquid phases, especially in the lactate method, reflects variations in the intensity of the P supply to plants and markedly enhances the usefulness of the conventional extraction value as a criterion of P status. This is strikingly demonstrated by the much superior yield correlations of the lactate and bicarbonate P_x values compared with the *L* value. Even so, the main possibility for improvement probably lies in this aspect. The two P_q values give a correlation of nearly 0·9 with the *L* value and the three values have practically identical crop relationships. There would therefore seem to be little scope for

improvement with respect to the quantity factor. Further, as explained earlier, the importance of the intensity factor in these soils is enhanced by the considerable variations in the relative and absolute contents of soluble aluminium and iron, and in the P-retention capacity and degree of P saturation.[4, 12, 13] Modification of some of the experimental factors, especially extractant/soil ratio and anion concentration, which govern the partition of P_q between the soil and the extract, might therefore enhance further the usefulness of the conventional P_x values. There may, however, be an inherent limitation in the abilities of soil suspensions to reflect the intensity of the P supply to plants, in that the low mobility of P in soils is conducive to heterogeneous distribution of P-rich zones of high intensity which are particularly favourable for uptake by roots. Laboratory suspensions tend to destroy such heterogeneity and this may restrict their usefulness.[3, 4] There are also several other considerations, such as rate of factors,[4, 5] salt effects[3] and the close proximity of roots and soil colloids,[24, 25] which can modify P-uptake and limit the crop correlations of soil-P values.

The crop relationships are fully compatible with the premise[4] that the quantity measurements, represented by the *L* value and P_q, should tend to relate better to P-uptake, which extends over the growth period, than to yield, which depends on high intensity and rapid uptake in the early stages. As discussed elsewhere,[4] quantity measurements can also be expected to be more significant for crops with extensive rooting systems, for slower growing crops, for less responsive crops, and where the soil conditions are favourable for root development. Conversely, intensity values should tend to be most significant in relation to the yield of highly responsive, rapidly growing crops with limited rooting systems, especially those which respond markedly to placement and those for which water-soluble P is important.[4] For example, the intensity factor is likely to be more important for crops like swedes and potatoes than for cereals and grass. Except for soils where the intensity and quantity factors run closely parallel, as frequently happens,[26] the choice of method for evaluating P status may therefore depend on the cropping system. With intensive grass production, for example, the emphasis is on the quantity factor.[4, 26] In some situations,[4] therefore, it may be better to use two methods, one, such as 0·01M-calcium chloride[2] or possibly a narrow-ratio lactate extraction, to evaluate intensity, and the other, such as the P_q estimation or perhaps a wide-ratio treatment with the lactate or bicarbonate reagents, to assess the quantity factor. The usefulness of a method, however, depends ultimately not only on the level of correlation with crop performance in the field, but also on the scatter of points, particularly for the soils of intermediate P status. In large-scale advisory soil testing, differences in the crop relationships of extraction methods have also to be balanced against various subsidiary considerations, especially simplicity, speed, cheapness, analytical convenience, and the desirability of evaluating another nutrient, especially potassium, in the same extract.[3, 4]

Conclusions

The best and most widely applicable results[3, 4] are to be expected, and are in practice found, from mild extractions, with an intermediate pH, fairly short extraction period, relatively inactive anions, and a fairly narrow extractant/soil ratio. All these characteristics both guard against attack on unavailable P and favour variation of the P_x/P_q ratio, but a change in one of them can usually be compensated to some extent by adjustments of the others. For example, low or high pH, and displacement of P by active anions, are counteracted by short extraction and narrow extractant/soil ratio. Many combinations can therefore give useful results, and have been adopted.[3, 4]

The magnitudes and relationships of the P_q and *L* values indicate that the lactate and bicarbonate methods probably extract available P only, and the lactate method, especially, shows a wide variation in P_x/P_q ratio. The latter factor also operates very favourably in the acetic method, but not in the Truog method, due to the very wide extractant/soil ratio, nor in the two ammonium fluoride methods, because of strong displacement of aluminium-bound P by fluoride. The crop correlations of these methods, however, especially the neutral ammonium fluoride and the acetic methods, are restricted by attack on unavailable P. Consequently the lactate and bicarbonate P_x values give the best crop relationships, especially with yield, and are considerably superior to the *L* value. The latter defines the quantity factor and correlates

better with phosphate uptake than yield, but lack of reflection of intensity limits the level to about 0·6. The P_q values behave similarly, confirming that they are quantity measurements. Consequently, the best P_q crop correlations, also given by the lactate and bicarbonate methods, are equal to, but in no case better than, the L value.

Acknowledgments

Grateful acknowledgment is made to numerous members of the laboratory staff for analytical assistance, to Mr. R. H. E. Inkson for the correlation coefficients, and, especially, to Mr. N. M. Scott who supervised most of the experimental work.

The Macaulay Institute for Soil Research
Craigiebuckler
Aberdeen

Received 18 February, 1963

References

[1] Wiklander, L., *LantbrHögsk. Ann.*, 1950, **17,** 407
[2] Schofield, R. K., *Soils & Fert.*, 1955, **18,** 373
[3] Williams, E. G., *Trans. int. Soc. Soil Sci.* (Commissions II & IV, Dublin), 1952, **1,** 31
[4] Williams, E. G., *Trans. int. Soc. Soil Sci.* (Commissions IV & V, New Zealand), 1962, in press
[5] Barber, S. A., *Soil Sci.*, 1962, **93,** 39
[6] Larsen, S., *Plant & Soil*, 1952, **4,** 1
[7] Larsen, S., *Bull. Docum. Ass. int. Superphos. Mfrs*, 1956, No. 20, 22
[8] Russell, R. S., Russell, E. W., & Marais, P. G., *J. Soil Sci.*, 1957, **8,** 248
[9] Andersen, A., Gregers-Hansen, B., & Nielsen, G., *Acta agric. scand.*, 1961, **XI,** 270
[10] Mattingly, G. E. G., & Pinkerton, A., *J. Sci. Fd Agric.*, 1961, **12,** 772
[11] Williams, E. G., Scott, N. M., & McDonald, M. J., *J. Sci. Fd Agric.*, 1958, **9,** 551
[12] Williams, E. G., *Agrochimica*, 1959, **3,** 279
[13] Williams, E. G., *Trans. 7th int. Congr. Soil Sci.* (Madison, Wisconsin), 1960, **3,** 604
[14] Stewart, R., *Bull. Imp. Bur. Soil Sci. Tech. Comm.*, 1932, No. 25
[15] Bray, R. H., & Kurtz, L. T., *Soil Sci.*, 1945, **59,** 39
[16] Bray, R. H., in ' Diagnostic Techniques for Soils and Crops ', H. B. Kitchen (Ed.), 1948, pp. 53–86 (Washington, D.C. : Amer. Potash Inst.)
[17] Williams, E. G., Reith, J. W. S., & Inkson, R. H. E., *Trans. int. Soc. Soil Sci.* (Commissions II & IV, Dublin), 1953, **2,** 84
[18] Truog, E., *J. Amer. Soc. Agron.*, 1930, **22,** 874
[19] Egnér, H., *Medd. Cent Anst. Försöksv. Jordbr., Stockh.*, 1932, No 425
[20] Olsen, S. R., Cole, C. V., Watanabe, F., & Dean, L. A., *U.S. Dept. Agric.*, 1954, Circ. No. 939
[21] Williams, E. G., & Saunders, W. M. H., *Trans. 6th int. Congr. Soil Sci.* (Paris), 1956, B.797
[22] Williams, E. G., & Saunders, W. M. H., *J. Soil Sci.*, 1956, **7,** 189
[23] Fitts, J. W., *et al.*, *N.C. agric. Exp. Sta. tech. Bull.*, 1956, No. 121
[24] Elgabalay, M. M., & Wiklander, L., *Soil Sci.*, 1962, **93,** 281
[25] Blanchet, R., Chaumont, C., & Studer, R., *Ann. agron.*, 1962, **13,** 21
[26] Mattingly, G. E. G., & Talibudeen, O., *Rep. Rothamsted exp. Sta.*, 1960, 246

PHOSPHORUS IN CALCAREOUS SOILS. III.*—' Available ' Phosphate in Calcareous Soils as Measured by Five Chemical Methods and Phosphate Uptake by Rye-grass in a Pot Test

By M. B. SEN GUPTA and A. H. CORNFIELD

The amounts of phosphate extracted by 0·2N-ammonium oxalate, 0·5M-sodium bicarbonate (pH 8·5), Morgan's reagent and carbon dioxide-charged water from 15 calcareous soils (0·10–49·8% $CaCO_3$) were all significantly correlated with each other. Phosphate extracted by 0·03N-ammonium fluoride in 0·1N-hydrochloric acid was not correlated with that obtained with the other methods. Total uptake of phosphorus by rye-grass in pot tests using these soils was significantly correlated only with phosphate extracted by 0·5M-sodium bicarbonate and carbon dioxide-charged water.

* Part II : *J. Sci. Fd Agric.*, 1962, **13,** 655

Introduction

Over the last three decades many chemical methods for assessing the so-called ' available ' phosphorus in soils have been developed.[1–5] Most of these have been developed for use with non-calcareous soils and some, particularly those employing unbuffered acids, are unsatisfactory for calcareous soils. Several methods have been developed specifically for use on calcareous soils, in particular the ammonium oxalate[6] and the carbonic acid methods.[7] The 0·5M-sodium bicarbonate method[10] is claimed to be applicable to both non-calcareous and calcareous soils.

The empirical results obtained with a particular chemical extract are only of value in diagnosing the phosphorus status of soils if they are correlated with uptake of phosphorus by crops. Ensminger & Larson[8] used carbon dioxide as an extractant and found good correlation with crop response in calcareous soils. Smith *et al.*[9] observed that 0·03N-ammonium fluoride in 0·025N-hydrochloric acid was a better extractant for indicating the phosphorus status of calcareous soils than was 0·5M-sodium bicarbonate.

The present paper reports work comparing the amounts of phosphate extracted from 15 calcareous soils by five chemical methods and the uptake of phosphorus by rye-grass in a pot test. The chemical methods were the ammonium oxalate and carbonic acid methods, developed mainly for calcareous soils, the 0·5M-sodium bicarbonate and the 0·03N-ammonium fluoride in 0·1N-hydrochloric acid methods which are considered satisfactory for calcareous as well as non-calcareous soils. A modified Morgan's reagent was used because its high buffer capacity may be suitable for the analysis of calcareous soils.

Experimental

Materials and methods

Fifteen calcareous soils from different parts of South-East England, which gave a wide range of extractable phosphate in a preliminary extraction with Morgan reagent, were used in this investigation (characteristics given in reference [9a]). One g. portions of air-dry 2 mm. sieved soil were used in duplicate for each soil for extraction of ' available ' phosphate by the five chemical methods :

(1) *The Morgan reagent method:*[5] Extraction with Morgan's reagent (0·5N-acetic acid buffered with 0·75N-sodium acetate at pH 4·8) using a soil/solution ratio of 1 : 10 and 1 min. shaking. This ratio is wider than the normal one (1 : 2) to ensure that acetic acid was not neutralised by the calcium carbonate in the soils.

(2) *The 0·2N-ammonium oxalate method:*[6] Extraction was with a soil/solution ratio of 1 : 25 and 2 h. shaking. The filtrate was freed of oxalate ion by evaporating 10 ml. to dryness with 2 ml. of concentrated nitric acid. The residue was extracted with 1 ml. of 0·1N-hydrochloric acid and 9 ml. of distilled water.

(3) *The 0·5N-sodium bicarbonate (pH 8·5) method:*[10] Extraction was with a soil/solution ratio of 1 : 20 and 30 min. shaking. The test solution was neutralised with hydrochloric acid before colour development.

(4) *The carbon dioxide-charged water method:*[7] Extraction by bubbling carbon dioxide for 10 min. through a soil suspension with a soil/water ratio of 1 : 10 and frequent shaking. The bubbling rate was the same for all the soils.

(5) *The 0·03N-ammonium fluoride in 0·1N-hydrochloric acid method:*[11] A soil/solution ratio of 1 : 20 was used with 40 sec. shaking. The test solution was treated with 0·8M-boric acid to avoid interference by fluoride ion.

Phosphate was determined in all cases by the molybdenum blue–aminonaphtholsulphonic acid method.[12] Extinction measurements were made with the Hilger Biochem Absorptiometer using the red filter.

Pot tests with rye-grass

The experiment was carried out in the greenhouse with the same 15 soils that were used for chemical extraction. Portions (100 g.) of air-dry 2 mm. sieved soil mixed with 150 g. of sand that had been washed with hydrochloric acid and water, were placed in polythene pots (4 in. dia. at the top). Italian rye-grass was sown at the rate of 0·2 g. of seed per pot. Sufficient

distilled water was added at the beginning to provide enough moisture for germination. The pots were subsequently watered regularly with distille dwater. Potassium nitrate at a rate equivalent to 50 lb. of nitrogen per acre and magnesium sulphate at a rate equivalent to 10 lb. of magnesium per acre (acre × 7 in. soil vol. = 2,000,000 lb.) were added in solution to all the pots 5 days after germination and later after each cutting.

In order to ensure good growth of the rye-grass and to magnify difference in phosphate uptake, three successive cuttings were taken. The experiment lasted from 24th March (date of sowing) to 27th June (final cutting). After the final cutting, the roots were removed from the pots and washed free of soil and sand with water, Stergene and distilled water. Each cutting was dried (60°) and the three cuttings were combined, weighed, ground and analysed for phosphorus. The roots were dried, weighed and analysed separately. For analysis, 0·1 g. portions of the ground materials were digested by the sulphuric acid–hydrogen peroxide digestion method[13] and phosphorus was determined in suitable aliquots.

Results

Values for ' available ' phosphate as determined by the five chemical methods are shown in Table I. The extractable phosphate generally decreased in the order, 0·03N-ammonium fluoride in 0·1N-hydrochloric acid, 0·2N-ammonium oxalate, 0·5N-sodium bicarbonate, Morgan's reagent, carbon dioxide-charged water.

The correlation coefficients between the values obtained by the different chemical methods are given in Table II. ' Available ' phosphate as determined by Morgan's reagent, ammonium oxalate, sodium bicarbonate and carbon dioxide-charged water were all significantly correlated with each other. The fluoride–hydrochloric acid method was poorly correlated with the other methods.

The last column of Table I shows the total uptake of phosphorus in the combined cuttings and roots of rye-grass: the values ranged from 3·73 to 8·36 mg. as P_2O_5.

Correlation coefficients between ' available ' phosphate extracted by the five chemical methods and total phosphorus uptake (tops and roots) by the rye-grass are shown in Table III. Only the 0·5M-sodium bicarbonate and carbon dioxide-charged water methods were significantly correlated with phosphorus uptake by the rye-grass. The correlation coefficient for the fluoride–hydrochloric acid method approached the significant level (the minimum correlation coefficient required for significance at the 5% level with 13 D.F. is 0·514).

Table I

' Available ' phosphate extracted by five chemical methods and total phosphorus uptake by rye-grass from 15 calcareous soils

(Soils arranged in increasing order of % $CaCO_3$)

Soil no.*	$CaCO_3$, %	Phosphorus extracted, as p.p.m. as P_2O_5					Total P uptake by rye-grass, mg.
		Morgan reagent	Ammonium oxalate	Fluoride–HCl	Sodium bicarbonate	Carbon dioxide–water	
1	0·10	1	25	60	40	1·0	8·36
2	0·15	10	37	56	46	3·0	5·44
9	2·95	25	112	250	63	8·2	7·98
16	13·5	45	62	15	73	8·0	7·44
17	13·9	50	75	108	80	10·0	7·49
18	16·5	20	87	160	46	2·5	5·89
20	18·4	20	87	140	53	2·5	5·64
21	22·3	43	100	140	73	7·0	7·55
24	27·3	23	75	80	53	1·0	5·57
25	28·7	20	37	40	40	1·0	3·73
26	30·4	33	47	40	40	1·0	3·73
29	41·9	36	92	92	53	5·5	5·74
30	46·3	43	112	26	60	7·0	5·16
31	47·2	36	87	10	53	2·5	5·20
32	49·8	31	97	34	46	5·5	4·81

* Soils numbered as in Part II of this series[9a]

Discussion

The widely-different amounts of phosphate extracted by the five chemical methods are due to their ability to dissolve different forms of soil phosphate. On average, the fluoride–hydrochloric acid reagent extracts about the same amount of phosphorus as do ammonium oxalate solutions, but large differences between results with individual soils indicate the possibility that different forms of phosphate are extracted by the two methods. The fluoride–hydrochloric acid extracted the widest range of phosphate because the free acid in the solvent was probably neutralised by the calcium carbonate present in some of the soils, thus causing precipitation of phosphate. The carbon dioxide–water reagent extracted much less phosphate from all soils than the other chemical reagents.

Although amounts of phosphate extracted by sodium bicarbonate, carbon dioxide–water, ammonium oxalate and Morgan's reagent were all significantly correlated with each other, only two of these (sodium bicarbonate and carbon dioxide–water) were significantly correlated with total phosphorus uptake by rye-grass. This points to the danger of replacing one chemical method by another, merely because the new method correlates well with the old. For a particular soil type, or even for a particular crop, it would appear important that the new method be compared with results of crop uptake.

Table II

Correlation coefficient ('r') between values obtained by the five chemical methods

Correlation between	Values of 'r'
(1) Morgan reagent and NH_4 oxalate	+0·537
(2) ,, ,, ,, fluoride–HCl	−0·124
(3) ,, ,, ,, $NaHCO_3$	+0·711**
(4) ,, ,, ,, CO_2–water	+0·710**
(5) NH_4 oxalate and fluoride–HCl	+0·430
(6) ,, ,, ,, $NaHCO_3$	+0·516*
(7) ,, ,, ,, CO_2–water	+0·559*
(8) Fluoride–HCl and $NaHCO_3$	+0·274
(9) ,, ,, ,, CO_2–water	+0·274
(10) $NaHCO_3$ and CO_2–water	+0·779**

* Significant at the 5% level ** Significant at the 1% level

Table III

Correlation coefficients ('r') between total phosphorus uptake by rye-grass and phosphorus extracted by five chemical methods

Correlation between P uptake by rye-grass and 'available' phosphate as measured by—	Values of 'r'
Morgan reagent	+0·033
0·2N-NH_4 oxalate	+0·117
0·03N-NH_4F in 0·1N-HCl	+0·477
0·5M-$NaHCO_3$	+0·594*
CO_2-charged water	+0·527*

* Significant at the 5% level

Although significant correlations exist between phosphorus uptake by rye-grass and both the sodium bicarbonate and carbon dioxide–water methods, the correlation coefficients are not very high. If the correlation coefficients are squared and expressed as percentages, the values obtained are 35% and 27% for the sodium bicarbonate and carbon dioxide–water methods respectively. This indicates that a relatively small amount of the variability in phosphorus can be attributed to the soil analysis values. With calcareous soils containing a wide range of calcium carbonate content none of the chemical methods for assessing availability of phosphorus was very precise. This may be due to wide differences not only in calcium carbonate content but also in the particle size of calcium carbonate present in the different soils.

Chemistry Dept.
Imperial College of Science and Technology
London, S.W.7

Received 17 January, 1963; amended manuscript 29 March, 1963

References

[1] Fraps, G. S., *Texas agric. Exp. Sta. Bull.*, 1909, No. 126
[2] Ghani, M. O., *Indian J. agric. Sci.*, 1943, **13,** 562
[3] Dyer, B., *Trans. chem. Soc., Lond.*, 1894, **65,** 115
[4] Truog, E., *J. Amer. Soc. Agron.*, 1930, **23,** 874
[5] Morgan, M. F., *Conn. agric. Exp. Sta. Bull.*, 1937, No. 392, 129 pp.
[6] Joret, G., & Hebert, *Ann. Agron.*, 1955, **6,** 233
[7] McGeorge, W. T., *Arizona agric. Exp. Sta. Tech. Bull.*, 1939, No. 82
[8] Ensminger, L. E., & Larson, H. W. E., *Soil Sci.*, 1944, **58,** 253
[9] Smith, F. W., Ellis, B. G., & Grova, C., *Proc. Soil Sci. Soc. Amer.*, 1957, **21,** 400
[9a] Sen Gupta, M. B., & Cornfield, A. H., *J. Sci. Fd Agric.*, 1962, **13,** 655
[10] Olsen, S. R., Cole, C. W., Watanabe, F. S., & Dean, L. A., *U.S. Dept. Agric. Circ.*, 1954, No. 939
[11] Bray, R. H., & Kurtz, T., *Soil Sci.*, 1945, **59,** 39
[12] Yuen, S. H., & Pollard, A. G., *J. Sci. Fd Agric.*, 1951, **2,** 36
[13] Yuen, S. H., & Pollard, A. G., *J. Sci. Fd Agric.*, 1952, **3,** 441

THE EFFECTS ON BIRDS OF CERTAIN CHLORINATED INSECTICIDES USED AS SEED DRESSINGS*

By E. E. TURTLE,† A. TAYLOR,† E. N. WRIGHT,† R. J. P. THEARLE,† H. EGAN,‡ W. H. EVANS‡ and N. M. SOUTAR‡

In England during the spring, commencing in the year 1956, numbers of deaths occurred amongst wild birds and the insecticides used to dress cereal seeds were suspected.

Pigeons were fed with dieldrin, aldrin, heptachlor and γ-BHC and the toxicities and residues in flesh and organs measured. Following this, birds from the field were examined during the 1960/61 sowing season. The results of analyses support the view that dieldrin, aldrin and heptachlor had been mainly responsible for the deaths. An ecological section includes an explanation for the predominance of casualties in the spring time.

Introduction

In the early spring of 1956, unusually large numbers of dead birds, particularly of wood-pigeons and pheasants, were noticed in various parts of England. The greatest number of incidents occurred in cereal-growing areas at, or soon after, the sowing period. Sometimes the birds were observed to drop dead to the ground whilst in flight and sometimes death was preceded by convulsions. Such incidents recurred in subsequent years with varying severity until the spring of 1960 when they were fairly widespread in the eastern counties. Information concerning incidents during spring 1960 and 1961 was published by the Royal Society for the Protection of Birds.[1]

During 1960 there was circumstantial evidence that deaths had occurred amongst foxes, badgers and farm dogs and cats from the consumption of poisoned birds. The matter attracted considerable comment in the press and elsewhere and the many suggestions made as to the causes of the incidents included :

(i) variations in sowing conditions had resulted in the exposure of different amounts of seed from year to year ;
(ii) the introduction of wet dressing formulations and machines had possibly resulted in higher contents of insecticides at the time of sowing ;
(iii) there had been increases in the total amounts of insecticidal dressings used, particularly against wheat bulb fly for which a higher dose of insecticide is employed ;

* Read before Pesticides Group, 19 March, 1962
† Ministry of Agriculture, Fisheries & Food, Infestation Control Laboratory
‡ Dept. Scientific & Industrial Research, Laboratory of the Government Chemist

(iv) new organo-mercuric fungicides had been introduced which are more toxic than those hitherto used ;

(v) dressed seeds had been distributed deliberately by farmers or other persons wishing to control pigeons.

The work described in this paper was commenced during the summer of 1960, with the primary object of ascertaining as precisely as possible the cause or causes of the incidents. From an examination of the toxicological literature and from information supplied by the interested companies, it was evident that the insecticides used represented a much greater potential hazard than the fungicides. In planning the work it was noted that some of the questions could only be answered by estimating the proportion of the natural populations of individual species being killed off and by ascertaining the range of species affected. Such studies were considered impractical, however, with the time and facilities available, and the work was confined to pigeons and foxes.

Although much of the toxicological evidence available concerned mammalian species, some work with birds had been previously reported. Carnaghan & Blaxland,[2] for example, showed that grain dressed with dieldrin was toxic to pigeons and pheasants. Grain dressings containing organomercurials and γ-BHC were, however, found to be relatively non-toxic to both of these species. Borg,[3] when investigating deaths amongst pheasants after spring sowing in Sweden, showed that pheasants had died after eating seed dressed with aldrin and mercurial fungicide. Experimental birds survived for about a month when fed solely on the grain treated with the fungicide alone. This paper also contains useful information about the residues of mercury in the organs of the experimental birds. Other authors[4, 5] give data on the acute oral toxicities to avian species of certain pesticides, notably DDT, aldrin, dieldrin, toxaphene and γ-BHC. These last authors found an LD_{50} figure of approximately 350–400 mg./kg. for γ-BHC for mourning doves, but only 15 birds were used. For quail they report a sex difference and give LD_{50} values for males as 120–130 mg./kg. and for females 190–210 mg./kg. DeWitt[6–8] gives the results of toxicity tests with various chlorinated insecticides on quail and pheasants.

None of the above workers had been able to observe marked changes in the organs of experimentally dosed birds, which might have served as a basis for identifying the causes of poisoning. Furthermore, the post-mortem pathological examinations, in the Central Veterinary Laboratory of the Ministry of Agriculture and elsewhere, of bodies obtained from the field revealed no characteristic change by which the causes of death could be determined. In these circumstances it was unfortunate that the means were not then readily available for analysing the carcasses for small residues of the chlorinated insecticides concerned.

A series of feeding trials, with associated chemical analysis of the bodies, was therefore undertaken. The primary objectives were to measure the toxicities to birds of the insecticides implicated and to provide means for the chemical examination of bodies obtained from the field. To enable the results to be applied during the 1960/61 sowing season, the work had to be undertaken quickly and on a limited scale. It was only possible to work with one species and pigeons were chosen as experimental subjects because deaths of these birds had been most commonly reported in the field and because they were obtainable at fairly short notice. Aldrin, dieldrin and heptachlor were selected for the initial studies because they were considered to offer the greatest hazard, but work on γ-BHC has since been undertaken. At the same time, with a view to obtaining information about the effects on predators, these chemicals and certain poisoned birds were fed to foxes by D. K. Blackmore of the Royal Veterinary College and flesh from these animals was analysed for residues of toxicant. (The detailed results of this work are to be published elsewhere.)

Feeding trials

Unfortunately the wood-pigeon is a difficult bird to keep in captivity and it was considered impracticable to carry out tests with this species. As a compromise, therefore, the domestic pigeon (*Columba livia* var.) was used. Birds were purchased from animal suppliers and were of heterogeneous stock and unknown history.

On arrival at the laboratory, birds were housed in a communal aviary until required. They

were transferred to individual cages for testing purposes and were weighed approximately 24 h. before administration of toxicants so that doses could be adjusted to the body weights. Test substances were force-fed to the birds in gelatin capsules after overnight deprivation of food and a further delay of up to 4 h. was allowed to elapse before food was replaced. Thereafter food was available in excess. Water was freely available at all times.

(1) *Dieldrin, aldrin and heptachlor*

Initially four tests were carried out with dieldrin and were intended primarily to provide relicta for chemical analysis but also to obtain some indication of the toxicity of dieldrin to pigeons. The birds were housed in temporary cages under makeshift conditions, as the experimental bird room used in all subsequent tests was not quite ready. It appeared from the literature that the LD_{50} of dieldrin might be expected to be between 40 and 60 mg./kg. For the first test, dosage levels of 20, 40 and 80 mg./kg. were chosen and four birds were dosed at each level. The second test replicated this procedure. In these tests the toxicant was administered as an acute oral dose and it was noted that birds receiving large doses often vomited shortly afterwards. In an attempt to overcome this problem, two further tests were carried out. In the first of these, four birds received the equivalent of 160 mg./kg., two birds being given acute doses and two receiving the same dosage, in four equal parts, on successive days. In the other test, six birds received the equivalent of 160 mg./kg. in three equal doses on successive days. This procedure was not particularly successful in preventing vomiting and was not used in subsequent tests.

The tests with aldrin and heptachlor were designed as bio-assays to give LD_{50} figures and provide relicta for chemical analysis. The birds were housed in individual cages arranged so that they could not see each other in a room maintained at approximately 18°. A minimum conditioning period of 6 days was allowed before commencement of testing. Preliminary tests were undertaken with six birds to determine the approximate dosage range to use for each chemical. In the bio-assays, groups of eight birds were used, all birds within a group receiving equivalent amounts of chemical administered as an acute oral dose. For aldrin the dosages were 40, 46, 53, 61, 70 and 80 mg./kg.; and for heptachlor 40, 54, 73, 99, 133, 180 and 243 mg./kg. As with dieldrin many birds were seen to vomit soon after dosing. Although some birds undoubtedly got rid of unknown amounts of toxicant in this way an analysis of mortality did not show any significant difference between birds that vomited and those that did not. It was therefore concluded that this factor did not seriously affect the calculation of the LD_{50} dosage; it may well have a greater bearing on the comparison of dosage rates with residue levels determined after death had occurred.

The behaviour of birds after dosing was carefully noted. Most birds took a little food immediately it was presented, but the greater part of this appeared to be regurgitated. Many of the birds that died made no further attempt to feed even though death did not occur for several days. A few birds did die after they had resumed feeding, but in most cases birds which regained their appetites survived.

Symptoms of poisoning were similar for all three chemicals, speed of onset and severity being roughly correlated with dose received. Moping, blinking, slight tremor and head turning were noted in most birds, even at the lowest dosage levels. Hypersensitivity, loss of equilibrium and convulsions with marked opisthotonos were characteristic of the higher dosage levels and only one bird known to have suffered convulsions subsequently survived. Convulsions lasted from several seconds to 20 min. or more. Bright green faeces were a common, but not invariable, feature during the first week or so after dosing. With aldrin and dieldrin the symptoms were more pronounced than with heptachlor, which was also more variable in its effects. Birds that died were weighed as soon as possible after death and placed in deep freeze at about −20°. All showed a loss in weight ranging from 3% to 37% (mean 20%). The time of death ranged from 2½ h. to 17 days (mean 5 days) after dosing. When the tests began, it was not known how long after dosing the birds might die and in the first test with dieldrin half of the surviving birds were sacrificed 9 days after dosing and the remainder 4 days later. In all subsequent tests the bio-assay lasted 21 days and no birds were sacrificed during this period. At the end of this time survivors were feeding heartily and showed no signs of the treatment. These

survivors were sacrificed after various intervals of time and stored in deep freeze either for chemical analysis or to be fed to foxes as part of the studies on secondary poisoning.

The LD_{50} figures, calculated from the bio-assay results by probit analysis, are for aldrin 55 mg./kg. (fiducial limits, at 5% level, 46 and 65 mg./kg.) and for heptachlor 167 mg./kg. (fiducial limits, at 5% level, 133 and 245 mg./kg.). Although the tests with dieldrin were not designed to give an LD_{50} figure, it has proved possible to analyse the results obtained to give a very rough LD_{50} estimation of 67 mg./kg. (fiducial limits, at 5% level, 44 and 115 mg./kg.). It is not possible to check these results precisely against those of previous workers because no-one appears to have undertaken acute toxicity tests with pigeons in the same way. However, most previous workers have recorded similar relative toxicities for these three chemicals to birds except that DeWitt[6–8] reported aldrin to be appreciably more toxic than dieldrin to quail and pheasants. This latter result is not supported by the observations of Radomski *et al.*,[9] Bann *et al.*[10] and ourselves that aldrin is rapidly converted to dieldrin within the animal body.

(2) *γ-BHC*

An attempt was made to determine the LD_{50} of γ-BHC to pigeons in a similar way. Three groups of eight birds each were used, the dosage levels being 290, 420 and 600 mg./kg. respectively. One bird died in the first group, two in the second and three in the third. These observations are insufficient for the calculation of an LD_{50} figure, but it was interesting to note that so few deaths occurred at the high dosage rates. The difficulty of administering even 600 mg./kg., and the excessive vomiting that followed, suggested that it would be useless to attempt to increase the acute dosage range still further. Attention was therefore turned to the semi-chronic toxicity of γ-BHC. Eight birds were given daily doses of 72 mg./kg. for 5 days. All birds showed slight symptoms of poisoning and went off their food. One died 7 days after the cessation of dosing. The remaining birds quickly recovered and six were sacrificed 14 days after the last dose was given. Flesh was removed from each of these birds immediately after death and analysed, by gas–liquid chromatographic methods, with the results shown in Table I. A further five birds, dosed at the same time and at the same rate, were sacrificed at daily intervals, beginning 3 days after the last dose was administered and the insecticide levels in flesh and livers were determined (Table II). In these tests, γ-BHC was administered in a flour pill, but in spite of this, and the lower dosage level, all birds vomited to some extent.

γ-BHC is not stored to the same extent in the animal body as are the other insecticides under consideration : the fate of the chemical in the flesh after death is uncertain. According to Davidow & Frawley,[11] with regular intake of BHC, storage in the tissue occurs, particularly in the fat. After exposure has ceased the α-, γ- and δ-isomers disappear from rat fat in 3 weeks. The results in Table III give the levels found after dosing pigeons with γ-BHC at 350 mg./kg. The birds were sacrificed after 7 days and sampled 7 and 14 days after death. It is thus possible to detect and measure residues of γ-BHC after death. The results as a whole confirm that the toxicity of this chemical is much lower than the other insecticides used in cereal seed dressings.

Table I

Residues in feral pigeons receiving 72 mg./kg. of γ-BHC for 5 days

(by gas–liquid chromatography : birds sacrificed 15 days after dosing)

Live wt., g.	γ-BHC found, p.p.m.	
	Flesh	Liver
534	26·2	36·0
540	28·1	37·1
515	23·2	35·0
447	24·5	19·8
468	3·9	4·2
573	21·9	19·5
458*	29·4	31·6

* Bird died 7 days after dosing

Table II

Residues in feral pigeons receiving 72 mg./kg. of γ-BHC for 5 days

(by gas–liquid chromatography)

Live wt., g.	Sacrificed after dosing, days	γ-BHC found, p.p.m.	
		Flesh	Liver
512	3	11·8	48·4
564	4	16·5	18·7
457	5	144·0	67·0
443	6	29·4	51·8
533	7	29·0	24·5

(3) *Significance of the toxicity figures*

The toxicity figures are of particular interest when related to the quantities of the respective insecticides which could be consumed if birds were to feed on grain treated at the recommended dosage rates. If a wood-pigeon were to eat 50 g. of cereals in a day, for example (30 g. is an adequate diet for captive birds), it would receive about 40 mg., or about 80 mg./kg., if the grain were treated at the maximum dosage recommendation of 2 oz. per bushel with a preparation containing 40% of the insecticide. This amount is greater than the LD_{50} figure for dieldrin or aldrin but much less than that for γ-BHC : some confirmation for the intake assumed in this calculation is obtained from the work of Murton, Isaacson & Westwood.[12]

Analytical methods

Both specific and non-specific methods are available for the determination of residues of chlorinated insecticides. The non-specific methods, such as those depending on the determination of total organic chlorine, can obviously give no information regarding the identity of residues. Relatively specific chemical methods of analysis may be used for samples known to contain residues of aldrin,[13] dieldrin,[14] heptachlor and its epoxide[15] or γ-BHC,[16] but it is impracticable to apply a succession of these methods with the object of detecting and measuring an unknown residue. At the time of commencement of the work under consideration, no satisfactory single method was available for the extraction and clean-up of all chlorinated insecticide residues from samples of either vegetable or animal tissue. Since it would obviously be advantageous to use a single technique for all of the residues and at the same time to be able to distinguish individual insecticides, special attention was paid to the paper chromatographic methods already developed by Mitchell and others.[17] As a result, a suitable end method of analysis was developed by Egan & Evans[18] and with appropriate extraction and clean-up methods this was applied to the detection and determination of residues in the field samples. To begin with, however, the residues in the dieldrin feeding trial were determined colorimetrically by the phenylazide method of O'Donnell *et al.*,[14] using an acetone–carbon dioxide freezing bath to facilitate the isolation of the aldrin intermediate in hexane following the zinc reduction stage.[19]

The following extraction and clean-up methods were used. For *dieldrin and aldrin*, 20 g. of prepared tissue was refluxed for 1 h. with 60 ml. of 95% ethanol and 12·5 ml. of 10N-potassium hydroxide, the cooled digest extracted with 5 × 30 ml. hexane, the combined extracts washed with five portions of 200 ml. of water and the hexane run through a short column of anhydrous sodium sulphate, then reduced to about 5 ml. and passed through a 30-g. magnesia column. For *heptachlor and heptachlor epoxide*, 20 g. of tissue incorporated with 40 g. of sand and 60 g. of anhydrous sodium sulphate was tumbled gently for 1 h. with 150 ml. of hexane in a stoppered flask, the hexane filtered, the solid material thoroughly washed with further hexane and the filtered washings combined with the original filtrate : the hexane phase was then treated with 25 ml. of 1 : 1 concentrated/fuming (20% SO_3) sulphuric acid mixture, as described by Sergeant & Wood for DDT residues,[20] passed through a 35-g. column of anhydrous sodium sulphate, reduced in volume to about 5 ml. and passed through a 30-g. magnesia column. A similar process was used for *γ-BHC* residues, but the final column treatments with anhydrous sodium sulphate and magnesia were replaced by a single Davidow column treatment.

Overall recovery values, with mean deviations from the mean, for the various insecticides added to control pigeon flesh at levels of 2–10 p.p.m., for the combined extraction, clean-up and paper chromatographic determination were : aldrin (HHDN) $94 \pm 12\%$; dieldrin (HEOD) $89 \pm 12\%$; heptachlor $86 \pm 12\%$; heptachlor epoxide $78 \pm 8\%$; γ-BHC $83 \pm 3\%$.

Since the extraction and clean-up method for one insecticide residue does not necessarily lead to the quantitative recovery of other residues, the examination of bodies of certain birds from the field survey was discontinued when a significant positive result was obtained for one insecticide. For this reason, Table IX shows the number of analyses which were undertaken for each insecticide, rather than the number of bodies examined. In this, as in certain other respects, the introduction of the gas–liquid chromatographic method of Goodwin *et al.*[21] represents a major development. It enabled analyses for a range of chlorinated hydrocarbon insecticides to be carried out together, and during the autumn of 1961 and the spring of 1962 it was

used exclusively for the examination of any bodies obtained from the field. An account of the use of the technique for this purpose has been prepared by Taylor.[22]

Analyses of experimentally fed birds

In accordance with the findings of previous workers with mammalian species, it was thought that the insecticides might be concentrated in fatty tissues or in organs such as the liver. With a few of the birds receiving aldrin, dieldrin or heptachlor in the feeding trials, therefore, an attempt was made to separate fat by dissection ; generally this was impossible owing to the emaciated condition of many of the birds at the time of death, but steps were nevertheless taken to ensure that such fat as was available was included with the muscle tissue for analysis.

With a few of the birds fed experimentally with aldrin or with dieldrin, the liver, kidneys and breast muscle flesh were analysed separately ; dieldrin residue results are shown in Table IV. This line of enquiry was not pursued since a simple technique was required for the examination of considerable numbers of bodies from the field. The impression obtained from these few tests, and from the subsequent examination of the separate organs of a few of the bodies obtained from the field, however, was that a wide variation could occur in the distribution of residues through the flesh and organs. The method of dosing and the timing between the intake of the poison and death, and possibly the condition of the bird receiving the dose, may each have an effect.

The method employed for the examination of most of the experimental birds and, subsequently, for the examination of birds from the field therefore was to remove the breast muscle ; this was macerated, extracted and determined by paper chromatography at the Laboratory of the Government Chemist or by gas–liquid chromatography in the Infestation Control Laboratory. In some cases a composite macerated sample of flesh, liver and kidneys was examined.

The examination of individual birds receiving aldrin (Table V) confirmed the observation of Bann *et al.*,[10] that only traces of this chemical can be found in the body because of its rapid epoxidation to dieldrin. For this reason, it was not possible to differentiate between aldrin and dieldrin as contributory causes of death in cases where residues of dieldrin were found in birds from the field. At the same time it could be deduced that when aldrin is found the bird has probably ingested the poison shortly before death. Experiments with relicta of birds receiving heptachlor (also shown in Table V) similarly indicated rapid conversion to the epoxide.

The results of analyses of a selection of the birds which had been used in the toxicity tests are given in Tables VI–VIII which refer respectively to birds which received dieldrin, aldrin and heptachlor. The time and manner of death are shown in each case. (The examination of domestic pigeons which had not received insecticides gave negative results for chlorinated hydrocarbons when analysed in the same way.) From the results it is evident that the birds which died generally carried higher residues than those which survived and which were therefore sacrificed for analytical work. Nevertheless there is only a rough correlation between the occurrence of death and the size of a residue in a body. A figure for a residue of one of the insecticides in a body obtained from the field, therefore, can only be used as presumptive evidence as to the cause of death. It needs to be considered alongside other, and particularly the circumstantial, evidence before firm conclusions are drawn.

Investigation of deaths in the field

Arrangements were made in the autumn of 1960 for members of the Regional Pests Staff of the Ministry of Agriculture to investigate the circumstances of any unusual incidence of bird deaths. Reports coming in to any of the offices of the Department, or to the notice of members of the Advisory Services, were to be passed on to the Regional Pests Officer. The latter officers were asked, when it was possible, to visit the localities from which reports came and, amongst other things, to check the truth of any statements about the numbers of deaths, to ascertain what agricultural activities had been taking place recently in the locality and, if cereal seeds had been sown, what preparations if any had been used to dress them. They were also asked to collect sample bodies and to send them to Infestation Control Laboratory. The arrangements were put into effect in October 1960 but during the autumn of that year only one or two allegations of deaths from this cause were made and no bodies were received for

Table III

Residues of γ-BHC (p.p.m.) in flesh of pigeons fed at 350 mg./kg., analysed 7 and 14 days after sacrificing

Bird no.	7 days	14 days
1	12·2	10·3
2	15·3	12·7
3	6·8	6·2
4	6·7	6·7

Table IV

Distribution of dieldrin residues between flesh, liver and kidneys of feral pigeons

(determination by phenylazide colorimetric method)

Dieldrin dose rate, mg./kg.	Death after dosing, days	Dieldrin found (p.p.m.) Flesh	Liver	Kidney
2 × 55	2	20·6	15·0	23·0
4 × 40	5	29·0	19·8	13·0
3 × 50	4½	24·8	24·4	22·0
3 × 50	3½	26·0	20·2	22·0

Table V

Examination of flesh of pigeons for aldrin and for heptachlor

(determination by paper chromatography)

Dose, mg./kg.	Death after dosing, h.	Chemical found, p.p.m.	
Aldrin		Aldrin	Dieldrin
143	2½	16	20
81·5	7	9	30
141	35	2	37
87	105	0	31
Heptachlor	days	Heptachlor	Heptachlor epoxide
113	2	1	16
68	4½	0	29
186	5½	0	7
179	7	0	31

examination. During the spring of 1961, however, very many reports were received and in some Regional Offices they were too numerous to enable each to be investigated in detail. About 80 incidents were so investigated and the 300 bodies which were received in the Laboratory represented only a small percentage of those that had been found. Reports began to be received at the end of the first week in March and they continued until the second week in May. In the majority of cases they coincided with and shortly followed a period of seed sowing. They were also most numerous in cereal-growing areas and particularly in the counties of Yorkshire, Cambridgeshire, Lincolnshire, Kent and Nottinghamshire.

The largest concentrations of dead birds were found in roosts, with as many as 500 woodpigeons in one or two cases. It often proved difficult for the investigating officer to ascertain where birds had been feeding. In any event it was not always possible to find out what dressings had been used for particular sowings. This was either because the farm records were inadequate or because the farmer did not know what dressing had been used on the seed. Furthermore, when the results of chemical analysis became available, it was not easy to correlate given incidents with specific sowings in a given locality.

Analytical work on bodies from the field during autumn 1960 to spring 1961

(i) *Birds*

Bodies were only accepted for analysis if they were in reasonably fresh condition and if the circumstantial evidence implicating dressed seed was fairly strong. Although the exact dates were not normally known, the majority of the birds had probably been dead some 5–14 days when received in the laboratory. The flesh or other part of the body was removed on receipt, and placed in cold storage pending chemical examination. The examinations were confined to seed-dressing insecticides and did not include examination for residues of mercurial fungicide.

Table VI

Residues in feral pigeons receiving dieldrin

(determination by phenylazide colorimetric method)

Live wt., g.	Dose, mg./kg.	Death after dosing, days	Dieldrin in flesh, p.p.m.
340	20	9*	3·4
335	20	13*	3·0
315	20	5	8·2
275	20	9*	3·8
350	20	21*	2·8
420	20	21*	11·5
245	20	21*	2·2
370	20	21*	2·8
240	40	9*	5·8
390	40	13*	10·2
335	40	13*	10·4
290	40	9*	5·6
340	40	2	9·0
310	40	21*	7·2
345	40	21*	2·6
295	40	21*	4·0
355	80	13*	13·5
290	80	4½	32·0
300	80	7½	21·0
225	80	3½	16·5
330	80	3½	21·5
340	80	2	6·2
290	80	4½	26·5
290	80	21*	4·2

* Sacrificed

Table VII

Residues in feral pigeons receiving aldrin

(determination by paper chromatography)

Live wt., g.	Dose, mg./kg.	Death after dosing, days	Dieldrin, in flesh, p.p.m.
389	40	17½	39
416	40	63*	4
338	40	3½	21
343	46	10	38
317	46	1½	10
409	53	10	26
340	53	5½	22
587	53	63*	8
312	53	2	25
457	61	63*	4
304	61	1	16
274	70	4	14
383	70	63*	3
276	70	3	17
283	70	4	16
297	70	3	22
278	80	2	13
252	80	2	9
411	80	5	9
287	80	4	19
431	80	3	35
388	80	1	16

* Sacrificed

Ninety-one bodies collected from the field in this way during the springtime were examined. Most of the analytical work was carried out by paper chromatography, but nine specimens were examined by gas–liquid chromatography, for which the apparatus only became operative towards the end of the period of the survey. As the paper chromatographic technique involved separate analyses for the individual insecticides, the findings are summarised in Table IX, which gives the results from the individual analyses. In certain cases there may be more than one

Table VIII

Residues in feral pigeons receiving heptachlor

(determination by paper chromatography)

Live wt., g.	Dose, mg./kg.	Death after dosing, days	Heptachlor epoxide in flesh, p.p.m.
428	40	28*	9
411	54	28*	5·5
412	73	28*	10
422	73	15	20
464	99	28*	4·5
311	133	6½	19
331	133	3½	22
398	133	10	46
446	133	4	33
502	133	28*	7
321	180	5½	31
370	180	13	48
475	180	28*	11
338	243	3	33
366	243	9	38
396	243	14	53
467	243	8½	49
478	243	28*	23

* Sacrificed

Table IX

Summary of analyses of breast muscle of birds from the field during the spring of 1961

(The 91 birds in this table included 68 wood-pigeons, 5 pheasants and 2 partridges)

Insecticide	Number of birds examined	No. of birds containing stated residues			
		Over 12 p.p.m.	Between 3 and 12 p.p.m.	Between 0·5* and 3 p.p.m.	Not detected
Dieldrin	67	20	20	10	17
Heptachlor	64	11	16	24	14
γ-BHC	22	0	0	7	15

* Column includes 3 entries for dieldrin and 3 for heptachlor which were only qualitative and which may have contained more than 3 p.p.m.

entry for a given bird if its body was examined for more than one insecticide. In some instances significant amounts of more than one of the insecticides were present. The maximum residue of dieldrin found was 41 p.p.m. and the maximum for heptachlor epoxide was 34 p.p.m.

(ii) *Main conclusions*

When an attempt was made to compare the results of analysis with the dressings reported in the corresponding field reports, the picture was found to be confused. Many of the reports recorded that more than one insecticide had been used in the district and many did not provide very certain information on what materials had been used. The main practical conclusion of the analytical work, therefore, was to confirm the circumstantial evidence that seed dressings were responsible. It also indicated that, of the seed-dressing insecticides used, aldrin, dieldrin and heptachlor were primarily responsible.

Action arising from the investigations

Although there was evidence that very large numbers of pigeons and other birds died, in certain districts, little or no information was obtained concerning the proportion of the population of any species which was affected, either throughout the country generally or in any given district. Neither were details obtained of the range of species affected, although these included both avian and mammalian predators. Surveys of a different character and wider in extent are needed to obtain a scientifically satisfactory overall assessment of the effects of toxic chemicals on wildlife. In the absence of such thorough investigations, however, it was concluded, on the evidence already available, that any recurrence of the events should be avoided. Means of doing this were therefore examined both independently and in conjunction with representatives of the interested companies and other organisations. The suggestion of incorporating a repellent substance into the formulation of each of the more toxic dressings was considered as an attractive means of minimising the hazard. At the present time, however, there appears to be no formulation or chemical which has been shown to be effective for this purpose. An alternative possibility was to modify the use of the respective insecticides. Evidence available for the relative values of the different insecticides as dressings for the control of wireworms and wheat bulb fly, the pests against which they are mainly used, has been discussed by Gough *et al.*[23] and by Maskell & Gair.[24] These have shown that for wheat bulb fly control, dieldrin, aldrin and heptachlor have advantages over γ-BHC if the grain is sown early in the autumn. For grain sown early in the New Year, however, the evidence showed that γ-BHC was slightly more effective for the control of this pest. For the control of wireworms, aldrin, dieldrin and heptachlor showed no very definite advantages. On these grounds it was considered that there was not a sufficient justification to warrant the continued use of aldrin, dieldrin and heptachlor during the spring sowing period. This view was accepted by the companies marketing the various products and by various interested naturalist and other organisations. An announcement was made by the Minister of Agriculture, Fisheries and Food in the House of Commons[25] that from 1st January, 1962, dressings containing dieldrin, aldrin and heptachlor would not be used for spring sowings, but that they would only be used for dressing autumn and winter wheat where there was a real danger of attack from wheat bulb fly.

During the autumn of 1961 the previous arrangement for investigating incidents reported to Regional Offices of the Ministry of Agriculture had been supplemented by field observations conducted on a planned basis irrespective of whether any reports came into offices. Numerous Field Officers acted as investigators. Between September and the end of the year some 30 alleged incidents involving about 120 birds were investigated. Although the results substantiate the conclusion that the insecticides in question represent some hazard to birds at this time, it is considered that the number of casualties is small having in mind the efforts which were made to discover them.

Some ecological aspects of the findings

Various authors have pointed out that the effects of toxic chemicals on wildlife depend not only on the toxicity and persistence of the chemical and the area treated, but also on the relationship between different species and the biotope.[26–28] The weather may also have a profound influence, both directly by its effect on the chemical, and indirectly by its effect on soil conditions and the behaviour of animals. It is interesting to pursue these ideas in the light of our findings and of what is known about bird behaviour.

Intensive studies of the feeding habits of wood-pigeons have shown that cereals are a preferred food item and will be taken whenever available.[29] They have also demonstrated that considerable amounts of seed are left on the surface during normal sowing operations. The practice of treating cereal seed with an insecticide, therefore, presents a particular hazard to wood-pigeons but the abundance of alternative food is an important factor in determining the extent to which they feed on the newly planted fields. During the autumn a wide selection of foods is freely available, including grain from stubbles and, in some years, abundant acorns and beechmast. Wood-pigeons may feed on these rather than on new sowings and, in spite of the fact that autumn-sown cereals may be dressed with the more toxic insecticides, few deaths may occur. Failure of the acorn and beechmast harvest, coinciding with conditions that favour early ploughing of stubbles, would increase the bird pressure on sowings and could result in more casualties. In spring the natural food of wood-pigeons is at a low ebb ; their main diet at this time is clover, but this is readily forsaken in favour of cereals when these become available from newly sown fields. It is not only likely that more of the birds will feed on seed grain in the spring than in the autumn, but also that it will form a bigger proportion of the total food consumed by individual birds. Moreover, the physiological condition of birds in the spring may render them more susceptible to the effects of toxic chemicals than at other times of year.

This outline of factors affecting the consumption of cereal seeds by wood-pigeons suggests why the observed effects of seed dressing chemicals can differ widely and why they have been most dramatic in the springtime. These observations refer to conditions in England. The potential hazards to birds may be different under different conditions, such as may well apply in other countries where insecticidally dressed seed may be sown. Studies of the habits of other species would also be expected to throw up similar information of value in assessing the hazard to which they may be subjected by agricultural chemicals.

(*Crown Copyright Reserved*)

Ministry of Agriculture, Fisheries & Food
Hook Rise
Tolworth, Surbiton
Surrey
and
Laboratory of the Government Chemist
Clement's Inn Passage
Strand, W.C.1

Received 9 November, 1962 ; amended manuscript 14 January, 1963

References

[1] 'Deaths of Birds and Mammals connected with Toxic Chemicals', *Rep. of the BTO–RSPB Commee on Toxic Chemicals*, 1960, No. 1, 20 pp.; 1962, No. 2, 24 pp. (London: Roy. Soc. for Protection of Birds)
[2] Carnaghan, R. B. A., & Blaxland, J. D., *Vet. Rec.*, 1957, **69,** 324
[3] Borg, K., *Proc. 8th Nordic vet. Conf.* (Helsinki), 1958, pp. 394–400
[4] Coburn, D. R., & Treichler, T., *J. Wildl. Mngmt*, 1946, **10,** 208
[5] Dahlen, J. H., & Haugen, A. O., *J. Wildl. Mngmt*, 1954, **18,** 477
[6] DeWitt, J. B., *J. agric. Fd Chem.*, 1955, **3,** 672
[7] DeWitt, J. B., *J. agric. Fd Chem.*, 1956, **4,** 863.
[8] DeWitt, J. B., & George, J. L., *U.S. Fish. & Wildl. Serv. Circ.* No. 84, 1960
[9] Radomski, J. L., & Davidow, B., *J. Pharmacol.*, 1953, **107,** 259
[10] Bann, J. M., De Cino, T. J., Earle, N. W., & Sun, Y. P., *J. agric. Fd Chem.*, 1956, **4,** 937
[11] Davidow, B., & Frawley, J., *Proc. Soc. exp. Biol. Med.*, 1951, **76,** 780
[12] Murton, R. K., Isaacson, A. J., & Westwood, N. J., *Brit. Birds* (in press)
[13] O'Donnell, A. E., Neal, M. M., Weiss, F. T., Mann, J. M., De Cino, T. J., & Lau, S. C., *J. agric. Fd Chem.*, 1954, **2,** 573
[14] O'Donnell, A. E., Johnson, A. W., jun., & Weiss, F. T., *J. agric. Fd Chem.*, 1955, **3,** 757
[15] Polen, P. B., & Silverman, P., *Analyt. Chem.*, 1952, **24,** 733; Meyer, C. F., Malina, M. A., & Polen, P. B., *J. agric. Fd Chem.*, 1960, **8,** 183
[16] Schechter, M. S., & Hornstein, I., *Analyt. Chem.*, 1952, **24,** 544; Hancock, W., & Laws, E. Q., *Analyst*, 1955, **80,** 665
[17] Mitchell, L. C., *J. Ass. off. agric. Chem., Wash.*, 1958, **41,** 781; McKinley, W. P., & Mahon, J. H., *ibid.*, 1959, **42,** 725; Mills, P. A., *ibid.*, 1959, **42,** 734
[18] Egan, H., *Abstr. XVIII Int. Congr. pure appl. Chem.*, 1962, p. 262; Evans, W. H., *Analyst*, 1962, **87,** 569
[19] Modesto Method Series, MMS–4/59 (Shell Development Co., Modesto, Cal., U.S.A.)
[20] Sergeant, C. A., & Wood, R., *Analyst*, 1959, **84,** 423
[21] Goodwin, E. S., Goulden, R., & Reynolds, J. G., *Analyst*, 1961, **86,** 697
[22] Taylor, A., *Analyst*, 1962, **87,** 824
[23] Gough, H. C., Woods, A., Maskell, F. E., & Towler, M. J., *Bull. ent. Res.*, 1961, **52,** 621
[24] Maskell, F. E., & Gair, R., *Bull. ent. Res.*, 1961, **52,** 683
[25] *Hansard* (*Commons*), 1961, **643,** (139), Col. 983 (London: H.M.S.O.)
[26] Cottam, C., *Proc. 13th Annu. Conf. South-eastern Ass. of Game & Fish Commissioners, U.S.A.*, 1959
[27] Hickey, J. S., State of Wisconsin, U.S.A., Report to the Governors of the Special Commee on Chemicals & Health Hazards, 1961
[28] Mills, H. B., *Trans. N. Amer. Wildl. Conf.*, 1959, **24,** 113
[29] Murton, R. K., Isaacson, A. J., & Westwood, N. J., (in preparation)

FUMIGATION OF AGRICULTURAL PRODUCTS. XVIII.*— Effect of Methyl Bromide on the Bent Grass Nematode, *Anguina agrostis* (Steinbuch 1799) Filipjev 1936, and on the Germination of Bent Grass, *Agrostis tenuis*

By N. G. M. HAGUE

Seed of *Agrostis tenuis* and seed galls of the nematode *Anguina agrostis* were fumigated with methyl bromide at three concentration–time products over a range of moisture contents. Satisfactory nematode control was obtained by fumigating seed of about 12% moisture content at concentration–time products between 600 and 800 mg. h./l.

Introduction

Goodey[1] has reviewed the information available about *Anguina agrostis* which is a serious pest where bent grass is grown for seed, notably in the U.S.A., Australia and New Zealand. Courtney & Howell[2] showed that *A. agrostis* could be controlled by interfering with the life

* Part XVII: *J. Sci. Fd Agric.*, 1961, **12,** 96

history through crop rotation and lawn cutting, the latter preventing the formation of the inflorescence. These authors showed that hot water treatment at 120° F for 15 min., following soaking at 75° F for 24 h., killed the galls in seed samples.

Previous work[3] has shown that desiccated fourth-stage larvae of *Ditylenchus dipsaci* in lucerne seed samples can be killed by methyl bromide : Lubatti & Blackith[4] have shown the effect of methyl bromide treatment on cereals conditioned at different moisture contents. It was therefore decided to determine the toxicity of methyl bromide to both bent grass seed and nematode galls at three dosage levels and at four moisture contents.

Experimental

Agrostis tenuis seed was obtained from Elsom & Sons Ltd., Spalding, Lincs., and *Anguina agrostis* galls from a heavily infested seed sample sent by Dr. W. Cottier, D.S.I.R., Nelson, New Zealand.

Known amounts of water were added to 100 g. of seed (original moisture content 9·7%) and the whole shaken thoroughly in an air-tight vessel in which it was stored to produce batches of seed with 11·9 and 13·9% moisture content. Seed at 8·0% moisture content was obtained by passing warm air over batches of 100 g. of seed for about 2 weeks.

To condition the eelworm galls to the same moisture content as the *Agrostis* seed, 20 intact galls were added to each 100-g. batch of seed.

Fumigation

Fumigations were carried out in air-tight cylindrical metal chambers of 20 l. capacity, provided with a suitable ampoule breaker and sampling taps, as described by Lubatti & Smith.[5]

The seed and nematode galls were placed in a muslin bag in the chamber to allow the seed and nematodes under test to condition the atmosphere of the fumigation chamber. After 24 h. a weighed amount of methyl bromide was introduced into the chamber from a glass ampoule, and the chamber was rolled gently on its side to distribute the vaporised fumigant evenly. The fumigation lasted 20 h.

The concentration of gas in the chamber was estimated by collecting gas samples about 15 min. before the end of the fumigation ; the sampling technique and the method of determination were similar to those described by Lubatti & Blackith,[6] and the concentration–time product was calculated as described by these authors.[7] The concentration–time products used are shown in Table I (nominal values 600, 800 and 1000 mg. h./l.).

Viability assessment

The biological responses of the seeds were tested 5 weeks after fumigation, during which period the seeds were maintained at their initial moisture contents. Three replicate batches of 100 seeds were placed on thick moistened filter-paper pads in Petri dishes and germination was recorded at intervals during 7 weeks. The germination tests were done at 20° in a growth room with the light necessary for the germination of the seeds supplied for 16 h. each day.

The kill of the nematode galls was also tested 5 weeks after fumigation. Six galls from each batch of 100 g. of seed were soaked in water for 24 h., then broken open and placed on a cottonwool milk filter placed on a raised sieve over a Petri dish of water. The second-stage larvae which migrated through the filter were considered to be viable and were counted.

$$\% \text{ Mortality} = 100\,(N_C - N_T)/N_C$$

where N_C and N_T are the numbers of nematodes migrating from untreated and treated galls respectively.

Results and discussion

At the highest moisture contents and the highest rate of fumigation there was a significant decrease in germination and a significant increase in nematode kill (Table II). Satisfactory nematode control was obtained by treatment of seed at a moisture content of approximately

Table I

Moisture contents and fumigation treatments of bent grass seeds and nematode galls

Moisture content, %	Concentration–time product, mg. h./l.
8·0	570, 740, 950
9·7	590, 770, 930
11·9	580, 740, 970
13·3	560, 740, 990

Table II

Effect of methyl bromide on the viability of Anguina agrostis *galls and the germination of bent grass,* Agrostis tenuis, *conditioned at different moisture contents*

Moisture content, %	Nominal concentration–time product mg. h./l.							
	0		600		800		1000	
	larvae/gall	% Germination	% kill	% Germination	% kill	% Germination	% kill	% Germination
13·3	312	81	100	80	100	70	100	55
11·9	312	89	100	87	100	82	100	66
9·7	407	87	98·5	81	83	84	91	87
8·0	552	87	25	88	59	83	49	90

12% and at concentration–time products between 600 and 800 mg. h./l. There was a significant interaction between moisture content and dosage: in general the results of fumigating bent grass are very similar to those obtained when cereals were fumigated with methyl bromide.[4]

There was delayed germination which, as Lubatti & Blackith[6] state, is of interest but probably of little economic importance.

Conditioning *Anguina agrostis* galls at moisture contents higher than those found in normal stored seed significantly decreased the viability of the second-stage larvae, a result for which there is no adequate explanation.

Acknowledgment

The author acknowledges the assistance of Mr. P. Clarke, who helped with methyl bromide determinations, and Miss S. Morse, who carried out the germination tests.

Imperial College Field Station
Ashurst Lodge
Sunninghill, Ascot
Berkshire

Received 21 December, 1962

References

[1] Goodey, J. B., *J. Sports Turf Inst.*, 1959, **10,** 35, 1
[2] Courtney, W. D., & Howell, H. B., *Plant Dis. Reptr*, 1952, **36,** (3), 75
[3] Page, A. B. P., Hague, N. G. M., Jakabsons, V., & Goldsmith, R. E., *J. Sci. Fd Agric.*, 1959, **10,** 461
[4] Lubatti, O. F., & Blackith, R. E., *J. Sci. Fd Agric.*, 1957, **8,** 691
[5] Lubatti, O. F., & Smith, B., *J. Soc. chem. Ind.*, 1948, **67,** 347
[6] Lubatti, O. F., & Blackith, R. E., *J. Sci. Fd Agric.*, 1956, **7,** 149
[7] Lubatti, O. F., & Blackith, R. E., *J. Sci. Fd Agric.*, 1956, **7,** 343

EFFECT OF STORAGE TEMPERATURE AND MOISTURE CONTENT ON THE GERMINATIVE ENERGY OF MALTING BARLEY, WITH PARTICULAR REFERENCE TO HIGH TEMPERATURE

By H. D. BURGES,* D. M. EDWARDS,† N. J. BURRELL* and M. E. CAMMELL*

The germinative energy of malting barley was determined after storage at two moisture contents, 12·8 and 13·3%, at three constant temperatures between 32 and 40°. The results link up well with other work at lower temperatures. When plotted against storage temperature and moisture content, the storage times during which germination falls to 95% form almost straight lines between 10 and 35°. Above 35°, a greater increase in the rate of deterioration is shown.

Introduction

After being dried in hot-air dryers, malting barley and other cereal grains are often still warm when put into store. Although the temperature of the stored grain can be reduced by aeration,[1] some time may elapse before the grain becomes cool enough throughout a bulk to prevent deterioration.[1] The first easily recognisable sign of deterioration is a fall in the germinative energy of the grain, which is of great concern to the malting industry. It is, therefore, important to know how long grain can be stored at different temperatures before the viability decreases. Some work has been described on the germination of barley after storage at moderate temperatures.[2–4] In good storage conditions, barley may retain its viability for 29 years.[5] We know of no work concerning storage at temperatures above 30°, which are fairly often found in practice. The purpose of the present paper is to describe work at such temperatures and to compare the results with the published information at lower temperatures.[2–4]

Experimental

Grain for experiment was taken from the surface of a large bulk of Proctor barley. Dormancy had been overcome by kiln-drying after harvest in 1960 and by storing until February, 1961, when the experiment was started. The surface of the bulk cooled to about 20° within 2 days of drying, and to about 5–10° during winter; these can be regarded as the pre-experimental storage temperatures.

Two levels of grain moisture content for experiment were obtained in the following way. A bushel of barley at 14·3% moisture content was divided into two portions, one of which was dried to 12·8% and the other to 13·3% moisture content by aeration at room temperature near 25° on a miniature platform dryer. After being dried, each portion was left to equilibrate in a sealed tin for 1 day, then it was thoroughly mixed and further divided into 56 replicate sub-portions, each of which was placed in a small linen bag. Seven bags of grain filled an airtight 4-lb. jar. One jar was stored at each of the two moisture levels at four different experimental temperatures comprising a total of 56 samples. Since the jars were sealed, loss of moisture during the experiment was prevented.

Because the rate of respiration of dry grain is very low,[6] the accumulation of carbon dioxide could be prevented by inserting a small glass tube containing 1 ml. of caustic potash solution in each jar. In order to avoid the possibility of slight changes in the moisture content of the grain, the strength of the solution was adjusted to give a relative humidity[7] in equilibrium with the grain (specific gravity of 1·31 at 15° for a moisture content of 12·8%, and 1·26 for 13·3%, calculated from points half-way between the absorption and desorption curves of equilibria[8] between humidity and barley moisture content at 25°). The jars were opened weekly for 1 min. to replenish any slight reduction in oxygen content.

The jars were stored in incubators or rooms at controlled temperatures, which were recorded

* Pest Infestation Laboratory
† Messrs Munton & Fison Ltd.

on thermographs and checked at least once weekly against mercury thermometers attached to each jar. The mean temperatures were 39·6, 34·9, 32·1 and 17·5°, with ranges of 39·0—40·3, 34·0–36·0, 31·5–32·9 and 17·1–17·8° respectively.

Bags of barley were removed at intervals for the determination of germination and moisture content (Fig. 1). Germinative energy was measured by keeping two samples of 100 grains from each bag at 20–21° for 3 days on two filter papers (Whatman No. 29) moistened with 4 ml. of water in 9 cm. Petri dishes, and removing the germinated corns every 24 h. Moisture contents were determined by drying two coarsely ground samples at 113° for 4 h. in a ventilated oven. The largest difference in moisture content between the two samples from any bag was 0·20%. Tests showed that this method gave the same results for barley as drying for 1 h. at 130° in a Brabender Semi-automatic Moisture Tester, which was the method used by Kreyger.[3]

Results

Moisture content

The moisture content of the barley in each jar remained satisfactorily stable over the duration of the experiment. At either moisture level, there were no significant differences between the mean values for jars at the three high temperatures. However, there were small differences between the mean values for jars at 17·5° and averages of jars at the three high temperatures combined. At the lower moisture content, the difference of 0·20% was significant statistically.* Kreyger[3] obtained increases of the order of 0·5% of moisture during storage at low temperatures and greater decreases at high ones, probably because his grain was not stored in jars but in bags of Polythene and Kraft paper, which were not complete barriers to water vapour.

Germinative energy

Germinative energy was clearly related to storage temperature and moisture content (Fig. 1). At 17·5°, it remained virtually constant for 26 weeks. At the three high temperatures, it remained almost constant for periods that decreased inversely with temperature and moisture content, and then it fell rapidly (Fig. 1).

In Fig. 2, these results are related to published work. The numbers against the symbols in this figure show the storage periods during which the germinative energy fell to 95%. These periods were obtained from Fig. 1 and from similar graphs prepared from the published work.[2–4]

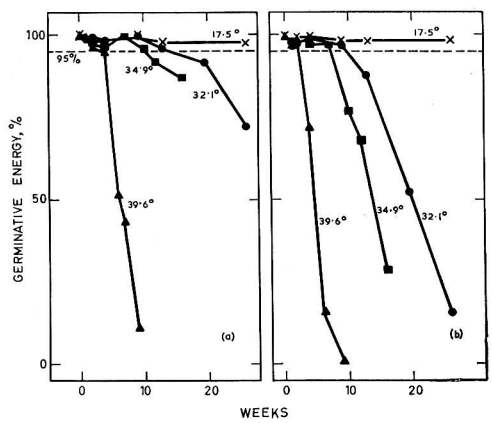


FIG. 1.—*Germinative energy of barley after storage at different temperatures and two levels of moisture content*
(*a*) 12·6 ± 0·3% moisture (*b*) 13·2 ± 0·3% moisture

* Lower moisture level, P = <0·001 between 17·5° (mean 12·83%, standard error 0·03%) and 32·1, 34·9 and 39·6° combined (mean 12·63%, standard error 0·03%)

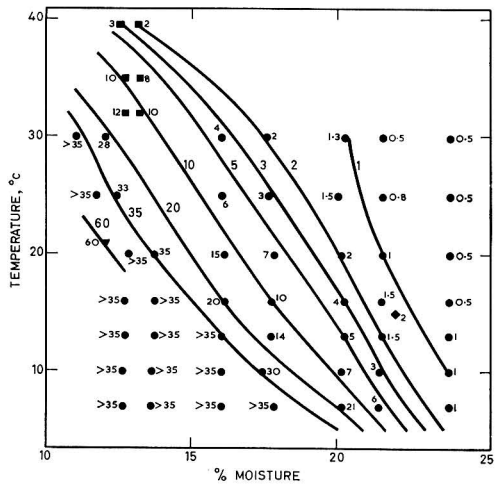


FIG. 2.—*Effects of temperature and moisture content of grain during storage on the germinative energy of barley*
(numerals on the figure are the storage periods in weeks during which the germinative energy fell to 95%)
■ present results ● ▲ ◆ results from literature (references 3, 4 and 5 respectively)

To determine the curves in Fig. 2, the storage periods against the symbols were first plotted against moisture content on separate graphs for each temperature, and against temperature on another set of graphs for each moisture content. These curves were smoothed by eye and the smoothed values used to construct the curves in Fig. 2, which were further smoothed. The value of 95% germination was used as the criterion of the start of deterioration, because it is very difficult to recognise earlier deterioration (Fig. 1). Although the moisture content changed very little during storage in the present work, it changed considerably in Kreyger's work.[3] Therefore the values of moisture content used are means of samples measured up to the fall to 95% germination.

The results of the present work combine well with the published data. In Fig. 2, the lines representing particular storage periods are almost straight between 10 and 35°. When plotted in the same way, the half-viability periods[9] of wheat also lie along straight lines below 35°. With barley, there is a suggestion of curvature above 35° (Fig. 2), indicating a greater increase in the rate of loss of germination.

Discussion

The results in Fig. 2 refer to constant temperatures. In storage practice, these results can be applied only to bulks of dry barley in which the temperature is not increasing due to the production of heat by granicolous moulds or granivorous insects. Such heating would obviously accelerate deterioration. Combinations of temperature, moisture content and insect infestation in which heating is likely to occur will be described in a later paper.[1] In bulks not heating, grain that is drier than 14% moisture content can be left at 25° and below for quite long periods without deterioration; at higher temperatures, however, store owners should exercise great care, because even grain that has less than 13% moisture content cannot be left without risk of deterioration for longer than 8 weeks at 30–35°, or for longer than 3 weeks above 35°. With higher moisture contents, the maximum permissible storage periods are far shorter.

Acknowledgments

The Pest Infestation Laboratory and Messrs Munton & Fison Ltd. collaborated in this work.

The authors wish to thank Mr. S. W. Pixton of the Pest Infestation Laboratory for carrying out the comparative moisture content tests with the Brabender tester and the ventilated oven.

Pest Infestation Laboratory
London Road
Slough, Bucks.
and
Munton & Fison Ltd.
Stowmarket, Suffolk

Received 4 February, 1963

References

[1] Burges, H. D., & Burrell, N. J., *J. Sci. Fd Agric.*, 1963, **14**, in the press
[2] Robertson, D. W., Lute, A. M., & Gardner, R., *J. agric. Res.*, 1939, **59**, 281
[3] Kreyger, J., *Petit J. Brass.*, 1958, **66**, 811, 832; 1959, **67**, 7
[4] Bewer, H. E., *Ber. Landtech. München*, 1957, No. 47, 53 pp.
[5] Haferkamp, M., *Proc. Ass. off. Seed Anal. N. Amer.*, 1949 (June 27–July 1), 111
[6] Milthorpe, J., & Robertson, R. N., *Bull. Coun. sci. industr. Res. Aust.*, 1948, No. 237, 9
[7] Solomon, M. E., *Bull. ent. Res.*, 1951, **42**, 543
[8] Finn-Kelcey, P., & Hulbert, D. G., *Tech. Rep. Brit. elect. Res. Ass.*, W/T33, 1957, 26 pp.
[9] Roberts, E. H., *Ann. Bot., Lond.*, 1960, **24**, 12

THE RÔLE OF PHYTIN IN THE TEXTURE OF COOKED POTATOES

By H. G. WAGER

The possibility that the precipitation of calcium by phytin is a major cause of the softening of potatoes during cooking is considered. Old potatoes do not soften after the removal of phytin nor at a pH below that at which calcium phytate is precipitated. New potatoes (i.e., immature ones) are not softened by phytin.

Introduction

Cooked potatoes vary in texture from floury to soapy, the distinction between them being the degree to which the individual cells of the cooked potato separate one from another. Little is known about the causes of this separation of the cells, but a general correlation has been established between the starch content of the tubers and the flouriness after cooking.[1]

The cells of the cotyledons of dried peas also separate from one another after cooking and Mattson[2] suggested that this was brought about by the removal of calcium ions from the pectin of the middle lamella, thus converting the pectin from a form which was insoluble to one soluble at pH 6–7. The calcium precipitant implicated was phytin, present in considerable quantities in the pea, and which starts to precipitate as the calcium salt at pH 5. Such a mechanism depends on the ratio of phytin to available calcium, on the pH of the tissue and on the pectin or other binding material of the middle lamella being rendered soluble by removal of ionisable calcium. Since potatoes also contain considerable amounts of phytin[3] and the softening during cooking is similar in type, i.e., cell separation, it seemed possible that the same mechanism operates and the present note contains some preliminary evidence.

The texture of cooked potatoes normally varies to only a small extent between the stem and rose end, and tubers of one stock usually have a similar texture. In consequence, if phytin

were the major factor controlling the texture of the cooked potato, one would expect its concentration to be similar at both ends of the same tuber and in different tubers of the same stock. Further the phytin content of ' new ' potatoes should be lower than ' old ' since they are always more ' soapy '.

Experimental

The phytin content of the stem and rose ends of tubers from a commercial stock of potatoes (var. King Edward) was estimated. The phosphate was extracted by blending 15-g. portions cut from the rose or stem end of the tuber in 5% trichloroacetic acid solution at 1°, keeping for 2 h. and filtering. The phytin was precipitated as the ferric salt by the method of Early & de Turk,[4] the precipitate was collected by centrifuging and warmed with N-sodium hydroxide solution, the ferric hydroxide was removed by again centrifuging and the phosphorus content of the supernatant estimated, after digestion with perchloric acid,[5] by Waygoods modification[6] of the method of Lowry & Lopez.[7] The total phosphorus content of the trichloroacetic acid extract was also estimated.

The results, given in Table I for a number of tubers, showed that the stem end contained less phytin and also less total phosphorus than the rose end and that there was considerable variation between tubers of the same stock. A similar difference in content of inorganic phosphorus between the stem and rose end was found in the tubers investigated by Hughes & Swain.[8]

To investigate the change in phytin with maturity, the mean phytin contents were determined of all the tubers from single plants harvested from the same plot at four stages from 8 weeks to 2 weeks before full maturity. The variations between plants proved too large for a change in phytin content with age to be shown. In some recent published results, however, the phytin content of potato tubers was shown to increase with age[9] as would be expected if phytin were playing a part in controlling the texture of cooked potatoes.

In spite of these rather negative findings the following experiment suggests that phytin does, nevertheless, play a part in the disintegration of mature potatoes on cooking.

Slices of tubers were frozen at −20° (to disrupt the cells), allowed to thaw, leached in water at 1° for 6 days and then divided into three groups. In the first group of slices, leaching was continued for the next 2 days, the second group was soaked in M/600-sodium phytate solution at pH 6 and the third group was soaked in M/200-calcium chloride solution. All groups and a control from −20° were then boiled in water for 20 min. (The pH of the cooking liquids was between 6·0 and 6·2.) With old potatoes the control slices after boiling were normally soft, those which were leached and then had the phytin replaced were beginning to disintegrate, being appreciably softer than the control, whereas the slices leached and those leached and then soaked in calcium chloride showed no softening at all.

The new (immature) potatoes tested responded quite differently in that the texture of the slices from all the treatments was similar and even after longer boiling (about 2½ h.) there was no separation of the cells even in the phytin replaced slices.

The control slices of the old and the new potatoes contained 8·80 and 7·60 mg./100 g. of phosphorus phytin respectively, whereas the leached slices did not differ from the reagent blank, showing that the leaching had been effective.

It is clear, therefore, that the presence of phytin in old potatoes does lead to the separation of their cells after cooking at about pH 6 and the contrary is also true, i.e., in the absence of

Table I

Content of phytin and total phosphorus of the stem and rose end of tubers of King Edward potatoes

(mg. P/100 g. fresh wt.)

	Phytin		Total phosphorus	
	Rose end	Stem end	Rose end	Stem end
Tuber 1	10·7	5·9	46·2	33·9
Tuber 2	4·0	1·9	36·3	25·7
Tuber 3	9·6	6·7	40·7	31·4
Tuber 4	12·1	2·7	35·0	21·4

phytin no cell separation occurs. There is, therefore, a *prima facie* case for suggesting that under normal conditions phytin plays a part in the separation of the cells of old potatoes during cooking.

The variation in the content of phytin shown in Table I does not preclude its acting as a calcium precipitant, since the effective removal of calcium ions will depend on the ratio of available calcium ions (perhaps also magnesium ions) to phytin after cooking as well as to the pH of the tissue. The pH of the stem end of a potato is higher than the rose end by 0·1–0·2 of a unit[8, 10] and this will tend to make the lower phytin content at the stem end a relatively more effective calcium precipitant (cf. Mattson[2]) and hence to make the difference in content of phytin between the two ends of the tuber of less importance. The calcium in the middle lamella is not likely to vary very much at the two ends of the tuber, but the calcium in the cytoplasm may well be related to the acid content which is larger at the rose end than the stem end.[8, 11] Clearly the interaction of these various factors might well lead to a fairly uniform texture in spite of some variation in phytin content.

Mattson[2] showed that the proportion of calcium precipitated by phytin fell rapidly as the pH was reduced from 6 to 5 and corresponding to this the softening of the peas during cooking decreased over the same range. This is also true of the softening of potato tissue during cooking, as shown by the marks awarded by a taste panel for the texture of strips of potato cooked at various pH values (Fig. 1). Thus the relationship between the texture and the pH of cooked strips of potato, also, is such as might be expected if phytin were playing a part in the process of cell separation.

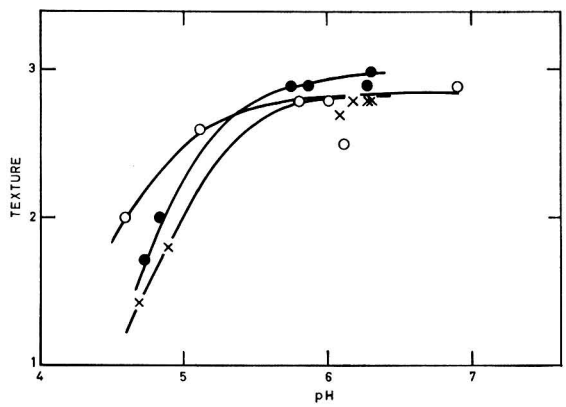


FIG. 1.—*Texture of cooked strips of three stocks of potato and the pH of the cooking liquor*

Ordinates—average of the marks for texture awarded by a taste panel, 4 perfect texture, 3 good, 2 fair and 1 poor. Abscissa—pH of cooking liquor after cooking

Conclusion

In the old potatoes, therefore, the hypothesis that phytin leads to softening after cooking by acting as a calcium precipitant is supported by the available facts. In the case of new potatoes, however, the cells appear to be bound together by a material which either is not rendered soluble by the removal of calcium ions at pH 6 or in which the dissociation of the calcium ion from the pectin at this pH is so slight that, when the phytin concentration is M/600 and the pH is 6·0–6·2, insufficient ions are released to reach the solubility product for calcium phytate. The difference in texture between new and old potatoes, therefore, must be sought in the properties of the material binding the cells together rather than in the content of phytin.

Acknowledgment

The experimental work of this paper was carried out by Mr. F. A. E. Porter.

Low Temperature Research Station
Downing St.
Cambridge

Received 31 January, 1963

References

[1] Burton, W. G., 'The Potato', 1948 (London: Chapman & Hall)
[2] Mattson, S., *Acta agric. suec.*, 1946, **2,** 185
[3] McCance, R. A., & Widdowson, E. M., *Spec. Rep. med. Res. Counc., Lond.*, No. 235, 1942
[4] Early, E. B., & de Turk, E. E., *J. Amer. Soc. Agron.*, 1944, **36,** 808
[5] Allen, R. J. L., *Biochem. J.*, 1940, **34,** 858
[6] Waygood, E. R., *Canad. J. Res.*, 1948, **26C,** 461
[7] Lowry, O. H., & Lopez, J. A., *J. biol. Chem.*, 1946, 421
[8] Hughes, J. C., & Swain, T., *J. Sci. Fd Agric.*, 1962, **13,** 229
[9] Samotus, B., & Schwimmer, S., *Nature, Lond.*, **194,** 578
[10] Wager, H. G., *J. agric. Sci.*, 1946, **36,** 214
[11] Juul, F., 'Studies over Kartoflens mørhfarning efter Kogning', 1945, pp. 152 (Copenhagen: Jul Gjellerups Forlag)

FUMIGATION OF PYRAMIDAL STACKS OF BAGGED DECORTICATED GROUNDNUTS WITH METHYL BROMIDE

By D. HALLIDAY and P. F. PREVETT*

An experiment to determine the distribution of methyl bromide gas in a pyramid-shaped stack of bagged decorticated groundnuts in Kano, Nigeria, is described. It was considered that, in view of the poor distribution of gas observed, a pyramid-shaped stack is probably unsuitable for a fumigation in which injection of the gas at a single point is used.

Introduction

For the past 15 years or so it has been the custom to store part of the Northern Nigerian groundnut crop for periods of up to one year or more, mostly in the Kano area. Originally this storage was necessitated by the lack of adequate transport facilities to evacuate the groundnuts to the ports, but nowadays nuts are retained in Kano for delivery to local oil expressers, who process 100–150 thousand tons of decorticated groundnuts per year, and to act as a buffer stock for export purposes.

As the result of investigations carried out by this Unit in collaboration with the Produce Inspection Division of the Northern Region Ministry of Agriculture, over a period of more than 10 years, the method of storage has now been standardised. Groundnuts are stored decorticated in B twill bags, as pyramidal structures of between 850 and 900 tons (11,000–11,700 bags), mounted on specially constructed raised square plinths of side length 54 feet. The pyramids have vertical sides which are 10 bags high and a total height of 42 bags. Originally the pyramids were constructed on a dunnage of groundnut husk and ashes, but in recent years a waterproof dunnage has been substituted. The first waterproof dunnage to be used was sisalkraft paper, but this is now being superseded by roofing felt. Pyramids are normally covered with overlapping tarpaulins several months before the beginning of the wet season. Nearly all groundnuts stored in Kano are now located at a recently constructed stacking area, which has provision for about 200 pyramids.

* Present address: Tropical Stored Products Centre, Post Infestation Laboratory, Slough, Bucks.

Storage of groundnuts in the open has resulted in serious infestation problems, which have necessitated the provision of facilities to fumigate complete pyramids with methyl bromide, under large specially manufactured gasproof sheets. Such fumigations have been carried out very successfully in Kano and other storage centres of Northern Nigeria for over 10 years.

An experiment to determine the distribution of methyl bromide within a pyramid during fumigation was described by Hayward[1] in 1954. However, at that time the most serious insect pest infesting stored groundnuts in Kano was *Trogoderma granarium* Everts, the larvae of which require higher concentration–time (c.t.) products of methyl bromide for near 100% mortality than for most other insect pests. For this reason very heavy doses of methyl bromide (1 lb. per 5 tons of nuts) were used for routine fumigations. *T. granarium* has now been eliminated from Nigerian groundnuts, but it is still necessary to carry out many fumigations of pyramids as a routine control measure against other major pests, viz., *Tribolium castaneum* (Herbst), *Corcyra cephalonica* (Staint.), *Cadra cautella* (Wlk.) and *Plodia interpunctella* (Hübn.), which are more susceptible to methyl bromide than *T. granarium*. Because of the greater susceptibility of these to methyl bromide, for the past 2 years the dose used has been cut to 1 lb. per 10 tons of nuts, and it was decided that a further experiment should be conducted to investigate distribution of the gas inside a pyramid during a fumigation at this lower dosage rate.

In addition, the size of pyramids has been slightly increased since the experiment of 1954 referred to above, and waterproof dunnage has replaced that of groundnut husk and ashes, and it was desired to see whether these factors affected methyl bromide distribution. With the development of the thermal conductivity meter[2] for determining concentrations of methyl bromide in air, it was possible to plan a far more detailed and comprehensive experiment than that previously carried out.

In recent years attention has been drawn to the possibility of groundnuts acquiring a high bromide residue after fumigation with methyl bromide, and it was therefore decided that bromide residues in samples taken from bags adjacent to the methyl bromide sampling points should be determined, after the pyramid had been broken down. It was hoped that these values might also supply some confirmatory evidence for conclusions based on the figures obtained for c.t. products.

Experimental

Copper gas-sampling tubing of dimensions 0·115 in. external dia. and 0·075 in. i.d., as recommended by Burns Brown,[3] was built into a pyramid during its construction. The pyramid was of the standard type described above, containing 886 tons of groundnuts, and of dimensions : base length 54 ft., height of vertical side 9 ft. and total height 38 ft., giving a total volume of 54,400 cubic feet (1,540,000 litres). After completion, and subsequent to the installation of all sampling tubes, the pyramid was covered with tarpaulin.

Sampling tubes were installed at positions on the outside bags, and between bags at positions 1 bag and 5 bags inside the pyramid and at the centre of the pyramid, as shown in Figs. 1–3. Immediately before fumigation the tarpaulins were removed from the apex of the pyramid, and the top bags restacked to make a channel of approximate dimensions 4 ft. × 3 ft. × 2 ft. Alkathene tubing to carry the fumigant was laid on to the pyramid, with a single terminal Bray jet nozzle at the centre of the channel, pointing downwards at an angle of 45° to the horizontal. The pyramid was covered with a gasproof envelope of neoprene on nylon, and the base sealed with sandsnakes. The copper sampling tubing was bunched together and led out under the sandsnakes in such a way that no leakages of fumigant were likely to occur.

Methyl bromide (89 lb.) was admitted over a period of about 1 h., and readings of methyl bromide concentration taken with two of the thermal conductivity meters (model A)[2], at suitable intervals for 24 h. after gassing had been completed. Readings were taken hourly for the first 12 h. when rapid changes in methyl bromide concentration were occurring, but less frequently for the second 12 h.

The fumigation experiment was carried out on 3–4 April, 1962, commencing at approximately 8 a.m. on the first day and terminating at the same time during the following day.

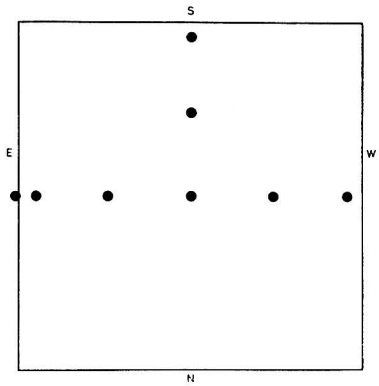


FIG. 1.—*Plan of bag layers* 1, 5 *and* 10 *to show gas sampling points*

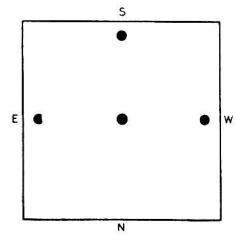


FIG. 2.—*Plan of bag layer* 26 *to show gas sampling points*

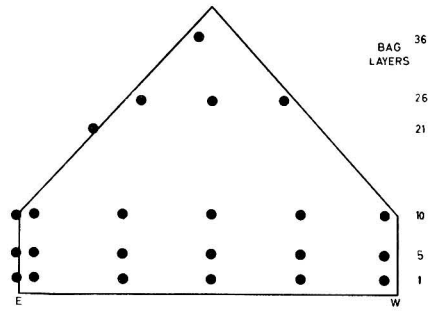


FIG. 3.—*Cross-section of pyramid on E–W axis to show gas sampling points*

For the first 12 h. of the experiment a strong north-easterly breeze was blowing, but for the latter half of the experiment there was little or no wind.

When the pyramid was broken down (during the month of October, 1962) representative samples of groundnuts were taken from bags adjacent to the fumigant sampling points as shown in Figs. 1–3 above. The contents of bromide residue in these samples were determined by ashing in the presence of alkali at temperatures below 500°, oxidising any bromide present to bromate with sodium hypochlorite solution, and determining bromate iodometrically.[4]

Results

Concentration–time products calculated for each sampling point as methyl bromide mg. h. per litre for the full 24-h. period of the experiment are given in Table I. Bromide residues, of samples taken from bags adjacent to the fumigant sampling points, expressed as p.p.m. bromide (Br^-) are given in Table II. Average figures for samples position ' 1 ' and ' 5 bags in ' at layers 26, 10, 5 and 1 are also given.

Concentration–time curves for outside positions, centre positions, and averages of the three positions ' 5 bags in ' and ' 1 bag in ' for each layer are given in Figs. 4–7.

Discussion

It will be seen from Figs. 4 and 5 that the concentration of methyl bromide at the surface and 1 bag in, reached an initial peak very quickly, and then fell gradually to a fairly steady value after 8–12 h. Table I shows that the c.t. products for the 24-h. period of the experiment for

Table I

Concentration of methyl bromide after 24 h. fumigation (mg. h./l.)

Position of bag	Layer 36	Layer 26	Layer 10	Layer 5	Layer 1
Outside, East	—	248*	236	249	231
1 bag in, East	289	196	244	208	192
,, ,, ,, South	—	207 } 247	262 } 226	257 } 253	255 } 233
,, ,, ,, West	—	339	171	293	253
5 bags in, East	—	—	185	143	143
,, ,, ,, South	—	—	189 } 178	155 } 149	147 } 144
,, ,, ,, West	—	—	161	150	142
Centre	—	>600	380	132	151

* Actually layer 21 because of impossibility of fixing sampling point at layer 26.

Table II

Residual bromide (p.p.m. Br^-) after 24 h. fumigation

Position of bag	Layer 36	Layer 26	Layer 10	Layer 5	Layer 1
Outside, East	—	66	59	58	57
1 bag in, East	77	77	52	62	65
,, ,, ,, South	—	63 } 85	58 } 50	79 } 69	54 } 62
,, ,, ,, West	—	115	40	66	66
5 bags in, East	—	—	38	35	65
,, ,, ,, South	—	—	42 } 46	41 } 42	40 } 64
,, ,, ,, West	—	—	59	51	86
Centre	—	152	60	41	43

the above positions did not in general differ very much in value, indicating that good mixing of gas had occurred on the pyramid surface, possibly due to the influence of the strong north-easterly breeze which was blowing during the first half of the experiment. It is interesting to note, however, that position west, ' 1 bag in ' of layer 10 (at the angle between the vertical side and sloping side), had a markedly lower c.t. product than the analogous south and east positions at layer 10, and probably due to billowing of the fumigation sheet by the wind at this point. It will be seen from Table II that figures for bromide residues for samples taken from bags adjacent to the above sampling points in general confirm these findings for c.t. products.

The c.t. curves for positions further inside the pyramid, i.e., ' 5 bags in ' and the centre positions, Figs. 6 and 7 respectively, show that the centre of layer 26 received a very large initial dose of methyl bromide, but the concentration then gradually fell to reach a fairly steady value after 8 h. This heavy concentration gradually diffused downwards to give peak concentrations at both the centre and ' 5 bags in ' positions of layer 10 after 6 h. The analogous position at layers 5 and 1 did not show the same marked peaks for methyl bromide concentration, but there is some evidence of a slight rise in concentration after 10–12 h., which may be due to diffusion from the central area of high methyl bromide concentration referred to above. It would appear, however, that at the inside positions at layers 5 and 1, the concentration of methyl bromide was at a fairly steady low level 3 h. after the commencement of the experiment. It will be noted that there is no evidence of loss of methyl bromide by diffusion through the base of the pyramid, and nor in fact were any leaks detected with halide lamps around the base of the pyramid during the course of the 24-h. fumigation period. The use of sisalkraft paper dunnage on this occasion should have reduced the loss through the base of the pyramid which Hayward[1] suggested occurred with the earlier forms of porous base.

It will be seen from Table I that the c.t. products for the centre position show considerable variation from top to bottom of the pyramid because of the high concentrations immediately below the point of gas injection. The c.t. products for the ' 5 bags in ' position are similar for layers 5 and 1 and comparatively high for layer 10, in correlation with the centre figures at these layers. Figures for the ' 1 bag in ' position are generally higher than at ' 5 bags in ' and it will be noted that there is more variation between sides at this position (particularly at

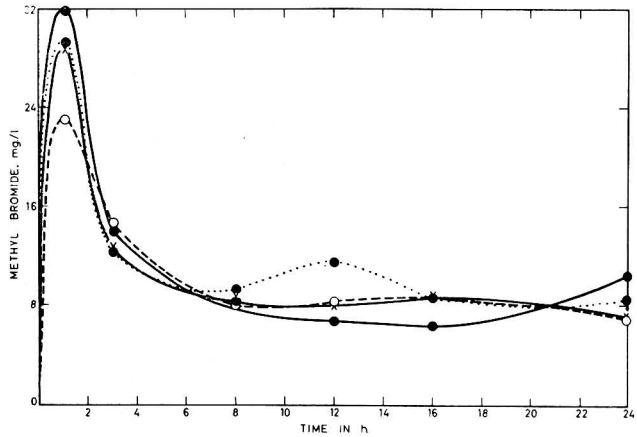


FIG. 4.—*Concentration–time curves for gas sampling points on the periphery of a groundnut pyramid*
○———○ layer 1 ●····● layer 5 ×——× layer 10 ●——● layer 26

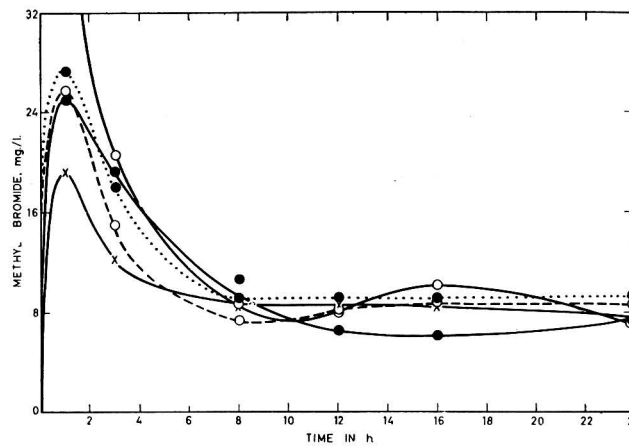


FIG. 5.—*Concentration–time curves for gas sampling points ' one bag in ' from the periphery of a groundnut pyramid* (average of three points)
○———○ layer 1 ●····● layer 5 ×——× layer 10 ●——● layer 26 ○——○ layer 36

layer 26) than at ' 5 bags in '. The corresponding figures for bromide residue (Table II) show rather less variation, but those for the centre positions follow the same distribution pattern. It should be remembered, however, that the level of bromide residue will depend on the speed of aeration of the pyramid after removal of the fumigation sheet in addition to the c.t. product to which it is exposed during the actual fumigation. In this connexion, the rather surprisingly high residue at the ' 5 bags in ' position of layer 1 may also be due to this factor.

It is considered that all the c.t. products recorded would be sufficient to cause near 100% mortality of any infestation present. In this connexion Burns-Brown[3] has suggested that at 30°, which is the approximate level of internal temperature likely to be experienced in the bottom 10 layers of the pyramid during April (Table III), a c.t. product of 65 mg. h. methyl bromide

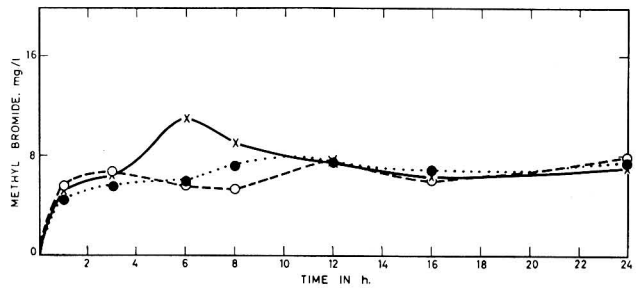


FIG. 6.—*Concentration-time curves for gas sampling points 'five bags in' from the periphery of a groundnut pyramid* (average of three points)
(legend as Fig. 5)

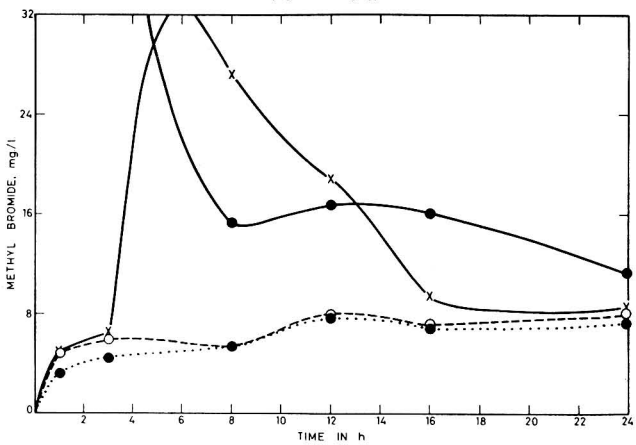


FIG. 7.—*Concentration-time curves for gas sampling points at centre of a groundnut pyramid*
(legend as Fig. 5)

per litre is sufficient to kill 99·9% of a variety of insect species. The lowest figure obtained is slightly more than double this estimate.

The most important feature of the results of this experiment is the wasteful manner in which methyl bromide persists initially in the centre of the pyramid below the single point of application. This would appear to be related to the pyramid shape which allows only a single point of gas injection, and it is very unlikely that any significant change could be made in fumigation technique to improve distribution of gas unless the stack shape is altered. In this connexion it is hoped to carry out similar distribution experiments with stacks of different shapes

Table III

Expected levels of temperature within a groundnut pyramid, early April (from Prevett & Halliday[5])

Position of bag	Temperature, °c (approx.)				
	Layer 36	Layer 26	Layer 10	Layer 5	Layer 1
Surface, 8.30 a.m.	43	43	45	37	36
Surface, 3.00 p.m.	54	49	50	46	42
1 bag in	37	33	32	31	30
5 bags in	—	—	27	27	28
Centre	—	—	27	27	28

N.B. Ambient temperatures in the region of 38° max., 25° min.

built on the same plinths during the 1962–63 storage season, to try to effect better gas distribution. These would include a pyramid with higher vertical sides and correspondingly shorter sloping sides, and possibly a stack with a convex top rather than a sharp peak. Both of these stacks will contain the normal quantity of groundnuts (approximately 850 tons).

In no case does the bromide residue exceed the United States Produce Limit of 200 p.p.m., and it is considered that further consideration of residues in relation to future experiments will be unnecessary.

Acknowledgments

The authors wish to acknowledge the help and encouragement given to them by Messrs. W. Burns-Brown, D. W. Hall, and H. K. Heseltine of the Pest Infestation Laboratory, Agricultural Research Council, Slough, England, in planning and assessing the results of this experiment. Thanks are also due to those members of the junior staff of W.A.S.P.R.U., Kano, who assisted with the field measurements and laboratory analyses, and to the Pest Control Unit, Produce Inspection Division, Ministry of Agriculture (Northern Region), Kano, for their co-operation.

This experiment constituted part of the work programme of the Kano Laboratory, West African Stored Products Research Unit, Federal Ministry of Commerce and Industry, Nigeria.

Federal Ministry of Commerce & Industry
West African Stored Products Research Unit*
Kano
Nigeria

Received 10 December, 1962; amended manuscript 15 February, 1963

References

[1] Hayward, L. A. W., *J. Sci. Fd Agric.*, 1954, **5**, 192
[2] Heseltine, H. K., *Pest Infestation Res. Bull.*, 1961, No. 2 (London: H.M.S.O.)
[3] Burns-Brown, W., *Pest Infestation Res. Bull.*, 1959, No. 1 (2nd edn) (London: H.M.S.O.)
[4] Private communication, Pest Infestation Laboratory, Agric. Res. Counc.
[5] Prevett, P. F., & Halliday, D., *W. Afr. Stored Products Research Unit Annu. Rep.*, 1961, Tech. Appendix No. 5

* Now 'Nigerian Stored Products Research Institute'.

MEASUREMENTS OF THE DIELECTRIC PROPERTIES OF FROZEN AND DEFROSTED MEAT AND FISH IN THE FREQUENCY RANGE 10–200 MHz

By N. E. BENGTSSON, J. MELIN, K. REMI and S. SÖDERLIND

Dielectric constant and loss tangent were determined for lean meat (beef) and for codfish over the temperature range $-25°$ to $+10°$ and frequency range 10–200 MHz. More limited determinations were made for pork, animal fats and herring. The measurements were made using a Boonton RX-meter for which a suitable test cell and measuring technique had been developed.

The measuring technique is described and the results obtained presented in curves and tables showing the effects on dielectric properties of temperature, frequency, frozen storage time and the variability of dielectric properties of the raw material.

The objective of the work done has been to obtain the raw material constants necessary for studying and evaluating the process of dielectric defrosting of meat and fish. The results are discussed from this standpoint.

Introduction

Work on dielectric defrosting of foods has been reported by several authors after the end of World War II, most recently by Jason & Sanders.[1] Similar work with dielectric defrosting of fish and meat is also in progress in these laboratories, a preliminary report of which has been presented elsewhere.[2] A good understanding of dielectric defrosting requires knowledge of the dielectric properties of the materials to be defrosted over a range of temperatures and frequencies. Such results for meat and fish have been available hitherto only to a very limited extent. Various authors have determined specific conductivity,[3, 5] specific resistance,[6] dielectric constant[3–8] and loss tangent[3–4, 7, 8] at various frequencies (up to 1000 MHz) for different foodstuffs (meat, fish, fat—foods of both high and low dielectric loss) at various temperatures (Ede[3] −30° to +100°). Several other workers have reported measurements on human tissues at frequencies above 1000 MHz and at room or body temperatures.

For measuring the dielectric properties of biological materials with high loss, such as meat and fish, several methods have been reported. These include a Marconi Q-meter,[3] equipped with a thermo-insulated coaxial test cell, and measured with series connexion of the test cell, the so-called Barretter method,[5] in which dielectric properties are determined by comparisons with solutions of known data, and a double wire system,[6] operating on a resonant principle. In another paper by Schwan[9] a number of different techniques and test cell designs for measuring biological impedances are described. For the present work a Boonton RX-meter was used.

Experimental

(a) *Instrumentation and measuring technique*

Instrumentation

The general principle for determining dielectric properties in the frequency range concerned is to place the sample in a test cell (condenser) in which the sample constitutes the dielectric material. The admittance of the full and the empty test cell is determined and the dielectric properties calculated.

Two types of instrument were available for the present measurements, a Marconi Q-meter type TF 1245 and a Boonton RX-meter type 250 A. The latter was chosen from considerations of simplified test cell construction and calculation of maximum error and greater precision and accuracy of measurement. This meter is in principle a modified Schering bridge circuit, in combination with oscillator and null detector.

Test cell construction.—The test cell is shown attached to the instrument in Fig. 1 and partly dismantled in Fig. 2.

The cell consists of two silver-plated copper electrodes with leads for connexion to the RX-meter and held at a fixed distance of 10·0 mm. by a Plexiglas ring to form a sample compartment of 25 mm. diameter. The electrodes are covered on the outside by Plexiglas discs, and the different cell elements are joined together with PVC screws.

To permit measurements down to −25° with acceptable temperature stability, good thermal insulation had to be provided. For this reason thick Plexiglas was used in the outer cell elements. To reduce heat conduction through the leads, these were also made of Plexiglas, covered with thin silver foil. Finally, a box of Foamplast was put around the cell during measurements.

To make resistance in the connexions between cell and instrument as low as possible, screw-on connexions were used and all contact surfaces silver-plated. To hold the inductance of the leads at a small value, these were made wide and flat (foil) and as short as possible.

The dimensions of the cell (in terms of the cell constants C_0, C_1, L_s, R_s and R_0 discussed below) were chosen in such a manner that the admittance of the filled cell would fall within the measuring range of the instrument for all temperatures, frequencies and materials studied in the investigation.

Measuring technique

Theory and procedure of calculation.—The admittance of the test cell can in principle be written as

$$Y = 1/R_0 + j.\omega(C_0 + \varepsilon'.C_1) \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where R_0 is a resistance representing loss in the Plexiglas of the cell and possible losses through radiation

ω is the angular frequency

ε' the complex relative dielectric constant of the sample

C_1 the capacitance of the empty sample compartment of the cell

C_0 the capacitance due to the displacement flow through the Plexiglas and the surrounding air. $\varepsilon.C_1$ corresponds to the displacement flow in the sample, where

ε is the relative dielectric constant of the sample.

For dielectric constant and loss tangent of the sample the following relationships hold:

$$\varepsilon' = \varepsilon - \mathrm{j}.\frac{\kappa}{\omega.\varepsilon_0} \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

and

$$\tan\delta = \frac{\kappa}{\omega.\varepsilon.\varepsilon_0} \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

where δ is the loss angle

κ the conductivity of the sample and

ε_0 the dielectric constant for vacuum.

The admittance of the cell can then be given as

$$Y = 1/R_0 + \mathrm{j}.\omega.C_0 + \kappa.C_1/\varepsilon_0 + \mathrm{j}.\omega.\varepsilon.C_1 \quad . \quad . \quad . \quad . \quad (4)$$

and is determined from measurements of parallel capacitance and resistance. To calculate ε and tan δ from Y the cell constants C_1, C_0 and R_0 must be known.

C_1 was obtained by determining the cell admittance with substances of known dielectric properties. It was also calculated from the geometric dimensions of the cell sample compartment, assuming homogeneous field distribution. C_1, being an air capacitance, is independent of frequency and also for practical purposes of temperature, since the linear expansion for Plexiglas is quite low. R_0 and C_0 were determined for each temperature and frequency used in the test series through measurements on the empty test cell.

The inductance L_s of the test cell, its leads and the binding posts of the bridge is of considerable importance at the higher frequencies. To make the necessary corrections, the value of the inductance had to be determined. Two methods were used for this purpose: from determinations of the admittance of the air-filled cell at high and low frequencies and from the determination of the admittance of the cell short-circuited with copper shavings. The latter method also gives an idea of the magnitude of the resistance in the cell leads, R_s, which was found to be negligible. The complete equivalent circuit for the test cell can then be represented as shown in Fig. 3 below, where C_0, C_1, R_s, R_0 and L_s are the different cell constants. Effects from electrode polarisation were considered negligible in the frequency range investigated.

Accuracy and precision.—The maximum errors in calculated dielectric constant ε and loss tangent, tan δ, caused by the measurement error and frequency error of the RX-meter and by the errors in determining the cell constants, were estimated for a number of different frequencies and temperatures of measurement as seen in Table I, which lists values calculated for codfish. It should be remembered that these maximum errors have been obtained through algebraic addition of several independent maximum partial errors. For this reason the true mean error is probably quite considerably lower.

Components of total variability in measurements are variations in temperature, sample, test cell, operator, and possibly others. Precautions were taken to keep these variations as low as possible by using standardised procedures for preparation and handling of samples, temperature adaption and actual measurement and by frequent control of empty cell data. That reproducibility of measurements was quite satisfactory, can be seen from Tables III–VIII, where 95% confidence limits are given.

Comparisons with literature values.—Table II lists some dielectric values reported in the literature for various materials, in comparison with the present measurements. Overall the agreement appears to be quite fair, considering the differences in samples, methods, time, operators, etc.

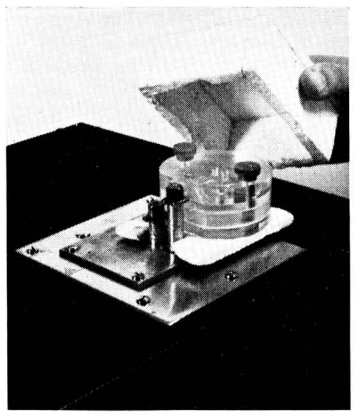


FIG. 1.—*Test cell attached to the Boonton RX-meter*

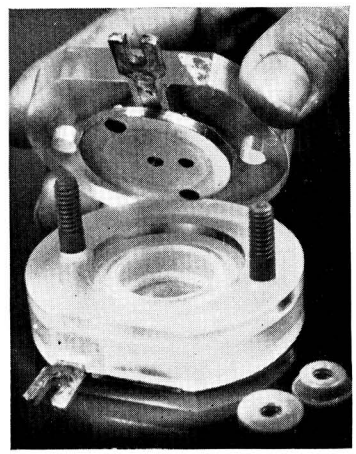


FIG. 2.—*Dismantled test cell*

(b) *Sample preparation and procedure*

Samples were minced, well mixed, frozen and stored at −30° in plastic bags. In preparation for measurement they were defrosted in a cold room overnight. Identical test cells (2–5) were filled from the sample, adjusted to near the temperature of measurement and put in constant-temperature boxes, the temperature of which could be set between −30° and room temperature. For measurement, the test cells were attached to the binding posts of the RX-meter, protected by a heat insulating box. The temperature of the sample immediately before and after measurement was determined by means of a thermocouple in one of the cells. Temperature rise during transfer from thermostat and during measurement was quite low, the time required for measurement being only 30 sec. The sample temperatures at the time of measurement were determined with an error of about 0·5°.

(c) *Raw materials*

Dielectric properties were determined for lean beef meat, lean pork meat, raw beef fat and pork fat, codfish, herring and sprats.

Four different lots of Swedish grade 1 (lean) cut beef meat were used, with times of slaughter ranging from September, 1961, to February, 1962. Average water and fat contents were 74% and 2·5% respectively. Determinations were also made for lean meat from some of the individual cuts used in this grade, approximately equivalent to rib, thick flank, brisket and top and bottom round cuts.

Two lots of lean pork meat of 74% water content and 3% fat content with times of slaughter in August, 1961, and May, 1962 were examined and one sample each of raw beef fat and pork fat.

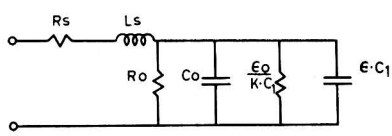


FIG. 3.—*Equivalent circuit for the test cell*

Table I

Estimated maximum errors in the determination of ε and tan δ for codfish

Temperature °C	Frequency MHz	ε	$\left[\frac{\Delta\varepsilon}{\varepsilon}\right]_{max}$	tan δ	$\left[\frac{\Delta \tan \delta}{\tan \delta}\right]_{max}$
−25	10	6·25	0·19	0·35	0·29
	35	5·45	0·20	0·24	0·25
	100	4·90	0·37	0·19	0·42
	200	4·45	0·95	0·17	1·12
−5	10	17·0	0·10	2·43	0·09
	35	13·2	0·12	1·33	0·11
	100	10·5	0·23	0·71	0·23
	200	9·1	0·42	0·56	0·60
+10	10	95·2	0·19	11·4	0·17
	35	75·4	0·25	4·46	0·23
	100	72·8	0·18	1·70	0·27
	200	68·9	0·26	0·88	0·97

Four different lots of codfish were used, caught off the Swedish west coast over a period from December, 1961, to March, 1962. The fish were of size grades 2 and 3 (40-60 cm. length and below 40 cm. length) and of 81% average water content. One lot of herring of 78% water content and 6% fat content and one lot of sprats (small herring) of 72% water content and 12% fat content were also examined. Both lots were caught in February–March, 1962.

All measurements reported were made on minced samples. In preliminary work, dielectric results for minced meat and whole meat were compared. Since those for minced meat were intermediate between (or equal to) the values for whole meat with fibres parallel to and for whole meat with fibres perpendicular to the electrode surfaces, it was decided to use minced material for all further testing.

(d) *Experimental plan*

The type of determinations carried out may be summarised as follows:

(*a*) The temperature-frequency dependence of dielectric constant and loss tangent were determined for lean beef and codfish by measurements at the frequencies 10, 35, 100 and 200 MHz and temperatures from −25° to +10°. Corresponding determinations were carried out to a more limited extent for beef fat, pork fat, lean pork meat and herring.

Table II

Comparison between literature data and present measurements

Reference	Material	Temperature °C	Frequency MHz	Conductivity, ohm^{-1}cm.$^{-1}$ × 10^3	Dielectric constant ε	Loss tangent, tan δ
Sharma[10]	Olive oil	25	32	—	3·06	0·0361
Present work	,, ,,	25	32	—	3·12	0·045
Von Hippel[4]	Ethylene glycol	25	1	—	41	0·03
Present work	,, ,,	25	1	—	37·9	0·029
Von Hippel[4]	Water (conductivity)	25	10	—	78·2	—
Present work	Water (distilled)	25	40	—	76·0	—
Ede & Haddow[3]	Lean beef	−10	20	0·09	—	—
	,, ,,	0	20	3	—	—
Present work	,, ,,	−10	35	0·092	—	—
,, ,,	,, ,,	0	35	3·0	—	—
Osswald[5]	Human muscle	20	100	5–7	71	1·5 (calculated)
Present work	Lean beef	10	100	4·7	70	1·3
Schwan & Li[6]	Human muscle	27	200	8·5	56	—
Present work	Lean beef	10	200	5·6	67	—
Ede & Haddow[3]	Beef fat	0	20	—	7·0	0·5
Present work	,, ,,	0	35	—	6·7	0·8

(*b*) Variability of the raw material was studied by comparing five different cuts of lean meat from one and the same animal at two temperatures and two frequencies, by comparing samples of equivalent cuts from three different animals, by comparing flesh from two different body areas of codfish from the same catch, and by comparing codfish of two different size grades and from two different fishing grounds.

(*c*) The effect of frozen storage on dielectric properties of meat and fish was determined over a 3-months period.

Results

(a) *Temperature and frequency dependence of dielectric constant and loss tangent*

Beef and pork

Results of measurements at different temperatures and frequencies on four different lots of grade 1 lean beef meat are shown in Fig. 4 A and B. As is seen from Fig. 4 A, dielectric constant remains low up to the region of incipient thawing, when a sharp increase occurs, after which further increase is again slow. Loss tangent (Fig. 4 B) shows a marked increase in the region of defrosting only at the lower frequencies.

Both loss tangent and dielectric constant show a decrease with increasing frequency, that of the loss tangent being most pronounced. This is shown more clearly in Fig. 5 A–D, where dielectric properties of several different materials are given as function of frequency at two temperatures. Fig. 5 shows very little difference between the measurements made on beef and on lean pork meat.

Results of more limited measurements on raw beef fat and pork fat are shown in Fig. 6 A–D. For these materials loss tangent and dielectric constant are quite considerably lower than for meat. The relatively sharp increase above $-5°$ at the lower frequencies probably reflects the water content of the raw fats.

Codfish and herring

Results from measurements on three different lots of codfish are shown in Fig. 4 C, D. The relationships between dielectric constant, temperature and frequency are very much the same as found for lean beef. The shape of the curves for loss tangent is similar to those for lean meat but show a more rapid increase with temperature at the lower frequencies. The limited number of results obtained for herring and sprats are shown only in Fig. 5 A–D, together with results for codfish and lean meat. There appear to be no striking differences in dielectric properties between these materials at $+2°$ or $-10°$ except for the lower dielectric constant for sprats at $+2°$, probably caused by the lower water content and higher fat content of the sprats.

(b) *Variability in dielectric properties of the raw material*

Lean beef

Variations in dielectric constant and loss tangent were determined for different cuts from the same animal and for equivalent cuts (rib) from different animals.

The results for cuts from the same animal are shown in Table III, which also lists the water content of the meats and the 95% confidence limits for a single mean ($\pm 2\sigma$ limits). An analysis of variance showed no significant difference between cuts, but a very significant difference between the different frequencies used. The averaged results from determinations on equivalent cuts (rib) from three animals are shown in Table IV together with data for moisture content and statistical significance criteria. At $+2°$ an analysis of variance showed a significantly lower ε-value for a meat sample with lower water content. At $-10°$ the difference was nearly significant.

Codfish

Table V shows the results from determinations comparing dielectric properties of flesh from the back and from the belly flap for three codfish from the same batch. No significant differences in the material were found in an analysis of variance. The results in Table VI for determinations comparing two different catches and two different sizes of cod likewise showed no significant

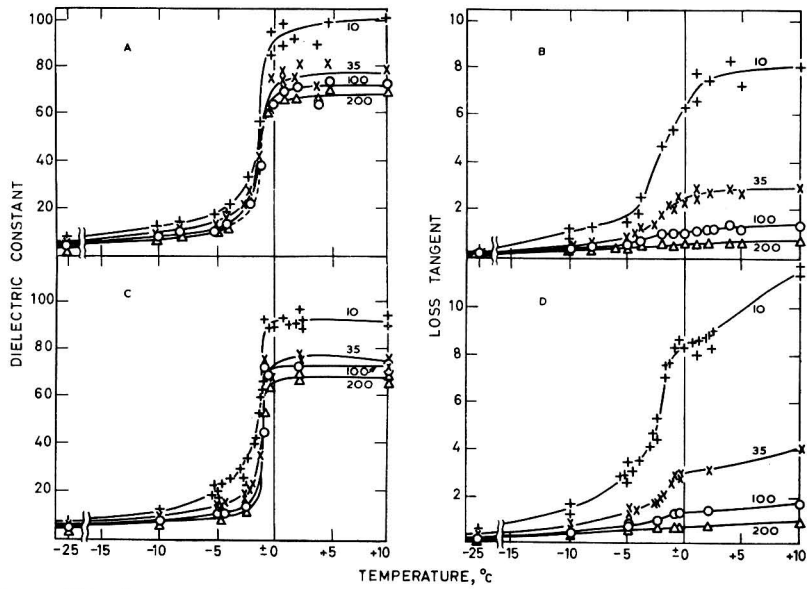


FIG. 4.—*Dielectric constant (ε) and loss tangent (tan δ) as function of temperature and frequency for lean beef and for codfish*

A lean meat, ε C codfish, ε
B lean meat, tan δ D codfish, tan δ
The points represent averages of 2–5 determinations
(numerals on curves are frequencies in MHz)

raw material differences. The moisture content of the fish ranged between 80·8 and 81·8% water.

(c) *Effect of frozen storage time*

Samples of minced meat of fish and beef were prepared and frozen in sealed polythene bags as described above, and stored at −30°. Samples were removed for testing after 1–2 days, after 1–2 weeks and after 1–3 months. The results of dielectric measurements at two temperatures and two frequencies, after different frozen storage times, are shown in Table VII for meat and Table VIII for cod. An analysis of variance showed no significant effect of frozen storage time for fish, but there was a significant difference for beef at −10°, where both dielectric constant and loss tangent decreased with prolonged frozen storage.

Discussion

(1) *Effect of temperature on dielectric properties*

For all raw materials with high water content, a sharp increase in dielectric constant was observed in the region of thawing, more pronounced at the lower frequencies. For loss tangent a similar increase with temperature was obtained but less marked than for ε, especially at the higher frequencies. Also, for raw fats, increases in dielectric constant and loss tangent were observed in this temperature region, but of lower magnitude than for meat or fish, in keeping with the much lower water content of the raw fats.

The observations are in good agreement with data reported by Ede *et al.*[3] For lean beef, lean pork and herring they found a very marked increase in specific conductivity between −10° and 0° at 20 MHz, for lean beef from 90 μmhos/cm. to 3 mmhos/cm., compared with values of 92 μmhos/cm. and 3 mmhos/cm. calculated from the present investigation at 35 MHz.

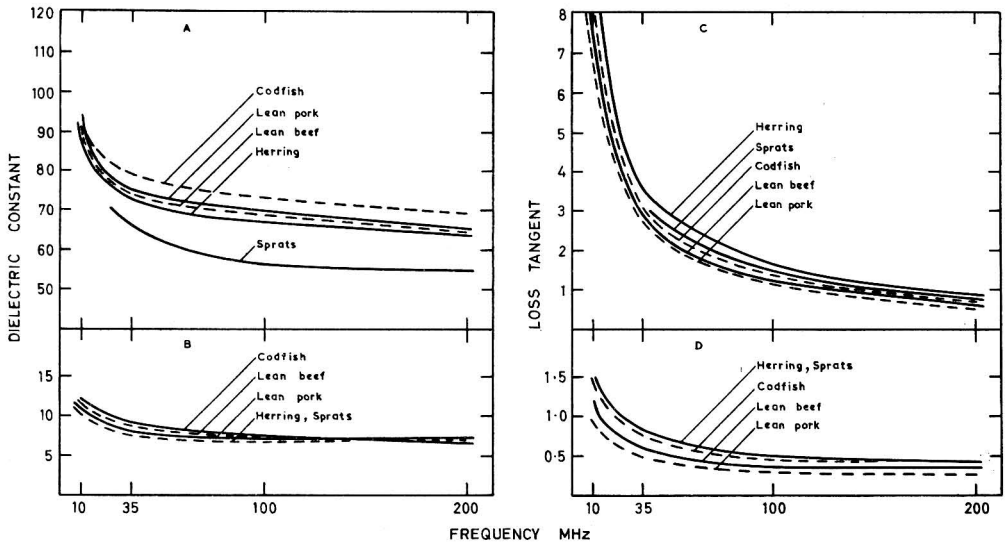


FIG. 5.—*Dielectric constant and loss tangent for lean meat of beef and pork, codfish, herring and sprats as function of frequency at +2° and −10°*

A dielectric constant at 2° C loss tangent at 2°
B " " " −10° D " " " −10°

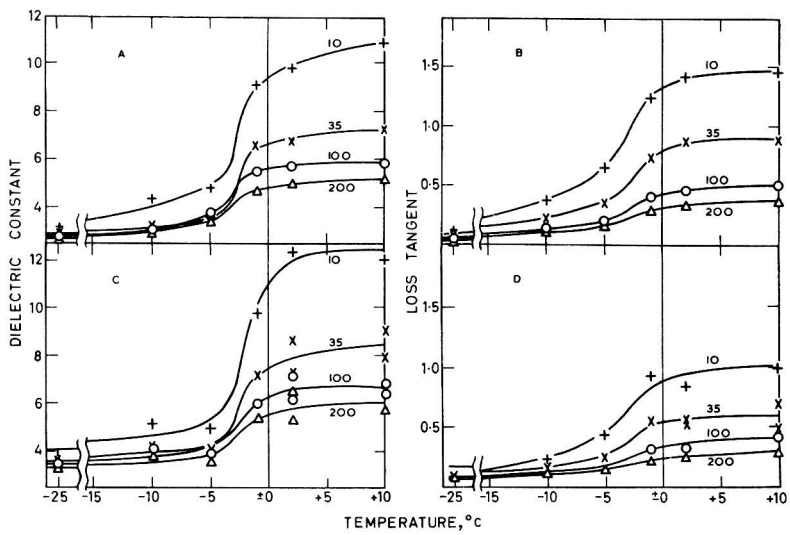


FIG. 6.—*Dielectric constant and loss tangent as function of temperature and frequency for raw beef fat and pork fat*

A, B Beef fat C, D Pork fat

Table III

Variability of dielectric properties between five different cuts of lean beef from the same animal at two temperatures and four frequencies

(results are averages of duplicate determinations)

Cut	Water content, %	Dielectric constant ε								Loss tangent, tan δ							
		−10°				+2°				−10°				+2°			
Frequency MHz		10	35	100	200	10	35	100	200	10	35	100	200	10	35	100	200
Rib	75·2	13·1	9·9	8·4	7·3	90·6	74·3	68·9	64·9	1·17	0·64	0·40	0·34	7·53	2·69	1·16	0·61
Brisket	74·1	13·5	10·2	8·9	7·8	92·6	74·9	69·2	64·9	1·07	0·58	0·39	0·33	7·26	2·65	1·16	0·62
Top round	73·5	13·1	10·2	8·8	7·9	94·0	76·3	71·1	68·5	1·19	0·58	0·39	0·33	7·50	2·71	1·17	0·59
Bottom round	73·9	12·2	9·4	8·3	7·1	93·7	75·1	70·2	65·7	1·04	0·57	0·39	0·33	7·25	2·67	1·15	0·60
Thick flank	73·8	12·8	9·8	8·7	7·4	92·0	74·7	69·8	66·0	1·11	0·59	0·42	0·34	7·56	2·69	1·18	0·62
Analysis of variance		Significant difference between frequencies only															
95% confidence limits of a single mean			±0·5				±2·0				±0·08				±0·08		

Table IV

Variation of dielectric properties of lean beef between equivalent cuts (rib) from three different animals at two temperatures and four frequencies

(averages of triplicate determinations)

Animal	Water content, %	Dielectric constant, ε								Loss tangent, tan δ							
		−10°				+2°				−10°				+2°			
Frequency MHz		10	35	100	200	10	35	100	200	10	35	100	200	10	35	100	200
A	75·2	12·4	9·4	7·9	7·1	91·2	74·2	69·3	65·0	1·26	0·61	0·40	0·33	7·45	2·70	1·15	0·64
B	69·9	11·7	9·0	7·6	6·8	82·8	68·2	62·2	62·0	1·24	0·61	0·39	0·32	7·40	2·65	1·16	0·62
C	73·2	11·9	9·4	7·9	7·1	89·4	73·0	67·7	64·3	1·12	0·61	0·39	0·31	7·50	2·70	1·16	0·64
Analysis of variance		Very significant difference between frequencies															
Sign. difference between samples										—				—			
95% confidence limits of a single mean			±0·4				±1·4				±0·07				±0·07		

Table V

Variability of dielectric properties between flesh from two body areas for three codfish, at two temperatures and three frequencies

(averages of six determinations)

Part of fish	Dielectric constant, ε						Loss tangent, tan δ					
	−10°			+2°			−10°			+2°		
Frequency MHz	35	100	200	35	100	200	35	100	200	35	100	200
Back	9·75	7·90	6·91	73·8	71·5	67·7	0·76	0·48	0·39	3·24	1·31	0·70
Belly flap	9·82	7·96	7·08	74·5	71·6	68·0	0·75	0·49	0·40	3·28	1·37	0·72
Analysis of variance	Significant differences only between frequencies											
95% confidence limits for a single mean		±0·2			±1·7			±0·02			±0·07	±0·02

For raw beef fat at 20 MHz, Ede reported ε-values of 3·0 and 7·0 and tan δ-values of 0·07 and 0·5 at −20° and 0° respectively. The corresponding data from the present investigation at 35 MHz are 2·8 and 6·7 for ε and 0·10 and 0·8 for tan δ. In agreement with the present work, the results of Ede showed only little difference between lean beef, lean pork and herring.

High water content probably is the most important reason for the sharp increase observed in dielectric properties of biological material in the thawing region, judging from literature data for dielectric constants for ice and water. Von Hippel[4] lists dielectric constants of 3·7 for ice at −12° and 87 for water at 0° and 10 MHz. In the present measurements differences in water content of the order of 5% corresponded to differences in the ε-values that were statistically significant.

With the melting of the ice, any salts, etc. present will be expected to increase both the conductivity and the loss tangent of the material.

(2) *Effect of frequency on dielectric properties*

In all measurements frequency was found to have a significant effect on dielectric properties.

Table VI

Variability of dielectric properties of codfish between two different catches and two different sizes, at two temperatures and three frequencies

(averages of triplicate determinations)

Size cod, cm.	Dielectric constant, ε						Loss tangent, tan δ					
	−10°			+2°			−10°			+2°		
Frequency MHz	35	100	200	35	100	200	35	100	200	35	100	200
Catch A												
40–60	10·1	8·40	7·2	77·0	72·5	70·5	0·78	0·48	0·40	2·98	1·25	0·66
<40	10·5	8·6	7·8	76·1	73·8	69·8	0·86	0·49	0·42	3·09	1·28	0·67
Catch B												
40–60	10·6	8·7	7·4	78·6	74·6	70·8	0·84	0·53	0·42	3·03	1·27	0·67
<40	10·3	8·6	7·4	78·3	73·4	69·2	0·81	0·50	0·41	3·02	1·26	0·69
Analysis of variance	Significant differences between frequencies only											
95% confidence limits for a single mean		±0·25			±2·3		±0·04	±0·03		±0·12		±0·03

Table VII

Effect of frozen storage time on dielectric properties of lean beef measured at two temperatures and two frequencies

(averages of triplicate determinations)

Frozen storage time at −30°	Dielectric constant, ε				Loss tangent, tan δ			
	−10°		+2°		−10°		+2°	
Frequency, MHz	35	100	35	100	35	100	35	100
1 day	10·5	8·4	75·0	69·2	0·70	0·42	2·81	1·20
1 week	9·6	8·1	74·3	68·3	0·58	0·37	2·79	1·20
1 month	9·0	7·7	76·6	70·4	0·56	0·38	2·73	1·19
3 months	8·7	7·4	76·4	70·3	0·49	0·34	2·82	1·21
Analysis of variance	Significant effect of storage time		No significance		Significant effect of storage time		No significance	
95% confidence limits for a single mean		±0·35		±1·4		±0·04		±0·07

Table VIII

Effect of frozen storage time on dielectric properties of codfish measured at two temperatures and two frequencies

(average of four determinations)

Frozen storage time at −30°	Dielectric constant, ε				Loss tangent, tan δ			
	−10°		+2°		−10°		+2°	
Frequency, MHz	35	100	35	100	35	100	35	100
2 days	10·3	8·6	76·2	73·0	0·84	0·52	3·14	1·28
2 weeks	10·0	8·4	78·9	72·8	0·80	0·51	3·10	1·30
1 month	10·6	8·4	75·8	73·0	0·76	0·51	3·23	1·32
Analysis of variance	No significant effect of storage time							
95% confidence limits for a single mean		±0·25		±2·0		±0·025		±0·12

Both ε and tan δ decreased with increasing frequency, the effect being most evident in tan δ, as seen from Figs. 4–6. This is in fair agreement with data reported by Osswald,[5] who, for human muscle at +20°, reported ε-values of 96, 85 and 71 at 25, 50 and 100 MHz, respectively. From Osswald's data for ε and for conductivity tan δ-values of 4·4, 2·5 and 1·5 can be calculated for the same frequencies. The corresponding data for 25, 50 and 100 MHz calculated from the present measurements on lean beef at +10° are 83, 73 and 70 for ε and 5·0, 2·0 and 1·3 for tan δ.

The data for ε are somewhat lower in the present investigation. On the other hand, the new values for ε of lean beef at 200 MHz and +10° are higher than those reported by Schwan & Li[6] for human muscle at +27°—viz. 67 compared with 56. The differences may in any case be judged as relatively small considering the differences in materials and methods used.

(3) *Variability of raw material*

It appears from the results reported that, for one and the same quality of meat or codfish, variability between and within batches is low, probably because of their fairly constant water content. Batches of codfish represented a time span of 4 months (December–March) between catches and batches of meat a period between times of slaughter of 6–9 months (August–May). It is possible that summer catches of cod, had such been included, might have resulted in more variability. According to Dahl[11] the total range of variation in moisture content in grade 1 cut beef meat is about 71–78% and for grade 2 beef about 71 76%.

In comparison the difference found between the samples of herring and sprats was quite noticeable as seen in Fig. 5 A, as was the difference in fat and moisture content. That variability in fat content of herring can be quite appreciable is also seen from Brandes & Dietrich[12] who report variations from 2% up to 28% in fat content.

(4) *Effect of frozen storage*

With fish no difference in dielectric properties was noticed after 1 month's frozen storage. With lean beef a relatively small but significant decrease in the dielectric properties measured at $-10°$ occurred, for which no ready explanation can be offered.

(5) *Significance of data for the dielectric defrosting of meat and fish*

The relatively small variations with raw material found in the dielectric properties of meat and fish in this investigation are important from the viewpoint of process control in continuous dielectric defrosting, since indications are that only small changes in equipment settings would be required during processing, provided regularly shaped, homogeneous blocks are used.

On the other hand, the great difference in dielectric properties between lean meat and fat and the marked effect of temperature on these same properties observed round the region of thawing would suggest that selective heating (runaway heating) might readily occur in dielectric heating of fat–lean mixtures and in material where temperature differences have for some reason developed.[3] This is seen from the formula for heating effect developed in a material placed between plane parallel electrodes, over which a high-frequency voltage is applied (see, e.g., [13]):

$$P = \varepsilon.\tan\delta.E^2/d^2.5{\cdot}56.f.10^{-7} \quad . \quad . \quad . \quad . \quad . \quad (5)$$

where P is the power dissipation in W/cm.^{3}
- ε dielectric constant of the material
- δ dielectric loss angle
- d material thickness in cm.
- E voltage over the electrodes in V
- f frequency in MHz

The measurements reported above indicate that the tendency to selective heating might be reduced by increasing the frequency, judging from the less pronounced rise in ε and tan δ with temperature found at the higher frequencies. Another possible advantage of high frequencies is seen from Fig. 7, showing log ($\varepsilon.\tan\delta.f$) as a function of temperature for beef and beef fat at 35 MHz and 200 MHz. For temperatures up to about $-2°$ it seems that a lower field strength could be used at the higher frequency for a given heating effect, with reduced tendencies towards arcing and electrical discharge.

The much lower values of ε and tan δ for fat compared with lean meat suggest a tendency to selective heating of the lean portion of the meat, as judged by equation (5). This is, however, contradicted by practical dielectric defrosting experiments with unhomogeneous meat grades in this laboratory,[2] in which a tendency towards selective heating of the fat was observed in surface layers. Schwan & Piersol[14] also claimed that fatty tissue in tissue with high water content is selectively heated in dielectric heating between parallel electrodes, the difference decreasing with increasing frequency. This may partly be explained by the lower heat capacity of the fatty tissues. A study of the voltage distribution between airgap and layers of fat and lean meat offers another explanation.

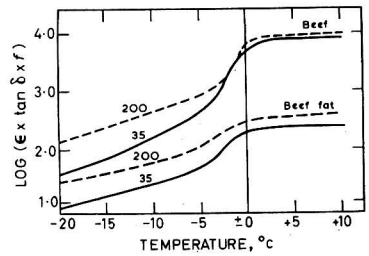


FIG. 7.—*Log* (ε.*tan* δ.f) *as function of temperature and frequency for beef and beef fat*
(f = frequency in MHz)

For layers of different materials between flat, parallel electrodes power density in each layer can be calculated from equation (5), provided E is substituted by the voltage over the layer in question. For three parallel layers (air, fat and meat), the following expression for the voltage over a layer can be derived, regarding each layer as equivalent to a capacitance and a resistance in parallel.

$$E_1 = E \cdot \frac{\varepsilon_1 . d_2 (1 - j . \tan \delta_1)}{\varepsilon_2 . d_1 (1 - j . \tan \delta_2) + \varepsilon_1 . d_3 (1 - j . \tan \delta_1)} \qquad (6)$$
$$\varepsilon_3 . d_1 (1 - j . \tan \delta_3)$$

where E is total voltage over the electrodes
E_1 the voltage over layer 1
ε_1–ε_3 dielectric constant for layers 1–3
tan δ_1–δ_3 loss tangent for layers 1–3
d_1–d_3 thickness of layers 1–3

In this way it can be shown that the field strength in a fatty surface layer can be very much higher than that in the lean meat, at temperatures near 0° and above (because of the lower values for the dielectric properties of fat), which results in much higher power dissipation in the fat layer and increased tendency towards selective heating. In the same manner it can be shown that, should the surface layer of a lean meat block be heated up to about +5°, while lower layers are still at around −2 to −3°, this will not necessarily result in runaway heating. Field strength and power density will be much lower in the surface layer and will tend to counterbalance the appreciable difference in heat capacity of meat between the two temperatures. In continuous dielectric defrosting, a temperature gradient must by necessity develop in the direction of travel. If the electrodes are in direct contact with the blocks to be defrosted, most of the heat developed will be concentrated near the exit end because of the much higher values for the dielectric properties of defrosted meat. The effect of using an airgap will be to reduce voltage and power density over the warmer sections of the meat block, in the same way as discussed above for parallel layers, and thus to reduce tendencies to runaway heating in the defrosted meat.

The above deductions, based on the measured dielectric properties for meat and fish, possibly explain why runaway heating has not been a very serious problem in practical experiments with dielectric defrosting of regularly shaped blocks of homogeneous material.[2,3]

Conclusions

A technique has been developed for determining dielectric constant and loss tangent for meat and fish, that showed good reproducibility and gave values in fair agreement with published data. With this technique for measurements on meat and fish at 10, 35, 100 and 200 MHz, it was found that:

(*a*) Values of dielectric constant and loss tangent show a sharp increase on defrosting, especially for materials of high water content, an exception being loss tangent at the higher frequencies.

(*b*) Values of dielectric constant and loss tangent decrease with increasing frequency.

(*c*) Variation in dielectric properties caused by variability in raw material and by frozen

storage is relatively small for meat and codfish. Dielectric properties are quite similar for lean meat, lean fish and herring, but of much lower value for fats.

Application of the results obtained for ε and tan δ for studying the process of continuous dielectric defrosting of regularly shaped blocks of material between parallel electrodes, with an airgap, appear to explain observations made in earlier practical defrosting experiments, such as a tendency to selective heating of fat layers but not in homogeneous meat and fish material. Some advantage was indicated from increasing frequencies for dielectric defrosting within the range studied.

Addendum

After submission of the above paper a translation has become available of a report by Vasil'ev, A. S., & Vologdin, V. V., on ' Defrosting of meat in a high frequency field ' published in ' Novie Fizicheskie Metody Obrabotki Pishchevzkh Produktov, Obrabotka Pishchevikh Produktov Elektricheskim Tokom ', 1958 (Moskva : Gosinti). The authors present results on the dielectric properties of meat, fat and bone in the temperature range $-17°$ to $-2°$ and frequency range 1–40 MHz. The values were determined on a Q-meter with special attachment, consisting of two disk-shaped electrodes with variable distance, probably connected in series with a low loss condenser. Judging from the brief description given, it seems that voltage over the sample, and over an inductance-free resistor in series with the sample, was measured for amplitude and phase difference with a double-beam oscillograph.

Results show good agreement with those in the present report at $-17°$. At $-9°$ and especially at $-2°$ the results of Vasil'ev are higher for ε and considerably lower for tan δ. Conductivity values calculated from his figures show very little rise with temperature in contrast to the data by Ede & Haddow[3] and in the present report, where a very marked increase in conductivity was observed between $-10°$ and $-2°$. Apart from differences in measuring technique one explanation for the discrepancies observed may lie in a possible difference of definition of the reported tan δ values.

Acknowledgments

The work was supported by a grant from the Swedish Deepfreezing Committee. The authors are indebted to Prof. H. P. Schwan of the University of Philadelphia for the loan of his manuscript on the ' Determination of Biological Impedance ', prior to its publication ; and to Dr. A. C. Jason of the Torry Research Station, Aberdeen, for helpful discussions on dielectric measurements on fish.

The work was carried out at the Swedish Institute for Food Preservation Research (SIK), as part of a research project on dielectric defrosting of foods.

Swedish Institute for Food Preservation Research (SIK)
Kallebäck
Göteborg S.
Sweden

Received 18 January, 1963

References

[1] Jason, A. C., & Sanders, H. R., *Food Tech.*, 1962, **16,** 101

[2] Bengtsson, N. E., *Proc. 1st Int. Congr. Fd Sci. Tech.* (London), September, 1962

[3] Ede, A. J., & Haddow, R. R., *Food Manuf.*, 1951, **26,** 156

[4] von Hippel, A. R., (ed.) ' Dielectric Materials and Applications ', 1954, p. 63 (New York : John Wiley & Sons Inc.)

[5] Osswald, K., *Hochfrequenztech. u. Elektroakust.*, 1937, **49,** 40

[6] Schwan, H. P., & Li, K., *Proc. Inst. Radio Engrs, N.Y.*, 1953, **41,** 1735

[7] Morse, P. W., & Revercomb, H. E., *Electronics*, 1947, **20,** 85

[8] Harper, J. C., Chichester, C. O., & Roberts, T. E., *Agric. Engng*, 1962, **43,** 78

[9] Schwan, H. P., ' Physical Techniques in Biological Research ', in press (New York : Academic Press)

[10] Sharma, M. N., *J. sci. industr. Res.*, 1960, **19,** 5

[11] Dahl, O., *Medd. Statens Inst. för Folkhälsan*, 1948, No. 7, 29 p. (Stockholm : Esselte A.-B.)

[12] Brandes, C. H., & Dietrich, R., *Fette Seifen Anstrichm.*, 1953, **55,** 533

[13] Maddock, A. J., *J. sci. Instrum.*, 1946, **23,** 165

[14] Schwan, H. P., & Piersol., G. M., *Amer. J. phys. Med.*, 1954, **33,** 371

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

AUGUST, 1963

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

INDEX OF AUTHORS' NAMES

INDEX OF AUTHORS' NAMES

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

AUGUST, 1963

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Punched-card system for soil profiles. J. W. Muir and H. G. M. Hardie (*J. Soil Sci.*, 1962, **13**, 249—253).—A punched-card system, designed to make data on a large no. of profiles readily available, is described. A. H. CORNFIELD.

Weathering of Scottish basic igneous rocks with reference to soil formation. W. W. Smith (*J. Soil Sci.*, 1962, **13**, 202—215).—The relative stabilities of the primary minerals in the fine sand fraction (0·2—0·02 mm.) of basic igneous rocks of Lower Carboniferous age are recorded. A. H. CORNFIELD.

Origin of the indurated B_3 horizon of podsolic soils in north-east Scotland. J. C. C. Romans (*J. Soil Sci.*, 1962, **13**, 141—147).—The possible origins of the indurated layer are discussed in the light of experimental data. A. H. CORNFIELD.

Terrace chronology and soil formation on the south coast of New South Wales, Australia. P. H. Walker (*J. Soil Sci.*, 1962, **13**, 178—186).—Characteristics of the soils are presented. A. H. CORNFIELD.

Soil layers on hill slopes: study at Nowra, N.S.W., Australia. P. H. Walker (*J. Soil Sci.*, 1962, **13**, 167—177).—Three separate soil systems occur on hill slopes in the area. Their formation is discussed on the basis of a recurrent cycle of stable and unstable phases of landscape evolution (K-cycle). A. H. CORNFIELD.

Hardpan in soils of semi-arid Western Australia. W. H. Litchfield and J. A. Mabbutt (*J. Soil Sci.*, 1962, **13**, 148—159).—The occurrence of hardpan in the area is described. The hardpan results from SiO_2 and clay deposition, probably due to leaching following episodic sheet flooding. Its formation has kept pace with deposition of alluvium and continues at the present time. A. H. CORNFIELD.

Characteristic and genesis of a ferritic brown earth. R. R. Storrier and A. Muir (*J. Soil Sci.*, 1962, **13**, 259—270).—Characteristics of a brown earth with a high content of apparently flocculated ferric hydroxides are described and the possible genesis of the soil is discussed. A. H. CORNFIELD.

Genetic transitional class of soils and certain types of dark coloured soils they comprise. T. L. Bystritskaya and A. N. Tyuryukanov (*Dokl. Akad. Nauk SSSR*, 1962, **147**, 935—937).—A description of the contours and soils occupying the intermediate position between the automorphous and hydromorphous classes occurring in the river valleys of the southern temperate zone of the USSR, their possible mode of formation, and the conditions fostering their development is given. These transitional soils constitute a definite genetic class for which is proposed the term ' slitozems ', or mingled soils. They are characterised by having a dark coloured humic horizon up to 1 m. in width, neutral to mildly alkaline reaction, a humus content varying between 2—5% but generally close to 4%. The soil forming rocks for this class of soils are characterised by mechanical composition and CO_3^{2-} content. (18 references.) A. S. LEVESLEY.

Clay minerals in Schleswig-Holstein soils. D. Schroeder and H. Dümmler (*Z. PflErnähr. Düng.*, 1963, **101**, 129—140).—Illitic clay minerals predominate in the $<2\mu$ clay fractions and, with the exception of podsols, all samples contain small amounts of kaolinite and montmorillonite. The clay mineral status of materials of glacial and marine origin is not basically different. Differences in clay mineral status arise mainly during soil development, increasing amounts of illite being first transformed into a swelling form and then into an illite which has alternating and blocked interlayers reflecting at 14 Å. M. LONG.

Clay minerals in Indian soils. M. Sarkar and B. Chatterjee (*J. Indian chem. Soc.*, 1962, **39**, 737—738).—Two laterite, two red, one black and three alluvial Indian soils have been studied to classify the individual clay minerals present, as kaolinite, illite or montmorillonite. Methods used were *X*-ray data, differential thermal analytical, chemical analysis, potentiometric titration, variation of viscosity of H^+-clays on progressive addition of bases. Classification is not invariably into the same group by the different criteria, but there is a predominance of one group in the different criteria. Conclusions based on the use of one single property may lead to erroneous results and a combination of several methods is necessary. J. I. M. JONES.

Clay mineral studies of Egyptian desert and Nile alluvial soils. M. M. Elgabaly and M. Khadr (*J. Soil Sci.*, 1962, **13**, 333—342).—Chemical, physical and mineralogical characteristics of the soils and clays are recorded. A. H. CORNFIELD.

Preparation of radioactive montmorillonite and its use in studies in soil chemistry and morphology. H. W. Scharpenseel, H. Gewehr and H. Beckmann (*Z. PflErnähr. Düng.*, 1963, **101**, 122—129).—$^{55/59}$Fe and ^{46}Sc are the best nucleides for the labelling of clays. The sp. activity of the clays obtained by the process, described, amounts to 73 c/g. and about 25 mg. of the Fe supplied is absorbed by 1·5 g. of dried material, i.e., about 40%. M. LONG.

Effects of rainfall, radiant drying and soil factors on infiltration under rainfall into soils. C. W. Rose (*J. Soil Sci.*, 1962, **13**, 286—298).—Infiltration under laboratory rainfall conditions was greater with better structural conditions in both undisturbed and disturbed soil samples. The infiltration rate of a soil surface damaged by intense rainfall decreased as rainfall continued. For a clay soil previously exposed to rainfall, the infiltration rate after radiant drying for 24 h. and rewetting was only half that of soil which was dried in the shade. Infiltration into Ca-soil was much greater than that into Na-soil. Ca-kaolin crumbs were stable on immersion in water but were broken down under the action of rainfall. Ca-illite and Ca-montmorillonite crumbs were stable even under rainfall. A. H. CORNFIELD.

Impedance of water movement in soil and plant. W. R. Gardner and C. F. Ehlig (*Science*, 1962, **138**, 522—523).—Pepper plants were grown in a sandy loam in the greenhouse and simultaneous measurements of soil suction, conductivity of saturated soil, diffusion pressure deficit of detached leaves and transpiration rates were made daily. For values of conductivity $>5 \times 10^{-3}$ cm. per day the impedance was relatively independent of the conductivity. As the conductivity decreased below 10^{-3} cm. per day the impedance increased. T. G. MORRIS.

Hot-water bottle lysimeter. R. M. Holmes (*Canad. J. Soil Sci.*, 1963, **43**, 186—188).—To measure the rate of evaporation of water from a soil column a cylinder filled with soil is clamped in a vertical position with its base resting on a partly filled plastic hot-water bottle, to the inlet of which is attached a graduated glass tube serving as a pressure gauge. As water evaporated from the soil the equivalent depth of free water lost is registered directly on the gauge. A. G. POLLARD.

Diffusion of water vapour through soil. P. T. John (*J. sci. industr. Res.*, 1962, **21B**, 539—541).—V.p. in the soil above the capillary head and breaks of adsorption in different types of soil were studied. V.p. and R.H. and vol. diffusion, surface diffusion and surface diffusion coeff. at different temp. and at different heights above the water table are shown. Moisture—and v.p.—gradients are produced by soil particles adsorbing water-vapour mol. in equilibrium with the vapour in the pore space surrounding them. As these gradients cause the transfer of moisture and vapour towards the surface of the soil column, it is assumed that evaporation takes place at all levels above the capillary head. (13 references.) C. A. P.

Effect of moisture content and soil moisture on self-diffusion of ^{86}Rb in soils. I. J. Graham-Bryce (*J. agric. Sci.*, 1963, **60**, 239—244).—The self-diffusion coeff. (**I**) rises rapidly with moisture content from 5 to 10%. This effect is not due solely to the increased vol. of water-filled pore space. No correlation of **I** is found with other soil properties. In most Ca-dominated soils a value of $1·0 \times 10^{-7}$ cm.^{2}/sec. is a fair estimate of **I**. M. LONG.

Influence of different depths of ploughing on the physical properties of a clay soil at Guyenne, Quebec. J. R. Lessard, S. J. Bourget, H. A. Hamilton and M. Levesque (*Canad. J. Soil Sci.*, 1963, **43**, 178—185).—The soil was ploughed to 6, 12 or 24 in. and subjected to a 4-course crop rotation. With 24-in. ploughing the bulk density was greater and the non-capillary porosite smaller with shallower ploughing, and the 18—24 in. layer of soil was less packed than when undisturbed by the 8- or 12-in. ploughing. The clay content of the 0—6 in. layer was increased by deep ploughing. A. G. POLLARD.

Fibre-glass electrical resistance moisture meters for long-term measurement *in situ* of soil moisture. A. K. Deb and A. Singh (*J. Indian Soc. Soil Sci.*, 1963, **11**, 65—69).—Use of fibre-glass resistance cells (cf. Colman and Hendrix, *Soil Sci.*, 1949, **67**, 425) for this purpose is described. The direct method for calibrating the cells is preferred. A. G. POLLARD.

Self-diffusion of rubidium as influenced by soil moisture tension. A. S. Patil, K. M. King and M. H. Miller (*Canad. J. Soil Sci.*, 1963, **43**, 44—51).—Two half cells (2·5 cm. dia. × 1·5 cm.) were filled with soil at predetermined moisture tensions, one half cell being labelled with ^{86}Rb. The two half cells were placed in contact and the rate of movement of the ^{86}Rb was observed. Self-diffusion coeff. recorded at moisture tensions in the range 0·16—15·0 atm. accord with the simple diffusion theory, the effects of physical and chemical interactions with Rb being apparently constant. A. G. POLLARD.

Effect of intensity of cropping on the efficiency of water use W. S. Ferguson (*Canad. J. Soil Sci.*, 1963, **43**, 156—165).—Loss of water from cropped soils by evapotranspiration was mainly dependent on the intensity of cropping. In a rotation of wheat and summer fallow 38·3% of the total rainfall was accounted for in evapotranspiration; with continuous cropping the value rose to 78% and, under lucerne, to 90·8%. The effects of fertiliser treatment on evapotranspiration were relatively small but wheat yields per 1 in. of evapotranspiration were increased. Summer fallowing to conserve soil moisture is unnecessary and may be disadvantageous. A. G. POLLARD.

Recent accumulations of salts in the soils of south-eastern Saskatchewan. A. K. Ballantyne (*Canad. J. Soil Sci.*, 1963, **43**, 52—58).—Movements of salts in sloping saline areas following heavy rainfall are examined. Salt accumulations originate mainly within the profile and are carried to lower levels beneath the surface. A. G. POLLARD.

Salt accumulation in a glacial till soil in the presence of saline groundwater at shallow depths. J. C. van Schaik and R. A. Milne (*Canad J. Soil Sci.*, 1963, **43**, 135—140).—In grassland soils in which a saline ground-water level was maintained at 3 ft. depths salt accumulation was considerable. The Na adsorption ratio $(Na/[\frac{1}{2}(Ca + Mg)]^{\frac{1}{2}}$ increased largely as a result of pptn. of Ca. In fallow soils, whether left bare or covered with plastic sheeting, there was no appreciable accumulation of salts above the water table. Salt accumulation in cropped soil is due to water uptake by the plants. A. G. POLLARD.

Effect of method of irrigation on the distribution of salts in a Chin loam soil. E. H. Hobbs and G. C. Russell (*Canad. J. Soil Sci.*, 1963, **43**, 65—69).—Sprinkler irrigation resulted in higher concn. of sol. salts (Na, K, Ca, Ca + Mg, HCO^-_3, SO_4^{2-}) at all depths to 5 ft. than did surface flooding. Concn. of Ca decreased with increase in depth in sprinkler-irrigated soil but changed little with depth under surface flooding. $[K^+]$ were generally low with no marked variation with depth. The Na absorption ratio, S.A.R. = $Na/[\frac{1}{2}(Ca + Mg)]^{\frac{1}{2}}$ was higher, at all depths and the increase with depth was greater, in sprinkler-irrigated than in surface-flooded soil. Cl^- was present in a few sprinkled soils but not in any surface flooded plots. No Na_2CO_3 was detected in either case. $[SO_4^{2-}]$ was relatively high especially in the second ft. layer; in the surface-irrigated soil it increased somewhat with depth but was below that in the sprinkled area at all depths to 4 ft. A. G. POLLARD.

Relationship between some indices of saline-sodic soils. J. S. Kanwar, J. L. Sehgal and D. R. Bhumbla (*J. Indian Soc. Soil Sci.*, 1963, **11**, 39—44).—Simple regression equations are established between the pH of pastes of these soils and the exchangeable Na and K, pH and % CO_3^{2-}, electrical conductivity and total sol. cations in saturated extracts and between the gypsum requirements and exchangeable Na and K. Data for pH, conductivity and gypsum requirement may be used to assess the salinity and alkalinity of saline soils. A. G. POLLARD.

Saline irrigation of vegetable crops at various growth stages. I. Effect on yields. J. Lunin, M. H. Gallatin and A. R. Batchelder (*Agron. J.*, 1963, **55**, 107—110).—Top growth of spinach and onions was reduced when the irrigation water (dil. synthetic sea-water) had an electrical conductivity of 5 mmhos per cm., of broccoli, tomato and pepper at 10 mmhos and of beet tops at 20 mmhos per cm. Only with tomato and pepper was top growth decreased by salinity to a smaller extent with increasing delay in application of saline water. Beet roots and pepper fruit growth were reduced to a greater extent than was growth of tops, whilst the reverse was true for onions. A. H. CORNFIELD.

Determination of elemental sulphur in soils. S. L. Chopra (*J. Indian Soc. Soil Sci.*, 1963, **11**, 33—38).—Unchanged residues of S used in the reclamation of alkali soils, are determined, after destruction of org. matter by 20-vol. H_2O_2, by extraction with benzene, treatment of the extract with Raney Ni alloy to convert the S into NiS which is then decomposed by 6N-H_2SO_4. The H_2S produced is determined by the colorimetric *p*-aminodimethylaniline–Fe method. A. G. POLLARD.

Gypsum as an ameliorating agent for solonetzic soils in Alberta. D. N. Graveland and J. A. Toogood (*Canad. J. Soil Sci.*, 1963, **43**, 1—6).—The Bn horizons of seven solonetzic profiles were sampled and leached four times with water or with aq. gypsum at the rate of 1 acre-ft. per leaching to provide 0, 2, 4 or 8 tons of gypsum/acre. In all cases gypsum lowered the exchangeable Na and increased that of Ca, lowered the modulus of rupture, raised the air/water permeability ratio and increased the hydraulic conductivity. Two of the soils were not sufficiently improved, even with the 8 ton/acre dressing to be regarded as suitable for cultivation. Effective amelioration of solonetzic soils in which the exchangeable Na content exceeds 20—25% of the total base-exchange capacity cannot be achieved by gypsum treatment. A. G. POLLARD.

Phosphate equilibria in acid soils. S. N. Chakravarti and O. Talibudeen (*J. Soil Sci.*, 1962, **13**, 231—240).—Phosphate equilibria in 54 acid soils are examined and referred to equilibria with variscite and strengite. The equilibrium PO_4^{3-} concn. in British soils is governed by PO_4^{3-} residues less basic than these compounds and is approx. similar to that obtained by treatments of montmorillonite with 10^{-5}M-PO_4^{3-} solutions for 9 months. In Indian soils, both compounds are effective in controlling PO_4^{3-} concn. over the pH range 3·8—5·7. Kaolinite and glauconite treated with 10^{-3}M-PO_4^{3-} for 9 months exhibited characteristics intermediate between those of the two groups of soils. A. H. CORNFIELD.

Phosphate equilibria in an acid soil. A. W. Taylor and E. L. Gurney (*J. Soil Sci.*, 1962, **13**, 187—197).—A study of the Al phosphate ion products in solutions equilibrated with an acid, P-deficient soil showed that the PO_4^{3-} status of the undisturbed soil is compatible with the existence of variscite ($AlPO_4.2H_2O$) in the soil but produced no evidence that this phase has any effect in controlling the composition of dil. solutions equilibrated with the soil for periods of less than 32 days. When the soil is acidified subsequent changes in the composition of the solution reflect the dissolution of $Al(OH)_3$ and decay of the clay minerals. Phosphates added to the acidified soil are precipitated rapidly, and the final PO_4^{3-} concn. is less than would be supported by variscite under these more acid conditions. A. H. CORNFIELD.

Comparison of several ways of measuring soil phosphorus availability. J. Hagin, J. Hillinger and A. Olmert (*J. agric. Sci.*, 1963, **60**, 245—249).—Max. P adsorption capacity and adsorbed P values of soils, calculated from the Langmuir isotherm, are similar to those obtained by leaching soil samples with a phosphate solution, and leaching with water respectively. P uptake by plants is not significantly correlated with P availability indices obtained from extraction methods, using $NaHCO_3$, NH_4F or citric acid. However, good correlations exist with some parameters from equilibration and leaching experiments. M. LONG.

Status of organic phosphorus in some Alberta soils. J. F. Dormaar and G. R. Webster (*Canad. J. soil Sci.*, 1963, **43**, 27—34).—Two profiles from each of six soil zones were examined. In all profiles the total org. P content diminished with increasing depth below the surface. The Kaila–Virtanen method (*Maataloust. Aikak.*, 1955, **27**, 104) and that of Mehta *et al.* (*Proc. Amer. Soil Sci. Soc.*, 1954, **18**, 443) gave generally similar results although the latter tended to remove somewhat the higher amounts of org. P from the surface horizons of the Thin Black, Black, Dark Grey and Grey Wooded sub-Groups in the soil zones. The org. P fraction probably forms an integral part of the soil org. matter. A. G. POLLARD.

Adsorption of inositol phosphates and glycerophosphates by soil clays, clay minerals and hydrated sesquioxides in acid media. G. Anderson and E. Z. Arlidge (*J. Soil Sci.*, 1962, **13**, 216—224).—Boehmite, a soil clay with a high sesquioxide content, ferric oxide gel, and montmorillonite were the most active of a no. of materials tested in adsorbing inositol phosphates and β-glycerophosphate in acid media. In general adsorption of the inositol phosphates increased with the no. of PO_4^{3-} groups in the molecule. Adsorption of inositol hexaphosphate (**I**) was favoured at pH 3—4 and in acid of 0·1N concn. Montmorillonite was active at pH ~4, apparently owing to its content of active Al. Montmorillonite, kaolinite and an illitic soil clay sorbed negligible amounts of β-glycerophosphate in strongly acid media. Ferric oxide gel removed considerable amounts of **I** from strongly acid solution, but was relatively ineffective at pH 3—4. Boehmite was very reactive from 4N-acid to pH 4, greatest adsorption occurring at pH ~1. The sesquioxide-rich soil clay was very active over a wide range of acid concn. Removal of active Fe and Al destroyed its activity at pH 4, but not

at pH 1. The adsorption of phosphate esters in equiv. concn. of HCl and H_2SO_4 was often markedly different. A. H. CORNFIELD.

Comparison of methods of estimating soil organic phosphorus. R. J. Hance and G. Anderson (*J. Soil Sci.*, 1962, **13**, 225—230).—The extraction method of Saunders and Williams (*J. Soil Sci.*, 1955, **6**, 254) tended to be superior to that of Mehta *et al.* (*Proc. Soil Sci. Soc. Amer.*, 1954, **18**, 443) with acid soils and inferior with calcareous soils, although in many cases similar values were obtained. A modification of the latter method (involving extraction of the soil with alkali before as well as after acid treatment) caused less hydrolysis than did the standard method with acid soils and gave the highest average values for the soils as a whole. Varying the nature of the acid pre-treatments did not increase the efficiency of the methods. Ignition values (Saunders & Williams, *v.s.*) were usually greater than extraction values, but in most cases the differences were unimportant. A. H. CORNFIELD.

Determination of total organic phosphorus in soils by extraction methods. J. F. Dormaar and G. R. Webster (*Canad. J. Soil Sci.*, 1963, **43**, 35—43).—The original Kaila–Virtanen method is modified by replacing the 18-h. extraction with 4N-H_2SO_4 by a 4-h. extraction using a mechanical shaker. Of org. solvents used to effect complete removal of org. matter, acetone (**I**), dioxan and ethanol (**II**) did not interfere with the determination of P by the Mo-blue method and improved the extraction of org. P. **I** and **II** were useful for extracting low-mol. unstable humic substances. Use of HF in the extraction of B-horizon soils is recommended. A. G. POLLARD.

Mineralisation of soil organic phosphorus with particular reference to the effect of lime. R. L. Halstead, J. M. Lapensee and K. C. Ivarson (*Canad J. Soil Sci.*, 1963, **43**, 97—106).—The total org. P content of some acid soils declined appreciably on incubation with $Ca(OH)_2$ sufficient to produce pH $\simeq$7. Simultaneously the amounts of inorg. P sol. in N-H_2SO_4 or in 4N-HCl increased somewhat and the total org. P sol. in $NaHCO_3$ (pH 8·5) diminished markedly. The latter effect may be due, in part, to the direct effect of $Ca(OH)_2$ in lowering the solubility of org. P in $NaHCO_3$. Incubation of soil in absence of $Ca(OH)_2$ may lead to slow mineralisation of org. P. The decline in extractable org. P following liming is associated with increases in no. of soil micro-organisms and in the formation of CO_2 and NO_3^-. Possible interrelationships between the observed microbiological changes and the transformations of org. P are discussed. A. G. POLLARD.

Release and fixation of potassium in different size fractions of some Canadian soils as related to their mineralogy. A. J. MacLean and J. E. Brydon (*Canad. J. Soil Sci.*, 1963, **43**, 123—134).—The amounts of non-exchangeable K removed from mechanical fractions of 11 soils by H^+-resin or by boiling N-HNO_3 are recorded. On average clay yielded approx. double the amount from fine silt, four times that from medium silt and eleven times that from coarse silt and sand. Medium and fine silts fixed considerable amounts of K added to the soil. The proportion of the total K removed from K-bearing minerals by these methods was in the order, feldspar (**I**) < illite (**II**) < muscovite < biotite. No relationship was apparent between the amounts of K fixed by or released from soils and their contents of **I**, **II**, vermiculite or mixed-layer minerals in the clay fraction or the proportion of **I** or mica in the non-clay fractions. A. G. POLLARD.

Effect of deep and shallow ploughing on nutrient release, moisture conservation and yield of pearl millet in *Goradu* soil of Anand. N. R. Talati and B. V. Mehta (*J. Indian Soc. Soil Sci.*, 1963, **11**, 8—16).—Ploughing to 14—15 in. resulted in greater retention of moisture, N mineralisation and exchangeable K content than did shallow (4—5 in.) ploughing. These characteristics were significantly correlated with increased grain yields of pearl millet. The available P content of the soils was not significantly affected by depth of ploughing. Increased crop yields were due mainly to the improved N and water status of the deeply ploughed plots. A. G. POLLARD.

Determination of free iron in soils and clays. D. E. Coffin (*Canad. J. Soil Sci.*, 1963, **43**, 7—17).—Free Fe is removed from soil or clay by a single extraction with 5% $Na_2S_2O_4$ in a 0·2M-citrate buffer, pH 4·75 at 50° for 30 min. or for 10 min. after the soil is completely bleached. Fe is determined in the filtered extract. Clay minerals other than nontronite suffer no decomposition by the extractant. A. G. POLLARD.

Copper-deficient soils in south-east Scotland. D. Purvis and J. M. Ragg (*J. Soil Sci.*, 1962, **13**, 241—246).—Copper-deficiency symptoms ('wither-tip') in cereals occurred on soils of low total Cu content (<6 p.p.m.). The condition occurred on soils of the Eckford series (coarse-textured, free-draining soils of fluvioglacial sand origin). Analysis of many soils of the region indicated that a large proportion of them are potentially Cu-deficient. A. H. CORNFIELD.

Available molybdenum status of some Indian soils. R. K. Chatterjee and C. Dakshinamurti (*J. sci. industr. Res.*, 1962, **21B**, 597—598).—Black and laterite soils are poor in both total and available Mo while alluvial soils are rich in both. The high clay content of black soils and the low pH of laterite soils are thought to be responsible for the non-availability of Mo. S. A. BROOKS.

Silica in soil solutions. I. Form and concentration of dissolved silica in aqueous extracts of some soils. II. Adsorption of monosilicic acid by soil and other substances. J. A. McKeague and M. G. Cline (*Canad. J. Soil Sci.*, 1963, **43**, 70—82, 83—96).—I. The rate and extent of the dissolution of soil-Si in water differed with the method and conditions of equilibration. In all extracts Si appeared to be in the monomeric form $Si(OH)_4$. On shaking medium- and coarse-textured soils with water the amounts of extracted Si increased progressively during a month and were much greater than when the soil was allowed to stand in water for a similar period. The concn. of Si (1—>20 p.p.m.) in water extracts of soils decreased with rise in pH and increased with temp. and with the soil/water ratio used. The bearing of these observations on soil-forming process and on chemical changes in soil and in natural waters is considered.

II. Freshly pptd. hydroxides of polyvalent metals readily adsorbed $Si(OH)_4$ from dil. solutions; some soils and Fe oxide minerals were less active in this respect and alkaline earth carbonate minerals showed no adsorption. Adsorption curves accorded with the Freundlich equation with rise in pH (4—9) adsorption increased but with a ferrugenous soil decreased again at pH >10. Possible results of the evaporation of soil solutions containing dissolved Si are considered. A. G. POLLARD.

Penetration of fallout fission products into an Indiana soil. D. Telfar and J. Luetzelschwab (*Science*, 1962, **138**, 829—830).—Soil samples from virgin forest in 1961 and 1962 were collected in 1-in. layers, dried, ground, mixed and the γ-activity measured. After compensating for natural radioactivity the amount of ^{137}Cs below the second inch of soil was about the same for both years. The concn. of Zn/^{95}Nb rose sharply in the fourth inch and then decreased again with depth. Earthworms may have carried active surface soil downwards. T. G. MORRIS.

Effect of γ- and neutron radiation on chemical and physical properties of soil. D. G. Cummins (*Dissert. Abstr.*, 1962, **23**, 1843—1844).—Yields of rye on soils were increased by radiation, as also was the amount of N mineralised in the soil, which contributed to the increased rye yield. The radiation did not appear to influence the cation-exchange capacity, particle size distribution, or moisture desorption properties of either of the two soils tested. F. C. SUTTON.

Degradation of humic substances. II. Oxidation with chlorine dioxide, hydrogen peroxide and periodate. N. C. Mehta, P. Dubach and H. Deuel (*Z. PflErnähr. Düng.*, 1963, **101**, 147—152).—Humic acids, isolated with EDTA at pH 7 from the B_h-horizon of a podsol are examined. Oxidation by ClO_2 under acid and slightly alkaline conditions, or by H_2O_2 and $NaIO_4$ leads to between 34 and 100% degradation to water and ether-sol. products. The ether-sol. fraction from ClO_2 oxidation subjected to paper chromatography contains 0·5% of maleic acid and 2% of oxalic acid (on original humic acid basis); that from H_2O_2 oxidation 1·5% of malonic acid and that from $NaIO_4$ oxidation 0·3% of phthalic acid and 0·5% of lactic acid. M. LONG.

Amino-compounds in acid hydrolysates and aqueous leachates of some soils of Rajasthan. S. Singh and G. S. Bharandi (*J. Indian Soc. Soil Sci.*, 1963, **11**, 1—7).—The distribution of 25 amino-compounds in hydrolysates (boiling 6N-HCl) of typical semi-arid and arid soils is recorded. A. G. POLLARD.

Study of organic soil horizons using electrophoretic techniques. A. F. MacKenzie and J. E. Dawson (*J. Soil Sci.*, 1962, **13**, 160—166).—Electrophoretic patterns indicated a marked difference in the saturated $Na_4P_2O_7$ extracts between muck and different types of peat. The high exchange capacities of the muck samples were associated with the amount of highly charged material rather than with an increase in the charge density of the material. A. H. CORNFIELD.

Interaction of oxide-free clay and humic acid. B. C. Sen (*J. Indian chem. Soc.*, 1962, **39**, 683—686).—Free oxides were removed from an illitic clay by the Al–oxalic acid method of Marshall and Jeffries (*Proc. Soil Sci. Soc. Amer.*, 1945, **10**, 397).—The base-exchange capacities of mixtures of humus with oxide-free clay determined experimentally were less than the theoretical but the divergencies were not quite as great as in mixtures with the original clay. The amount of combined humic acid (non-extractable with Na_2CO_3) in the clay complex, however, was the same for both oxide-free and original clays. The presence of metal ions results in an increase in combined humic acid and it is suggested that the exchangeable H^+

or metal ions present in clay are more effective in forming clay–humus complexes by co-ordination with OH and COOH groups in humic acid, than are the metal oxides. J. I. M. JONES.

Measurement of the polysaccharide content of soils. C. J. Acton, E. A. Paul and D. A. Rennie (*Canad. J. Soil Sci.*, 1963, **43**, 141—150).—Preliminary dispersion of the soil sample in 0·5N-NaOH in a Waring Blendor prior to hydrolysis with 3N-H_2SO_4 materially increased the amount of polysaccharide determined by the colorimetric anthrone method. Incubation of soil with wheat straw and NH_4NO_3 increased the accumulation of microbial gum, the polysaccharides in which may be determined by a similar procedure. The less specific phenol–sulphuric acid method for polysaccharides indicated up to 35% higher values. Of the total polysaccharides in the soils examined approx. 10% occurred in the microbial gum, 15—25% in acid-sol.-, base-sol.-fulvic acids, 55—70% in the alkali-insol. humin and 4—12% in the humic acid fraction. A. G. POLLARD.

Carbon-bonded sulphur in selected Quebec soils. L. E. Lowe and W. A. DeLong (*Canad. J. Soil Sci.*, 1963, **43**, 151—155).—The Quebec soils examined especially the highly org. types contained appreciable amounts of C-bonded S as determined by digesting the sample with NaOH and Raney's Ni catalyst in a stream of N_2, distilling the product with HCl and measuring S in the distillate by the method described by DeLong and Lowe (*ibid.*, 1962, **42**, 223). Data for a no. of soil types are recorded. A. G. POLLARD.

Effect of cropping on some chemical properties of a sphagnum peat soil. L. R. Townsend and D. C. MacKay (*Canad. J. Soil Sci.*, 1963, **43**, 171—177).—Changes occurring in a strongly acid sphagnum peat due to liming, cropping and fertiliser use are examined by a conventional fractionation of the main org. constituents. For this purpose a preliminary treatment with 0·1N-HCl (4 successive extractions) was needed to remove the large accumulation of mineral matter prior to the hydrolysis of the hemicellulose. Cropping decreased the % of cellulose and hemicellulose and the C/N ratio of the peat and increased the bitumen, lignin–humus complex and cation-exchange capacity, the general trend being an approach to the composition of adjacent muck soils. A. G. POLLARD.

Mineralisation of crop residues as affected by the nitrogen content. U. Schmidt and G. Schmidt (*Z. PflErnähr. Düng.*, 1963, **101**, 99—109).—An increase in the rate of decomposition, limited to the initial stages, occurs when N is applied to materials of low N content. Later this increase is compensated for by a decrease in rate which finally changes into a preserving effect, this being the more marked at higher N applications. Where N is added to material of low N content to bring the level up to that of a high N content material, the decomposition rates of the two are substantially the same. With materials of high N content no clear evidence for a preserving action exists. M. LONG.

Importance of micro-organisms for soil productivity. L. Ettlinger (*Schweiz. landw. Forsch.*, 1962, **1**, 231—251).—The effects of inoculating leguminous and non-leguminous plants are extensively reviewed. The inoculation of legumes with root nodule bacteria to promote N fixation is of particular importance. Humus in soil probably has a direct growth-prompting action on plants. Some of the humic acids produced synthetically have similar activity to that of the naturally-produced acids. (341 references.) J. V. RUSSO.

Inhibition of germination of fungus spores in soil. H. C. Weltzier (*Zbl. Bakt.*, 1963, II, **116**, 131—170).—Germination of many fungal and bacterial spores (including many pathogens) was inhibited to varying extents by 20 soils examined. This action was partly or wholly counteracted by heating the soils, by fumigation with chloropicrin, by addition of nutrients including carbohydrates and amino-acids or plant residues. The isolation of a water-sol. inhibitory agent is described; it was strongly adsorbed on filter paper and migrated to the anode on gel-electrophoresis. A. G. POLLARD.

Effect of dicyanodiamide on transformation, loss and plant uptake of fertiliser nitrogen from Georgia soils. G. R. Reddy (*Dissert. Abstr.*, 1962, **23**, 1844—1845).—Sucrose apparently provided a quickly available energy source for soil micro-organisms that utilised the dicyanodiamide as a source of N and prevented its inhibiting effect on nitrification. It did not inhibit ammonification of urea nor did it increase the loss of NH_3 by volatilisation from surface-applied urea. Leaching studies showed that dicyanodiamide did not affect total N loss by leaching. F. C. SUTTON.

Reaction between monocalcium phosphate and calcium carbonate. S. Larsen, D. J. Parton and I. Svensson (*Nature, Lond.*, 1963, **197**, 317).—When air saturated with water vapour was passed down a column containing $Ca(H_2PO_4)_2$ on top of $CaCO_3$ the product after 10 days was 80% $CaHPO_4.2H_2O$ and some calcite. A further experiment indicated that the initial reaction leading to the formation of $CaHPO_4$ terminated within 3—4 days. S. A. BROOKS.

Use of concentrated sulphuric acid in production of granular normal superphosphate. L. D. Hand, jun., J. M. Potts and A. V. Slack (*J. agric. Fd Chem.*, 1963, **11**, 44—47).—To overcome difficulties in normal methods of production of superphosphate granules of correct size in the final removal of water, added to accomplish this and in increasing the removal of F, a modified method is devised in the laboratory using either 96% acid with finely ground rock or fuming acid with standard-grind rock. Local conditions would determine relative economy of these processes. Advantages claimed are elimination of acid dilution equipment and cutters; granulation before curing and therefore less handling and reduced drying costs. F recovery is doubled. J. B. WOOF.

Solubility and fineness of grinding of various borate fertilisers. G. J. Ouellette (*Canad. J. Soil Sci.*, 1963, **43**, 166—170).—Various B sources [borax (**I**), colemanite (**II**), 'safte-bor' (**III**)] ground to pass 35, 65 and 100 mesh/in. were applied to soils to provide 6·6 lb. of B_2O_3/acre. Some damage to oats was caused by **II** but not by **I** or **III**. The damage was proportional to the fineness of grinding but the effect of **II** in correcting B-deficiency was of much longer duration than that of **I** or **III**. Only **III**, ground to 100-mesh, increased yields of lucerne over 3 years. Concn. of B >0·35 p.p.m. in the arable layer of soils lowered oat yields; amounts <0·20 p.p.m. in years of normal rainfall (Quebec) or 0·30 in drier seasons caused deficiency symptoms in lucerne. The examination of surface layers of soil may not afford reliable indications of the B status of the soil. A. G. POLLARD.

Action of sewage sludge on soils and micro-organisms. H. Glathe and A. A. M. Makawi (*Z. PflErnähr. Düng.*, 1963, **101**, 109—121).—The total bacterial count and development of cellulose decomposers and *Azotobacter* are increased by fresh and autoclaved sewage sludges, *Azotobacter* being particularly activated by the latter. There is no difference between the actions of sludges, sterilised by autoclaving and by treatment with ethylene oxide, on soil microflora. Even after 30 days the no. of *Escherichia coli*, increased by addition of fresh sludge, is higher than that at the time of application, the untreated soil initially containing none. Sterilising the sludge by autoclaving or by ethylene oxide treatment destroys all *E. coli*. CO_2 formation is increased by addition of both sterilised and fresh sludges, the latter giving the most, and autoclaved sludge the least amount. Both NH_4^+ and NO_3^- concn. are increased when sludges are applied. M. LONG.

Conditioning of soil. A. Katchalsky, I. Bursztyn and D. Vofsi (B.P. 889,216, 15.10.59).—Arid soil is conditioned by producing *in situ* discrete grains or lumps of soil agglutinated by a nitrogenous binder applied thereto in aq. solution or dispersion. The binder may be a condensation product of CH_2O with urea, melamine, dicyanodiamide or guanidine. F. R. BASFORD.

Fertilisers. Imperial Chemical Industries Ltd. (Inventors: A. J. Lowe, B. N. C. Fenton and J. H. Hudson) (B.P. 890,569, 31.12.58 and 19.1., 8. and 31.7.59).—Urea is admixed with ≤0·2 mol. of an acidic substance containing ≤1 H replaceable by NH_4, (e.g., $NH_4H_2PO_4$, KH_2PO_4, $CaHPO_4$, NH_4HSO_4, $KHSO_4$) to provide a granular fertiliser, especially suitable for use as fertiliser in the combine drilling of cereals in sandy soil. The composition, which preferably has a N : P_2O_5 : K_2O ratio of 20 : 10 : 10 can be applied at the rate of >60 lb. of urea per acre without undue ammonolysis occurring. F. R. BASFORD.

Plant Physiology, Nutrition and Biochemistry

Temperature as an environmental factor in plant culture. F. Schwendimann (*Schweiz. landw. Forsch.*, 1962, **1**, 252—270).—The paper reviews the following topics: heat absorption in the soil from incoming radiation and the effect of type of soil; factors affecting plant temp.; the influence of temp. on metabolic processes; the relationship between temp. and phasic development and the effect of extremes of temp. on plants. (24 references.) J. V. RUSSO.

Formation of carbon monoxide during seed germination and seedling growth. S. M. Siegel, G. Renwick and L. A. Rosen (*Science*, 1962, **137**, 683—684).—Cucumber seedlings were grown from seed in rhyolitic mineral perlite at 25° in sealed jars in an atm. of 5% O_2 + 95% A. After 4 days incubation the atm. was adjusted to contain 5% O_2 + 0·002% CO_2 and the jars placed in darkness for 7 days. At the end of this time the O_2 content was unchanged but the CO_2 had risen to 3·5%. The atm. contained approx. 6000 p.p.m. of CO. Seedlings were achlorophyllous and had gained 12 g. fresh wt. and had elongated 30 mm. No aldehydes were detectable in the atm. or the substratum. CO was also formed from *Euphorbia clandestina* in sealed containers subjected to a diurnal variation of +20° to −20°. Similarly, seeds of a variety of plants in sealed containers having an A/O_2 atm. formed CO. T. G. MORRIS.

Anion-exchange properties of plant root surfaces. D. E. Williams (*Science*, 1962, **138**, 153—154).—Eosin dye, previously adsorbed on to root surfaces, was replaced by different Na salts. Different quantities of dye were replaced by different anions, the order being $F^- < Cl^- < Br^- < I^- < NO_3^- < SO_4^{2-} < HPO_4^{2-}$, CO_3^{2-}, HCO_3^- and OH^-. The amount of dye released increased with concn. in each salt. With alkaline salts, e.g., Na_2CO_3 or $NaHCO_3$ in 0·0001N solution, slightly more eosin was replaced than with neutral salts. With 0·001—0·1N solutions a plateau was reached and further increases in concn. did not release more eosin. A possible exchange site may be the NH_2-group of proteins. Acid solutions of NaCl maintained eosin, adsorbed in oat roots, in the bound condition. T. G. MORRIS.

Mineral nutritional disorders of plants in Uttar Pradesh. I. Iron deficiency in Lucknow and neighbourhood. S. C. Agarwala and N. K. Mehrotra (*J. Indian Soc. Soil Sci.*, 1963, **11**, 51—63).—The occurrence of chlorosis in 73 species of plants in this area is examined. In some species chlorotic leaves contained as much or more Fe than did those of normal appearance. No relationship was apparent between chlorosis and the amount of Fe extracted from the soils by N-NH_4OAc at pH 3. A. G. POLLARD.

Leaf analysis in the assessment of nutritional status of apple trees. I. Variation in leaf nitrogen, phosphorus, potassium and magnesium with fertiliser treatment, within seasons and between seasons. E. G. Bollard, P. M. Ashwin and H. J. W. McGrath. **II. Variation in leaf nitrogen, phosphorus, potassium, magnesium and calcium content between commercial orchards.** E. G. Bollard, F. Hurst and H. J. W. McGrath (*N.Z. J. agric. Res.*, 1962, **5**, 373—383, 383—388).—I. Differences in leaf nutrient levels were as would be expected of the different treatments except that when N was given in combination with other elements this did not result in a higher N % in the foliage. Variation of nutrient levels in foliage during any season means that for comparative results sampling must be restricted to a given period within the season. Variation in nutrient levels in leaves between seasons made comparison of treatments from season to season difficult as this may be due to such variable factors as rainfall and crop size. In general the results obtained agreed well with critical nutrient levels reported previously. (16 references.)

II. Apple leaf samples from 67 commercial orchards from one fertile and one not so fertile districts were analysed for nutrient levels over two seasons. Little difference was found between the two districts. K, Mg and Ca generally fell between the suggested critical limits but N was often above and P was often below these limits, in the case of the low P contents this may be due to an excess application of N. W. ELSTOW.

Uptake of strontium-85 by lucerne. C. C. Lee (*Science*, 1962, **138**, 41—42).—Lucerne plants were grown in pots in a loam of pH 7·2. To each pot of soil (400 g.) was added 0·1 mc of carrier-free ^{85}Sr. The crop was harvested at 60 days and thereafter at 4-week intervals. The uptake of ^{85}Sr in successive crops was not significantly different, but the uptake in the fifth crop was significantly less than that of the second, indicating a slight amount of fixation. $NH_4H_2PO_4$ caused no reduction in ^{85}Sr uptake. Of other nutrients only K at the rate of 1 mequiv. per 100 g. of soil gave a significant reduction. T. G. MORRIS.

Post-vernalisation seed treatment with vitamins on *Vigna catjang*. P. K. Mohanty and D. Mishra (*Science*, 1962, **138**, 902—903).—Seeds of *Vigna catjang* var. *pushaphalguni* were soaked in distilled water for 24 h. and then subjected to a temp. of 3—5° for 1 week. The seeds were then treated with riboflavin (**I**) and ascorbic acid (**II**), alone and in combination, at concn. ranging from 10^{-3}M to 10^{-5}M for 48 h., and then washed and sown. In the vernalised seedlings the elongation of the root and shoot was significantly higher in plants from vitamin-treated seed than in the controls when **I** and **II** were applied singly or in combination at lower concn., but **I** alone at 10^{-3}M was inhibitory. In unvernalised seeds low concn. of the two vitamins alone or in combination stimulated growth but the effect was less pronounced than in vernalised seeds. T. G. MORRIS.

Biosynthesis of coumarin and herniarin in lavender. S. A. Brown (*Science*, 1962, **137**, 977—978).—Solutions of radioactive compounds were administered through the roots of *Lavandula officinalis* plants which had been grown hydroponically under artificial illumination. After further growth of 6—7 days under the same conditions the plants were harvested and immediately homogenised in hot EtOH, and the evaporated residue used for testing. When L-phenylalanine was administered, free coumarin and herniarin were isolated from the plant. When *o*-coumaric acid or its glucoside were applied they were used in the plant with high specificity for the synthesis of coumarinyl glucoside ; when *p*-coumaric acid was given it was used with similar specificity for the synthesis of the glucoside which yields herniarin on hydrolysis with emulsin. Glucose was used by the plant with lower specificity for both syntheses. T. G. MORRIS.

Fatty acids in the pollen of some coniferous species. Te May Ching and Kim K. Ching (*Science*, 1962, **138**, 890—891).—The fatty acid content of viable pollen from Douglas fir, Formosan Douglas fir, big-cone Douglas fir, Ponderosa pine and Lodgepole pine was estimated by gas chromatography. Oleic, palmitic and linoleic acids are the major components of the Douglas fir pollens, and linoleic, oleic, palmitic and stearic acids of the pines. T. G. MORRIS.

Sources of error in the wheat cylinder bioassay for auxin. K. E. Cockshull and O. V. S. Heath (*Nature, Lond.*, 1963, **197**, 362—364).—Statistical analysis of data from experiments with different no. of wheat coleoptile sections and known concn. of indol-3-ylacetic acid (**I**) in corked assay tubes has shown that the concn. of **I** producing the greatest growth rate decreased with increase in the no. of sections. Probably the least error variance is obtained with single sections in replicate uncorked tubes. S. A. BROOKS.

Changes in permeability induced by victorin. H. Wheeler and H. S. Black (*Science*, 1962, **137**, 983).—Cut stems of resistant and susceptible oat plants were placed in diluted solutions of crude victorin, the toxin produced by the fungus *Helminthosporum victoriae*, for 2 h., after which the tissue was washed and shaken in distilled water. Permeability was then measured. With susceptible plants the victorin-treated tissues lost electrolytes to the ambient solution more rapidly than did controls, the effect increasing with increasing victorin treatment. With resistant plants no effect was noticed. T. G. MORRIS.

Increased tolerance of bean plants to soil drought by means of growth-retarding substances. A. H. Halevy and B. Kessler (*Nature, Lond.*, 1963, **197**, 310—311).—Bean plants treated with (2-chloroethyl)trimethylammonium chloride or 2,4-dichlorobenzyltributylphosphonium chloride remained turgid for at least 30 days after the last watering whereas control plants were completely desiccated after this time. S. A. BROOKS.

Concentration of a floral-inducing entity from plant extracts. D. L. Mayfield, R. G. Lincoln, R. O. Hutchins and A. Cunningham (*J. agric. Fd Chem.*, 1963, **11**, 35—38).—A prep. from *Xanthium strumarium* initiated flowering in other *Xanthium* plants normally prevented from flowering. Extraction and purification procedures are described. Methanol extract from freeze-dried leaf is chromatographed on paper using butanol/acetic acid/water and the active component located in the R_F 0·42—0·65 region. A technique for concentrating crude extract by acid-base solubility differences is described. (11 references.) J. B. WOOF.

Improvement of the Titan Yellow method for the determination of magnesium in plant tissue. K. Riebartsch (*Z. PflEnähr. Düng.*, 1963, **101**, 141—146).—The Titan Yellow method is improved by varying the type of polyvinyl alcohol and the concn. of polyvinyl alcohol and Titan Yellow. M. LONG.

Crops and Cropping

Effect of fertiliser on growth, nutrient uptake and moisture use of wheat on two soils in south-western Saskatchewan. F. G. Warder, J. J. Lehane, W. C. Hinman and W. J. Staple (*Canad. J. Soil Sci.* 1963, **43**, 107—116).—On a loam and on a clay soil of low $NaHCO_3$-sol. P content, wheat responded to N + P fertiliser by increased early growth, earlier maturation and increased no. of mature heads at harvest, and with greater water consumption between sowing and heading. Positive responses in yield were not related directly to available moisture levels at sowing or to rainfall during the season, but were associated with more efficient utilisation of water. The fertiliser increased the total P uptake of the crop but did not affect the P content of the grain. A. G. POLLARD.

Barley quality tests. II. Responses to lime and phosphatic fertilisers. R. Stern and G. M. Wright (*N.Z. J. agric. Res.*, 1962, **5**, 510—511).—Application of superphosphate gave substantial increase in yield and kernel wt. of barley. When the plots were top dressed with lime the higher rates of phosphate application resulted in a decreased β-amylase activity of the barley. W. ELSTOW.

Evaluation of weed competition and the effects of weed extracts and leachates on the development of field maize (*Zea mays*, L.) and oats (*Avena sativa*, L.). H. A. Collins (*Dissert. Abstr.*, 1962, **23**, 1851).—The competitiveness of various weed species, and the intensity of weeds necessary to effect significant dry-matter losses of maize are examined. The effects of leachates from sand in which weeds were growing on the development of greenhouse-grown maize and oats and also the effects of water extracts from several annual weed species on the elongation of maize and oat radicles are recorded. F. C. SUTTON.

Effect of flooding on the availability of phosphorus and on the growth of rice. I. G. Valencia (*Dissert. Abstr.*, 1962, **23**, 1845—1846).—Fractionation studies to determine the effect of flooding on the transformation of both native and applied PO_4^{3-} showed significant changes in the distribution of the various soil inorg. P fractions. The increase in the Fe-bound and Al-bound PO_4^{3-} was attributed to surface reactions in which the sol. PO_4^{3-} was sorbed on the solid phases of the hydrous oxides of Al and Fe on the clay mineral surfaces. F. C. SUTTON.

Rice growing in British Guiana. II. Field experiments to test effects of fertilisers, lime and cultivations on yield, composition and nutrient uptake. J. K. R. Gasser (*J. Soil Sci.*, 1962, **13**, 321—332).—Application of N increased rice yields most on the most fertile soil type. P did not increase yields on the most fertile soil type, increased yields moderately on the less fertile soils, and prevented crop failure on soil containing much exchangeable Al; water-sol. PO_4^{3-} was more effective than basic slag in the last respect. K improved early growth at one site but did not increase straw and grain yields. $CaCO_3$ did not increase yields even on the very acid soil containing much exchangeable Al. Application of N and K had little effect on the composition of straw or grain. P increased the P and decreased the N contents of straw and grain on P-deficient sites. P also decreased K content of the straw, increased that of the grain at one site, and also increased the Ca content of straw and grain. $CaCO_3$ increased the Ca content of the straw and grain at one site. Uptake of nutrients was affected more by yield than by composition. A. H. CORNFIELD.

Effects of fertilisers and farmyard manure [FYM] on swedes and turnips. J. W. S. Reith and R. H. E. Inkson (*J. agric. Sci.*, 1963, **60**, 145—157).—Top growth is substantially increased by applying N, but only slightly by P and still less by K. Whilst response to N is variable, some yield increase of roots is produced by N. P has a much larger effect on roots than either N or K. The K effect is much smaller than that of N. No NP interaction exists. Small positive interactions are found for NK and PK. FYM generally increases yields. When FYM is used, response to N is increased at sub-optimal P levels; that to P decreased when FYM is placed at the bottom of the ridge and that to K is always greatly reduced. The mineral content of the root is generally increased by the application of a nutrient, especially by that of N or P. Dressings of N 65, P_2O_5 125 and K_2O 125 lb. per acre are recommended as the most profitable when FYM is omitted, and 50, 110 and 55 lb. respectively when FYM is applied. M. LONG.

Interactions of phosphate and sulphate on irrigated pasture. J. Lammerink (*N.Z. J. agric. Res.*, 1962, **5**, 450—459).—On a stony silt loam soil, responses to superphosphate were due to both P and S. Large initial responses to P + S were recorded in the clover component of the sward with subsequent increases in grass production. In the third year clover and rye-grass still responded to P + S together but not when applied singly. Cocksfoot % decreased with increasing P application whilst timothy decreased with increasing S %. W. ELSTOW.

Effect of liming and potassium fertilisation on soil solution and on yield and composition of lucerne and orchard grass mixture. L. B. MacLeod (*Dissert. Abstr.*, 1962, **23**, 1853—1854).—Sintered glass siphons, placed in soil at the time of potting, comprised part of a closed system for extracting soil solution under a vac. The soil was saturated with distilled water and allowed to equilibrate for 24 h. prior to extraction under a vac. of 12 cm. of Hg. This technique affords an effective means of evaluating concn. of ions existing in the soil solution and of characterising the environment of plant roots. Harvest yield was correlated with wt. of the respective storage organs of each species and with total available carbohydrate content. F. C. SUTTON.

Potassium–boron relationships in the growth of lucerne. G. J. Ouellette (*Canad. J. Soil Sci.*, 1963, **43**, 59—64).—Lucerne was grown in soil and in sand culture with various levels of K and B. Additions of K tended to accentuate B-deficiency and to lower the intake of B by the plants. Additions of substantial amounts of borax somewhat favoured the uptake of K. The critical level of B in the crop, when the K supply was adequate, was 16—20 p.p.m. B-deficiency was associated with a K/B ratio in the plants of >1200 : 1. Satisfactory growth resulted with ratios 650 : 1 and 1150 : 1. A. G. POLLARD.

Soil changes in ley–arable experiments. A. J. Low, F. J. Piper and P. Roberts (*J. agric. Sci.*, 1963, **60**, 229—238).—The water stability of air-dry soil aggregates, resistance to simulated raindrops and rate of drainage increase with periods under ley; the drawbar pull during ploughing and the force required to crush aggregates decrease. The effects of 3 years under ley still show after 2 years of arable cultivation, whilst those of 2 years under ley largely disappeared. Two years under ley is adequate to maintain total soil N in a four-course rotation. CO_2 production increases with the length of ley. M. LONG.

Effects of lime, nitrogen and phosphorus on the response of ladino clover to molybdenum. G. J. Ouellette (*Canad. J. Soil Sci.*, 1963, **43**, 117—122).—Excessive applications of superphosphate caused a chlorosis in the clover, corrected by additions of molybdate or NO_3^- or limestone. Evidence is advanced that Mo deficiency restricted the formation of plant-available N. A. G. POLLARD.

Artificial freezing as a routine test of cold hardiness of young apple seedlings. K. Lapins (*Proc. Amer. Soc. hort. Sci.*, 1962, **81**, 26—34).—There were significant differences in cold hardiness among progenies of 6 crosses and 2 standard varieties subjected artificially to 25 freezings with a min. temp. of −43°. The estimates of recovery of the cold-treated apple shoots, following a 3-week forcing period in a growth chamber, were more reliable in showing differences in hardiness than was the electrical conductivity of the exosmosis liquid of the shoot sections. There was poor correlation between tree maturity in the autumn and hardiness. A. H. CORNFIELD.

Effects of heavy annual applications of potassium on Red Delicious apple trees. J. A. Barden and A. H. Thompson (*Proc. Amer. Soc. hort. Sci.*, 1962, **81**, 18—25).—Application of KCl (2·5—10·0 lb./acre) to a gravelly loam for 6 years increased available K in the 0—6 and 6—12 in. soil layers but not in the 12—24 in. layer. The treatments had no effect on yields or fruit size. K % in the leaves and fruit, but not in the wood, bark or roots was increased by the treatments which had no effect on available soil Mg, usually reduced leaf-Mg %, but had no effect on Mg, Ca or N % in any other tissue. The higher level of KCl increased the total titrable acidity of the fruit in one of two years. The treatments had no effect on fruit colour or storage life or on total sol. solids, reducing sugars, total sugars or dry matter % of the fruit. A. H. CORNFIELD.

Effects of photoperiod, light quality and growth regulators on growth and flowering of Jonathan apple trees. E. A. Stahly and A. A. Piringer (*Proc. Amer. Soc. hort. Sci.*, 1962, **81**, 12—17).—Growth of young Jonathan apple trees decreased as the supplemental incandescent lighting was increased from 4 to 16 h. per day over the 8 h. of natural light. Growth was significantly increased when 12 h. of extra fluorescent lighting per day was given. The most flowers were formed with the extra 12 h. incandescent lighting. No flowers were formed with the 8 h. photoperiod or with supplemental fluorescent lighting unless trees received naphthylacetic or 2,3,5-tri-iodobenzoic acid. A. H. CORNFIELD.

Effect of N^6-benzyladenine on the respiration and keeping quality of apples. R. M. Smock, D. Martin and C. A. S. Padfield (*Proc. Amer. Soc. hort. Sci.*, 1962, **81**, 51—56).—Application of N^6-benzyladenine to apples accelerated respiration in the preclimacteric and depressed it in the postclimacteric phase. In Tasmanian studies the treatment retarded yellowing in 2 of 5 varieties, but this did not occur in New York studies. A. H. CORNFIELD.

Effect of gibberellic acid on flowering of apple trees. R. Marcelle and C. Sironval (*Nature, Lond.*, 1963, **197**, 405).—Although the absolute no. of flower buds is increased by spraying apple trees with gibberellic acid, the % of flower buds is reduced due to the great increase in the no. of buds present on the lateral shoots. S. A. BROOKS.

Mechanical behaviour of apple fruit as related to bruising. N. Mohsenin, H. Goehlich and L. D. Tukey (*Proc. Amer. Soc. hort. Sci.*, 1962, **81**, 67—77).—The development of a no. of instruments for investigating the behaviour of fruit under various forces is described. A compression tester was the most promising for evaluating the mechanical behaviour of fruit as related to bruising. A. H. CORNFIELD.

Irradiation of fruit and simultaneous measurement of respiration. R. J. Romani and J. B. Bowers (*Nature, Lond.*, 1963, **197**, 509).—By attaching air inflow and outflow lines to a watertight cabinet containing the samples and then lowering the cabinet into a constant field of irradiation ($\sim 3 \times 10^5$ rads/h.), the respiratory rate of fruit can be followed at 20° by the Claypool–Keefer method (*Proc. Amer. Soc. hort. Sci.*, 1942, **40**, 177) before, during and after irradiation. Respiration rate of oranges, lemons and cherries rises with increasing dose to $\sim 10^6$ rads, but shows an intermediate transition level ($\sim 5 \times 10^5$ rads for cherries). Fruit receiving doses above this plateau-level decrease faster in post-irradiation respiration (4—16 h.) than does fruit receiving lower doses. Irreparable damage to respiratory mechanism at high dosage is postulated, with repair reactions ensuring continued respiration at lower dosages. W. J. BAKER.

Nitrogen studies with apple and cranberry. L. P. Somogyi (*Dissert. Abstr.*, 1962, **23**, 1856).—A liquid–liquid extraction, concentration

and distillation process yielded a typically odorous isolate, which was separated by gas–liquid chromatography. Ten different apple varieties were analysed by this method. Volatile components from the high-boiling compounds probably contribute most to actual flavour. A study was also made of the effect of slowly and readily available forms of N on growth, flowering, fruiting and N content of cranberry, grown in substrates of different org. matter content. Differences in leaf-N content were inversely related to vegetative growth. F. C. SUTTON.

Nutritional balance as related to leaf composition and fire blight (*Erwinia amylovora*) susceptibility in the Bartlett pear. L. N. Lewis and A. L. Kenworthy (*Proc. Amer. Soc. hort. Sci.*, 1962, **81,** 108—115).—One-year-old Bartlett pear trees were grown in sand cultures receiving low and high levels of both major and trace elements. Variation in the supply of major elements had a greater effect on leaf nutrient composition than had variations in trace elements supply. The lowest susceptibility to fire blight occurred in trees receiving a high level of Ca or a low level of B. Both low and high levels of most other treatments usually resulted in increased susceptibility to fire blight in comparison with medium levels of nutrients. A. H. CORNFIELD.

Effect of dilute and concentrated sprays of 1-naphthyl *N*-methylcarbamate (Sevin) on fruit set, size and seed content of Bartlett pears. W. H. Griggs and B. T. Iwakiri (*Proc. Amer. Soc. hort. Sci.*, 1962, **81,** 93—97).—Application of Sevin in high or low-vol. sprays did not result in fruit-thinning of Bartlett pear trees in a comparison with Guthion [*OO*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-ylmethyl) phosphorodithioate] sprays. Fruit was slightly larger after high-vol. Sevin sprays. The low-vol. Sevin spray produced the lowest seed per fruit and the most seedless fruit. Sevin caused more russet than did Guthion. Neither material inhibited flower-bud formation. A. H. CORNFIELD.

Internal atmosphere in Bartlett pears stored in controlled atmospheres. M. W. Williams and M. E. Patterson (*Proc. Amer. Soc. hort. Sci.*, 1962, **81,** 129—136).—A method is described for determining the CO_2 and O_2 in the core area of the pear without destroying the fruit. The internal atm. of fruit stored at −0·5° in storage conditions of varying CO_2 content contained ≯2—3% more CO_2 than did the storage atm.; the O_2 content was always >15%. During ripening in air at ordinary temp. the O_2 level dropped to about 11% and CO_2 increased to about 10%. A. H. CORNFIELD.

Effect of 2,4-D on the nitrogen fractions of Bartlett pear tissues. C. G. Woodbridge and A. L. Kamal (*Proc. Amer. Soc. hort. Sci.*, 1962, **81,** 116—122).—Application of 2,4-D (4—16 p.p.m.) to Bartlett pear trees did not affect chemical composition in the first year of application. In the second year the treatments usually increased total and protein N in the leaf and decreased them in the stem, but had no significant effect on amino-N in any of the tissues. The treatments had variable effects on sol. NH_3 and amide-N contents of both young and old leaves. A. H. CORNFIELD.

Effects of treating old peach soils on performance of young peach trees in the greenhouse. A. L. Havis (*Proc. Amer. Soc. hort. Sci.*, 1962, **81,** 147—152).—The performance of young peach trees on old acid peach soils was improved to the greatest extent by treatment with high-Ca or high-Mg liming materials. Fumigation with MeBr improved performance even more. $CaSO_4$ was ineffective. A. H. CORNFIELD.

Effect of high rates of nitrogen and potassium on the performance of peaches. G. E. Stembridge, C. E. Gambrell, H. J. Sefick and L. O. van Blaricom (*Proc. Amer. Soc. hort. Sci.*, 1962, **81,** 153—161).—High rates of N (208 lb.) and K (146 lb./acre) applied to mature peach trees failed to produce outstanding differences in yield during heavy-crop years. High rates of N increased yields in the light-crop year, probably as a result of increased fruit set. Maturity was delayed by N applications. K applications increased foliar K and decreased foliar N. High N rates increased foliar N and decreased foliar K. Desirable skin and flesh colour of fruit was associated with low foliar N and high foliar K. Canned peaches from high-N plots were ranked superior in texture and inferior in flavour to those from low-N plots. A. H. CORNFIELD.

Comparative water-use of softwood plantations and bamboo forest. H. C. Pereira and P. H. Hosegood (*J. Soil Sci.*, 1962, **13,** 299—313).—Continuous canopies of 120-ft.-high Radiata pine, 50-ft. Monterey cypress, 40-ft. bamboo thicket and 20-ft. Patula pine all used about the same amount of water, which was approx. 90% of that evaporated from an open water surface. Tall woody weeds which invaded clearings and young pine plantations before the latter had achieved closed canopies were equally effective in removing water. In the area both evaporation and rainfall (mainly seasonal) averaged 45 in. per annum. Water was saved over 2—3 years and soil-moisture tensions remained low enough throughout the profile to permit recharge of ground-water when the felling and replanting were accomplished under conditions of clean cultivation. A. H. CORNFIELD.

Uptake of nutrients from sterilised forest-nursery soils. G. M. Will (*N.Z. J. agric. Res.*, 1962, **5,** 425—432).—In seven forest nurseries in New Zealand the uptake of nutrients by conifer seedlings is increased by soil sterilisation. Chloropicrin is more effective than formaldehyde. Greatest response is in light-textured low-fertility pumice soils. The increased uptake of nutrients is to the detriment of succeeding crops of seedlings and a second sterilisation results in a further decrease of nutrient uptake. Combining compost application and sterilisation gives a greater response than either alone. (16 references.) W. ELSTOW.

Soil- and fertiliser-P uptake by *ragi*. N. P. Datta and G. Dev (*J. Indian Soc. Soil Sci.*, 1963, **11,** 45—50).—In field trials with ^{32}P-labelled fertiliser, *ragi* utilised more soil-P than fertiliser-P. Superphosphates and $CaHPO_4$ were utilised to similar extents by the crop. A. G. POLLARD.

Mustard seed processing: bland protein meal, bland oil and allyl isothiocyanate as by-product. G. C. Mustakas, L. D. Kirk and E. L. Griffin, jun. (*J. Amer. Oil Chem. Soc.*, 1962, **39,** 372—377).—The ground meal is treated in a laboratory cylindrical cooker fitted with an agitating mechanism, a spray nozzle for introducing moisture, and a steam-distillate outlet. A detoxified product suitable for rerolling and filtration-extraction by the process of Graci *et al.* can be obtained as follows: the meal, with 15—16% of moisture is kept at 55° for 15 min. for the hydrolysis (by the natural enzyme) of the glucoside with the production of allyl isothiocyanate (**I**). The meal is then heated to 85° and, more slowly, to 105° in order to steam-distil off the **I**; during this process, lasting 45 min., sufficient moisture is introduced to give a final product containing ~13% of moisture. Traces of **I** (0·01—0·04%) can be removed from the crude oil by the usual refining, bleaching and deodorising. The initial flavour score of the final oil is approx. the same as that of soya-bean oil, and slightly inferior after keeping at 60° during 4 days. The final meal contains <0·01% of **I**. The volatile oil contains 88—93% of **I**. P. S. ARUP.

Pest Control

Physiological basis of disease resistance. H. Kern (*Schweiz. landw. Forsch.*, 1962, **1,** 271—280).—Parasites depend for their nutrition on substances available in the host. Some plants naturally contain substances which are toxic to parasites (e.g. rye contains benzoxazolone) and others, e.g. the tuber of *Orchis militaris*, produce toxic substances after a primary infection which resist any further infection. Mineral fertilisers can decrease or increase resistance to attack depending on whether they favour the formation of parasite nutrients or inhibitors. (18 references.) J. V. RUSSO.

Fungicides. VI. Antifungal activity of certain dithiocarbamic and hydroxydithioformic acid derivatives. G. A. Carter, J. L. Garraway, D. M. Spencer and R. L. Wain (*Ann. appl. Biol.*, 1963, **51,** 135—151).—The structure-antifungal relationships of 137 S-containing compounds were studied by observing their effects on mycelial growth of six fungi and on spore germination of one fungus. Derivatives of alkoxydithioformic acids showed higher toxicity than those of *N*-alkyl-substituted dithiocarbamic acids, except for a series of β-(*N*-alkylthiocarbamoylthio)acrylic acids. The fungitoxicity of esters of alkyl-substituted dithiocarbamic or hydroxydithioformic acids showed little change on increasing the size of the alkyl substituent or ester grouping. The fungitoxicity of compounds derived from *N*-aryldithiocarbamic acids varied considerably with the nature of the *N*-substituent. A. H. CORNFIELD.

Effects of piperonyl butoxide on insecticidal potency. A. B. Hadaway, F. Barlow and J. Duncan (*Bull. ent. Res.*, 1963, **53,** 769—778).—The synergic action of piperonyl butoxide (**I**) was highly effective with natural pyrethrins and with carbamates on house flies; its action was much smaller on mosquitoes. The toxicity of the synthetic allethrin and Dimethrin was potentiated to a smaller extent than that of natural pyrethrins by **I**. The toxicity of malathion to *Musca domestica* and to some degree that to *Anopheles stephensi* was lowered whereas that to *Aedes aegypti* was increased by **I** which had little influence on the activity of parathion or diazinon (**II**) or their O-analogues in respect of these insects, except that it increased the toxicity of **II** and diazinoxon to *Ae. aegypti*. A. G. POLLARD.

Reaction of certain phosphorothionate insecticides with alcohols and potentiation by certain breakdown products. J. E. Casida and D. M. Sanderson (*J. agric. Fd Chem.*, 1963, **11,** 91—96).—2-Methoxyethanol is used as a solvent for formulating dimethoate prep. but it may cause decomposition to toxic products. At high temp. it is

shown that six ionic P compounds, seven neutral phosphate esters and the disulphide of *N*-methylmercaptoacetamide are formed. The toxicity of these compounds is investigated. An impurity in some batches potentiated oral toxicity to the rat. (22 references.)
J. B. WOOF.

Effect of didecyldimethylammonium bromide against powdery mildews and other fungal plant diseases. A. H. M. Kirby, E. L. Frick, R. T. Burchill and M. H. Moore (*Nature, Lond.*, 1963, **197,** 514).—Evidence is given of the outstanding effectiveness of didecyldimethylammonium bromide (0·025—0·05%) (**I**) in wetting foliage (without damage) and controlling apple mildew, strawberry mildew and apple scab, and in suppressing spores of *Gloeosporium perannans*, Zeller. In many instances **I** is more effective than dinocap, captan, phenylmercuric chloride, lime–S, etc., max. concn. of 0·2% being safe for rootstocks *in vitro*. W. J. BAKER.

Fungitoxicity of 2,4-dichloro-3,5-dinitrobenzoates. L. A. Summers and J. A. W. Turner (*Nature, Lond.*, 1963, **197,** 495—496).—Me, Et and Pr^i esters of 2,4-dichloro-3,5-dinitrobenzoic acid in concn. of 100—500 p.p.m. are effective protectant foliage-sprays for control of *Alternaria solani* on tomatoes, *Erysiphe graminis* on oats, and *Peronospora tabacina* on tobacco, all grown *in vitro*. The Me ester is most effective (ED_{95} = 0·01 vs. *A. solani*, in comparison with 0·08 for tetramethylthiuram disulphide). There is no phytotoxicity or deleterious plant growth. Fungitoxicity is ascribed to reactivity of halogen groups. W. J. BAKER.

Insecticidal and acaricidal chlorinated hydrocarbons. K. Liesche (*Disch. ApothZtg*, 1963, **103,** 152—157).—Discussions are given of the chemical structures of the various chlorinated hydrocarbons (and their deriv.) used as plant-protecting insecticides and acaricides—together with their trade names and the content of active ingredient in the commercial product. The compounds or products are classified in terms of structure, degree of chlorination and activity. Compounds or products discussed include the various chlorinated bicyclics, aldrin, dieldrin, endrin, Telodin, Thiodan, toxophene and hexachlorocyclohexane, DDT (and its deriv.), and compounds containing two chlorinated benzene rings joined together via —CH_2·S—, —S—, —SO_2— or —SO_2·O— groups. H. L. WHITEHEAD.

Fungicidal and insecticidal activity of some organic fluorine compounds containing aryloxy, benzamido, acetamido and thiazole ring systems. K. C. Joshi, S. Giri and S. C. Bahel (*J. sci. industr. Res.*, 1962, **21C,** 315—317).—Org. F compounds (54) including fluoroaryloxy acids and their Hg and amide derivatives, *N*-substituted fluorobenzamides and 2-arylamino-substituted thiazoles were screened for fungicidal and/or insecticidal activity. No antifungal activity (against *Alternaria solani*) was shown at 50 and 500 p.p.m., except by five Hg deriv. of fluoroaryloxy acids. Twelve of the compounds showed very poor activity compared with DDT against adult and larval mosquitoes. J. L. WALPOLE.

Effectiveness of methyl isothiocyanate as a general soil fumigant under tropical conditions. L. Roth and C. Bruhn (*Phytopathology*, 1963, **53,** 25).—Methyl isothiocyanate (**I**) (20%) was injected into sandy soil at 20 cm. depth (100—150 ml./sq. in.) with temp. at this depth of 30°. Under these conditions penetration of **I** did not exceed 40 cm. A plastic cover over the soil surface was necessary. Soil required aeration for five days after treatment before planting. Applications of 100 ml./sq. in. eliminated *Meloidogyne* spp. in tomato and celery and at 150 ml./sq. in. soil fungi and weeds were killed.
A. G. POLLARD.

Decomposition of sodium *N*-methyldithiocarbamate, vapam, in soil. N. J. Turner (*Dissert. Abstr.*, 1962, **23,** 1867).—Vapam decomposes in dil. solution at pH 9·5 to methyl isothiocyanate (MS) and S. High O_2 tensions and environmental conditions that favour soil aeration increase decomposition of vapam to the highly fungitoxic MS, which may persist in limited quantities considerably longer (weeks) than would be expected from its high chemical reactivity.
F. C. SUTTON.

Parathion residue in greens. L. H. Rolston and R. R. Walton (*J. econ. Ent.*, 1963, **56,** 169—172).—Analysis of five types of untreated turnip greens showed that they all contained natural substances that record as parathion when determined colorimetrically, particularly in autumn. The effect of dosage and time interval between applications on residues in different crops is discussed.
C. M. HARDWICK.

Cholinesterase: inhibition method of determining the distribution of organophosphorus insecticides in soils. R. Bardner, K. A. Lord and S. R. B. Solly (*Chem. & Ind.*, 1963, 123—124).—A sensitive method of determining the distribution of organophosphorus insecticides and their metabolites in the soil is based on their anti-cholinesterase activity. The insecticide diffuses into a gelatin-coated slide for a period of 48 h.; it is oxidised with Br vapour and a moist piece of filter paper soaked with an extract of insect esterase applied to it. The paper is developed by spraying with a chromogenic substrate (indoxyl acetate) when the inhibited (colourless) areas indicate the presence of insecticide. Practical details of the method are given together with the precautions to be taken. J. L. WALPOLE.

Insecticide treatment as determining factor for maintenance of balanced rotation in intensive cereal cultivation at le Berry, Champagne. M. H. Richard (*C. R. Acad. Agric. Fr.*, 1962, **48,** 865—874).—In long-term experiments lucerne has proved a satisfactory crop in rotation with wheat, barley and beet, provided that measures are taken for the control of *Contarina medicaginis* Kieffer. Two applications to the soil (before the flowering season) of dieldrin powder (500 g. of active matter per hectare) are recommended. Good yields are obtained with moderate annual N-manuring. Lucerne during 2—3 years in a 6-year rotation is superior to maize or colza as an intermediate crop. P. S. ARUP.

Systemic insecticides for control of insects attacking potatoes. J. K. Knoke (*Dissert. Abstr.*, 1962, **23,** 1844).—Several systemic insecticides, including phorate, were applied to potatoes by numerous techniques. Factors influencing the plant uptake of any particular systemic insecticide, included placement in soil, granule size and type, concn. of the toxicant on the granular carrier, rate applied, soil type, and type of fertiliser used. Soil-applied phorate gave adequate insect control on five potato varieties and, in those which were susceptible to purple top wilt and common tuber scab, a fair measure of disease control was obtained. F. C. SUTTON.

Relation of chlorogenic acid and total free phenols in potato plants to resistance to infection by *Verticillium albo-atrum*. S. S. Patil (*Dissert. Abstr.*, 1962, **23,** 1862—1863).—Young potato plants are resistant to the infection, because the invading pathogen is inhibited by the oxidised products of chlorogenic acid which are formed on tissue injury. F. C. SUTTON.

Control of potato aphids with systemic insecticides. D. D. Pond (*J. econ. Ent.*, 1963, **56,** 227—230).—One application of schradan or demeton (2 lb./acre) at planting time was as effective as 9 weekly foliar applications of malathion and better than foliar applications of DDT. Diazinon and dimethoate seed-piece dips were unsatisfactory. The average aphid-free period obtained was 77 days. Yields were mostly unaffected. Residues in the tubers were 0·03 p.p.m. in 1960 and 0·06 p.p.m. in 1961. Plants grown from these tubers after 9—10 weeks' storage were not toxic to aphids.
C. M. HARDWICK.

Control of *Pleospora betae* in sugar beet. W. J. Byford (*Ann. appl. Biol.*, 1963, **51,** 41—49).—Steeping sugar-beet seed in EtHg phosphate (EMP) (40 p.p.m.) for 20 min. before sowing controlled *Pleospora betae* and increased the emergence of seedlings better than did spraying seed with EMP solution or steeping in or spraying seed with other fungicides. A. H. CORNFIELD.

Influence of environment on diseases of turf grass. III. Effect of nutrition, pH, soil temperature, air temperature and soil moisture on *Pythium* blight of Highland bent grass. L. D. Moore, H. B. Couch and J. R. Bloom (*Phytopathology*, 1963, **53,** 53—57).—*Agrostis tenuis* was grown in sand culture with various supplies of nutrients and water and at different pH. Seedlings were inoculated with *Pythium* 100 days after sowing. The subsequent incidence of disease at the same pH was greater with higher nutrient levels. High air temp. (day–night range) favoured the blight (max. 85°, min. 65°). Susceptibility to the disease was highest in soils initially allowed to dry to the wilting point before irrigation to field capacity; under these conditions the % of N in the plants was greatest.
A. G. POLLARD.

Correlation between lipid content and per cent mortality of lucerne weevil to heptachlor and malathion. S. E. Bennett and C. A. Thomas, jun. (*J. econ. Ent.*, 1963, **56,** 239—240).—With heptachlor the % mortality decreased as the % of fat in the weevils increased over 10—35 days of age. From 35 to 54 days mortality and fat content remained constant for heptachlor and malathion. From 7 to 14 days of age the fat content increased from 9·4% to 31·4%; the % mortality decreased from 95% to 30% for heptachlor and 95% to 75% with malathion. Moisture contents decreased as fat increased.
C. M. HARDWICK.

Effect of phosphorus on the phototoxicity of tricalcium arsenate as manifested by bluegrass and crabgrass. C. F. Everett (*Dissert. Abstr.*, 1962, **23,** 1851—1852).—The effects of pH, P and 'low-lime tricalcium arsenate' (**I**) treatments on yield and on As and P contents of bluegrass were measured from an established sward on sandy-loam soil. The effect of P and period of exposure to As in nutrient solution on the uptake and toxicity of **I** and Na arsenite was measured with bluegrass and crabgrass in the greenhouse. Crabgrass regrowth in high P solutions treated with **I** for 17 days decreased the solubility of the As in the solution more than did bluegrass by a factor 4 : 1. Increasing P from 1 to 100 p.p.m. with crabgrass decreased the sol. As in the nutrient solution from 6·4 to 1·1%.
F. C. SUTTON.

Greenhouse evaluation of chemicals for control of powdery mildews. I. Method suitable for apple and barley. II. Factors affecting artificial infection of apple foliage. A. H. M. Kirby and E. L. Frick (*Ann. appl. Biol.*, 1963, **51**, 51—60, 61—68).—I. A tower for the artificial inoculation of potted apple rootstocks or barley seedlings with conidia of the appropriate powdery mildew is described. The method was successful for studying both the protective and curative effects of chemicals applied by a dipping technique.

II. Various factors affecting the extent of infection of apple foliage in the infection tower are reported. A. H. CORNFIELD.

Diphenylamine residues in apples in relation to scald control. C. A. Bache, R. M. Smock, L. Yatsu, C. Mooney and D. J. Lisk (*Proc. Amer. Soc. hort. Sci.*, 1962, **81**, 57—60).—Usually no scald developed during storage when diphenylamine residues (**I**) were 4—5 p.p.m. or more. However, in 1959 in two cases residues of 5·5 p.p.m. did not give complete control of scald. Spraying the fruit on the tree gave lower residues of **I** than did spraying during sorting or in the crates or dipping. A. H. CORNFIELD.

Evaluation of lead arsenate for control of apple maggot, *Rhagoletis pornorella*, in New Brunswick. C. W. Maxwell, W. T. A. Neilson and F. A. Wood (*J. econ. Ent.*, 1963, **56**, 106—161).—Poor maggot control followed the use of Pb arsenate sprays due to slow kill and rapid weathering. Addition of DDT increased the control of moderate infestations but was not sufficient to control severe infestations. C. M. HARDWICK.

Woolly aphid of apple (*Eriosoma lanigerum*) and its control in Southern Rhodesia. A. J. M. Carnegie (*Bull. ent. Res.*, 1963, **53**, 609—619).—Good control of above-ground infestations of the aphid was obtained by spraying with methyl demeton (**I**) (0·052—0·078%) or diazinon (0·065% active material) or by injection into trunks of **I** (0·5 fl. oz. of a 50% prep.) or of dimethoate (0·5 fl. oz. of a 40% prep.). Subterranean infestations were eliminated by application to soil of V-C 13 (*OO*-diethyl *O*-2,4-dichlorophenyl phosphorothioate) (100 ml. of a prep. containing 75% of **I**, in 1 gal. of water per tree). A. G. POLLARD.

Effects of insecticides and 2,4-D on the sugar content of Bartlett pear tissue. C. G. Woodbridge (*Proc. Amer. Soc. hort. Sci.*, 1962, **81**, 123—128).—Of six insecticides used at one location only DDT lowered the total sugar content of spur leaf tissue and then only in the first of two years. At another location DDT, parathion, malathion and Systox increased the total sugar content of young leaves during the first year, whilst malathion was the only material which failed to increase total sugars in older leaves. In the second year results were variable. In the first year 2,4-D increased the sugar content of both young and old leaves, but effects were less marked in the second year. The 2,4-D treatment tended to increase sugars in branches and decrease those in the roots. A. H. CORNFIELD.

Accumulation of ^{14}C from carboxyl-labelled 2,4,5-trichlorophenoxyacetic acid in fruit of Tilton apricot. E. C. Maxie, M. V. Bradley and B. J. Robinson (*Proc. Amer. Soc. hort. Sci.*, 1962, **81**, 137—146).—The ^{14}C from COOH-labelled 2,4,5-T accumulated in the mesocarp of apricot fruit for about 3 weeks after application at the beginning of pit hardening. The seed did not accumulate significant amounts of the applied material until about 3 weeks after the beginning of pit hardening. Measurable amounts of ^{14}C did not appear in the embryo until after these tissues were fully developed morphologically. 2,4,5-T persisted on the surface of leaves for at least one month after application. No evidence was found for metabolism of 2,4,5-T in either leaves or fruit. A. H. CORNFIELD.

Effect of various insecticides in the control of caterpillars attacking tomato in California. W. W. Middlekauff, C. Q. Gonzales and R. C. King (*J. econ. Ent.*, 1963, **52**, 155—158).—In small plot experiments over 2 years, toxaphene, TDE and Sevin gave good control of seven species of caterpillar. Thuricide, methoxychlor with or without diazinon gave less satisfactory control. Guthion gave excellent control but caused damage if it drifted on to lucerne, as there is no tolerance level for it. C. M. HARDWICK.

[A] **Effect of sulphur-containing amino-acids and related substances on *Aphanomyces* root-rot of peas.** [B] **Effect of amino-acids and related substances on *Aphanomyces* root-rot of peas.** G. C. Papavizas and C. B. Davey (*Phytopathology*, 1963, **53**, 109—115, 116—122).—[A] Peas, grown in SO_4^{2-}-free media or in a complete nutrient, developed *Aphanomyces* rot after inoculation with suspensions of mycelium or zoospores of the organism. No relationship was apparent between the severity of the rot and the state of oxidation of the S source. L- or DL-methionine (60—80 p.p.m.), added to an SO_4^{2-}-free or to a complete nutrient $\geqslant$24h. after inoculation of the plants prevented pathogenesis, the D-isomer being less effective. A 4-day delay in adding methionine to the nutrient practically prevented control. Development of pathogenesis was prevented by application, immediately after inoculation, of DL-methionine (80 p.p.m.), or of DL-ethionine, DL-isopropionine, DL-methionine methyl, sulphonium chloride or *S*-methyl-L-cysteine, but not by L-cysteine or DL-cystine hydrochloride. All effective S-compounds examined contained Me and NH_2 groups in the mol.; none of those lacking these groups lowered the severity of the disease.

[B] Further examination of a range of NH_2-compounds is described and data obtained is utilised in a discussion of the mechanism of various effects recorded. A. G. POLLARD.

Effect of DDT on *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.) in relation to the spread of cauliflower mosaic and cabbage black ring-spot viruses. G. D. Heathcote and J. Ward (*Bull. ent. Res.*, 1963, **53**, 779—784).—Treatment of healthy or infected brassicae with DDT reduced but did not entirely prevent transmission of the viruses from infected to healthy plants. A. G. POLLARD.

Susceptibility of European fruit lecanium, *Lecanium corni*, Bouché, to oil. J. H. H. Phillips and E. H. Smith (*J. econ. Ent.*, 1963, **56**, 175—180).—Oil of 70 sec. viscosity at different concn. in aq. emulsion was applied to the scales during the growing period. Susceptibility decreased sharply at slow growth stage, but more slowly after the final moult when wt. per scale was increasing rapidly. Sealings of the margins of the scale to tree bark with latex stopped oil penetration but limited growth and no eggs were laid. C. M. HARDWICK.

Community effort in boll weevil control. H. M. Taft and A. R. Hopkins (*U.S. Dept. Agric., agric. Res. Serv.*, 1963, ARS-33-82, 15 pp.).—Early-season insecticide programmes were carried out on overwintered boll weevils with a view to reducing the amount of applications required later in the season. The frequency of applications was important to prevent oviposition. Twelve fields received early-season treatments of Guthion emulsion spray at 0·25 lb./acre, some with DDT (0·5 lb./acre) added. Four or five treatments were applied. Another field received five applications of Sevin and BHC or DDT in the early season. Three control fields did not receive early application. All fields received varying late applications except one of the control fields. Early spraying held the boll weevil population in check for 3—5 weeks afterwards, and if combined with late-season treatment, the population could be reduced to a level where other methods could be applied. G. MACKENZIE.

Chemically induced resistance in the cotton plant to attack by the boll weevil. J. W. Matteson, H. M. Taft and C. F. Rainwater (*J. econ. Ent.*, 1963, **56**, 189—192).—None of the extracts from 358 species or 400 fermentation filtrates from bacteria, moulds, etc. caused any systemic resistance in cotton plants to attack by *Anthonomus grandis*. There was no effect on fecundity or larval development in squares. Of nine chemically identified compounds Bayer 39007 (*o*-isopropoxyphenyl methylcarbamate) imparted repellancy to the weevils in young cotton plants in the laboratory. C. M. HARDWICK.

Absorption and metabolism of dimethoate in the bollworm and bollweevil. D. L. Bull, D. A. Lindquist and J. Hacskaylo (*J. econ. Ent.*, 1963, **56**, 129—134).—Topically applied ^{32}P-labelled dimethoate was rapidly absorbed by fifth-instar *Heliothis zea* and adult *Anthonomus grandis* and rapidly excreted. The metabolism in both species and cotton seedlings was analysed by paper chromatography, autoradiography and standard radio-assay procedures. Dimethoate and 11 metabolites were found. C. M. HARDWICK.

Control of pink bollworm and a method for estimating losses in cotton yield. Ching H. Tsao and W. L. Lowry (*J. econ. Ent.*, 1963, **56**, 158—160).—A plot of cotton sprayed with DDT and a control plot were grown inside a screen cage. After 5 weeks there was no significant difference in the no. of eggs of *Pectinophora gossypiella* on the two plots but the treated area had a significantly higher yield. By counting the damaged and undamaged locks on representative plants and the use of a formulae given, potential yields and actual losses can be calculated. C. M. HARDWICK.

Insecticides tested for control of Douglas-fir cone midge. N. E. Johnson (*J. econ. Ent.*, 1963, **56**, 236—237).—Control of *Contarinia oregonensis* was greatest with Guthion in X-77 rather than in Z1 (spreader-activators). Endosulfan, dimethoate, Bayer 29493 [*OO*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate] and phorate were promising. Sevin gave variable results. C. M. HARDWICK.

Oak wilt development and its reduction by growth regulators. I. Production and activity of oak wilt fungus pectinase, cellulase and auxin. II. Effect of halogenated benzoic acids on oak trees, oak wilt disease and oak wilt fungus. T. F. Geary (*Dissert. Abstr.*, 1962, **23**, 1858—1859).—I. Gums and tyloses form in water-conducting vessels of oaks infected with *Ceratocystis fagacearum* (Bretz) Hunt. These compounds may be formed by action on vessel cell walls of pectinase, cellulase and auxin produced by the fungus. The possible

production of these metabolites by the fungus and their activity upon selected substrates were examined.

II. Growth regulators are examined as systemic protectants in oaks against the oak wilt disease. The most effective chemical treatments for reducing the incidence of oak wilt were : a single pre-springwood application of an emulsion of 2,3,5-tri-iodobenzoic acid ; and 3 weekly applications in the spring of an emulsion of a poly-chlorobenzoic acid isomer mixture to 3-in. bands of outer bark.

F. C. SUTTON.

Control of nematodes in outdoor rose plantings. A. F. Schindler and T. J. Henneberry (*Plant Dis. Reptr*, 1962, **46**, 610—613).—Pre-plant application of 1,2-dibromo-3-chloropropane (DBCP) or *OO*-diethyl *O*-2-pyrazinyl phosphorothioate at twice the recommended rate did not reduce nematodes significantly. Although application of the materials at 8 times the recommended rates resulted in significant control, the extent of control was not great.

A. H. CORNFIELD.

Control of root-knot nematodes in *Dioscorea* tubers. C. Bruhn and W. Koch (*Phytopathology*, 1963, **53**, 24).—Comparative trials with a range of insecticides and of hot water treatments are reported. Best results were obtained by dipping the tubers in water at 45° for 15 min. or in aq. SD 4965 for 30 min. A. G. POLLARD.

Influence of spring and autumn application of nematicides on *Pratylenchus penetrans* and on the quality of flue-cured tobacco grown with various forms of nitrogen. J. M. Elliot and W. B. Mountain (*Canad. J. Soil Sci.*, 1963, **43**, 18—26).—The nematode was controlled by spring or autumn applications of D-D (20—30) or Telone (16—24 gal./acre) injected in bands 12 in. apart and 8 in. deep. Autumn treatment which followed 3 weeks after the disking-in of rye straw and application of NH_4NO_3 (N 75 lb./acre), increased the total N and alkaloids and lowered the reducing sugar and ethanol extractives in the cured leaf. Nitrification of the fertiliser applied to the preceding summer crop and N to the current crop was excessive. Spring fumigation had no ill-effects on leaf composition. A. G. POLLARD.

Lepidopterous leafminers on sweet potato in Fiji. A. D. Hinckley (*Bull. ent. Res.*, 1963, **53**, 665—670).—Use of dieldrin as a pre-planting dip for the tubers may result in outbreaks of damage by leafminers. Evidence indicates this to be due to the elimination of predators, e.g. an ant, of the leafminers. A. G. POLLARD.

Some variations in response of two-spotted spider mite to acaricides. F. F. Smith, R. A. Fulton, and A. L. Boswell (*J. econ. Ent.*, 1963, **56**, 224—227).—*Tetranychus telarius* and *T. cinnabarinus* were treated with org. P insecticides, Aramite, Dimite, Eradex (quinoxaline-2,3-dithiol cyclic trithiocarbonate), and Hooker HRS-16 (decachlorobi-cyclopenta-2,4-dien-1-yl). The time elapsing before death and the symptoms associated with death are described.

C. M. HARDWICK.

Effect of seventeen chemicals on *Cytospora cincta*. A. E. Harvey and A. W. Helton (*Plant Dis. Reptr*, 1962, **46**, 593—596).—Of 17 chemicals tested *in vitro* five cycloheximide derivatives, four Omadine derivatives, oxyquinoline citrate, 8-hydroxyquinoline benzoate, Gerox and mycostatin were highly effective against the disease.

A. H. CORNFIELD.

Germination of grain sorghum and Sudan grass seeds after fumigation with methyl bromide and hydrocyanic acid. R. G. Strong and D. L. Lindgren (*J. econ. Ent.*, 1963, **56**, 144—149).—HCN did not damage seeds but those with high moisture content needed good aeration. Germination of seed after MeBr fumigation decreased with increased dosage when other variables were constant. Decreased germination caused by increased temp. was greater between 50° and 70°F than between 70° and 90°F. There was little difference between grain sorghum varieties in this respect but in general Sudan grass seeds were less susceptible than sorghum. (14 references.) C. M. HARDWICK.

Hydrogen cyanide and benzaldehyde produced by millipedes. H. E. Eisner, T. Eisner and J. J. Hurst (*Chem. & Ind.*, 1963, 124—125).—The defensive secretion of five species of polydesmoid millipedes is shown to contain HCN. One of them, *Apheloria corrugata* (Wood), examined in more detail by gas- and thin-layer chromatography, also contains benzaldehyde but free sugars appear to be absent. (10 references.) J. L. WALPOLE.

Field studies on the effects of insecticides on some aquatic wildlife species. M. S. Mulla, L. W. Isaak and H. Axelrod (*J. econ. Ent.*, 1963, **56**, 184—188).—Effects of 29 insecticides at multiple dosages on *Gambusia affinis* for up to 10 days after treatment, are recorded. At rates used for mosquito larvicides, 11 new compounds as well as Naled (Dibrom) methyl parathion and ronnel were non-toxic to fish. Guthion, ethyl Guthion, diazinon, parathion and trithion [*OO*-diethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate)] showed varying degrees of toxicity. Of 18 materials tested against larval bullfrogs, three were highly toxic to them. (32 references.)

C. M. HARDWICK.

Behaviour of variants of *Gilbertella persicaria* arising in media containing 2,6-dichloro-4-nitroaniline. J. M. Ogawa, R. H. Ramsey and C. J. Moore (*Phytopathology*, 1963, **53**, 97—100).—When grown in a medium containing 2,6-dichloro-4-nitroaniline (**I**), *G. persicaria* produced a variant with greatly increased (×20) tolerance of **I**. In peach fruit inoculated with the parent strain **I** at 1000 p.p.m. suppressed the development of the organism but had no effect on the variant at this concn. A. G. POLLARD.

Competitive effect of common weed species. P. J. Welbank (*Ann. appl. Biol.*, 1963, **51**, 107—125).—The competitive effect of several weed species on kale, sugar beet and wheat studied in pot tests at two levels of added N using dry-matter yields as a measure of competitive ability. All results were corrected with *Chenopodium album* used as a standard. The order of competitive ability of the weeds on the crop plants was similar, with some exceptions. Weed effects were generally not decreased by high compared with low N supply. Leaf N % of kale and sugar beet was greatly increased by high N supply, but usually only slightly affected by weeds.

A. H. CORNFIELD.

Agricultural chemical compositions. U.S. Rubber Co. (B.P. 890,191, 27.4.60. U.S., 14.5.59).—There are claimed plant-growth regulant and fungicidal compositions in which the active agent is a sydnone substituted at N and optionally at C by aliphatic, cycloaliphatic or aromatic radicals, e.g., *N*-phenyl-*C*-methylsydnone.

F. R. BASFORD.

Fungicidal composition. U.S. Rubber Co. (B.P. 889,706, 16.7.60).—A composition effective for the control of fungi, e.g., *Uromyces phaseoli* var. *typica* Arth on Pinto beans, comprises a 2-disubstituted-aminoiminazoline with H or Me on position 4, compounded with a wetting agent or a powdered solid carrier. The prep. is described of 2-*p*-anilinophenylaminoimidazoline, m.p. 212—213°. H. S. R.

Unsaturated percyano cyclic sulphides. E. I. Du Pont de Nemours and Co., Assee of H. E. Simmons, jun. (B.P. 891,093, 30.9.59. U.S., 1.10.59).—The prep. is described of tetracyanodithiin and 5,6,5′-tricyano-*p*-dithiino-[*c*]-isothiazole. The latter decomposes in aq. solution to give HCN and is useful as an insecticide. H. S. R.

Bis-dithiocarbamates. Montecatini Società Generale per l'Industria Mineraria e Chimica (B.P. 889,675, 13.6.60. It., 17.6.59).—Compounds with high fungicidal properties comprise the Zn and Cd salts of bis-dithiocarbamates of the general formula $CS_2H{\cdot}NH{\cdot}CHR{\cdot}CHR'{\cdot}NH{\cdot}CS_2H$ (R and R′ are alkyl, or R is H). In an example, directions are given for the prep. from 1,2-propylenediamine of propane-1,2-bis. dithiocarbamic acid, the Zn salt of which is more active than the Zn salt of the corresponding ethylene compound against *Peronospera*. F. R. BASFORD.

Substituted mercaptoamidine hydrochlorides, their derivatives, and fungicidal compositions prepared therefrom. Union Carbide Corp., Assee of J. A. Lambrech and W. H. Henesley (B.P. 889,002, 19.8.59. U.S., 28.8.58).—Compounds claimed have the general formula $Y(CH_2{\cdot}S{\cdot}Z)_n$[Z is amidino, 4,5-dihydroiminazol-2-yl, or 3,4,5,6-tetrahydropyrimid-2-yl (in hydrochloride form) ; *n* is 1—2 ; Y is monovalent or divalent residue (according to whether *n* is 1 or 2) derived from thiophen (in which $CH_2{\cdot}S{\cdot}Z$ is in the 2- or 2- and 5-position) or from thianaphthen (in which $CH_2{\cdot}S{\cdot}Z$ is in the 2- or 2- and 7-position)]. They have fungicidal properties and can be obtained by condensing CH_2O with thiophen or thianaphthen in presence of HCl at −10° to +60°, then reacting the intermediate product with thiourea, ethylenethiourea or trimethylenethiourea. One product prepared is (*thien-2-ylmethyl*) *isothiouronium hydrochloride*, m.p. 194—196°. F. R. BASFORD.

Dithiophosphonic acid anhydrides. Farbenfabriken Bayer, A.-G. (B.P. 889,085, 28.7.62. Ger., 21.8.59).—Fungicides and intermediates for insecticide prep. comprise compounds RPS_2 (R is alkyl). In an example, $MePSCl_2$ is treated with H_2S at 141° to give *methylthionophosphine sulphide* ($MePS_2$), m.p. 72·5°, in almost quant. yield.

H. S. R.

Thiophosphonic and thiophosphinic acid esters. Farbenfabriken Bayer A.-G. (B.P. 890,424, 20.5.60. Ger., 26.5.59).—Compounds $R(R'){\cdot}PY{\cdot}S{\cdot}R''{\cdot}CZX$, useful as insecticides (effective against aphids), are obtained by reacting $R(R'){\cdot}PY{\cdot}R''{\cdot}Br$ with $CZX{\cdot}SM$, or with $CZX{\cdot}SH$ in presence of alkali metal alkoxide (R is alkyl or alkoxy of 1—4 C ; R′ is alkyl of 1—4 C ; R″ is alkylene of 1—4 C ; X is alkoxy of 1—4 C or is NR'_2 ; Y and Z are different and are O or S). Full details are given for the prep. of O-*Et* S-(2-*ethoxythiocarbonylthioethyl*) *methylphosphonothiolate*, a pale yellow oil.

F. R. BASFORD.

Organo-phosphorus acaricidal and ovicidal agents. Farbenfabriken Bayer A.-G. (B.P. 890,320, 2.10.59. Ger., 3.10.58).—There are claimed acaricidal and ovicidal compositions in which the active ingredient is a compound of the general formula $(OR)_2PO{\cdot}NR'R''$ [R is halogenophenyl; R′ and R″ are H, alkyl, alkenyl, or together with the N form a 5- or 6-membered hetero-cyclic ring optionally containing other hetero atoms, or R′ is C_{1-4}-alkylene substituted by another $NR''{\cdot}PO(OR)_2$ group]. A preferred agent, which is active against spider mites, is di-(*p*-chlorophenyl)*NN*-diethylphosphoramidate. F. R. BASFORD.

Substituted amino-sulphonic acids. E. I. Du Pont de Nemours and Co. (B.P. 890,194, 14.7.60. U.S., 15.7.59).—*Methyl-methoxysulphamic acid* ($MeO{\cdot}NMe{\cdot}SO_3H$) and its salts are obtained by treating alkali metal nitrite with alkali metal bisulphite and SO_2 at 5°, methylating the product, hydrolysing the methoxyimidosulphonate produced to a *N*-methoxyaminosulphonate, and further methylating this in presence of a strong base. The compounds are useful as pesticides, herbicides (lethal to woody vines, e.g., poison ivy), and as intermediates (e.g., afford 1-methoxy-3-arylureas by subjecting to hydrolysis and subsequent treatment with aryl isocyanate). F. R. BASFORD.

Urea derivatives. Farbenfabriken Bayer A.-G. (B.P. 890,540, 14.1.60. Ger., 15.1.59).—Compounds claimed (useful as herbicides) have the general formula $NR''R'''{\cdot}CO{\cdot}NR'{\cdot}[CHR]_n{\cdot}X$(R and R′ are H or alkyl of 1—4 C; R″ and R‴ are alkyl of 1—4 C, or R″ is H, or R″ and R‴ together with N comprise a 5- or 6-membered heterocyclic ring optionally containing other hetero-atoms; *n* is 0—2; X is bicyclo[2,2,1]-heptyl or -heptenyl, optionally substituted by halogen or alkyl of 1—4 C). Interaction of dimethylcarbamoyl chloride with bicyclo[2,2,1]hept-2-ylamine in benzene gives NN-*dimethyl*-N′-*bicyclo*[2,2,1]*hept*-2-*ylurea*, m.p. 154·5—155°. F. R. BASFORD.

Herbicidal compositions. Leek Chemicals Ltd. (Inventor: W. H. Evans) (B.P. 889,152, 2.10.59).—There is claimed a herbicidal composition containing as active ingredient the *ethylenediamine salt* of 4-*chloro*-2-*methylphenoxypropionic acid*, formed by heating the amine with the acid and dilution with water. F. R. BASFORD.

Animal Husbandry

Importance of starch on the microscopical identification of cereal grains in feeds. M. M. MacMasters and D. H. Waggle (*Stärke*, 1963, **15**, 7—11).—Microscopical characteristics of particles of ground grains that are pertinent to identification of the grains are reviewed and illustrated. There are differences among particles of pericarp and endosperm that are distinctive for various grains. E.g., bran particles readily differentiate sorghum from other grains. Samples of maize, sorghum, wheat, barley, rye, oats, rice starches (ungelatinised) at a magnification of 530 show the individual characteristics of the starch grains. E. M. J.

Nutritive value of New Zealand dairy pastures. II. Herbage intake and digestibility studies with dry cattle. J. B. Hutton (*N.Z. J. agric. Res.*, 1962, **5**, 409—424).—One member of each set of non-lactating identical twin cattle was fed fresh pasture herbage to appetite and the other was given approx. 60% of this amount in a 6-month indoor experiment. Contrary to most previously published work, although the animals allowed some choice ate proportionately less fibre than the others they had no particular preference for the high-protein or energy-containing parts of the herbage. A relationship exists between the apparent digestibility and voluntary intake for herbages below 70% digestibility and at high intakes mean digestibility was lower than when feeding was restricted. (20 references.) W. ELSTOW.

Protein cycle in ruminants. J. Landis (*Schweiz. land. Forsch.*, 1962, **1**, 327—339).—The utilisation of the nitrogenous fraction of the fodder is affected largely by the bacterial activity in the first and second stomachs of ruminants. Proteins, amino-acids and amides are broken down and new proteins are synthesised by these bacteria. This newly synthesised bacterial protein is of good nutritional value. Because of the bacterial transformation of the fodder proteins the biological value of the feed is not directly related to its essential amino-acid content as it is for non-ruminants. Absorption of amino-acids proceeds continuously in ruminants. (18 references.) J. V. RUSSO.

Benzene–ethanol extracts of forage and faeces as indicators of digestibility. D. A. Shearer (*J. agric. Fd Chem.*, 1963, **11**, 33—35).—Plant pigment is used to measure digestibility of forage crops. Dried forage and faecal samples are extracted with a 2 : 1 mixture of benzene and ethanol and the pigment determined gravimetrically. Results are obtained rapidly and agree with chromogen and methoxyl methods. The determination can be carried out along with regular proximate constitution analysis. (12 references.) J. B. WOOF.

Evaluation of various indicator techniques in estimating forage intake and digestibility by range cattle. R. R. Wheeler (*Dissert. Abstr.*, 1962, **23**, 1848).—Cr_2O_3 administered via an encapsulated cellulose—indicator mix twice daily to steers grazing range forage appears promising in estimating the faecal production of such animals. The faecal N method provided good results in estimating forage digestibility. F. C. SUTTON.

Reviews of the progress of dairy science. Sect. F. Milk-borne disease. P. W. Bothwell (*J. Dairy Res.*, 1963, **30**, 109—159).—A comprehensive review covering brucellosis, tuberculosis, staphylococcosis, salmonellosis, Q fever, virus diseases, effects of pesticides and antibiotics, relation of milk fats to heart disease, radionuclides in milk together with data on milk consumption. (About 250 references.) A. G. POLLARD.

Zinc deficiency in the dairy calf. J. K. Miller (*Dissert. Abstr.*, 1962, **23**, 1847).—Seven Holstein calves fed a low-Zn purified diet in two experiments developed severe parakeratosis. Five comparable animals receiving the same diet supplemented with Zn (40 p.p.m.) remained normal and made satisfactory wt. gains. Addition of Zn (260 p.p.m.) to the rations of three deficient calves beginning at 15 weeks of age resulted in rapid and dramatic recovery. F. C. SUTTON.

Radio-iodine in milk of cows consuming stored feed and of cows on pasture. B. Kahn, C. P. Straub and I. R. Jones (*Science*, 1962, **138**, 1334—1335).—The ^{131}I levels in the milk of cows on open pasture and those eating only stored food under cover have been compared over the period Dec. 2nd, to 18th, 1961. The milk of the cows on the pasture contained ^{131}I, ^{140}Ba and ^{137}Cs. Cows under cover had no detectable ^{131}I or ^{140}Ba. The results are discussed in relation to radioactivity levels in air. T. G. MORRIS.

Properties of New Zealand butterfat. VI. Comparison of properties and vitamin-A potencies of butterfats produced by clover-fed and rye-grass-fed dairy cows. VII. Effect of the stage of maturity of rye-grass fed to cows on the characteristics of butterfat and its carotene and vitamin A contents. F. H. McDowall and W. A. McGillivray. **VIII. Fatty acid composition of the milk of cows grazing on rye-grass at two stages of maturity and the composition of the rye-grass lipids.** J. C. Hawke (*J. Dairy Res.*, 1963, **30**, 47—57, 59—66, 67—75).—VI. Monozygotic twin cows were given short-rotation rye-grass (*R*) or white clover (*C*) in indoor feeding trials. *R* produced butterfat of lower I val., *n*, and vitamin-A and carotene content and higher Reichert and sap. val. than did *C*; blood-carotenoids were also lower. In outdoor trials in which *R* was compared with *R–C* pastures having various proportions of *C*, similar trends but relatively smaller differences were apparent in spring and early summer. During autumn the characteristics of the butterfat and the levels of blood-carotenoids under the two feeding regimes were reversed.

VII. Immature short-rotation rye-grass, fed to cows during spring and early summer in outdoor trials, produced butterfat of lower saturation and higher carotene and vitamin-A content and also higher carotene levels in the blood than did comparable feeding with mature rye-grass. Differences in the stage of maturity of herbage may be a more important factor than its botanical composition in controlling vitamin-A and carotene levels of butterfat. Pasture lipids are probably associated with this effect of maturity.

VIII. The lipid content of S. Dakota rye-grass was greater in the short succulent stage and also included larger amounts of linolenic and less linoleic and palmitic (**I**) acids than at maturity. The milk-fat of cows grazing the young growth contained larger proportions of oleic and other C_{18}-acids and less myristic and **I** acids than when fed the mature grass. Among short-chain acids the amounts of butyric acid were greater and those of hexanoic and octanoic acids were smaller on the milk-fat from cows grazing the young grass; the total short-chain acid content was similar under both feeding regimes. A. G. POLLARD.

Threonine requirement of the weanling pig. R. E. Evans (*J. agric. Sci.*, 1963, **60**, 259—266).—A level of 0·45% L-threonine (**I**) in air-dry food is adequate for good growth and economy of food conversion and promotes as good retention of N as do higher levels. Normal rations supply sufficient **I** to prevent it being the limiting essential amino-acid. Beyond 120 lb. live-wt. requirements are lower. A diet supplying 0·47% of lysine is adequate beyond 120 lb. wt., higher amounts being uneconomic, as also is feeding animal protein. M. LONG.

Effect on the performance of growing pigs of the level of meal fed in conjunction with an unrestricted supply of whey. K. G. Mitchell and P. H. Sedgwick (*J. Dairy Res.*, 1963, **30**, 35—45).—A meal mixture was fed to 8–9-week pigs at rates of 2·5 or 3·0 lb./head/day continuously or with changes of rate within these limits at different periods. Unrestricted whey was available throughout the trial. No significant differences in rate of growth, efficiency of feed utilisation

or carcass quality at bacon wt. were apparent although there were significant differences in the proportions of whey and meal consumed. A. G. POLLARD.

Composition of Iraqi sheep's milk. H. T. Nejim (*J. Dairy Res.*, 1963, **30**, 81—85).—Bulk samples of milk from a flock of Awassi sheep examined over 2 years showed the following average analysis: sp. gr. 1·0366; titratable acidity 0·217%; pH 6·65; fat 6·88; protein 6·18; lactose 5·75; solids-not-fat 12·99; ash 0·928; CaO 0·29; P_2O_5 0·32; CaO in ash 31·5; P_2O_5 in ash 34·3%. A. G. POLLARD.

Restricted feeding of pullets. I. Value of pasture and self-selection of dietary components. H. L. Fuller (*Poultry Sci.*, 1962, **41**, 1729—1736).—When birds had access to pasture there was a 6% saving in total feed consumed over conventional mash-grain feeding, a 13% saving where pullets were permitted to select the grain, mineral and protein–vitamin components at will, and a 20% saving when the birds were provided only with grain and minerals. When supplied, free-choice, with a powdered mineral mixture the birds consumed sufficient to provide themselves with 0·52—0·60% Ca and 0·43—0·49% P (feed basis). Egg production and feed efficiency were slightly higher with birds that were permitted to select their feed components than with those fed in the conventional manner. A. H. CORNFIELD.

Magnesium metabolism of chickens. N. M. St. D. Nugara (*Dissert. Abstr.*, 1962, **23**, 1847).—The effects of Ca and P on Mg metabolism on growth, intestinal absorption, excretion and skeletal mineralisation in chickens were studied. Purified diets of a glucose monohydrate–soya-bean protein type were used. The results obtained indicated that when the supplemental Ca and P in the diet was increased at various levels of Mg, there was an increased mortality and a depression of growth. F. C. SUTTON.

Sulphur-amino-acid requirement of the chick from 4 to 8 weeks of age as affected by temperature. R. L. Adams, F. N. Andrews, J. C. Rogler and C. W. Carrick (*Poultry Sci.*, 1962, **41**, 1801—1806).—Growth and feed consumption of chicks from 4 to 8 weeks of age were reduced when the environmental temp. was increased from 21·1° to 29·4°. 0·5% sulphur amino-acids (**I**) supported max. growth whilst 0·6—0·7% was necessary for optimal feed efficiency at both temp. The reduced growth at the higher temp. was not due to lowered intake of **I**. A. H. CORNFIELD.

Organic and inorganic supplements in a purified type diet for chicks. C. R. Creger, M. A. Zavala, R. H. Mitchell, R. E. Davies and J. R. Couch (*Poultry Sci.*, 1962, **41**, 1928—1931).—An inorg. constituent(s) necessary for max. growth of broiler strain chicks to 4 weeks of age was found in corn steepwater and the ash of corn steepwater and was dialysable through a semi-permeable membrane. A. H. CORNFIELD.

Response of fat-deficient laying hens to maize oil supplementation. J. E. Marion and H. M. Edwards, jun. (*Poultry Sci.*, 1962, **41**, 1785—1792).—Addition of 10% of maize oil to a fat-deficient diet increased egg. wt., but had no significant effect on egg production over 70 days, or on dry matter, total lipid content or major lipid components in the egg. The treatment significantly reduced plasma lipids, total wt., dry matter and lipid content of the liver, glyceride content of plasma and liver and cholesterol content of the liver. The treatment increased hatchability of fertile eggs and growth of progeny. A. H. CORNFIELD.

Effect of maize oil added to practical diets on egg weight from young pullets. J. V. Shutze, L. S. Jensen and J. McGinnis (*Poultry Sci.*, 1962, **41**, 1846—1851).—Addition of 5% of maize oil to practical diets containing different cereal grains (maize, wheat, oats, barley, milo) increased egg wt. from pullets during the first 6—8 weeks of production. Egg wt. was not increased by maize oil when compared with an isocaloric diet containing tallow. A. H. CORNFIELD.

Influence of levels and sources of calcium and vitamin D_3 on the biological half-life of ^{45}Ca in the chick. D. B. Bragg, W. G. Martin and H. Patrick (*Poultry Sci.*, 1962, **41**, 1797—1801).—The biological half-life of ^{45}Ca in chicks fed 1·2% of Ca and receiving adequate vitamin D_3 was 36 days; it was increased by reducing vitamin D_3 and/or Ca level and decreased by raising these levels. The extent of variation was not additive when both factors were changed. The source of added Ca (CO_3^{2-}, SO_4^{2-}, lactate or gluconate) affected its rate of absorption by the bird but did not significantly affect its biological half-life. A. H. CORNFIELD.

Additive effects of nucleic acids and antibiotics as individual growth promotants for chicks. H. Eyssen (*Poultry Sci.*, 1962, **41**, 1822—1828).—Addition of the antibiotic virginiamycin (**I**) (20 p.p.m.) to a semi-synthetic casein–sucrose diet increased chick wt. by 15—30%. Nucleic acids and yeast extracts improved growth by 10—25%, without interfering with the effect of **I**. The growth rate of chicks on a practical-type diet was improved by 10% by **I**, but not by the nucleic acids. A. H. CORNFIELD.

Nutritive value of soya-bean meal as measured by chemical and physical methods. A. Anwar (*Poultry Sci.*, 1962, **41**, 1915—1918).—The nutritive value of 10 samples of soya-bean meal, from various parts of the world, as assessed by gross protein value, was poorly correlated with Lund–Sandström fractions (*J. agric. Res.*, 1943, **66**, 349), N solubility in 6N-HCl, 0·02N-NaOH or 0·5M-NaCl (*J. Nutr.*, 1953, **49**, 479), or α-amino-N released during pancreatin digestion. A. H. CORNFIELD.

Supplemental value of fish meal protein in relation to other sources of protein in the test diet. H. N. Waterhouse and H. M. Scott (*Poultry Sci.*, 1962, **41**, 1936—1939).—Two fish meals, which were markedly different in promoting chick growth when they were the sole protein source, did not differ significantly when assayed in diets containing other sources of protein. This indicates that assays designed to evaluate fish meals as protein sources should be conducted with the specific ration in which they are to be used. A. H. CORNFIELD.

Nutritional evaluation of fish meals using four short-term chick tests. L. E. Ousterhout and D. G. Snyder (*Poultry Sci.*, 1962, **41**, 1753—1757).—Four types of diet for use in 4—10-day chick growth tests are described for testing fish meals. The diets test the availability to the chick of the protein in the fish meal, the metabolisable energy of the meal or the presence of toxic factors, the content of available S-containing amino-acids, and the availability of the second limiting amino-acid in the meal. A. H. CORNFIELD.

Unidentified growth factor activity and supplemental feeding value of commercial fish meals of known processing history. H. R. Bird, F. H. Steinke and T. D. Runnels (*Poultry Sci.*, 1962, **41**, 1740—1744).—Six menhaden and one redfish meal had unidentified growth factor (UGF) activity of 50—90% of that in condensed sardine solubles. Addition of the fish meals to supply 3% protein increased wt. gains and feed efficiency (except for one lot) of chicks on a vegetable diet. The two fish meals with the highest UGF potency also had the highest supplemental feeding value. A. H. CORNFIELD.

Evaluation of the nutritive content of fish meals by chemical methods. D. G. Snyder, L. E. Ousterhout, H. W. Titus, K. Morgareidge and S. Kellenbarger (*Poultry Sci.*, 1962, **41**, 1736—1740).—The proximate composition, minerals, vitamins and amino-acid composition of the protein of six menhaden meals and one redfish meal of known processing history are presented. Two measures of protein quality, viz., available lysine and pepsin-digestible protein, of the fish meal samples were poorly correlated with each other. A. H. CORNFIELD.

Metabolisable energy and digestibility evaluation of fish meal for chickens. L. M. Potter, W. J. Pudelkiewicz, L. Webster and L. D. Matterson (*Poultry Sci.*, 1962, **41**, 1745—1752).—The metabolisable energy of six menhaden fish meals ranged from 2·469 to 3·121 kcal. per g. (dry basis). The coeff. of digestibility of the protein in the fish meals ranged from 65·3 to 76·3%, and that of the fat from 74·6 to 86·3%. Digestible protein contributed 4·197 and digestible fat 8·769 kcal. per g. to the chick. A. H. CORNFIELD.

Vitamin B_6 and cholesterol metabolism in the chick. N. J. Daghir and S. L. Balloun (*Poultry Sci.*, 1962, **41**, 1868—1879).—The addition of soya-bean oil (4%) to the diet of chicks improved wt. gains whether or not vitamin B_6 was adequate. Addition of 1% of nicotinic acid to vitamin B_6-adequate, fat-free diets reduced wt. gains. Serum-cholesterol and aorta wt. were higher in chicks fed vitamin B_6-deficient diets than in those receiving adequate amounts of the vitamin. A deficiency in vitamin B_6 resulted in lower total lipid and cholesterol content of heart tissue in one of two tests, but had no effect on total lipid and cholesterol content of the liver. A. H. CORNFIELD.

Effect of acrylic acid salts on growth of chicks. R. H. White-Stevens, J. M. Pensack, E. L. R. Stokstad and J. M. Sieburth (*Poultry Sci.*, 1962, **41**, 1909—1915).—Growth of chicks to 25 days of age was increased by addition of Na acrylate (**I**) (333—3000 p.p.m.) to the diet. The extent of increased wt. gains due to **I** was usually greater and occurred with lower levels of the chemical in the presence of added chlortetracycline (**II**) (100 p.p.m.) than in its absence. When added at the 500 p.p.m. level the Na, K and Mg salts of acrylic acid increased wt. gains of chicks only in the presence of **II** (100 p.p.m.), whilst the Zn and Ca salts were ineffective. A no. of acrylic esters were also ineffective. With 500 p.p.m. in the diet **I** reduced mortality and increased the growth of chicks artificially infected with pleuro-pneumonia-like organisms. The treatment was complementary to **II** in this respect. A. H. CORNFIELD.

Tritium for the determination of residual oestrogen in the tissues of broilers fed 3,4-dianisyl-2-T-3-hexene. D. DeSteiguer, E. M. Hodnett, R. D. Morrison and R. H. Thayer (*Poultry Sci.*, 1962, **41**, 1815—1822).—Chicks were fed with 3,4-dianisyl-2-T-3-hexene (labelled with tritium) at 0·05 per lb. of ration for 21 days. Oestrogen residues were found in the breast, thigh-leg muscle, liver, blood,

skin and adipose tissue immediately on terminating the treatment. There were no significant decreases in the concn. of the oestrogen following a 3-day depletion period. A. H. CORNFIELD.

Effect of diethylstilboestrol on choline deficiency in the chick. R. M. Leach, jun., L. C. Norris and M. L. Scott (*Poultry Sci.*, 1962, **41,** 1828—1832).—Administration of diethylstilboestrol (0·05 g. injection) to chicks receiving choline-deficient diets increased wt. gains and reduced the incidence and severity of bone normalities. The oestrogen had only slight effects in overcoming Zn deficiency and had no effects in overcoming Mn deficiency. A. H. CORNFIELD.

Relationships of organ weight to body weight in chickens. R. E. Burger, F. W. Lorenz and C. E. Gates (*Poultry Sci.*, 1962, **41,** 1762—1773).—Analyses of relations of organ wt. to body wt., and of their alteration by hormone treatments, have shown the importance of proper interpretation of the interrelationships. In particular the fallacies inherent in indiscriminately reporting organ wt. in terms of unit body wt. are considered. A. H. CORNFIELD.

Fatty acid composition of embryonic fat organ lipids. G. L. Feldman, H. T. Jonsson, T. W. Culp and R. H. Gowan (*Poultry Sci.*, 1962, **41,** 1851—1857).—The fatty acid composition of embryonic adipose tissue bears a striking resemblance to that of egg fat and not to the fat of the adult chicken. The triglyceride fatty acids are relatively constant and do not reflect the changes associated with embryonic development. The phospholipid fatty acids fluctuate widely and appear to be related to the onset of triglyceride synthesis and pipping of the shell. An unidentified fatty acid occurs in all of the phospholipids, in which it comprises a major component. A. H. CORNFIELD.

Carbonic anhydrase, diuretics and egg shell formation. W. J. Mueller (*Poultry Sci.*, 1962, **41,** 1792—1796).—Shell thickness was not correlated with carbonic anhydrase activity of the uterine tissue. The carbonic anhydrase content of the uterine tissue during shell deposition was no higher then when the forming egg was in the magnum or isthmus. Mercuhydrin, cardalin and acetazolamide interfered with shell deposition and caused diuresis, whilst neohydrin had no effect. A. H. CORNFIELD.

Tyrosine, protein and ascorbic acid effects on egg shell thickness from chickens subjected to heat stress. P. A. Thornton (*Poultry Sci.*, 1962, **41,** 1832—1835).—Shell thickness of eggs was decreased when birds were subjected to heat stress whether 13% or 17% protein diets were fed or whether or not tyrosine (2·7 g. per kg. of feed) was supplied. Tyrosine + ascorbic acid (0·044 g. per kg. of feed) prevented reduction in egg shell thickness with the 13%- but not with the 17%-protein diet. A. H. CORNFIELD.

Relationship of the egg formation cycle to antibiotic blood level in laying hens. R. H. Harms and P. W. Waldroup (*Poultry Sci.*, 1962, **41,** 1932—1936).—The oxytetracycline content of the blood serum of laying pullets increases as the shell is calcified and decreases 4—6 h. before the egg is laid. Higher serum levels of the antibiotic were obtained when it was supplied in the morning than when in the evening. Injection of progesterone (0·0064 g. per bird) reduced the serum antibiotic level, whilst addition of dienoestrol diacetate (0·016 g. per lb. of feed) to the diet had no effect. A. H. CORNFIELD.

Shell and egg interior quality of the indigenous poultry of Uganda compared with five imported breeds and crosses. J. C. M. Trail (*Poultry Sci.*, 1962, **41,** 1887—1891).—Albumin quality of the eggs from the indigenous breed was midway between that of the five imported breeds (Rhode Island Red, Light Sussex, Black Austrolorp, Light Sussex × Rhode Island Red and White Leghorn × Rhode Island Red). The indigenous breed produced eggs with significantly thicker shells than did all the imported breeds and also showed the lowest incidence of blood and meat spots, although the incidence of these was not significantly lower than those of all the other breeds. A. H. CORNFIELD.

Effect of certain dietary ingredients on the incidence of bloodspots in chicken eggs. J. B. Ward (*Dissert. Abstr.*, 1962, **23,** 1848).—Three strains of Single Comb White Leghorn hens were used. The % of red blood cells (haematocrit value) was different for strains, periods and treatment while prothrombin times (blood clotting potential) were no different for strains or treatments. Haematocrit values were significantly depressed by a high level of Co while a low level of Co caused a non-significant increase in haematocrit values. F. C. SUTTON.

Effects of cottonseed oil and cottonseed oil derivatives on the quality of eggs stored at −1·1° and 15·5°. W. F. Pepper, E. S. Snyder, I. R. Sibbald and S. J. Slinger (*Poultry Sci.*, 1962, **41,** 1943—1946).—Cottonseed oil, acidulated cottonseed soapstocks and cottonseed still bottoms were supplied at the 3% level in the feed to pullets for 20 days. Eggs collected during the last 10 days were stored at either −1·1° or 15·5° for 1—113 days. All treatments resulted in the production of undesirable yolk and/or white characteristics within 1—8 days of storage. A. H. CORNFIELD.

Effect of gradual and abrupt lengthening of photoperiod on reproductive response of turkeys. S. J. Marsden, N. S. Cowen and L. M. Lucas (*Poultry Sci.*, 1962, **41,** 1864—1868).—Abruptly increasing the photoperiod of 31—35-week-old birds to 11—15 h. per day in Dec. (9 h. natural daylight) resulted in improvement in egg production, in the time between initiation of lighting and date of first egg, in hatch or total eggs set, and in no. of poults produced than did a gradual increase in lighting. The best results were obtained with the abruptly started 13-h. photoperiod. A. H. CORNFIELD.

Effect of phosphorus on growth and hock disorder of turkeys 8—23 weeks of age. M. L. Jones, C. W. Deyoe, R. E. Davies and J. R. Couch (*Poultry Sci.*, 1962, **41,** 1925—1928).—Turkeys receiving 0·43% of org. P in their diet grew better from 8 to 23 weeks of age when the diet contained 0·6—0·7% than when it contained 0·3—0·5% of inorg. P. The level of inorg. P had no effect on feed efficiency, mortality, leg weakness or breast blister incidence. A. H. CORNFIELD.

Carcass composition of young turkeys as affected by diethylstilboestrol and thiouracil. B. H. Davis and C. C. Brunson (*Poultry Sci.*, 1963, **42,** 102—107).—Treatment of turkeys at 7 weeks of age with thiouracil retarded wt. gains to 10 weeks of age when the birds were growing rapidly, but not to 13 or 16 weeks of age. Treatment of birds with diethylstilboestrol increased wt. gains at all three ages. The treatments reduced carcass moisture content, particularly at 10 weeks of age. The treatments reduced carcass protein %, but had no significant effect of carcass fat % at any age. At all ages male carcasses were higher in moisture, protein and ash and lower in fat content than were female carcasses. The treatments had no effect on taste panel scores. Carcasses 13 weeks old rated lower than those 10 and 16 weeks. A. H. CORNFIELD.

Effect of restricting light during the adolescent period on reproductive performance in turkeys subsequently exposed to 12-, 14- and 20-hour day. F. X. Ogasawara, W. O. Wilson and V. S. Asmundson (*Poultry Sci.*, 1962, **41,** 1858—1863).—Hens were given 14 h. light per day from 5 to 20 weeks of age and then 6 or 10 h. to 23 weeks of age. Subsequent egg production was better with the 6 h. than with the 10 h. pre-conditioning treatment and was also better with 14 h. than with 12 h. or with 14 h. lengthened by 1 h. per week to 20 h. Turkey males had a lower threshold for light as indicated by age at sexual maturity, semen vol. and sperm concn. A. H. CORNFIELD.

Effect of ionic interactions on the water metabolism of chicks. A. K. Kondo and E. Ross (*Poultry Sci.*, 1962, **41,** 1132—1136).—Addition of 0·5—4·0% of NaCl to chick rations containing 0, 15 and 30% of molasses resulted in increasing water consumption and faecal moisture with increasing level of added NaCl. Water consumption and faecal moisture were directly related to the Na and/or K content of the feed. 2% of NaCl added to the feed caused slight, whilst 4% caused severe, mortality. Addition of extra Ca ($CaCO_3$) to the feed did not affect the increasing faecal moisture due to high levels of K in the feed. A. H. CORNFIELD.

Citrus bioflavonoids in broiler diets. C. W. Deyoe, L. E. Deacon and J. R. Couch (*Poultry Sci.*, 1962, **41,** 1088—1090).—Addition of 1—2·5% of citrus bioflavonoids to the diet of broilers to 8 weeks of age did not affect wt. gains or feed efficiency. Addition of the material at the 5% level reduced wt. gains and feed efficiency. There were no gross differences in appearance of dressed broilers due to the treatments. A. H. CORNFIELD.

***Cassia tora*, L. leaf meal as a component in poultry rations.** V. N. Murty (*Poultry Sci.*, 1962, **41,** 1026—1028).—Addition of the meal (5% of the diet) had no effect on the performance of birds. Addition of 10% of the meal reduced the digestibility of the non-protein org. nutrients. A. H. CORNFIELD.

Dietary phosphorus levels and calcium/phosphorus ratios needed by growing turkeys. E. J. Day and B. C. Dilworth (*Poultry Sci.*, 1962, **41,** 1324—1328).—With dietary Ca/P ratio ranging from 1/1 to 2/1 a level of total P of 0·6% (~0·33% available P) was adequate (as indicated by growth, feed efficiency, and toe ash) for optimum growth of turkeys from 9 to 16 weeks of age. Performance from 17 to 24 weeks of age was satisfactory with 0·45% of total P (~0·21% available P) in the diet over the above Ca/P ratio range. A. H. CORNFIELD.

Availability of phytic acid phosphorus to the chick. R. H. Harms, P. W. Waldroup, R. L. Shirley and C. B. Ammerman (*Poultry Sci.*, 1962, **41,** 1189—1191).—Phytic acid was as effective a source of P to chicks, as measured by wt. gains to and tibia ash at 4 weeks of

age, as was $CaHPO_4$ when the added Ca and P was in the ratio of 1 : 1, but was not quite as effective when the added ratio was 2 : 1. A. H. CORNFIELD.

Effect of feeding various levels of calcium and phosphorus on the performance of and absorption of chlortetracycline by caged layers. H. J. Eoff, R. E. Davies, T. M. Ferguson and J. R. Couch (*Poultry Sci.*, 1962, **41**, 1071—1078).—Reducing the Ca content of the basal diet (Ca 2·37, P 1·06%) of caged layers to 1·35% for 28 days followed by feeding the basal diet for 56 days reduced egg production over an 84-day period. Reducing the Ca content to 0·45% and P content to 0·40% for 9 days reduced egg production and increased leg weakness, fatigue and mortality. When the surviving birds were placed on the basal diet for 75 days egg production returned to near normal. Most of the antibiotic present in the serum of birds fed chlortetracycline had disappeared 21 h. after administration was discontinued. The antibiotic treatment resulted in egg shell discoloration. A. H. CORNFIELD.

Relationship between dietary phosphorus level and the level of plasma inorganic phosphorus of chicks. E. E. Gardiner (*Poultry Sci.*, 1962, **41**, 1156—1163).—Chicks required ≮0·42% of inorg. P in their diet for optimum growth, bone ash % and plasma inorg. P level to 4 weeks. These three factors were directly related to the level of dietary P, and were not affected by increasing the Na level of the diet by 0·2%. Withdrawal of feed or reduction of inorg. P content to below the optimal resulted in a rapid reduction in plasma inorg. P level. Differences in the availability of P from three sources were reflected in differences in plasma inorg. P levels. Na_2HPO_4 and $CaHPO_4$ were more effective than ' soft phosphorus '. Plasma inorg. P level may be used in place of bone ash % as a criterion in P studies with the chick. A. H. CORNFIELD.

Effect of source of calcium and phosphorus on antibiotic potentiation and egg production of caged layers. H. J. Eoff, R. E. Davies, T. M. Ferguson and J. R. Couch (*Poultry Sci.*, 1962, 41, 1065—1070).—With a diet containing chlortetracycline (200 g. per ton of feed), the serum-antibiotic level was reduced initially when 1% of the Ca present as oyster shell flour ($CaCO_3$) was replaced by $CaSO_4$ when defluorinated rock phosphate or Na_2HPO_4 was the source of added P; when colloidal phosphate was fed together with $CaSO_4$ the serum-antibiotic level was increased initially; with prolonged feeding the level was increased by $CaSO_4$ irrespective of the source of added P. Feeding colloidal phosphate plus $CaSO_4$ reduced egg production and wt. and feed intake compared with the other treatments. $CaSO_4$ caused increased water consumption and wet droppings. These effects were even worse when colloidal phosphate was used in addition to $CaSO_4$ and vomiting also resulted. A. H. CORNFIELD.

Comparison of feed grade tallow and a mixture of tallow and acidulated soapstocks in practical chicken roaster rations. W. F. Pepper, S. J. Slinger and I. R. Sibbald (*Poultry Sci.*, 1962, **41**, 1163—1168).—Increasing the level of either tallow or tallow-acidulated soapstocks (1 : 3) from 4% to 12% in the diet whilst maintaining constant kcal./protein or kcal./methionine ratios did not improve wt. gains in cockerels to 15 weeks of age. Feed efficiency to 5 weeks of age was similar with both materials but from 6 to 15 weeks of age tallow was superior in this respect. A. H. CORNFIELD.

Fat tolerance in laying hens. Effects of restricted intake of a high animal fat diet. W. E. Donaldson (*Poultry Sci.*, 1962, **41**, 1060—1065).—Feeding a balanced diet containing 30·4% of animal fat *ad lib.* to pullets resulted in reduced egg production, increased energy intake and increased body wt. in comparison with a control maize–wheat–soya-bean oil meal diet. Restricting the intake of the fat-containing diet to nutrient intakes comparable with the control diet reduced wt. gains to the level of the control but did not maintain egg production so effectively. Thus increased intake and wt. gains are not responsible for poorer egg production when high-fat diets are fed. The N intake of hens fed the fat diet *ad lib.* was higher, whilst N retention was lower, than corresponding values for the control diet. A. H. CORNFIELD.

Response of deutectomised chicks to dietary fat supplementation. H. M. Edwards, jun., J. E. Marion and J. C. Driggers (*Poultry Sci.*, 1962, **41**, 1050—1052).—Removal of the yolk sac from 12-hour-old chicks did not increase their requirement for dietary fat for their first 3 weeks. However, yolk sac removal decreased the growth rate of the chicks fed low-fat diets and these chicks showed increased growth rate when supplied with fat. A. H. CORNFIELD.

Response of chicks to dietary animal fat in new and old environments. W. E. Donaldson (*Poultry Sci.*, 1962, **41**, 1106—1108).—Chicks showed increased wt. gains on addition of 10% of animal fat to a maize–soya-bean oil meal diet when reared in a new (clean) environment but not when reared in an old (dirty) environment. Environment did not affect wt. gains on the control diet. This indicates that the type of intestinal microflora influences the ability of chicks to respond to fat. A. H. CORNFIELD.

Effect of type of dietary fat on the fatty acid composition of eggs and tissues of the hen. L. J. Machlin, R. S. Gordon, J. Marr and C. W. Pope (*Poultry Sci.*, 1962, **41**, 1340—1343).—Hens were fed purified diets containing either 15% of hydrogenated coconut oil or 15% of safflower oil, as the sole source of fat, for 12 weeks. The coconut oil produced eggs containing considerable quantities of lauric, myristic and myristoleic acids and significantly less arachidonic acid than did the safflower oil. The depot fat, liver and heart of hens fed the coconut oil contained significantly more lauric and myristic acids and significantly less linoleic acid than did those of hens fed safflower oil. A. H. CORNFIELD.

Responses of chicks to the dietary inclusion of materials which might be assumed to possess beneficial extra-caloric properties. I. R. Sibbald, S. J. Slinger and W. F. Pepper (*Poultry Sci.*, 1962, **41**, 1254—1261).—' Still bottoms ', the material remaining when fatty acids are distilled from hydrolysed fats, possessed a nutritive value similar to that of feed grade tallow, and, in view of its low metabolisable energy, possessed extra caloric properties. Addition of 2—4% of mineral oil to chick diets depressed wt. gains, 2—4% still bottoms had no effect, whilst 2—4% tallow, soapstocks, soya-bean oil and vegetable fats stimulated wt. gains more than could be explained in terms of variation in dietary energy or kcal. : protein ratios. This extra-caloric effect increased with level of the added material. The compositions of the materials gave no indication of the cause of the growth response. The extra-caloric effect was also reflected in feed efficiency data. A. H. CORNFIELD.

Restricted feeding of pullets. II. Effect of duration and time of restriction on 3-year laying house performance. H. L. Fuller and W. S. Dunahoo (*Poultry Sci.*, 1962, **41**, 1306—1314).—Growth of White Leghorn pullets was retarded by restricting feed intake (to that at 6 weeks of age) during 6—12, 6—18, 6—24 and 12—24 weeks of age, but recovery was rapid on being placed on normal diets. Feed restriction delayed sexual maturity by 0—4 weeks, but the deficit in early egg production was overcome in less than 12 months. Restricting pullets during 6—24 weeks of age tended to increase egg production in the first and third laying years. Egg size was greater for birds restricted up to 24 weeks of age, but only during the first few weesk of production. Egg shell thickness and Haugh units were not affected by the treatments after the birds had been in production for 12 months. A. H. CORNFIELD.

Maize–soya-bean laying diets. V. Seasonal patterns of performance at marginal levels of dietary protein. D. J. Bray and D. J. Morrissey (*Poultry Sci.*, 1962, **41**, 1078—1081).—Egg production by pullets fed a maize–soya-bean diet containing 12% of protein was somewhat lower during the first 12 weeks than that by pullets fed an 18% protein diet. During the next 24 weeks there were no differences in egg production between the two protein levels fed, but during the final 6 weeks of the trial egg production was again somewhat lower with the 12% protein diet. Egg wt. was significantly lower with the 12% than with the 18% protein diet only during the 1–4 week period. A. H. CORNFIELD.

Effect of calcium level of the developing and laying ration on hatchability of eggs and on viability and growth rate of progeny of young pullets. L. R. Berg, G. E. Bearse and L. H. Merrill (*Poultry Sci.*, 1962, **41**, 1328—1335).—Fertility of eggs was high and was not affected by level of Ca in the developing diet (0·5—2·0% Ca) or the laying diet (1·75—3·25% Ca) of the pullets. At 30%-lay hatchability of fertile eggs decreased and chick mortality increased with increasing level of Ca in the laying ration. The high levels of Ca in the laying ration also resulted in reduced growth rate and abnormal wing feather development (similar to that described for Zn deficiency) of the chicks. With eggs produced 4 weeks after 30%-lay was attained there was no effect of Ca level of the laying diet on hatchability of eggs or viability or growth rate of the progeny. Ca level of the developing diet had no effect on any of the factors measured. A. H. CORNFIELD.

Effect of excessive amounts of dietary vitamin A on egg production in White Leghorn hens. J. Biely, J. D. Wood and J. E. Topliff (*Poultry Sci.*, 1962, **41**, 1175—1177).—Addition of 22,000 i.u. of vitamin A per lb. of feed to the diet of laying hens had no effect on egg production over 5 weeks compared with a normal level of vitamin A in the feed. Addition of 44,000 i.u. per lb. of feed reduced egg production slightly, whilst 220,000 i.u. or more reduced egg production considerably, particularly in the later weeks. Lingcod liver oil supplying the high levels of vitamin A caused a greater reduction in egg production than did an equal level of vitamin A acetate. A. H. CORNFIELD.

Effect of terephthalic acid and broad-spectrum antibiotics on egg production and egg shell coloration. H. J. Eoff, R. E. Davies, T. M. Ferguson and J. R. Couch (*Poultry Sci.*, 1962, **41**, 1036—1041).—Addition of chlortetracycline or oxytetracycline (50 g. per ton of

feed), alone or in combination with 0·4% of terephthalic acid, to the diet of laying hens over 252 days had no significant effect on egg production, feed efficiency or incidence of cracked eggs, but maintained body wt. better during hot weather. Addition of chlortetracycline (600—800 g. per ton of feed) to the hens' diet caused a yellowish colour in the egg shells; this colour was greatly intensified by the further addition of 0·4% of terephthalic acid. A. H. CORNFIELD.

Artificial control of egg production in turkeys by photoperiods. W. O. Wilson, F. X. Ogasawara and V. S. Asmundson (*Poultry Sci.*, 1962, **41,** 1168—1175).—Photoperiods of 6 h. resulted in a more consistent improvement in egg production by turkeys than did those of 4 h. A 5-weeks' restriction period was better than a 3-week period. Response to reduced photoperiod occurred with birds initially 26—32 weeks of age. A. H. CORNFIELD.

Effect of furazolidone on egg production, egg quality, fertility and hatchability under commercial farm conditions. P. G. Stiles (*Poultry Sci.*, 1962, **41,** 1336—1338).—Addition of furazolidone (25 g. per ton of feed) to the diet of hens for 3 months had no significant effect on egg production, mortality, interior egg quality, blood spot incidence, meat spot incidence or egg shell thickness. Furazolidone at 50 g. per ton of feed added to the diet of breeding chickens significantly increased the % hatch of total eggs set by 1·95%, but had no effect on fertility. A. H. CORNFIELD.

Interrelationships between antibiotics and terephthalic acid in diets for laying hens. S. J. Slinger, W. F. Pepper and I. R. Sibbald (*Poultry Sci.*, 1962, **41,** 1241—1247).—Egg production and feed efficiency with respect to egg production were increased by addition of terephthalic acid (0·35%), but not of oxytetracycline or chlortetracycline (50 g. per ton of feed) to an all-vegetable diet. The antibiotics significantly decreased egg wt. There were no significant interactions between terephthalic acid and the antibiotics. A. H. CORNFIELD.

β-apo-8′-Carotenal as an egg yolk pigmenter. R. H. Bunnell, W. L. Marusich and J. C. Bauernfeind (*Poultry Sci.*, 1962, **41,** 1109—1115).—Supplying hens with β-*apo*-8′-carotenal (0·002 g. per hen per day or 4·36 g. per ton of dry feed) was an effective yolk pigmenter for three breeds. Dry stabilised prep. of the material were better utilised, as measured by extent of yolk pigmentation, than were oil solutions. Trace elements, antibiotics and other additives did not reduce the effectiveness of the material as a yolk pigmenter. A. H. CORNFIELD.

Egg shell quality during hot weather with calcium gluconate in the diet. B. W. Heywand and R. W. Lowe (*Poultry Sci.*, 1962, **41,** 1213—1215).—Addition of up to 0·86% of Ca as gluconate, in place of $CaCO_3$, to the diet of hens during hot weather had no significant effect on egg production, egg wt., dried shell wt./whole egg wt. ratio or shell thickness. A. H. CORNFIELD.

Effect of cottonseed oil on discoloration of cold-storage eggs. A. R. Kemmerer, B. W. Heywang, H. E. Nordby and R. A. Phelps (*Poultry Sci.*, 1962, **41,** 1101—1103).—Supplying hens with 0·003—0·006 g. of gossypol per hen per day did not produce a sufficient level of olive colour in the yolks during storage (1—3 months at 1·6°) to be of practical significance. The gossypol treatment did not produce any pink whites. Addition of 2·5—5·0% of cottonseed oil to the diet of birds not receiving gossypol did not produce olive yolks but produced pink whites. Cottonseed oil intensified the level of olive colour in the yolks due to gossypol treatment. A. H. CORNFIELD.

Effects of feeding Haloanisone on the growing chicken. R. Marsbroom and G. Sierens (*Poultry Sci.*, 1962, **41,** 1346—1347).—The addition of Haloanisone (for controlling cannibalism) at the rate of 0·04 g. per kg. of feed to the diet of broiler chicks from 2 to 10 weeks of age had no effect on growth rate or feed efficiency. A. H. CORNFIELD.

Nihydrazone and the *Salmonella* infections in chicks and poults. B. W. Bierer and B. D. Barnett (*Poultry Sci.*, 1962, **41,** 1291—1294).—Addition of 0·011% of Nihydrazone (**I**) to the diet of chicks gave moderate control, whilst 0·022% gave good control, of *Salmonella typhimurium* as indicated by mortality. With 0·011% of **I** mortality of chicks infected with *S. gallinarum* was greatly reduced. Mortality was reduced from 75% to 14% by 0·011—0·0165% of **I** in the diet of poults infected with *S. gallinarum*. A. H. CORNFIELD.

Growth and feed utilisation of turkeys as affected by reserpine. W. D. Morrison (*Poultry Sci.*, 1962, **41,** 1210—1213).—Addition of reserpine (0·2—1·0 p.p.m.) to the diet of turkeys aged 8—20 weeks, had no significant effect on wt. gains. Feed efficiency was depressed slightly by the treatments only during the last 4 weeks. A. H. CORNFIELD.

Control of cattle grubs by pour-on, injection and spray. P. H. Kohler and W. M. Rogoff (*J. econ. Ent.*, 1962, **55,** 539—544).—Ruelene as a spray or a pour-on prep. gave grub reductions of 99·6% and 96·3% respectively. Co-ral was more effective as a pour-on than a spray. Bayer 29493 (*OO*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate) was 100% effective by all methods. Cyanamid 38023 (*OO*-dimethyl *O*-*p*′-(dimethylsulphamoyl)phenyl phosphorothioate) was >99% effective as an injection. Only Co-ral gave a cholinesterase depression significant at the 5% level. Injection of insecticides affected wt. gains to the greatest extent. Toxic symptoms occurred at some test locations. In the worst case 25% of the animals suffered severe bloat. C. M. HARDWICK.

Spray and pour-on application of systemic insecticides for control of cattle grubs in Virginia. E. C. Turner, jun. (*J. econ. Ent.*, 1962, **55,** 564—565).—Dylox, Bayer 29493 (*OO*-dimethyl *O*-[4-methylthio-*m*-tolyl] phosphorothioate) and Ruelene as sprays gave excellent grub control. Dylox, Co-ral and Ruelene were effective by the pour-on technique. Bayer 29493 had poor hair penetration. C. M. HARDWICK.

Effect of delayed spraying on cankerworm control. H. E. Thompson (*J. econ. Ent.*, 1962, **55,** 558—559).—Of eight sprays tested 'thuricide' (spores of *Bacillus thuringiensis*) gave 88% reduction of *Paleacrita vernata*. Sevin at 1 lb./100 gal. gave 70% control but at 0·5 lb./100 gal. only 29%. Sprays were most effective if applied before there was much leaf feeding. C. M. HARDWICK.

Co-ral as a litter and nest dust to control the chicken body louse. F. W. Knapp (*J. econ. Ent.*, 1962, **55,** 571—572).—Co-ral dust applied to litter and nests gave 100% control of *Menacanthus stromineus* within 4 days and this remained for at least 52 days. After 54 days enough Co-ral remained to prevent re-infestation. C. M. HARDWICK.

Poultry tolerance to excessive amounts of Co-ral dust. F. W. Knapp (*J. econ. Ent.*, 1962, **55,** 560—561).—Laying hens were dusted once, thrice or seven times weekly with 0·02 g. of (active) Co-ral. There was no difference in egg no., feed consumption or wt. gain attributable to the dust. Some residue was present 5 days after the last application in meat but not in giblets or eggs. C. M. HARDWICK.

Horn fly control using spray, dust and pour-on formulations. C. K. Dorsey, J. O. Heishman and C. H. Taylor (*J. econ. Ent.*, 1962, **55,** 425—430).—In general, dusting and pour-on techniques gave greater control than did spray applications. Pour-on Dylox and Co-ral were excellent for 14 days and Ruelene for 28 days. Dusts of 2% and 25% diazinon were effective for 21 days. Sprays of 0·1% diazinon were good for 14 days and 1% Dimethoate for 7 days. C. M. HARDWICK.

Residues of Sevin in whole milk from sprayed and dusted cows. J. F. Eheart, E. C. Turner and J. Dickinson (*J. econ. Ent.*, 1962, **55,** 504—505).—An extraction and chemical assay method, sensitive to 0·05 p.p.m., is described. Milk from cows sprayed and dusted with 50% Sevin was analysed but only milk obtained within an hour of application contained residues. One tablespoon of Sevin dust per cow gave 15 days' control of horn flies. C. M. HARDWICK.

Horn fly control on beef cattle by use of cable rubbers. R. C. Dobson and R. C. Peterson (*J. econ. Ent.*, 1963, **56,** 230—234).—Methoxychlor, toxaphene, DDT and ronnel on cable rubbers effectively controlled *Haematobia irritans*. Mineral seal oil was no more effective than diesel oil. The addition of 3% Tabutrex did not increase control. C. M. HARDWICK.

Control of face fly on cattle with Co-Ral [applied] in grain and on pasture. C. M. Jones and J. G. Medley (*J. econ. Ent.*, 1963, **56,** 214—215).—Co-Ral (0·5 mg./kg live-wt.) was fed to cattle daily for 6 weeks with their rations. After 6 days the development of face flies in the faeces was inhibited and after 2 days only 46% of housefly larvae survived to pupate. Brome grass pasture was sprayed weekly with Co-Ral to give a similar intake. After 8 days, 100% mortality of face flies was recorded and only 29% of houseflies completed development in the faeces. C. M. HARDWICK.

Stachybotryotoxicosis of chicks. G. Schumaier, H. M. DeVolt, N. C. Laffer and R. D. Creek (*Poultry Sci.*, 1963, **42,** 70—74).—*Stachybotrys atra*, previously shown to be toxic to animals other than the chick, produced in chicks a lipid-sol. toxin which reduced growth and created necrotic lesions in the mouth and crop of the chick. The toxin was not destroyed by autoclaving for 1 h. at 121°. A. H. CORNFIELD.

Response of the feral pigeon when offered the active ingredients of commercial repellents in solution. C. J. Duncan (*Ann. appl. Biol.*, 1963, **51,** 127—134).—The most marked repellent action to pigeons was obtained with solutions of β-naphthol, although solutions of diphenylguanidine, naphthalene, thiram and K and NH_4 alums were also repellent. A. H. CORNFIELD.

Mode of action of Nihydrazone against caecal coccidiosis in chickens. C. Johnson and R. J. van Ryzin (*Poultry Sci.*, 1964,

41, 1918—1924).—Timed medication feeding experiments, histological studies of infected caeca, and haematocrit determinations indicate that nihydrazone is active against the first and second asexual phases in the life cycle of *Eimeria tenella*. Delayed appearance of sex cell precursors indicates that the drug is coccidiostatic. Gross and histologic observations in experiments employing unilateral caecal ligation suggest that nihydrazone reaches the parasitised cells by way of the vascular system. A. H. CORNFIELD.

Activity of vitamin K_1 and menadione sodium bisulphite complex when measured by mortality of chicks with caecal coccidiosis. R. H. Harms, P. W. Waldroup and D. D. Cox (*Poultry Sci.*, 1962, **41,** 1836—1839).—Vitamin K_1 was three times as effective as menadione–$NaHSO_4$ complex in preventing mortality of chicks artificially infected with caecal coccidiosis. The adequate amounts for max. protection were 0·44 g. of the former and 1·2 g. of the latter per ton. Lucerne meal (1·5%) also gave adequate protection, whilst 3% of menhaden meal was ineffective. Coccidiosis did not increase the chicks' requirement for vitamin K_1.
A. H. CORNFIELD.

Non-toxic castor-cake. E. Darzins (B.P. 890,258, 31.12.59).—A non-toxic castor-cake, of high nutritious value, and suitable for use as animal feed, is produced by preparing a homogeneous mixture of comminuted, substantially oil-free natural castor-cake ; removing part of the water and water-sol. carotenoid pigments dissolved therein ; adding a strong culture of a proteolytic bacteria obtained from decomposed org. substance (e.g., sewage) ; allowing the mixture to ferment at 20—45°/pH 7·5—9 (for ⩽10 h., e.g., 50—72 h.) ; then heating at 100—120° (for ⩽1 h.) ; and drying.
F. R. BASFORD.

Antibacterial agents. Beecham Research Laboratories Ltd. (B.P. 889, 168, 10.11.60. U.S., 16.11.59).—Compounds claimed comprise penicillanic acids (and salts thereof) substituted in the 6-position by $NH{\cdot}CO{\cdot}[CH_2]_n{\cdot}NH{\cdot}COR$ (*n* is 1—20 ; R is Ph optionally substituted by 1—3 radicals). They are useful as antibacterial agents (especially active against benzyl-penicillin-resistant micro-organisms), supplements in animal feed, agents for the treatment of mastitis in cattle, infections in poultry, etc., and are obtained by interaction of 6-aminopenicillanic acid (or preferably a salt thereof) with $R{\cdot}CO{\cdot}NH{\cdot}[CH_2]_n{\cdot}COCl$ or its functional equivalent. A detailed example describes the prep. of the *K salt* of 6-(*ω-benzamidocaproamido*)*penicillanic acid*.
F. R. BASFORD.

Veterinary compositions comprising prednisolone 21-trimethylacetate. CIBA Ltd. (B.P. 889,049, 2.6.59. U.S., 2.6.58).—There is claimed a veterinary composition (for use in the treatment of imbalance in the adrenal cortical function of animals) in which the active ingredient is prednisolon e21-trimethylacetate.
F. R. BASFORD.

Veterinary chemotherapeutic compositions. Whitmoyer Laboratories Inc. (Inventors : A. M. Brubaker and J. R. Wiley) (B.P. 889,137, 18.12.59).—A composition for use in the treatment of enterohepatitis (blackhead) in poultry contains as active ingredient 0·01—1 wt.-% of *p*-ureidobenzenearsonic acid (or a water-sol. salt thereof).
F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Motion of granular material on an oscillating conveyor. C. E. Schertz (*Dissert. Abstr.*, 1962, **23,** 1638—1639).—Tests with maize were conducted in which mass rate of flow measurements were used to determine the rate of material movement. Results indicate that the mean η of the conveyed material is not always linear with respect to depth of grain layer. Extrapolation of the experimental data to zero depth of layer indicated that the predicted results were within 10% of the observed values. F. C. SUTTON.

Production of fodder yeast from barley. I. Preliminary studies on the use of the Waldhof fermenter. K. J. Goering and M. J. Houle (*Cereal Chem.*, 1963, **40,** 18—30).—Yields of over 50% of dry yeast (based on the carbohydrate content of the barley used) were obtained in continuous fermentation in a 20-l. Waldhof fermenter. Studies on the effects of rate of feeding and aeration, concn. of N and P, and of re-use of spent beer on the yield and protein content of the yeast (*Candida utilis* NRRL Y-900) are reported. Several other yeasts were also examined, but gave inferior results. (10 references.) E. C. APLING.

Rice quality factors. VIII. Physico-chemical characteristics of starch and its fractions : their changes on ageing. E. Primo, A. Casas, S. Barber and C. Benedito de Barber. **IX. Influence of protein fractions on the cooking quality.** E. Primo, A. Casas, S. Barber, C. Benedito de Barber, J. Alberola and F. Piñaga (*Rev. Agroquim. Technol. Aliment.*, 1962, **2,** 343—353, 354—359).—VIII. Changes in the I-binding characteristics (measured by potentiometric titration and by spectrophotometry) and limiting η of the separable starch and its amylose and amylopectin fractions during storage of rice were studied in relation to accompanying improvements in cooking quality. For the three varieties under test limiting η of the starch and its amylose A_1 fraction reduced and that of the amylopectin fraction increased with improvement in cooking quality. (21 references.)

IX. Determinations of the proportions of albumin, globulin, prolamine and glutelin in the protein of ten varieties of white rice are reported. Glutelin was present in higher proportion than the other fractions, with significant varietal differences which showed positive correlations with total protein, protein in the outer layers of cooked grains and consumer preference ; negative correlation with η of the cooked paste, but no correlation with cohesiveness of the cooked grain. The results suggest that glutelin plays an important rôle in the cooking behaviour of rice. E. C. APLING.

Biochemical and nutritional investigation on rice and rice products of East Pakistan. I. Differential study of the change of the size of the paddy and rice and photomicrographic investigations of the alteration of starch constituents due to parboiling. M. Q. Khuda, H. N. De and M. Rahman. **II. Differential autoamylolytic activity of parboiled and unboiled rice for evaluation of their storage and cooking character.** M. Q. Khuda, H. N. De and J. C. Debrath (*Pakist. J. sci. industr. Res.* 1962, **5,** 238—246, 247—250).—I. Autoamylolytic activity persists in unboiled rice. This may be taken as an explanation for longer storage life and better working properties of parboiled rice. (12 references.)

II. Parboiling results in a change in length and dia. of a paddy. Photomicrographic studies have shown that this change is associated with the degradation of the starch granules. (10 references.)
A. JABBAR.

Chemical differentiation of proteins of wheat and rye. VI. Detection and identification of a glycoprotein in wheat and rye flour. M. Rohrlich, W. Essner and I. Lichtenfels (*Z. LebensmittUntersuch.*, 1963, **119,** 118—123).—In continuation of previous work (cf. *ibid.*, 1962, **116,** 101), the presence of a glycoprotein is confirmed in certain fractions obtained by the column-chromatography of aq. extracts of the flours. The solution of the glycoprotein, freed from dialysable material, contains no free amino-groups or coagulable protein, and is shown by electrophoretic analysis to be practically homogeneous at pH >4·6 ; on hydrolysis with dil. HCl it yields coagulable protein, pentoses and hexoses. The amino-acid composition of the protein constituent has a proline : glutamic acid ratio of 0·7 which exceeds the corresponding ratios for glutenine, gliadin and gluten. (15 references.) P. S. ARUP.

Isolation of endosperm protein and aleurone cell contents from wheat and determination of their amino-acid composition. D. J. Stevens, E. E. McDermott and J. Pace (*J. Sci. Fd Agric.*, 1963, **14,** 284—287).—Endosperm protein and aleurone cell contents were isolated from flour by air classification and differential centrifugation in non-aq. media. In the isolated protein fraction the proportion of the glutenin/gliadin group of proteins is higher than in the parent flour. This partial segregation seems to occur in the air-classification step. The protein isolated from Manitoba flour on comparison with that of Hybrid 46 showed that the overall amino-acid composition of the two types is closely similar. The overall amino-acid composition of the protein in the aleurone cells is quite different, being much closer to the composition of some of the sol. fractions which have been extracted from flour. It has a relatively high arginine content. (16 references.) E. M. J.

Trypsin inhibitor in wheat flour. E. Mitchell Learmonth and J. C. Wood (*Cereal Chem.*, 1963, **40,** 61—65).—Aq. extracts of wheat flour inhibit the proteolytic action of trypsin on a gelatin substrate. The inhibitor is present in wheat flours from widely different sources, is not attributable to chemical treatment or additives, and is not associated with the bran or germ fraction. A gelation test for trypsin activity is described. E. C. APLING.

Free fatty acids of wheat flour : their rôle in simple flour–water mixtures. W. R. Morrison (*J. Sci. Fd Agric.*, 1963, **14,** 245—251).—A direct quant. study of the changes which occur in flour lipids during aerobic mixing is made ; a system of lipid extraction and analysis of flour–water mixtures is described. Aerobic mixing causes loss of free fatty acids (FFA) within 10 min. but no loss of other P-free lipids. It is suggested that the FFA losses are due to lipoxidase oxidation of essential fatty acids and concurrent enzymic oxidation of all FFA. The same pattern of FFA oxidations was also observed in lower grades of flour from the same type of grist. (48 references.) E. M. J.

Brabender Quadruplex and commercially milled flours compared. R. K. Bequette and R. B. Potts (*Cereal Sci. Today*, 1962, **7**, 354—355, 358).—The moisture, ash and protein contents, and farinograph adsorption, peak, stability and valorimeter values of 21 flours produced by a Brabender Quadruplex laboratory mill, were compared with those of unmalted, commercial, straight-grade flours milled from the same wheats. Some differences were observed. The laboratory unit can be used to test wheat mixes before commercial use provided that the relationship between the properties of the flours from the two mills is first determined.
S. G. AYERST.

Measuring the oil-binding characteristics of flour. W. C. Shuey, O. S. Rask and P. E. Ramstad (*Cereal Chem.*, 1963, **40**, 71—78).—The affinity of flours for oils generally rises with increased protein content, but is also affected by starch properties, as shown by a marked increase in the oil-binding capacity of wheat starch following bleaching with chlorine. Two methods for the measurement of oil-binding capacity are described, depending on (*a*) centrifuging a flour–oil–water suspension in a Babcock bottle and measuring the vol. of uncombined oil, and (*b*) Amylograph measurements on a flour/mineral oil slurry. Comparable oil-binding measurements are obtained regardless of whether the oil used is a saturated or unsaturated triglyceride oil or a hydrocarbon oil. E. C. APLING.

Indigenous starches of Pakistan. IV. Comparative photomicrographic investigations of the digestive disintegration of starch granules. M. Q. Khuda, H. N. De and M. Rahman (*Pakist. J. sci. industr. Res.*, 1962, **5**, 251—256).—Salivary digestion of heated swollen granules of various indigenous starches was studied by following the discharge of the iodine-stained blue colour on a microscope slide. With potato, cassava and wheat, the walls of the granules are first ruptured and the digestion proceeds towards the centre. In banana the saliva enters the centre through some pores and action proceeds in the reverse direction. With shati the enzyme penetrates through a hole at the hilum end (formed by swelling during boiling) and towards the opposite end.
M. H. KHUNDKAR.

Colorimetric determination of amino-nitrogen in maize syrups. B. L. Scallet (*Stärke*, 1963, **15**, 50—52).—The method is based on that of Folin for determination of amino-N in blood. Maize syrup liquor (≡2·5 g. dry substance) samples were used, aq. Na_2CO_3, water and Na naphthaquinone-4-sulphonate were added and the mixture set aside in darkness, AcOH + AcONa were added + Na thiosulphate, the solution made up to vol. and read immediately in a photoelectric colorimeter. A standard curve for D-glutamic acid was prepared. About half of the N in the maize syrup liquor was present as amino-N. The method was checked against the modified Harral–Van Slyke method and gave slightly but consistently lower results. E. M. J.

Specific surface of flour and starch granules in a hard winter wheat flour and its five subsieve-size fractions. Rezsoe Gracza and S. I. Greenberg (*Cereal Chem.*, 1963, **40**, 51—61).—Measurements, by the Whitby centrifugal sedimentation method, of the specific surface of the starch separated by proteolysis from a Minnesota hard winter wheat patent flour and its fractions are reported. Specific surface of the parent flour was 0·1249 m.²/g., and that of the separated starch (corrected for swelling factor) was 0·7272 m.²/g., (ratio 4·5 : 1). In the subsieve-size fractions the ratio of starch granule surface to total flour surface varied from 1·34 (finest fraction) to 5·45 (coarsest fraction), indicating the increasing contribution of the starch granules to the total surface on reduction of flour particle size. (16 references.) E. C. APLING.

Hydration of starch. VII. Effect of removal of water from starch on the rheological behaviour of its dispersion. F. Schierbaum and K. Täufel (*Stärke*, 1963, **15**, 52—56).—Samples of potato, wheat, maize and rice starch were dried to different water contents in a drying cupboard or in a vac. at room temp. The pasting properties were measured after dispersion in boiling water or N-NaOH at room temp. The η as measured by the falling ball viscometer, decreased in potato starch containing <10% and in the three cereal starches containing <5%, i.e., only during dehydration of the last water which is very firmly bound. (18 references.) E. M. J.

Comparison of viscosity measurements in starches and starch products with different measuring apparatus. H.-H. Grun (*Stärke*, 1963, **15**, 60—65).—Data are given on (*a*) native starches with potato starch as representative, (*b*) starches prepared in various ways, (*c*) dextrin. The η is measured by six different procedures including the use of the Brabender Viscograph, the Brabender Plastograph and the Brookfield Viscosimeter. It is shown that before accurate η measurements can be made, the starch must be prepared under constant mechanical conditions, e.g., rolled dried starch, or dextrin, and results will be valid only for the product concerned. E. M. J.

Effect of amylopectin on the properties of starch gels. H. J. Cornell (*Stärke*, 1963, **15**, 43—49).—Variation in properties of wheat starch (**I**) caused by addition (of, e.g., 1—30%) of amylopectin (**II**) prepared from potato starch is discussed. Because of the complexity of its mol. **II** can act as an effective preventive for intermol. H bonding of amylose mol. and assist in the waterholding properties of the amylose and the starch gel in general. This would explain the low water seepage from gels prepared from mixtures of **II** and **I**, the reduced tendency to form tough skins and the improvement in η stability on storage of such gels. The mechanism of the process of retrogradation is discussed. (14 references.) E. M. J.

Treatment of rye for the inactivation of α-amylase. G. Ström and O. Quist (*Getreide u. Mehl*, 1963, **13**, 7—12).—The biochemical basis of the problem of excessive α-amylase activity in the baking of rye bread is reviewed and the results obtained in experiments in the reduction of α-amylase activity by heat treatment of the grain are described. The grain was subjected to superheated steam for up to 105 sec. and then rapidly cooled to 30—40° with air and water. The treatment resulted in heat inactivation of a significant proportion of the amylase with consequent improvement in baking quality.
E. C. APLING.

Location and possible rôle of esterified phosphorus in starch fractions. M. W. Radomski and M. D. Smith (*Cereal Chem.*, 1963, **40**, 31—38).—The results of determinations of P on various amylose and amylopectin fractions are reported and discussed. In potato starch there is a sharp difference between the P contents of the amylopectin, intermediate and amylose fractions (0·165%, 0·083% and 0·008% respectively), and a positive correlation was found (seven samples) between the limits of β-amylase conversion and the P content of the amylose. Hydrolysis resulted in concentration of P in the β-limit dextrin, suggesting that esterified P is responsible for the incomplete hydrolysis of amylose by enzymes. Amylolysis (α or β) of waxy maize starch also resulted in concentration of P in the limit dextrin, suggesting an association between esterified P and the branch points in amylopectin. (25 references.)
E. C. APLING.

Determination of chromium and lead in periodic acid solution and dialdehyde starch. L. T. Black, E. B. Lancaster and H. G. Maister (*Cereal Chem.*, 1963, **40**, 66—71).—The colorimetric determination of Cr and Pb (by reaction with diphenylcarbazide and dithizone respectively), following destruction of periodate with Na_2O_2 or starch with H_2SO_4 is described. E. C. APLING.

Utility of the tetrazolium method for the evaluation of the baking value of wheat. R. Belderok (*Getreide u. Mehl*, 1963, **13**, 1—7).—Response of wheat to staining with tetrazolium (cf. Seibel, *Getreide u. Mehl*, 1958, **8**, 25—28) is proposed as a rapid quality test for samples of milling wheat. Staining % is reduced by heat damage, microbial attack, sprout and by prolonged storage, and on samples damaged by heat or micro-organisms the results show a good general relationship with baking quality. Staining % as low as 70 may result from sprout or long storage without necessarily indicating loss of baking quality, but % <60 indicate significant damage by heat or micro-organisms. (15 references.) E. C. APLING.

Modification of flour proteins by dough mixing: effects of sulphydryl-blocking and oxidising agents. D. K. Mecham, E. G. Cole and H. A. Sokol (*Cereal Chem.*, 1963, **40**, 1—9).—The rate and extent of conversion of wheat protein insol. in 0·01N-acetic acid to extractable form, which occurs in dough mixing, is increased by addition of the sulphydryl-blocking reagent *N*-ethylmaleimide (**I**) to doughs. KIO_3 shows a similar, but smaller, effect. The increase in extractable protein due to additions of **I** occurs during mixing development of doughs and before the effect of **I** is evident on the Farinograph mixing curve. (16 references.) E. C. APLING.

Continuous dough processing. G. W. Trum and E. G. Snyder (*Cereal Sci. Today*, 1962, **7**, 344—346, 348, 358).—The specification and quality requirements of flour, sugar, milk and milk products, and shortenings, in relation to the techniques of continuous dough processing are discussed. Modifications of laboratory equipment to simulate the conditions of continuous dough processing are described. S. G. AYERST.

Inclusion of various flour types in the production of loaf bread. E. Drews (*Brot u. Gebäck*, 1963, **17**, 7—10).—Statistics of flour usage for breadmaking in the various states of W. Germany during 1962 are presented. Changes in the pattern of use compared with that in the previous 2 years are slight, but a slight trend towards greater employment of wheat and rye flours of lower ash content is revealed. E. C. APLING.

Effect of L-arabinose and D-xylose on dough fermentation and crust browning. J. G. Ponte, jun., S. T. Titcomb and R. H. Cotton (*Cereal Chem.*, 1963, **40**, 78—86).—L-Arabinose and D-xylose inhibit gas production in flour/water doughs, but show little effect in the

presence of 10% of D-glucose. Baking tests, employing 2·5 and 5% of the pentoses in a formula containing 5·5 and 8% of total sugar, showed the pentoses to have no effect on loaf vol., but to increase proof time, reduce crumb brightness and to produce a markedly thicker and darker crust. (10 references.) E. C. APLING.

Evaluation of lactase preparations for use in breadmaking. Y. Pomeranz and B. S. Miller (*J. agric. Fd Chem.*, 1963, **11**, 19—22).—β-Galactosidases from various sources and their application to breadmaking are studied. Assay of enzymes from yeast, fungi and bacteria is performed under conditions comparable with those found in panary fermentation. The enzyme is incubated with lactose and a yeast suspension in N base medium added. Hydrolysed sugar allows fermentation and the CO_2 liberated is measured manometrically. Effects of digestion time and temp. and substrate concn. and pH on activity are measured. Gas evolution is directly related to activity of lactases. The method is used to evaluate enzymes from other sources. (18 references.) J. B. WOOF.

Characteristics of yolk solids affecting their performance in cake doughnuts. I. Effects of yolk type, level and contamination with white. II. Variability in commercial yolk solids. M. L. Bean, T. F. Sugihara and L. Kline (*Cereal Chem.*, 1963, **40**, 10—18, 38—50).—I. Comparison of the effects of liquid yolk and spray-dried yolk on the characteristics of a commercial cake-doughnut mix and on the resulting cooked goods, showed significant differences only in batter fluidity. At a given level of yolk solids, liquid yolk consistently yielded more fluid batters than spray-dried yolk, but showed no significant advantage in eating quality or staling rate. Egg-white solids have little effect on batter fluidity or doughnut vol., but decrease fat absorption and reduce eating quality. Normally occurring levels of contamination with egg-white in commercial yolk show only slight effects.

II. A study of the relationships between the effects of yolk solids on batter fluidity and the changes occurring during processing or on storage at elevated temp. is reported. Batter η increases with reduction in protein solubility and increase in reconstitution η of the dried yolk. Slight variations in these factors can be compensated by increasing the water content of the batter, but major reductions in protein solubility can not. A turbidimetric procedure is proposed for the rapid routine measurement of protein solubility. (10 references.) E. C. APLING.

Microstructure of baked products and doughs. R. G. Gerdes and R. E. Simson (*Food Technol., Aust.*, 1963, **15**, 184—185, 187, 190—191).—The difficulties of preparing baked products and doughs for microscopical examination, owing to dispersion or distortion during sectioning, are discussed. Advantages offered by embedding in paraffin wax, polyethylene glycol or Carbowax, gelatin, methyl methacrylate and Araldite are described. Freeze sectioning for doughs is also described. (15 references.) E. M. J.

Leavened baked products. Howards of Ilford Ltd. (Inventors: E. W. M. Fawcett and R. H. Lock) (B.P. 891,458, 24.1.57).—The properties of a leavened baked product (buns, cakes, biscuits, pastry, etc.) are improved by incorporating with the dough an emulsifying agent comprising a partial ester of sorbitol with a saturated or unsaturated fatty acid of 8 C. A typical emulsifier is sorbitol monolaurate or mono-oleate. F. R. BASFORD.

Baked food products. Howards of Ilford Ltd. (Inventors: F. Schild and R. J. Wicker) (B.P. 890,205, 18.4.58).—A dough or baking mixture, which affords a baked product (especially bread or other leavened or fermented baked food such as buns, cakes, biscuits) of improved properties, contains up to 5 wt.-% of a partial sucrose ester of a fatty acid of <18 C (oleic, lauric, stearic or palmitic acid) as emulsifying agent. F. R. BASFORD.

Sugars and confectionery

Separation and identification of carbohydrates from coconut by circular paper chromatography. A. S. Bhown (*Indian J. appl. Chem.*, 1962, **25**, 97—98).—With n-butanol–acetone–water (2 : 7 : 1) as solvent and aniline–diphenylamine–phosphoric acid as detecting agent, sucrose, galactose and glucose have been detected in coconut milk and raffinose, sucrose, glucose, fructose, rhamnose and an unidentified sugar have been detected in the endosperm of the coconut. W. ELSTOW.

Possible glucose/glycine browning intermediates and their reactions with sulphites. D. J. McWeeny and H. S. Burton (*J. Sci. Fd Agric.*, 1963, **14**, 291—302).—At 50° and initial pH 5·5—6·0, browning of aldose/amino systems via the formation and reaction of hydroxymethylfurfural (**I**) is very slow. The glycine-dependence of browning reactions of monofructose glycine, difructose glycine (**II**) and 3-deoxyhexosone (**III**) was shown. These reactions are not accelerated by adding glucose. Addition of limited amounts of bisulphite to model mixtures containing **I**, **II**, **III** showed that bisulphites do not react directly with these compounds in retarding browning. One of the compounds with which bisulphites react in retarding browning may be 3,4-unsaturated 3,4-dideoxyhexosone which is readily derived from **III**. (15 references.) E. M. J.

Specifications of honey. S. N. Mitra, P. N. Sengupta, T. V. Mathew and A. K. Dhar (*J. Instn Chem. India*, 1962, **34**, 267—269).—The following standards for honey were laid down by the 'Prevention of Food Adulteration Rules, Government of India, Ministry of Health Publication, New Delhi, 1959'. Max. water 25, ash 0·5, sucrose 10% and min. reducing sugars 60%, Fiehe's test should be negative. Of 30 samples (*Apis indica* variety) analysed the results generally supported the standard fixed for honey under the Food Rules. I. DICKINSON.

Detection of methyl anthranilate in Spanish orange-flower honey by thin-layer chromatography. J. Deshusses and A. Gabbai (*Mitt. Lebensm. Hyg., Bern*, 1962, **53**, 408—411).—A light petroleum extract of the diluted honey is concentrated to a small bulk by spontaneous evaporation; portions of the residual solution are chromatographed (with reference substances) on thin layers of Kieselgel G (containing 5% of rice starch) with hexane–AcOEt (9 : 1) as ascending solvent. After ~1 h. the spots can be located in u.v. light. Me and Et anthranilates (but not Me methylanthranilate or β-napththylmethylketone (**I**) give a yellow coloration when sprayed with *p*-dimethylaminobenzaldehyde in N-HCl. **I** (but not the other substances) gives an orange coloration when sprayed (on another chromatogram) with 2,4-dinitrophenylhydrazine in dil. HCl. The R_F values of the above-mentioned substances are given. Spanish honey contains ~0·4—0·5 p.p.m. of Me anthranilate. P. S. ARUP.

Chemistry of protopectin: a critical review of historical data and recent developments. M. A. Joslyn (*Adv. Fd Res.*, 1962, **11**, 1—107).—The most likely explanation of the relative insolubility of protopectin is that it exists as a polygalacturonide in which the hydroxyl groups on C atoms C4 and C5 are masked by glycosidic and ring formation and the carboxyl group on C1 is either free, esterified with methanol, or esterified with araban, galactan or other polysaccharide. The OH on C atoms C2 and C3 may be free, esterified with acetyl groups, or linked by ether-like linkage to polysaccharides or lignins. Non-uronide sugars occur probably in the main and in branched chains. Side chains may be formed by occasional ester linkage between carboxyls and free hydroxyls of polysaccharides, by hemiacetal linkage between the terminal functional reducing group of a polysaccharide chain and the free hydroxyl of polygalacturonide, or by ether linkages between hydroxyls of polysaccharide and polygalacturonide. The effects of controlled oxidation and acetylation are discussed. (300 references.) E. M. J.

Utilisation of natural polysaccharide gums in the food industry. M. Glicksman (*Adv. Fd Res.*, 1962, **11**, 109—200).—The following are discussed: Seaweed extracts, agar, carrageenan, alginates, furcellaran; plant seed gums, locust bean gum, guar gum; tree exudates, gum arabic, gum tragacanth, gum karaya. The areas in the food industry where these natural polysaccharide gums have been and are being used, are reviewed to show how the individual properties of each gum are used in particular applications suited to the inherent characteristics of the gum, viz., thickening, stabilising, gelling and imparting textural properties to individual food products. (266 references.) E. M. J.

Purification of sugar. Rohm & Haas Co. (B.P. 889,949, 19.5.58. U.S., 5.6.57 and 6.1.58).—Sugar solution contaminated with org. impurities derived from the natural source of sugar (including products not normally suitable for human consumption) is purified by passing it through a bed of ion-exchange resin particles in salt form in at least that half of the bed which extends interiorly from the effluent end (substantially the remainder of the resin particles in that portion of the bed which extends interiorly from the influent end having a different form of exchangeable ions such as are derived from a regeneration treatment with a solution of a non-electrolyte); then eluting the bed with water; and collecting a selected fraction of the effluent sugar solution which contains a higher ratio of sugar to org. impurities than does the input liquor. The used bed may be regenerated with water. Flow sheets are appended. F. R. BASFORD.

Purification of sugar solutions. American Sugar Refining Co. (B.P. 889,823, 13.6.60. U.S., 22.6.59).—A continuous process for the purification of sugar solutions is claimed. Apparatus is figured. F. R. BASFORD.

Purification and decolorisation of pre-treated technical sugar solution. Sugar Chemical Co. Etablissement (B.P. 889,647, 22.9.59.

Switz., 23.9.58).—A technical sugar solution, which has already been partly purified by treatment with lime and CO_2 and which still contains sol. impurities, is further purified by successively subjecting to treatment with anion-exchange material (to replace at least part of the anions present in solution for anions which form difficultly sol. compounds with alkaline-earth metal cations); with cation-exchange material (to replace cations present by NH_4 ions); and then with alkaline-earth metal hydroxide (to convert anions now present into alkaline-earth metal compound and to liberate NH_4 present).
F. R. BASFORD.

Confectionery coating compositions. E. F. Drew & Co. Inc. (B.P. 891,205, 3.5.60. U.S., 8.5.59).—A confectionary coating composition of improved properties (e.g., no blooming) comprises a hard fat containing in dispersion 0·5—10 wt.-% of soap-free sugar esters of fatty acids of 10—24 C (and containing 1—8 fatty acid radicals per mol.); e.g., sucrose di- to octa-stearates.
F. R. BASFORD.

Fermentation and Alcoholic Beverages

Preliminary note. Cold desulphitation of grape must. E. Primo, B. Lafuente and F. Gasque (*Rev. Agroquím. Technol. Aliment.*, 1962, **2**, 363—366).—The sulphite content of must was reduced from 1200 p.p.m. to from 400 to 700 p.p.m. by a single passage through a bed of ion-exchange resin (Zerolit-E) previously saturated with tartaric acid. Using two columns in series the sulphite content was reduced to 160 p.p.m. in the first bed-vol. of effluent, rising to 370 p.p.m. after 10 bed-vol. Partial oxidation of the must with oxygenated water to an SO_2 content of 250 p.p.m. raised the SO_4^{2-} concn. to 3·75 g. per l. (permitted max. 2 g. per l.), but one passage through a tartaric acid–ion exchange column reduced the SO_4^{2-} concn. to $<1·5$ g. per l. even after the passage of 20 bed-vol., but without any further reduction of the SO_2 content of the must.
E. C. APLING.

Acidity and wine yeasts. B. Inigo Leal and F. Bravo Abad (*Rev. Cienc. apl.*, 1962, **16**, 481—485).—This is the start of a series of articles concerning the fermentation of grapes. In this first article are examined the problems of the org. acids of the ferment. Materials and methods used for the qual. determination of the acids are given as well as the chromatographic technique for determining the quant. org. acidity. (13 references.) B. F. FULLAN.

Sensory examination of four organic acids added to wine. C. S. Ough (*J. Fd Sci.*, 1963, **28**, 101—106).—Difference thresholds for four org. acids, determined at a reference concn. of 0·615 (as g. of tartaric acid (**I**)/100 ml.) were $\pm$0·13 g./100 ml. for **I**, $\pm$0·18 g./100 ml. for citric acid (**II**), $\pm$0·10 g./100 ml. for fumaric (**III**) and $\pm$0·13 g./100 ml. for adipic (**IV**). When the acids were compared directly by addition of equal molar amounts of each to a wine (equal to 0·20 g. of **I**/100 ml. on a mol. basis), **II** was judged most sour, **III** and **I** about equal and **IV** the least sour. Trends were in favour of **II** and **I** over **III** and **IV**. E. M. J.

Rapid determination of tartaric acid in wine. J. Schneyder and G. Pluhar (*Mitt. Wein u. Obstbau, Wien*, 1963, **13A**, 40—43).—The acid is precipitated as KH tartrate by adding to the sample (10 ml.) AcOH, aq. KCl (nearly saturated) containing small amounts of Rochelle salt and K oxalate, and finally EtOH. (The Rochelle salt is added to promote pptn. and to compensate for the KH tartrate remaining in solution, and the K oxalate to prevent the Ca being precipitated as tartrate.) The mixture, in a centrifuge tube, is kept at 8° during 2 h., the pptn. being aided by scratching. The ppt. is collected and washed with dil. aq.-ethanolic KCl by centrifugation, after which it is dissolved in water and titrated with 0·1N-NaOH with bromothymol-blue as indicator. Differences between theoretical results and recoveries and between the results by this method and those by the Halenke and Möslinger method are within $\pm$0·2 g./l. P. S. ARUP.

Oxidation processes in wine. VI. Oxygen uptake by sparkling wine during preparation by the tank-fermentation process. E. Kielhöfer and G. Würdig (*Mitt. Wein u. Obstbau, Wien*, 1963, **13A**, 18—35).—A method for collecting and measuring the gases in the headspace of the bottled wine is described; the gases are analysed by gas-chromatography. The dissolved O_2 is determined by the modified Tödt method (cf. *Weinwissenschaft*, 1962, **17**, 217). The uptake of O_2 by tank-fermented wine during filtration can largely be avoided by the use of CO_2- instead of air-pressure, but a marked increase in uptake during the course of bottling is unavoidable; this is due to the accumulation of air from the bottles in the pressure chamber. The initial total O_2 content of 30 bottles was 5—20 mg. per 1-l. bottle, chiefly in the headspace. The amount of free SO_2 required to counteract the undesirable effects of the O_2 would be prohibitive, but this object could probably be achieved by the combined use of SO_2 and ascorbic acid in suitable proportions. The presence of O_2 (and its effects) constitutes the only known difference between tank-fermented wine and wine prepared by the champagne process. Wine which has been partly fermented in bottles and then transferred to tanks for further treatment (instead of disgorging) should (on account of the uptake of O_2) not be described as 'bottle fermented'. P. S. ARUP.

Analysis of wine. IV. Treatment of wine with cation-exchangers. W. Diemair and G. Maier (*Z. LebensmittUntersuch.*, 1963, **119**, 123—135).—The literature of the subject is reviewed. In laboratory experiments on the action of four sulphonated resins on natural and synthetic wines, the best results as regards removal of K^+ and min. interference with the amino-acid content are obtained with Lewatit S-115, partly in the H^+ form and partly in the Na^+ form. Whilst the amounts of protein removed are small or negligible, the amounts of amino-acids and NH_4^+ removed average ~40% of the total content. Some of the amino-acids (especially the basic compounds) are not removed from the resin by the normal regeneration with HCl and NaCl; these must first be removed from the resin by treatment with aq. NH_3 and further with HCl. Whilst Ca^{2+} and Mg^{2+} are completely removed from a synthetic wine containing no citric acid, they are only partly removed from natural wine. (31 references.) P. S. ARUP.

Peonidin-3-monoglucoside in [*Vitis*] *vinifera* grapes. R. F. Albach, R. E. Kepner and A. D. Webb (*J. Fd. Sci.*, 1963, **28**, 55—63).—The pigments of most of the red wine varieties of *V. vinifera* show eight discrete pigment bands when paper chromatographed. With *V. vinifera* var. Freisa, band 4 is present in high relative concn. This has a rose or red orange colour. By hydrolysis, partial hydrolysis and alkaline microdegradation of the pure pigment it was identified as peonidin-3-monoglucoside. (20 references.) E. M. J.

Micromethod of determination of hexachlorocyclohexane in wine. Experimental study of the passage of this insecticide into the wine. P. Castel, G. Gras and J. Michel (*Ann. pharm. franç.*, 1962, **20**, 777—795).—Standard methods of determining hexachlorocyclohexane (**I**) are discussed, and sources of error in Schechter and Hornstein's method (*Analyt. Chem.*, 1952, **24**, 544) are investigated. Further modifications are proposed to Zeumer and Neuhaus' modifications to this method (*Chem. Ztg.*, 1953, **77**, 105). Interferences and suitable solvents for extraction of **I** from wine are studied. The most suitable solvent for extraction of **I** from the wine is found to be CH_2Cl_2, and a procedure for this is described. The determination is effected by dechlorination of **I** with Zn and AcOH, and distilling the benzene formed into nitrating acid in a modified absorber. The *m*-dinitrobenzene formed is determined colorimetrically in presence of methylethyl ketone and KOH at 565 mμ. The effect of the presence of **I** on the fermentability, taste and odour of the wine is studied and it is shown that the max. concn. of **I** to be expected in the worst conditions is 3·76 p.p.m., while the taste is undetectable below 6 p.p.m. The fermenting properties are unaffected. The possible public health implications are discussed. (43 references.)
E. J. H. BIRCH.

Question as to reliability of analytical and organoleptic tests for detecting watering of wine. B. Weger (*Mitt. Wein u. Obstbau, Wien*, 1963, **13A**, 36—39).—Analyses are given of eight genuine wines. A large proportion (~50%) of the samples show results, especially for glycerin % 100 per 100 g. of EtOH and for sugar-free extract, which are very close to or under the prescribed limits (6% and 15 g./l. in the above instances). Strict adherence to these and other limits (official in Austria and other countries) will result in unjust prosecutions. Members of tasting panels should be kept ignorant of suspicion attaching to any of the samples.
P. S. ARUP.

Gas-chromatographic determination of trichloroethylene in pastes. W. Kleber and P. Schmid (*Brauwissenschaft*, 1963, **16**, 36—39).—The basic principles of the gas-chromatographic technique are described, and its possibilities in connexion with brewing chemistry are pointed out. Operational details are given by which 0·005% of residual C_2HCl_3 (**I**) can be detected in solution in Et_2O. In prep. for the determination, the sample is shaken with Et_2O and water; the Et_2O phase containing the **I** is distilled to remove most of the Et_2O, and the residue is then steam-distilled; the phases of this (second) distillate are mixed and then allowed to separate. The Et_2O solution of **I** thus obtained is dried with Na_2SO_4 and used for the determination in a Gasofract Junior apparatus with a column of Silicongummi SE 30 on Chromosorb. Planimetric measurements in comparison with results from solutions of **I** in Et_2O of known concn. give a reproducibility within 1·5%. P. S. ARUP.

Continuous malting of grain. Dominion Malting (Ontario) Ltd. (Inventors: W. E. Stoddart, F. R. Graesser, D. L. Thompson and N. Kier) (B.P. 891,289, 28.8.58).—A process for malting of grain comprises continuously forming and moving a bed of grain in a

predetermined path; subjecting the moving grain bed to intermittent periods of water spray, periods of humid aeration, and intervening periods of rest (all at 10—38°); and finally moving the bed through a drying zone at high temp. Apparatus is figured and claimed. F. R. BASFORD.

Fruits, Vegetables, etc.

Controlled atmosphere storage of New Zealand fruit: Effects of low oxygen and varying carbon dioxide levels. C. A. S. Padfield (*N.Z. J. agric. Res.*, 1962, **5**, 485—500).—Apples and pears were stored in controlled atm. containing 2 to 3% O_2 and 3 to 5% CO_2 and examined for condition after periods of 4 to 8 months at about 32°F and again after 1 to 2 weeks at room temp. Of the New Zealand grown varieties examined, Dougherty, Frimley Beauty, Golden Delicious, Rome Beauty and Sturmer Pippin benefited from this type of storage but Ballarat, Delicious, Granny Smith and Kidd's Orange Red apples and Williams' Bon Chretien pears did not. (14 references.) W. ELSTOW.

Extraction of pectins from apple marc preparations. M. A. Joslyn and H. Deuel (*J. Fd Sci.*, 1963, **28**, 65—83).—Marked differences were found in extractability of pectins present in marcs prepared in various ways from the same lot of apples. When enzymic browning occurred, the solubility of the pectins decreased, but the composition of the extracted pectins did not alter. Oxidation of apple tissue constituents and drying of apple pomace reduced the solubility of pectins. With different solvents yields of pectins differed. The pectins extracted were precipitated with alcohol and characterised by determining the anhydrogalacturonide content and degree of esterification. (51 references.) E. M. J.

Liquid–solids separation, a problem in processed apple sauce. V. Toldby and R. C. Wiley (*Proc. Amer. Soc. hort. Sci.*, 1962, **81**, 78—92).—Lyophoresis (the extent of separation of solids and liquids in apple sauce) was closely related to the viscosity of the free liquid, which in turn was influenced by the pectin and starch contents and pH of the fruit. Addition of acids increased lyophoresis in sauces made from immature apples and reduced it in those made from mature apples. Addition of 0·5—0·6% of a 1 : 1 mixture of high and low methoxyl pectins and adjustment of pH to 3·4 before packing reduced lyophoresis in apple sauce made from mature apples and improved the appearance and quality of the product. A. H. CORNFIELD.

Volatile esters of Bartlett pear. II. W. G. Jennings and R. K. Creveling (*J. Fd Sci.*, 1963, **28**, 91—94; cf. J.S.F.A. Abstr., 1962, i, 235).—By gas-chromatographic studies, i.r. and u.v. spectroscopy, and C–H balance, the C-10 unsaturated acid previously reported to be a major constituent of pear essence hydrolysates was identified as 2,4-decadienoic acid. An alcohol contaminant was identified (2-ethylhexanol-1); freshly prepared essence hydrolysates indicated the presence of ethyl, n-propyl and probably n-amyl esters. E. M. J.

Fungi associated with softening of bisulphite-brined cherries. J. C. Lewis, C. F. Pierson and M. J. Powers (*Appl. Microbiol.*, 1963, **11**, 93—99).—Softening of sound $Ca(HSO_3)_2$-brined cherries was induced fairly rapidly by brining them with cherries rotted with *Aspergillus niger*, *Cytospora leucostoma* and *Penicillium expansum*; this effect was not obtained with *Alternaria* sp., *Aspergillus oryzae*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Cladiosporium* sp., *Mucor racemosus*, *Rhizopus stolonifer* and *Sclerotinia fructicola*. This softening was correlated with the presence of a bisulphite-stable polygalacturonase. (13 references.) C. V.

Pectin content of raisins. M. A. Brown, J. R. Woodward and F. De Eds (*J. Fd Sci.*, 1963, **28**, 64).—The content of pectin substances in Thompson seedless raisins: (*a*) laboratory (mg./g.), (*b*) commercial (mg./g.). (*a*) water-sol. pectin 2·25—2·38; total pectic substances 9·0—10·4. (*b*) water sol. pectin 2·14—2·20; total pectic substances 8·2—9·6. E. M. J.

Rapid hydration of dried fruits. F. S. Nury, H. R. Bolin and J. E. Brekke (*Food Technol.*, 1963, **17**, No. 3, 98—99).—Hydration procedures by steam and boiling water for prunes, figs and raisins are described: the process is followed by short immersion in cold water. The rate and extent of hydration are greater by this method, heat damage is less. E. M. J.

Colour studies on processed dried fruits. F. S. Nury and J. E. Brekke (*J. Fd Sci.*, 1963, **28**, 95—98).—A colour extraction procedure and studies of rates of change in colour and of apparent activation energies for darkening or browning of processed raisins are reported. Data are given on 1 lb. bags of raisins and golden bleached (sulphured) raisins held at 50, 70 and 90°F for periodic sampling. Such information may be useful as a criterion of quality and in predicting shelf-life of commercially processed and packaged golden bleached and sun-dried raisins. (10 references.) E. M. J.

Preparation and quality evaluation of processed fruits and fruit products with sucrose and synthetic sweeteners. D. K. Salunkhe, R. L. McLaughlin, S. L. Day and M. B. Merkley (*Food Technol.*, 1963, **17**, No. 2, 85—91).—Cherries, apricots, peaches processed in 40% sucrose were highly acceptable to taste panels; acceptability decreased progressively as the concn. of the cover syrup increased to 50 or 60%. The acceptability of cover syrups containing sucrose 10% + Ca cyclamate 0·1% or sucrose 10% + Na saccharin 0·02% was equal to that of the 40% sucrose. Low-calorie jellies and syrups (apple, sour cherry, grape and black raspberry) were preferred when compared with those sweetened with 65% sucrose. (16 references.) E. M. J.

Non-volatile acids of blueberries. P. Markakis, A. Jarczyk and S. P. Krishna (*J. agric. Fd Chem.*, 1963, **11**, 8—11).—Acidic aq. extracts of ripe and unripe fruit are purified by adjusting pH and Pb pptn. and, after concentration, subjected to gradient elution from Dowex 1 resin. Fractions were identified by comparison of retention vol. and R_F with authentic acids and determined quant. by titration. Glutamic, aspartic, shikimic, quinic, galacturonic, glyceric, glycollic, succinic, glucuronic, citramalic, malic, citric, malonic, chlorogenic, caffeic, phosphoric and oxalic acids were found. Malic, chlorogenic and phosphoric increased on ripening whilst citric and quinic decreased. (21 references.) J. B. WOOF.

Volatile components of bananas. P. Issenberg and E. L. Wick (*J. agric. Fd Chem.*, 1963, **II**, 2—8).—Isolation, separation and identification of volatile compounds contributing to the odour of ripe Gros Michel bananas are described. Large (10 kg.) samples of bananas are pulped and distilled, the vapour being collected in traps maintained at 0°, −78° and −196° respectively. Samples of the vapour over each of these fractions, together with those over whole homogenate and stripped honogenate for comparison, were subjected to gas chromatography. Repeating the separation on a preparative scale combined with i.r. spectra allowed the identification of eight compounds of which isobutyl acetate and isoamyl butyrate have not previously been reported in bananas. Physical data but no positive identification are given for several other components. (11 references.) J. B. WOOF.

Analytical chemistry of biphenyl. I. S. W. Souci and G. Maier-Haarländer (*Z. LebensmittUntersuch.*, 1963, **119**, 217—222).—The method of Gunther *et al.* for the determination of biphenyl and *o*-phenylphenol on and in citrus fruits (cf. *Analyst*, 1963, **88**, 36) is examined and considered satisfactory. Recoveries of biphenyl added to the fruits (six samples) were +4·1 to −2·5% of the amounts added. Some minor modifications are proposed. P. S. ARUP.

Industrial quality of tomato varieties. II. Suitability for juice and concentrate of some varieties used for canned peeled tomatoes. L. Durán Hidalgo and A. R. Roig Valero (*Rev. Agroquím. Technol., Aliment.*, 1962, **2**, 325—330).—From 12 varieties tested in Spanish Levante the highest yields of juice per hectare were given by the varieties Roma, A.C.E. and Queens. A.C.E. is selected as the most suitable variety for juice and concentrate manufacture. E. C. APLING.

Carob bean sugars. VII. Optimum purification treatments. E. Primo Yúfera, B. Lafuente Ferriols and V. Cortés Navarro (*Rev. Agroquím. Technol. Aliment.*, 1962, **2**, 337—342).—The course of purification of carob bean juice by successive defecation, ion-exchange, decolorisation and final concentration under the optimum conditions previously described for each stage was studied. More than 99% of the original colour and 98—99% of the butyric acid and tannins were eliminated, producing high purity edible syrups in which residuals of butyric acid and tannins were below the organoleptic detection thresholds. (11 references.) E. C. APLING.

Disappearance of endrin residues on cabbage. L. R. Mattick, D. L. Barry, F. M. Antenucci and A. W. Avens (*J. agric. Fd Chem.*, 1963, **11**, 54—55).—An emulsion containing endrin was sprayed on to maturing cabbage in various amounts and samples analysed up to 21 days. Gas chromatography showed that even at high dose rates, only 0·13 p.p.m. remained after this time. J. B. WOOF.

Extraction and cleanup studies for parathion residues on leafy vegetables. C. H. van Middelem, R. E. Waites and J. W. Wilson (*J. agric. Fd Chem.*, 1963, **11**, 56—58).—Effective extraction of insecticide from plant materials often limits the validity of determinations. Blending in benzene followed by tumbling is more effective than tumbling alone for extracting parathion residues and further improvement can be obtained using isopropanol as co-solvent. Additional purification of the extract is achieved by passing through a column containing layers of anhydrous Na_2SO_4 and mixtures of Hyflo Supercel, Florisil and Nuchar C-190-N. J. B. WOOF.

Effect of germination on the fat of the soya-bean. B. E. Brown, E. M. Meade and J. R. Butterfield (*J. Amer. Oil Chem. Soc.*, 1962, **39**, 327—330).—Soya-beans were germinated in the dark at 25° and total dry matter and crude fat were determined on the seedling axis and cotyledon each day for 12 days. The cotyledon crude fat was analysed for free fatty acids, neutral fat and neutral non-triglycerides. The I val. and fatty acid composition of the neutral fat were also determined. Total dry wt. and crude fat decreased with germination time and there was a significant decrease in the oleic acid content of the cotyledon neutral fat. The results are discussed in relation to those obtained by other workers. (27 references.) J. V. Russo.

Soya-bean unsaponifiables: chromatographic separations and characterisation. R. L. Hoffmann, H. A. Moser, C. D. Evans and J. C. Cowan (*J. Amer. Oil Chem. Soc.*, 1962, **39**, 323—327).—Unsaponifiables were extracted from soya-beans by three methods and they were fractionated by liquid–liquid chromatography on silicic acid into three fractions. The three fractions were further analysed by gas–liquid chromatography and thin-layer chromatography. Oxidation led to an overall increase in unsaponifiables with a decrease in the least polar fraction (hydrocarbons). The organoleptic effects of oxidised and non-oxidised unsaponifiables were evaluated by adding them to cottonseed oil. (12 references.) J. V. Russo.

Extraction and precipitation of nitrogenous constituents of dry Navy beans (*Phaseolus vulgaris*). R. J. Evans and M. H. Kerr (*J. agric. Fd Chem.*, 1963, **11**, 26—29).—Investigation of protein extraction from dried beans showed that max. efficiency, as shown by Kjeldahl determinations, was achieved with HCl at pH 1·5 or NaOH above pH 7 or by 1—8% NaCl. Solutions at pH 3·8 gave min. extraction efficiency, removing mainly albumins. Adjusting the pH from 1·5 to 3·8 causes erratic and incomplete pptn. but at this pH all but 27% of the protein extracted at pH 7 is precipitated. Protein from 2% NaCl after dialysis has at least four components as shown by electrophoresis and chromatography on DEAE-cellulose. (22 references.) J. B. Woof.

Non-alcoholic beverages

Effect of processing conditions on the consistency of tomato juice. A. Ephraim, H. C. Mannheim, J. B. S. Braverman and L. Shelef (*Bull. Res. Counc. Israel*, 1962, **11C**, 286—294).—The effects of hot break and mechanical treatment on the consistency of tomato juice and on juice proteins, cells and pectins was studied. Although pectolytic enzyme inactivation tended to increase the η during hot break, a net decrease occurred due to partial denaturation of proteins and cell flattening. Mechanical treatment also caused a decrease in consistency. (12 references.) S. A. Brooks.

Use of cation-exchange capacity of citrus and tomato juices as a rapid screening test for the detection of adulterations. J. J. Monselise (*Bull. Res. Counc. Israel*, 1962, **11C**, 283—285).—Results obtained by a method for detecting adulteration in citrus juices by the cation-exchange capacity are discussed. Although the method may be used for general screening of orange and grapefruit juices, it is not reliable for lemon or tomato juices. S. A. Brooks.

Analysis of orange juice for total carotenoids, carotenes and added β-carotene. W. K. Higby (*Food Technol.*, 1963, **17**, No. 3, 95—98).—A recently devised simplified analytical method for total carotenoids and carotenes showed that tinctorially significant amounts of β-carotene can be detected if added to orange juice to improve colour. E. M. J.

Influence of some carotenoid pigments on non-enzymic browning in some fruit products. V. K. Satyavati, B. S. Bhatia, L. V. L. Sastry and Girdhari Lal (*Indian J. appl. Chem.*, 1962, **25**, 66—70).—β-Carotene is shown to exert some protective influence on the non-enzymic browning of orange and lime squashes and in a synthetic sucrose–glycine system. No such effect was observed with tomato serum. W. Elstow.

Chemical composition of natural and processed orange juices. R. Sawyer (*J. Sci. Fd Agric.*, 1963, **14**, 302—310).—A scheme of analysis was devised after consideration of the results of analysis of juices extracted from fresh oranges and of concentrates of guaranteed quality. These results were compared with those of prepared 'pulp extracts' and manufactured concentrates of varied origin. Samples (112) of conc. juice from the same growing region as the fresh fruits were examined. Indication of adulteration of trade concentrates was given by mineral figures and citric acid contents, e.g., Na salts in excess of expected amount from sulphiting agents. A significant negative correlation between nicotinic acid and log (total invert sugar/total sol. solids ratio) was obtained for 101 concentrates not shown to contain liquid glucose. (31 references.) E. M. J.

Numbers of tasters required to determine consumer preferences for fruit drinks. A. Kramer, F. W. Cooler, J. Cooler, M. Modery and B. A. Twigg (*Food Technol.*, 1963, **17**, No. 3, 86—91).—For accurate results with reasonably good precision, consumer-preference panels of 40—80 tasters are sufficient. In general a Brix level of 15—16 and a pH of 3·0—3·2 were preferred. A fruit content (natural fruit equiv.) of 37% was preferred to 25% but a 50% was not preferred over a 38% formulation. E. M. J.

Tea, coffee and cocoa

Chemistry of tea and tea manufacturing. W. H. Stahl (*Adv. Fd Res.*, 1962, **11**, 201—262).—This review covers very briefly some of the historical knowledge of tea chemistry and tea manufacture, but, more importantly, current chemical research on tea. Agricultural phases, e.g., genetics, soil conditions, yields, etc., are not covered. The following are dealt with: Essentials of methods of processing, (*a*) types and grades of tea, (*b*) steps in the manufacture of black tea. Chemical constituents in tea leaves, (*a*) general composition, (*b*) phenolic substances, and (*c*) non-phenolic substances, (*d*) volatile constituents, (*e*) enzymes. Chemical composition of parts of tea plant other than leaves. Changes in composition on manufacturing black tea, (*a*) withering process, (*b*) initiation of fermentation by rolling, (*c*) chemical changes in fermentation. Evaluation of tea quality, (*a*) tea tasting and definition of terms, (*b*) chemical evaluation and standards, (*c*) correlation of chemical composition and organoleptic evaluation. Use of isotopes in tea research. (204 references.) E. M. J.

Micro method for the determination of traces of copper residues in green tea leaf. M. S. Ramaswamy (*J. sci. industr. Res.*, 1962, **21B**, 578—580).—The method is based on the Cu. catalysed reduction of ferric salts by thiosulphate, the rate of reduction being proportional to Cu concn. It was tested with tea leaves dusted and sprayed with copper fungicide and gave accurate results, particularly with a Cu concn. <8 μg. S. A. Brooks.

Tempering triglycerides by mechanical working. R. O. Feuge, W. Landmann, D. Mitcham and N. V. Lovegren (*J. Amer. Oil Chem. Soc.*, 1962, **39**, 310—313).—Mechanical tempering of cocoa butters and stearines by rapid cooling of the molten glycerides followed by repeated extrusion through small orifices is compared with conventional tempering methods. *X*-ray diffraction measurements showed similarities between the two methods. The method was also applied to chocolate and chocolate products and hardness tests showed its effectiveness. (10 references.) J. V. Russo.

Interactions between proteins and polyphenols of cacao beans during fermentation. B. Biehl (*Z. LebensmittUntersuch.*, 1963, **119**, 105—118).—A preliminary extraction of the polyphenols from the comminuted and defatted unfermented beans with 50% $COMe_2$ at −5° increases the proportion of protein sol. in 0·2% KOH from ~63 to 86%. If the original material is kept moist during 3 h. at 25—30° under aerobic conditions, the proportion of insol. protein is increased to 34—40%, but if the moist material is kept under anaerobic conditions, the resulting insol. proteins amount only to 7—8%. The actual content of protein insol. in 0·2% KOH is small (possibly negligible). The proteins of the fermented beans contain ~30% of protein hydrolysates sol. in 50% COMe. The electropherograms of the (dialysed) proteins which have been extracted with 0·2% KOH from the untreated unfermented beans show three distinct fractions; those of the unfermented beans which had undergone the moist aerobic treatment, and those of the untreated fermented beans show a decreased protein content with no fractionation. These results and the probable mechanism of the action of the polyphenols on the proteins are considered with reference to other published data. (32 references.) P. S. Arup.

Softening point of cocoa butter, in relation to chocolate fat-bloom. R. Whymper (*Confect. Prod.*, 1963, **29**, 54, 56, 58—60; 113—115).—There are some 40 di- and tri-glycerides present in cocoa butter (**I**) and much of the recorded data are conflicting; this results from too frequent sampling from the upper m.p. range, this being regarded as that at which max. stability occurs. This lies between 30—35°. The softening point, which lies some 10° lower, is of much greater importance in the development of bloom. Two points are chosen: the complete fusion point (CF) and that of incipient fusion (IF) and a series of different **I** is examined from different sources or with different histories of melting, cooling, stirring, etc., the CF and IF values being given. C. V.

Continuous fermentation and drying of processed tea leaf. P. Crombie (Inventor: I. McTear) (B.P. 889,741, 22.4.59).—Apparatus is figured and claimed. F. R. Basford.

Milk, Dairy Products, Eggs

Efficiency of modern milk cooling processes. J. Hesselbach (*Kältetechnik*, 1963, **15,** 45—51).—High quality of milk can be safeguarded only when the milk is cooled on farms to at least 10° within 2 h. and maintained at this temp. The efficiency of modern cooling equipment, particularly immersion coolers and cooling coils, is evaluated and commercial types are illustrated.
M. SULZBACHER.

Comparison of the caseins of buffalo's and cow's milk. R. Aschaffenburg and A. Sen (*Nature, Lond.*, 1963, **197,** 797).—Milk samples from individual buffaloes, zebus, Jersey cows and some zebu-Jersey F_1 crosses have been compared by paper electrophoresis. The β- and κ-casein components had comparable electrophoretic mobilities but the α-caseins showed quite marked differences in their rates of migration. S. A. BROOKS.

Preparation of κ-casein. R. D. Hill (*J. Dairy Res.*, 1963, **30,** 101—107).—Centrifuged raw milk (9000 r.p.m. at 35°) from which the fat was subsequently removed by freezing (3°) was acidified with N-HCl to pH 4·5 at 30°. The ppt. was filtered, washed, redissolved in N-NaOH, keeping pH <8·0, re-precipitated with HCl, again re-dissolved and re-precipitated with 4M-$CaCl_2$ at 3° (pH 6·7—7·2). After 1 h. the suspension was centrifuged at 35° for 15 min. The supernatant liquid was dialysed against water and the dialysed solution was evaporated to small bulk (vac. at <40°). After addition of 4M-$CaCl_2$ the suspension was warmed to 35° and centrifuged (60,000 r.p.m. for 15 min.). The clear liquid was again dialysed against water, followed by 0·05N-acetic acid buffered (NaOH) at pH 6·25. The dialysed solution may be further purified chromatographically on DEAE-cellulose using a two-stage elution process with 0·05N-acetic acid buffer (pH 6·25) and also 0·1N-acetic acid (pH 6·25) to which was added $CaCl_2$ to give 0·5M followed by N-HCl to produce pH 4·5. A. G. POLLARD.

Preparation of calcium caseinate with calcium hydroxide in sucrose solution. M. Srinivasan, P. N. Achuta Murthy, A. Sreenivasan and V. Subrahmanyan (*Food Technol.*, 1963, **17,** No. 3, 112—113).—A description is given of the prep. of Ca caseinate by adding a solution of $Ca(OH)_2$ in sucrose to a solution of casein in aq. NH_3 at a 1% level of Ca. A completely water-dispersible product, acceptable in all respects for human metabolism studies, was obtained.
E. M. J.

Direct chromatographic analysis of milk. R. Bassette, S. Ozeris and C. H. Whitnah (*J. Fd Sci.*, 1963, **28,** 84—90).—Modifications of the previously employed head-space gas-methods were used for studying off-flavours of milk. Org. compounds were detected at <0·1 p.p.m. Off flavours were characterised by the development of certain chromatographic peaks. Acetaldehyde, propionaldehyde, acetone and 2-butanone were identified. E. M. J.

Residues in milk from dairy cows fed low levels of toxaphene in their daily ration. G. Zweig, E. L. Pye, R. Sitlani and S. A. Peoples (*J. agric. Fd Chem.*, 1963, **11,** 70—72).—Dairy cows had 0—20 p.p.m. of toxaphene incorporated into their feed for 77 days to determine the insecticide level necessary to produce detectable concentrations in the milk. Toxaphene was measured as total chloride. 1·0 p.p.m. in the feed gave 0·03 p.p.m. in the milk and in all but one of the animals none was detected 14 days after removal of contaminated diet. J. B. WOOF.

An improved method for Kelthane residue analysis with applications for determination of residues in milk. C. F. Gordon, L. D. Haines and J. L. Martin (*J. agric. Fd Chem.*, 1963, **11,** 84—86).—Modified methods are described in which Kelthane residues from plant material are hydrolysed by a base and the resulting $CHCl_3$ and halogen swept free from other plant material by a N_2 stream in a specially designed apparatus. $CHCl_3$ is then determined by the Fugiwara technique. The method is rapid, needs no steam heating stage and does not require elaborate processing of the sample. Milk may also be examined in this way. J. B. WOOF.

Occurrence of bromides in the milk of cows fed sodium bromide and grain fumigated with methyl bromide. G. E. Lynn, S. A. Shrader, O. H. Hammer and C. A. Lassiter (*J. agric. Fd Chem.*, 1963 **11,** 87—91).—Bromide ions derived from methyl bromide or occurring naturally in fodder products may be secreted in the milk of dairy cows. If there is a steady ingestion rate, equilibrium milk levels are established in 20—30 days. Bromide in the milk is related to that in the diet and a correlation is observed between blood and milk levels. J. B. WOOF.

Caesium-137 from fall-out in human milk. A. Åarkrog (*Nature, Lond.*, 1963, **197,** 667—668).—Human milk, obtained from one mother from Nov. 1961—Sept. 1962, showed a variation in $^{137}Cs/K$ ratio with time, increasing in summer when cows were grazing. The $^{137}Cs/K$ ratio was higher in the mother's milk than in her diet. About 7% of a large ingested dose of ^{137}Cs from reindeer meat was excreted in the milk within the first week. S. A. BROOKS.

Residues in butterfat and body fat of dairy cows fed at two levels of Kelthane (1·0 and 2·0 p.p.m.). G. Zweig, E. L. Pye and S. A. Peoples (*J. agric. Fd Chem.*, 1963, **11,** 72—74).—Cows were fed 1·0 and 2·0 p.p.m. of Kelthane in their feed for periods up to 71 days. Samples of butterfat taken at intervals and analysed for the pesticide by alkaline degradation to $CHCl_3$ and subsequent colorimetric measurement by the Fugiwara reaction. The technique does not require preliminary fat separation, recovery is good and sensitivity is satisfactory. The 2·0 p.p.m. dosage gave up to 0·4 p.p.m. in the butterfat but at lower rates none was detectable. Kelthane is stored in body fat up to 2·7 p.p.m. (10 references.)
J. B. WOOF.

Nature of the C_{18} polyethenoic fatty acids of butterfat. K. Sambasivarao and J. B. Brown (*J. Amer. Oil Chem. Soc.*, 1962, **39,** 340—344).—Me esters were prepared from two samples of butterfat and the C_{18} esters were prepared by low-pressure distillation and crystallisation. The C_{18} concentrate was fractionated on a silicic acid column and the fractions were analysed by i.r. spectrophotometry and gas chromatography. The fractions were further characterised by bromination and lipoxidase enzyme methods; 65% and 73% (respectively) of the non-conjugated dienes were linoleic acid and 79% and 71% of the trienoates were linolenic acid. No *trans–trans* linoleic acid was identified but a major proportion of the non-conjugated dienoic fraction appears to have a widely separated *cis–trans* configuration. (25 references.)
J. V. RUSSO.

Programmed flow gas chromatography of butter fatty acids. II. S. Valussi and G. Cofleri (*Riv. ital. Sostanze grasse*, 1962, **39,** 617—619).—A new procedure for examining products of a wide range of volatility in a single experiment in which the flow rate is programmed, e.g., from 30 to 110 c.c./min. with a polyester, at 10 to 60 c.c./min. and a non-polar stationary phase, has been applied to fatty acids from butterfat. The technique is compared with programmed temp. gas chromatography. L. A. O'NEILL.

Incidence of bacteria in cheese milk and Cheddar cheese and their association with flavour. J. G. Franklin and M. E. Sharpe (*J. Dairy Res.*, 1963, **30,** 87—99).—The distribution of the principal species of non-starter lactic acid bacteria, of lipolytic organisms and of the serological Group-D streptococci in milks and in cheeses prepared from them is examined. Cheese flavour was correlated with the starter cultures used and also with the pH of the cheese but not with its moisture, salt or fat contents. A. G. POLLARD.

Thermal inactivation of heat-resistant bacterial spores in milk concentrate. W. P. Segner (*Dissert. Abstr.*, 1962, **23,** 1876—1877).—This study was initiated to indicate the ultra-high thermal treatments that might be required for the sterilisation of milk concentrate containing approximately 36% total solids. Rates of inactivation of spores of *Bacillus coagulans* WH-9, *Bacillus stearothermophilus* 1518, and *Clostridium sporogenes* (PA 3679) were determined in 3 : 1 milk concentrate and in 1 : 15M phosphate buffer at pH 6·2. Aerobic spore-forming bacteria, isolated from 23 of approximately 200 lots of the processed milk concentrate tested for sterility, according to their identity and the heat resistance of their spores, were concluded to represent post-sterilisation contaminants.
F. C. SUTTON.

Evaluation of egg albumin quality. E. J. Eisen and B. B. Bohren (*Poultry Sci.*, 1963, **42,** 74—83).—Relationships between albumin height, egg wt. and vol. of inner thin, outer thin, and thick albumin were determined on 416 eggs from White Leghorns completing their first laying year. Partial correlations between residual variation in albumin height and albumin vol. (with egg wt. constant) showed that albumin height adjusted for egg wt. accounted for 5·4% of the variation in thick albumin vol. adjusted for egg wt., and for 14·6% of the adjusted vol. of thick + inner thin albumin. Variation in Haugh units accounted for 5·9% and 16·8% of the variation in the adjusted vol. of thick albumin and thick + inner thin albumin respectively. A. H. CORNFIELD.

Development and use of colour standards for egg yolks. H. E. Ashton and D. A. Fletcher (*Poultry Sci.*, 1962, **41,** 1903—1909).—The development of permanent standards for measuring the colour of egg yolks is described. A. H. CORNFIELD.

Gelation of egg yolk. W. D. Powrie, H. Little and A. Lopez (*J. Fd Sci.*, 1963, **28,** 38—46).—Quant. data on the rate of apparent η increase of yolk frozen for short periods at various temp. were obtained. In all cases the heat energy was removed uniformly from yolk samples. The effectiveness of sucrose and NaCl as inhibitors and the influence of urea on the η of native and frozen egg yolk were studied. Cysteine added to native yolk partially inhibits the η

increment caused by freezing and thawing. The cysteine may rupture the intramol. S–S bonds in the lipoproteins and bring about a partial uncoiling of the protein mol. (27 references.) E. M. J.

Oiled *versus* unoiled eggs for short storage periods. I. Effect of time and method of oiling. G. W. Froning and M. H. Swanson (*Poultry Sci.*, 1962, **41**, 1880—1886).—When eggs were sprayed with oil before being stored at 12·8° for 15—30 days the treatment could be given immediately after the eggs were laid. When eggs were dipped in oil it was necessary to delay the treatment 8—12 h. after laying in order to maintain satisfactory quality during storage at 12·8°. Regardless of the oiling method used, eggs stored at 0° could be oiled immediately after laying. A. H. CORNFIELD.

Powdered yeast product. General Milk Co. (Inventor: R. B. Parker) (B.P. 889,979, 4.2.59).—Individual particles of dried yeast (cells of any suitable strain of *Saccharomyces cerevisiae* or *Torulopsis utilis*) are moistened with 16—35% of water to render them sticky and cause the particles to adhere together in the form of random aggregates of a size substantially larger than that of the individual particles, then the product is dried in a current of air at 290°F, to provide a non-viable, free-flowing dehydrated food product (of 0·5—8% of water content), further characterised by high wettability and dispersibility in water. The product is suitable for dispersing in water (to form an acceptable beverage) or compounding with 5—20 wt.-% of powdered milk, to form a pill. F. R. BASFORD.

Edible Oils and Fats

Chromatographic separation of glycerides of olive oil. Z. Kwapniewski and J. Sliwick (*Riv. ital. Sostanze grasse*, 1963, **40**, 11).—The glycerides of olive oil have been separated by circular-paper chromatography using liquid paraffin impregnated paper and AcOH as developing solvent. The chromatogram shows three rings, corresponding to three types of glyceride. L. A. O'NEILL.

Component fatty acids of the glyceride and phospholipid fractions of the baobab seed (*Adansonia digitata*). S. H. W. Cmelik (*J. Sci. Fd Agric.*, 1963, **14**, 287—291).—Glycerides from the decorticated seed were extracted with acetone and the fatty acid composition examined by reversal-phase paper chromatography. Pure lecithin and cephalin were isolated from the alcohol–ether (3 : 1) extract and their fatty acids studied. In all fractions isolated, the most predominant saturated acid is palmitic and the main unsaturated acid is oleic acid. Linoleic acid is present in all fractions (21·7—30·6) (% by wt. of fraction). (35 references.) E. M. J.

Pro- and anti-oxidants in the field of fats. VIII. Effect on the physiological process. Cholesterol metabolism. Synthesis of cholesterol esters of hydroxy- and keto-fatty acids. H. P. Kaufmann, H. Garloff and F. Deicke (*Fette Seif. Anstrichm.*, 1962, **64**, 1104—1109).—Cholesterol linoleate undergoes the ' water reaction ' in aq. suspension in the presence of air and haemoglobin. The raw oxidation product may be fractionated by extraction with ether and ethanol–water. The former may be subdivided into hexane and water-sol. fractions. Infra-red spectra and other properties of each of the fractions are examined. The model system also shows that the Fe-porphyrin-protein-catalysed oxidation of cholesterol and esters in aq. medium may be inhibited to a greater or lesser degree by antioxidants like tocopherol and bilirubin. The significance of this in the biological process is considered. (25 references.) J. B. WOOF.

Fatty raw materials of margarine. I. Native fats and oils. II. Hardened fat and a new method of continuous fat hardening. H. P. Kaufmann (*Fette Seif. Anstrichm.*, 1962, **64**, 1168—1178).—After discussing fats as raw materials for margarine manufacture, a new fat hardening method is outlined. The continuous plant is described with flow diagrams and the quality of product is related to throughput, hydrogenation temp. and raw material composition. J. B. WOOF.

Margarine additives. H. Pardun (*Fette Seif. Anstrichm.*, 1963, **65**, 25—35).—A wide range of margarine additives is reviewed, and the properties and function of emulsifiers, aroma-producing substances, flavours, dyestuffs, vitamins and identification materials are discussed. A number of typical constitutional analyses of commercial additives are also given. (30 references.) G. R. WHALLEY.

Nature of fats produced by *Rhodotorula gracilis*. F. Allegrini, L. Cieri and E. Ciranni (*Ric. sci., RC*, 1962, **2B**, 258—263).—The effect of the sucrose medium on the composition of the fat produced by fermentation with *Rhodotorula gracilis* has been studied by gas chromatography. Sucrose gave fatty acids covering a wide range of chain lengths from formic to linoleic, whereas cane molasses gave mainly oleic and palmitic acids. Beet molasses, in addition to these two acids gave smaller amounts of lactic and linolenic acid. (13 references.) L. A. O'NEILL.

Derivatives of fats for use as foods. R. O. Feuge (*J. Amer. Oil Chem. Soc.*, 1962, **39**, 521—527).—A review of reactions involving deriv. of fats which are used as edible products. The esterification and interesterification processes are discussed with the common reaction mechanisms involved. Finally prep. and properties of surface-active deriv. and some examples of specialised products are dealt with. (38 references.) J. B. WOOF.

Physiological properties of blown oils. W. Kieckebusch, K. Jahr, G. Czok, W. Grein, K.-H. Bässler, D. C.-H. Hammar and K. Lang (*Fette Seif. Anstrichm.*, 1962, **64**, 1154—1167).—The toxicity of the oxypolymeric compounds produced from polyunsaturated acids by blowing oils at high temp., towards rats is investigated. Fat absorption and the effects on the intestine and bile are examined as are enzymes systems derived from cyclophase and liver mitochondria. Toxicity increased with temp. to a max., then declined. The limiting dose is 0·45—0·90 g./kg. body wt. Amongst the physiological effects noted were reduced food and protein efficiency, increased sensitivity, obstruction of the intestinal tract and increased capillary permeability. (38 references.) J. B. WOOF.

Screening method for chlorinated pesticide residues in fat without clean-up. L. F. Krzeminski and W. A. Landmann (*J. agric. Fd Chem.*, 1963, **11**, 81—83).—Total chlorinated hydrocarbon residue in fat is measured by a potentiometric technique (Furman and Low). Water-sol. inorg. chloride is removed by heat rendering and the residue treated with Na in liquid NH_3 to reduce bound org. chlorine to the ion. Using a 10 g. sample of fat with a 10 p.p.m. DDT content, 90% recovery may be achieved. J. B. WOOF.

Quantitative preparation of methyl esters of fatty acids for gas chromatography. C. W. Gehrke and D. R. Goerlitz (*Analyt. Chem.*, 1963, **35**, 76—80).—A method has been developed for the analysis of carboxylic acids in biological material which involves gas chromatography of their esters. Macro- and micro-procedures are described in detail. The esterification stage involves reaction of iodomethane with the Ag salts of the acids, which produces quant. ester formation with little or no side-reactions or loss of volatile esters. The methods are accurate and precise and have been applied routinely for the analysis of fatty acids in milk fat, and to identify fatty acids present in lipid fractions from bovine semen and unesterified fatty acids in human blood serum. S. M. MARSH.

Meat and Poultry

Polarographic studies on storage of meats. XXII. Influence of proteolytic enzymes on the polarographic wave of beef protein solutions. T. Obara and Y. Ogasawara (*J. Fd Sci.*, 1963, **28**, 8—14).—The influence of pepsin, irradiated pepsin, trypsin and papain on the polarographic wave of native and denatured beef protein solutions was studied. When beef samples irradiated with 148×10^4 to 200×10^4 r were stored at 20° there was a pronounced and characteristic change in the polarographic wave of their water extract. This change is quite similar to that observed with beef protein solutions (**I**) incubated with pepsin, particularly irradiated pepsin. **I** incubated with trypsin or papain show less significant polarographic changes than those incubated with pepsin. (27 references.) E. M. J.

Studies on beef quality. X. Effect of temperature, freezing, frozen storage, thawing and pH on the rate of hypoxanthine production. C. A. Lee and H. L. Webster (*Commonw. sci. industr. Res. Org. Aust. Div. Fd Pres.*, 1963, Tech. Paper No. 30, 10 pp.).—An increase in temp. and in ultimate pH, increased the rate of hypoxanthine (**I**) production, as also did the thawing out of frozen samples. No increase was noted on freezing or during frozen storage. The process of ripening and the production of **I** to a level of 1·5—2 μmol./g. are affected to a similar extent by temp. The possible value of the **I** content in meat as an index of ripening is discussed. E. M. J.

Comparison of the press method with taste-panel and shear measurements of tenderness in beef and lamb muscles. L. J. Bratzler and H. D. Smith (*J. Fd Sci.*, 1963, **28**, 99—100).—The *longissimus dorsi* muscle was taken from seven beef ribs, 15 beef short-loins, 129 lamb loins, and the *semitendinosus* muscle from 51 beef rounds. Press and shear methods used on cooked meat gave comparable relations with sensory-panel scores. E. M. J.

Biochemical properties of pork muscle in relation to curing. C. L. Walters and A. McM. Taylor (*Food Technol.*, 1963, **17**, No. 3, 118—123).—To determine whether surviving enzyme systems in pork muscle were capable of effecting the specific reductions essential for colour formation, purified myoglobin was prepared from pig hearts.

The pigment was isolated as metmyoglobin and this combined directly with NO to form a nitric oxide–metmyoglobin complex. (The absorption spectrum was similar to that of nitrosylmyoglobin, but with absorption max. displaced towards the blue end of the spectrum.) This complex decomposed rapidly on contact with air giving a brown product showing no characteristic absorption bands. (15 references.) E. M. J.

Comminuted meat emulsions: factors affecting meat proteins as emulsion stabilisers. C. E. Swift and W. L. Sulzbacher (*Food Technol.*, 1963, **17**, No. 2, 106—108).—The emulsifying capacity (**I**) of water-sol. proteins increased in increasing concn. of NaCl, their capacity being max. at pH 5·2. **I** in 0·5M solutions decreased in the order KSCN, KI, KNO_3, KBr, KCl and K_2SO_4. In the pH range approaching the isoelectric point of the salt-sol. proteins **I** of the proteins increased as concn. of NaCl was increased. Similarly at these pH values **I** of the meat increased with increasing concn. of NaCl. Increase in **I** of the meat on increasing pH from 7 to 8 was attributed to an increased extraction of proteins. E. M. J.

Detection of thickening materials in meat products and possible interference by other polysaccharides. R. Grau and A. Schweiger (*Z. Lebensmitt Untersuch.*, 1963, **119**, 210—216).—Two improvements are made on the procedure of Becker and Eder (cf. *Anal. Abstr.*, 1957, **4**, 1675). (*a*) The solution in 40% MeOH of the products of acid hydrolysis of the sample (previously freed from fat and sol. sugars) is purified by passage through a column of the cation-exchanger Merck I in order to remove traces of Ba^{2+} and other ions which interfere with the subsequent chromatographic analysis. (*b*) Thin-layer chromatography (on cellulose) is substituted for paper chromatography for the detection of the hexoses, pentoses and uronic acids derived from the additives. Some spices, condiments or other vegetable products used in the prep. of sausages yield recognisable amounts of certain hexoses and pentoses, but not in sufficient quantity or assortment to interfere with the evaluation of the chromatograms. (12 references.) P. S. ARUP.

Rôle of micro-organisms in the fermentation and colour development of summer sausage. D. F. Wessley (*Dissert. Abstr.*, 1962, **23**, 1880—1881).—Acceptable summer sausage could be produced with polysaccharide-forming lactobacilli as starter organisms. Since nitrate-reducing bacteria were not found at levels sufficient to provide an adequate amount of nitrite from nitrate, the addition of nitrite to the sausage emulsion should insure proper colour development in the product. F. C. SUTTON.

Residues in body tissues of livestock sprayed with Sevin or given Sevin in the diet. H. V. Claborn, R. H. Roberts, H. D. Mann, M. C. Bowman, M. C. Ivey, C. P. Weidenbach and R. D. Radeleff (*J. agric. Fd Chem.*, 1963, **11**, 74—76).—Several types of domestic animals were sprayed four times with 1% Sevin suspensions. Hereford steers were fed 50 and 100 p.p.m. in their diet for 27 days. Animals were slaughtered at intervals and their tissue examined for Sevin, 1-naphthol and conjugates of 1-naphthol by the Union Carbide method. No residue was detected after feeding and only the fat and brain of a goat accumulated insecticide sprayed on the animal. J. B. WOOF.

Determination of Sevin insecticides and its metabolites in poultry tissue and eggs. D. P. Johnson, F. E. Critchfield and B. W. Arthur (*J. agric. Fd Chem.*, 1963, **11**, 77—80).—Tissue and egg samples are extracted with methylene chloride in a blender and filtered. After passing through a Florisil column, the solution is assayed for free naphthol by reaction with *p*-nitrobenzenediazonium fluoborate and measuring absorption at 475 mμ. Sevin and conjugates are first hydrolysed to give the free 1-naphthol. Dusting with insecticide caused the level in skin tissue to rise to 2·15 p.p.m. Seven days after treatment, eggs and birds were entirely free from Sevin. J. B. WOOF.

Effect of method of freezing on survival of micro-organisms on turkey carcasses. A. A. Kraft, J. C. Ayres, K. F. Weiss, W. W. Marion, S. L. Balloun and R. H. Forsythe (*Poultry Sci.*, 1963, **42**, 128—137).—Freezing by brine immersion followed by air blast, or by air blast alone, resulted in 98—99% destruction of the total surface flora. Blast freezing was somewhat more effective than was brine immersion–air blast freezing in reducing no. of coliforms, enterococci and staphylococci on inoculated carcasses and total aerobes on uninoculated carcasses. Both methods were equally effective in destroying fluorescent organisms. A. H. CORNFIELD.

Effects of polyphosphates on water uptake, moisture retention and cooking losses in broiler carcasses. E. P. Schermerhorn, R. L. Adams and W. J. Stadelman (*Poultry Sci.*, 1963, **42**, 107—110).—Addition of Na tripolyphosphate or a commercial polyphosphate (4%) to the carcass cooling water had no effect on, whilst 8—12% of the materials reduced, water uptake. The treatments decreased moisture losses during a 16-h. holding period, and also reduced cooking losses. A. H. CORNFIELD.

Sausage products. Unilever Ltd. (Inventor: A. J. H. Sale) (B.P. 891,175, 21.5.59).—A skinless sausage product is produced by exposing to microwaves (of wavelength 3—50 cm.) raw sausage meat shaped into sausage form (and optionally enveloped in pastry dough). Apparatus is figured. F. R. BASFORD.

Fish

Biochemical changes in fish muscle during rigor mortis. D. J. Nazir and N. G. Magar (*J. Fd Sci.*, 1963, **28**, 1—7).—Mullets (*Mugil dussumieri*), Bombay ducks (*Harpodon nehereus*) and groupers (*Epinephalus malabaricus*) were stored in crushed ice at 2°; the fish were in full rigor for 9, 4, 11 h. respectively. Immediately after death pH decreased slowly to about 6·4 to 6·5 in full rigor. Glycogen decreased, but measurable quantities remained at the end of the experiment. Lactic acid increased but no direct relationship was observed. Inorg. P increased slowly, creatine phosphate and ATP decreased. The Ba acetate-non-precipitable ribose increased throughout storage; as did also the ratio of the non-precipitable ribose to acid-sol. ribose. Trimethylamine values rose gradually but were still low at the end of the experiment. (38 references.) E. M. J.

Post mortem changes in chilled and frozen muscle. W. Partmann (*J. Fd Sci.*, 1963, **28**, 15—27).—Conditions in rigor development in fish of different species, in whole fish and isolated beef muscles are discussed. The rapid phase of ATP breakdown and increasing rigidity of the muscles is initiated by inactivation of the Marsh–Bendall factor in the post mortem period. Contraction occurs when ATP is added to fibre fragments of aged meat, the actomyosin complex formed during rigor development becomes dissociated and tenderness changes in the aging period are correlated with this process. In frozen muscle tissue stored below −18°, ATP-ase activity and contractability decrease very slowly showing that the actin and myosin filaments are not greatly changed structurally by freezing and thawing. (52 references.) E. M. J.

Nucleotide degradation in the muscle of iced haddock (*Gadus aeglefinus*), lemon sole (*Pleuronectes microcephalus*) and plaice (*Pleuronectes platessa*). Bung-Orn Kassemsarn, B. Sans Perez, J. Murray and N. R. Jones (*J. Fd Sci.*, 1963, **28**, 28—37).—The muscle of trawler-caught haddock, lemon sole and plaice contained little adenosine 5′-triphosphate (ATP) and much inosine 5′-monophosphate (IMP) at death. ATP, adenosine 5′-diphosphate and adenosine 5′-monophosphate changed rapidly after death. IMP was lost more slowly, liberating inosine which was degraded to hypoxanthine. A little adenine was formed by an alternative pathway of ATP degradation in lemon sole. A relatively high initial level of guanine was found in plaice muscle. Traces of xanthine were found in spoiling muscle from the three species. Quality testing and flavour changes in iced fish are discussed. (60 references.) E. M. J.

Effects of sub-sterilisation doses of radiation on the storage life extension of soft-shelled clams and haddock fillets. J. T. R. Nickerson, S. A. Goldblith and E. B. Masurovsky (*U.S. atomic Energy Comm. Rep.*, 1962, NYO 10,412, 86 pp.).—Methods for obtaining max. aerobic and anaerobic bacterial counts using this material are described with special reference to clostridia. Three samples were taken daily from three plants during July and August 1961. The data obtained suggest that haddock fillets can be kept under refrigerated conditions for two to three months when treated with 700,000 to 800,000 rad. Irradiation with ^{60}Co γ-rays at 500,000 to 650,000 rad is effective for 4—6 weeks while 400,000 to 500,000 reduces the period to under a month. In general the soft-shelled clams give similar results. (60 references.) C. V.

Spices, Flavours, etc.

Essential oil of *Schinus molle*: the terpene hydrocarbon fraction. R. A. Bernhard and R. Wrolstad (*J. Fd Sci.*, 1963, **28**, 59—63).—The essential oil obtained by steam distillation of the fruit of the California pepper (*S. molle*) was re-examined. By gas–liquid chromatographical analysis of the terpene hydrocarbon fraction 11 components were obtained, viz., α-pinene, β-pinene, α-phellandrene, β-phellandrene, myrcene, D-limonene and *p*-cymene were tentatively identified; six hydrocarbons not hitherto reported in the oil and three other unidentified compounds. (16 references.) E. M. J.

Sesquiterpene fraction of the essential oil of *Olearia paniculata*. R. E. Corbett, G. A. Jamieson and J. Murray (*J. Sci. Fd Agric.*, 1963, **14**, 349—351).—The sesquiterpene fraction of the essential oil

was separated by distillation into three fractions (i) aromadendrene, (ii) ±-*ar*-curcumene and (iii) (±)-γ-curcumene. The physical constants of the fractions are given. E. M. J.

Determination of the quality of essential oils of lemon by infra-red and ultra-violet spectrophotometry. F. Navarro Albaladejo (*Quím. e Ind., Bilbao*, 1962, **9**, 199—206).—The results of examination of 59 commercial samples of Spanish oil of lemon and of mixtures of genuine lemon oil with distilled essential oil of orange are reported. Spectrophotometric examination makes possible the distinction between different methods of preparation and the detection of admixture with citral and/or other products of deterpenisation. The absorption ratios (×10) of citral (5·96 μ)/*d*-limonene (6·08 μ) and of carbonyls (5·73 μ)/*d*-limonene were over 5 and 3·8 respectively for over 90% of the samples examined and are significantly reduced by additions of distilled orange oil (>5%). The CD value, defined as the area bounded by *E* 370 mμ, *E* 285 mμ and *E* at $\lambda_{max.}$ (>313 mμ), provides an additional criterion of purity and was >0·75 for 86% of the samples examined. E. C. APLING.

Gas chromatography and the adulteration of essential oils. Addition of citral from lemon grass to lemon essences. A. L. Montes (*An. Asoc. quím. Argent*, 1962, **50**, 111—119).—The detection of additions of citral from lemon grass to lemon essence is possible because of the different ratio of geranial (citral a) to neral (citral b) in the citrals from lemon essence and lemon grass oils, average values for natural samples being 3·43 and 2·50 respectively. The geranial/neral ratio may be determined using a sucrose diacetate-hexaisobutyrate stationary phase at 150°. Additions of 1—2% may be detected and reconstituted lemon essences can be recognised. L. A. O'NEILL.

Paper chromatography of essential oils. L. Hörhammer, G. Richter and H. Wagner (*Analyt. Chem.*, 1963, **10**, 108—110).—A paper impregnated with paraformaldehyde was used for the separation of essential oils and their constituents. The solvent systems n-hexane–n-heptane–glacial acetic acid (15 : 15 : 2); cyclohexane–ethyl acetate (97 : 3) and trichloroethylene gave satisfactory separations in most cases. G. P. COOK.

Gas–liquid chromatography in the control of mint essential oils. T. A. A. Manfredini and A. L. Montes (*An. Asoc. quím. Argent.*, 1962, **50**, 198—213).—The essential oils of peppermint, spearmint, Japanese mint, penny royal and peperina have been examined by gas chromatography, generally with a sucrose diacetate–hexaisobutyrate stationary phase at 150°. The relative retention vol. of the components responsible for peaks have been calculated with reference to menthone. The use of the method for quality control of the oils is indicated. L. A. O'NEILL.

Factors affecting the flavour of sodium caseinate. M. N. Cayen and B. E. Baker (*J. agric. Fd Chem.*, 1963, **11**, 12—14).—Sodium caseinate samples were prepared by treating fresh skim milk with HCl and from caseins obtained by freeze-drying, tunnel drying and washing curd thoroughly and reprecipitating. Direct tasting of these and commercial samples showed very low rating for most of them. Fairly tasteless specimens can be prepared from well washed, freshly precipitated casein. (10 references.) J. B. WOOF.

Enzymic enhancement of flavour. E. J. Hewitt (*J. agric. Fd Chem.*, 1963, **11**, 14—19).—Restoration of flavour by enzyme action on processed food is investigated. After treatment with enzymes from fresh cabbage and mustard, processed cabbage is subjectively evaluated and extracts examined by paper chromatography and volatile components by gas chromatography. Prep. from fresh onions showed sulphoxidase activity and enhanced the flavour of dried onions. An enzyme and substrate derived from raspberries together gave both volatile deriv. and aroma of the fresh fruit. Wide application of these 'flavour' enzymes is envisaged. (16 references.) J. B. WOOF.

Preservatives

Action of benzoic and salicylic acids on the metabolism of micro-organisms. I. Bosund (*Adv. Fd Res.*, 1962, **11**, 331—353).—The following are covered: General aspects. Influence of pH on the growth-inhibiting effect. Relative effectiveness of different benzoic acids. Inhibition of various processes in intact cells, (*a*) oxidation of glucose, pyruvate and lactate, (*b*) metabolism of the acetyl, group, (*c*) oxidation of TCA-cycle intermediates, (*d*) assimilatory processes, (*e*) formation of adaptive enzymes. Effect on oxidative phosphorylation in isolated mitochondria. Inhibition of cell-free enzymes. There exists a striking resemblance in all characteristics between benzoic and salicylic acids in the effects on bacterial cells. (46 references.) E. M. J.

Use of pentachloronitrobenzene in the storage of cabbage. M. Feuersenger (*Dtsch. Lebensmitt Rdsch.*, 1963, **59**, 14—15).—Cabbages, carrots and celery were treated with from 44 to 100 p.p.m. of KP2 fungicidal spray (equivalent to from 6·6 to 15 p.p.m. of pentachloronitrobenzene) and residuals determined after storage. Celery and cabbage showed only negligible residues after storage for 3 months and normal prep. for consumption, but on cabbage there was no significant reduction in residuals after 2 months storage and after 3 months from 0·2 to 1 p.p.m. remained after cleaning and removal of the outer leaves. E. C. APLING.

Food Processing, Refrigeration

Heat transfer analysis of heat exchanger plate. S. A. Hassan, C. W. Hall and G. M. Trout (*Pakist. J. sci. industr. Res.*, 1962, **5**, 219—221).—Overall heat transfer coeff. over a single plate was evaluated and the inlet and outlet temp. of hot pasteurised milk and cold raw milk were measured, 38 pairs of thermocouples being soldered to the test plate for temp. measurement. In addition, four thermocouples were placed in each of the four ports for inlet and outlet of hot and cold milk to the test plate. Contours of temp. and the overall heat transfer coeff. were plotted over the plate area. Results were discussed and conclusions drawn. M. A. NAWAB.

Freeze-drying of foodstuffs. G. Nemitz (*Dtsch. Lebensmitt Rdsch.*, 1963, **59**, 1—4).—A brief description of the process and review of current industrial developments. (12 references.) E. C. APLING.

State of development of irradiated foods. J. J. Macfarlane (*Food Technol. Aust.*, 1963, **15**, 118—119, 121, 123, 125, 210—211, 213, 223).—The general effects of ionising radiation and principal areas of application to foodstuffs, corresponding to three dose levels, e.g., sterilisation, pasteurisation, insect disinfestation and sprouting treatments are reviewed. Meats, fruits and vegetables, etc., are dealt with and the wholesomeness of irradiated foods is discussed. Details of known applications at the research stage of development and the need of pilot plant studies to show that the process can be successfully adapted to commercial conditions are considered. E. M. J.

Radiation pasteurisation of fresh fruits and vegetables. T. A. Truelsen (*Food Technol.*, 1963, **17**, No. 3, 100—103).—In tests on fresh strawberries, raspberries, cauliflower, tomatoes and asparagus given pasteurising doses of γ-radiation, only with strawberries was it possible under certain circumstances to obtain a considerable increase in stability. No series colour changes were observed in either strawberries or raspberries, but in all other products, which were unsuitable for preservation by irradiation, discoloration occurred. In tomatoes there was defective development of lycopene which is possibly connected with delay in ripening. (11 references.) E. M. J.

Packaging

Application of gas chromatography to the measurement of gas permeability of packaging materials. M. Karel, P. Issenberg, L. Ronsivalli and V. Jurin (*Food Technol.*, 1963, **17**, No. 3, 91—94).—The modified concentration-increase method was used to determine the O_2 and CO_2 permeability of a large no. of samples of several food packaging films including polypropylene, Mylar and fluorocarbon films. The most important advantages are: possibility of repeated sampling of the gases in the test cell; possibility of determination of the rate of approach to steady-state transfer of the gases; increased sensitivity and decreased time expenditure. E. M. J.

Diffusion of hydrogen through tinplate containers packed with grapefruit juice. G. Serra and G. A. Perfetti (*Food Technol.*, 1963, **17**, No. 3, 114—117).—Results show that a considerable amount of H_2 generated during the corrosion of tin-plate containers by grapefruit juice diffuses through the container and that the amount of H_2 diffusing is proportional to the amount remaining in the headspace of the container. The shelf life of tinplate containers as measured by time to 'springer failure' will depend on the H_2-diffusion characteristics of the tin-plate. E. M. J.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Atherosclerosis in relation to nutrition and other environmental factors—a review. A. S. Aiyar and A. Sreenivasan (*J. sci. industr. Res.*, 1962, **21A**, 559—571).—Chemical and animal experimental investigations emphasising the importance of environmental factors, particularly nutrition, in the etiology of atherosclerosis have been reviewed. (388 references.) S. A. BROOKS.

Changes in composition of the fatty acids in the nutritional fatty liver. A. S. Feigenbaum (*Dissert. Abstr.*, 1962, **23**, 1902).—Experi-

ments were designed to study the species difference between rats and chickens during starvation with regard to liver fat accumulation. Neither growing chickens nor adult roosters developed fatty livers during the 5-day starvation period. Fat did accumulate in rat liver. In the final portion of this study, day-old chickens were fed diets containing various levels of ethionine for two-week periods. Chickens, like rats, develop a fatty acid liver when treated with ethionine. F. C. SUTTON.

Influence of temperature, heating time and aeration on the nutritive value of fats. C. E. Poling, W. D. Warner, P. E. Mone and E. E. Rice (*J. Amer. Oil Chem. Soc.*, 1962, **39,** 315—320).—Oils subjected to temp. of 200° for days or aerated at 60° for 16 days or more led to less growth in rats than control oils and produced liver enlargement. These treatments are much more drastic than conventional cooking methods and there is no evidence that normal cooking leads to nutritional changes. (23 references.)
J. V. RUSSO.

Biosynthesis and function of messenger RNA [ribonucleic acid]. D. P. Burma (*J. sci. industr. Res.*, 1962, **21A,** 572—576).—The evidence is reviewed for the concept of messenger RNA transcribing the genetic information from DNA mol. and carrying this information from the nucleus to the ribosomes. (53 references.)
S. A. BROOKS.

Influence of dehydration of foods on the digestibility and the biological value of the protein. A. P. de Groot (*Food Technol.*, 1963, **17,** No. 3, 103—107).—Twelve different cooked foods and corresponding cooked products after dehydration were fed to rats as the only source of protein; digestibility and biological value of the protein were determined by the N balance method of Thomas–Michell. No serious damage to the protein by hydration was revealed. In general, dehydration in hot air showed more indications of slight protein damage than did spray drying or freeze drying (**I**). By contrast with **I** the canning of green beans caused a decrease in protein quality. By frying fish patties in deep fat for 2½ min. at 200° slight damage to the protein was caused. The utilisation of protein was not affected whether or not dehydrated fish patties, green beans and kale were rehydrated prior to feeding. An excess of water in the diet may result in slightly lower biological value and digestibility of the protein. (26 references.) E. M. J.

Relationship between the sulphur/nitrogen ratio and the protein value of diets. D. S. Miller and G. Donoso (*J. Sci. Fd Agric.*, 1963, **14,** 345—349).—Evidence is presented that the protein values of most human diets are limited by their content of the S amino-acids and a chemical method is proposed for the measurement of nutritive value based on S and N contents. For practical purposes the protein scores of mixed diets are equal to 1000 S/N. The net dietary protein values may be estimated for natural mixed diets by simple chemical procedures. The calculated protein values are in good agreement with the results of bioassay. (16 references.)
E. M. J.

Influence of varietal and ecological factors on the composition of amino-acids of the grains of *Pennisetum* and of sorghum. F. Busson, P. Lunven, M. Lanza, R. Aquaron, A. Gayte-Sorbier and M. Bono (*Agron. trop.*, 1962, No. 9, 752—764, Reprint).—Data are given on the amino-acids, determined by paper chromatography, in two species of *Pennisetum* and 11 species of sorghum. The difference in the species is reflected in the amino-acids of the grains of the cereals, as much in the millets as in the sorghums. Ecological factors appear to have no influence on the quality of the proteins. E. M. J.

Determination of amino-acids with ion-exchangers. E. L. Duncan and H. Mohler (*Mitt. Lebensm. Hyg., Bern,* 1962, **53,** 399—408).—The results obtained with the Hannig modification (described) of the automatic apparatus of Spackmann *et al.* are satisfactorily reproducible by different analysts. The range of concn. of the test solution within which it is practicable to work with the 578 mμ extinction curve is limited; if the concn. is too high, use can be made of the 436 mμ curve; the optimum concn. (within 1—2 μmol.) should be selected for each sample. The observed interference by peptides with the results for amino-acids is a problem awaiting solution. P. S. ARUP.

Biochemical and nutritional studies on East Pakistan vegetables. I. Total ascorbic acid, its co-existence with plant pigments and the effect of traditional cooking habit on its retention. M. Q. Khuda, H. N. De and M. A. H. Shariff (*Pakist. J. sci. industr. Res.*, 1962, **5,** 232—237).—Five vegetables, 17 fruits, seven tubers of local origin were examined for vitamin C content. The pigmented varieties have higher % of the vitamin. The duration of cooking and the nature of the utensils used determined the extent of loss of the vitamin during cooking. (28 references.) A. JABBAR.

Decomposition of vitamin C and its inhibition in foodstuffs. V. Effect of the main catalysts responsible for the decomposition of vitamin C and reduction of their activity. VI. Efficiency of natural protecting substances and their rôle in the enrichment of ascorbic acid in industry. VII. Food preservation by ionising radiation with regard to vitamin C content. F. Balla and M. Kiszel (*Acta chim. hung.*, 1963, **35,** 119—129, 130—132, 132—135).—V. Small- and large-scale experiments on the production of various vegetable and fruit products showed a system of oxidising enzymes principally responsible for the decomposition of vitamin C. This system has max. activity at 30° at pH 4 and at 50° at pH 5·5 but is inactivated at 60°, when kept for some time, and completely at 85—90° during 30—60 sec. When this heat treatment was applied to the prep. of paprika purée, the losses of vitamin C were reduced to a low level, i.e., to ½ or ¼ of the losses in conventional plant. In every experiment, the presence of 5 mg. or more of Cu(II) in 100 g. caused marked decomposition of vitamin C while similar amounts of Fe(III) did not impair the total vitamin quantity although the oxidation of ascorbic acid to dehydroascorbic acid was promoted. Fe(III) increased also the catalytic effect of Cu(II). SO_2, even in amounts stoicheometrically much below those corresponding to the quantity of dissolved O_2, stabilised greatly.

VI. The amount of vitamin C that can be stabilised over the original content by natural protective substances was established in vegetable and fruit products. In fibre-containing prep., this value was up to 50% of the original vitamin C content. Filtered apple juice did not protect added amounts of the vitamin.

VII. When identical dosage rates but different intensities of ionising radiation were applied, changes of the vitamin C content were independent of the intensity. At high intensities, however, the % of ascorbic acid increased and that of dehydroascorbic acid decreased. As dehydroascorbic acid is less stable, higher intensities of radiation (600 rad/min.) are favourable. (16 references.) (In Russian; from English summary.) M. SULZBACHER.

Polarographic determination of total ascorbic acids in foods. H. G. Lento, C. E. Daugherty and A. E. Denton (*J. agric. Fd Chem.*, 1963, **11,** 22—26).—A method is described in which total ascorbic acid is determined polarographically. The plant sample is extracted in a blender with 2% HPO_3 under N_2 and filtered. Dehydroascorbic acid is reduced at pH 6·8 to ascorbic acid with homocysteine, excess of which is removed with *N*-ethylmaleimide since it interferes with the determination. Total ascorbic acid may be calculated from the wave height before and after addition of standard solution. Dehydroascorbic acid is given by difference between reduced and non-reduced samples. This method is more specific but generally agrees well with the 2,6-dichlorophenolindophenol and 2,4-dinitrophenylhydrazine methods. (16 references.)
J. B. WOOF.

Determination of traces of vitamin B_{12}. D. Monnier, Y. Ghaliounghi and R. Saba (*Anal. chim. Acta,* 1963, **28,** 30—40).—Traces of ionic Co are removed by an ion-exchange resin and the purified vitamin B_{12} is decomposed and the liberated Co determined colorimetrically with nitroso-R-salt. Losses are determined by the addition of vitamin B_{12} labelled with ^{60}Co. The error is less than 6% and only homologues of vitamin B_{12} containing Co interfere.
A. J. BENNETT.

Tocopherol content of some fats and oils. II. Distribution of tocopherols in selected products. K. Taufel and R. Serzisko (*Nahrung,* 1962, **6,** 413—422).—Conflicting reports of differences in biological and antioxidant properties of the various tocopherols are reviewed. Analytical methods, including the $FeSO_4$/ferricyanide method and qual. and quant. paper chromatography, are considered, in relation to their suitability for different fats and oils. It is noted that paper chromatograms always show an unexplained tocopherol-positive spot at the origin. Total tocopherol content and the % of each component are quoted for a range of natural substances. Rice germ oil has an unusually high tocopherol content and may be used to enrich margarine. (15 references.)
J. B. WOOF.

Tocopherols in nuts. G. Lambertsen, H. Myklestad and O. R. Brækkan (*J. Sci. Fd Agric.*, 1962, **13,** 617—620).—Results of analyses of the contents of α- and γ-tocopherol in nuts (eight varieties) and the presence of other tocopherols are reported. The tocopherols were determined spectrophotometrically on chromatographic fractions. Thin-layer chromatography was applied to study the tocopherol pattern. Filberts and almonds were rich in α-tocopherol, 210 and 150 μg./g., respectively; groundnuts and brazil nuts showed medium values; walnuts, pecans and coconuts were poor sources, 15 to 7 μg./g. γ-Tocopherol occurred in walnuts and pecans at 205 and 170 μg./g., respectively, groundnuts at 110 μg./g., chestnuts at 70 μg./g. and coconuts at 2·5 μg./g., respectively. (13 references.) E. M. J.

Vitamin analysis: vitamin E. W. Feldheim (*Ernährungsforschung,* 1962, **7,** 375—376).—An historical review.
P. S. ARUP.

Vitamin E and essential fatty acids. F. Weber, U. Gloor and O. Wiss (*Fette Seif. Anstrichm.*, 1962, **64**, 1149—1153).—Increased absorption of essential fatty acids, especially linoleic and arachidonic, from the diet results in an increased vitamin E requirement. ^{14}C-labelled tocopherol is fed to rats in conjunction with linoleic acid and the activity distribution in cell nuclei, mitochondria and microsomes determined. The increased tocopherol requirement results from its consumption by the unsaturated acid in the animal. Vitamin E content of edible fats is examined and desirable levels considered in the light of the experimental findings. (24 references.) J. B. WOOF.

Unclassified

Nature and rôle of fluid consistency in food engineering applications. S. E. Charm (*Adv. Fd Res.*, 1962, **11**, 355—435).—The review covers: Elements of consistency, (*a*) sliding friction, (*b*) viscous friction. Liquid structure (*a*) Eyring theory of coeff. of η, (*b*) mol. structure of water, (*c*) suspensions, (*d*) colloidal suspensions, (*e*) Staudinger's Viscosity 'Law', (*f*) thixotropy and dilatancy. Shear stress, rate of shear and consistency, (*a*) shear stress, (*b*) rate of shear, (*c*) consistency, (*d*) apparent η. Determination of fundamental consistency constants for food material (five viscometers are described). Time-dependent non-Newtonian fluids, thixotropic and rheopectic fluids. Application of rheology to chocolate coating, (*a*) calculation of coating thickness for vertical and (*b*) horizontal surfaces. Tensile strength of fluids. Spreading coeff. Bread dough rheological properties, (*a*) analysis of dough stresses in proofing, (*b*) evaluating the important rheological properties of dough. Application of rheological properties to determinations of pumping requirements. Bingham plastic flowing in tubes. Method suggested for estimating conditions for onset of turbulence in pseudoplastic fluids. Flow of suspensions. Heat transfer characteristics of non-Newtonian fluids. Non-Newtonian mixing. Influence of consistency in filtration. (61 references.) E. M. J.

3.—SANITATION, WATER, etc.

Behaviour of ethylene dibromide [EDB] methyl bromide [MB] and their mixtures. I. In columns of grains and milled materials. S. K. Majumder, M. Muthu and K. S. Narasimhan (*Food Technol.*, 1963, **17**, No. 3, 108—111).—The stratification of EDB and MB and their mixtures in 5-ft. columns of jowar and wheat semolina was studied. In semolina columns, out of mixtures of EDB with MB 1 : 1, 1 : 2 and 1 : 3 (W/W), 1 : 2 was the best at 68 and 100°F as evidenced by intergranular gas concn. and insect mortality. The bromide residues were lower at 68 than at 100°F and increased with the increased proportions of MB. Differential analysis of gas samples with monoethanolamine and ethanolic KOH showed elution of EDB to the bottom of the columns along with MB, with progressive enrichment of MB at the bottom. (17 references.) E. M. J.

Oil-in-water emulsion for cleaning eggs. D. G. Bigbee, L. E. Dawson, J. A. Davidson, W. L. Mallmann and G. A. Houghtby (*Poultry Sci.*, 1962, **41**, 1947—1954).—Eggs washed in a detergent-sanitiser and then oiled by spraying lost significantly less wt. and showed less increase in albumin storage during subsequent storage than did eggs washed in 10% mineral oil emulsions. However, Haugh unit quality was no different between the treatments. The presence of 1% *o*-phenylphenol in the oil emulsion was not as effective as NaOCl (200 p.p.m. Cl_2) in controlling the microbial population in the wash solution over 15 days, although the former treatment resulted in the lowest internal contamination of eggs. A. H. CORNFIELD.

Second report joint FAO/WHO expert committee on meat hygiene. (*FAO Agric. Stud., Rome*, 1962, No. 58, 87 pp.).—The report covers public health aspects; hygiene in relation to quality and standards; diseases caused by ingestion of or contact with meat; methods of slaughter; meat inspection; poultry meat hygiene; hygiene of refrigerated and manufactured meat; transport of meat; sanitation of retail shops and restaurant kitchens; hygiene of meat handlers; newer developments: radionuclides, irradiation, antibiotics; hygiene in tropical countries. The annexes cover food-borne disease outbreaks in England and Wales 1953—60 associated with meats; design of abattoirs, methods of electrical stunning used in the USSR; post mortem inspection; inspection procedures for poultry; storage of meat and poultry and their products. (27 references.) E. M. J.

Deuterium isotope rate effect in free-radical reactions of t-carbon-deuterated DDT and its analogues. A. C. Dachauer (*Dissert. Abstr.*, 1962, **23**, 1514).—As a means of testing the hypothesis that DDT and its analogues undergo a chemical reaction in the toxic process which involves the t-carbon–hydrogen bond, it was suggested that DDT and several of its analogues be synthesised with deuterium in the t-C position in order that any chemical reaction involving this site in the mol. would then be subject to the deuterium isotope rate effect. The resulting experimental data support the view that at least one mode of resistance to DDT by flies is chemical in nature and that the t-C–H group is involved. The data also indicated that susceptible houseflies contain some mechanism that can detoxify DDT and that this detoxication is chemical. F. C. SUTTON.

Metabolism of glycerol 1-phosphate in resistant and susceptible houseflies (*Musca domestica* L.) and effect of dieldrin. J. P. Heslop and J. W. Ray (*Biochem. J.*, 1963, **87**, 31—34).—As the symptoms of poisoning become more advanced, dieldrin progressively decreases the amount of thoracic glycerol 1-phosphate in resistant and susceptible houseflies under aerobic and anaerobic conditions. This effect is not associated with normal differences in concn. of glycerol 1-phosphate between strains nor with presence of dieldrin alone. Neither the sarcosomal glycerol 1-phosphate oxidase system nor the cytoplasmic glycerol 1-phosphate dehydrogenase is inhibited in thoracic prep. from houseflies poisoned with dieldrin. (11 references.) J. N. ASHLEY.

Significance of BHC-degradation in resistant houseflies. J. R. Busvine and M. G. Townsend (*Bull. ent. Res.*, 1963, **53**, 763—768).—Rates of elimination of BHC by resistant and susceptible houseflies after treatment indicate the association of resistance with a higher rate of decomposition of the insecticide. A. G. POLLARD.

Comparing resistance of houseflies to the eight stereoisomers of allethrin. R. J. Barker and L. N. Edmunds, jun. (*J. econ. Ent.*, 1963, **56**, 152—155).—The toxicity of 8 allethrin isomers to susceptible and resistant flies was compared. In all cases values for females were double those for males. The asymmetric atom on the chrysanthemumate was dominant. Dosage–response slopes were not parallel for different isomers and show no obvious changes correlated with resistance. C. M. HARDWICK.

Control of houseflies with chemosterilant techniques. G. C. La Brecque, D. W. Meifert and R. L. Fye (*J. econ. Ent.*, 1963, **56**, 150—152).—Baits containing 0·5% of Metepa [Methaphoxide; tris-(2-methyl-1-azirdinyl)phosphine oxide] were applied to poultry droppings at nine weekly intervals. Baits based on vermiculite and granulated sugar were less effective than those based on maizemeal. Oviposition was slightly reduced and viability decreased to <10% when an effective bait was used. Male fertility was also reduced. Results were influenced by infiltration of flies from other areas. C. M. HARDWICK.

Oviposition in DDT-resistant and -susceptible strains of *Aedes aegypti* (L.): egg-laying on open-water surfaces. R. J. Wood (*Bull. ent. Res.*, 1963, **53**, 755—790).—Oviposition by strains of *A. aegypti* on water surfaces or on white paper was unrelated to their relative resistance or susceptibility to DDT or to light intensity. A. G. POLLARD.

Pyrethrum extracts. East African Extract Corp. Ltd. (Inventor: J. A. Stevens) (B.P. 891,166, 28.8.58).—An improved extract is obtained by extracting fresh or dried pyrethrum flowers or a crude pyrethrum extract with a water-immiscible org. liquid (isohexane or light petroleum) in presence of a non-ionic or anionic surface-active agent, e.g., Lissapol NX or Teepol (30—130% on crude extract, 0·1—2% on fresh flowers or 0·5—10% on dried flowers). Most of the pyrethrum but little of the colour remains in the solvent extract. F. R. BASFORD.

Water, wastes and sewage

Radioactivity of rainwater. B. S. Sood and A. K. Talwar (*J. sci. industr. Res.*, 1962, **21B**, 595).—The gross radioactive content of some samples of rainwater collected at Chandigarh during March–August 1962 was estimated using an end window Geiger counter. The activity of the samples collected during July–August was found to be weaker than those from March–June. S. A. BROOKS.

Oil in groundwaters. C. Z. Maehler and A. E. Greenberg (*J. Wat. Pollut. Control Fed.*, 1962, **34**, 1262—1267).—Wells sampled were heavily polluted with org. compounds which were related to the compounds in the oil field wastes. I.r. spectra were used mainly to determine the various well water pollutants. B. F. FULLAN.

Properties of aquatic humus fractions. M. A. Shevchenko, G. B. Barashenkov and R. S. Kas'yanchuk (*Ukr. khim. Zh.*, 1962, **28**, 879—883).—The properties of crenic, apocrenic and humic acids, taken from the water of the Dnieper and the Desna, were studied with regard to their pH values, colour, optical properties and $KMnO_4$ and $K_2Cr_2O_7$ oxidisability, and it was shown that these are medium strength acids, with equiv. wt. between 100—300. Apocrenic acid is more highly coloured, has a higher content of easily oxidised groups, and is more readily absorbed, particularly by $Al(OH)_3$, than crenic acid. The properties of humic acid are intermediate between

those of the other two acids, although frequently they approach close to those of apocrenic acid. The relationship established between the properties of aquatic humus and the composition of the fractions of which it is composed makes it possible to predict how these properties will change with the changing hydrologic conditions of watercourses. A. S. LEVESLEY.

Complexiметric method for the determination of dissolved oxygen in water. R. Th. Roskam and D. de Langen (*Anal. chim. Acta.*, 1963, **28,** 78—81).—Dissolved O_2 oxidises Fe^{II}-ethylenediamine sulphate at pH 7·5 and after adjusting the pH to 2·4 the Fe^{III} formed is titrated with 0·02M-EDTA in the presence of salicylic acid as indicator. The method is more accurate than the Winkler method for polluted waters. A. J. BENNETT.

Biological effects of synthetic detergents in the River Lee, Hertfordshire. H. B. N. Hynes and F. W. Roberts (*Ann. appl. Biol.*, 1962, **50,** 779—790).—No marked changes in the flora and fauna of the River Lee, which consists primarily of sewage effluent, occurred over the period 1950 to 1931 when the detergent content of the effluent increased from about 1 p.p.m. in 1950 to 3—4 p.p.m. in 1958 and then fell to 2 p.p.m. in 1960. A. H. CORNFIELD.

Quantitative determination of blood in residual waters by estimation of protoporphyrins. P. Lejeune, J. Bouquiaux and A. Lafontaine (*Trib. Cebedeau*, 1962, **15,** 600—604).—The determination of protoporphyrin (**I**) and deuteroporphyrin (**II**) in water (~22·5 mg. of **I** per l.) is described. Haemin is extracted from pptd. oxyhaemoglobin (**III**) with oxalic acid dissolved in methanol and the extract treated with HCl gas to yield **I** which is then purified by extracting with $CHCl_3$, precipitating and dissolving the ppt. in 1·5N-HCl. The filtrate remaining after pptn. of **III** is evaporated to dryness, and the proporphyrins extracted with AcOH–AcOEt. **I** and **II** are then removed from this extract with 1·5N-HCl; the extinction of the combined acid solution is measured at 409 mμ. The limit of detection is 0·05 μg. of **I** per ml. of this solution. (In French.) P. D. PARR-RICHARD.

Past, present and future of B.O.D. [biochemical oxygen demand]. G. van Beneden (*Trib. Cebedeau*, 1962, **15,** 615—624).—Methods for the determination of B.O.D. are reviewed (24 references). Subjects discussed include: (i) method of dilution, (ii) measurement of reoxygenation, (iii) method without dilution, (iv) measurement of putrescibility (rate of consumption of O_2), (v) determination of org. N. For heavily contaminated waters, measurement of the stability relative to methylene blue, of the amino-acid content (by ninhydrin reaction) and of the redox potential are among the tests recommended. (In French.) P. D. PARR-RICHARD.

Organic composition of various domestic sewage fractions. J. V. Hunter (*Dissert. Abstr.*, 1962, **23,** 1906).—Fractions were obtained from sewage by chemical coagulation, which yields one particulate and one sol. fraction; and by physical separation by consecutive sedimentation, centrifugation and filtration, which yields three particulate fractions and one sol. fraction. Chemical coagulation gave a particulate fraction with a lower org. content than particulate and sol. fractions obtained through physical separation procedures. F. C. SUTTON.

4.—APPARATUS AND UNCLASSIFIED

Identification of 3,4-methylenedioxyphenyl synergists by thin layer chromatography. M. Beroza (*J. agric. Fd Chem.*, 1963, **11,** 51—54).—Thin-layer chromatography on silica gel was used to separate all the methylenedioxyphenyl synergists. Corrosive chromogenic reagents like chromotropic–H_2SO_4, not possible with paper, could be used. Several solvent systems were tested, and 2·5% acetone in benzene gave the widest separation. Satisfactory loadings can be as little as 0·1 μg. or as large as 100 μg. (23 references.) J. B. WOOF.

Residue analysis of phorate by cholinesterase inhibition after oxidation. T. E. Archer, G. Zweig, W. L. Winterlin and E. K. Francis (*J. agric. Fd Chem.*, 1963, **11,** 58—63).—Chromatographic techniques are described for purification of plant extracts prior to determination of phorate and its O analogues by cholinesterase inhibition. If phorate is oxidised with peracetic acid, inhibition is enhanced and a product with i.r. spectrum similar to that of phorate phosphorothiolate sulphone is obtained. Results of residue analysis of cottonseed, sugar beet and potatoes are reported. (16 references.) J. B. WOOF.

Colorimetric method for the estimation of dimethoate residues. P. A. Giang and M. S. Schechter (*J. agric. Fd Chem.*, 1963, **11,** 63—66).—Chloroform extracts of plants are chromatographed on Nuchar–alumina–Celite columns and the appropriate fraction analysed for dimethoate. Alkaline hydrolysis gives thioglycollic acid and this is reacted with sodium phospho-18-tungstate. Absorption of the resultant solution is measured at 720 mμ. Recovery of added insecticide is never less than 90% and 0·05 p.p.m. can be detected in a 200-g. sample of treated milk or plant. The O analogue is measured at the same time but cannot be distinguished, breakdown products of the two compounds do not interfere. (17 references.) J. B. WOOF.

Colorimetric method for the determination of Ethion of residues. J. R. Graham and E. F. Orwoll (*J. agric. Fd Chem.*, 1963, **11,** 67—69).—Ethion is estimated by a modification of Norris's method. After extraction of the plant tissue with hexane and concentration, the Ethion is hydrolysed with ethanolic 6N-NaOH to give diethyl phosphorodithioic acid. $CuSO_4$ is added and the complex extracted into cyclohexane, filtered and the absorption measured at 418 mμ. If blanks are high, preliminary chromatographic purification is necessary. Special extraction and hydrolysis procedures makes the method specific for Ethion in the presence of related thiophosphate insecticides. J. B. WOOF.

Rapid determination of hexachlorocyclohexane in DDT by Kofler's microdetermination of the refractive index. T. Kartnig (*Mikrochim. ichnoanal. Acta*, 1963, No. 1, 88—92).—A mixture of the sample (2—3 g.) with neutral Al_2O_3 (2—3 g.) is placed on top of a column of neutral Al_2O_3 and the HCH and DDT are eluted with ether or n-hexane. The solution is evaporated *in vacuo* and the residue, after drying in a vac. desiccator, is weighed and heated to 130°. The mass is cooled on a block and the m.p. and n are determined. The % composition is found from standard curves prepared from the two insecticides. (In German.) M. SULZBACHER.

Analytical chemistry of monobromoacetic acid. E. Mergenthaler (*Z. LebensmittUntersuch.*, 1963, **119,** 144—155).—The literature of the subject is reviewed. Monobromoacetic acid (**I**) is quant. removed from acid aq. solutions by continuous extraction with Et_2O for 24 h. The **I** obtained after evaporation of the Et_2O is converted into glycollic acid (**II**) by boiling with N-NaOH. The **II** is purified from other org. acids by passing the solution (at pH 7) through a column of Dowex 2 × 8 (CO_3^{2-} form) and selectively eluting with 0·25N-$(NH_4)_2CO_3$. The **II** is determined in the (conc.) eluate by spectrophotometric measurement at 580 mμ of the colour developed on heating with H_2SO_4 and the chromotropic acid reagent; the coloration is due to the formaldehyde quant. produced by the action of H_2SO_4 on **II**. The **I** added to foods is more or less rapidly converted into **II** on keeping; as **II** cannot be quant. extracted from aq. solutions with Et_2O, the test will, in such cases, be of qual. significance, only. Immediate recoveries of **I** added to solutions containing sucrose, fructose and tartaric, citric and malic acids were 95—119% and from apple juice or sweet wine 93—122%. (63 references.) P. S. ARUP.

Chemical modification of dithiocarbamates during storage. G. Petrosini, F. Tafuri and M. Businelli (*Ric. sci., RC*, 1962, **2B,** 248—257).—The stability of the dithiocarbamate fungicides of Zn on storage under various conditions has been examined. The final degradation products of Zn ethylenebisdithiocarbamate are ethylenethiourea (probably formed via ethylenethiuram monosulphide), CS_2 and ZnS. Degradation increased with humidity but temp. under dry conditions had little effect. Zn dimethyldithiocarbamate was by contrast much more stable. Insol. Cu salts enhanced the decomposition of the former fungicide under warm damp conditions but not the latter. (11 references.) L. A. O'NEILL.

Determination of low fluorine content in plants. M. Buck (*Z. anal. Chem.*, 1963, **193,** 101—112).—The method comprises a combination of the Willard–Winter distillation with an improved colorimetric determination of F. Green plant parts are calcined at 450—500°, the ashes are fused with NaOH and the aq. solution of the melt is steam distilled in the presence of 70% $HClO_4$. The distillate is kept alkaline by the addition of NaOH. The colorimetric determination is based on the reaction between F^-, Ce^{3+} and alizarin complexan, i.e., 3-aminomethyl-alizarin-*NN*-diacetic acid. The extinction coeff. is measured in a photometer at 587 mμ and compared with a standard curve. There are no interferences when plant parts are analysed. The average deviation is 4% when the F-content is $1 \cdot 10^{-3}$%F^-. (23 references.) M. SULZBACHER.

[A] Liquid scintillation counting of plutonium-239, [B] separation and determination, in biological samples. [A, B] T. Y. Tombara, C. Predmore, [A] D. A. Murkin and [B] P. A. Hargrave (*Talanta*, 1963, **10,** No. 2, 205—208, 209—214).—[A] After ion-exchange separation ^{239}Pu is counted in a liquid scintillator [1 ml. of aq. solution, 10 ml. of ethanol, 10 ml. of 0·005% 1,4-di-2-(5-phenyloxazolyl)benzene and 0·4% 2,5-diphenyloxazole in toluene] using a LPI scintillation spectrometer.

[B] The sample is ashed, extracted with 8N-HCl, any insol. matter fused with K_2CO_3 and dissolved in 8N-HCl. Pu is retained on a column of Amberlite XE-117 and eluted with saturated SO_2 solution before scintillation counting. T. R. ANDREW.

SOCIETY OF CHEMICAL INDUSTRY

MONOGRAPH NO. 3

RECENT STUDIES IN YEAST AND THEIR SIGNIFICANCE IN INDUSTRY

Comprising papers (with discussion) read at a Symposium organised by the Dublin and District Section, The Microbiology Group and the Food Group, held in Dublin 17—19 September, 1956

Price : **15s. 6d.**

Price to members : **11s. 7d.**

Orders should be sent to :

The Publications Department,
Society of Chemical Industry,
14 Belgrave Square,
London, S.W.1. (Tel : Belgravia 3681)

Journal of Applied Chemistry

The following papers are appearing in the August, 1963, issue

The purification of lithium by vacuum distillation
By A. G. Ward and G. H. Broomfield

Separation of emulsions formed in the dinitration of toluene
By Tarik G. Somer

Alkyl quaternary ammonium compounds as inhibitors of the acid corrosion of steel
By R. J. Meakins

An approach to applicable inorganic polymers. IV. Interaction of boron or silicon halides with esters of phosphonic acids
By G. Chainani, W. Gerrard, J. K. Patel and R. Twaits

Effect of a miscible solvent on the Clemmensen reduction
By G. E. Risinger and J. A. Thompson

Surface properties of hydrolysed titania. III. Titania prepared from titanium chloroalkoxides
By M. R. Harris and G. Whitaker

Silica powders of respirable size. IV. The long-term dissolution of silica powders in dilute hydrofluoric acid: an anisotropic mechanism of dissolution for the coarser quartz powders
By I. Bergman

Preparation of uranium peroxide hydrates
By Taichi Sato

J. Sci. Fd Agric., 1963, Vol. 14, August

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

CONTENTS

Printed in Great Britain by Butler & Tanner Ltd., Frome and London

76583
18 OCT 63