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THE PHOSPHOLIPIDS OF FISH*

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Of the relatively few investigations that have been made of the phospholipids of fish tissues, many have yielded inexact results, for a variety of reasons.

Detailed studies have now been made of the phospholipids of the flesh of cod and haddock. The component phospholipids of this tissue show a general similarity in the two species. A complex mixture of phospholipids is present, in which lecithins predominate (50–60% of the total phospholipids). There is also a group of unidentified phospholipids, temporarily named A, B and C, with A greatly predominating. Two of these lipids (A and C) may be derivatives of a bis-phosphatidic acid. Three types of inositol phosphatide have been detected. Phosphatidyl-ethanolamine is a minor component, there are traces of plasmalogens, sphingomyelin is doubtful, and phosphatidyl-serine is absent.

The fatty acids of the lecithins are remarkable for their high content of C_{20} and C_{22} polyethylenic acids and for the virtual absence of hexadecenoic acid. Part of the lecithin fraction is soluble in cold acetone, and this material is more unsaturated than the acetone-insoluble lecithins. Phospholipid-A contains an unusually high proportion of stearic acid, while phospholipid-B is virtually devoid of C_{22} acids. The fatty acids of phospholipid-C resemble those of the lecithins.

Phospholipid content of fish tissues

THE phospholipids of fish have, in general, been very inadequately studied. Some investigators have been interested primarily in the much more plentiful triglycerides of fish tissues, but have investigated the fatty acids of the mixed phosphatides incidentally extracted along with the triglycerides. The extraction techniques used in such cases, although satisfactory for depot fat, have usually been inadequate for complete extraction of phospholipids. The crude phospholipids have often contained considerable proportions of impurities, as shown, for example, by their low phosphorus contents. In other cases, where the investigation has been primarily concerned with the phospholipids, extraction techniques are more satisfactory. The same cannot always be said, however, of the procedures used to determine the proportions of individual phospholipids (e.g. lecithins, cephalins, sphingomyelins) in the total phosphatides. These factors probably account for most of the discrepancies in published data on the phospholipid content of fish tissues, although some variation according to species and season may be expected. A selection of such data is given in Table I.

Table I

Phospholipid content of fish tissues, as % of wet weight

Tissue	Fish	Total phospholipid %
Liver[2]	Groper	0·16–1·5
Liver[3]	Snoek	2·0
Liver[4]	Shark	0·3
Liver[5]	Sardine	0·49*
Roe[6]	Ling	0·4
Roe[7]	Salmon	4·0†
Intestine[5]	Sardine	0·41*
Intestine[3]	Snoek	1·0
Flesh[1]	Various species	0·1–0·7
Flesh[3]	Sardine	0·35–0·43*
Flesh[8]	Salmon	0·85
Flesh[1, 8, 9]	Cod	0·4 ; 0·85 ; 0·35
Flesh[1, 10]	Haddock	0·17–0·48 ; 0·35

* Choline-containing phospholipids
† Lecithin fraction

* Read at Joint Conference of the Food and Oils & Fats Group, 9–10 February, 1956.

As with higher vertebrates, the brain and spinal chord of fish are relatively rich in phospholipids. On a wet-weight basis, assuming 80% of water in both tissues, fish brain contains about 4% of total phospholipids[11, 12, 13] and spinal chord about 6%.[14]

Composition of total phospholipids

The analysis of a crude preparation of the total phospholipids of a tissue into its individual constituent lipids, phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, etc., is not a simple operation. Typical procedures include determination of choline and its allocation between lecithin and sphingomyelin on the basis of ease of hydrolysis or the direct determination of sphingomyelin as the reineckate. The latter method is not reliable.[15, 16] Total ' cephalin ' is often considered as the total non-choline-containing phospholipids. Total phospholipids themselves are often determined by multiplying total ether-soluble phosphorus by a conventional factor. There is no simple or quantitative procedure for the sharp separation of individual classes of phospholipid and it is seldom that an investigator has determined all the products of hydrolysis, e.g., the proportion of the total phosphorus which occurs as glycerophosphoric acid is seldom reported. Crude lipid extracts, completely soluble in organic solvents, may contain appreciable proportions of non-lipid forms of choline and phosphorus. Unless a quite large amount of raw material is examined, subsequent fractionation procedures will be hampered by yields becoming too small for adequate chemical analysis.

For reasons such as the above, most of the scanty published accounts of the content of individual phospholipids in fish tissues can only be regarded as approximations. Some data are collected in Table II.

Table II

Components of fish phospholipids as % of total phospholipids

Tissue	Fish	Lecithin	'Cephalin'	Sphingomyelin
Flesh[8]	Cod	77	12	11
Flesh[8]	Salmon	58	42	nil
Brain[11]	Various species*	27–39 (33)	41–59 (52)	5–24 (15)
Spinal chord[14]	Various species*	28–36 (32)	24–53 (43)	15–40 (25)

* Average values, for seven species, in parentheses

In these laboratories detailed studies of the lipids of the flesh of cod (*Gadus callarias*) and haddock (*Gadus aeglefinus*) have been undertaken. These fish are species of economic importance and have the additional advantage for present purposes of concentrating their depot fat almost exclusively in the liver, leaving the flesh lipids relatively free from triglycerides. The lipids have been quantitatively extracted, using a series of solvents,[17] the crude extracts largely freed from impurities simultaneously extracted,[9, 10] and the purified extracts fractionated by counter-current distribution between aqueous ethanol and light petroleum. Groups of the resulting fractions, and/or hydrolysates of them, have been analysed for all known lipid constituents. On the basis of proportions of fatty acids, total unsaponifiable matter, free and esterified cholesterol, higher aliphatic aldehydes, glycerophosphoric acid, glycerol, inositol, choline, ethanolamine and serine, it has usually been possible to achieve a fair balance sheet, indicative of the composition of any particular fraction, and hence of the total lipids. At the same time, this method has revealed not only the complexity of the mixture of lipids in these tissues, but the presence in them of novel lipids. Some fractions have been found to show an excess of one or more component after the other components have been equated on the basis of known lipid structures. If an excess of fatty acids is also present in such fractions, a novel lipid is suggested as one component of the fraction. It has been confirmed that the segregation of such fractions by counter-current distribution is reproducible, and analytical data on them are consistent.

In Table III are shown the approximate phospholipid compositions of cod and haddock flesh, respectively. The approximate nature of the data arises largely from the fact that arbitrary conversion factors have had to be used, e.g., for converting ' excess ' fatty acid into novel lipids and inositol into inositol phosphatides. Further, some of the novel lipids may not be phospholipids, and the material estimated as higher aliphatic aldehyde may not all be such.[18]

Nevertheless, it is considered that Table III gives a reasonably quantitative picture of these two lipids. It is considerably different from that suggested for cod in Table II.

Table III

Phospholipids of the flesh of cod and haddock, as % of total phospholipids

Fish	Lecithin	Phosphatidyl ethanolamine*	Inositol phosphatides	Plasmalogens	Novel phospholipids
Cod	54	11	3	present	32
Haddock	62	8	6	2	22

* No phosphatidyl serine present

Counter-current distribution resulted in the segregation of three types of individual inositol lipid, one going to the extreme ethanol end, one to the extreme light petroleum end, and one towards the middle of the distribution chain. The relative proportions of these sub-fractions of the total inositol lipids were different in haddock and cod, and these differences were reflected in different chromatographic behaviour on a cellulose column.[9] Counter-current distribution similarly distinguished three novel phospholipids. These substances are discussed in more detail below.

No reference is made in Table III to sphingomyelin, the analytical determination of which is not simple. Many fractions of the haddock lipids were assayed for sphingosine nitrogen, by the method of McKibbin & Taylor,[19] and appreciable amounts were sometimes found. However, the counter-current distribution behaviour of the ostensible sphingolipid did not agree with that of sphingomyelin, nor did its solubility properties, e.g., in acetone, nor was there usually phosphorus available equivalent to the supposed sphingosine. Cerebrosides cannot have been present, since fractions rich in this ostensible sphingosine often contained no sugar.[10] It is believed that the method is not sufficiently specific, and that this nitrogen was not sphingosine-nitrogen in most cases. The presence of very small proportions of sphingomyelin cannot be ruled out, but certainly it is not present in much more than traces.

Another phospholipid not mentioned in Table III, occurring only in trace proportions, is an acidic substance found in both haddock and cod extracts.[9, 10] It is not a simple phosphatidic acid, but possibly a complex of several lipids, with the acidity derived from excess phosphoric acid units.[20]

The unidentified phospholipids of haddock flesh contained three components, named temporarily A, B and C, present in the approximate ratios of 70, 15 and 15%, respectively, of the total. The data on the unidentified lipid fractions in the cod are not so amenable to quantitative examination, but lipid-A undoubtedly predominates, perhaps amounting to 85% of the total, while lipid-C does not appear to exceed about 6%. Phospholipids-A and -C were both found to contain ratios of fatty acid and glycerol to phosphorus in excess of those of the usual phosphatidyl esters. Although not obtained pure, it appears, from detailed hydrolytic studies,[9, 21] that both lipids may be fatty acid esters of a polyglycerol phosphate, with a probable fatty acid : glycerol : phosphorus ratio of 4 : 2 : 1. This corresponds to a simple bis-phosphatidic acid. However, neither lipid possesses acidic properties. Crude preparations of them contain nitrogen, which has not so far been characterized, and which may be an impurity rather than a lipid component.

Unidentified lipid-C, in both cod and haddock, is more difficult to extract from the tissue than the other phospholipids, since, while they are largely extractable by acetone, lipid-C appears almost exclusively in a subsequent extract obtained with an ethanol–ether mixture.[9, 10] There is no reason to attribute this behaviour to solubility differences, since much of the lecithin, for instance, although extractable with acetone in the first place, is insoluble in acetone after partial purification. It appears more probable that lipid-C is bound more firmly than most of the other lipids into lipoprotein complexes.

The inositol lipids, like lipids A, B and C, have not been obtained pure, but hydrolysis studies on the richest concentrates of them, from both cod and haddock, suggested a molecular glycerol : inositol ratio of 2 : 1,[9, 10] in contrast, for instance, to the unimolecular ratio in the

diphosphoinositide of brain. The inositol lipids are rather resistant to extraction, an appreciable proportion remaining even after extraction with both acetone and ethanol–ether.

Fatty acids of phospholipids

Several of the phospholipid fractions of haddock flesh have been obtained in sufficient quantity for fatty acid analysis by fractional distillation of the methyl esters. The lecithin fraction was partially soluble in cold acetone, and the soluble and insoluble fractions were examined separately. Some of the fatty acid analyses are shown in Table IV. It must be emphasized that none of the preparations was free from other lipid types—they were rather concentrates of the lipids being studied. Further, the analyses had to be carried out on very small amounts of fatty acid, with consequent reduction in accuracy. Nevertheless, the data in Table IV show some outstanding features.

Table IV

Composition of the fatty acids of some phospholipids of haddock flesh (wt.-%)

Lipid	Saturated acids			Unsaturated acids*		
	C_{16}	C_{18}	C_{20}	C_{18}	C_{20}	C_{22}
Acetone-soluble lecithins[22]	15	5	2	9 (5·4)	38 (8·0)	31 (11·5)
Acetone-insoluble lecithins[22]	22	8	1	19 (3·6)	32 (7·3)	18 (9·9)
Total lecithins[18, 22]	18	7	2	14 (4·2)	35 (7·6)	24 (10·8)
Lipid-A[22]	6	18	2	8 (2·7)	42 (5·5)	24 (11·4)
Lipid-B[18]	16	5	6	28 (4·0)	45 (8·0)	—
Lipid-C[18]	8	7	6	16 (4·0)	45 (8·0)	18 (10·0)

* Average unsaturation in parentheses, as deficiency of hydrogen atoms

Firstly, all the lipids are remarkable for the virtual absence of hexadecenoic acid, which is a characteristic major component of fish depot fats in general, including haddock depot (liver) fat. The only haddock flesh lipid found to contain major proportions of hexadecenoic acid is the extremely small amount (about 0·01% of the wet tissue weight) of triglycerides present which, indeed, show a general close chemical resemblance to the depot fat.[23]

Secondly, the lecithins are remarkable for their high content of C_{20} and C_{22} acids, and for the high degree of unsaturation of these groups. The lecithins soluble in cold acetone show an enhancement of both these trends. Reduced proportions of saturated acids, and of C_{18} unsaturated acids, together with higher mean unsaturation of all unsaturated groups, combine to make the fatty acids of the acetone-soluble lecithins considerably more unsaturated than those of the acetone-insoluble ones. The mean iodine values of the fatty acids, calculated from Table IV, would be 275 and 188, respectively.

Since lecithin is the predominant component of the phospholipids, and since the other major phospholipid components have fatty acid mixtures of the same general type as the lecithins (Table IV), it is reasonable to compare these lecithin results with those of Shorland and co-workers on certain total phosphatide preparations from fish tissues. The phospholipids from the roe of the New Zealand ling (*Genypterus blacodes*) contained more C_{20} and C_{22} acids and less hexadecenoic acid than did the triglycerides.[6] The phospholipids and triglycerides from the liver of the groper (*Polyprion oxygeneios*) showed a similar difference for the C_{20} and C_{22} acids, with only a slight difference in the amount of hexadecenoic acid.[2] The phospholipids and triglycerides of the head and body tissues of the school shark (*Galeorhinus australis*) showed no consistent trend in this direction.[24] It should be noted that removal of all triglycerides from the fraction requires repeated precipitation from acetone, which was not always done. Klenk's qualitative study[25] of the phospholipids from the liver of a different shark, *Etmopterus spinax*, showed that they were rich in C_{20} and C_{22} polyethylenic acids, whereas the liver triglycerides of this species were

almost devoid of them. Nevertheless, the data on the school shark suggest that no generalization on the phospholipids of fish compared with the triglycerides from the same tissue would be safe.

The absence of C_{20} and C_{22} polyethylenic acids from a purified lecithin of salmon eggs, reported by Anno,[7] in contrast to the presence of these acids in large proportions in the triglycerides of the same tissues, is probably attributable to the cadmium chloride purification procedure used yielding a concentrate of the more saturated (less soluble) lecithins, an effect noted by Sinclair.[26] The same comment applies to other work[27] on the purified lecithin from the eggs of a shark, *Squalus sucklii*, which contained only 13% each of C_{20} and C_{22} polyethylenic acids and, on the other hand, 34% of palmitic acid plus 9% of stearic acid. The shark egg lecithin, however, was devoid of hexadecenoic acid, whereas the egg triglycerides contained 13% of it.

The fatty acids of phospholipid-A are notable for the high content of stearic acid, confirmed in two analyses of rich concentrates of the lipid and a third of a more mixed preparation. Phospholipid-B is remarkable for the apparent absence of C_{22} acids. Phospholipid-C, although low in palmitic acid and high in arachidic acid, shows a general similarity to lecithin in its fatty acid composition. Hence its completely different counter-current distribution behaviour,[18] and its greater resistance to extraction from the tissues,[10] cannot be attributed to its fatty acids. Since its glycerol-phosphate skeleton appears to resemble that of phospholipid-A, which shows a counter-current distribution behaviour only a little different from that of lecithin,[22] the different nitrogenous components[9] may be responsible.

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THE FATTY ACID COMPOSITION OF MILK FATS FROM BEEF COWS FED ON DIFFERENT WINTER RATIONS

By G. A. GARTON and W. R. H. DUNCAN

The component fatty acids of four composite milk fat samples from groups of stall-fed beef cows in the Inverness area were determined by the ester-fractionation procedure. The milk fat from cows fed on grass silage resembled in composition that from dairy cows at pasture, whilst the milk fats from cows fed wholly or mainly on roots were more saturated; a feature of these fats was their relatively high content of palmitic acid accompanied by a correspondingly low content of oleic acid. In all four milk fats a reciprocal relation was observed between the proportions of oleic acid and palmitic acid present, whilst the proportion of stearic acid in these fats varied directly with their content of oleic acid. The influence of diet on the composition of the milk fats is discussed.

Introduction

This study formed part of an investigation into the etiology of the enzoötic muscular dystrophy which occurs in spring in suckling beef cattle in the North of Scotland,[1] particularly when the dams have been stall-fed exclusively or mainly on roots and straw during most of their pregnancy; this type of ration provides the cow, and in turn the calf, with very little vitamin E. It is known that dystrophic lesions of the muscles can be prevented by the administration of α-tocopherol,[2] although the tocopherol requirement is conditioned by the nature of the dietary fat. Thus unsaturated dietary fats, such as cod liver oil (a rich source of polyethenoid fatty acids), increase the amount of α-tocopherol required to prevent the occurrence of the disease.[3] It was considered possible that the degree of unsaturation of the milk fat received by the calves might be a further factor, additional to a low tocopherol intake, accounting for the incidence of the disease. This supposition was supported by the findings[4–6] that calves given fat-free rations containing very little α-tocopherol, did not develop muscular dystrophy.

It was found, however, that although cows given rations of turnips and straw produced milk fats having a very low content of α-tocopherol, the fats themselves were less unsaturated than the milk fat of animals fed on grass silage (a good source of α-tocopherol[7]); these findings, *inter alia*, have been summarized in a recent communication.[8] It was concluded that, under normal feeding practice, there was no association between the unsaturated fatty acid content of the milk fat and the incidence of muscular dystrophy.

Nevertheless, it is of interest to record the detailed analyses of the four milk fats, one from cows fed on silage, one from animals fed on roots and straw with a silage supplement and two from animals fed almost exclusively on roots and straw, since as far as is known there is no published information on the composition of the milk fat of beef cows as opposed to dairy cows. Much of the information on the component fatty acids of the milk fat of dairy cows and factors which may influence its composition has been summarized and reviewed by Hilditch[9] and Hansen & Shorland.[10] With regard to winter stall-feeding, Hilditch & Jasperson[11] found that the composition of the milk fat from a group of silage-fed dairy cows in Cheshire more closely resembled that reported in earlier studies of milk fats from cows fed on hay, roots and concentrates than it resembled milk fat from cows at pasture. Since the daily intake of carbohydrates, proteins and lipids of the pasture-fed and the silage-fed cows was almost the same, Hilditch & Jasperson concluded that the fall in the oleic acid content of milk fat frequently observed during winter was probably due to an environmental, rather than to a dietary effect.

Experimental

Animals and diet.—The cows, on farms in the neighbourhood of Inverness, were stall-fed from November until milk samples were obtained at the end of March, 2–3 weeks after calving. In Table I is shown the breed, number and diets of the cows and the groups from which composite milk samples were obtained.

Milk samples.—Bulked samples of milk (2–3 gallons) were obtained from each group of cows. The proportion of milk contributed from each farm to the bulked samples is shown in Table I.

Table I

Breed and number of cows used and diets fed and proportion of milk contributed from each farm

Group providing composite milk sample	Farm	Breed and number of cows	Proportion of milk in bulk sample (%, v/v)	Diet/cow/day
I	A	Shorthorn (7)	60	Oat straw *ad lib.* 52 lb. grass silage
	B	Aberdeen Angus (6)	40	Oat straw *ad lib.* 40 lb. grass silage
II	C	Shorthorn × Aberdeen Angus (6)	25	15 lb. oat straw 30 lb. turnips 20 lb. grass silage
	D	Ayrshire × Shorthorn (6)	75	12 lb. oat straw 35 lb. turnips 6 lb. oats 22 lb. arable silage (every other day)
III	E	Shorthorn × Aberdeen Angus (4)	25	16 lb. oat straw 55 lb. turnips
	F	Shorthorn × Aberdeen Angus (6)	50	16 lb. oat straw 60 lb. swedes
	G	Shorthorn × Aberdeen Angus (5)	25	16 lb. oat straw 50 lb. turnips
IV	H	Aberdeen Angus (6)	40	Oat straw *ad lib.* 60 lb. swedes or swedes and turnips 4 lb. hay 28 lb. cabbages (during Feb. and March)
	J	Shorthorn × Aberdeen Angus (5)	60	16 lb. oat straw 60 lb. turnips 8 lb. grain and draff

It should, perhaps, be pointed out that the figures given do not represent the exact proportions of milk fat provided by each group of cows, since the amount of fat in the milk probably varied somewhat from farm to farm.

Preparation of milk fats.—Butter, prepared in the churn from the milk samples, was melted and kept at 60° for 30 min. to permit the separation of water, salts and curd. The clear milk fat was then decanted through a pre-warmed paper filter and stored under nitrogen in the dark at −1° until analysis was commenced.

Analytical characteristics of milk fats.—The general analytical characteristics of the four fats are recorded in Table II; iodine values were determined by the Wijs method as described by Hilditch.[12]

Table II

Analytical characteristics of the milk fats

Milk fat	I	II	III	IV
Iodine value	41·0	36·0	36·6	30·4
Saponification equivalent	254·3	246·0	243·3	242·6
Free fatty acid (as oleic %)	0·2	0·2	0·2	0·7

Component fatty acids of the milk fats

The fats were saponified by refluxing for 2 h. with excess of ethanolic potassium hydroxide. A preliminary separation of the steam-volatile fatty acids from each fat was effected as described by Hilditch and co-workers.[13–15] The non-volatile acids of each fat were then resolved into two groups (mainly saturated and mainly unsaturated acids) by crystallization of the more saturated acids from peroxide-free ether (10 ml./g. of non-volatile acids) for 5 hr. at −40°. The results of these preliminary separations are shown in Table III.

Treatment of steam-volatile acids.—The acids were recovered by means of ether from the steam distillate and from inside the condenser. After drying over anhydrous sodium sulphate, the ether was carefully removed leaving the free fatty acids which were fractionally distilled

Table III

Preliminary separation of the fatty acids of the milk fats

Fraction	Weight		Iodine value	Sap. equiv.
	g.	% of total		
		Milk fat I		
V	8·28	5·79	—	—
A	56·20	39·22	3·5	263·0
B	78·80	54·99	74·8	267·1
		Milk fat II		
V	8·98	7·75	—	—
A	54·20	46·77	4·3	263·9
B	52·70	45·48	74·4	264·0
		Milk fat III		
V	11·03	7·87	—	—
A	65·42	46·66	7·3	259·8
B	63·78	45·47	75·6	258·7
		Milk fat IV		
V	6·50	8·84	—	—
A	33·50	45·58	2·5	257·7
B	33·50	45·58	70·9	258·5

V = Volatile in steam
A = Non-volatile in steam, insoluble in ether at −40°
B = Non-volatile in steam, soluble in ether at −40°

from a Vigreux flask in a semi-micro distillation apparatus. Ether recovered from the dried acids and also the extracted aqueous distillate were titrated against 0·1N-sodium hydroxide to determine the residual acidity which was calculated as butyric acid.

Treatment of solvent-segregated acids.—Each group of acids was converted to its methyl esters by refluxing with methanol containing 1% (w/w) concentrated sulphuric acid. The esters were then fractionally distilled *in vacuo*, as described by Hilditch,[9] through an electrically-heated column packed with single- and multi-turn glass helices. The methods used for the fractional distillation of the methyl esters and for the analytical examination of the ester fractions were those described by Hilditch ;[9] spectrophotometric analysis of appropriate C_{16} and C_{18} unsaturated ester fractions were performed by the method of Hilditch, Morton & Riley,[16] using the $E^{1\%}_{1\,cm.}$ reference values given by Meara.[17]

Calculation of fatty acid composition of milk fats.—The composition of each ester fraction and each steam-volatile acid fraction was then calculated by methods described by Hilditch[9] and the percentage composition of each group of acids was thus derived. These compositions and the resultant composition of the total fatty acids in each milk fat are given in Table IV.

Discussion

For purposes of comparison the molar percentages of the fatty acids in the four fats are given in Table V.

In this discussion, owing to the wide range of molecular weight of the fatty acids, all percentages refer to values expressed on a molar basis.

The fatty acid composition of the four milk fats may be discussed firstly from the point of view of certain individual fatty acids and secondly from a consideration of groups of fatty acids. The most striking differences in composition in respect of individual fatty acids relate to the major components oleic and palmitic acids, and to stearic acid. The fatty acid composition of milk fat I (from animals fed on grass silage) closely resembles that usually found in the milk fats of dairy cows at pasture (see, for example, Hilditch & Jasperson[11]). Whilst milk fat I contains 28·7% oleic acid, the other milk fats contain less, especially milk fat IV in which oleic acid comprised only 16% of the fatty acids. In the many published analyses of bovine milk fats only one example could be found containing less than 20% of oleic acid ; Jack & Henderson[18] reported a value of 16·4% for a mixed sample of Californian milk fat from animals on unspecified diets. Although milk fats II and III are similar in fatty acid composition, the former (from animals given silage in addition to roots and straw) contains 1·6% more oleic acid than the latter.

Table IV

Component fatty acids in groups V, A, B (as % by wt. of the group) and the whole milk fats (separation into groups V, A and B is shown in Table III)

Acid	Milk fat I V (5·79)	A (39·22)	B (54·99)	Total	Fatty acids in the whole fat % (w/w)	% (mol.)	Milk fat II V (7·75)	A (46·77)	B (45·48)	Total	Fatty acids in the whole fat % (w/w)	% (mol.)
Butyric	47·66	—	—	2·76	2·8	7·7	39·23	—	—	3·04	3·0	8·0
Caproic	34·20	—	—	1·98	2·0	4·2	38·19	—	—	2·96	3·0	6·1
Caprylic	11·57	—	—	0·67	0·7	1·2	11·23	—	—	0·87	0·9	1·5
Capric	6·22	—	2·45	1·71	1·7	2·4	10·32	—	2·90	2·12	2·1	2·9
Lauric	—	—	4·03	2·22	2·2	2·7	—	—	5·56	2·53	2·5	2·9
Myristic	—	2·21	8·28	5·42	5·4	5·7	—	7·36	7·55	6·87	6·9	7·1
Palmitic	—	63·52	8·67	29·69	29·7	28·1	—	67·80	9·24	35·91	35·9	33·0
Stearic	—	25·60	0·86	10·50	10·5	9·0	—	16·78	—	7·85	7·9	6·5
Arachidic	—	4·86	—	1·91	1·9	1·5	—	3·33	—	1·56	1·6	1·2
Decenoic	0·35	—	0·11	0·08	0·1	0·1	1·03	—	0·16	0·15	0·2	0·3
Dodecenoic	—	—	0·45	0·25	0·3	0·4	—	—	0·58	0·26	0·3	0·4
Tetradecenoic	—	—	2·52	1·39	1·4	1·5	—	—	2·48	1·13	1·1	1·1
Hexadecenoic	—	—	5·86	3·22	3·2	3·0	—	—	6·09	2·78	2·8	2·6
Hexadecadienoic	—	—	0·40	0·22	0·2	0·2	—	—	0·41	0·19	0·2	0·2
Octadecenoic	—	3·81	57·87	33·32	33·4	28·7	—	4·73	54·57	27·03	27·1	22·6
Octadecadienoic (conjugated)	—	—	0·51	0·28	0·3	0·3	—	—	0·63	0·29	0·3	0·3
Octadecadienoic (conjugatable)	—	—	1·10	0·60	0·6	0·5	—	—	2·48	1·12	1·1	0·9
Octadecatrienoic	—	—	1·03	0·56	0·6	0·5	—	—	1·30	0·59	0·6	0·5
Unsaturated C_{20}–C_{22}	—	—	5·45	3·00	3·0	2·3	—	—	5·47	2·49	2·5	1·9
Unsaponifiable	—	—	0·41	0·22	—	—	—	—	0·58	0·26	—	—

Acid	Milk fat III (7·87)	(46·66)	(45·47)				Milk fat IV (8·84)	(45·58)	(45·58)			
Butyric	51·71	—	—	4·07	4·1	10·8	42·76	—	—	3·78	3·8	9·9
Caproic	30·26	—	—	2·38	2·4	4·8	29·07	—	—	2·57	2·6	5·1
Caprylic	5·97	—	—	0·47	0·5	0·8	8·26	—	—	0·73	0·7	1·1
Capric	11·05	—	2·79	2·14	2·1	2·8	18·66	—	3·41	3·20	3·2	4·3
Lauric	—	—	7·15	3·25	3·3	3·8	—	—	5·64	2·57	2·6	3·0
Myristic	—	8·51	7·85	7·54	7·5	7·6	—	9·30	8·17	7·96	8·0	8·0
Palmitic	—	67·12	9·43	35·61	35·7	32·2	—	73·00	17·45	41·23	41·3	37·0
Stearic	—	14·35	—	6·70	6·7	5·4	—	12·25	1·11	6·09	6·1	4·9
Arachidic	—	3·04	—	1·42	1·4	1·0	—	3·58	—	1·63	1·6	1·2
Decenoic	1·01	—	0·25	0·19	0·2	0·3	1·25	—	0·23	0·21	0·2	0·3
Dodecenoic	—	—	1·22	0·56	0·6	0·7	—	—	0·84	0·38	0·4	0·5
Tetradecenoic	—	—	3·44	1·56	1·6	1·6	—	—	2·91	1·33	1·3	1·2
Hexadecenoic	—	—	10·90	4·95	4·9	4·5	—	—	12·03	5·49	5·5	5·0
Hexadecadienoic	—	—	0·74	0·34	0·3	0·3	—	—	0·82	0·37	0·4	0·4
Octadecenoic	—	6·98	49·10	25·57	25·6	21·0	—	1·87	40·96	19·52	19·6	16·0
Octadecadienoic (conjugated)	—	—	0·74	0·36	0·4	0·3	—	—	0·79	0·36	0·4	0·3
Octadecadienoic (conjugatable)	—	—	1·81	0·81	0·8	0·6	—	—	1·29	0·59	0·6	0·5
Octadecatrienoic	—	—	1·50	0·68	0·7	0·6	—	—	1·90	0·87	0·9	0·7
Unsaturated C_{20}–C_{22}	—	—	2·57	1·17	1·2	0·9	—	—	1·84	0·84	0·8	0·6
Unsaponifiable	—	—	0·51	0·23	—	—	—	—	0·61	0·28	—	—

(Values in parentheses are % of total acids in the groups)

There appears to be a reciprocal relationship between the content of oleic acid and that of palmitic acid in these milk fats, although it is not suggested that these acids are metabolically interconvertible ; the combined amount of these two acids in milk fats I, II, III and IV accounts for 56·8, 55·6, 53·2 and 53·0%, respectively, of the total fatty acids. Compared with the palmitic acid content of the milk fats of dairy cows which approaches some measure of constancy at about 24–26% in English milk fats[9] and about 22–24% in New Zealand milk fats,[10] the palmitic acid content of milk fats II, III and IV is abnormally high. The stearic acid content of the four fats runs parallel with the content of oleic acid, showing a fall from 9·0% in milk fat I to the low value of 4·9% in milk fat IV.

Table V

Fatty acid composition of milk fats, expressed on a molar basis, excluding unsaponifiable matter

Milk fat	I	II	III	IV
Diet (excluding oat straw)	Silage	Roots plus some silage	Roots alone	Roots alone
Acid				
Butyric	7·7	8·0	10·8	9·9
Caproic	4·2	6·1	4·8	5·1
Caprylic	1·2	1·5	0·8	1·1
Capric	2·4	2·9	2·8	4·3
Lauric	2·7	2·9	3·8	3·0
Myristic	5·7	7·1	7·6	8·0
Palmitic	28·1	33·0	32·2	37·0
Stearic	9·0	6·5	5·4	4·9
Arachidic	1·5	1·2	1·0	1·2
Decenoic	0·1	0·3	0·3	0·3
Dodecenoic	0·4	0·4	0·7	0·5
Tetradecenoic	1·5	1·1	1·6	1·2
Hexadecenoic	3·0	2·6	4·5	5·0
Hexadecadienoic	0·2	0·2	0·3	0·4
Octadecenoic	28·7	22·6	21·0	16·0
Octadecadienoic (conjugated)	0·3	0·3	0·3	0·3
Octadecadienoic (conjugatable)	0·5	0·9	0·6	0·5
Octadecatrienoic	0·5	0·5	0·6	0·7
Unsaturated C_{20}–C_{22}	2·3	1·9	0·9	0·6

In addition to the reciprocal relationship noted between the palmitic and oleic acid content of the milk fats examined in the present study, a similar relationship seems to hold in respect of the saturated fatty acids C_4–C_{14} which, as a group, account for 23·9, 28·5, 30·6 and 31·4%, respectively, of the total fatty acids of the four milk fats. Although the oleic acid content of the milk fats differs so much, their content of C_{18} dienoic acids (conjugated and conjugatable) and C_{18} trienoic acid is very similar; on the other hand, unsaturated acids higher than C_{18} (calculated as C_{20}–C_{22}) account for 2% of the fatty acids of milk fats I and II (rations containing silage), whilst less than 1% was found in the fatty acids of milk fats III and IV from animals fed on roots and straw.

When the saturated fatty acids C_4–C_{16} are considered as a group, milk fat I stands apart with a content of 52·0%, whilst this group of acids accounts for 61·5, 62·8 and 68·4%, respectively, of the total fatty acids of milk fats II, III and IV. This may reflect, in some measure, the extent to which these acids were synthesized from acetate in the udder tissue and incorporated into milk glycerides, since Popják *et al.*[19], who investigated the synthesis of milk fatty acids from radioactive acetate in a lactating goat, found that whereas the saturated acids C_4–C_{16} could be synthesized and showed significant specific activities, a pronounced fall in specific activity occurred between palmitic acid and the C_{18} acids, stearic and oleic, suggesting that the main bulk of these acids in milk fat were not of glandular origin. In this connexion it should be noted that Duncombe & Glascock[20] recently demonstrated that C_{18} acids of milk fat come mainly from blood lipids.

Although almost all the shorter-chain saturated fatty acids C_4–C_{10} of milk fat arise *de novo* in the gland, it seems that the saturated acids C_{12}–C_{16} have a dual origin (from blood lipids and from acetate condensation in the gland), whilst stearic and arachidic acids may be derived almost entirely from blood lipids. That fatty acid chain elongation in the udder virtually ceases when palmitic acid is reached is supported by the findings of one of us[21] that palmitic acid accumulated to the extent of 41·3% of the total fatty acids of bovine mammary gland fat at the termination of lactation.

The composition of blood lipids is affected by the nature of absorbed dietary lipids and thus it seems possible that the proportions of oleic and stearic acids in milk fat I (and, to a limited extent, milk fat II) may have been influenced by the presence of C_{18} fatty acids in the diet. In both cases the diet (containing grass silage) afforded linoleic and linolenic acids which, as Shorland *et al.*[22] have recently shown, can undergo hydrogenation in the rumen to yield oleic

and stearic acids which become available for intestinal absorption and pass into the blood lipids. It is of interest to note that Hilditch & Jasperson[23] found that feeding diets containing oleic and linoleic acids (as groundnut oil, iodine value 88, or as partially hydrogenated groundnut oil, iodine value 45) resulted in increased proportions of oleic and stearic acids, but not of linoleic acid, appearing in the milk fat.

On the other hand, milk fats II, III and IV from cows fed wholly or mainly on turnips and straw (very poor sources of dietary fatty acids) resemble each other in that they all contain an unusually high content of palmitic acid and a correspondingly low proportion of oleic acid. In that milk fats III and IV were obtained from cows fed on apparently similar diets, it might have been expected that they would resemble each other more closely in composition, although it should be noted that it is only in the relative proportions of oleic and palmitic acids that they differ to any marked extent. Although milk fat IV is abnormal it may only reflect a more marked effect on metabolism of an influence also affecting milk fats II and III. We consider that this influence may reside in the α-tocopherol status of the lactating cows and experiments are in progress to investigate this possibility. The findings of Bratzler *et al.*[24] lend support to this suggestion; these workers found that the iodine value and oleic acid content of the body fats of young pigs fed for 75 days from weaning on a fat-free diet containing α-tocopherol were significantly higher than the values found for the fats of animals which had received the same ration without α-tocopherol.

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NOTES ON THE 2 : 4-DINITROPHENYLHYDRAZINE METHOD FOR PYRETHRUM ASSAY

By B. P. MOORE

Experiments are described which have clarified the chemistry of the D.N.P.* method[1] for pyrethrum and allethrin assay. The fate during the course of analysis of a number of the non-insecticidal contaminants has been determined and the scope and limitations of the method are reviewed in the light of the new findings.

Introduction

In an earlier communication[1] the writer put forward a method for the assay of 'pyrethrin' and allethrin concentrates, based upon reaction with 2 : 4-dinitrophenylhydrazine (D.N.P.). The procedure finally adopted contained empirical features; in particular, the calculation of 'pyrethrins' values involved important assumptions. Nevertheless, the results of tests on a wide range (though limited number) of standards showed promise.

Recently a variant of the new method, involving a different practical procedure, has been proposed[2] for allethrin assay and further work in this Laboratory has clarified most of the outstanding points. In the following notes, the details of the three main phases of the assay—the formation, isolation, and estimation of the 2 : 4-dinitrophenylhydrazones—will be reviewed in the light of this additional information.

The formation of the 2 : 4-dinitrophenylhydrazones

The 'pyrethrins' and allethrin react rapidly with D.N.P. in the presence of alcoholic mineral acid, but the resulting derivatives readily undergo alcoholysis of the ester linkage and the conditions for their quantitative formation are therefore critical. It is clear that under the reaction conditions originally proposed, namely hot 10% v/v sulphuric acid in ethanol, the alcoholysis is virtually complete, but the resulting 2 : 4-dinitrophenylhydrazones, which serve as the basis for the colorimetric estimation, are derivatives of the rethrolone† ethyl ethers rather than those of the free keto-alcohols themselves.

By using milder and rigidly standardized conditions, Green & Schechter[2] have obtained nearly quantitative yields of crystalline α-(±)-*trans*-allethrin 2 : 4-dinitrophenylhydrazone. When applied to commercial allethrin, these conditions gave rise to a non-crystalline mixture of isomeric derivatives which could be segregated for the purpose of gravimetric or colorimetric estimation by chromatography on a silica column. The 'pyrethrins' also reacted satisfactorily, but the derivatives of 'pyrethrins I' and 'pyrethrins II' were not separable on their silica columns.

From the practical point of view, the alcoholytic technique has the advantage that with both 'pyrethrins' and allethrin it gives rise to a less complicated mixture of derivatives, for the acidic moiety, the chief source of variation, is removed. This undoubtedly contributes to the greater precision of the final colorimetric estimation. On the other hand, free rethrolones† present in the original concentrates, or substances giving rise to them (such as rethrins† degraded in the acidic moiety) will interfere, for they give rise to the same derivatives as do the rethrins† under conditions of alcoholysis. The resulting errors are unlikely to be serious with allethrin estimations, since allethrolone is a very minor impurity in the usual commerical preparations[3] and it may in any case be removed by a preliminary percolation through alumina along the lines proposed by Brown, Phipers & Singleton[4] for pyrethrum extracts. Such a treatment may prove essential for the reliable assay of severely degraded 'pyrethrins'.

The non-alcoholytic method is not subject to interference from free rethrolones† present as minor impurities but the poor solvent properties of the reaction medium (cold methanolic hydrochloric acid) cause difficulties with crude concentrates, particularly with the pyrethrum oleoresins, which remain largely undissolved throughout the reaction period.

* In this paper 2 : 4-dinitrophenylhydrazine is referred to as D.N.P.

† For convenience, the stem names '-rethrolone' and '-rethrins' suggested by Harper (*Chem. & Ind.*, 1949, p. 636) are used here in a generic sense.

The isolation of the 2 : 4-dinitrophenylhydrazones

In view of the complex nature of commercial pyrethrum preparations in particular, adequate purification of the 2 : 4-dinitrophenylhydrazone(s) is essential. The author has employed column chromatography on standardized alumina for this purpose, whereas the American workers preferred silica as adsorbant. The 2 : 4-dinitrophenylhydrazones of ' pyrethrins I ' and ' pyrethrins II ', prepared under non-alcoholytic conditions, are readily separated on alumina (but not on silica) columns but the separation of cinerins and pyrethrins is only partial under these conditions. Experiments at this Laboratory indicate that ' pyrethrins I ' and ' pyrethrins II ' may thus be estimated separately after the manner already described[1] for total ' pyrethrins ', but using a reference curve prepared from pure α-(±)-*trans*-allethrin under non-alcoholytic conditions. However, it appears unlikely that this refinement will be adaptable to routine analysis of crude oleoresins, for in this case, the separation of the two bands and their quantitative elution in pure condition requires careful attention to detail and is expensive in both time and materials.

Now that the chemistry of the reaction with 2 : 4-dinitrophenylhydrazine is more fully understood, it is possible to determine the fate of some of the inactive constituents of pyrethrum oleoresin during the course of the analysis. The most important of these are undoubtedly the materials which result from degradation of the various ' pyrethrins '. It has already been shown[1] that the ' polypyrethrins ' produced by heat-treatment of crude oleoresin do not interfere with the alcoholytic technique, presumably because the ' pyrethrolone ' moiety is degraded under these conditions. Recently, Brown & Phipers[5] have obtained evidence that under the influence of ultra-violet radiation, the degradation of the ' pyrethrins ' involves preferential attack upon the acidic moiety of the molecule. The resulting false ' pyrethrins ' would interfere with an alcoholytic assay but they may be removed satisfactorily by prepercolation through a small column of alumina.

There is evidence[6] that insecticidally inactive esters of pyrethrolone and/or cinerolone with palmitic and linoleic acids occur (probably in small amounts only) in pyrethrum oleoresin. The isolation of these substances has not yet proved possible, but tests with the related (±)-allethrolone palmitate, prepared as a model substance, indicate that interference is to be expected with either variant of the D.N.P. analytic technique. Under alcoholysis, the latter substance affords a high yield of the 2 : 4-dinitrophenylhydrazone of (±)-allethrolone ethyl ether and it would thus register as about 78% of its weight of allethrin. On the other hand, the 2 : 4-dinitrophenylhydrazone resulting from mild reaction conditions is not readily separable on the column from the corresponding derivatives of allethrin and the ' pyrethrins '.

The chief impurities present in commercial allethrin concentrates are simple derivatives of chrysanthemic acid derived from an excess of the acid chloride employed in the synthesis ; these do not interfere with either variant of the D.N.P. assay.

Allethrolone would interfere with an assay by alcoholysis as it is readily converted to the 2 : 4-dinitrophenylhydrazone of its ethyl ether. However, as already noted, errors from this source are unlikely to be important and they may in any case be eliminated by a preliminary chromatographic treatment.

3 : 8-Nonadiene-2 : 5-dione has occurred as a minor impurity in all allethrin concentrates examined at this Laboratory. It may be detected by the formation of a deep magenta colour on heating with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) in ethanolic sulphuric acid (allethrin, allethrolone, and chrysanthemic acid do not form colours under these conditions). Tests with the pure diketone, prepared according to the directions of Schechter, Green & LaForge,[7] indicate that the coloured derivatives obtained under conditions of alcoholysis are very strongly held upon alumina ; no interference is therefore to be expected.

The colorimetric estimation

In the original method[1] the eluted 2 : 4-dinitrophenylhydrazones were estimated by comparing the optical densities of aliquot portions, determined on a Spekker absorptiometer (blue-green filter), with a standard curve prepared from α-(±)-*trans*-allethrin, carried through the same procedure. Total ' pyrethrins ' equivalents were calculated using a molecular weight factor

(1·13, not 1·07 as printed), a conversion which involved the assumption that the various 2 : 4-dinitrophenylhydrazones had similar absorption spectra. The validity of this assumption has now been confirmed for the 2 : 4-dinitrophenylhydrazones of pyrethrolone ethyl ether and (±)-allethrolone ethyl ether. The absorption spectra (Fig. 1), determined in benzene solution on a Unicam quartz spectrophotometer, are identical over the range 330–530 mμ, and the ε molar values at the maximum (380 mμ) are in good agreement. Similar agreement has been noted between the absorption spectra of the 2 : 4-dinitrophenylhydrazones of ' pyrethrins I ' and (±)-*trans*-allethrin.

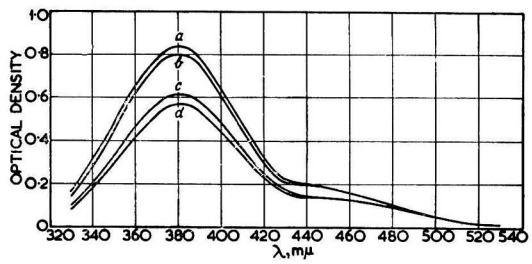

FIG. 1.—*Absorption spectra (benzene solution) of* 2 : 4-*dinitrophenylhydrazones of* (a), α-(±)-trans-*allethrin* (1·428 *mg./*100 *ml.*), (b), ' *pyrethrins I* ' (1·496 *mg./*100 *ml.*), (c), (±)-*allethrolone ethyl ether* (0·828 *mg./*100 *ml.*), (d), *pyrethrolone ethyl ether* (0·824 *mg./*100 *ml.*)

These findings show that the choice of wave-length for the colorimetric estimation is not critical and it may therefore be modified to suit the facilities of individual laboratories.

Experimental

[All melting-points are uncorrected. Microanalyses are by Miss M. Corner and Mrs. A. Grant of the Chemical Research Laboratory, Teddington.]

(±)-*Allethrolone palmitate.*—(±)-Allethrolone (300 mg.) and pyridine (0·3 ml.) in dry benzene (5 ml.) were added to palmitic acid chloride (1·1 g.) in dry benzene (10 ml.) and the mixture set aside overnight at room temperature. The product, isolated in the usual manner, was freed from volatile impurities by heating to 150° at 0·05 mm. and then percolated in 1 : 1 benzene–light petroleum (b.p. 40–60°) through a column (diam. 12 mm.) of neutral alumina (10 g., grade III activity). About 50 ml. of solvent mixture was required. Evaporation of the eluate yielded (±)-*allethrolone palmitate* as a colourless oil which soon solidified. When recrystallized from light petroleum, the product formed colourless waxy rosettes, m.p. 47–48° (Found : C, 77·1, 77·3 ; H, 10·9, 10·7. $C_{25}H_{42}O_3$ requires C, 76·9 ; H, 10·8%).

The 2 : 4-*dinitrophenylhydrazone* formed orange plates from ethanol, m.p. 93–94° (Found : C, 65·4, 65·5 ; H, 8·1, 8·4. $C_{31}H_{46}O_6N_4$ requires C, 65·2 ; H, 8·1%).

α-(±)-trans-*Allethrin* 2 : 4-*dinitrophenylhydrazone.*—α-(±)-*trans*-Allethrin (200 mg.) was dissolved as rapidly as possible in a warm solution of D.N.P. (160 mg.) in carbonyl-free ethanol[8] (20 ml., containing two drops concentrated sulphuric acid) and the mixture kept at room temperature. The product separated as orange plates, in almost quantitative yield, but repeated recrystallization from ethanol failed to yield an analytically pure specimen. Accordingly, the product was chromatographed in 2 : 1 benzene–light petroleum mixture on column (diam. 2·5 cm.) of alumina (50 g. Grade III), the eluate from the front third of the single orange band being rejected arbitrarily. The remaining eluate was evaporated and the residue, on being recrystallized twice from light petroleum, formed orange platelets, m.p. 128° (Found : C, 62·0, 62·2 ; H, 6·2, 6·2. Calc. for $C_{25}H_{30}O_6N_4$: C, 62·2 ; H, 6·3%).

(±)-*Allethrolone ethyl ether* 2 : 4-*dinitrophenylhydrazone.*—α-(±)-*trans*-Allethrin (200 mg.) and D.N.P. (150 mg.) were dissolved in ethanolic sulphuric acid (20 ml. of 10% v/v) and the mixture heated in an oven at 80° for 30 minutes. On cooling, the product crystallized.

(±)-*Allethrolone ethyl ether* 2 : 4-*dinitrophenylhydrazone* formed orange felted needles from ethanol, m.p. 134° (Found : C, 56·8, 56·8 ; H, 5·9, 5·6. $C_{17}H_{20}N_4O_5$ requires C, 56·7 ; H, 5·6%).

The same product resulted from similar treatment of (±)-allethrolone, (±)-allethrolone palmitate, or commercial allethrin.

'Pyrethrins I' 2 : 4-*dinitrophenylhydrazone.*—Purified 'pyrethrins'[1] (300 mg.) was dissolved as quickly as possible in a warm solution of D.N.P. (250 mg.) in carbonyl-free ethanol (30 ml., containing 3 drops of concentrated sulphuric acid) and the mixture allowed to stand overnight at room temperature. The mixture was diluted with water, the product isolated with ether and dried *in vacuo* at room temperature. The resulting mixture of 2 : 4-dinitrophenylhydrazones was applied in 1 : 1 benzene–light petroleum (b.p. 40–60°) to a column (diam. 2·5 cm.) prepared from a slurry of alumina (60 g., grade III) in the same solvent, and developed with 2 : 1 mixed solvent. The chromatogram was soon resolved into four coloured bands. A pale-yellow forerun which rapidly left the column was discarded. It was followed by the orange 'pyrethrins I' fraction, which was eluted without change of solvent. The 'pyrethrins II' fraction formed a deep orange band about an inch below the top of the column, just separate from the immobile impurities.

The 'pyrethrins I' fraction was collected in 5 × 30 ml. and 1 × 50 ml. portions, each of which was evaporated *in vacuo*. On recrystallization from carbonyl-free ethanol, the separate fractions afforded needles of m.p. ranging from 115–116° (first fraction) to 127–128° (last fraction). Material of m.p. 125° or higher was combined and rechromatographed under similar conditions, four arbitrary fractions being collected. Material from the last fraction was recrystallized from ethanol to give orange needles, m.p. 131·5° (50 mg.). The m.p. was not raised by rechromatography or recrystallization [Found : C, 63·9, 63·9 ; H, 6·6, 6·9. Calc. for $C_{27}H_{32}O_6N_4$ (pyrethrin I derivative) : C, 63·8 ; H, 6·3 ; Calc. for $C_{26}H_{32}O_6N_4$ (cinerin I derivative) : C, 63·0 ; H, 6·5%].

Pyrethrolone ethyl ether 2 : 4-*dinitrophenylhydrazone.*—The accumulated 2 : 4-dinitrophenylhydrazone from a number of alcoholytic assays of purified 'pyrethrins' was recrystallized repeatedly from carbonyl-free ethanol. Ultimately, orange felted needles, m.p. 136–137° were obtained and analysis indicated that these were essentially pure *pyrethrolone ethyl ether* 2 : 4-*dinitrophenylhydrazone* [Found : C, 58·9, 59·1 ; H, 5·9, 5·9. $C_{19}H_{22}O_5N_4$ (pyrethrolone derivative) requires C, 59·0 ; H, 5·7. $C_{18}H_{22}O_5N_4$ (cinerolone derivative) requires C, 57·7 ; H, 5·9%].

Specimen assay of purified pyrethrum oleoresin under non-alcoholytic conditions

Purified pyrethrin concentrate[1] (80 mg., alcoholytic assay : 78·7, 77·2% 'pyrethrins'), pure D.N.P. (100 mg.) and methanolic hydrochloric acid 10 ml., prepared from a mixture of carbonyl-free methanol[8] (28 ml.) with concentrated hydrochloric acid (0·1 ml.) were shaken together for 2½ hours at 20°. The mixture was then diluted with ether (30 ml.), worked up according to the original method,[1] and applied in 1 : 1 benzene–light petroleum (b.p. 40–60°) to a column (diam. 2·5 cm.) prepared from a slurry of alumina (80 g., grade III) in 1 : 1 mixed solvent. The chromatogram was developed with 2 : 1 mixed solvent until the pale-yellow forerun had left the column. The 'pyrethrins I' fraction was eluted with 3 : 1 mixed solvent and the 'pyrethrins II' with pure benzene. Large volumes of solvent were necessary and even development was essential for clean separation of the bands, particularly that of the 'pyrethrins II' which was closely followed by excess of D.N.P.

The colorimetric estimation of each main fraction was carried out according to the original procedure,[1] the comparisons being made against a standard curve derived from α-(±)-*trans*-allethrin under similar conditions. The conversion factors were 1·07 for 'pyrethrins I' and 1·21 for 'pyrethrins II' (Found : 'pyrethrins I', 46·0, 46·8 ; 'pyrethrins II', 26·6, 25·9 ; total 'pyrethrins', 72·6, 72·7%).

Separation of (±)-*allethrin and* (±)-*allethrolone*

(±)-Allethrin (redistilled, 360·5 mg.) and (±)-allethrolone (redistilled, 51 mg.) were dissolved in 1 : 3 ether–light petroleum (b.p. 40–60°) (10 ml.) and applied to a column (diam. 12 mm.)

prepared from a slurry of alumina (10 g., grade III) in the same solvent. The chromatogram was developed with the 1 : 3 solvent until exactly 200 ml. of eluate had been collected. The eluate was well shaken and a 20-ml. aliquot portion withdrawn for analysis by the alcoholytic method (Found: allethrin, 35·8 mg., 99·3% recovery).

Discussion

With the increase in knowledge of the composition of pyrethrum oleoresin, the problem of chemical analysis becomes manifestly more complicated. Ideally, the four pyrethrins would need to be isolated quantitatively and estimated separately, but in view of their close relationship, their lability, and the complex nature of the concomitant impurities, the possibility of developing a suitable technique seems remote. The D.N.P. method appears to offer promise for the collective assay of the 'pyrethrins' but the choice of optimum experimental conditions could be made only after extensive collaborative work.

The practical advantages and disadvantages of the author's original technique, the alcoholytic method, have already been discussed.[1] The method suffers from the drawback that only total 'pyrethrins' are determined, whereas earlier methods afforded separate, though very inaccurate, estimates of the 'pyrethrins I' and 'pyrethrins II' fractions. From the point of view of the pyrethrum user, this is unimportant, since the two fractions are still heterogeneous, but the calculation of total 'pyrethrins' values involves small additional errors consequent upon the use of a hybrid molecular weight factor. The value chosen, namely 340, assumed average pyrethrin/cinerin ratios of 1 : 1 and 'pyrethrins I'/'pyrethrins II' ratios of approximately 6 : 4. Deviations from the former ratio will be unimportant, since the molecular weights of the pure compounds differ by less than 4%. Variations in the latter ratio between 4 : 6 and 8 : 2 (inclusive) would be covered by a tolerance of $\pm 3\%$ and the maximum error possible, resulting from an assay of an oleoresin containing only pyrethrin II (a most unlikely eventuality), would amount to -11%.

The development of satisfactory non-alcoholytic reaction conditions[2] has permitted the separate estimation of 'pyrethrins I' and 'pyrethrins II'. However, the necessity for the separation of two pure bands on the column greatly complicates the chromatographic step. Large amounts of (non-recoverable) mixed solvent are necessary and it appears unlikely that this variant of the D.N.P. method will prove adaptable to routine analysis.

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FACTORS AFFECTING THE UPTAKE OF PHOSPHORUS BY CROPS IN SOUTH-EAST SCOTLAND

By K. SIMPSON

A series of more than fifty field experiments carried out in south-east Scotland showed superphosphate to be a more efficient phosphate fertilizer than any other with which it was compared. The apparent recovery of phosphorus by crops showed highly significant correlations with exchangeable calcium (positive), easily-soluble soil phosphorus (negative), and summer rainfall (positive). The low (apparent) recovery of applied phosphorus in dry seasons appears to be due to the increased yield on the control plots, resulting from a higher availability of soil phosphorus. This may be caused by an increase in soil temperature in dry seasons. The optimum dressing of superphosphate for swedes on phosphate-deficient soils was approximately 4·5 cwt. per acre.

Introduction

This paper presents the results of a series of experiments carried out with phosphate fertilizers under conditions prevailing in S.E. Scotland between 1941 and 1955. The object of the investigation was four-fold : (1) the comparison of superphosphate as a standard with other phosphate fertilizers ; (2) the correlation of the amount of phosphorus recovered by the crop (usually swedes) with factors such as easily soluble phosphate, pH, exchangeable calcium, and rainfall ; (3) the calculation of the efficiency of the various fertilizers in terms of production of dry matter per acre ; (4) the estimation of residual effects of phosphate fertilizers applied in a particular season on the following crop.

A report by Smith & Simpson[1] on the earlier experiments in this series was published in 1950. It was not possible at that time, owing to the small number of results available, to reach more than tentative conclusions. More than fifty experiments have now been carried out, including those for estimation of residual values, and some of the correlations and trends observed in the preliminary paper have been greatly strengthened.

Experimental

Soils

Many of the experimental soils are derived from parent material of glacial origin. Most of the soils (40 sites) are based on heavy or medium-heavy glacial till of very mixed origin. Six other soils are derived from fluvio-glacial sands and gravels and the remaining five are mostly alluvial in nature.

The work was concerned with the phosphate-deficient soils of ' marginal farms ' in the Lothians, Peebles, Selkirk, and Roxburghshire.

The fields with few exceptions lie between 450 and 1000 feet above sea level. Most of them were in permanent grass until the war when they were ploughed and cropped. They have remained in cultivation since that time, usually in a six- or seven-course rotation, including three or four years' grass. Drainage is seldom free on these soils, but sites were chosen where the water table was always well below the surface.

These marginal areas are recognized as being generally in need of lime and the soils selected were all acid, the majority falling in the pH range 5·5 to 6·5. In only three cases was acidity considered to be a limiting factor in crop growth.

Samples of soil, to plough depth, were taken from every plot on each site before the application of fertilizers. Sub-samples were taken from each plot for the estimation of pH, exchangeable calcium,[2] and easily-soluble phosphate, estimated by four different methods : (i) extraction with 0·2N-HCl or (ii) with 1% citric acid ; (iii) by use of *Aspergillus niger* ;[3] and (iv) extraction with 0·5N-acetic acid. Very good agreement was obtained between the results using the different methods, and the figures from methods (i) and (ii) only are used in subsequent comparisons.

Rainfall

The average annual rainfall of the area ranges from below 30 in. on the east coast to more than 45 in. in parts of West Lothian. This range affords a good opportunity to compare the

effects of rainfall on the efficiency of phosphate fertilizers at the different experimental sites. The area has a large population of meteorological stations. It was thus possible in almost every case to obtain rainfall data for a station within a mile or so of the experimental area. The annual data were, for the purpose of correlation with phosphorus uptake, sub-divided into Spring (January to April), Summer (May to October), and ' Total ' (January to October). Precipitation in the months of November and December was not taken into account.

Crops

Thirty-four of the experiments were carried out with swedes, five with oats, four with potatoes and three with grass. In three of the earlier experiments it was possible to estimate residual effects in the year following application of fertilizers. Oats was the second-year crop in these cases. This crop was found to give very low responses to residual phosphorus. In five later experiments, therefore, fertilizers were applied to oats and residual effects measured on swedes in the following season.

Treatments and design of experiments

The design was usually a 5 × 5 or 6 × 6 Latin square, except for potatoes, where a randomized block lay-out was used. The results from different experiments are comparable as superphosphate was invariably applied at two rates: 0·33 and 0·66 cwt. of P_2O_5 per acre, so as to obtain a yield curve. All other phosphate fertilizers were applied at an intermediate rate of 0·50 cwt. of P_2O_5 per acre. Farmyard manure was not applied on any of the experimental sites. A basal dressing of ammonium sulphate and potassium chloride was applied in each case. This dressing varied according to the requirements of the crop grown.

Fertilizers were always applied after the final cultivation and before (usually one day) the seed was sown or planted. Fertilizers for swedes, oats and grass were broadcast by hand. In the potato experiments the dressings were applied in the split drills immediately before planting. Swedes were usually sown in late April or early May, oats in late March or early April, and potatoes were planted in mid-April.

Visual observations

Visual observations were made at regular intervals on each experiment. The most striking observation made was that, in the early part of the season, crops treated with superphosphate were nearly always visually superior to those on other plots. This effect was often not so obvious later in the season, but it is felt that the vigorous early root growth promoted by the readily-available phosphorus from superphosphate is largely responsible for the superiority of this fertilizer in the area. At four centres (swedes) the crop on the control plots brairded satisfactorily, but afterwards the plants, although not diseased, made little or no progress. At one other centre ' finger and toe ' seriously affected the yield of swedes, particularly on the control plots. It was possible on only one of the experiments on oats to see any difference between treatments. In all potato experiments, the plots treated with superphosphate were superior to the control throughout the season.

Harvest

Before the swedes were lifted, samples of leaves and cores of roots were taken from fifty plants in each plot for determination of dry matter and phosphorus content. The roots were then lifted, topped and tailed, and the roots and leaves weighed separately.

The oat crops were harvested by a technique of taking random samples of several small areas within each plot. The crop was threshed and the yield of grain and straw obtained. Samples of grain and straw were then taken for analysis. Grass was sampled at harvest and weighed fresh in the field. In the potato experiments, samples of haulms, roots and tubers were taken at intervals during the season. The tubers were harvested, weighed and sampled for the determination of dry matter and phosphorus. The uptake of phosphorus by the whole plants was calculated by adding the amount of P_2O_5 in the tubers at harvest to the amount in the haulms at the latest sampling, which was carried out before the plants began to die back.

Results

Yield

A selection of the results showing the effects of superphosphate on the yield of swedes (roots) and the ' apparent recovery ' of phosphorus from the fertilizer are presented in Table I. The yield of tops, although determined, is omitted from this table.

Table I

Yield of swedes in tons per acre and percentage ' apparent recovery ' of phosphorus from superphosphate

Soil No.	P_2O_5 added, cwt./acre			S.E. ±	% recovery of P_2O_5 from	
	nil	0·33	0·66		0·33 cwt. treatment	0·66 cwt. treatment
1	22·3	25·8	27·6	0·9	24	18
3	0·6	3·8	6·3	0·6	7	6
6	4·2	11·7	15·0	0·6	36	27
8	13·9	17·6	18·2	0·4	21	11
12	2·0	4·5	5·5	0·4	11	9
13	9·0	15·5	17·5	0·6	38	27
15	17·5	17·9	18·9	0·4	6	3
17	7·1	7·9	8·9	0·4	10	8
20	17·3	18·8	18·8	0·6	8	4
23	25·6	26·3	28·0	0·7	18	12
25	22·8	25·1	25·3	1·4	6	6
27	19·2	25·7	30·6	1·2	22	25
30	3·9	20·5	21·5	0·9	51	32
31	0·5	12·4	16·3	0·7	48	39
39	8·9	9·3	7·8	0·4	3	Nil
41	26·6	30·2	29·8	1·2	7	Nil
Average for all swede experiments	13·6	17·3	18·6	—	18·6	14·4

There were very large differences in the control yields and in the responses to fertilizer. Under such circumstances, figures for percentage increase in yield are liable to be misleading or difficult to compare. It was decided, therefore, to rely upon differences in the apparent percentage recovery of the phosphorus added as a measure of the relative efficiency of superphosphate treatments in different experiments. This factor was calculated from the formula $100(U_p—U_c)/F$, where U_p and U_c are the uptake in lb. per acre of P_2O_5 by the crop on the phosphate treated and control plots respectively and F is the rate of the application of P_2O_5 in lb. per acre.

Recent work with ^{32}P has given reason to believe that only part of the extra phosphorus taken up on treated plots comes from the fertilizer. Several investigations[4–6] have shown that the proportion of plant phosphorus derived from the fertilizer applied increases with the rate of dressing. It seldom exceeds 70%, however. Nevertheless, the application of fertilizer is indirectly responsible for the uptake of greater quantities of soil phosphorus. The ' apparent recovery ' may therefore be regarded as a good index of fertilizer efficiency.

The yield increase due to 0·33 cwt. of P_2O_5 per acre as superphosphate was significant in 26 of the 34 swede experiments. In 22 experiments, however, the difference in effect resulting from the application of 0·33 and 0·66 cwt. of P_2O_5 per acre was not significant.

Curves were plotted showing the increase in the yield of roots for different dressings of superphosphate.

At five centres a steep sloping curve similar to that given by soil No. 31 was obtained, and in fourteen other cases the curves were similar to those for No. 8 and 25, where the maximum of the graph had almost been reached. A rather flat sloping curve such as given by soils No. 1 and 17 occurred in five experiments, and at the remaining ten sites the maximum yield had been reached at a lower level than 0·66 cwt. of P_2O_5 per acre, as in experiments 39 and 41.

Fig. 1 shows the average effect of superphosphate on swedes, oats, potatoes and grass. Responses in potatoes and oats were good in all cases, but grass did not respond well in any case to phosphorus.

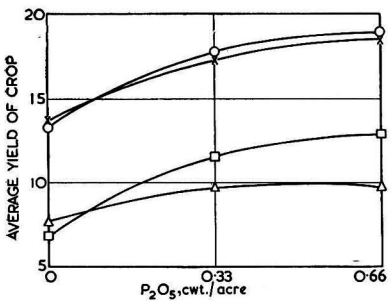

FIG. 1.—*Effect of superphosphate on the average yield of swedes, potatoes, grass and oats*

× Swedes (roots) in tons/acre
□ Potatoes in tons/acre
△ Fresh grass in tons/acre
○ Oats (grain) in cwt./acre

The values shown in Table I show how much the responses to fertilizer varied even when control yields were similar. They leave no doubt, however, about the value of a small application of superphosphate. On the average (Fig. 1), dressings of approximately 1·5 and 3 cwt. of superphosphate per acre increased the yield of swedes by 3·7 and 5·0 tons, respectively. In experiment 31, the increase in yield from 1·5 cwt. of superphosphate was 12 tons and in experiment 30, 16 tons. The present value of 16 tons of swedes is about £40 and the net cost of the superphosphate applied was 15 shillings.

The shape of the majority of the curves shows that the maximum yield would not be much larger than that obtained from an application of 0·66 cwt. of P_2O_5 per acre in the form of superphosphate. Indeed, in more than one-third of the experiments, the maximum had been reached below this rate of application.

Comparison of superphosphate with other fertilizers

Comparisons of the increase in yield of dry matter per acre produced by superphosphate with that produced by a number of other fertilizers are shown in Table II, along with comparative figures for recovery of phosphorus. These two factors were very closely related. (Correlation coefficient for 32 swede experiments = + 0·90.)

Table II

Average increase in dry matter yield per acre produced by various phosphate fertilizers and percentage recovery compared with superphosphate

Fertilizer at 0·5 cwt of P_2O_5/acre	Number of comparisons	Increase in yield of dry matter (lb./acre)		% recovery	
		Super-phosphate	Other fertilizers	Super-phosphate	Other fertilizers
Silicophosphate	11	1777	1449	20·3	17·8
Basic slag	8	1232	1026	16·7	13·1
Ground mineral phosphate	17	1801	1029	16·1	9·4
Ammoniated superphosphate	13	980	855	15·4	12·0
Dicalcium phosphate	20	1725	1481	18·2	13·7
Nitrophosphate	19	1011	804	15·3	13·8
Finely ground Gafsa mineral phosphate	8	1979	1318	15·5	8·1

In the whole series of experiments, not a single case was recorded of another fertilizer giving significantly superior results to superphosphate. Silicophosphate, nitrophosphate and ammoniated superphosphate gave the best comparative recovery figures. Gafsa mineral phosphate generally produced crops significantly inferior to those produced by superphosphate. This was also true for dicalcium phosphate, except in the dry season of 1955. The yields produced by Gafsa mineral phosphate were often not significantly different from the control yield.

Recovery of phosphorus

A selection of results for the recovery of phosphorus by the crop from superphosphate is

given in Table I. The recovery from 0·33 cwt. per acre of P_2O_5 varied from 3·0 to 51·4%, and for the 0·66 cwt. level, the range was from nil to 39·0%. The recovery was inversely related to control yield. [Correlation coefficient for 34 pairs (swedes) = — 0·57, significant at 1% level.]

Recoveries were low on the richer soils where the experiment was carried out at a point too near the maximum of the growth curve and high where the control yield was low. There were a few exceptions to this, where some other factor was involved, e.g., acidity.

Some of the factors giving rise to the very large variations in recovery of phosphorus are discussed below.

Residual effects

In eight experiments it was possible to estimate residual effects of fertilizers on the crop grown during the season after their application. In three cases the crop was oats and in five cases swedes. The results are summarized in Table III.

Table III

Residual effects of superphosphate on second crop

P_2O_5, cwt. per acre	0	0·33	0·66
Average yield of oats grain (cwt./acre)	6·1	7·6	8·6
Average yield of oats straw (cwt./acre)	7·4	8·2	10·0
Average % recovery by oats	—	6·6	2·7
Average yield of roots (tons/acre)	12·1	14·7	17·0
Average % recovery by roots	—	10·4	10·2

The recoveries in Table III were calculated as a percentage of fertilizer phosphate estimated as remaining in the soil after accounting for the apparent uptake by the first crop. In one swede experiment the crop recovered 20% of the fertilizer phosphorus (residual). The average recoveries obtained with swedes as the second crop were very satisfactory. Oats in the earlier experiments did not prove to be a very satisfactory crop for testing residual values.

A comparison of the residual value of superphosphate with that of other fertilizers showed the following decreasing order of merit: basic slag, superphosphate, silicophosphate, dicalcium phosphate and Gafsa mineral phosphate.

It is realized that these data are too few to draw any definite conclusions and further experiments are being carried out, specifically to estimate residual values.

Discussion of results

Yield and response

The steeply rising response curves obtained in many of the experiments show the undoubted value of *small* dressings of phosphorus in soluble form on the acid glacial drift soils of south-east Scotland. The average response to phosphate over the series of experiments is sufficient to merit the application of at least 0·66 cwt. of P_2O_5 per acre as superphosphate in this part of the country, but not much more. The shape of most of the curves indicates that the maximum yield will be reached below the level of 1 cwt. of P_2O_5 per acre. In a dry season the maximum yield may be reached at a much lower rate of application than this, as the yield from 0·33 cwt. of P_2O_5 per acre is often higher than that from 0·66 cwt. For the average curve (all swede experiments) it appears that little more increase in yield may be expected from applications greater than 90 lb. of P_2O_5 per acre (about 4 cwt. of superphosphate). In several experiments carried out on soils with higher amounts of ' available ' soil phosphorus, there was no response to phosphorus. This lack of response was evident even on soils containing very little ' available ' phosphate in the very dry season of 1955. Similar conclusions may be drawn for the oats and potato experiments.

These findings agree very well with those of Williams & Reith[7] for phosphate-deficient soils in the north-east of Scotland. They found that dressings higher than 80–100 lb. of P_2O_5 per acre did not give any further response in the swede crop. Crowther[8] quotes an average response in swedes, without dung, for Scotland of 5·9 tons per acre for an application of 0·5 cwt. of P_2O_5 per acre. The figure interpolated from the average curve drawn from the result given in Table I

is slightly less than this, viz. 4·3 tons per acre. The high response to small dressings of phosphorus strongly supports the statement made by Stewart[9] that, on such soils as those concerned in this series of experiments, any attempt to build up a reserve of phosphate in the soil quickly, by means of heavy applications, is fundamentally unsound and uneconomic. The residual values shown in Table III are also not high enough to support the practice of heavier dressings at less frequent intervals.

Crowther & Yates[10] calculated the 'optimal' dressing of superphosphate for swedes in Scotland to be 6·5 cwt. per acre with an average crop response of 9·3 tons of roots per acre. There is little evidence from the present investigation to support that statement, as the response from a dressing of this order, extrapolated from the average curve, would be no more than 6 tons per acre. Crowther[8] later calculated the most profitable dressing of P_2O_5 for swedes under 'standard' conditions to be 1·4 cwt. per acre and suggested adding a further 0·2 cwt. for deficient soils, i.e., an application of almost 8 cwt. superphosphate. This might apply to very wet conditions in the west of Scotland, but it would certainly appear to be a luxury dressing for the conditions concerned here.

Comparison of superphosphate with other fertilizers

The results quoted in Table II agree with the findings of many workers that no other phosphate fertilizer is consistently superior to superphosphate. Under our conditions, in terms of dry-matter production, ammoniated superphosphate, dicalcium phosphate, and basic slag, in that order, are only about 20% less effective. Silicophosphate and nitrophosphate are about 25% less effective and mineral phosphate is of little use for swedes even in the wetter part of the area. Williams & Reith[7] in north-east Scotland found that Gafsa mineral phosphate, although sometimes effective for swedes, was much less effective than superphosphate. For Great Britain as a whole, Crowther[8] found that for swedes, but not for potatoes, Gafsa mineral phosphate (G.M.P.) was often as satisfactory as superphosphate. In this series of experiments, the yields produced by G.M.P. often did not differ significantly from the control yield, and it is not to be recommended even in the wettest parts of the area. An extra-finely-ground mineral phosphate (ex 300 mesh sieve) has been used in some of the more recent experiments. Whilst giving better results than the normal G.M.P., this material was inferior in each case to superphosphate. It will, however, be necessary to carry out many more experiments with the finely ground material before coming to definite conclusions.

Recovery of phosphorus

The average recovery of phosphorus from superphosphate was 18·6 and 14·4% from 0·33 and 0·66 cwt. of P_2O_5 per acre, respectively. The most striking observation is the extraordinarily high percentage recovery figures of 51 and 48, respectively, from 0·33 cwt. of P_2O_5 per acre in experiments 30 and 31 carried out in 1954. As far as the author is aware, these figures have not been paralleled in this country. They were associated with extremely high summer rainfall (in experiment 30, the May–October rainfall was 30·3 inches) and further discussion of this fact will be made later.

Residual effects

The difficulties involved in carrying through an experiment for a second year on a commercial farm are great. As a result of this, very few figures are available. In the early years the crop on which the residual effects were tested was oats, the recovery in these experiments being low. In recent years it has been the practice to apply the fertilizers to lea oats and to harvest the following root crop. Results here have been more encouraging and have shown that residual effects although small are not negligible.

Williams,[11] using large dressings of superphosphate, showed that applications of 10 and 25 cwt. per acre found considerable residual values after two years, but the residues are ineffectual compared with a new dressing. In the majority of our experiments this appears to be the case also, although, in experiments 24 and 33, the recovery by swedes in the second season was 17·3 and 12·8%, respectively.

Correlation of recovery with rainfall and soil factors

The correlation coefficients for the recovery of phosphorus from 0·5 cwt. per acre of P_2O_5 as superphosphate are given in Table IV.

Table IV

Correlation coefficients of recovery with rainfall and soil factors

Factor	Number of pairs	Correlation coefficient	Level of significance
pH	46	+ 0·280	N.S.
Exchangeable Ca	44	+ 0·530	0·01
Citric acid-soluble P_2O_5	44	− 0·597	0·01
0·2N-HCl-soluble P_2O_5	44	− 0·542	0·01
Spring rainfall	46	+ 0·129	N.S.
Summer rainfall	46	+ 0·597	0·01
Total rainfall	46	+ 0·549	0·01
Control yield (swedes)	34	− 0·574	0·01

All the correlation coefficients (except that with control yield) in Table IV refer to experiments carried out with all crops. Correlations were calculated for swede experiments alone and, in most cases, the degree of significance of the correlation coefficients was only slightly different from those given above.

The correlation between recovery and pH is not quite significant at the 5% level. This comparatively poor relationship is due to the very large variation in recoveries in the middle range of pH values (5·5–6·0). It was necessary therefore to find some other factor to explain these variable results.

The points giving rise to the highly significant correlation between recovery and exchangeable calcium (Table IV) are shown in Fig. 2.

It is interesting to compare this result with those of Birch[12, 13] working with acid soils in Kenya, who found an inverse correlation between phosphate response (as measured by the percentage increase in yield over the control yield) and exchangeable calcium (expressed as a percentage of the total base-exchange capacity). Birch also states that under conditions obtaining in these soils, phosphate responses are not to be expected when the saturation of the base-exchange capacity exceeds 80%. His results obviously differ very greatly from those reported here, but further inspection shows that for some of the more unsaturated soils in the Kenya experiments, it would be possible to draw a positive correlation between exchangeable calcium and response. There is a great deal of evidence that exchangeable bases, particularly calcium, can retain appreciable amounts of phosphorus in readily available form.[14] It may be, therefore, that in our phosphate-deficient soils a considerable amount of newly applied fertilizer-phosphorus is held in this way.

Good negative correlations were obtained between recovery and easily soluble phosphorus estimated by various methods. The best of these was with citric acid-soluble phosphorus. This correlation is shown in Fig. 3.

The strength of this correlation is reduced to some extent by several points where, despite low contents of citric acid-soluble phosphorus, low recoveries were obtained. The soils in these cases were all very acid and it appears that, unless acidity is a limiting factor, easily-soluble phosphorus is a good guide to response in these soils. Cooke[15] came to similar conclusions, and also found a good correlation between easily-soluble phosphate estimated by two different methods.

Crowther & Yates[10] reported large regional variations in response to phosphate fertilizers in Britain, the wetter regions showing higher responses. Average responses in south-east England were only about two-thirds of those in north-central England. In the wetter areas of Wales, western England, and Scotland, responses were about one-and-two-thirds times as great as those in north-central England. These very large differences in response were attributed to rainfall, but little precise work on this factor has been carried out in this country.

In this series of experiments there is practically no relationship between spring rainfall and recovery. A very good positive correlation was found, however, between the recovery and both

summer and total rainfall. Fig. 4 shows the relationship between summer rainfall and recovery.

On examining the results from the thirteen seasons during which experiments have been carried out, most of the points in the top right-hand corner of Fig. 4 were obtained in two particularly wet seasons, 1948 and 1954. The two ringed points represent experiments carried out in the extraordinarily wet season of 1954. It appears therefore that high rainfall increases recovery considerably. There were only two very dry seasons during the series of experiments, 1946 and 1955. During these years twelve experiments were carried out and in nine of them the recovery was low. The correlation coefficient, for thirteen pairs of observations, between the yearly average recovery and the yearly average summer rainfall is +0·53 (almost significant at the 5% level).

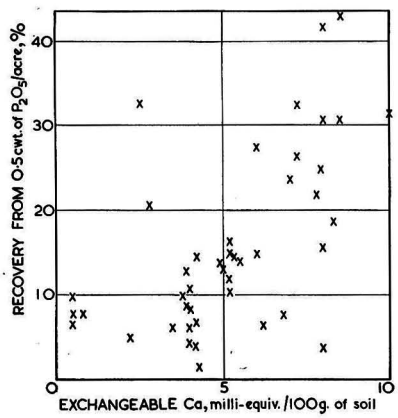

FIG. 2.—*Percentage recovery and exchangeable calcium*

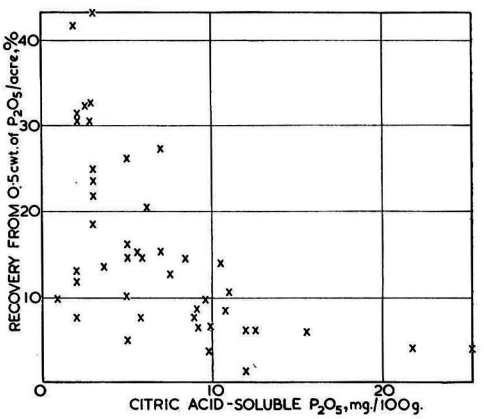

FIG. 3.—*Percentage recovery and citric acid-soluble* P_2O_5

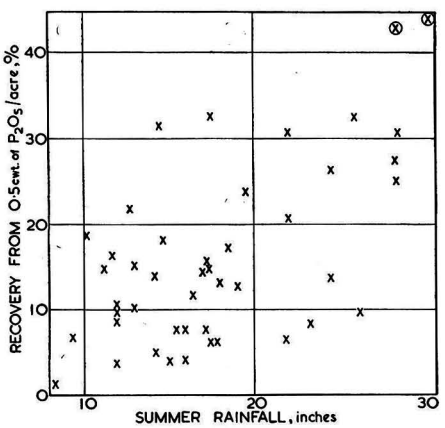

FIG. 4.—*Percentage recovery and summer rainfall*

The low recovery of phosphorus in crops in the dry seasons of 1946 and 1955 is particularly interesting. In only one, possibly two, of the experiments was drought considered to be a limiting factor to crop growth. The low responses in these seasons were due mainly to the high yield on the control plots, even on soils which, by our methods of estimation, had very low 'easily-soluble phosphate' figures. A good example of this is in experiment 42 in 1955 where

a soil with citric acid-soluble P_2O_5 of 5 mg. per 100 g. produced a control yield of 28·6 tons of swedes per acre. In the previous wet season of 1954 on experiment 30, about one mile away from the site of experiment 42, the control yield was only 3·9 tons per acre for a citric acid-soluble P_2O_5 value of 2 mg. per 100 g.

For 34 swede experiments, the correlation coefficient for summer rainfall and control yield was −0·425 (significant at the 2% level). *Soil* phosphorus was, therefore, apparently more available in drier seasons.

The average soil temperature at the fairly high elevations at which these experiments were carried out, will be fairly low and the moisture content high. Even in a dry season the soils are not likely to suffer from drought, but it is reasonable to expect that, with increased hours of insolation, there will be appreciable increases in soil temperatures. For example, in the wet season of 1954, the average *air* temperature was 5–10° below that in the dry season of 1955 and the hours of sunshine were 40–70% of the 1955 figure. It may be that the resultant increase in soil temperature accelerates the release of soil phosphorus in available form. Further work is being carried out on this point.

Conclusion

The yield of swedes, oats and potatoes on the marginal soils of south-east Scotland may be considerably increased by moderate dressings of phosphorus, particularly in the form of superphosphate. The optimum dressing for swedes is approximately 4·5 cwt. per acre.

Superphosphate is, on these soils, a more efficient fertilizer than any with which it was compared, but ground mineral phosphate is of very little value.

Exchangeable calcium shows a high positive correlation with recovery of phosphorus. Easily-soluble soil phosphorus shows a high negative correlation. The best guide to predicting the probable response to phosphate fertilizers on a particular soil would be obtained by determining both these values. At the same time it is necessary to take into account the rainfall during the months of May to October which shows a highly significant positive correlation with recovery of phosphorus.

The low recovery of applied phosphorus by crops in dry seasons appears to be due to the increased yield on the control plots, resulting from a higher availability of soil phosphorus. This may be caused by an increase in soil temperature in a dry season.

Acknowledgments

The author wishes to thank the Scottish Meteorological Office for putting at his disposal the many rainfall data. Without the land made available by more than fifty co-operative farmers these experiments could not have been carried out. Thanks are also due to Dr. A. M. Smith for his constant advice and guidance and to all members of the Chemistry Department who helped so much in both laboratory and field.

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FLOUR TESTING. I.—A Comparison of the Brabender Extensograph, Chopin Alveograph and Simon Extensometer Methods of Testing Bread Flours with Particular Reference to the Effect of Various Forms of Flour Treatment

By RUTH BENNETT and J. B. M. COPPOCK

An outline is given of the standard procedures of dough testing on the Brabender, Chopin and Simon instruments and a comparison is made of the essential differences in technique, especially in the methods of preparation and treatment of the dough. Data from each instrument on several series of flours are compared with baking behaviour. The flours examined include samples which had been treated in various ways, e.g., with agene, oxidising improvers, etc.

The effect of the presence of yeast on the dough is important especially when comparing untreated with treated flours in relation to fermentation time requirements. It was concluded that for reliable testing of bread flours of unknown treatment the technique should ensure that the dough (1) contains the amount of water to bring it to the correct consistency at the end of fermentation, (2) should include yeast, (3) should be allowed at least 3 hours rest before the final moulding and (4) should be given adequate moulding.

The Simon technique meets these requirements, and the Brabender technique can be modified to include them. The Chopin apparatus, though useful in studying untreated wheat flours and also in experimental work, does not permit such modifications to be made to its mode of use.

Introduction

It is well-known that valuable information on flour quality can be obtained by the use of flour testing instruments. This paper describes the results of an examination of a series of flours of varying quality and treatments, on three modern instruments situated in a single laboratory.

The information which it was felt particularly desirable to obtain is as follows:

(*a*) which instrument gives the most reliable data for forecasting the baking behaviour of bread flours of unknown origin and treatment?

(*b*) is the action of the various methods of flour and dough treatment revealed to the same extent by each instrument?

(*c*) does the existing technique or 'standard method' for each instrument reveal flour differences to the best advantage or would modification make such tests more valuable?

(*d*) do the instruments reveal the same or different physical properties of dough, and if different, is any one instrument more suitable for revealing a particular and important property?

Extensive literature has been published especially on the use of the Brabender Extensograph. Farinograms, Extensograms and Alveograms of various flours have been compared by Aitken *et al.*,[1] with particular reference to protein content, grade and reproducibility of the instrumental measurements. Other workers have varied the standard technique in many ways to suit the investigation, thereby enhancing the value of the instrument for a particular purpose, but it is not intended here to review such work. As an outcome, however, of the comparative tests here described, modifications to the method of using the Brabender Extensograph have been devised and are described in Part II (following paper).

In all the tests recorded herein, temperature has been maintained at 27° so that the operation of all instruments has been compared under the same temperature conditions. It will be observed that the standard methods differ somewhat in the temperature chosen.

Experimental

Materials and methods

The standard methods of using the three instruments are briefly as follows:

Brabender Extensograph.—A flour/salt/water dough is used. The amount of water is adjusted according to the resistance of the dough to mixing in the Farinograph, the doughs being brought to a standard consistency of 600 Brabender Units (B.U.). Salt (2%) is included in the dough which is mixed for 3½ minutes. When mixed, the dough is divided into duplicate

150-g. pieces which are moulded and rolled on special devices, allowed 45 minutes rest at 30° in the carriers provided, and then stretched. After stretching, the same dough piece is then remoulded and allowed 45-minutes' resting period before stretching again. This process is repeated as often as required, but frequently three stretchings only are given, i.e., the last mould being 90 minutes after doughing and the last stretching 135 minutes after doughing. A chromium-plated mixing bowl was used throughout. The resistance of the dough to the applied force is recorded in the height of the curve and the degree of stretch in the length of the curve.

Chopin Alveograph.—In preparing the dough normally each flour is given the same amount of water, 50% of 2·5% salt solution based on flour weight and corrected to 14% moisture content. In the makers' standard method, no moulding is given to the dough pieces and only 20 minutes are allowed between mixing and stretching. A disc of dough is stretched by air pressure into a thin sphere. The dough temperature is 25°.

In all the tests now given, the ' moulded ' method previously described[2] has been used. This was designed more clearly to reveal the influence of flour treatment. The dough which is given more saline solution (54%) is mixed for 7 minutes and is kept for 3 hours at 27°, after which 20-g. pieces are weighed, moulded, flattened and the discs so formed are allowed to rest for 20 minutes before stretching. The resistance towards this and the extent to which the dough film is stretched before rupture are recorded as the height and length of the curve.

Simon Extensometer.—This instrument was devised by Dr. P. Halton[3] of The Research Association of British Flour Millers.

The amount of water required by each flour to give a yeasted salt-water dough of standard consistency 3 hours after mixing is first determined by measuring the rates at which doughs containing different amounts of water extrude through a hole. Each flour is then mixed with this pre-determined amount of water for 6 minutes using the equivalent of 3·5 lb. (1·25%) of yeast and 4 lb. (1·4%) of salt per sack of 280 lb. of flour. The dough is kept under cover in a constant-temperature room for 3 hours at 27°. It is then divided into 75-g. pieces and moulded on a ' shaper '. The standard resting period is 45 minutes, but 20 minutes is preferred, after which the ball of dough is impaled centrally on split pins and stretched by the lower pin travelling downwards at constant speed. The pull on the upper pin, or resistance to being stretched is recorded as the height of the curve, and the time before the dough breaks is recorded as the length of curve or extensibility.

Baking.—In the standard test baking procedure used, dough is mixed on an Artofex laboratory model mixer from 1000 g. of flour, 18 g. of yeast (equivalent to 5 lb./280 lb. sack of flour) and 14 g. of salt (equivalent to 4 lb./sack). The water required is determined by the Simon extrusion meter. Dough and fermentation temperature is 27°, the fermentation time 3 hours, with a knock back at 2 hours. The dough is hand-scaled to 454 g., passed through a ' Talbot ' moulder once, allowed 10 minutes' recovery, then remoulded and tinned. Final proof is 45 minutes at 27° and baking time 30 minutes at about 230°. Three loaves are made from each batch.

In addition to this basic procedure, a larger quantity of dough is often mixed and a fermentation time test is made by taking doughs for dividing at say, hourly bulk dough fermentation times.

Essential differences in standard methods

The standard techniques involve differences in flour/water ratio, salt content, temperature, resting time and mechanical treatment, but a more fundamental difference is the use of a fermenting dough on the Extensometer and the absence of yeast in the Extensograph and Alveograph doughs. It has long been accepted that if a test is to portray *the behaviour of a flour in a bakery* then the technique of the test must simulate as far as possible the conditions to which flour is subjected in the breadmaking process. It may be predicted, therefore, that of the three tests under examination, the one in which a fermenting dough is used, namely, the Extensometer test, would provide the best assessment of the baking value of a flour. It is also deduced that if the other two tests could be performed with fermenting doughs they would provide more informative assessments of the behaviour of flours in the breadmaking process; the use of a yeasted dough is not successful in the Alveograph test because the fermentation causes the dough films to rupture irregularly when stretched, but a method has been devised

(Part II) which enables fermenting doughs to be used with the Extensograph. The results of the tests which are to be described provide experimental confirmation of the soundness of the foregoing deductions based on general knowledge and experience and also reveal the magnitude of the effect of the other differences in the standard methods mentioned earlier. The nature of these latter differences are as follows:

(*a*) *Flour/water ratio.*—The most important difference in the various methods lies in the proportion of flour to water, which may well obscure significant differences in flour properties. For example, if a flour has an unduly high water absorption, e.g., owing to heavy milling or damaged starch, then in the Chopin method this flour appears more resistant to stretching than in the Simon method in which the amount of water has been adjusted before making the Extensometer test. Thus, unless water absorption is separately known, a high Chopin curve could be erroneously interpreted as indicating toughness.

Similarly, consistency of a dough as indicated at the mixing stage may be different from consistency at the moulding stage because some doughs slacken more than others (those containing malted flours especially slacken more during fermentation). Furthermore, consistency of the dough judged by resistance to mixing differs from consistency determined by extrusion of the dough, and in some cases in the opposite direction.[4]

The selection of a suitable amount of water is not easy in laboratories having only one instrument. It might be assumed that it is theoretically desirable to vary the amount of water to suit the flour in the same way as the baker does in practice. Using the Chopin instrument, however, a reliable method of accomplishing this has not yet been found, and there is some evidence that if the water is adjusted in this way then the detection of differences between flours is much reduced. Thus the Chopin test is in itself partly a measure of dough consistency superimposed on elastic and extensible properties.

Near & Sullivan[5] have reported an adaptation of the Farinograph for determining water absorption although it does not meet all the needs of the varying flours in use in Great Britain because no account is taken of the varying rates of slackening of the dough during fermentation.

A method of using the Brabender Farinograph to determine water absorption is described in Part II and has been developed to overcome the criticism of using resistance to mixing as a measurement comparable to the baker's judgment.

(*b*) *Mixing.*—Different types of mixing bowls and times of mixing are used (double **Z**-blades in the Farinograph and single **Z**-blades in Chopin and Simon mixers).

(*c*) *Reaction and relaxation times.*—Using the terms 'reaction time' for the time between mixing and moulding and 'relaxation time' for that between moulding and stretching,[6] in Brabender tests reaction times of 0, 45, 90 or more minutes are given and relaxation times of 45 minutues; in the Chopin 'moulded' and Simon tests, reaction times of 3 hours with relaxation times of 20 minutes are given. The standard Chopin test has 20 minutes' reaction time only.

(*d*) *Temperature.*—As stated in the tests here reported the temperature has been standardized at 27°, but each instrument normally has a different operating temperature indicated earlier in the description of the standard techniques.

(*e*) *Moulding or shaping.*—In the Brabender standard method the dough is moulded immediately after it has been either mixed or stretched, i.e., already in a state of tension. In the Chopin 'moulded' and Simon methods the dough is moulded after a resting period.

(*f*) *Method of stretching.*—In the Brabender method the cylinder of dough varies in diameter according to the elasticity and is stretched at right angles to the length. In the Chopin instrument the dough is stretched into a spherical thin film and in the Simon test a ball is stretched by central split pins parting.

(*g*) *Salt.*—Salt is used in each of the standard techniques; 2% in the Brabender tests, about 1·4% in the Chopin and 1·4% in the Simon tests.

It will be appreciated that many users of dough-testing instruments alter the basic technique better to reveal the particular features they are seeking and one advantage of such instruments is their flexibility in use. Nevertheless standard methods are an essential basis for comparative work between different laboratories and the correct interpretation of the results is of considerable importance. The following comparative data further illustrate this and in particular supply answers to the questions put at the beginning of the paper. The flours used include as far as

possible an untreated and unbleached, a flour treated with a gaseous improver, either agene or chlorine dioxide, and an untreated flour to which a powder improver, such as potassium bromate has been added in known amount. Much of this work was done however before the use of agene was discontinued commercially as a flour improver, but this in no way detracts from the value of the comparative data.

Results

(a) Comparison of untreated, gas-treated and bromated flours

The untreated flour (*a*) was of 80% extraction, the gas-treated flour (*b*) was from the same grist and treated with agene (7·5 g. per sack), and the bromated flour (*c*) was flour (*a*) containing 0·0015% of potassium bromate. This and similar percentage levels of treatment are based on the flour weight. Preliminary tests had shown that this level of bromate was needed to give comparable baking behaviour to the agene-treated flour.

These three flours were examined on the Brabender, Chopin and Simon instruments by the standard procedures except that reaction times of 0, 1, 2, 3, 4 and 5 hours were allowed on the Chopin and Simon instruments and the dough was stretched every 45 minutes on the Brabender Extensograph.

The same flours were also baked allowing 0, 1, 2, 3, 4 and 5 hours' bulk fermentation.

Fig. 1 shows the curves obtained at 1, 3 and 5 hours for the Chopin and Simon instruments and at $\frac{3}{4}$, 3 and $4\frac{1}{2}$ hours on the Brabender tests. The three methods clearly reveal different behaviour at differing times.

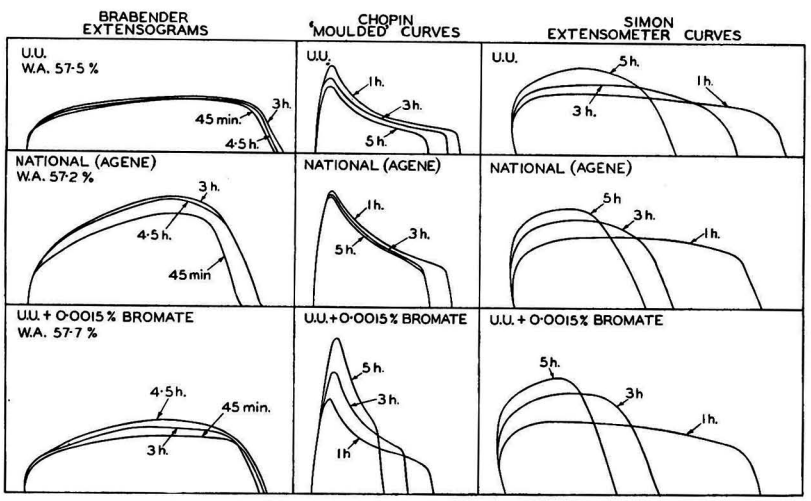

FIG. 1.—*Comparison of Brabender Extensogram, Chopin 'moulded' curves and Simon Extensometer curves on untreated, commercially treated and on bromated flours each tested at increasing periods of time after doughing. The curves are $\frac{1}{8}$ normal size*

U.U. = untreated flour
W.A. = water absorption

The untreated flour curve shows negligible change on the Brabender instrument, decrease in size of curve on the Chopin moulded test, and a progressive toughening on the Simon test.

The agene-treated flour shows only slight toughening on the Brabender and Chopin tests, most of the toughening having already taken place in the first hour. The Simon tests show progressive toughening with time, though at each hour the agene-treated flour is considerably tougher than the untreated flour.

The bromate-treated flour tested on the Chopin instrument shows greater change with time

than the untreated and the agene-treated flours, and this change is more evident than with the same flour tested on the Brabender or Simon instruments.

On baking, using a 3-hour fermentation process, flours (*b*) and (*c*) gave very similar bread. The untreated flour progressively improved with fermentation time up to 5 hours when the bread was similar to the two treated loaves after 3 hours' fermentation. Flours (*b*) and (*c*) progressively improved up to 3 or 4 hours after which over-fermentation signs were evident, the bromated flour deteriorating more at 5 hours' fermentation than agene-treated flour.

Comparing the various curves at 3 hours, (1) the Brabender method suggested that bromate treatment gave less effect than was evident from baking tests and compared with agene treatment, whereas the Chopin and Simon tests showed comparable effect; (2) in the Brabender and Chopin methods the effect of agene is revealed in the dough as soon as mixed and there is little further toughening with time. In the Simon method the progressive tightening of dough made from agene-treated flour is shown and coincides with baking behaviour. Even at 1 hour the tightening effect is evident compared with the behaviour of the untreated flour; (3) the bromate action appears on the Brabender and Chopin tests to be much slower than that of agene, and on the Brabender test the bromated-flour dough has not nearly reached the condition of the agenized flour dough even 4½ hours after mixing. In the Chopin method the bromated dough appears similar to the agenized flour dough at 3 hours but then surpasses it in toughness and at 5 hours shows much tighter characteristics; thus the change in the dough with time is very much more emphasized. The effects found with flours treated with chlorine dioxide are in general similar to gaseous improvement with agene.

A comparison of the curve for each flour using each method, with the bread baked at the same time, showed clearly that the Simon method was the only one which followed correctly fermentation changes with time. From the curves obtained on this instrument the baking behaviour at any specified time could be predicted. The Brabender and Chopin curves could not be used to predict baking behaviour at corresponding times, although they can be used to throw light on the action of gaseous and powder improvers in the dough.

It is later shown that the presence of yeast in the test dough is essential if stretching tests on untreated and variously treated flours at progressive fermentation times are to run parallel with baking changes.

(*b*) *Comparison of tests by the standard methods for the three instruments and the effect on flour of treatment with improvers, etc.*

Preliminary tests on the control flour were made to select the amount of each of the powder oxidizing improvers and A.C.P. (acid calcium phosphate) to give about comparable baking results (bread of the best quality). A test was also made using sodium sulphite to widen the scope of the tests and to check the ability of each instrument to detect dough softening as well as dough toughening agents.

Tables I–III give resistance and extensibility data on the untreated flour and this flour with additions of each improver at the level corresponding to the best baking effect. The results are arranged in decreasing order of resistance measured by the Brabender standard method (taking the 135-min. curve), the Chopin moulded method and the Simon standard method.

Table I

Testing of treated flours in Brabender Extensometer

In order of resistance at 135 minutes

Improver	Level	45 min.		135 min.	
		Resistance	Extensibility	Resistance	Extensibility
Ascorbic acid	0·0015%	470	18·5	675	15·5
Ammonium persulphate	0·02%	460	18·5	600	15·2
Acid calcium phosphate	0·35%	420	18·8	500	19·0
Agene or chlorine dioxide	7·5 g./sack or 3·25 g./sack	400	20·0	470	18·7
Potassium bromate	0·0015%	300	20·5	375	18·5
Sodium sulphite	0·003%	320	19·5	355	20·0
Untreated (UU)	—	310	20·5	330	20·6

Table II

Testing of flours by Chopin moulded method

Improver	In order of height Level	Height	Area
Ammonium persulphate	0·02%	129	56
Ascorbic acid	0·0015%	111	45
Bromate	0·0015%	98	40
Agene or chlorine dioxide	7·5 g./sack or 3·25 g./sack	89	41
Acid calcium phosphate	0·35%	74	36
Untreated	—	66	33
Sodium sulphite	0·003%	47	21

Table III

Testing of flours by Simon Extensometer

Improver	In order of resistance Level	Resistance	Extensibility
Bromate	0·0015%	540	10·7
Ammonium persulphate	0·02%	490	11·0
Agene or chlorine dioxide	7·5 g./sack or 3·25 g./sack	460	13·0
Ascorbic acid	0·0015%	450	14·0
Acid calcium phosphate	0·35%	400	13·7
Untreated	—	400	16·8
Sodium sulphite	0·003%	340	16·3

It is clear from these results that no two instruments place the variously treated flours in the same order. The Simon method gives results probably more nearly representative of baking behaviour. It is also noted that extensibility (length of curve) appears to be a more important measure of dough changes whether due to improvers or fermentation than does this property in the other methods.

It is believed that these results have some bearing on the action of these various improvers. For example, they illustrate the need for time in the dough and dough moulding for bromate to be fully effective, compared with the more immediate action of ascorbic acid (cf. Tables I and III), although both finally behave in a very similar manner in baking at the same levels. Another point illustrated is the danger of using tests made without adequate dough time and moulding for predicting the amount of improver necessary to give comparable baking results.

The instrument tests can only be used to predict the level of improver to use in baking, if ingredients, time in the dough and procedure follow the baking procedure as nearly as possible.

(*c*) *Effect of yeast on the changes in dough properties with time as measured on the Brabender Extensograph and Simon Extensometer.*

The Chopin instrument was excluded as the three-hour moulded method does not give satisfactory results in the presence of yeast.

Untreated and agene-treated flours were examined and to the former an addition of 0·002% bromate was made.

The amount of water required by each flour was determined by the Simon extrusion meter and confirmed by baking, and this level of water was given in both Simon and Brabender tests. Doughs were moulded after 1, 3 and 5 hours, allowing on the Brabender Extensograph 45 minutes resting and on the Simon Extensometer 20 minutes resting before stretching.

Tests were made (*a*) without yeast, and (*b*) with 1·25% (3·5 lb. per sack) of yeast. Salt was added at the normal level for each instrument, i.e., 2% in the Brabender and 1·4% in the Simon tests.

The results are shown graphically in Fig. 2 which gives the change in resistance, in Brabender and Simon units, with time.

In the absence of yeast, both methods indicate that the change in the dough made from untreated flour is negligible at moulding times of 1–5 hours. Gas-treated flour yields a more resistant dough at 1 hour and toughens slowly afterwards, reaching its maximum at 3 hours on

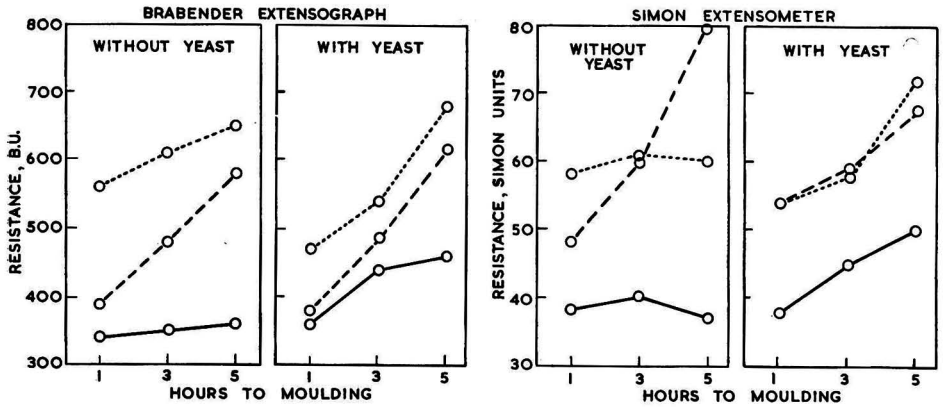

FIG. 2.—*Change in resistance of dough with time, using unyeasted and yeasted doughs. Comparison of untreated flours with flours given* (1) *gas and* (2) *bromate treatment*

the Simon method. Bromated dough on both instruments is less resistant at 1 hour than the dough made from gas-treated flour and toughens continuously up to 5 hours. The difference between the instruments is that on the Brabender Extensograph, the bromated dough has not reached the same condition as the dough from gas-treated flour even in 5 hours, whereas on the Simon instrument it is equal in 3 hours and has exceeded in 5 hours the toughness of the dough from gas-treated flour.

In the presence of yeast, these changes in the dough with time are altered. The tightening of the dough made from untreated flour as fermentation proceeds is now clearly revealed. On the Brabender instrument gas- and bromate-treated flour doughs tighten with time in a more parallel manner than without yeast, although the bromate-treated dough is still not quite so tough. On the Simon method both bromate- and gas-treated flours produce similar dough properties at each period.

This test confirms that yeast is essential to reveal fully the progressive toughening effect with time of fermentation of untreated and gas-treated flour doughs: a bromate-treated flour dough shows progressive toughening even without yeast if time is sufficiently extended; this time factor is very important.

The improved trend towards agreement between the two methods is in part due to the dough pairs containing the same amount of water. If water had been determined by resistance to mixing in the Farinograph, bringing each dough to the same level as the dough made from the gas-treated flour, then the untreated and the bromate-treated flours would have required 1·4% more water than they were given in the above tests, rendering them softer. This is clear from Table IV.

Table IV

Comparison of water absorption of three flours obtained on the Simon and Brabender instruments

Flour	Water absorption determined by	
	Simon extrusion	Farinograph to 660 B.U.
Untreated	55·7%	57·1%
Gas-treated	55·3%	55·3%
Untreated + bromate	55·7%	57·1%

It will have been observed that the only difference between the dough pairs in the two methods was in the amount of salt included. Further tests were therefore made to ascertain the effect of salt level on the bromate response.

(*d*) *Effect of amount of salt on Brabender Extensograph and Simon Extensometer curves, comparing untreated, gas- and bromate-treated flours in the presence of yeast.*

Bulk doughs were mixed on a laboratory Artofex mixer using water determined by the Simon extrusion meter, 1·25% yeast and (*a*) 1·4% salt (normal for Simon tests and laboratory test baking) and (*b*) 2% salt (normal in Brabender tests). The dough was kept at 27° for 3 hours, divided and a part moulded and stretched on the Brabender Extensograph 45 minutes after moulding and a part moulded on the Simon shaper and stretched 20 minutes later.

From the results shown in Table V, it is seen that the additional salt tightened all doughs. Doughs made from bromate- and gas-treated flours gave in all these tests similar curves showing the expected toughening. At the higher salt level there was, however, a tendency for bromated dough to show less resistance than the dough from gas-treated flour.

Table V

Effect of salt on the resistance to extension of yeasted dough moulded 3 hours after mixing

Flour	1·4% salt		2% salt	
	Brabender Units	Simon Units	Brabender Units	Simon Units
Untreated	330	44	360	51
Gas-treated	490	60	560	79
Untreated + 0·002% bromate	490	63	520	75

It may be concluded that the difference in the quantity of salt used in the two standard methods is an additional, although relatively small, contributory factor in the smaller response to bromate on the normal Brabender method.

Further factors affecting the validity of comparative measurements are now outlined.

(*e*) *Comparison of bromated and agenized flours on the Brabender Extensograph*

As bromate treatment gave less than the anticipated response on the Brabender Extensograph, the method could not reliably be used to predict the quantity of bromate required by a flour.

A contributory factor is the method of determining water. In the above tests using the Brabender method the untreated and bromated flour had about 0·5% more added water than the agenized flour, compared with about 1·7% less water in the Simon test and the same quantity of water added to all doughs in the Chopin tests. This would tend to give the bromated flour less resistance than the agenized flour on Brabender Extensographs, but it is not however the whole explanation. Thus further tests were made as follows:

(1) *Varying proportions of water to flour.*—Brabender dough tests as normally made to give a Farinograph curve on the 600 line yield a slacker dough containing a higher percentage of water than is customarily used in baking or in Chopin and Simon tests.

In an effort to overcome this factor as a cause of the differences found between instruments, tests were made on each instrument using the water requirement normally used for the others. Although the response to bromate in the Brabender method was improved, slacker doughs were not apparently the cause of the difference. This was confirmed by examining the bromate response in a slack dough and a firmer dough. Brabender tests were made with (*a*) the quantity of water ascertained as correct for baking by the Simon Extrusion Meter and (*b*) with 3% additional water in all doughs. The response was not materially different.

(2) *Brabender tests with increased bromate.*—Quantities up to 0·0035% which gave visible overtreatment in bread, still did not reveal the anticipated toughening effect on the Brabender curve.

(3) *Varying relaxation times.*—Allowing both 20 minutes and 40 minutes in all three methods did not alter the relation between the three instruments at correct treatment levels of bromate.

(4) *Varying mixers and time of mixing.*—Further information on the apparent failure of the Brabender instrument fully to reveal the bromate effect also included mixing studies. Doughs were mixed in the Simon and Chopin mixers for stretching on the Extensograph and corresponding interchanges of mixers for each instrument were made, but the general pattern of results was

little changed. Tests were also made with added powder improvers giving 1½, 3 and 6 minutes' mixing to each dough in the Farinograph. Extra mixing increased the change in resistance of the dough with time which in part is due to air oxidation. It did not however affect the placings of the different improvers in relation to their effect on dough properties. (See later.)

(5) *Presence of yeast.*—Brabender tests with yeast present were made at reaction times up to 135 minutes, that is, giving 180 minutes from mixing to stretching. Increased response to bromate was evident, the curve becoming nearer the expected shape in relation to baking properties.

From these tests it was concluded that the deficiency of the Brabender Extensograph in showing bromate response was not due to differences in mixing, slackness of dough, or relaxation time. The improved response with added yeast cannot, however, be entirely attributed to its presence, for without yeast Chopin tests fully reveal the bromate effect and so does the Simon instrument.

Further tests showed that when the same dough is used, and conditions are maintained as identical as possible, the three instruments can place bromated and agenized flours in similar positions relative towards each other, as indicated by baking tests. In order to reveal the correct response of an untreated flour on the three instruments, it is necessary to prepare the dough on each instrument with the same quantity of water, to allow 3 hours' reaction time, and to mould the dough at the end of this reaction period before stretching.

(f) Malt flour supplement and its effect on dough measurements on the several instruments, particularly on judgment of water requirements

Malt flours vary in their dough-softening effects, but all reduce the consistency of yeasted dough measured by extrusion 3 hours after mixing, although they have little effect on consistency of dough measured during initial mixing.

Malt flour at three different levels was added to a control flour. The water absorptions determined are given in Table VI.

Table VI

Effect of malt flour on water absorption of flours

	Flour	Malt flour added		
		0·18%	0·36%	1·08%
Brabender Farinograph	57%	56·5%	56·0%	56·0%
Simon Extrusion Meter	54%	51·3%	49·2%	49·0%
Maltose figure	2·0	2·6	3·0	4·0

It is evident that when judging water by extrusion at a time equal to the dividing stage in baking, a reduction in water requirements of 2·7%, 4·8% and 5% for these successive additions appeared necessary, whereas estimates at mixing stage (i.e., Farinograph) suggested only a 1% reduction for the higher levels. Baking tests made at the two levels of water absorption confirmed that the greater reduction in water was necessary. Doughs made without such reduced water were sticky at the dividing stage and difficult to handle, yielding coarse-crumbed bread.

This emphasizes the need for considering the diastatic level of flours to be compared (whether differing naturally according to wheats milled or supplemented by malt products) because the system of deciding the amount of water to add in doughing affects the results of the stretching tests.

If the maltose figure is used as the measure of diastatic activity as is a common practice, this figure is affected not only by the diastatic activity of the flour but also by the susceptibility of the starch or the extent to which the starch has been damaged in milling.

(g) The effect of severity of milling

A partially milled flour, known as 'C' stock was obtained, divided into two portions and further milled. Half was milled lightly and the other half heavily on a laboratory Miag mill,*

* This was prepared in the laboratories of H. Horace Ward of Aynsome Laboratories, Grange-over-Sands, Lancs.

including the whole stock in each case. The flours so obtained were examined by the Chopin 'moulded' and Simon Extensometer methods.

Heavy milling increased the water absorption of the flour by 3·5%, i.e., from 58·3% to 61·8% as determined by extrusion.

In the Chopin method all flours are given the same amount of water and so effects are superimposed on other physical characteristics, measured in the stretching tests. The heavily milled flour gave much greater height and less length of curve. With an increased amount of water as determined by extrusion, the heavily milled flour gave a Chopin 'moulded' curve showing only slightly greater toughening than the lightly milled flour. Baking tests confirmed that there was little difference between the two flours in the dough and in the bread, provided water requirements were appropriately adjusted. Normally, however, the Chopin instrument is used at a fixed level of water and these tests reveal, therefore, one of its disadvantages.

When testing unknown flours by the Chopin method it is difficult to use the maltose figure for making the necessary allowances in interpreting the curves. A high maltose content due to damaged starch increases water absorption and would tend to give stiffness in the Chopin 'moulded' curve. On the other hand it has just been seen that a high maltose content due to high diastatic or enzyme activity decreases water absorption, and this would tend to give a weaker, less resistant Chopin curve.

It might be thought that Chopin tests could be made with water adjusted to the needs of the flour, e.g., according to the Simon Extrusion Meter or other method, but such modifications to the method have not met with real success. It is believed that the properties measured by the Chopin method of stretching include some of those measured by extrusion of a dough; adjustment of water on this instrument tends to lessen the differences between flours until some flours of known baking difference give almost identical curves.

Conclusions

It is clear from this work that different laboratories in which different techniques and instruments are used will differ in their judgment of flour quality. Each instrument measures a complicated mixture of physical properties, the data obtained is largely empirical[7] and until the many factors contributing to baking behaviour are known, baking tests are considered advisable as a confirmation of the trends revealed by instrument tests. The basic baking test should as nearly simulate normal commercial practice as possible, but variant tests such as the multiple fermentation test are frequently essential, and are especially useful for revealing the nature of the strength of the flour sample.

The results of these studies amply confirm that the technique that gives the best information for relating the instrument curve to baking behaviour should include: (1) the adjustment of water requirements according to the needs of the flour, (2) the inclusion of yeast, (3) the addition of salt at a level comparable with baking procedure, (4) a resting period for the dough comparable with that given in the baking process before moulding, resting and stretching.

The standard Simon equipment and method fulfil these requirements and under carefully controlled operating conditions in a single laboratory reproductibility of results by this instrument is reasonably satisfactory. The Extrusion Meter gives a convenient method of estimating water requirement, which should be measured at a time similar to the dividing stage in the bakery, as this is the critical stage so far as softness or stickiness of the dough is concerned. Judgment of the correct water to use in doughing is vital in the preparation of good bread, and equally in making physical tests on the dough.

An alternative technique has been developed using the Brabender equipment, based on the results here described, and this yields as accurate information as the Simon method.

In an earlier paper[2] it was shown that useful information on the action of improvers could be obtained on the Chopin instrument, especially using the 'moulded' method at successive reaction times. Further recent modifications[8] enhance this view. Similarly, the standard Brabender method of repeated moulding and stretching of unyeasted doughs can give useful information. When investigating improver effects these methods are valuable, provided it is realized that the curves obtained cannot always be closely correlated with baking behaviour.

Nevertheless, these comparative tests have enabled us to interpret the results on each instrument more accurately. They reveal the factors involved in selecting test procedures and the pitfalls which can occur in their application and relation to baking behaviour.

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FLOUR TESTING. II.*—An Alternative Method of Using the Brabender Farinograph and Extensograph for Testing Bread Flours

By RUTH BENNETT and J. B. M. COPPOCK

A method is described for measuring the water absorption of flour, using a yeasted dough 3 hours after mixing, by determining the minimum mobility on remixing in the Brabender Farinograph. This enables a correction to be applied to derive the water necessary for the dough subsequently stretched on the Extensograph. A yeasted dough is given 3 hours rest before moulding and then a further 30 minutes before stretching. The advantage over the standard method is that untreated and variously treated bread flours are shown in a more correct relation to each other in close agreement with their placings in baking practice.

Introduction

The standard method suggested by the makers of the Brabender instrument is used in many laboratories for flour testing. It gives useful information on the strength of different wheat flours especially on untreated flours before receiving bleaching and/or oxidizing treatment.

If, however, this method is used for testing bread flours from mixed grists that have been treated with various ' improvers ', the type and amount of which is not known by the operator, then in certain cases misleading results can be obtained.

In Part I[1] are described the factors involved in improving the response of the Brabender instrument to oxidizing improvers and in particular to potassium bromate.

The defects of the standard method principally lie in : (*a*) the measurement of water absorption by resistance to mixing on the Farinograph in which dough stickiness increases resistance. A sticky dough causes the water absorption determination to give a misleading and too high water requirement of a flour in relation to the water desirable for satisfactory baking into bread ; (*b*) the water absorption, being measured at the mixing stage, takes no account of dough softening during the fermentation process. It is essential in making good bread that dough consistency should be correct at the dividing and moulding stage, i.e., the time that the dough is tinned prior to proving and baking ; (*c*) the Extensograph stretching test being given insufficient time in the dough fully to reveal the effect of certain flour treatment ; and (*d*) the absence of yeast so that a dough made from unbleached and untreated flour reveals little change with time.

* Part I : preceding paper

Thus the essential requirements in overcoming these difficulties are (1) inclusion of yeast in the dough (see Fig. 2, Part I[1]), (2) measurement of water absorption at the end of a fermentation period and (3) moulding of the dough after fermentation and prior to its stretching. These factors are included in the Simon method previously described and as originally devised by Halton.[2]

A method has now been devised using the Brabender equipment, to meet these requirements.

Experimental

Method

The following equipment additional to the standard instrument is required:

(1) Jars with lids to hold 450–500 g. of dough and sufficient space to allow for its expansion during 3 hours' fermentation. For this 12-in.-high glass jars as used for storing boiled sweets, etc., are convenient.

(2) A fermentation cabinet maintained at a constant temperature for holding these doughs.

The same amount of salt and yeast as used in commercial baking should be present in the dough. The temperature of the fermentation cabinet should be equal to the average fermentation conditions in the bakery. A typical basic method would be to prepare a dough from flour 300 g., salt 4·5 g., yeast 3·75 g. equivalent to a mix of 280, 4·2 and 3·5 lb. respectively, fermenting at 27° for 3 hours after mixing with the appropriate quantity of water.

Water absorption.—A dough is mixed in the Farinograph for 3½ minutes adding an amount of water (calc. on the weight of flour) approximating to the correct amount, e.g., 55% for flour in England and Wales (15·4 gallons per sack); 57% for flour in Scotland and Northern Ireland (16·0 gallons per sack); 50% for flour in Eire (14·0 gallons per sack). The quantity of water added should be noted. The dough is then left in a covered jar at 27° for 3 hours to ferment.

450 g. dough are then weighed and remixed in the Farinograph until the minimum mobility (the lowest part of the curve) has been just passed. The centre of the curve at this minimum mobility is read off in Brabender Units (B.U.). From the consistency of the dough in B.U. and the water known to have been given to this dough, the correction in this amount of water to give dough of standard consistency (510 B.U.) can be found from Table I. This table shows

Table I

Correction table for converting water given to required water absorption at standard consistency

3 h. B.U.	Water to be increased by		3 h. B.U.	Water to be decreased by	
	% of flour wt.	gallon per sack		% of flour wt.	gallon per sack
650	3·6	1·0	*510*		
640	3·4	0·95	500	0·1	0·05
630	3·2	0·9	490	0·5	0·15
620	2·9	0·8	480	0·8	0·2
610	2·7	0·75	470	1·1	0·3
600	2·4	0·7	460	1·4	0·4
590	2·0	0·6	450	1·7	0·5
580	1·8	0·55	440	2·0	0·6
570	1·6	0·5	430	2·5	0·7
560	1·3	0·4	420	2·7	0·8
550	1·0	0·3	410	2·9	0·85
540	0·8	0·25	400	3·0	0·9
530	0·7	0·2			
520	0·4	0·1			
510		—			

Note: 55% dough water = 15·4 gallons per sack (280 lb.)
57% ,, ,, = 16·0 ,, ,, ,, ,, ,,
50% ,, ,, = 14·0 ,, ,, ,, ,, ,,

the amount of water to be added to the amount actually given, for each B.U. above 510, and similarly to be deducted from the amount given if the reading is below 510. If the water selected for actual test is more than 3% (1 gallon per sack) different from that required for a consistency of 510 B.U., a repeat test should be made, giving dough water nearer the correct figure.

The table was originally prepared by taking several types of flour and preparing doughs from each at five or six different levels of water, and measuring consistency after 3 hours. Consistency in B.U. was plotted against the known water given. From a graph representative of these several tests, the values in the table were read off.

Water absorption levels obtained in this way have been found to agree closely (*a*) with the baker's opinion of the requirements of a flour and (*b*) with the figure obtained by extrusion on the Simon Meter.

As each Farinograph may have slightly different correction factors from those given, construction of individual correction tables is advisable. It should be stated, however, that closely similar figures have been obtained on another instrument.

Extensograph.—A dough is prepared containing the amount of salt and yeast stated above, and the quantity of water obtained from the water absorption test. The dough is fermented for 3 hours in a covered jar at 27°.

Two 150-g. pieces are weighed, moulded and shaped in the usual way, and allowed 30 minutes' resting at 27° before stretching in the usual manner. (If preferred 45 minutes' resting can be allowed, but the shorter resting time permits the performance of a greater number of tests per day.)

Further information on flour quality can be obtained by fermentation tolerance tests. In this method the dough is divided after a 1-hour fermentation in the jar and is then moulded and stretched 30 min. later. Each dough piece is pressed together by hand and returned to the constant-temperature cabinet of the Extensograph until 2 hours after mixing, when the process is repeated, and again at 3, 4, 5 hours, etc.

The ratio of extensibility to resistance gives a useful guide to fermentation time requirements, the larger the ratio the longer the fermentation time necessary to give the best bread.

Example

Water absorption.—If 55% water (15·4 gallons per sack) was used in doughing and gave at 3 hours, on remixing, a minimum mobility figure of 550 B.U., to this 55% is added an additional 1·0% (0·3 gallons per sack) giving a ' correct ' water absorption of 56% (or 15·7 gallons per sack).

Extensograph.—To illustrate how this method gives more reliable information than the standard method two sets of tests are quoted. (1) An untreated flour, a gas-treated commercial flour and the untreated flour with additions of potassium bromate and ascorbic acid at levels adjusted to give equivalent bread to the commercial treatment, were all baked, tested by the standard method and also tested by the suggested procedure. Extensograph curves and breadprints are given in Fig. 1. It can be seen that in the standard method the three treatments give very different curves, contrary to the baking behaviour of these flours. The suggested method, with yeast in the dough, gives curves that can be more directly interpreted to baking behaviour.

(2) An untreated and a commercially treated flour were each baked allowing 1, 3 and 5 hours' fermentation before dividing and moulding and were each tested by both the standard and suggested methods at these same times. Breadprints and Extensograph curves are given in Fig. 2. It is seen from the breadprints that the untreated flour gives progressively improving bread up to 5 hours, whereas the treated flour gives better bread than the untreated flour at 3 hours, but poorer bread at 5 hours.

In the standard method without yeast, the untreated flour shows no such progressive change as is evident in the bread. The suggested method, however, does show this change. The treated flour shows some change, when tested by both methods. A comparison of the two loaves baked at 1, 3 and 5 hours, respectively, with the corresponding Extensograph curves at 1, 3 and 5 hours indicates the closely parallel changes revealed in the suggested method that are not indicated by the standard method.

The replication of the procedure is satisfactory: for example, (1) using yeasted doughs 3 hours after fermentation, Extensograph curves on duplicate dough pieces agree as well or better than those for the unyeasted doughs on the standard method ; (2) a series of flours each tested in triplicate on the same day gave a variation between triplicates in resistance of 20–30

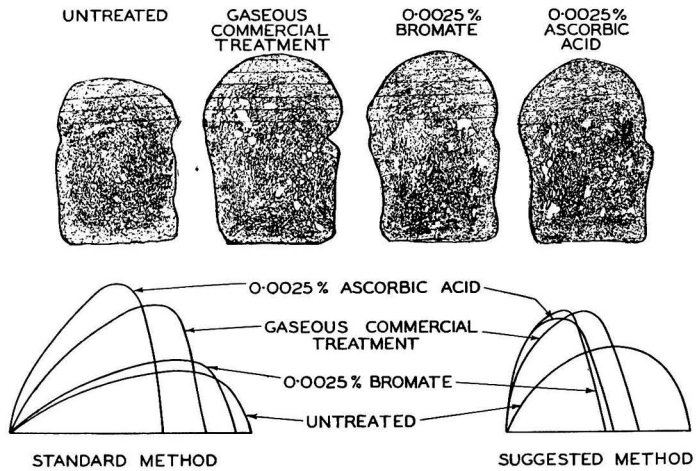

FIG. 1.—*Untreated flour and flour treated with bromate and ascorbic acid equivalent to commercial treatment. Comparison of the standard 135-min. Extensographs with the suggested method with yeast at 3 hr. The curves are $\frac{1}{8}$ normal size*

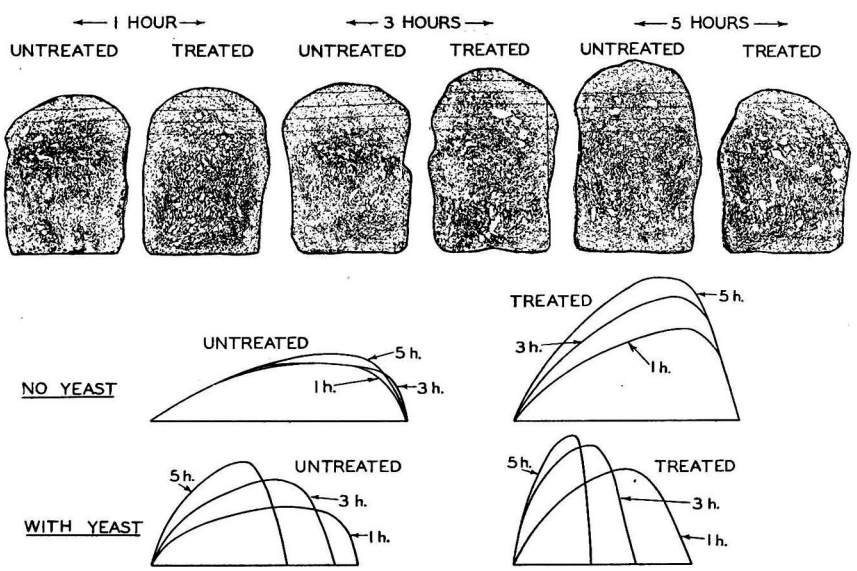

FIG. 2.—*Untreated flour and commercially treated flour. Comparison of Extensograph tests with and without yeast at 1, 3 and 5 hours, with bread baked after 1, 3 and 5 hours*

units and extensibility of 1 unit; (3) no larger variation was obtained on replicates tested on different days during a period of about a month.

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NUTRITIVE VALUE OF LEAF PROTEIN CONCENTRATES. I.—Effect of Addition of Cholesterol and Amino-acids

By S. J. COWLISHAW, D. E. EYLES, W. F. RAYMOND and J. M. A. TILLEY

The value of leaf protein concentrates as supplements to cereal diets for chicks has been studied using the ' gross protein value ' technique. Protein concentrates from different crops have been found to vary widely in nutritive value, but have in all cases been inferior to casein. Concentrates from lucerne contained a factor depressing chick growth, the effect of which was counteracted by adding cholesterol to the diets. This factor was not found in concentrates from Italian ryegrass or white clover. The addition of lysine increased the value of diets containing leaf protein concentrates, possibly because the lysine present in the latter was partly unavailable through low digestibility of the proteins or chemical combination.

Introduction

The extraction of protein concentrates (' leaf proteins ') from fresh green crops, and the use of these concentrates either as supplements for cereal rations fed to pigs and poultry, or in human nutrition, has been discussed by Slade,[1] Pirie,[2] Deijs & Sprenger,[3] Tallarico[4] and others. Some 30–40% of the crude protein (C.P.) in leaves can readily be recovered as a concentrate (35–55% C.P.) by various processes which have been developed, of which at least two[4, 5] are commercially available. Whilst protein can be extracted from the majority of green plants, most of the experimental studies have been made with herbage, from which annual yields of concentrate containing up to 400 lb. of crude protein per acre are possible.[6]

Considerable advances have been made on the mechanical aspects of the process, but few studies have been reported on the nutritive value of the products. It has been suggested that the proteins extracted from leafy material, concerned as they are with the metabolic processes of the plant, may be superior to vegetable seed proteins.[7] The results of a small number of feeding experiments with leaf protein concentrates have not confirmed this. Thus Carpenter, Duckworth & Ellinger[8] found low values for these concentrates as supplements to cereals in chick diets, from which Ellinger[9] concluded that leaf protein concentrates were unlikely to be better than groundnut meal as a protein source. Davies, Evans & Parr[10] reported low digestibilities and biological values for a number of extracted concentrates when these were fed to rats. In experiments at this Institute, Hughes & Eyles[11] obtained reasonable chick growth when a grass/lucerne protein concentrate was fed as the main supplementary protein in a practical diet (18·8% C.P. on dry matter basis). Production of eggs from diets containing a ryegrass protein concentrate was similar to that from a fish-meal diet. However, in further experiments (unpublished) a lucerne protein concentrate gave consistently poorer growth than did fish-meal, when both were fed to chicks in diets containing 15–16% crude protein.

As a result of experiments at this Institute with protein extraction machinery, Tilley, Barnes & Raymond[6] concluded that, if leaf protein concentrates have a nutritive value equivalent only to that of the oil-seed meals their production is unlikely to be economic. Thus the study of their nutritive value, and of possible methods of increasing this value, is of greater immediate importance than further development of the mechanical extraction process.

When prepared on a large scale, leaf protein concentrates contain much non-protein soluble matter, including bitter and possibly toxic components, as well as pigments, fats, waxes, etc., which are mostly indigestible. Much of the protein is present as intact or fragmented chloroplasts, which may not be readily digestible. The nutritional value of the proteins may have been affected in the heat drying of wet curds.

In the present paper the effects of the addition of cholesterol and of amino-acids (lysine and methionine) to leaf protein concentrates are discussed. The effect on the nutritive value of leaf protein concentrates of various methods of processing is reported in Part II.

In these experiments the ' gross protein value ' (G.P.V.) technique of Heiman, Carver & Cook,[12] as modified by Carpenter, Ellinger & Shrimpton,[13] was used to measure the supplementary value of protein concentrates with cereal diets for chicks.

Some of the concentrates tested were prepared from lucerne, which is known to contain water-soluble toxic constituents which can be inactivated by cholesterol,[14] and are probably saponins.[15] The low nutritive value of the lucerne protein concentrates may be due in part to the presence in them of toxic saponins. In Experiments 1–4 cholesterol was added to diets containing concentrates prepared from lucerne (*Medicago sativa*), as well as those from Italian ryegrass (*Lolium italicum*) and white clover (*Trifolium repens*). Preliminary results from these experiments have already been reported.[16]

Diets which contain proteins from whole cereals only are nutritionally inadequate for growing chicks, lysine and the sulphur amino-acids being the main deficiencies. Animal proteins contain high levels of these amino-acids and are therefore better supplements to cereal diets than are vegetable proteins. Green herbage and protein concentrates prepared from such herbage contain levels of lysine and methionine + cystine above those of most vegetable proteins.[17, 18] The availability of these amino-acids will be reduced if the proteins have a low digestibility, and lysine in particular might be rendered unavailable by heat during the preparation of the concentrates. In order to determine whether the low G.P.V. of the protein concentrates was due to low availability of lysine or methionine, these acids were added to diets containing lucerne cytoplasmic protein concentrate (Experiment 5 ; for preparation of this fraction see Part II, following paper). In Experiment 6 lysine was added to diets containing protein concentrates from lucerne, white clover and Italian ryegrass, which were known to differ widely in G.P.V.

Experimental

Preparation of leaf protein concentrates

All the leaf protein concentrates were produced by methods similar to those described by Tilley *et al.*[6] Fresh herbage (leaves and stems) was partly disintegrated in a fixed-hammer mill and further macerated and pressed in a specially designed screw expeller[5] so that nearly half the fresh weight of the crop was separated as juice, leaving behind a fibrous residue.* After screening to remove fibre fragments, the juice was heated by steam to 80° to coagulate the proteins. The coagulum was separated as a wet curd (15–25% dry matter) from the clear mother liquor by filtration and stored at −10° C until required. Normally this curd was oven- or roller-dried before incorporation into chick diets. Further processing will be described under each experiment. Details of the crops used, their botanical analysis and N content, and the chemical analysis of the concentrates prepared from them, are given in Table I.

Chick feeding trials

The ' gross protein value ' technique[12, 13] tests the value of a protein as a supplement in a cereal diet containing 11% of crude protein (8 parts of cereal, yeast, and whey protein and 3 parts of test protein). This is well within the range of linear response of live-weight increase of chicks to protein concentration in the diet. A low-protein ' depletion ' diet is fed for the first 14 days after hatching, followed by a 14-day experimental period. In this a ' positive ' control diet containing casein (to replace test protein) and a negative ' control ' diet containing only 8% of cereal protein are fed in addition to those containing test proteins. In the experiments reported here crude protein levels were calculated on a dry matter basis.

Composition of diets

Details of the depletion diets and basal cereal mixes are given in Table II. These differed from those used by Carpenter *et al.*[13] in that penicillin and vitamins B_2, B_{12}, and E were added, and vitamins A and D_3 were supplied as a stabilized concentrate. The depletion diets were fed during the first fortnight after hatching and contained crude protein levels ranging in different experiments from 8·4 to 10·5%, approximately 8% of crude fibre to prevent cannibalism, and high levels of vitamins to allow storage to cover any deficiencies in the test diets. The experimental diets were prepared by adding to the basal mixes either starch to give the low-protein

* In the earliest tests juice was squeezed from the milled herbage by a roller press made by the National Institute of Agricultural Engineering, Silsoe.[19]

Table I

Details of crops processed and of protein concentrates extracted

Crop	Date cut	Stage of growth	% dry matter* as sown species	% dry matter* as other species	% crude protein in dry matter of crop	Diet numbers (D)	Processing (sequence of operations)	Analysis of concentrates % of dry matter: Crude protein	Calcium	Phosphorus
Lucerne A	8/53	Second cut, late bud	93·1	3·3	18·1	3, 4, 30, 31	Frozen, roller dried	48·4	2·42	0·63
Lucerne B	6/53	First cut, early flower	54·7	45·3	15·6	5, 6	Frozen, oven dried	42·8	2·01	0·75
						36	Frozen, roller dried	42·5	1·68	0·48
						37	Frozen, freeze dried	44·7	1·76	0·47
						38	Frozen, roller dried, washed, oven dried	46·0	1·29	0·47
Lucerne C	6/54	First cut, early bud	—	—	21·9	9, 10	Oven dried, washed, oven dried	58·1	0·69	0·28
Lucerne D	10/53	Third cut, late bud	83·4	9·1	20·6	32	Roller dried whole juice	35·6	3·06	0·52
						13, 14	Roller dried	43·4	2·25	0·51
						33	Roller dried, washed, oven dried	52·3	1·89	0·57
Lucerne (cytoplasmic)	8/54	Second cut, late bud	—	—	—	23, 24, 25	Frozen, washed, oven dried	60·4	3·47	1·55
						39	Frozen, oven dried	47·5	5·95	2·28
Italian ryegrass A	8/53	First cut, early flower	—	—	15·6	17, 18	Frozen, roller dried	40·0	1·16	0·53
Italian ryegrass B	7/54	First cut, pre-flower	85·3	0·0	11·9	28, 29, 42	Frozen, oven dried	34·5	1·31	0·58
						43	as R28 etc., extracted with 90% ethyl alcohol	47·5	1·92	0·61
						44	as R28 etc., extracted with 85% acetone	42·6	1·89	0·63
						45	as R44, extracted with carbon tetrachloride	44·6	1·74	0·56
						48	Frozen, oven dried, washed, oven dried	36·1	1·40	0·69
						49	(pH 4·5), frozen, washed, oven dried	34·3	0·95	0·59
						50	(pH 8·2), frozen, washed, oven dried	37·4	1·97	1·01
						51	Frozen, oven dried, alkali soluble, oven dried	48·9	trace	0·08
White clover	8/53	First cut, early flower (sown 3/53)	52·1	32·6	22·5	19, 20, 26, 27	Frozen, roller dried	37·7	1·80	0·57

* Dead material not included

Table II

Composition (as % of air-dry material) of depletion diet and basal mix

Ingredient	Depletion diet	Basal mix
Maize meal	15·0	25·0
Sussex-ground oats	10·0	25·0
Fine bran	20·0	10·0
Barley meal	—	20·0
Dried grass meal	7·5	—
Dried distiller's yeast	2·0	5·0
Dried whey	2·5	8·0
Condensed fish solubles	2·5	—
Vitamins A and D_3 (' Nuclo ')	0·5	1·0
Penicillin concentrate (' Distafeed ')	0·1	0·2
Riboflavin ,, ,,	0·1	0·2
Vitamin B_{12} ,, ,,	0·1	0·2
Vitamin E in starch*	1·0	1·0
Salt	0·5	1·0
$CaHPO_4.2H_2O$	1·6	—
$CaCO_3$	0·5	—
Starch	36·1	3·4

* 30 mg. of α-tocopheryl acetate per 100 g. of starch

negative control, or casein (Glaxo ' C ') and starch to give the positive control diet, or leaf protein concentrate and starch for a test diet. These diets were balanced for calcium, phosphorus and crude fibre (approx. 1%, 0·6% and 7%, respectively). The high levels of cereals and starch in all the diets ensured that energy levels were above optimum. Details of diets fed in Experiments 1 to 3 are given in Table III, and in Experiments 4 to 6 in Table IV.

Experimental methods

Rhode Island Red × Light Sussex day-old pullets were fed on the depletion diet for 14 days. After this period variability due to size of yolk sac and time of hatching had been considerably reduced. The birds were then weighed and allocated to groups of twelve chicks, with three groups per treatment, each group having the same initial mean weight and weight distribution. The groups were then allocated to the compartments of three three-tier brooders (McMaster Indoor Brooder, Model A), each tier of which was divided lengthwise to form two compartments. An ordinary randomized block design was used in all the experiments reported here. Brooder temperatures were reduced daily from 95° F at day-old to 60° F at 4 weeks of age.

After the period on the depletion diet, the test diets were fed *ad lib.* for 14 days. Group feed intakes (dry matter) and individual chick weights were recorded at 7 days and 14 days in the experimental period. The weight gain of each group above the mean gain of the three negative control groups during the experimental period was calculated. The mean weight gain (per chick) of each group above that of the negative control groups, per g. of supplementary protein consumed, was calculated. The value for each group was expressed as a percentage of the mean value for the three positive (casein) control groups. The average for each test treatment was taken to be the ' gross protein value ' of the leaf protein concentrate fed for that treatment. In calculating the results, proportionate allowances were made for food consumed by chicks which died during the experiment : mortality (number of chicks dying between 14 and 28 days of age) among the 2000 chicks used was less than 2% in all experiments except in Experiment 9 where it was less than 4%.

Results and discussion

The results of the experiments made are given in Tables III and IV, and in Table V the G.P.V. relating to the feeding of cholesterol are summarized. In each case cholesterol was found to increase the G.P.V. of chick diets containing lucerne protein concentrate,[16] by as much as 30% in the cases of lucernes A and C, but with B and D the increase was not significant ($p<0·05$). In terms of live-weight gains and dry-matter consumption the response of lucernes A and C was also greater. Carpenter *et al.*[8] have also reported differing responses when cholesterol

Table III

Composition (dry matter basis) of diets and results of feeding lucerne protein concentrates with and without cholesterol

Experiment No.	1							2					3				
Diet No.	D1	D2	D3	D4	D5	D6		D7	D8	D9	D10		D11	D12	D13	D14	
Basal mix	60·3	60·6	60·7	60·7	60·7	60·7		59·4	59·5	59·7	59·7		59·3	59·4	59·6	59·6	
Oat feed	10·0	10·0	10·0	10·0	10·0	10·0		13·6	13·6	13·6	13·6		10·0	10·0	10·0	10·0	
Maize starch	26·7	23·2	21·4	21·1	19·7	19·4		25·8	22·6	20·5	20·2		27·6	24·4	20·8	21·5	
$CaHPO_4,2H_2O$	1·30	1·21	1·13	1·13	1·11	1·11		0·93	0·78	0·84	0·84		1·30	1·14	1·15	1·15	
$CaCO_3$	1·70	1·79	1·57	1·57	1·48	1·48		0·27	0·32	0·20	0·20		1·80	1·86	1·54	1·54	
Casein	—	3·20	—	—	—	—		—	3·20	—	—		—	3·20	—	—	
Protein concentrate	—	—	6·20[a]	6·20[a]	7·01[b]	7·01[b]		—	—	5·16[c]	5·16[c]		—	—	6·91[d]	6·91[d]	
Cholesterol	—	—	—	0·30	—	0·30		—	—	—	0·30		—	—	—	0·30	
% crude protein in dry matter	8·0	11·2	11·3	11·3	11·1	11·2		8·5	11·5	11·4	11·4		8·4	11·5	11·6	11·5	
Mean live-weight gain, g. per chick in 2 weeks	37·4	91·8	63·8	77·3	68·0	72·5	4·6†	39·4	87·9	61·7	69·4	5·3†	29·9	84·6	35·2	37·4	5·8†
Mean dry matter intake, g. per chick in 2 weeks	184	259	217	250	232	240	12†	189	265	223	233	13†	151	242	157	158	20†
'Gross protein value'	—	100	57	74	65*	70	7†	—	100	56	72	11†	—	100	15	21	15†

Table IV

Composition (dry matter basis) of diets and results of feeding Italian ryegrass and white-clover protein concentrates with and without cholesterol (Expt. 4), and of feeding protein concentrates with and without supplementary lysine or methionine (Expt. 5 and 6)

Experiment No.	4							5						6						
Diet No.	D15	D16	D17	D18	D19	D20		D21	D22	D23	D24	D25		D26	D27	D28	D29	D30	D31	
Basal mix	56·0	56·1	56·3	56·3	56·4	56·4		56·4	56·6	56·6	56·7	56·7		57·9	57·9	58·0	58·0	57·9	57·9	
Oat feed	10·0	10·0	10·0	10·0	10·0	10·0		11·7	11·7	11·7	11·7	11·7		11·5	11·5	11·4	11·4	11·5	11·5	
Maize starch	31·3	28·0	23·8	23·5	23·5	23·2		29·9	26·5	25·3	25·4	25·2		21·1	21·3	20·4	20·6	22·6	22·8	
$CaHPO_4,2H_2O$	1·50	1·38	1·27	1·27	1·24	1·24		1·13	1·00	0·73	0·73	0·73		0·85	0·86	0·75	0·76	0·87	0·88	
$CaCO_3$	1·20	1·32	1·13	1·13	1·01	1·01		0·87	1·00	0·71	0·71	0·71		0·67	0·68	0·75	0·76	0·60	0·62	
Casein	—	3·20	—	—	—	—		—	3·20	—	—	—		—	—	—	—	—	—	
Protein concentrate	—	—	7·50[e]	7·50[e]	7·95[f]	7·95[f]		—	—	4·96[g]	4·50[g]	4·73[g]		7·98[f]	7·62[f]	8·70[h]	8·34[h]	6·20[a]	5·83[a]	
Amino-acid	—	—	—	—	—	—		—	—	—	0·26[v]	0·23[w]		—	0·14[v]	—	0·14[v]	—	0·14[v]	
Cholesterol	—	—	—	0·30	—	0·30		—	—	—	—	—		—	—	—	—	0·33	0·33	
% crude protein in dry matter	8·0	11·0	11·0	11·0	11·1	10·9		8·0	11·3	11·2	11·2	11·3		11·1	11·1	10·8	11·2	11·0	10·8	
Mean live-weight gain, g. per chick in 2 weeks	30·0	84·6	52·0	51·6	42·5	41·8	3·5†	38·0	94·3	70·9	88·6	71·2	7·6†	47·0	72·2	58·9	79·2	72·4	77·4	5·1†
Mean dry matter intake, g. per chick in 2 weeks	161	251	198	201	179	180	13†	195	285	243	274	243	17†	199	243	217	253	237	254	14†
'Gross protein value'	—	100	51	49	32	30	5†	—	100	73	100	70	13†	(23)	(78)	(57)	(88)	(83)	(94)	11†

* Quoted in error as 67 by Cowlishaw *et al.*[16] † Least significant difference at 5% level

a lucerne A *b* lucerne B *c* lucerne C
d lucerne D *e* Italian ryegrass A *f* white clover
g lucerne cytoplasmic protein, water-washed *h* Italian ryegrass B
v L-lysine hydrochloride *w* DL-methionine

Table V

Effect of addition of cholesterol on the 'gross protein value' of protein concentrates extracted from lucerne, Italian ryegrass and white clover

Protein concentrate	Diet No.	'Gross protein value'	
		without cholesterol	with cholesterol
Lucerne A	D3, D4	57	74
,, B	D5, D6	65	70
,, C	D9, D10	56	72
,, D	D13, D14	15	21
Italian ryegrass A	D17, D18	51	49
White clover	D19, D20	32	30

was added to diets containing lucerne leaf proteins, the G.P.V. of two samples being raised from 0 to 47 and from 11 to 19. In the case of lucerne meals, only half the samples tested by Wilgus & Madsen[20] caused growth depression with chicks.

From the results shown in the tables it is seen that the addition of cholesterol to the diets containing concentrates prepared from Italian ryegrass A and white clover did not significantly (5% level) affect G.P.V., live-weight gain or feed consumption. In the case of the grass protein concentrate these results are in accord with those of Carpenter *et al.*[8] who found no benefit from feeding cholesterol with grass meals. Although the white-clover protein concentrate was produced from a ley containing some 33% of grasses, there is no evidence that preparations from pure white clover would contain a growth-depressing factor similar to that found in lucerne protein concentrates.

Protein concentrates prepared from lucerne contain varying amounts of a factor depressing chick growth, which is counteracted by the addition of cholesterol to the diets. When considered in relation to the utilization of leaf protein concentrates on a large scale, the feeding of cholesterol is quite uneconomic. However, Peterson[14] has shown that the growth-depressing factor is water-soluble, and, in Part II the effect of water-washing on the nutritive value of lucerne protein concentrates is reported.

Supplementation of the diet containing lucerne cytoplasmic protein (Experiment 5) with methionine was without effect, but there was a marked response to lysine, the G.P.V. being increased from 73 to 100. In Experiment 6 lysine was fed with three protein concentrates, the level of supplementation being half that of Experiment 5. In each case there was an increase in the live-weight gain per g. of supplementary protein consumed over that obtained when lysine was not added: these correspond to estimated increases in G.P.V. of 23 to 78 with white clover, 57 to 88 with Italian ryegrass B and 83 to 94 with lucerne A (plus cholesterol). These G.P.V. have been calculated by assuming mean data for positive and negative controls from previous experiments. The G.P.V. previously obtained with these three types of proteins were 32, 67 and 74, respectively. Thus supplementation with lysine of the four leaf protein concentrates tested has led in each case to an increase in G.P.V. The 'cereal' proteins (including yeast and whey) supplied 8% crude protein in each test diet. This crude protein contained approximately 4·3% lysine (much of this being from yeast and whey). This level is lower than the 4·8% lysine quoted by Almquist[21] as being optimal for chick growth in an 11% crude-protein diet. The 3% of crude protein added as leaf protein concentrate should contain 6% of lysine in order to raise the lysine content of the diet to this optimal level. This is within the range of values reported for the lysine content of leaf protein.[17, 18, 22] Since in both Experiments 5 and 6 there was a considerable response to added lysine, it may be that some of the lysine supplied by the leaf protein concentrates was in an unavailable form. This explanation is supported by the observations of Davies *et al.*[10], Carpenter *et al.*[23] and Jones[24] that the proteins in leaves are poorly digested by rats. Bender[25] has discussed reactions between lysine and carbohydrates or amino-acids which render lysine unavailable, and it is possible that condensations of this sort occurred during the production of the leaf protein concentrates.

The reported figures for the methionine + cystine contents of leaf proteins (3·8 to 4·0%)[17, 18, 22] and the calculated figure for the ' cereal ' diet (3·0%) are both below the optimal level of 4·5% quoted by Almquist[21] for chick growth. The lack of response to methionine supplementation in Experiment 5 suggests that available lysine was the limiting amino-acid in this diet. March, Biely & Young[26] reported a similar effect with meat meals. Stephenson[27] has suggested that unless the lysine : methionine ratio in a chick diet is greater than 2 : 1, no response to methionine supplementation can be expected.

Although the amino-acid composition of leaf proteins does not appear to vary much with species and stage of growth,[17, 28] these factors may affect the digestibility of the protein, and consequently the availability of the amino-acids. However, it is evident that the ' gross protein value ' technique, while giving an evaluation of a feed under practical conditions, is not suitable for the more critical examination of digestibility and amino-acid deficiencies. Any further studies should determine the biological values and digestibilities and compare these with the amino-acid composition of the leaf protein concentrates.

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NUTRITIVE VALUE OF LEAF PROTEIN CONCENTRATES. II.*—Effects of Processing Methods

By S. J. COWLISHAW, D. E. EYLES, W. F. RAYMOND and J. M. A. TILLEY

Leaf protein concentrates were treated in various ways in an attempt to improve their nutritive value, as measured by the ' gross protein value ' technique with chicks. Washing with hot water improved the value of some lucerne concentrates by removing a water-soluble toxic factor. No appreciable improvement was obtained by : (*a*) varying pH of precipitation of curd, (*b*) different methods of drying the wet curd, or (*c*) solvent extraction of pigments, waxes, etc. Dried whole juice and alkali-soluble protein had low nutritive values. The leaf protein concentrates differ considerably in ' gross protein value ', but all are inferior to the better animal protein concentrates.

Introduction

The results of experiments reported in Part I[1] showed that the nutritive value, as measured by the ' gross protein value ' (G.P.V.) technique, of chick diets containing some lucerne protein concentrates was increased by the addition of cholesterol (0·3% of the total diet). This suggested the presence in these concentrates of a growth-depressing factor similar to the hot-water-soluble factor found in dried lucerne meals by Peterson.[2] As hot-water extraction, unlike the addition of cholesterol, can be used in practice for treating large quantities of material, the effect of washing lucerne concentrates has been studied in the present paper.

The crude leaf protein concentrates produced by heat precipitation and filtration from leaf juice contain only 35–50% of protein (N × 6·25), but considerable amounts (approx. 20%) of pigments, fats, waxes, etc., which act as low-digestibility diluents and may also reduce the nutritive value of the concentrates. In preliminary experiments (unpublished), partial extraction of a lucerne protein concentrate with alcohol or acetone appeared to have little effect, except to increase slightly the consumption of feed. More exhaustive solvent extraction has now been investigated.

In Part I it was shown that the addition of lysine to chick diets containing leaf protein concentrates led to increased G.P.V., the increase being greatest in the case of a concentrate prepared from white clover. This response to lysine was greater than might be expected on the basis of average published lysine contents for leaf proteins, suggesting low availability of the lysine in leaf proteins. The possibility of improving the nutritive value of the proteins by various methods of processing appeared worthy of investigation.

Myburgh, Louw & Groenewald[3] have reviewed the variable effects of heat on proteins, and Bender[4] has shown that, in certain cases, heating will prevent the release of some amino-acids during enzymic hydrolysis. On the other hand, the heating of soya-bean proteins improves their nutritive value by destroying a trypsin inhibitor.[5] The possibility that the nutritive value of the concentrates produced by the present method was being affected by heat during the drying process has been studied, using samples of a lucerne protein concentrate, which had been either roller-dried or freeze-dried.

In another experiment a comparison has been made between concentrates prepared from Italian ryegrass juice by precipitation at pH 6·7 and at pH 4·5, approximately the isoelectric point of the proteins, and in addition a batch made by precipitation at pH 8·2 was also tested. Slade, Branscombe & McGowan[6] claimed that, at pH above 7, the protein curd from grass was palatable and free from the usual bitter taste.

The possibility of separating protein fractions of higher nutritive value from leaves has also been investigated by dissolution of a concentrate in aqueous sodium hydroxide followed by precipitation of protein by acid from the filtered extract. Chibnall[7] states that the cytoplasmic proteins of leaves are richer in lysine than the chloroplastic protein, whilst Davies, Evans & Parr[8] showed that a cytoplasmic fraction prepared from cocksfoot had a higher digestibility and

* Part I : see preceding paper.

biological value than a chloroplast-rich fraction. A cytoplasmic protein fraction produced from lucerne juice has been examined both before and after washing with water to remove much water-soluble material.

Experimental

Details of the crops processed, and the chemical analyses of the leaf protein concentrates have been given in Table I, Part I.[1]

Preparation of concentrates

The mechanical process of extraction of leaf juice and coagulation of a protein-rich curd has been described in Part I. Further processing was carried out as under.

(*a*) *Water-washing.*—A wet curd produced from lucerne D was extracted with boiling water in the laboratory until the washings were colourless, and then oven-dried at 80°. A cytoplasmic fraction washed by the same method was also compared with the unwashed material. Protein concentrates from lucerne B, lucerne C and Italian ryegrass B were washed in a continuous-flow extractor with water at 70°. Washing in this pilot-scale equipment was less efficient than the laboratory extraction.

(*b*) *Drying methods.*—Samples of lucerne B concentrate were either roller-dried or freeze-dried. The roller-dryer heated the wet curd to 120–130° for a few seconds, producing a dry flaky material which, unlike oven-dried concentrate, could be powdered without milling. For Experiment 7 a sample of whole (unprecipitated) lucerne juice D was roller-dried. The dried juice was hygroscopic and was therefore sealed in an air-tight tin until required for mixing with the basal mix.

(*c*) *Solvent extraction.*—A dried concentrate prepared from Italian ryegrass B was extracted in the laboratory with 90% aqueous ethyl alcohol, or 85% aqueous acetone, or 85% aqueous acetone followed by carbon tetrachloride. After solvent extraction, the concentrates were air-dried before being incorporated into test diets. The crude protein contents of the extracted concentrates (Table I, Part I) showed that 90% alcohol was the most efficient solvent for removing non-nitrogenous constituents (including probably simple sugars, which are not soluble in the other solvents), while the carbon tetrachloride removed certain constituents not soluble in 85% acetone.

(*d*) *Precipitation at different pH.*—Protein curds were prepared from juice from Italian ryegrass B by precipitation with steam at normal pH (6·7), or after adjusting to pH 8·2 (150 g. of anhydrous sodium carbonate added per 10 gal. of juice), or to pH 4·5 (85 ml. of concentrated hydrochloric acid added per 10 gal. of juice). Each curd was filtered on cloth, oven-dried, water-washed in the continuous-flow extractor to remove acid or alkali and then redried. In all other experiments the juice (without adjustment of pH) was heated to 80° by steam and the curd filtered on cloth before being dried, either immediately or after storage at −10°.

(*e*) *Alkali-soluble protein.*—A sample of Italian ryegrass B concentrate (coagulated at normal pH) was oven-dried and then mixed with boiling 0·25N-sodium hydroxide. After keeping for 10 minutes, insoluble material was removed by centrifuging, and 6N-sulphuric acid then added to the extract until precipitation occurred. The coagulum was filtered off and thoroughly water-washed before oven-drying at 80°.

(*f*) *Cytoplasmic protein.*—Fresh lucerne juice was heated by steam to 65°, at which temperature the chloroplasts coagulated. By filtration a clear brown liquor was produced, from which cytoplasmic protein was precipitated by further heating. Because large volumes of juice had to be heated, local over-heating could not be avoided with the equipment available, and much of the cytoplasmic protein co-precipitated with the chloroplasts. Yields of cytoplasmic protein were, therefore, very much lower than those obtainable in the laboratory. However, a wet curd of cytoplasmic protein containing some 20 lb. of dry matter was produced and freeze-dried. A further sample was oven-dried, water-washed and then redried. The washed cytoplasmic fraction was still very impure, containing only 60% crude protein, compared with 90% crude protein found in laboratory preparations.

Chick feeding trials

Composition of diets.—The depletion diets and basal mixes were similar to those fed in Experiments 1 to 6 (Table II, Part I). The composition of the complete diets for Experiments 7 to 11 are given in Tables VI and VII.

Experimental methods.—The experimental methods used were the same as those described in Part I.

Results and discussion

The results of Experiments 7 to 11 are presented in Tables VI and VII, and the results of water-washing are summarized in Table VIII. In Experiment 7 the water-washing of lucerne D protein concentrate raised the G.P.V. from 15 to 41, considerably more than the increase found when cholesterol was added (G.P.V. = 21, Experiment 3, Part I). A similar response was found when the lucerne cytoplasmic protein concentrate fed in Experiment 8 (D39) was water-washed and fed in Experiment 11 (D23), the G.P.V. being 57 and 73, respectively. These experiments show that the addition of 0·3% cholesterol did not inactivate all the toxic factor present in these samples. In turn, washing in the continuous-flow extractor did not remove all the toxic material present, as the addition of cholesterol to a diet containing lucerne C concentrate washed in this extractor raised its G.P.V. from 56 to 72 (Experiment 2, Part I). It may have been for the same reason that washing of lucerne B concentrate in this extractor led to a non-significant increase in G.P.V. (from 48 to 53) in Experiment 8. It was noted that this extraction only raised the crude protein content of the concentrate from 42·5 to 46·0% (Table I, Part I) compared with the increase from 43·4 to 52·3% when lucerne D concentrate was thoroughly washed with boiling water. The latter method thus appeared more efficient in removing non-nitrogenous soluble material, including the water-soluble toxic factor.

In Experiment 7 chicks fed the diet containing the dried lucerne juice (D32) grew very slowly, and ate little; growth rates and feed consumption were both much less than those of chicks on the negative control diet, leading to a G.P.V. of −36. The lucerne juice may have contained either toxic or unpalatable factors, but the data do not enable these effects to be

Table VI

Composition (dry matter basis) of diets and results of feeding lucerne protein concentrates processed in different ways

Experiment No.	7						8					
Diet No.	D11	D12	D32	D13	D33		D34	D35	D36	D37	D38	D39
Basal mix	59·3	59·4	59·6	59·6	59·6		55·5	55·6	55·7	55·6	55·6	55·6
Oat feed	10·0	10·0	10·0	10·0	10·0		10·0	10·0	10·0	10·0	10·0	10·0
Maize starch	27·6	24·4	19·6	20·8	21·9		32·7	29·5	25·8	26·3	26·4	27·2
$CaHPO_4,2H_2O$	1·30	1·14	1·10	1·15	1·13		1·00	0·82	0·81	0·79	0·80	0·18
$CaCO_3$	1·80	1·86	1·27	1·54	1·63		0·80	0·88	0·63	0·60	0·68	0·37
Casein	—	3·20	—	—	—		—	3·20	—	—	—	—
Protein concentrate	—	—	8·43[i]	6·91[d]	5·74[j]		—	—	0·76[k]	6·71[l]	6·52[m]	6·32[n]
Cholesterol	—	—	—	—	—		—	—	—	—	—	0·33
% crude protein in dry matter	8·4	11·5	11·3	11·6	11·4		8·4	11·3	11·3	11·3	11·3	11·4
Results												
Mean live-weight gain, g. per chick in 2 weeks	29·9	84·6	21·0	35·2	46·4	5·8†	22·3	75·8	43·8	47·9	45·7	52·9	3·2†
Mean dry matter intake, g. per chick in 2 weeks	151	242	121	157	181	20†	160	238	202	196	202	237	13†
'Gross protein value'	—	100	−36	15	41	15†	—	100	48	59	53	57	6†

† Least significant difference at 5% level

i lucerne D, dried whole juice *d* lucerne D *j* lucerne D, water-washed
k lucerne B, roller dried *l* lucerne B, freeze-dried *m* lucerne B, water-washed, oven-dried
n lucerne cytoplasmic protein

Table VII

Composition (dry matter basis) of diets and results of feeding lucerne cytoplasmic protein and Italian ryegrass protein concentrates processed in different ways

Experiment No. ..	9							10						11				
Diet No	D40	D41	D42	D43	D44	D45		D46	D47	D48	D49	D50		D21	D22	D51	D23	
Basal mix	56·3	56·5	56·7	56·6	56·6	56·6		56·6	56·8	56·9	56·9	56·9		56·4	56·6	56·9	56·6	
Oat feed	11·7	11·7	11·7	11·7	11·7	11·7		11·7	11·7	11·7	11·7	11·7		11·7	11·7	11·7	11·7	
Maize starch	30·0	26·7	21·3	23·8	23·1	23·4		29·7	26·4	21·5	21·0	22·0		29·9	26·5	23·3	25·3	
$CaHPO_4,2H_2O$	1·14	0·96	0·87	0·90	0·93	0·90		1·06	0·89	0·83	0·83	0·58		1·13	1·00	1·10	0·73	
$CaCO_3$	0·86	0·94	0·73	0·68	0·63	0·67		0·94	1·01	0·76	0·82	0·80		0·87	1·00	0·87	0·71	
Casein	—	3·20	—	—	—	—		—	3·20	—	—	—		—	3·20	—	—	
Protein concentrate	—	—	8·70[h]	6·32[o]	7·04[p]	6·73[q]		—	—	8·31[r]	8·75[s]	8·02[t]		—	—	6·13[u]	4·96[g]	
% crude protein in dry matter	8·1	11·3	11·2	11·0	10·8	11·0		7·9	10·9	10·8	11·0	11·0		8·0	11·3	11·3	11·2	
Results																		
Mean live-weight gain, g. per chick in 2 weeks	31·7	86·0	62·4	55·5	63·5	62·8	6·2†	39·1	91·3	60·6	61·4	66·2	3·7†	38·0	94·3	51·2	70·9	7·6†
Mean dry matter intake, g. per chick in 2 weeks	173	261	226	214	226	226	13†	205	279	233	239	246	7†	195	286	225	243	17†
'Gross protein value'	—	100	67	57	78	72	13†	—	100	51	48	57	9†	—	100	30	73	13†

† Least significant difference at 5% level

h Italian ryegrass B
q Italian ryegrass B, acetone- and carbon tetrachloride-extracted
o Italian ryegrass B, alcohol-extracted
r Italian ryegrass B (pH 6·7), water-washed
t Italian ryegrass B (pH 8·2), water-washed
g lucerne cytoplasmic protein, water-washed
p Italian ryegrass B, acetone-extracted
s Italian ryegrass B (pH 4·5), water-washed
u Italian ryegrass B, alkali-soluble

Table VIII

Effect of water washing on the 'gross protein value' of lucerne protein concentrates

Protein concentrate	Diet No.	Unwashed	Washed
B	D36, D38	48	53
C	D9	—	56
C + cholesterol	D10	—	72
D	D13, D33	15	41
D + cholesterol	D14	21	—
Cytoplasmic	D23	—	73
Cytoplasmic + cholesterol	D39	57	—

distinguished. McDonald[9] has shown that a greatly increased yield of product can be obtained by drying whole juice, compared with that from drying heat-coagulated and filtered protein curds. On the other hand, in the case of lucerne juice and lucerne protein concentrates, the removal of water-soluble material is evidently desirable since this leads to higher nutritive values. In the case of Italian ryegrass B concentrate, water-washing led to a decrease in G.P.V. from 67 (D42) to 51 (D48). Thus it is possible that water-washing removes materials of nutritive value, and in the absence of a toxic factor drying of the whole juice appears desirable.

In Experiment 8 the G.P.V. of a freeze-dried concentrate (D37, G.P.V. = 59) was significantly higher than that of a roller-dried concentrate (D36, G.P.V. = 48). The consumption of food by the chicks on the two diets was the same, but the live-weight gain of the chicks on the diet containing freeze-dried material was higher. This indicates that heat-drying may cause some damage to the proteins. Some heat damage may also occur during the heat coagulation, but no other method of coagulation on a large scale is at present available, and this factor has not been investigated.

The effect of solvent extraction of a dried Italian ryegrass B concentrate was studied in Experiment 9. Extraction with 85% acetone (D44, G.P.V. = 78), or with 85% acetone followed by carbon tetrachloride (D45, G.P.V. = 72) had little effect on nutritive value or feed intake. Extraction with 90% alcohol reduced the G.P.V. to 57 (D43). This was due to a reduction in the growth rate of chicks, and not to a change in feed consumption, which suggests that alcohol removed fractions of nutritive significance. The lipoid materials and pigments associated with the chloroplasts appear to have little effect on the nutritive value of diets containing leaf protein concentrates.

In Experiment 10, the effect of the pH of precipitation on the nutritive value of Italian ryegrass B concentrate was studied. Chicks, when fed diets containing a protein concentrate precipitated at pH 8·2 (D50), grew faster and ate more than those having proteins precipitated at pH 4·5 (D49) or pH 6·7 (D48) but the G.P.V. was only raised from 51 to 57. Slade *et al.*[6] also found that concentrates precipitated at pH above 7 were more palatable. Unfortunately the filtration of leaf protein concentrates precipitated at high pH is more difficult than it is at normal pH, and this in practice might outweigh the advantage of greater palatability. Acid precipitation had no significant effect compared with precipitation at normal pH.

The nutritive value of a protein preparation made by acidification of a filtered alkaline extract of Italian ryegrass B concentrate was studied (D51). It was thought that this preparation, containing no cell fragments, would have been highly digestible. While the consumption of the diet containing this material was similar to that of the untreated protein concentrate fed in D48, the growth rate was lower and there was a fall in G.P.V. from 51 to 30. This reduction in nutritive value may have been due to the destruction of amino-acids during the alkaline digestion.

The water-washed cytoplasmic-rich fraction of lucerne protein fed in Experiment 11 (D23) had a G.P.V. of 73, which was no higher than that of the best sample of unfractionated material (D4, G.P.V. = 74). This material was expected to have a higher nutritive value, as both the digestibility,[8] biological value[8] and lysine content[7, 10] of cytoplasmic proteins have been shown to be higher than those of the corresponding chloroplastic proteins. The marked response to

lysine supplementation of this cytoplasmic fraction (Experiment 5, Part I) indicates that, as prepared by the present method, the cytoplasmic protein was deficient in available lysine.

With the exception of the water-washing of lucerne protein concentrates, the effects of the methods of processing studied here have been small compared with the effects of supplementation found in Part I. As a supplement to the cereals fed here, the nutritional failing of the leaf protein concentrates, produced by the present methods, was mainly due to a deficiency of lysine (at least in an available form) together with the presence of a toxic factor in concentrates produced from lucerne. The effect of the latter can be reduced either by feeding cholesterol in the diets or by water-washing the concentrates. Except when supplemented with lysine, the G.P.V. of the protein concentrates has always been lower than that of soya-bean meal (G.P.V. = 75[11]), and the average value has been closer to that of groundnut meal (G.P.V. = 40–50[11]). This conclusion is in agreement with that of Carpenter *et al.*[12]

The results reported here refer to leaf protein concentrates as supplements to cereal diets for chicks. Because of their deficiency in available lysine, they are not likely to be superior to oilseed proteins when fed to other non-ruminant species.

Pirie[13] has estimated the cost of production of crude protein as undried leaf protein curd at 1*s.* 6*d.* per pound, which is much higher than that of crude protein from soya-bean meal (9*d.* per pound, November 1955). On the evidence at present available, leaf protein concentrates appear unlikely to compete economically during peace time with other vegetable proteins as protein supplements in non-ruminant diets.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

DECEMBER, 1956

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

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

DECEMBER, 1956

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Grasslands Research Station, Marandellas, Rhodesia. H. D. L. Corby (*Rhod. agric. J.*, 1955, **52,** 302—314).—A report of recent work carried out at the station. A. H. CORNFIELD.

Fertilising food and general crops in Southern Rhodesia. Anon. (*Rhod. agric. J.*, 1955, **52,** 315—329).—Fertiliser recommendations are given. A. H. CORNFIELD.

Clay minerals of some upland soils in Japan. T. Egawa, Y. Watanabe and A. Sato (*Bull. nat. Inst. agric. Sci.*, 1955, Ser. B, No. 5, 39—107).—Data presented (*X*-ray diffraction, differential thermal analysis, electron micrographs, ethylene glycol retention) show that the principal mineral in subsoils derived from volcanic ash was allophane. Minerals in the surface layer became re-silicated by SiO_2 from plant residues and allophane was partly converted into endellite or halloysite. In soils derived from diluvium, halloysite or endellite dominated and in those from Tertiary beds the principal clay minerals were halloysite, illite and, in some cases, montmorillonite. A. G. POLLARD.

Synthetic soil conditioners. J. A. Beattie and P. H. Walker (*Agric. Gaz. N.S.W.*, 1955, **66,** 640—644).—Application of soil conditioners improved emergence of nearly all crops tested and resulted in earlier maturation and increased yields in some cases. Tilth was improved and irrigation water was absorbed much more rapidly. A. H. CORNFIELD.

Soil conditioners. F. Scheffer and A. Kloke (*Plant & Soil*, 1956, **7,** 269—280).—Addition of 0·025—1·0% Flotal to a clay soil had no effect on water-stable aggregation. The treatments had no effect on uptake of P and K in rye seedling tests, except that the heaviest dressing resulted in slightly reduced uptake of P and K. Addition of 0·025—0·200% of Krilium (hydrolysed polyacrylonitrile) or Kapokril (a German material, composition unknown) increased water-stable aggregation in prop. to the amount added. Krilium had no effect on the availability of P to rye but reduced the availability of K and increased that of Na. Kapokril had no effect on uptake of P or Na but increased that of K. A. H. CORNFIELD.

Laboratory experiments with Krilium. H. Klintworth and K. B. Norton (*Fmg S. Afr.*, 1956, **31,** 193—196, 206).—Application of the conditioner to a moistened soil nearly doubled the proportion of stable aggregates >0·59 mm. in diameter. When applied to the dry soil which was subsequently moistened the breakdown of aggregates on further wetting was diminished. The stability of aggregates was considerably increased by water-logging the soil after mixing with Krilium; it was still further increased by puddling the wet soil. A. G. POLLARD.

Soil moisture measurement by neutron moderation. C. H. M. van Bavel, N. Underwood and R. W. Swanson (*Soil Sci.*, 1956, **82,** 29—41).—An Ra-Be source of neutrons is used with a ^{10}B-lined counter and a stable, portable rate meter. The source and counter may be placed on the soil surface or lowered into a hole in the soil. The latter method is useful where information on vertical heterogeneity is not required. T. G. MORRIS.

Capillary fringe and flow of water in soil. II. Experimental results. D. Swartzendruber and D. Kirkham (*Soil Sci.*, 1956, **82,** 81—95).—Results of tests made with a laboratory model have confirmed the theoretical conclusions of the authors (*ibid.*, 1956, **81,** 473—484) that flow from the capillary region can take place. When the capillary region was at a min. the water table was linear between inlet and outlet points. As the capillary flow increased the water table bent upwards nearest the outlet end. T. G. MORRIS.

Effects of heat and brush burning on the physical properties of certain upland soils that influence infiltration. V. H. Scott and R. H. Burgy (*Soil Sci.*, 1956, **82,** 63—70).—Brush (111 tons per acre) burned on the soil surface raised the temp. of the top 0·125 in. to over 300° for a period of 30 min. Following the burning there was a significant increase in the infiltration rate of water through a soil derived from shale. There was little change when the soil was derived from basic igneous rocks. T. G. MORRIS.

Veld burning. D. C. H. Plowes (*Rhod. agric. J.*, 1955, **52,** 380—394).—Reasons for and methods of burning veld are discussed and described. A. H. CORNFIELD.

Cation-exchange in soils. Origin of negative charges carried by soils and their absorbing powers for ammonium and calcium. T. Harada and K. Kutsuna (*Bull. nat. Inst. agric. Sci. Japan*, 1955, Ser. B, No. 5, 1—26).—Highly org. soils absorbed $Ca^{\cdot\cdot}$ and $Na^{\cdot}$ in ratio approx. 4 : 1 from mixed solutions (0·1N. each) of Ca and Na acetates. With mineral soils the absorption ratio under these conditions was approx. 1 : 1 although the minerals in these soils were quite different. The absorbing power of the H- and Ca-soils for $NH_4^{\cdot}$ from aq. NH_4 acetate or chloride was greater for the org. than for the mineral soils. Relationships between absorption of individual bases and concn. of salts in the external liquid are examined. In org. soils absorbed $NH_4^{\cdot}$ was much more readily leached than that from mineral soils. A. G. POLLARD.

Ammonium fixation by residual soil from crystalline schists at Yahatahama: mineral responsible for fixing ammonium. T. Harada and K. Kutsuna (*Bull. nat. Inst. agric. Sci., Japan*, 1955, Ser. B, No. 5, 27—37).—In soil derived from chlorite schist the mineral capable of absorbing $NH_4^{\cdot}$ was produced during the weathering of the schist and resembled vermiculite. A. G. POLLARD.

Rôle of chalk in assimilation of ammoniacal nitrogen. P. Gouny (*C. R. Acad. Sci., Paris*, 1955, **241,** 95—97).—*Zea mays*, *Pisum sativum* and *Lupinus albus* were grown in inert sand with nutrient solutions flowing through. The action of chalk was studied by mixing 30 g. $CaCO_3$/1 kg. sand in various culture pots. Nitric and ammoniacal N nutrients were used separately and compared. Ammoniacal N nutrition, in the absence of $CaCO_3$, decreased growth and caused various vegetative defects, a marked reduction of the penetration of K, Ca and Mg cations during accumulation of N, and a very marked diminution in the org. anion content. The penetration of the NH_4 ion in the plant causes the appearance of H ions at the root tips and the absorption of metallic cations is reduced by the competition of NH_4 and H ions. The presence of $CaCO_3$, finely divided and in intimate contact with the roots, immediately neutralises H ions as they appear and enables the penetration of cations in the plant to revert to normality, thus eliminating the undesirable effects of ammoniacal nutrition. J. S. C.

Influence of soil moisture on phosphate absorption as measured by an excised root technique. L. A. Dean and V. H. Gledhill (*Soil Sci.*, 1956, **82,** 71—79).—A technique for growing uniform root mats from rye seeds is described. After washing and removal of tops the mats were pressed on to the surface of the soil, treated previously with ^{32}P, and contained in small tins. After 8 hr. the mat was removed and the ^{32}P content measured. On a sand, the P absorbed decreased with increasing moisture content (initial) if the time of contact was up to 4 hr. With 8 hr. contact there was a max. at an intermediate moisture content. At higher temp. absorption increased. Roots preconditioned to low moisture stress and placed in contact with dry soil absorbed P rapidly, but water was lost simultaneously from the roots. Roots conditioned to high moisture stress absorbed P at low rates. T. G. MORRIS.

Phosphate retention in some Australian soils. J. S. Kanwar (*Soil Sci.*, 1956, **82,** 43—50).—The P retention of podsolic soil containing much lateritic gravel was very high (up to 15,000 p.p.m.). Most of the retention capacity was in the coarser fractions, the clay fraction (12% of the soil) contributing only 20%. Removal of the reactive sesquioxides (especially Al_2O_3) caused a marked reduction in the retention capacity. T. G. MORRIS.

Availability of phosphate carriers to small grains and subsequent clover in relation to: I. Nature of soil and method of placement. II. Concurrent soil amendments. R. A. Olson, A. F. Dreier, G. W. Lowrey and A. D. Flowerday (*Agron. J.*, 1956, **48,** 106—111, 111—116).—I. Considering yields and uptake of P with small grains the effectiveness of the P fertilisers was as follows: $(NH_4)_2HPO_4$ > conc. superphosphate > ordinary and ammoniated superphosphate, metaphosphate, and high water-sol. nitrophosphate > Rhenania phosphate, low water-sol. nitrophosphate and fused $Ca_3(PO_4)_2$ > rock phosphate. The P in the low water-sol. materials was much less available in calcareous than in acid soils. Phosphate availability to clover following oats was similar irrespective of the P fertiliser used.

II. In contact with fertiliser P, NH_4^+-N increased the uptake of P by wheat and oats in the case of super- and meta-phosphates. K had no appreciable effect on P uptake. Liming acid soils reduced the effectiveness of most P carriers to oats, but increased slightly their residual value for following sweet clover. Treatment of calcareous soils with S eight weeks prior to sowing had little effect on utilisation of fertiliser P, but increased the uptake of P from all carriers by following sweet clover. A. H. CORNFIELD.

Effect of dryer inlet air temperature, phosphate rock particle size and surfactants in the quick curing of superphosphate. G. L. Bridger and J. L. Kearns (*J. agric. Food Chem.*, 1956, **4**, 526—531).—Laboratory studies are reported of the effect of various factors on the composition and physical properties of superphosphate made by a quick-curing process which consists of mixing phosphate rock with 55% H_2SO_4 and drying in a Roto-Louvre dryer. Dryer inlet air temp. has no effect on P_2O_5 conversion but has a marked effect on product particle size. Moisture content is the most important factor affecting conversion. Decreasing rock particle size improves conversion but excessive grinding is required to obtain significant results. Surface-active agents added during mixing have no advantage. N. M. WALLER.

Quick curing of superphosphate. Pilot plant studies. G. L. Bridger and W. Drobot (*J. agric. Food Chem.*, 1956, **4**, 532—536).—Normal superphosphate is made on a pilot-plant scale by mixing phosphate rock and 55% H_2SO_4, denning, disintegrating and drying in a Roto-Louvre dryer. The process can be carried out continuously to produce a product suitable for either direct application or mixed fertiliser production. The available P_2O_5 content is equivalent to that of superphosphate made by the storage-curing process. N. M. WALLER.

Magnesium status of soils in the Suwannee Valley area of Florida. W. G. Blue and C. F. Eno (*Soil Sci.*, 1956, **82**, 51—61).—Mg deficiency was apparent on oats, millet and clover grown on two fine sands from the area. Plant Mg levels were low. Applications of $MgSO_4$ corrected the deficiencies and usually increased yields. Leaching of Mg is considered to be a factor of importance. T. G. MORRIS.

Chemistry of soil arsenic. James Richard Thomas (*Dissert. Abstr.*, 1955, **15**, 2379—2380).—Arsenic compounds in contaminated soils were characterised by comparing the solubility of residual As with that of known compounds added to non-contaminated soils. Factors and chemicals affecting the solubility, movement and distribution of As in soils are determined. The pH–solubility curves for residual As were of similar shape for the Pb and Ca salts and the free acid. Sol. As in soils treated with Pb arsenate was less than in those treated with Ca arsenate or the free acid. Residual As in soils contaminated with Pb arsenate sprays probably occurs as Fe- or Al–Pb arsenate complexes. O. M. WHITTON.

Decomposition of amino-acids in soils. I. Survey of techniques. D. J. Greenwood and H. Lees (*Plant & Soil*, 1956, **7**, 253—268).—The decomposition of the common amino-acids in a sandy loam (pH 6·5) was examined by measurement of O_2 uptake or CO_2 release as well as of mineralised N There was fair agreement between the two methods. Most of the amino-acids decomposed rapidly and in a similar manner. There was very rapid deamination (usually complete in 24—36 hr.) accompanied by almost as rapid CO_2 release and O_2 uptake. Some of the C and N present was not mineralised; this portion had a C–N ratio of ~3·5. Decomposition of threonine was very slow. With methionine a certain amount of de-thiolation occurred rapidly, but decomposition of the residue was slow. A. H. CORNFIELD.

Gaseous loss of nitrogen from soil. H. Loewenstein (*Dissert. Abstr.*, 1955, **15**, 2377—2378).—Conditions favouring gaseous loss of N from soil include the following: liming, application of P fertiliser to acid soil. No evidence was obtained of formation of N_2O from NH_2OH and NO_2' in soil. O. M. WHITTON.

Non-symbiotic nitrogen fixation in soil. C. C. Delwiche and J. Wijler (*Plant & Soil*, 1956, **7**, 113—129).—Factors affecting the non-symbiotic fixation of N_2 by a soil were studied using ^{15}N-labelled N_2 gas. The N_2 fixed over 48 days increased from 0·7 to 1·85 lb. per acre as added straw increased from 0 to 4 tons per acre. Concn. of NO_3' > about 1·5 mequiv. per g. of soil suppressed N_2 fixation but not the growth of *Azotobacter*. Large amounts of N_2 were fixed when glucose or sucrose were added to the soil. Fixed N appeared largely as NO_3', NH_3 and amide. Growing grass did not enhance fixation. Addition of grass cuttings or lucerne meal caused only slight increases in fixation, whilst inoculation of soil with high concn. of *Azotobacter* did not affect fixation. A. H. CORNFIELD.

Resistance to salt of *Azotobacter*. N. N. Sushkina (*Mikrobiologiya*, 1956, **25**, 35—40).—Limiting concn. of single salts in an Ashby medium compatible with growth of two strains of *Azotobacter* isolated from ordinary and salty soils, were, respectively: NaCl 5·1 and 0·6, Na_2SO_4 12·56 and 1·57, Na_2CO_3 0·08 and 0·04, K_2SO_4 0·007 and 0·86, KCl 0·009 and 0·7, K_2CO_3 0·009 and 0·26, $MgCl_2$ 3·0 and 1·0, $MgSO_4$ 11·0 and 1·84 and $CaCl_2$ 1·1 and 3·3%. It is probable that the organisms would withstand higher concn. of salts in mixtures containing antagonistic cations. R. TRUSCOE.

Biodynamics of the rhizosphere of foliaceous and coniferous stands in the forest-steppe zone. S. A. Samtsevich (*Mikrobiologiya*, 1956, **25**, 49—56).—Under comparable conditions of soil and climate, the density and composition of the root microflora of stands of oak, ash, larch and fir are identical. The counts are about 50% more than in the bulk of the soil, and tend to vary parallel with soil humidity. About 40% of the saprophytic forms present are spore-forming bacteria, the density of which equals that in the surrounding soil, whilst the density of actinomycetes is twice as great. The effects observed are related to secretion into the soil of water-sol. org. substances from the sucking rootlets. R. TRUSCOE.

Plant root excretions in relation to the rhizosphere effect. I. Nature of root exudate from oats and peas. II. Properties of root exudate and its effect on the growth of micro-organisms isolated from the rhizosphere and control soil. III. Effect of root exudate on the numbers and activity of micro-organisms in soil. A. D. Rovira (*Plant & Soil*, 1956, **7**, 178—194, 195—208, 209—217).—I. When grown aseptically in quartz sand pea roots excreted considerable amounts of amine material, containing 22 different amino-compounds, over 21 days. Oats excreted less material, containing 14 amino-compounds. The proportions of the amino-compounds varied between the two root species. Fructose, glucose, u.v.-absorbing and fluorescent compounds were also excreted.

II. Addition of pea or oat root exudate to liquid media increased the growth of micro-organisms in the control soil and (more markedly) in that from the rhizosphere of pea plants three weeks old. The growth-promoting ability of the pea root exudate was similar to that of yeast extract for some, but not for other, organisms. The exudate was not adequately replaced by mixtures of amino-acids, vitamins, or a synthetic mixture of known growth factors. The effects of various physical and chemical treatments on the growth-promoting ability of the root exudate are also reported.

III. Treatment of soil with pea root exudate resulted in increased no. of Gram-negative bacteria, but had no effect on the release of phosphate from soil org. matter or from yeast nucleic acid added to soil. Nitrification in and O_2 uptake by soil were increased by root exudate additions only in the presence of added glucose or peptone. A. H. CORNFIELD.

Comparative studies of streptomyces populations in soils. M. Misiek (*Dissert. Abstr.*, 1955, **15**, 2386).—The distribution of predominating types of streptomyces populations inhabiting similar and dissimilar soil series follow a seasonal cycle. Correlation is established between soils in a given series and between similar series and predominating streptomyces populations. Some types of streptomyces are associated with a particular soil series. O. M. WHITTON.

Application of bacterial fertilisers to growth of vegetable seeds in peat-compost nutrient cubes. E. N. Mishustin and A. N. Naumova (*Mikrobiologiya*, 1956, **25**, 41—48).—The bacterial content of cubes made up of peat 3, sawdust 1, dried blood 0·5, and mineral salt solution 4·5 parts was greatly increased after inoculation with *Azotobacter* and *Bacillus megatherium phosphaticum*. Sprouting of cabbage and tomato seeds and growth of the seedlings were distinctly better in inoculated cubes, and the cabbage roots were free of *Plasmodiophora brassicæ* infection. R. TRUSCOE.

Mulch-tillage and pasturing: effect on soil productivity and physical properties. W. E. Holmes (*Dissert. Abstr.*, 1955, **15**, 2377).—With proper fertilisation on a well-drained soil, mulch till-planting may be superior to mouldboard ploughing for preparing seed-beds for maize. With some autumn cultivation, a field-cultivated seed-bed may give a yield equal to that from a ploughed seed-bed. In pasture soils the mean wt., diameter of water-stable aggregates are a poor measure of the physical condition of the compacted soil. O. M. WHITTON.

Potassium frit as a special purpose fertiliser. O. R. Lunt and B. Kwate (*Soil Sci.*, 1956, **82**, 3—8).—Orthoclase felspar fused at 1200° with KNO_3 or K_2CO_3 gave a glass frit with 36% of K_2O. When air quenched the weathering rate was high and the solubility low. The weathering rate was controlled by the fineness of grinding and the amount of impurity present. When incorporated into soil the frit supplied K steadily over a long period. When left on the soil surface the frit supplied less K. The frit is a ready source of K and large applications may be made without increasing the soil salinity or leaching losses. T. G. MORRIS.

Plant Physiology, Nutrition and Biochemistry

Ages and developments of soil landscapes in relation to climatic and vegetational changes. R. V. Ruhe and W. H. Scholtes (*Proc. Soil Sci. Soc. Amer.*, 1956, **20**, 264—273).—The types of vegetation (dated by the radiocarbon method) and climate in past ages are discussed. A. H. CORNFIELD.

Applications of the thermoelectric method for measuring water flow rates in plants. M. E. Bloodworth, J. B. Page and W. R. Cowley (*Agron. J.*, 1956, **48**, 222—228).—Modifications of the thermoelectric method (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 411) are described. Max. transpiration rates for cotton plants occurred when R.H. varied between 42% and 50%, temp. between 35° and 37·8°, and wind velocity between 1 and 4 m.p.h. Absorption and movement of water in the plants was dependent on soil moisture tension. A. H. CORNFIELD.

Effect of the soil moisture stress and oxygen concentration on the growth of maize roots. J. R. Gingrich (*Dissert. Abstr.*, 1956, **16**, 202).—With increase in soil moisture tension and in presence of adequate O_2 the rate of growth (dry matter) of maize embryos declines. Osmotic stress has no such effect. Root growth is sensitive to moisture stress in the range 3—1 atm. but is unaffected in the range 0·3—1·0 atm. Other soil and environmental factors are examined. O. M. WHITTON.

Toxic influences of sodium and sulphate ions on citrus seedling. P. Zusman (*Bull. Res. Counc. Israel*, 1956, **5D**, 210—218).—Seedlings grown in soil supplied with increasing amounts of SO_4'' were poisoned if the concn. was >1000 p.p.m., 1·05% SO_3 in the dry substance of the leaves being found in sour orange and about 0·9% in sweet lime. Sweet lime seedlings grown in soil containing 1·1 mequiv. of Na per 100 g. soil (22% exchangeable Na) developed typical symptoms of injury, while sour orange seedlings remained healthy under the same conditions. Development of the seedling was correlated positively with the Ca/Na ratio in the leaves. (11 references.) E. G. BRICKELL.

Influence of cation ratio, temperature and time on adsorption and absorption of calcium and potassium by citrus and other plant species. R. L. Smith and A. Wallace (*Soil Sci.*, 1956, **82**, 9—19).—In solution cultures containing ^{45}Ca or ^{42}K, adsorption and absorption of Ca by plant roots was a function of the concn. and the root exchange capacity. Species differences increased with Ca concn. $>10^{-4}$N; at 10^{-1}N-Ca the adsorption was in the order orange > beans > barley and the absorption, beans > orange > barley. There was a high degree of correlation between cation-exchange capacity and both types of sorption. Presence of K markedly reduced both sorptions. The temperature coeff. Q_{10} (change in reaction rate at T + 10 to that at T) was generally 1—1·5; the uptake of K or Ca (in the presence or absence of the other cation) and their translocation were controlled by diffusion. The nutrient concn. had no effect on the Q_{10} values. T. G. MORRIS.

Uptake of sodium and other cations by five crop species. E. O. McLean (*Soil Sci.*, 1956, **82**, 21—28).—Plants were grown in gravel with a nutrient medium in which K was progressively replaced with Na. The cation-exchange capacity, activities and bonding energies of the cations on the plant roots were determined. Reed grass and maize roots bonded K 3—5 times as strongly as Na, whereas in oats, lespedeza and celery the bonding energy of Na was not weaker than that of K. No correlation of bonding energies and cation exchange capacity was found. Replacement of K by Na increased root yields initially but as the K replacement increased root yields decreased. Na accumulated in all roots with increasing amounts in the solution; some but not all species also accumulated Na in the tops. K uptake generally decreased with K supply but Ca and Mg uptakes were not similarly affected. Relationships between cation-exchange capacity, mean free bonding energies and Na uptake are examined. T. G. MORRIS.

Correlation of phosphorus and magnesium contents of plants grown in synthetic ion-exchange resins. A. Wallace and R. T. Ashcroft (*Agron. J.*, 1956, **48**, 219—222).—Rough lemon, avocado, barley and bush beans were grown on sand–synthetic ion-exchange resin media with varying levels of P and Mg. Increasing the Mg level in general had no effect on uptake of P by the plants, although for species with low Mg requirement increasing both P and Mg supply resulted in higher uptake of P only. The distribution of P between leaves, stems and roots of the avocado was unaffected by Mg supply. A. H. CORNFIELD.

Effects of soil treatments on the occurrence of marsh spot of peas and on manganese uptake and yield of oats and timothy. S. G. Heintze (*Plant & Soil*, 1956, **7**, 218—236).—Applications of Mo or Zn to Mn-deficient soils increased the incidence of marsh spot in peas and reduced the total Mn content of the seeds in some soils. Marsh spot increased the concn. of amino-acids in peas. Addition of Mo also increased the amino-acid concn. of peas. Although application of various Mn compounds failed to prevent grey speck in oats, grain and leaf yields were increased by the treatments. Steaming the soil increased grain yields more than did any Mn treatment. Steam treatment or Mn applications induced Cu deficiency in oats grown in Mn-deficient soils. Applications of Mn compounds to a Mn-deficient soil failed to increase the yields of successive cuts of timothy, although the Mn uptake was related to reducibility and to the size of the ultimate particles of the insol. Mn materials. A. H. CORNFIELD.

A leaf breakdown of tobacco associated with the copper status of the soil. R. Thomson and H. O. Askew (*N.Z. J. Sci. Tech.*, 1956, **37, A**, 584—599).—A breakdown of the leaves as they approach maturity was corrected by applying 50 or 100 lb. of $CuSO_4$ per acre; 20 lb. was ineffective and more than 50 lb. gave little extra benefit. Affected leaves are rich in total-N and protein-N. The application of $CuSO_4$ reduces the N content and increases the sugar content. Sound leaves contain at least 9% of reducing sugars, and not more than 2·05% of total N and 0·90% of protein N. Unsound leaves are high in Mn and low in Cu; sound leaves contain not more than 130 p.p.m. of Mn and not less than 6 p.p.m. of Cu; the Mn : Cu ratio should be less than 28. R. H. HURST.

Response of subterranean clover to molybdenum. Anon. (*Agric. Gaz. N.S.W.*, 1955, **66**, 399).—Application of Mo (in addition to superphosphate + lime) to an acid shale soil increased the yields of green matter from subterranean clover from 0·35 tons to 10 tons per acre. Plots receiving Mo + lime or Mo + superphosphate only showed poor growth. A. H. CORNFIELD.

Chemical factors limiting growth of phytoplankton in Lake Victoria. G. R. Fish (*E. Afr. agric. J.*, 1956, **21**, 152—158).—Evidence is presented to show that, as far as test algæ are concerned, sulphates are a limiting factor as well as nitrates and phosphates. (15 references.) E. G. BRICKELL.

Orange leaf transpiration under orchard conditions. IV. Contribution to the methodology of transpiration measurements in citrus leaves. V. Influence of leaf age and changing exposure to light on transpiration on normal and dry summer days. A. Halevy (*Bull. Res. Counc. Israel*, 1956, **5D**, 155—164, 165—175).—IV. It is suggested that when measuring the transpiration of citrus leaves on summer days it is essential to accomplish the initial and final weighings within 100 sec. after plucking. (29 references.)

V. Transpiration losses on Sharav days, which are very hot and dry, were no higher than those on a normal summer day and a direct correlation could mostly be established between rates and stomatal aperture. In general young leaves had the highest transmission rates but young, light coloured leaves, had rates lower than those of mature leaves. (33 references.) E. G. BRICKELL.

The viability of citrus seeds and certain properties of their coats. A. Cohen (*Bull. Res. Counc. Israel*, 1956, **5D**, 200—209).—A method for testing the viability of citrus seeds by means of resazurin is described. Removal of seed coats assists germination; removal of both testa and tegmen being significantly more efficient than removal of the testa only. (11 references.) E. G. BRICKELL.

[A] **Frost effects 1956 and vine cultivation.** H. Breider and E. Wolf. [B] **Winter frost damage and behaviour of several species [of vines] grown in 1956.** H. Konlechner (*Mitteilungen, Klosterneuburg*, Ser. A, 1956, **6**, 205—222, 223—243).—[A] In Würzburg certain new rootstocks of vines, especially the varieties *Riesling, Perle von Alzey, S* 1—28 and *Mainriesling*, under test in the local soil and climatic conditions, were more resistant than standard varieties to the severe frosts of Feb. 1956. These varieties were resistant to *Phylloxera vastatrix*. Recommendations are made to select vines suited to the soil and climate, etc., in which they are to be grown, and having also hereditary resistance to frost.

[B] Frost damage in Feb. 1956 in the Klosterneuburg vineyards where the min. temp. was −22·6° is reported. In general total death of the vines was limited, but in most varieties the buds were damaged where not covered with snow. Factors affecting frost resistance were: variety, soil, fertilisation, age, rootstock, etc. Some very sensitive high-culture varieties were seriously damaged, others did better than those with different training. E. M. J.

Auxins fail to stimulate rooting of yellow poplar cuttings. B. J. Huckenpahler (*Bot. Gaz.*, 1955, **117**, 73—75).—A case of failure to induce rooting by indolyl-butyric and -acetic and by naphthyl-acetic acids is reported. A. G. POLLARD.

Relation of herbicidal activity to the amide moiety of *N*-substituted α-chloroacetamides. P. C. Hamm and A. J. Speziale (*J. agric. Food Chem.*, 1956, **4**, 518—522).—The herbicidal activities of a variety of *N*-substituted α-chloroacetamides are determined in relation to their

structural configurations. As a class they are effective in the selective control of annual grasses in pre-emergence application. A no. of heterocyclic derivatives were among the most active tested. The furfuryl-, tetrahydrofurfuryl- and 2-thenyl-α-chloroacetamides are highly effective at 2·5 to 5 lb./acre. Aromatic compounds are almost inactive. N. M. WALLER.

Relative effectiveness of mono-, di- and tri-chlorophenoxyacetic acids in retarding abscission of mature apples. P. C. Marth, W. H. Preston, jun., and J. W. Mitchell (*Bot. Gaz.*, 1955, **117**, 51—55).—Sixteen chlorophenoxyacetic acids (1% in lanolin) were applied in narrow bands to the abscission zone of six varieties of apples about three weeks prior to the normal harvesting time. Abscission was effectively retarded by 3 : 4 : 5-trichlorophenoxyacetic acid in all six varieties, by the 4-chloro- and 2 : 5-dichloro-acids in five varieties, by the 3-chloro-, 3 : 4-dichloro-, 2 : 3 : 5- and 2 : 4 : 5-trichloro-acids in four varieties, by 2 : 4-dichloro-acids in two and by the 2-chloro-acid in one variety. The 2 : 3 : 5-trichloro-acid had a partial action on all six varieties. There was no response to the 2 : 6-, 2 : 3- and 3 : 5-dichloro- and the 2 : 3 : 6- and 2 : 4 : 6-trichloro-, the 2 : 3 : 4 : 6-tetra- and the pentachloro-acids. The concept that two unsubstituted positions *para* to each other in the ring are necessary for high physiological activity is not entirely supported. A. G. POLLARD.

Induction of abscission of olive fruits by maleic hydrazide. H. T. Hartmann (*Bot. Gaz.*, 1955, **117**, 24—28).—Winter application of maleic hydrazide (1% as Na salt) caused later abscission of about 95% of the fruit and some of the older leaves. Oil from fruit from sprayed trees contained small amounts of the hydrazide (0·15 p.p.m.). A. G. POLLARD.

Responses of plant cells to gibberellin. Y. Kato (*Bot. Gaz.*, 1955, **117**, 16—24).—Gibberellin (**I**) solutions in the concn. range 0·01—10 mg./l. accelerated the growth of *Allium fistulosum* coleoptiles, the germination of pollen of *Lilium longiflorum* and the spore germination and protonemal growth in fern. Higher concn (100 mg./l.) induced mitosis and formation of pseudochiasmata in roots of *Allium cepa*. Treatment of seedlings of *Vigna sesquipedalis* with lanolin containing 0·01% of **I** stimulated growth by increasing cell elongation rather than cell multiplication. Effects of indolylacetic acid differed markedly from those of **I**. A. G. POLLARD.

Crops and Cropping

Agricultural Experiment Station, Salisbury, Annual Reports, 1953—54. A. G. H. Rattray (*Rhod. agric. J.*, 1955, **52**, 246—261.)—The report discusses crop rotations and manurial trials with maize, beans, cowpeas, groundnuts and potatoes. A. H. CORNFIELD.

Spacing and fertilisation of winter *versus* spring companion crops. C. R. Blackmon (*Dissert. Abstr.*, 1955, **15**, 2374—2375).—The alternate 7- and 14-inch spacing of winter wheat companion crops (red clover, lucerne) coupled with a spring top dressing of 30 lb. of N per acre gave best yield of both the companion crop and forage. Seven-inch spacing was more effective with a spring oat companion crop. O. M. WHITTON.

Spring small grain : anatomical response to top growth clipping. C. Thakur, H. L. Shands and R. I. Evans (*Agron. J.*, 1956, **48**, 123—126).—Stems of clipped field plants of wheat, oats and barley had vascular bundles more closely arranged and smaller in diameter than did those of unclipped plants. High temp. (25·5°) reduced stele diameter, no. of xylem strands and no. of metaxylem vessels. No single causal factor for lodging was isolated, although several associations were observed. A. H. CORNFIELD.

Inheritance of leaf rust reaction in crosses of *vulgare* wheats. M. Hashim (*Proc. Indian Acad. Sci.*, 1956, **44, B**, 38—46).—Plants (Thatcher × Frontana cross) which are resistant to stem or leaf rust at the seedling stage may or may not be resistant at the adult plant stage, under greenhouse conditions. Testing of breeding material in both seedling and adult plant stages is suggested. E. M. J.

Uptake of minerals by maize as affected by nitrogen fertiliser. E. G. Giffard (*Rhod. agric. J.*, 1955, **52**, 262—267).—Yields of tops and total uptake of N and P increased with rate of application of N (300—600 lb. $NaNO_3$ per acre). Yields of grain, and % of protein, P and K in the grain also increased with N application. A. H. CORNFIELD.

Effect of plant population and rates of fertiliser nitrogen on average weight of ears and yield of maize in the South. W. Thomas (*Agron. J.*, 1956, **48**, 228—230).—The wt. of maize ears decreased as the plant population increased from 6000 to 18,000 per acre and was not affected by N applications (40—160 lb. per acre). Yields of ears were higher with 12,000 than with 6000 or 18,000 plants per acre at all levels of N application. Yields were increased by application of 40 lb. of N, but were not increased further by heavier applications. A. H. CORNFIELD.

Nitrogen fertiliser for maize production on an irrigated chestnut soil. F. V. Pumphrey and L. Harris (*Agron. J.*, 1956, **48**, 207—212).—Studies with irrigated maize showed that in the year of N application and in the following year increased yields were influenced by season, soil nutrient status, and time and rate of application. Time of application had little effect on yields on low-productivity soils. N was utilised more efficiently during the year of application when applied before ploughing, at planting time, or side-dressed when maize was 6—12 in. high than when side-dressed when maize was 20—36 in. high. The % of crude protein in the grain increased with the N application, but was not consistently affected by time of application. A. H. CORNFIELD.

Maturation and yield of maize as influenced by climate and production technique. R. H. Andrew, F. P. Ferwerda and A. M. Strommon (*Agron. J.*, 1956, **48**, 231—236).—Yields of two maize hybrids were much higher when grown at Wageningen, Holland, than at Spooner, Wisconsin. Higher plant populations, more uniform rainfall distribution, higher soil fertility and lower disease incidence contributed to the higher yields at Wageningen. The growing season at Wageningen was nine weeks longer than at Spooner for a comparable stage of maturity to be reached. A. H. CORNFIELD.

Date of ear emergence in rice. I. Relation between sowing time and date of ear emergence. H. C. Gangulee (*Bot. Gaz.*, 1955, **117**, 1—10).—All Aman varieties of rice are probably short-day types. There is no general trend in photoperiodic reaction among non-Aman varieties. A. G. POLLARD.

Influence of variety on the specific gravity–mealiness relationship of potatoes. A. E. Schark, C. E. Peterson and F. Carlin (*Amer. Potato J.*, 1956, **33**, 79—83).—Differences between mealiness scores for varieties, averaged over all sp. gr. classes, were highly significant. There was some factor other than sp. gr. which influenced the evaluation of mealiness by the judges, indicating that sp. gr. should be used in conjunction with some other test in assessing mealiness of baked potatoes. There was a significant linear relationship between mealiness scores and sp. gr. of tubers. A. H. CORNFIELD.

Effect of nutrition of the potato plant on the content of free amino-acids and on the amino-acid composition of the protein of the tubers. E. G. Mulder and K. Bakema (*Plant & Soil*, 1956, **7**, 135—166).—Although varying mineral nutrition of the potato markedly affected the protein content of the tubers, the amino-acid composition of the protein was similar whether N, P or K was deficient during growth. Ample N and deficiency in P or K increased the proportion of amides in the sol. non-protein fraction. Both asparagine and glutamine were associated with N shortage. The relative content of γ-amino-butyric acid was much lower in P-deficient than in normal tubers. The tyrosine content of P-deficient tubers was lower, whilst that of K-deficient tubers was higher than normal. A. H. CORNFIELD.

Production of "new" after-season potatoes, their utilisation as seed. G. Mathieu-Collot (*C. R. Acad. Agric. Fr.*, 1956, **42**, 431—433).—Small-scale trials are described of planting potatoes which had been specially protected against pests, and were of good cultural value, as late as, e.g. July 15, the crop being harvested in November. This technique provides a means of obtaining "new" after-season potatoes in regions of suitable climate and also good seed for the following spring cultivation. E. M. J.

Seedling competition in compounding forage seed mixtures. R. E. Blaser, W. L. Griffeth and T. H. Taylor (*Agron. J.*, 1956, **48**, 118—123).—The mechanism of competition in seed mixtures of lucerne, orchardgrass and red clover in spring and summer sowings was studied. Lucerne seedlings developed faster when sown in summer than in spring, whilst the reverse was true for red clover. Stands of lucerne were similar with both spring and summer sowings, whilst those of red clover were poorer with summer sowing. Seedling growth and stands of orchardgrass were similar with both times of sowing. Red clover seriously suppressed lucerne stands and root wt. from spring but not from summer sowings, and also suppressed orchardgrass in spring sowings. Lucerne was moderately suppressed by orchardgrass when spring sown. A. H. CORNFIELD.

Legume top and root yields in the year of seeding and subsequent barley yields. W. Kroontje and W. R. Kehr (*Agron. J.*, 1956, **48**, 127—131).—There were no significant differences in forage or root yields between two hardy and four non-hardy varieties of lucerne. There were only small differences in the total N produced by the different varieties. Madrid and Hubam sweetclover produced similar amounts of forage although Madrid produced much more root growth and more N than did Hubam. Barley yields following the various lucernes were quite similar, and in some cases were slightly higher than in check plots. Hubam sweetclover had an effect similar to that of the lucernes, whilst Madrid depressed barley yields. Vetches increased barley yields in all cases. A. H. CORNFIELD.

Influence of autumn cutting in the seeding year on the dry matter and nitrogen yields of legumes. D. Smith (*Agron. J.*, 1956, **48,** 236—239).—Cutting lucerne, red clover and alsike clover in Sept. and/or Oct. of the seeding year reduced the dry matter and N yields produced by all of the legumes, particularly of lucerne, which was weakened by crown and root rot diseases. A. H. CORNFIELD.

Effects of moisture, nitrogen fertiliser and clipping on yield and botanical composition of ladino clover-orchardgrass pastures under irrigation. C. E. Nelson and J. S. Robins (*Agron. J.* 1956, **48,** 99—102).—Over three years greater yields of forage were obtained with summer irrigation given every 7—11 days than with that given every 15—20 or 20—30 days. Clipping when the forage was 12 in. high gave greater yields than did clipping at 6 in. Yields of forage increased and % of clover in the forage decreased with increasing N fertilisation. The 7—11 day irrigation treatment gave the highest % of clover in the forage. There was no difference in botanical composition between the two clipping heights. Yields were greater when N was applied in April than in June. Split applications of 150—200 lb. N did not give higher yields than did split application of 100 lb. N. A. H. CORNFIELD.

Boron requirement for crimson clover seed production, its accumulation in soils, and residual effects of sensitive crops. J. I. Wear (*Agron. J.*, 1956, **48,** 132—134).—Annual application of borax (10—30 lb. per acre) over four years resulted in considerable accumulation of water-sol. B in the 0—6- and 6—12-in. soils layers in fine-, but not in coarse-textured, soils. On coarse-textured soils fair yield increases of crimson clover seed were obtained with 10 lb. of borax per acre; higher rates gave no further increase. On fine-textured soils yields were not affected by any rate of application of B. Soya-beans and cotton following clover on soils which had received the 30-lb. rate of borax for four years showed no toxic symptoms. A. H. CORNFIELD.

Improving the inoculation of crimson clover planted under unfavourable moisture conditions. G. W. Burton (*Agron. J.*, 1956, **48,** 142—144).—Where moisture was applied soon after planting, water was as good an adherent as was black strap molasses for the *Rhizobium trifolii* inoculum carried in peat. Under these conditions forage and root yields of crimson clover were similar with both treatments. Where the soil remained comparatively dry for 1—4 weeks after sowing molasses was a more satisfactory adherent. A. H. CORNFIELD.

Effect of calcium sulphate on yield and composition of grass-clover pasture. T. W. Walker, A. F. R. Adams and H. D. Orchiston (*Plant & Soil*, 1956, **7,** 290—300).—Application of $CaSO_4$ (25—200 lb. per acre) to a pasture on a young loessial sandy loam resulted in marked increase in growth of both clover and grass and of total N uptake. The extra N in the grass was probably derived by underground transference from the clover. SO_4'' was leached fairly readily from the soil and recovery by the plant of the heavier applications was poor. Nearly all the S in the clover was org., whilst about 20% of that in the grass was present as SO_4''. The importance of applying S, particularly in regions where return of atm. S to the soil is low, to promote optimum N-fixation by legumes and hence high production from grass-clover associations, is stressed. A. H. CORNFIELD.

Factors causing errors in the determination of dry matter and nitrogen in forage crops. W. H. Hesse and W. K. Kennedy (*Agron. J.*, 1956, **48,** 204—207).—Moisture losses from bagged forage samples were serious, particularly when air temp. exceeded 21·1° and when samples were left exposed. These losses could be reduced considerably by piling green forage over the bags. Dry matter contents of forage were measured more accurately by protecting samples from loss of moisture and weighing accurately indoors than by weighing immediately in the field with less accurate scales. Dry matter losses due to respiration occurred when samples were held for more than one day. Such samples had a greater concn. of total N than had those not suffering from such losses. There were no differences in total N content between different methods of grinding. A. H. CORNFIELD.

Factors causing inaccuracies in determination of dry matter and nitrogen in forage crops. W. H. Hesse (*Dissert. Abstr.*, 1955, **15,** 2376—2377).—The magnitude of losses in dry matter and total N during forage harvesting was determined and factors causing variations in the analytical results were studied. In dry matter determination, these factors were, evaporation and respiration, use of different containers, and placing additional samples in the oven with those already present. In nitrogen determinations, the effects of mechanical grinding equipment, and of respiration losses are noted. O. M. WHITTON.

Manurial needs of fruit crops. T. Wallace (*Agric. Rev., Lond.*, 1956, **1,** 14—22).—A review of recent information. The importance of K, Mg and trace elements in the nutrition of tree and soft fruits is stressed. Symptoms of deficiency and of excess of mineral nutrients, together with current manurial practices. are summarised. A. G. POLLARD.

Control of seed-coat splitting in beans. S. Rehm (*Fmg S. Afr.*, 1955, **30,** 507—510, 523—524).—Seed splitting was favoured by vigorous growing conditions, notably excessive supplies of N, and diminished by restricted P and water supplies and by " short-day " conditions. A. G. POLLARD.

Border effects in cotton variety trials. J. M. Green (*Agron. J.*, 1956, **48,** 116—118).—In tests over three years at three locations (3-row plots, 3 ft. between rows, using yield data from the middle row) there were significant differences in yields of two of the four varieties arising from border effects in four of the nine tests. There was no relationship between competitive ability and either earliness or yields. A. H. CORNFIELD.

Reducing the hard seed problem in cotton. V. T. Walhood (*Agron. J.*, 1956, **48,** 141—142).—Hot-water treatment (85° for 1 min.) of cotton seed increased greatly both rate and final % of germination. Results were similar whether the seed was acid-delinted immediately before treatment or delinted, dried and stored before treatment. The treatment was effective in maintaining high germination for at least two months. A. H. CORNFIELD.

Relationship between the growth and mineral composition of the foliage of Japanese larch (*Larix leptolepsis*, Murr.). L. Leyton (*Plant & Soil*, 1956, **7,** 167—177).—Height of growth of young Japanese larch was significantly correlated with concn. of N, P, K in the needles. Probably the height of growth was limited by deficiencies of both N and K. An equation relating height of growth to concn. of N and K in the needles is presented. A. H. CORNFIELD.

Molybdenum status of the laminæ of *Hevea brasiliensis* as determined by bioassay and chemical methods. E. W. Bolle-Jones (*Plant & Soil*, 1956, **7,** 130—134).—*H. brasiliensis* was grown in sand culture at deficiency or low levels of each of the macro-nutrients and of Fe, Mn and B. Deficiency of SO_4'' in the nutrient increased the Mo concn. in the laminæ as determined by both chemical and bioassay (*Aspergillus niger*) procedures. Bioassay, but not chemical analysis, indicated a significantly decreased concn. of Mo in the laminæ when K was deficient; lack of other nutrients had no effect on the Mo concn. A. H. CORNFIELD.

Chemical composition of seed from different portions of the soya-bean plant. F. I. Collins and J. L. Cartter (*Agron. J.*, 1956, **48,** 216—219).—Seed from the lower half of the plant was higher in oil and lower in protein than that from the upper half. Beans near the tip of long terminal racemes had less oil than had those farther down. Seed in the tip of the pod was slightly higher in oil and lower in protein than that in the middle or base. A. H. CORNFIELD.

Pest Control

Literature on plant pathology. M. M. Baldwin and K. S. Chester (*J. agric. Food Chem.*, 1956, **4,** 562—563).—A review. (17 references.) N. M. WALLER.

Tests to develop non-explosive mixtures of solvents in formulations for use in aërosol generators. A. H. Yeomans (*J. econ. Ent.*, 1956, **49,** 415—416).—The explosiveness of aërosol formulations containing various proportions of 10 different commercial flammable solvents in admixture with the non-inflammable solvent tetrachloroethylene was determined. Results showed that the proportion of flammable solvents must fall to 27·7—15% by wt. to avoid the risk of explosion. A. A. MARSDEN.

Dichlorodiphenyltrichloroethane: chemistry, manufacture and application. P. Müller (*Industr. y Quím.*, 1955, **17,** 333—340, 343).—A review. (41 references.) D. LEIGHTON.

Synthesis and application of insecticides and herbicides. K. A. Hassall (*Industr. y Quím.*, 1955, **17,** 344—346, 348).—A review with 18 references. D. LEIGHTON.

Lindane content of tissues of plants grown in soil or nutrient solution. R. G. Haines (*Dissert. Abstr.*, 1955, **15,** 2376).—Lindane is absorbed and translocated throughout the plant-tissues and accumulates in several plant organs. O. M. WHITTON.

Variation in effectiveness of derris dusts against the pea aphid. F. H. Harries (*J. econ. Ent.*, 1956, **49,** 363—367).—Temp. and R.H. were not important factors in the effectiveness of rotenone (1%) dusts. Moisture on the plants increased the residual action of these dusts, but the effect was lost after drying. Talc-derris extract was more toxic than other derris-talc dusts. The kind of diluent, its particle size and that of the derris root, had a marked effect on toxicity. A. A. MARSDEN.

Breakdown of DDT in granulated formulations. P. R. Sferra (*J. econ. Ent.*, 1956, **49**, 414—415).—Fused DDT formulations were more unstable than solvent-impregnated ones, particularly with the carrier attapulgite. The heat (100—104°) applied during the fusion process intensifies the incompatability of attapulgite with DDT. DDT fused on tobacco, and all the solvent-impregnated materials (except attapulgite) showed a negligible amount of breakdown. A. A. MARSDEN.

Organic insectofungicides. XVIII. New method of preparation of esters of chloro- and dichloro-thiophosphoric acids. Z. M. Bakanova, Ya. A. Mandel'baum, N. N. Mel'nikov and E. I. Sventsitskiĭ (*Zh. obshch. Khim.*, 1956, **26**, 494—495).—The reactions $3PSCl_3 + 2Al(OEt)_3 = AlCl_3 + 3(OEt)_2PS{\cdot}Cl$ and $3PSCl_3 + Al(OEt)_3 = AlCl_3 + 3OEt{\cdot}PSCl_2$ take place at 50—60° (2—3 hr.); the products are obtained in about 40% yield. R. TRUSCOE.

Organic insectofungicides. XIX. Synthesis of [substituted] amino-alkyl esters of dithiophosphoric acid. K. D. Shvetsova-Shilovskaya, N. N. Mel'nikov and N. I. Martem'yanova (*Zh. obshch. Khim.*, 1956, **26**, 496—498).—A series of esters of general formula $(OR)_2PS{\cdot}S{\cdot}CHR'''{\cdot}NR'{\cdot}CO_2R''$ were prepared by the Mannich reaction. R was Me, Et, Pr^n, Pr^i, Bu^n or Bu^i; R' was H, Me or Et; R'' was Et or Pr^i; R''' was H or Me. Physical constants are given for the esters. Most have weak contact insecticidal action, and some, not specified, act as systemic insecticides, approaching pyrophosphoric octamethyltetramide in activity and persistence. R. TRUSCOE.

Structure and composition of plant cuticle in relation to environmental factors and permeability. J. D. Skoss (*Bot. Gaz.*, 1955, **117**, 55—72).—A method of isolating plant cuticle in quantity is described. The possible use of the product in testing the penetrability of insecticides, etc., is noted. A. G. POLLARD.

Plant and soil nematodes of the Federation of Rhodesia and Nyasaland. G. C. Martin (*Rhod. agric. J.*, 1955, **52**, 346—361).—Nematodes are catalogued under hosts or associated plants. A. H. CORNFIELD.

Insects attacking red clover in Rhode Island and their control. T. W. Kerr, jun., and I. H. Stuckey (*J. econ. Ent.*, 1956, **49**, 371—375).—Lindane and malathion effectively controlled aphids and DDT reduced various species of weevils, beetles and leafhoppers. Chlordane greatly increased leaf-hopper populations. Dusts and sprays of lindane, DDT and malathion gave increased clover yields, often in the succeeding crops. Residues of malathion were small at harvest, but those of DDT were relatively large. A. A. MARSDEN.

Control of insects affecting vetch seed production. N. M. Randolph (*J. econ. Ent.*, 1956, **49**, 403—404).—Malathion and parathion gave excellent control of pea aphids but a toxaphene–DDT spray gave the best control of all the chief injurious insects (pea aphids, armyworms and cutworms), and a significant increase in the yield of vetch seed per acre. A. A. MARSDEN.

Parathion spray residue on apples, beans and quinces. H. E. Barr, P. J. Clark and H. Jacks (*N.Z. J. Sci. Tech.*, 1956, **37**, 623—625).—Average residues from parathion applications on fruit are below 1 p.p.m. (the suggested safety limit). J. S. C.

Effect of oil spray application timing on juice quality yield and size of Valencia oranges in a southern California orchard. L. A. Riehl, R. T. Wedding and J. L. Rodriguez (*J. econ. Ent.*, 1956, **49**, 376—382).—The effect of single applications of oil sprayed annually in different months of the year showed that reduction in juice quality (% of total sol. solids) or yield of oranges was least from applications in late summer. Total sol. solids may be reduced significantly by oil sprays from Nov. to June. Fruits from oil-sprayed trees were larger than those from HCN-fumigated trees or those sprayed with a non-oil acaricide. A. A. MARSDEN.

Wilt disease of banana. T. S. Ramakrishnan and S. Damodaran (*Proc. Indian Acad. Sci.*, 1956, **43**, 213—222).—The wilt disease of banana, caused by *Fusarium oxysporum* var. *cubense*, was studied in respect of the varying incidence of attack on some 20 varieties of banana grown in Madras state. The cultural characters of the fungus are described and its enzyme production is studied. J. S. C.

Control of *Tetranychus hicoriæ* McG. on pecans. D. R. King and D. W. Rosberg (*J. econ. Ent.*, 1956, **49**, 404—405).—Addition of S (6 lb. per 100 gal.) to a DDT spray applied for control of pecan nut casebearer prevented the development of severe infestations of mites, *Tetranychus hicoriæ*. Zineb (2 lb. per 100 gal.) used in pecan scab control also inhibited the development of mites. A. A. MARSDEN.

Residues and flavours of asparagus treated with malathion. L. P. Ditman, R. C. Wiley and P. A. Giang (*J. econ. Ent.*, 1956, **49**, 422).—Immediate residue deposits from a spray application of malathion were <8 p.p.m. and no malathion was detected on fresh asparagus three days after treatment. All malathion was removed in processing both canned and frozen spears, and no off-flavours were noted. A. A. MARSDEN.

Colorimetric test and symptoms permitting the detection of virus diseases in potatoes. C. Martin and J. Quemener (*C. R. Acad. Agric. Fr.*, 1956, **42**, 426—428).—The colour test described, depending on the formation of a blue colour with the juice of diseased but not of healthy potato plants and a solution of 2 : 6-dichlorophenol-indophenol previously decolorised with $NaHSO_3$ (the colour formation being independent of the nature of the virus) was in 95% agreement with corresponding serological tests in a large no. of trials with several varieties of potato. The sprouts of diseased potato tubers infected with virus X and Y have in general less intense colour in the pigmented zones as compared with those of healthy tubers. E. M. J.

Control of virus diseases of beetroot and potato by the application of aphicides. L. Bonnemaison (*C. R. Acad. Agric. Fr.*, 1956, **42**, 509—512).—Trials made to control virus diseases of beetroot and potato during the last 25 years are discussed including the use of parathion, schradan, isolan and demeton and the difficulty arising in connexion with persistence of a systemic insecticide in the tubers. E. M. J.

Control of black bean aphids. J. G. Gellatley (*Agric. Gaz. N.S.W.*, 1955, **66**, 494—495).—A single top-spray of 0·13—0·26% schradan on young bean plants controlled the wingless form of the black bean aphid (*Aphis craccivora*) for 3—4 weeks and reduced the incidence of virus disease symptoms. Parathion (0·03%) gave poor control. A. H. CORNFIELD.

An evaluation of newer insecticides for control of DDT-resistant cabbage loopers. F. L. McEwen and G. E. R. Hervey (*J. econ. Ent.*, 1956, **49**, 385—387).—DDT-resistant cabbage loopers (*Trichoplusia ni*) were satisfactorily controlled with endrin, Isodrin and Shell OS-2046, whilst parathion–toxaphene gave 75% control of this pest. A. A. MARSDEN.

Control of onion thrips and its tolerance to certain chlorinated hydrocarbons. B. H. Richardson and G. P. Wene (*J. econ. Ent.*, 1956, **49**, 333—335).—The suspected tolerance of onion thrips to heptachlor, dieldrin, aldrin and toxaphene when used alone, was confirmed. The org. P compounds were effective and retained control for 5—7 days. $C_6H_6Cl_6$–DDT, dieldrin–parathion, and Perthane gave good control of this pest. A. A. MARSDEN.

Practical application of chemical controls of root maggots in rutabagas. A. R. Forbes and K. M. King (*J. econ. Ent.*, 1956, **49**, 354—356).—Heptachlor and aldrin applied at standard rates by band, furrow or spray methods gave effective and economical control of *Hylemya brassicæ* attacking rutabagas. A. A. MARSDEN.

Control of tomato pests. P. C. Hely (*Agric. Gaz. N.S.W.*, 1955, **66**, 441—443).—An 0·025% DDT spray gave satisfactory control of the tomato caterpillar, potato moth, green vegetable bug and jassids and reduced losses from Bronze wilt on tomatoes. Addition of 0·1% of nicotine to the DDT spray did not improve its action; 0·01% of parathion was somewhat less effective. A. H. CORNFIELD.

Yellow top disease of tomatoes. W. S. Sutton (*Agric. Gaz. N.S.W.*, 1955, **66**, 655—658).—The disease (incidence and symptoms described) is probably of virus origin. Spraying for insect control has not controlled the disease. A. H. CORNFIELD.

Toxicity of widely used insecticides to beneficial insects in California cotton and lucerne fields. R. van den Bosch, H. T. Reynolds and E. J. Dietrick (*J. econ. Ent.*, 1956, **49**, 359—363).—Against insects of the following genera: *Orius*, *Geocoris*, *Nabis*, *Chrysopa* and *Hippodamia*, parathion and toxaphene–DDT combinations were highly toxic; toxaphene, endrin and DDT were moderately effective, and demeton had only limited toxicity. *Chrysopa* larvæ and *Orius* sp. were relatively tolerant to all insecticides tested. A. A. MARSDEN.

Control of the southern garden leafhopper, a new pest of cotton in southern California. H. T. Reynolds and A. S. Deal (*J. econ. Ent.*, 1956, **49**, 356—358).—Although DDT was at first highly effective against *Empoasca solana*, the insect quickly became resistant. Perthane, demeton, parathion and Diazinon gave excellent control: malathion and Chlorthion gave fair results on cotton but very good control on other crops. A. A. MARSDEN.

Hessian and stored tobacco pests. J. A. Whellan (*Rhod. agric. J.*, 1955, **52**, 502—504).—The control of the cigarette beetle, *Lasioderma serricorne*, F., and the tobacco moth, *Ephestia elutella*, Hbn. in hessian used for wrapping tobacco, by heat treatment or fumigation with CS_2 is described. A. H. CORNFIELD.

Broadcast treatments with insecticides and soil fumigation for tobacco wireworm control. F. E. Guthrie and R. L. Rabb (*J. econ. Ent.*, 1956, **49**, 344—347).—Excellent control of heavy infestations

of wireworms attacking tobacco was obtained with heptachlor, dieldrin and aldrin. Chlordane gave good results in moderate infestations, but endrin was less effective. Row fumigation of tobacco gave some decrease in wireworm injury, but control was not considered practical. A. A. MARSDEN.

Production and prevention of frenching of tobacco in the greenhouse. R. A. Steinberg (*Plant & Soil*, 1956, **7**, 281—289).—Partial steam sterilisation of soil followed by addition of lime, $CaHPO_4$ and cellulose resulted in frenching of tobacco and gave high counts of *Bacillus cereus*, Frankland and Frankland, the assumed causal agent. Soil capacity for frenching built up with successive plantings. Frenching was also associated with low soil N. Increasing acidity and NO_3' supply reduced or prevented frenching. A. H. CORNFIELD.

Control of hornworms and flea beetles on tobacco with endrin and TDE. C. B. Dominick (*J. econ. Ent.*, 1956, **49**, 425—426).—Dusts of TDE (10) and endrin (1 and 1·5%) effectively controlled both tobacco hornworm and flea beetle. An endrin (19·5%) or TDE (25%) spray gave excellent kill of hornworms, but the TDE was much less effective against flea beetles than was endrin. A. A. MARSDEN.

Fungicides for the control of damping-off in pine seedlings. II. Field trials. I. A. S. Gibson (*E. Afr. agric. J.*, 1956, **21**, 165—166).—Dithane Z-78, Perenox, thiram 63%, Phelam, Leytosol B and Crag 658 were tested but none of the treatments were sufficiently uniform or effective for general nursery practice in Kenya. E. G. BRICKELL.

Control of the pine spittlebug and the pine needle miner. T. W. Kerr, jun. (*J. econ. Ent.*, 1956, **49**, 426).—Wettable powder sprays of DDT, methoxychlor, dieldrin, lindane and malathion effectively controlled nymphs of the pine spittlebug, *Aphrophora parallela*. Dieldrin was more effective than DDT or methoxychlor in preventing attack by the pine needle miner, *Exobeleia pinifoliella*. A. A. MARSDEN.

Control of the army worm with endrin. J. A. Whellan (*Rhod. agric. J.*, 1956, **53**, 102).—Application of endrin (0·5—1 pint of 19·5% emulsion in 50 gal. per acre) gave complete kill of the army worm in 2 ft. high maize. A. H. CORNFIELD.

Stem-break of sunn hemp, *Crotolaria juncea*, in Southern Rhodesia. J. O. Whiteside (*Rhod. agric. J.*, 1955, **52**, 417—425).—Characteristics of the disease, due to *Colletotrichum curvatum*, are described. Infected trash in commercial seed has probably accounted for much of the dissemination of the disease. Seed treatment with TMTD (4 oz. per 100 lb.) has given good control. Cultural methods for reducing the severity of the disease are discussed. A. H. CORNFIELD.

Control of aphids. E. H. Zeck (*Agric. Gaz. N.S.W.*, 1955, **66**, 659—660).—Details of methods used for aphid control are described. A. H. CORNFIELD.

Control of false-loopers. E. H. Zeck (*Agric. Gaz. N.S.W.*, 1955, **66**, 662).—False-looper caterpillars, *Plusia* spp., were controlled with 0·1% DDT sprays. A. H. CORNFIELD.

Control of gladiolus thrips. E. H. Zeck (*Agric. Gaz. N.S.W.*, 1955, **66**, 437—439).—Gladiolus thrips (*Tæniothrips simplex*) were controlled by 0·1% DDT sprays or 2% DDT or $C_6H_6Cl_6$ dusts applied at weekly intervals when thrips were numerous. A. H. CORNFIELD.

Response of *Heliothis zea*, (Boddie) and *H. viriscens*, (F.) to DDT and endrin in laboratory toxicity studies. J. E. McPherson, L. D. Newsom and J. S. Roussel (*J. econ. Ent.*, 1956, **49**, 368—371).—In the laboratory the first three instars of both species of insects were easily controlled with DDT (10%) dust. Many fourth instar larvæ survived but few fifth of sixth instar larvæ were killed with 10% DDT (15 lb. per acre) or $C_6H_6Cl_6$–DDT–S (3–5–40). *H. zea* was more susceptible to topical applications of DDT and endrin than were *H. viriscens* larvæ. A. A. MARSDEN.

Measurements of distribution of weed-killing spray applied to gorse (*Ulex europæus*) by helicopter and aeroplane. P. B. Lynch, L. J. Matthews and F. B. Thompson (*N.Z. J. Sci. Tech.*, 1956, **37, A**, 505—522).—The distribution of a spray mixture based on 2 : 4 : 5-trichloroacetic acid was measured by sensitised cards placed within the gorse, and by paper strips erected about 6 ft. above the ground. Spraying by helicopter resulted in a narrower swath and greater density of deposit, more even distribution of droplet sizes and less wind-drift than that by aeroplane. R. H. HURST.

Subterranean clover and skeleton weed spraying. E. Tindale (*Agric. Gaz. N.S.W.*, 1955, **66**, 649—651).—Under normal conditions, with sufficient spring rain, subterranean clover survived 2 : 4-D 0·75 lb. of acid equiv. per acre, applied for skeleton weed control, even though a temporary reduction in growth rate occurred. The germinating capacity of subterranean clover seed was not affected by this rate of application. A. H. CORNFIELD.

Effect of maleic hydrazide on growth and reproduction of the sandbur. E. V. Miller, A. E. Miller and E. Mesiano (*Bot. Gaz.*, 1955, **117**, 76—78).—Sandbur seedlings were destroyed by spraying, within two weeks of emergence, with maleic hydrazide (4000—8000 p.p.m. as Na salt). Other seeds in the bur may germinate after the emergence of the first plant and, in practice, spraying should be repeated. Similar applications to mature plants retarded growth and diminished fruiting but did not destroy the plants. A. G. POLLARD.

Morphology and root anatomy of squash and cucumber seedlings treated with *iso*propyl *N*-(3-chlorophenyl)carbamate (CIPC). M. A. Scott and B. E. Struckmeyer (*Bot. Gaz.*, 1955, **117**, 37—45).—Roots of squash seedlings were injured by CIPC in concn. 1—10 p.p.m. With 15 p.p.m. visible injury was accompanied by abnormal modifications. Growth of seedling roots of cucumber was adversely affected by concn. 1 p.p.m. CIPC inhibited mitosis and caused abnormal enlargement of cells and maturation of tissue near the root apex. A. G. POLLARD.

Eradication of *Lantana* and thorn trees. C. P. Naude and J. Serfontein (*Fmg S. Afr.*, 1956, **31**, 209—210).—Max. efficiency of the amyl ester of 2 : 4 : 5-T was attained in sprays containing 3·5 oz. of the ester in 2 gal. of paraffin or diesel oil. More conc. solutions gave no better results. A. G. POLLARD.

Control of *Terminilia sericea* (mangwe) and *Acacia rehmanniana* (common thorn). A. D. McKay (*Rhod. agric. J.*, 1955, **52**, 505—512).—Application of 40% As_2O_3 (16 ml. per in. circumference gave the best kill, although this averaged only 75% with *Terminilia* and 43% with *Acacia*. 2 : 4-D (5—10 lb. acid equiv. per 100 gal.) and diesel oil were ineffective, whilst 2 : 4 : 5-T and 2 : 4-D + 2 : 4 : 5-T were somewhat more effective. Control according to method of treatment decreased in the order freshly-cut stumps, stab wound, and basal bark. No one date of application was superior to others. A. H. CORNFIELD.

Bird-control apparatus for experimental plots. R. P. Pfeifer (*Agron. J.*, 1956, **48**, 139—141).—The equipment consists of two wires spaced about 2 in. apart with insulators and suspended 10—14 ft. above the entire length of the plots. Application of 30,000 v. across the wires results in periodic snapping and arcing. Blackbirds caused no damage to crops within 150 ft. and sparrows no damage within 75 ft. of the wires. A. H. CORNFIELD.

Animal Husbandry

Feeding values of local barley, maize and oat straws. A. Rogerson (*E. Afr. agric. J.*, 1956, **21**, 159—160).—Tabular data from experiments carried out with Masai-type wethers are presented. E. BRICKELL.

Nutritive values of locally prepared pollards and dried brewers' grains. A. Rogerson (*E. Afr. agric. J.*, 1956, **21**, 161—162).—Tabular data from digestibility trials conducted with Merino-type wethers are reported. E. G. BRICKELL.

Nutritive value of pineapple residues (dried). A. Rogerson (*E. Afr. agric. J.*, 1956, **21**, 163).—A digestibility trial, using Masai-type sheep, is reported. E. G. BRICKELL.

Calcium metabolism, with special reference to parturient paresis (milk fever), in dairy cattle. J. M. Boda and H. H. Cole (*J. Dairy Sci.*, 1956, **39**, 1027—1054).—A review with 254 references. S. C. JOLLY.

Large-scale production of leaf-protein. M. Byers, D. Fairclough and N. W. Pirie (*Biochem. J.*, 1956, **63**, Proc. xxxiii).—To separate leaf protein from fibrous and other material, fresh, lush green leaves are pulped in a drum containing beaters fixed to an axial shaft. About 33% of the protein is present in the juice from the pressed pulp, and more is obtained if the extraction is repeated. The proteins are coagulated by heating the juice to 75—80°, and are filtered off, pressed, suspended in water and re-pressed. After extraction with acetone or methanol the residue is pale green or fawn, tasteless, and contains 10—13% of N, i.e., is 60—80% protein; the rest is starch, fat, fibre and inorg, material. J. N. ASHLEY.

Prussic acid poisoning on pastures. J. H. Visser (*Fmg S. Afr.*, 1956, **31**, 216—217).—Risk of HCN poisoning of animals grazing on sorghum pastures is minimised by maintaining a suitable P : N balance in fertilisers used, by avoiding grazing on pastures less than 18 in. high and by feeding roughage prior to the grazing. A. G. POLLARD.

Metabolism of bull semen. III. Relation of lactic acid and its accumulation during incubation with other semen quality measurements and non-returns. F. H. Flerchinger, R. E. Erb, L. E. Mikota and M. H. Ehlers (*J. Dairy Sci.*, 1956, **39**, 1006—1014).—Within bulls, there were high positive correlations between lactic acid concn.

and increase after 1-hr. incubation at 37° and fructose loss, spermatozoa concn., total P, and motility before and after incubation. There were high negative correlations between fructose concn. after incubation, resazurin reduction time and motility decrease after incubation. Initial lactic acid concn. was not highly correlated with any measurement. There was highly significant between-bull correlation between non-return rates and lactic acid concn. after incubation ($r = 0.43$) and lactic acid increase ($r = 0.79$). There was no relation for samples within bulls. Fructose loss during incubation recovered as lactic acid averaged 63·5%. High lactic acid accumulation was associated with above-average maintenance of motility during incubation. S. C. JOLLY.

Importance of water in the management of cattle. M. H. French (*E. Afr. agric. J.*, 1956, **21**, 171—181).—Experiments with Zebu cattle are described. Consumption of water varies appreciably with frequency of drinking, environmental conditions including air temp. and R.H., and with the amount of walking in search of both food and water. Water plays an intimate rôle in nutrient dissolution and absorption, is necessary for the removal of noxious metabolic products, for the maintenance of body temp. and the retention of normal O.P. and turgidity in tissue cells. (40 references.) E. G. BRICKELL.

Comparison of silages made from field maize (Ohio M15) and silage maize (Eureka) for milk production. C. F. Huffman and C. W. Duncan (*J. Dairy Sci.*, 1956, **39**, 998—1005).—Milk production was not significantly different when either mature (Ohio M15) or immature (Eureka) maize silage, both with and without supplementary grain, was fed to milking cows. Most of the grain-equiv. in immature maize silage is present in the vegetative part of the plant (stalks and leaves). The importance of using varieties of maize for silage that mature before frost is stressed. S. C. JOLLY.

Dried whey and lactose as supplements to a vegetable milk replacer. C. H. Noller, C. F. Huffman, G. M. Ward and C. W. Duncan (*J. Dairy Sci.*, 1956, **39**, 992—997).—The addition of 5% of dried whey or 3·5% of lactose to an all-vegetable milk replacement starter for calves had no significant effect on the amount of whole milk consumed or on the average daily wt. gains. The critical period in the life of a calf occurs until 25 days of age, after which feed consumption and body wt. increased, appearance improved, and colour and odour of fæces changed. Calves receiving whey had more costive fæces and smoother hair-coat and were more alert. Lactose had no beneficial effects. S. C. JOLLY.

Use of dried buttermilk in meal mixture as a feed for dairy calves. M. R. Patchell (*N.Z. J. Sci. Tech.*, 1956, **37, A,** 538—541).—Calves were reared on whole milk for the first three weeks, and the diet was then gradually changed to a mixture of buttermilk powder, maize or wheat meal, and pollard, fed either as a gruel or in the dry form. Calves receiving the gruel made faster wt. gains than did those fed on the dry meal. R. H. HURST.

Tributyrin lard and tallow in feeding of "filled milk" for veal production. S. J. Ritchey, J. Hopper, K. E. Gardner and B. Connor Johnson (*J. Dairy Sci.*, 1956, **39**, 1070—1071).—There was no significant difference in wt. gains made by calves receiving *ad libatum* whole milk and those made by calves receiving emulsified "filled milk" containing tributyrin lard (lard containing 3·5% of tributyrin) and tallow. S. C. JOLLY.

Interrelationships between carotene from artificially dehydrated lucerne and vitamin A from a dry carrier when fed simultaneously to Holstein calves. K. L. Dolge (*Dissert. Abstr.*, 1955, **15**, 2371—2372).—The ration of vitamin-A-deficient calves was supplemented with four levels of carotene. After 12 weeks, the supplement was omitted for four weeks. During the last eight weeks of the supplemented oral feeding, the average plasma-vitamin-A concn. was linearly related to the log of the carotene and vitamin-A intakes, and the average plasma carotenoid values were linearly related to the carotene intake and were significantly depressed by higher intakes of vitamin A. At slaughter, liver stores of vitamin A were linearly related to the log vitamin A intake and to the carotene intake; liver stores of carotenoids were extremely low. Body stores of vitamin A were linearly related to log carotene and log vitamin-A intakes. O. M. WHITTON.

Effects of dietary arsanilic acid on the growth and well-being of young dairy calves. E. E. Bartley, F. W. Atkeson, H. C. Fryer and F. C. Fountaine (*J. Dairy Sci.*, 1956, **39**, 989—991).—The feeding of 50 mg. of arsanilic acid daily from birth to 23 weeks of age had no significant effect on rate of wt. gain, feed efficiency, incidence of disease or physical appearance of calves. S. C. JOLLY.

Effect of synthalin A on blood sugar in dairy cattle. C. R. Richards and H. G. Weaver (*J. Dairy Sci.*, 1956, **39**, 983—988).—Blood-glucose levels in immature and mature cattle were significantly affected by intramuscular injection of synthalin A (decamethylene-di-*N*-guanidine). In calves, 1·0 mg. per lb. of body wt. had no effect; 1·3 mg. caused initial hyperglycæmia followed by marked hypoglycæmia; 1·5 mg., or more, reduced blood glucose to <40 mg. per 100 ml. (in some cases to near 10 mg. per 100 ml.). In a mature cow, 1·0 mg. per lb. of body wt. had an apparent hyperglycæmic but no hypoglycæmic effect; 1·4 mg. caused a pronounced initial hyperglycæmia followed by a marked hypoglycæmia. S. C. JOLLY.

Effect of feeding various levels of *NN'*-diphenyl-*p*-phenylenediamine to lactating dairy cows and its detection in the milk. R. Teichman, J. E. Rousseau, jun., M. E. Morgan, H. D. Eaton, P. MacLeod, M. W. Dicks and R. E. Johnson (*J. Dairy Sci.*, 1956, **39**, 1064—1069).—Feeding *NN'*-diphenyl-*p*-phenylenediamine (**I**) at levels of 0·0001, 0·001, 0·01 and 0·1% of the total ration fed (90% dry matter basis) for five weeks to cows producing milk which developed oxidised flavour 72 hr. after addition of 5 p.p.m. of Cu^{++} resulted in significant increases in the fat and vitamin-A levels of the milk. Oxidised flavour caused by 1 p.p.m. of Cu^{++} was reduced by all levels of **I** and that caused by 5 p.p.m. of Cu^{++} was reduced by the two higher levels of **I**. **I** was detected in the milk of cows given the three highest levels. S. C. JOLLY.

Food intake of baconers on pasturage. S. A. Oosthuizen, W. A. Verbeek and T. D. Potgieter (*Fmg S. Afr.*, 1956, **31**, 34—36).—The daily intakes of pasturage by pigs of live-wt. approx. 200 lb. on young oat pasturage alone, on the same with a supplement of 3 lb. of concentrates and on lucerne pasturage alone were 15·1, 19·5 and 20·8 lb. respectively. Digestibility data are recorded. A. G. POLLARD.

Fertilising capacity of fowl semen as affected by time and temperature of storage. W. G. Hunsaker, J. R. Aitken and G. S. Lindblad (*Poultry Sci.*, 1956, **35**, 649—653).—The fertilising capacity of undiluted fowl semen declined with length of storage (up to 9 hr.). The least decline occurred with storage at 15°. Semen stored at 0° maintained a high fertilising capacity for 2 hr., but declined rapidly in fertilising power with longer periods of storage. With storage at 30° fertilising capacity was low even after 2 hr. storage. A. H. CORNFIELD.

Condensed whale solubles in chick starter rations. F. N. J. Milne (*Qd J. agric. Sci.*, 1955, **12**, 21—31).—Addition to chick starter rations of condensed whale solubles (8—16%), stored for 5—18 months, as substitute for meatmeal, resulted in dermatosis, depressed growth and/or high mortality. Reduced growth rate following use of solubles stored for five months was corrected by addition of 4% of livermeal to the ration. Condensed whale solubles are probably deficient in B-complex vitamins, particularly biotin. Storage of solubles may increase fat rancidity which in turn may inactivate biotin. A. H. CORNFIELD.

Evaluation of Cuban "High Test" syrup in chick rations. M. M. Rosenberg (*Poultry Sci.*, 1956, **35**, 558—562).—Addition of 7·5—34·5% of Cuban "High Test" syrup (sucrose 24%, reducing sugars 51%, ash 2·48%) to the diet of chicks to 42 days of age had no effect on growth rate or mortality. Feed efficiency tended to decrease with more than 16·5% of syrup in the feed. The syrup was superior to Hawaiin cane final molasses (sucrose 33%, reducing sugars 19%, ash 10·98%) at comparable levels of addition. The syrup and molasses treatments increased fæcal moisture content, the latter being more effective in this respect. A. H. CORNFIELD.

Trichloroethylene-extracted feeds. VIII. Relative resistance of avian species to the toxic factor in trichloroethylene-extracted soya-bean oil meal. E. G. Hill, K. P. Misra, T. H. Canfield, E. L. Johnson, V. Perman, W. R. Pritchard, J. H. Sautter and M. O. Schultze (*Poultry Sci.*, 1956, **35**, 686—692).—In comparison with hexane-extracted soya-bean oil meal, trichloroethylene-extracted meal of known high bovine toxicity slightly reduced growth rate and delayed egg production somewhat when fed to growing chickens, poults, goslings and laying pullets in amounts ranging from 24·8—47·5% of the ration. No definite toxicity symptoms could be ascribed to any of the samples tested. A. H. CORNFIELD.

Effect of certain cations in cane final molasses on fæcal moisture of chicks. M. M. Rosenberg and A. L. Palafox (*Poultry Sci.*, 1956, **35**, 682—686).—The increase in fæcal moisture content of chicks supplied with cane final molasses was traced to the KCl present in the molasses. The sugar, Mg salts, and water present in the molasses bid not affect fæcal moisture content. Feed efficiency was depressed by adding KCl or water, but not by adding sugar or MgO, to an experimental ration. A. H. CORNFIELD.

Manganese in chicken nutrition. R. D. Creek (*Dissert. Abstr.*, 1955, **15**, 2371).—With a diet marginal in Mn, perosis incidence was increased by omitting glycine or arginine but was unaffected by thiouracil and by stilbœstrol. Thiouracil depressed the Mn content

of the liver. $MnSO_4$, $MnCl_2$ and $MnCO_3$ were equal, on a Mn-equivalent basis, in growth promotion and perosis prevention. Perotic symptoms were produced in one leg, if it was made to carry the total body weight of the chicken, at normally safe levels of Mn. Technical $MnSO_4$ did not affect vitamin stability in a whole mixed feed, or concentrate when stored for 4—6 months. A choline or niacin deficiency produced perosis much more quickly and severely than a Mn deficiency. Neither alanine nor glycine replace glutamic acid in the chick diet. The chick is unable to grow at a max. rate on a diet high in free amino-acids. O. M. WHITTON.

Growth and reproduction in swine and rats as influenced by certain identified and unidentified dietary factors. E. E. Goodwin (*Dissert. Abstr.*, 1955, **15,** 2372).—The influence of vitamin B_{12} and nicotinic acid on reproduction, lactation and early growth of swine, and the influence of vitamin B_{12}, a crude "animal protein factor" supplement (containing vitamin B and aureomycin), fish meal and fish solubles, in natural and purified rations on growth and reproduction of rats were investigated. O. M. WHITTON.

Fat studies in poultry. V. Effect of dietary fat level on the choline requirement of the chick. B. March and J. Biely (*Poultry Sci.*, 1956, **35,** 545—549).—Chicks receiving a maize-soya-bean oil meal–meat meal diet supplemented with vitamin B_{12} and adequate methionine required 0·6 g. of choline per lb. of feed for normal growth to five weeks of age. Addition of 6—12% of tallow to the diets increased the choline and methionine requirements of the chicks. A. H. CORNFIELD.

Utilisation of phosphorus from various phosphate sources by chicks. I. Motzok, D. Arthur and H. D. Branion (*Poultry Sci.*, 1956, **35,** 627—649).—The availability of the P in 27 phosphatic materials to chicks to five weeks of age is reported. A. H. CORNFIELD.

Dicalcium phosphate as a source of phosphorus for chicks. I. Comparison of dicalcium and tricalcium phosphates as a source of phosphorus in chick and poult rations. B. G. Creech, B. L. Reid and J. R. Couch (*Poultry Sci.*, 1956, **35,** 654—658).—Growth and bone ash values at four weeks of age indicated that chicks and poults utilised the P in $CaHPO_4$ better than that in $Ca_3(PO_4)_2$. Chicks required 0·55% of total P and poults $\geqslant$0·8% of total P in the diet for optimum bone ash values. A. H. CORNFIELD.

Distribution of intravenously injected radiophosphate among turkey tissues. A. H. Smith, G. H. Bond and C. M. Winget (*Poultry Sci.*, 1956, **35,** 576—581).—Phosphorus uptake, following intravenous injection of ^{32}P-labelled PO_4''', by the various tissues of mature turkeys decreased in the order liver, kidney, heart, spleen, lung, gastrocnemius and brain. A. H. CORNFIELD.

Growth-promoting activity of ash when fed in practical diets to chicks. A. A. Camp, B. L. Reid and J. R. Couch (*Poultry Sci.*, 1956, **35,** 621—627).—Addition, to an all-vegetable diet, of the ash of fish solubles, dried whey and distiller's dried solubles, in amount equivalent to a 3% level of the original material, significantly improved wt. gains and feed efficiency of chicks to 10 weeks of age. The growth response obtained was 50% of that obtained with unashed material in the case of fish solubles, and equalled that obtained with the ashed materials in the case of dried whey and distillers' dried solubles. Addition of a mixture of inorg. compounds (containing 22 elements) at levels equal to those in an addition of 1·5% each of fish meal and dried whey also increased growth rates significantly. A. H. CORNFIELD.

Effect of grit feeding on growth and feed utilisation of chicks and egg production of hens. S. L. Balloun and R. E. Phillips (*Poultry Sci.*, 1956, **35,** 566—569).—Supplying grit to chicks from 3—6 or 4—8 weeks of age improved wt. gains and feed efficiency. All three grits tested (grey granite grit, red quartzite grit, river sand) were equally effective and the birds showed no particular preference for any one type. Supplying grit to laying hens improved egg production and feed efficiency with respect to egg production, but had no effect on shell thickness. The improvements due to grit feeding were greater where a mash-grain than where an all-mash ration was supplied. A. H. CORNFIELD.

Choline and tallow in breeder hen diets. S. L. Balloun (*Poultry Sci.*, 1956, **35,** 737—738).—The choline requirement of breeder hens was not greater than 0·5 g. per lb. of feed in the presence of adequate vitamin B_{12}. Addition of choline to a low-choline (0·4 g. per lb. of feed) diet did not affect egg size, egg quality (Haugh units) or hatchability. Addition of 2—4% tallow to a diet containing adequate choline and vitamin B_{12} did not affect hatchability. A. H. CORNFIELD.

Protein supplements for laying rations high in wheat. J. O. Anderson and C. I. Draper (*Poultry Sci.*, 1956, **35,** 562—566).—Addition of maize gluten meal (a relatively rich source of leucine) or 0·3% of L-leucine to a ration containing 56% of wheat slightly improved egg production and feed efficiency with respect to egg production and slightly reduced mortality of laying hens. A. H. CORNFIELD.

Niacin and tryptophan requirements of chicks. E. B. Patterson, J. R. Hunt, P. Vohra, L. G. Blaylock and J. McGinnis (*Poultry Sci.*, 1956, **35,** 499—504).—The niacin requirement for optimum growth of chicks (fed maize-soya-bean-gelatin diets) to four weeks of age was $\geqslant$0·008—0·009 g. per lb. of feed when the diet contained 0·24% of tryptophan and 0·013—0·015 g. per lb. when the diet contained 0·14% of tryptophan. The tryptophan requirement was $\geqslant$0·14% with adequate niacin (0·013—0·015 g. per lb.) and $\geqslant$0·24% with min. amounts of niacin (0·008—0·009 g. per lb.). Tryptophan completely compensated for a partial niacin deficiency. Incidence of perosis was negligible when the diet contained tryptophan 0·24% and niacin 0·010—0·011 g. per lb. A. H. CORNFIELD.

Folic acid requirements of turkey breeder hens. F. H. Kratzer, P. N. Davis and U. K. Abbott (*Poultry Sci.*, 1956, **35,** 711—716).—Hatchability of eggs from turkeys placed on a diet low in folic acid (0·00031 g. per kg. of feed) began to fall off after 50 days and reached 10% after 90 days. Egg production was not reduced and the hens appeared normal, but embryonic mortality was high and increased incidence of abnormalities of the extremities occurred. Optimum hatchability was obtained with 0·0007 g. folic acid per kg. in the dam's diet. A. H. CORNFIELD.

Folic acid supplementation of high protein—high fat diets. B. March and J. Biely (*Poultry Sci.*, 1956, **35,** 550—551).—Chicks fed diets containing sub-optimal levels of folic acid showed reduced growth rate to five weeks of age when the protein and/or fat content of the diet was increased. Addition of folic acid (0·00015—0·00065 g. per lb. of feed) to the diet increased growth rate, particularly with high-protein (28%) diets and those containing 7·5% of tallow. A. H. CORNFIELD.

Failure of thioctic (lipoic) acid to stimulate chick growth. A. B. Morrison and L. C. Norris (*Poultry Sci.*, 1956, **35,** 739—740).—Addition of thioctic acid (10—10,000 g. per kg. of feed) to the diet of chicks had no significant effect on growth rate or feed efficiency. The diet used was identical to that used by Debusk and Williams (*Arch. Biochem. Biophy.*, 1955, **55,** 587), who found a significant growth response to addition of thioctic acid. A. H. CORNFIELD.

Study of New Hampshire × barred Columbian chicks from two days to ten weeks of age. II. Effect of coccidiostats. C. E. Schoettle, E. F. Reber, H. W. Norton and J. O. Alberts (*Poultry Sci.*, 1956, **35,** 596—599).—Addition of 0·0075% of 3-nitro-4-hydroxyphenylarsonic acid (**I**) to the chicks' feed from two days to 10 weeks of age significantly increased body wt. and % of fat in the tibia. Addition of 0·0175% of sulphaquinoxaline had no effect upon growth rate and increased the tibia wt. to about the same extent as did **I**. Addition of 0·02% of 3 : 3′-dinitrodiphenyl disulphide depressed growth rate, wt. of spleen, livers, tibias and femurs and % fat in livers and femurs. A. H. CORNFIELD.

Body weight gains and dressing loss as affected by breed in diethylstilbœstrol-treated male chickens. R. E. Moreng and R. L. Bryant (*Poultry Sci.*, 1956, **35,** 672—674).—Diethylstilbœstrol pellet implants (0·012 g. at eight months of age) increased body wt. gains of the four breeds or varieties studied. There were no marked differences in response to the treatment due to breed or variety. Overall losses in dressing wt. were similar for treated and untreated birds. A. H. CORNFIELD.

Effects of multiple pellet implants of diethylstilbœstrol in 9-week-old chickens. R. M. Fraps, H. A. Sohn and M. W. Olsen (*Poultry Sci.*, 1956, **35,** 665—668).—The effects of implanting from one to eight diethylstilbœstrol pellets (each 0·015 g.) in males and females at nine weeks of age on subsequent performance were studied. Viability and growth rate were not appreciably affected by any dosage. The age at sexual maturity of females was retarded, but not proportionally to dosage levels. Max. production in laying birds occurred later with treated than with control birds, and compared favourably with that of control birds during the 5th—6th month following treatment. The treatment delayed the onset of semen production, and semen vol. decreased with increased dosage. Hatchability was only slightly affected by a single pellet, but was appreciably reduced at higher dosages. A. H. CORNFIELD.

Effects of antibiotics on the incidence of spoilage of shell eggs. O. J. Cotterill and P. Hartman (*Poultry Sci.*, 1956, **35,** 733—735).—Addition of aureomycin, chloromycetin, neomycin, polymixin or dihydrostreptomycin (50 p.p.m.) to the wash water (containing 0·25 oz. Dreft per gal.) did not reduce the incidence of microbiological spoilage of eggs during subsequent storage for 7—10 days at 32° and 80—85% R.H. A. H. CORNFIELD.

Effect of feeding antibiotics on the intestinal tract of the chick. H. G. Jukes, D. C. Hill and H. D. Branion (*Poultry Sci.*, 1956, **35,** 716—723).—The effects of supplying chicks with oxytetracycline or procaine penicillin on the dry wt. of the intestinal tract and on the thickness of the components of the gut wall are reported. A. H. CORNFIELD.

Chlortetracycline–aureomycin in poultry production. R. White-Stevens, H. G. Zeibel and N. E. Walker (*Cereal Sci.*, 1956, **1,** 101—108).—Continuous or prophylactic feeding of aureomycin at levels of 50—200 g. per ton of total diet produced significant increases in growth rate, livability, meat yield, quality and feed conversion among growing chickens, turkeys and ducks, and improved egg production and feed-to-egg ratios in laying and breeding fowls and turkeys. These advantages were specially noted under conditions of endemic diseases of an acute and/or a chronic ætiology. (42 references.) E. M. J.

Unidentified growth factors for poultry. L. R. Berg (*Dissert. Abstr.*, 1955, **15,** 2370—2371).—Fish solubles, soya-bean oil meal, salmon eggs, Difco yeast extract, whole liver meal, and a streptomycin fermentation product contained unidentified growth factors. None was found in brewers' yeast, a butyl fermentation product or a Wilson's liver residue. Sufficient growth-depressing factor was found in certain samples of dehydrated lucerne to counteract the effect of any growth-promoting factor. O. M. WHITTON.

Unidentified factor required in a purified diet for normal hatchability. A. A. Kurnick, R. L. Svacha, B. L. Reid and J. R. Couch (*Poultry Sci.*, 1956, **35,** 658—662).—Hatchability of eggs from laying birds fed a purified sucrose–soya-bean–protein type diet for 10—16 weeks decreased by 30—40%. Embryonic mortality during the first week of incubation was high in eggs from deficient birds. Addition of liver prep., condensed fish solubles and antibiotic and vitamin-B_{12} fermentation residues to the basal diet brought hatchability back to normal. A. H. CORNFIELD.

Inadequacy of certain salt mixtures used in studies of unidentified growth factors for chicks. G. M. Briggs (*Poultry Sci.*, 1956, **35,** 740—742).—Published experiments on unidentified growth factors for chicks are criticised on the basis that the basal diets often contained inadequate amounts of known mineral nutrients. A. H. CORNFIELD.

Unidentified growth factor produced by *Streptomyces* species B-1354. J. C. Fritz, F. D. Wharton, jun., R. M. Henley and R. B. Schoene (*Poultry Sci.*, 1956, **35,** 552—557).—Addition of dried fermentation product, produced by aërobic culture of *Streptomyces* species B-1354, to chick and poult diets, containing all known nutrients, vitamins and growth factors, resulted in improved growth rate and feed efficiency of the birds. Max. growth stimulation occurred with addition of 0·35—1·5% of the material to the feed. The treatment did not affect mature wt. of pullets or egg production. The vac.-condensed fermentation product was somewhat more effective than was an equiv. amount of dried (100°) material. A. H. CORNFIELD.

Growing rations for turkeys reared in confinement. J. W. Wyne, H. Yacowitz, V. D. Chamberlin, R. D. Carter and M. G. McCartney (*Poultry Sci.*, 1956, **35,** 735—736).—Growth rate, from 9—24 weeks of age, of turkeys reared in confinement on a 28%-protein ration containing all known vitamins was not affected by adding 2·5% of dried whey alone or with 4% of fish meal to the diet. Both treatments slightly improved feed efficiency. A. H. CORNFIELD.

Effects of farm refrigeration on marketable quality of eggs. L. E. Dawson (*Poultry Sci.*, 1956, **35,** 586—592). The performance of an egg cooler is described. The candled quality of eggs held in the cooler (14·4° ± 1°) was much higher than that of eggs held in a basement (22·2—25·0°) or in a feed room (0·6—29·4°). A. H. CORNFIELD.

Effect of *X*-irradiation of the oviduct on egg production and egg quality in the fowl. A. H. Smith, T. J. Hage, L. M. Julian and D. M. Redmond (*Poultry Sci.*, 1956, **35,** 539—545).—Oviducts were exposed by laparotomy and subjected to *X*-irradiation (50—5000 r with dosage rate of 425 r per min. in air). The inhibition of albumin formation in the egg was related logarithmically to the radiation dosage. The quality of broken-out eggs was decreased following irradiation. The appearance of eggs from irradiated birds was similar to that obtained following partial resection of the oviduct or following respiratory disease. A. H. CORNFIELD.

Vitamin-A deficiency in chicks produced by adding high levels of bentonite to synthetic diets. G. M. Briggs and M. R. Spivey Fox (*Poultry Sci.*, 1956, **35,** 570—576).—Addition of 2—3% or more of Na- or Ca-bentonite (200-mesh material) to a synthetic diet containing otherwise ample vitamin A in unstabilised form resulted in deficiency of vitamin A in chicks. Chemical analysis of the feed confirmed the disappearance of vitamin after addition of bentonite. No deficiency occurred when stabilised vitamin A was used or when higher levels of vitamin A or carotene were supplied. Where 90-mesh bentonite was used no vitamin-A deficiency occurred unless 20% bentonite was added to the diet. A. H. CORNFIELD.

Blood glutathione levels and egg production in inbred lines of chickens. E. C. Stutts, W. E. Briles and H. O. Kunkel (*Poultry Sci.*, 1956, **35,** 727—728).—Blood glutathione levels in the three lines of White Leghorns were significantly and negatively correlated with egg production during the 66 days following glutathione determination. There was a particularly high negative correlation between egg production and the blood glutathione/hæmoglobin ratio. H. A. CORNFIELD.

Intermittent light stimuli in egg production of chickens. W. O. Wilson and H. Abplanalp (*Poultry Sci.*, 1956, **35,** 532—538).—A no. of tests were made to compare the effect of continuous lighting (ranging from 1·5 hr. to 24 hr. in a 24-hr. period) with intermittent lighting (6 cycles in 24 hr.) on egg production. Intermittent lighting generally gave higher egg production than did the same amount of continuous lighting. Under short photoperiods egg production was not proportional to the amount of light given. Hens were more susceptible than pullets to light changes. Good layers were more resistant to shocks from light changes than were poor layers. The time of oviposition was influenced by light and management factors. A. H. CORNFIELD.

Control of the northern fowl mite and two species of lice on poultry. R. A. Hoffman (*J. econ. Ent.*, 1956, **49,** 347—349).—Dusting with lindane, Lauseto neu, Bayer 21/199, Bayer L 13/59, Am. Cyanamid 4124, Chlorthion and malathion, but not DDT, effectively controlled the northern fowl mite. Malathion (≤0·5%) dusts gave complete control of the body louse and the shaft louse. Litter treatments with malathion (4) and lindane (1%) dusts were effective at 1 lb. per 150 sq. ft., but DDT had little or no effect. A. A. MARSDEN.

Anthelmintic effect of three piperazine derivatives on *Ascaridia galli* (Schrank, 1788). C. Horton Smith and P. L. Long (*Poultry Sci.*, 1956, **35,** 606—614).—Piperazine adipate (**I**), piperazine citrate (**II**) and piperazine-CS_2 (equimol. complex) completely eliminated adult *Ascaridia* from chickens when administered in single doses ranging from 0·1—0·6 g. per kg. body wt. A considerable no. of larvæ were also removed by the treatment. Oil of chenopodium and phenothiazine were relatively ineffective. Addition of **I** or **II** to the wet mash (0·3 g. per 100 g. mash) or drinking water (0·15 g. per 100 ml.) also effectively controlled the disease. A. H. CORNFIELD.

Phenylalanine and the resistance of ascarid infections in chickens. B. B. Riedel and J. W. West (*Poultry Sci.*, 1956, **35,** 662—664).—Growth rate of artificially-infected birds from 0—5 weeks of age, no. of birds infected with ascarids, and total no. and average length of worms harboured by the birds were similar with diets low or high in phenylalanine. A. H. CORNFIELD.

Copper sulphate for control of moniliasis (crop mycosis) of chickens and turkeys. P. C. Underwood, J. H. Collins, C. G. Burbin, F. A. Hodges and H. E. Zimmerman, jun. (*Poultry Sci.*, 1956, **35,** 599—605).—Two commercial remedies, containing $CuSO_4$, recommended for treatment of moniliasis (due to *Candida albicans*) were ineffective for treating or preventing the disease in artificially infected chicks and poults. In two of five tests the treatment increased the severity of the symptoms. Addition of 0·06—0·1% of $CuSO_4$, $5H_2O$ to the drinking water was likewise ineffective. A. H. CORNFIELD.

Susceptibility of different breeds of chickens to experimental *Salmonella gallinarum* infection. H. W. Smith (*Poultry Sci.*, 1956, **35,** 701—705).—There were considerable differences in susceptibility to experimental *S. gallinarum* infection between chickens of different breeds, crossbreds and hybrids. Heavy breeds were generally more susceptible than were light breeds. A. H. CORNFIELD.

I. Toxicity of Diazinon vapours to horn flies. II. Control of horn flies on cattle with treated rubbing devices. W. J. Goodwin (*J. econ. Ent.*, 1956, **49,** 406—407, 407—408).—I. Field tests showed that the vapours from a 0·5% Diazinon wettable powder spray were highly toxic to horn flies on cows which were milked in treated barns.

II. Rubbing devices treated with DDT, methoxychlor, Perthane or malathion, in fuel oil were all effective in controlling horn flies on beef and dairy cattle. Addition of butoxy polypropylene glycol to DDT and methoxychlor in emulsifiable formulations slightly increased their effectiveness. A. A. MARSDEN.

Iodometer dip-tester for testing arsenical dips. Dept. of Agric. Div. Vet. Services (*Fmg S. Afr.*, 1955, **30,** 518—523).—New official instructions are given for the construction of dipping tanks, for testing the arsenical liquor (As_2O_3 and total As) and for the correct use of the dip. A. G. POLLARD.

Improvements in hydroponics. Nederlandse Centrale Organisatie voor Toegepast-Natuurwetenschappelijk Onderzoek (B.P. 741,493, 1.7.53. U.S., 2.7.52).—Apparatus for feeding nutrient solution to a hydroponic bed is figured and claimed. F. R. BASFORD.

Compositions for enhancing plant growth. H.-A. und E. Schweigart (B.P. 741,378, 6.3.53. S. Afr., 17.3.52).—A (dust or powder) composition for application to seed, tuber, bulb or roots of a seedling or plant (to enhance growth) contains at least nine macro-trace elements (Ca, Mg, Fe, Mn, Cu. B, Zn, Si, S, K, P, N, Na and Cl), at least four integrating trace elements (I, Cd, V, F, Li, Ba, Sr, Co, Mo, Au), and optionally some trace activators (As, Pb, Se, etc., and rare-earth elements). F. R. BASFORD.

Manufacture of insecticidal compositions containing organic phosphorus compounds. Murphy Chemical Co., Ltd. and M. Pianka (B.P. 741,662, 11.7.52. Addn. to B.P. 723,104, J.S.F.A. Abstr., 1955, ii, 168).—Activators described in B.P. 723,104 are used in conjunction with bis-dimethylaminofluorophosphine oxide, to give a synergistic insecticidal composition. F. R. BASFORD.

Fungicidal preparations containing a basic copper salt. Pest Control, Ltd. (Inventor: M. N. Gladstone) (B.P. 741,620, 24.4.53).—The prep. comprises alkaline earth metal carbonate particles coated with a continuous film of a fungicidal basic Cu salt. F. R. BASFORD.

Medicated feed compositions. Allied Laboratories, Inc. (Inventor: H. E. Blair) (B.P. 741,704, 4.11.53).—A composition for use in the control of large roundworm infections of poultry and domestic animals comprises an orally ingestible, non-toxic feed containing Cd 0·003—10 (0·003—0·04)% (as CdO or $CdCl_2$) and optionally another anthelmintic, e.g., NaF, phenothiazine, or nicotine sulphate. F. R. BASFORD.

2.—FOODS

Quantitative determination of fibre present in tapioca starch and sago globules. M. Narayana Rao, G. Rama Rao, M. Swaminathan and V. Subrahmanyan (*J. sci. industr. Res.*, 1956, **15B**, 202—204).—A colorimetric method based on the aniline acetate colour-test for pentosans is described for the detection and approximate quantitative estimation of fibre in tapioca starch and sago globules. Fibre to the extent of 1% in the starch can be detected by the method. I. JONES.

Effects of prolonged culture of *Aspergillus niger* AN-5 in mineral salt–starch solution. A. I. Fateyev (*Mikrobiologiya*, 1956, **25**, 84—89).—Repeated passaging during 15 months of the mould in media containing starch as the sole org. nutrient did not result in increase in amylolytic power of the organisms. The mould obtained after 40 passages grew more slowly in wort or sugar solutions than did the original stock. R. TRUSCOE.

Influence of hydrochloric acid on hydrolytic decomposition of starch and starchy raw materials in connexion with determination of starch. M. Krajčinović and J. Djukić-Jovanović (*Z. Lebensmitt-Untersuch.*, 1956, **103**, 350—355).—The products of hydrolysis by HCl of various concn. (1—8%) of maize, maize-starch, and distiller's wash are examined. The observed failure of methods based on hydrolysis by HCl to give starch values in accordance with those obtained by the standard methods of Lintner or of Evers is explained by the presence in the HCl hydrolysate of unhydrolysed dextrin and hydrolytic products of cellulose and pentosans. P. S. ARUP.

Spectrophotometric method for quantitative evaluation of early stages of hydrolysis of branched components of starches by α-amylases. J. W. Van Dyk and M. L. Caldwell (*Analyt. Chem.*, 1956, **28**, 318—320).—The method is an adaptation of that of McCready and Hassid in which spectrophotometric measurements were made on the iodine complexes with substrates and certain of their hydrolysis products, from the amylase hydrolysis of linear components of starches. This technique was successfully adapted for the study of the early stages of hydrolysis of fat-free waxy maize starch by crystalline pancreatic amylase. It was also applicable to the study of other starches. The standard deviation is ±0·09% theoretical glucose or $\pm 3{\cdot}5 \times 10^{-7}$M. of aldehyde groups per litre and the limiting concn. is 0·00625% of waxy maize starch. G. P. COOK.

Microscopical examination of modified starches. T. J. Schoch and E. C. Maywald (*Analyt. Chem.*, 1956, **28**, 382—387).—The examination includes a preliminary inspection, granule counting in a hæmacytometer and granule size distribution. The species of a pregelatinised starch is determined by destroying the material with an enzyme and examining the ungelatinised residue; the gelatinisation temp. is measured using the Koffer hot stage. Ionised starches are identified by dye adsorption techniques. G. P. COOK.

Strain retardation in starch jelly candy. C. Sterling (*Food Res.*, 1956, **21**, 491—501).—The rheological behaviour of starch jelly candy from the standpoint of strain retardation is considered. Under stress and recovery some rubber-like behaviour was noted. A reticulate framework of starch mol. with H bondings at the junction points, in which are dispersed, in a brush heap arrangement, free or less-strongly bonded starch mol. is assumed by way of explanation, together with a suggestion of a crystallisation effect (parallelisation of starch mol. to form new micelles or stronger old micelles), with increased amount of stress. (23 references.) E. M. J.

Automation in feed milling. W. R. Flach (*Cereal Sci.*, 1956, **1**, 88—90).—Automation applied to feed mixing, single or continuous batch, and continuous line methods is discussed. E. M. J.

Physical chemistry of wheat proteins in connexion with baking quality of flour. A. H. Bloksma (*Chem. Weekbl.*, 1956, **52**, 345—351).—A review with 85 references. P. S. ARUP.

Comparison of baking quality of frozen condensed and spray-dried skim milk. L. V. Rogers, T. J. Mucha and R. W. Bell (*J. Dairy Sci.*, 1956, **39**, 965—970).—Skim milk condensed to 36 and 45% of milk solids-not-fat developed high η, poor texture and undesirable appearance when stored for long periods at −17° and −27°. The η of condensed milk pretreated for 30 min. at 82° was higher than that of milk heated at 63°. The denaturation rate was slower at the lower storage temp., but all condensed milks were unsuitable as beverages. Storage temp. had no effect on the baking quality of bread produced when the milk was included at the rate of 6 parts of milk solids to 100 parts of flour. Heat treatment was the primary factor controlling baking quality; high-heat-treated skim milk, preserved in either the frozen condensed or dried state, produced larger loaf vol. than did low-heat-treated milk. Loaf vol. with dried skim milk were slightly larger than were those with frozen condensed solids. S. C. JOLLY.

Chromatographic identification of saccharin and dulcin in biscuits and chocolate. M. Vietti-Michelina (*Chim. e Industr.*, 1956, **38**, 392—393).—Identification of saccharin and dulcin in biscuits and chocolate is obtained by a chromatographic separation from the alcoholic liquid resulting from dialysis of the sample with ethanol at 95°. Experimental procedure is given. C. A. FINCH.

Trehalose in bakers' yeast. I. Formation and decomposition of trehalose in glucose fermentation. M. Elander (*Arkh. Kemi*, 1956, **9**, 191—224).—Using acetone-dried top yeast and yeast free from trehalose, in glucose fermentation with phosphate concn. of 0·05M., in two of three tests, the ratio between newly formed trehalose and fermented glucose was almost constant (50—60% and 40—74%); in the third the trehalose synthesis was greater at the beginning. With concn. of phosphate of 0·10 and 0·15M. and with that of 0·05M., the max. value of newly formed trehalose was independent of the phosphate concn., but in presence of 0·10M. phosphate the synthesis was retarded. Crystallised trehalose was obtained from each type of yeast fermentation. Concn, of 2×10^{-3}M-NaF, inhibited glucose fermentation and trehalose synthesis. Concn. of 5×10^{-3}M-arsenate inhibited the synthesis of trehalose to 40% of that without arsenate. (52 references.) E. M. J.

Acidobutyrometric determination of fat in fat-producing yeasts. J. Škoda and L. Šlechta (*Chem. Listy*, 1955, **49**, 1097).—To a mixture of H_2SO_4 (10 ml., *d* 1·810) and a suspension of fat-producing yeasts (11 ml.), contained in a dairy butyrometer calibrated 0—6%, amyl alcohol (1 ml.) is added, the contents are well mixed and centrifuged. Readings are taken after the apparatus has been kept at 60—65° for 5 min. The method is equally accurate and appreciably faster than the extraction procedure of Kleinzeller and Škoda (*ibid.*, 1950, **44**, 184). G. GLASER.

Formation of volatile acid by yeast cells under different fermentation conditions. J. A. van Zyl (*Fmg S. Afr.*, 1955, **30**, 495—502, 524).—Conditions conducive to slow fermentation and the associated increase in volatile acid production are examined. Fermentation period and the amount of volatile acid formed are directly related. A. G. POLLARD.

Colorimetric method for determination of sugars and related substances. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith (*Analyt. Chem.*, 1956, **28**, 350—356).—The method is based on the colour reaction of simple sugars, oligosaccharides, polysaccharides and their derivatives with phenol in the presence of conc. H_2SO_4. Absorption data for 30 carbohydrates are listed and under the conditions described the accuracy is within ±2%. When used in conjunction with partition paper chromatography the accuracy is within ±5%. G. P. COOK.

Effect of ultra-violet light on alkaline solutions of glucose and certain other sugars. T. C. Laurent (*J. Amer. chem. Soc.*, 1956, **78**, 1875—1877).—During u.v. irradiation of alkaline solutions of different monosaccharides, compounds are formed which absorb

between 200—300 mμ. The absorption max. of irradiated glucose at 265 mμ. shifts to 245 mμ. after acidification to pH <3. The possible nature of the u.v. absorbing substances is discussed. M. DAVIS.

Paper chromatography of sugar in foods. E. Becker (*Z. Lebensmitt-Untersuch.*, 1956, **104**, 122—126).—A verified method for sugar determination using *n*-butanol–pyridine–water and developing colour of (*a*) reducing sugar with phthalic acid/aniline and (*b*) non-reducing sugar with naphthoresorsin/trichloroacetic acid is described. The chromatographed sugars are generally taken up in water. The method is semi-quant.; not only the size, but also the intensity of the spot is considered. E. M. J.

Colorimetric determination of betaine in glutamate process end liquor [sugar beet]. R. L. Focht, F. H. Schmidt and B. B. Dowling (*J. agric. Food Chem.*, 1956, **4**, 546—548).—In the simple control method described betaine is determined by measuring the colour of the reineckate ion in 70% acetone at 525 mμ. after the betaine reineckate has been separated from an acid solution of the sample. The betaine is determined by reference to standard curves. N. M. WALLER.

Effect of different factors on the ascorbic acid content in citrus fruits. II. Relationship between species and variety and the ascorbic acid content of the juice. A. Cohen (*Bull. Res. Counc. Israel*, 1956, **5D**, 181—188).—Vitamin C content of the juice of 62 varieties of four citrus species was determined, oranges being richer than grapefruit or lemon and mandarins being lowest. Taxonomically related varieties had similar contents. (11 references.) E. G. BRICKELL.

Some physico-chemical changes in canned jack-fruit during storage. B. S. Bhatia, G. S. Siddappa and Girdhari Lal (*J. sci. industr. Res.*, 1956, **15C**, 91—95).—Changes in vacuum, head space, drained weight, internal corrosion, pH, soluble solids, sugars, carotene, colour and organoleptic quality of canned jack-fruit during storage for 63 weeks are reported. The canned product retained its normal colour, characteristic taste and aroma throughout at room temp. (24—30°)and at 2—5°. Deterioration was observed in 19 weeks in the product stored at 37°. I. JONES.

Species of *Clostridium* associated with zapatera spoilage of olives. Toshio Kawatomari and R. H. Vaughn (*Food Res.*, 1956, **21**, 481—490).—The taxonomy of the anaërobic clostridia isolated and factors which associate them with "zapatera" spoilage are studied. The characteristics of 270 cultures of *Clostridium* isolated from "zapatera" olive brines are given; two saccharolytic-proteolytic sp., *Cl. bifermentans* and *Cl. sporogenes* predominated among the cultures isolated. Representative isolates caused malodorous fermentation of olive brines under suitable conditions for growth. The sp. of *Clostridium* studied are one of a no. of groups of micro-organisms involved in the malodorous "zapatera" spoilage of olives. (20 references.) E. M. J.

Hydrolysis of pectic materials and oligouronides by tomato polygalacturonase. B. S. Luh, S. J. Leonard and H. J. Phaff (*Food Res.*, 1956, **21**, 448—555).—The reactions of partially purified preparations of tomato polygalacturonase on pectic acid and on the oligouronides tetra-, tri-, and digalacturonic acids were followed by paper chromatography. The first 25—30% of hydrolysis produces oligo-uronides but not free galacturonic acid; from 30—50% of hydrolysis galacturonic acid accumulates; at 50%, tri-, di-, and galacturonic acids, and at 80% only di- and monogalacturonic acids are found. (24 references.) E. M. J.

Carbohydrate components of the potato tuber. D. Le Tourneau (*J. agric. Food Chem.*, 1956, **4**, 543—545).—Russet Burbank potatoes were extracted with 80% ethyl alcohol and the free sugars in the extract identified, by paper chromatography, as sucrose, glucose and fructose. The alcohol-insol. residue was further fractionated and the fractions hydrolysed. The fractions isolated included an araban-galactan sol. in 50% ethyl alcohol, starch, pectin, small quantities of araban galactan extracted during pepsin hydrolysis, hemicellulose, and a cellulose fraction. (19 references.) N. M. WALLER.

Properties of polyphenolases causing discoloration of sweet potatoes during processing. J. C. Arthur, jun., and T. A. McLemore (*J. agric. Food Chem.*, 1956, **4**, 553—555).—The mechanism of the enzymic discoloration of sweet potatoes during dehydration is examined. The presence of monophenolase, catecholase and cytochrome *c* oxidase activities in various extracts is demonstrated by the differential effects of inhibitors, electron donors and mediators. It is suggested that discoloration is due to the balance between rate of oxidation and subsequent reduction of chlorogenic acid, being upset by accelerated oxidation rate or decreased quantity of available reducing agent, ascorbic acid. (16 references.) N. M. WALLER.

Freezing of sweet potatoes. M. W. Hoover and G. J. Stout (*Food Technol.*, 1956, **10**, 250—253).—Of various methods tested for preparing sweet potatoes for freezing by (*a*) baking with dry heat at 350°F., (*b*) precooking with steam under 10 lb. pressure for 10 min. and finishing off in dry heat at 350°F., (*c*) precooking in free steam for 15—30 min., then dry heat at 350°F., (*d*) steam under 10 lb. pressure and (*e*) free steam, cooking in free steam plus dry heat at 350°F. gave results most preferred, but all methods produced a high quality frozen product. E. M. J.

Sweet potato dehydration. Time and temperature of storage related to organoleptic evaluations. M. G. Lambou (*Food Technol.*, 1956, **10**, 258—264).—Storage temp. of the raw sweet potato has a profound effect on the palatability of the reconstituted dehydrated products. Reconstituted products made from raw sweet potatoes stored at 60° and 70—75°F. for 2—5 months were palatable, but those products made after storage at 50°F. were not. (16 references.) E. M. J.

Amino-acid composition of some Indian vegetables as determined by paper chromatography. S. G. Majumder, R. N. Dutta and N. C. Ganguli (*Food Res.*, 1956, **21**, 477—480).—Detection and quant. values of amino-acids were determined by chromatographic technique in vegetables available in the Indian daily market. All samples analysed were of good nutritional value, but spinach, tomato (ripe or green), onion, drumstick, cabbage and neem tender are better sources for both essential and non-essential amino-acids both in no. and quality. E. M. J.

Formation of alcohol, acetaldehyde and acetoin in frozen broccoli tissue. P. A. Buck and M. A. Joslyn (*J. agric. Food Chem.*, 1956, **4**, 548—552).—The carboxylase activity of broccoli tissues was investigated to determine the rôle of pyruvic carboxylase in the production of volatile aldehydic and ketonic compounds which might result in abnormal flavours in underscalded frozen broccoli. Broccoli carboxylase catalyses synthesis of acetoin and diacetyl from added pyruvate and acetaldehyde and is involved in their synthesis in both anaërobic and frozen tissues. Acetaldehyde inhibits broccoli carboxylase activity. The concn. of acetaldehyde, acetoin or diacetyl is not related to the development of abnormal flavours, but the accumulation of ethyl alcohol in the tissue is so related. (15 references.) N. M. WALLER.

Soya in the field of nutrition. E. M. Learmonth (*Chem. & Ind.*, 1956, 360—367).—A review. (54 references.) J. S. C.

Dielectric scalding of spinach, peas and snap beans for freezing preservation. M. M. Hard and E. Ross (*Food Technol.*, 1956, **10**, 241—244).—Of the dielectric, water and steam scalding of spinach, peas, and snap beans, the dielectric scalding procedure had no clear-cut advantage over water and steam methods combined with water-dip and air-cooling. (15 references.) E. M. J.

Chemical studies on Indian seaweeds. I. Mineral constituents. V. Krishna Pillai (*Proc. Indian Acad. Sci.*, 1956, **44, B**, 3—29).—Variations in the ash, Na, K, Ca, Mg, Cl, etc., and the amounts and variations of trace elements in Chlorophyceæ, Rhodophyceæ and Phæophyceæ are discussed. Max. amounts of Fe are found in mature plants of Phæophyceæ. Mn, B, Mo are found in the agarophytes, e.g., *Gracilaria lichenoides*, in max. quantities in July and Aug. I content is at a max. in Feb. in young, non-fruiting plants. (19 references.) E. M. J.

Reaction between iron salts and tannins from the chestnut (*Castanea sativa Scop.*). G. Thomas and J. Brossard (*Chem. & Ind.*, 1956, 440—441).—The blue colour given by reaction between $FeCl_3$ and gallic acid was studied with a Beckmann spectrophotometer and a single absorption max. at 470 mμ. found. The decolorising action of Sn^{II} and Sn^{IV} ions, and of other possible inhibitors such as Na hexametaphosphate, was also studied in connexion with the factors causing black discoloration in tinned chestnut purée. J. S. C.

B vitamin content of grapes, musts and wines. A. P. Hall, L. Brinner, M. A. Amerine and A. F. Morgan (*Food Res.*, 1956, **21**, 362—371).—Red grapes, musts and wines (except for pantothenic acid) were richer in thiamine, riboflavin, niacin, vitamin B_6, pantothenic acid than the corresponding white grapes and musts. Thiamine and pantothenic acid of the grapes disappeared chiefly in the vinification process, but riboflavin and folic acid disappeared in the extraction of the musts. Niacin and vitamin B_6 decreased during both processes. (20 references.) E. M. J.

Vitamin B_{12} in wines. E. Peynaud and S. Lafourcade (*C. R. Acad. Sci., Paris*, 1955, **241**, 127—129).—A mutant of *Escherichia coli* (113—3) was used as test organism to detect and estimate vitamin B_{12} in samples of grape musts, and white and red wines, variations of growth rate being determined nephelometrically. The mean amounts of vitamin B_{12} activity found were: musts, 0·75 μg./l.; white wines, 0·075 μg./l.; and red wines, 0·062 μg./l.

The vitamin B_{12} content appears to diminish during ageing. That of grape must increases by ~100% during fermentation. J. S. C.

Hygienic aspects of sulphurous acid in wine. H. Schanderl (*Z. LebensmittUntersuch.*, 1956, **103**, 379—386).—Experiments with synthetic stomach-juices reveal considerable decomposition of compounds of glucose with SO_2, causing copious liberation of SO_2, in non- and sub-acidic juices. The liberation of SO_2 is much retarded in normal or hyperacidic juices. Highly sulphited wines are unsuitable for subacidic subjects. P. S. ARUP.

Mechanism of copper casse formation in white table wine. I. Relation of changes in redox potential to copper casse. M. A. Joslyn and A. Lukton. **II. Turbidimetric and other physico-chemical considerations.** A. Lukton and M. A. Joslyn. (*Food Res.* 1956, **21**, 384—396, 456—476).—I. Data on redox potential changes which occur during copper casse formation are presented. The influence of pH was marked ; the change in potential per pH unit in one wine was ~69 mV. in both the oxidised and reduced states of the wine. The effects of chelating metal ions with Versene, of small amounts of Cu and Fe salts, of temp., of the rôle of sunlight energy on redox potential are discussed. (40 references.)

II. The mechanism of Cu casse formation as given by Ribéreau-Gayon is discussed. Data on the rôles of SO_2, protein and several reducing agents in Cu casse formations are presented and examined and the physical and chemical properties of the ppt. are studied. (27 references.) E. M. J.

Copper complexes causing cloudiness in wines. I. Chemical composition. C. E. Kean and G. L. Marsh (*Food Res.*, 1956, **21**, 441—447).—The undesirable cloudiness caused by Cu complexes in wines was found to consist of protein–tannin, Cu protein and Cu–S complex (probably CuS). (16 references.) E. M. J.

Separation and identification of aromatic substances in wine distillations. I. Separation and identification of free and esterified fatty acids. A. Frey and D. Wegener (*Z. LebensmittUntersuch.*, 1956, **104**, 127—136).—Acetic and butyric acids were found in the first fraction chiefly in the form of esters ; acids with >6 C atoms distilled over as free acid or ester, the small quantity occurring in the last fraction being in ester form chiefly. Butyric acid was also found in the last fraction. E. M. J.

Detection of lipins [in brewing materials and beer]. F. Knorr (*Brauwissenschaft*, 1956, **9**, 122—123).—Brief descriptions are given of the adaptation of known paper-chromatographic methods, whereby the presence of choline-, amino-, and acetal-lipins and phosphoric esters is demonstrated in malt and yeast. The last-mentioned esters are the only lipins found in beer. P. S. ARUP.

Relations between husk and protein content, grain size, and manuring of brewing barley. Ed. Hofmann (*Brauwissenschaft*, 1956, **9**, 118—121).—Husk and protein contents depend largely on interrelationships between climatic conditions and the variety cultivated and are independent of grain size. Manuring with N is highly effective in reducing husk content ; K-, and especially P-manuring are less effective ; their usefulness depends on the presence of adequate supplies of N. Grain size is increased by the use of K as KCl, and reduced by use as K_2SO_4. P. S. ARUP.

Changes during kilning of light malt. P. Kolbach (*Mschr. Brauerei, wissen. Beil.*, 1956, **9**, 63—67).—The chemical and physical changes occurring during the later stages of kilning of "light" malt are dependent on the treatment the malt receives during the earlier stages of the kilning process, when the changes are largely enzymic. In the final stages of kilning, the reactions are formation of colouring matter, production of aroma, coagulation of proteins, destruction of enzymes, formation of opalescence-producing substances, destruction of germinating power and acid formation. The moisture content at commencement of the final kilning, as well as the temp. and duration of the process, influences each of these reactions. There is presumably a connexion between the formation of opalescence-producing substances and the capacity of a malt to give a good foam, both being favoured by high kiln-temp. If the moisture content of the malt before the final kilning is low (2·1%) the final product may retain a germinating power as high as 31% even when the temp. has been 90°. (17 references.) J. C. EARL.

Value and limitations of hop analysis for judging the quality of hops. A. Ruppert (*Brauereitechniker*, 1956, **8**, 129—131).—In spite of the many advances in the detailed chemistry of hop constituents, the α-fraction of the analysis still provides the best practical guide to the brewing quality of the hops. The β-fraction makes a minor contribution and the formula $\alpha + \beta/9$ is still useful if the age of the hops as indicated by the hard resin content is taken into account. Aroma can as yet be only assessed by the judgement of the buyer. The "point system" of Kolbach (*Mschr. Brauerei, wissen. Beil.*, 1954, **7**, 27) is of great value. J. C. EARL.

Paper chromatographic analysis of carbohydrates in worts and beers. A. Stockli (*Brauwissenschaft*, 1956, **9**, 125 ; cf. J.S.F.A. Abstr., 1956, ii, 32). E. M. J.

Preservation of large masses of micro-organisms with retention of vital properties. I. Yeasts. H. Fink and H. Füsser (*Brauwissenschaft*, 1956, **9**, 90—97).—The genetic and enzymic properties of various yeasts can be preserved during ⩽3 years by incorporating the moist yeast mass in suitable proportions with a salt capable of taking up water of crystallisation, e.g. Na_2SO_4 (anhyd.) or Na_2HPO_4, $2H_2O$. The mixture is allowed to set in thin layers, after which it is collected and preserved in powdery form at 5°. Satisfactory preservation is possible at 25° during ⩽3 months. Regeneration takes place on transference of the powder to nutrient solutions. P. S. ARUP.

Readily controllable method for preparation of single-cell yeast cultures. F. Klinkhammer (*Brauwissenschaft*, 1956, **9**, 123).—The capillary method for the prep. of unicellular algal cultures is adapted for the prep. of yeast cultures. With the use of a micro-manipulator, the method admits of control by microscopical observation. P. S. ARUP.

Microbial origin of diacetyl and acetoin in beer. II. A. Kocková-Kratochvílová, A. Vavruchová and D. Vopátková-Nováková (*Brauwissenschaft*, 1956, **9**, 98—104 ; cf. J.S.F.A. Abstr., 1956, ii, 67).—The harmful effects of pediococci in beer are regarded not as due to a special property of the species, but to their disturbing effect on the dissimilation of the micro-organisms of beer. The pediococci are attracted to the yeast cells by a dissimilarity in electrical charges, thrive on certain yeast vitamins, interfere with the normal functions of the yeast, and promote yeast autolysis. Diacetyl, the principal beer spoilage product and a yeast poison, is produced by the pediococci by way of acetaldehyde and acetoin. Factors promoting the formation of diacetyl and the dissimilation of pediococci are examined. A polarographic method for the determination of minute amounts of acetoin and diacetyl, and a new method for isolating pediococci are described. P. S. ARUP.

Redox titration of beer in practice. B. D. Hartong (*Brauwissenschaft*, 1956, **9**, 105—106).—The rapid method of Klopper (cf. *ibid.*, 70) is examined and found satisfactory. Minor modifications are proposed. P. S. ARUP.

Titrimetric determination of carbon dioxide in beer. G. Nowak (*Brauwelt*, 1956, **96B**, 865—866).—A measured quantity of beer is treated with excess NaOH solution of known concn., and the mixture (10 ml.) is titrated with N/5 acid using phenophthalein to pH 8·3 (faint pink), the CO_2 being converted into $NaHCO_3$, (*a*). Another sample of beer is boiled, cooled, and using 10 ml. is titrated with N/5-NaOH and phenolphthalein to pH 8·3. This value gives the other acids present, (*b*). If the amount of NaOH solution used at first $\equiv x$ ml. N/5, then the CO_2 of the beer has combined with $x - (a + b)$ ml. N/5-NaOH and is thus the measure of CO_2 in g./l. of beer. E. M. J.

Brewery brushes with perlon bristles. E. Schild and H. Kammermayer (*Brauwelt*, 1956, **96B**, 1017—1019).—Perlon, in bristles of a roughened, waved kind, offers many advantages over natural bristles, or those of the plastic Pe-Ce-U, in making brushes for cleaning operations in breweries. Perlon bristles have long life compared with that of natural bristles ; they can be used in brushes for cleaning hot surfaces, are not disadvantageously affected by acids or alkalis, and the stability of the brush (wood, screws, binding wire) is increased. E. M. J.

Brewery effluents and their purification. A. Kaess (*Brauwelt*, 1956, **96B**, 913—918 ; cf. J.S.F.A. Abstr., 1956, ii, 144). E. M. J.

***Clostridium acetobutylicum* bacteriophage.** B. M. Nakhmanovich, S. G. Malinkin and V. V. Senkevich (*Mikrobiologiya*, 1956, **25**, 77—83).—Fermentation proceeds normally for 6—17 hr. after introduction of phage, and then stops abruptly ; the length of the latent period rises parallel with the age of the culture. The max. titre of phage suspensions found was 10^{12}. The phage is stable for at least 2—3 weeks at 0—3°, but retains only a fraction of its activity after two days at room temp. or 30 min. at 70—100°, and is totally inactivated after 20 min. at 120°. Renewal of fermentation may occur 12—40 hr. after cessation, and is due to growth of surviving phage-resistant *Clostridia* ; these differ in morphology from, and are inferior in fermenting power to, the original stock. R. TRUSCOE.

Lactose crystallisation in frozen milk. H. Tessier, D. Rose and C. V. Lusena (*Canad. J. Technol.*, 1956, **34**, 131—138).—Studies of lactose crystallisation in frozen milk are reported, using a dilatometric method by which crystallisation can be followed continuously in one sample. The most important controlling factors are the activity of suitable nuclei, temp., and degree of supersaturation.

Since lactose crystallisation is closely related to the pptn. of casein these factors have some bearing on the storage of frozen milk. N. M. WALLER.

Effect of heat and of prefreezing storage on the stability of frozen milk. D. Rose and H. Tessier (*Canad. J. Technol.*, 1956, **34,** 139—144).—Moderate heat treatment (up to 150°F. for 30 min.) before freezing has a stabilising action on pasteurised and conc. milk, while severe heat treatment (180°F. for 30 min.) and prefreezing storage greatly decrease storage life. Effects of prefreezing and moderate heat are related to the crystallisation of lactose while the action of severe heat treatment is related to the presence of heat-denatured serum protein. N. M. WALLER.

Influence of sugars and glycerol on casein precipitation in frozen milk. D. Rose (*Canad. J. Technol.*, 1956, **34,** 145—151).—Casein–calcium phosphate complex was used to study the effect of sugars and glycerol on the stability of casein in frozen milk. All sugars and glycerol have a marked stabilising action providing they remain in solution. This action is probably due to the retention of sufficient unfrozen water by the sugar to delay development of salt concn. that ppt. casein, and to the increase in viscosity of the liquid phase. N. M. WALLER.

Composition of the liquid portion of frozen milk. H. Tessier and D. Rose (*Canad. J. Technol.*, 1956, **34,** 211—212).—The composition of unfrozen liquid separated centrifugally from an ultra-filtrate of conc. milk during freezing is reported. In solution remained ~80% of the Na and K, 50% of the Ca, 84% citrate and 61% phosphate. These results are representative of frozen milk at the beginning of storage and although not themselves sufficient to coagulate casein, these salt concn. and acidities do decrease the stability of casein suspensions. N. M. WALLER.

Causes for varying xanthine dehydrase activity in cows' milk from different districts. F. Kiermeier and K. Vogt (*Z. LebensmittUntersuch.*, 1956, **103,** 355—361).—Considerable regional variations occur in the enzymic activity measured by the capacity of the milk to reduce methylene-blue in the presence of formaldehyde (Schardinger test). A high order of positive correlation is found between the enzymic activity and soil alkalinity. The possibility of a connexion between the activity and the Mo content of the fodder is discussed. (33 references.) P. S. ARUP.

Effect of *Streptococcus lactis* and of filtrates of its cultures on growth of *Str. paracitrovorus* in milk. E. N. Deryabina (*Mikrobiologiya*, 1956, **25,** 72—76).—Production of volatile fatty acids and growth of *Str. paracitrovorus* in summer and autumn milk are stimulated by addition of cell-free filtrates of *Str. lactis* cultures; the effect is much smaller with winter milk, which evidently lacks some other growth factor. Analogous effects are observed in mixed cultures of *Str. lactis* and *paracitrovorus*. R. TRUSCOE.

Biochemistry of cheese ripening. XVIII. Respiration and fermentation in course of ripening of sour milk cheese. J. Schormüller and H. Huth (*Z. LebensmittUntersuch.*, 1956, **103,** 361—379).—Respiration in aq. suspensions of the cheese increases from low initial values to a max. at 6—8 days of age, after which it declines to the original values. Respiration is slightly affected by alterations in pH (5—8), and shows temp. coeff. of 1·4—1·7, and R.Q. 0·8—0·9. Suspensions of the cheese made without NaCl or ripening salts show a similar, but more intense type of respiration. Decarboxylation under aërobic conditions is slight. Respiration is strongly inhibited by HCN, Na azide, or penicillin, slightly stimulated by NaCl, considerably stimulated by DL-lactic acid, glucose, or DL-alanine, and moderately so by AcOH, EtOH, and several other amino-acids. Fermentation, viz. CO_2 production (accompanied by H_2 production) under anaërobic conditions, sets in at the stage when respiration has reached the max. intensity, and is, in comparison with respiration, too slight in extent to affect the main ripening process. (57 references.) P. S. ARUP.

Determination of dry matter in emulsion-liqueurs by means of infra-red radiation. J. Kottász (*Z. LebensmittUntersuch.*, 1956, **103,** 386—387).—Drying by means of i.r. radiation affords a convenient and accurate means for determination of solids in "egg-brandy", ice cream and similar products. P. S. ARUP.

Determination of fat in meat and meat products by Gerber procedure. M. S. Pohja, S. E. Komulainen and F. P. Niinivaara (*Z. LebensmittUntersuch.*, 1956, **103,** 333—341).—The van Gulik method for determining fat in cheese can be used with minor modifications for determining fat in meat and meat products. Single results show max. deviations of ±0·4% from results obtained by the Schmid–Bondzynski–Ratzlaff method. (67 references.) P. S. ARUP.

Quick-freezing of meat. I. H. Burke (*Kältetechnik*, 1956, **8,** 155—160).—The effect of air-cooling and a number of other factors on cooling time and loss of wt. of beef and pork carcasses is studied. Some suitable procedures for use in the slaughterhouse are recommended on a basis of these experiments, which are described in some detail. The results obtained are examined theoretically, and a complicated mathematical expression is obtained for the cooling time. C. A. FINCH.

Meat tenderisation. II. Factors affecting the tenderisation of beef by papain. A. L. Tappel, D. S. Miyada, C. Sterling and V. P. Maier (*Food Res.*, 1956, **21,** 375—382; cf. J.S.F.A. Abstr., 1956, ii, 154).—Histological and dye tracer studies indicated that papain penetration into beef is limited to 0·5 to 2 mm. The greatest hydrolysis of beef proteins catalysed by papain occurred at 60° and 80°, tenderisation being ascribed to the hydrolysis of all structural and functional components. E. M. J.

Chemical responses of connective tissue of bovine skeletal muscle. M. Miller and J. Kastelic (*J. agric. Food Chem.*, 1956, **4,** 537—542).—The responses of various fractions of bovine muscle to extraction, autoclaving and to enzymic digestion are examined. An extracting solution of 0·6M-KCl is compared with 0·1N-NaOH solution. The protein content of the residual connective tissue is shown to be dependent on the solvent used; 15—20% of the total N remains insol. when KCl is used, but only 5—10% is not extracted by NaOH. The factors involved in the tenderness of meat are discussed. (23 references.) N. M. WALLER.

Effects of three levels of nutrition and age of animal on the quality of beef. I. Palatability, cooking data, moisture, fat and nitrogen. II. Colour, total iron content and pH. III. Vitamin B_{12} content. M. Jacobson and F. Fenton (*Food Res.*, 1956, **21,** 415—426, 427—435, 436—440).—I. With increase in level of nutrition the wt. of the muscles and fat increased in the raw meat, the moisture content decreased. With increase in age, the carcass wt., fat and N-content increased in the raw meat, and in cooked meat, scores for aroma, flavour, juiciness and tenderness tended to decrease after 48 weeks of age. (11 references.)

II. The redness and Fe content of raw beef were increased by both level of nutrition and age of the animal, particularly in the *semimembranosus* muscle.

III. The *longissimus dorsi* and the *semimembranosus* muscles from animals slaughtered at 64 weeks of age contained significantly more vitamin B_{12} than did these muscles from animals slaughtered at 32 or 48 weeks of age. E. M. J.

Meat extract—a revaluation. A. E. Bender and T. Wood (*Food Manuf.*, 1956, **31,** 223—227).—A review of the literature relating to the composition, properties and preparation of meat extract. (22 references.) J. S. C.

Residual microflora of tinned meat and fish. M. Panaiotova and D. Bail'ozov (*Mikrobiologiya*, 1956, **25,** 211—216).—An examination of the products of five Bulgarian canneries showed that of 1293 tins containing meat or fish products 189 were not hermetically sealed. Of the remainder, 2·26% contained viable spores or bacteria, of which *Clostridium putrificum* Bienstock, *Cl. sporogenes* Metschnikoff, *Bacillus subtilis* Cohn, *Escherichia coli*, and *Achromobacter album* were identified. The spores withstood heating at 120—125° for 5—35 min., whilst the vegetative organisms did not survive 30 min. at 100°. R. TRUSCOE.

Research of fish preservation and processing. H. L. A. Tarr (*Food Manuf.*, 1956, **31,** 239—243).—An account is given of the work of the Pacific Fisheries Experimental Station, Vancouver, on microbiological deterioration of fish, fish preservation with antibiotics (particularly in the use of aureomycin), use of refrigerated sea water, salmon canning, "browning" of white fish, and fat oxidation. J. S. C.

Literature review on oils and fats 1954. M. N. Krishnamurthi (*Counc. sci. industr. Res. New Delhi*, 1956, 55 pp.).—The review covers trends in research (1949—54) on fats, oils and oilseeds; statistical survey; cultivation, storage and processing of oil seeds; processing of oils and fats; spoilage and characteristics, composition and synthesis of fats; nutrition and metabolism; and an appendix. (546 references.) E. M. J.

Determination of *O*-(3-chloro-4-nitrophenyl)*OO*-dimethyl phosphorothioate (Chlorthion) residues in cottonseed. M. J. Kolbezen and H. T. Reynolds (*J. agric. Food Chem.*, 1956, **4,** 522—525).—The method described for the determination of Chlorthion residues in cotton seed is sensitive to 0·02 p.p.m. in 200 g. The cotton seed is mechanically ginned, delinted with conc. H_2SO_4, ground, and extracted with pentane. Chlorthion is separated from extracted cottonseed oil by partition between pentane and acetonitrile. Interfering materials are removed from the acetonitrile solution by chromatography through activated alumina. Finally the coloured compound is developed by reduction with Zn and phosphoric acid followed by treatment with $NaNO_2$ solution, ammonium sulphamate and *N*-1-naphthylethylenediamine dihydrochloride. The Chlor-

thion is determined spectrophotometrically by comparison with standard curves. This method of reduction at the colour development stage removes the difficulty of yellow colour developing. Cottonseed from a plot treated with high doses of Chlorthion was found to contain no residues by this method. N. M. WALLER.

The effect of vitamin B_{12} on the unsaturated fatty acid content of castor oil. N. Y. Mary, R. H. Bohning and J. L. Beal (*J. Amer. pharm. Ass., Sci. Edn.*, 1956; **45**, 347).—No significant change in the composition of the unsaturated fatty acids present in 6-months-old plants of *Ricinus communis* var. Baker 195 was observed after the administration of 0·5—10 mg. quantities of vitamin B_{12}. No other changes were noted. G. R. WHALLEY.

Quantitative estimation of vitamins D_2 and D_3 in pure solution. D. H. Laughland and W. E. J. Phillips (*Analyt. Chem.*, 1956, **28**, 817—819).—The method is based on the formation of colour when the vitamins D are treated with furfuraldehyde and H_2SO_4. Distinctive absorption curves are obtained for the two forms and binary mixtures can be analysed by differential spectrophotometry at two wavelengths. As little as 15 μg. of total vitamin can be estimated G. P. COOK.

Methods and limitations of chromatographic determination in protein hydrolysates. F. Parisi, C. Dalombi and G. Funes (*Chim. e Industr.*, 1956, **38**, 398—403).—Methods for the identification and determination of protein amino-acids are developed and improved. The identification is performed with two-dimensional paper chromatography, and determination of most of the amino-acids by single-dimension paper chromatography with buffered solvents. For quant. determination of lysine, arginine and histidine, selective retention on buffered carboxylic resins is used: tyrosine and tryptophan have been determined in alkaline protein hydrolysates by u.v. spectroscopy. By these methods, deviations of only 2% are obtained. Analytical data obtained on pure amino-acid solutions and on *Saccharomyces cerevisiæ* are presented. (74 references.) C. A. FINCH.

Non-enzymic browning reactions and their physiological sequence. II. Methods to define changes. E. Menden and H. D. Cremer (*Z. LebensmittUntersuch.*, 1956, **104**, 105—121).—Data are presented on various methods of producing non-enzymic browning reaction changes in individual amino-acids and in protein. Short heating at high temp. leads to irreversible changes in greater measure than longer heating at low temp. Statistical differences were obtained in the enzymic cleavage between heated and non-heated casein–sugar mixtures after short-time *in vitro* digestion with pancreatin. E. M. J.

Chemical additives in foods. P. E. Johnson (*Cereal Sci.*, 1956, **1**, 94—96).—A general discussion of additives to foods, to include, e.g., texture appeal, preservation, enhancement of nutritive values, improvement through acidity, incidental chemical additives, tests for safety, etc. E. M. J.

Chronic toxicity studies on food colours. II. Toxicity of FD & C Green No. 2 (Light green SF yellowish), FD & C Orange No. 2 (Orange SS) and FD & C Red No. 32 (Oil red XO) in rats. M. G. Allmark, H. C. Grice and W. A. Mannell (*J. Pharm., Lond.*, 1956, **8**, 417—424).—The administration of FD & C Orange No. 2 (**I**), FD & C Red No. 32 (**II**) and FD & C Green No. 2 (**III**) food colours at concn. of 0·03% of the diet of rats, does not affect their growth, food consumption or food efficiency, although 100% mortality occurred with concn. of 0·7 and 1·5% of **I** and **II**. At 3% of **III** growth was retarded, but 1·5% gave no effect. A decline in blood hæmoglobin is observed with 200 and 400 mg./kg. oral doses of **I** and **II** after 20 weeks. Rats receiving 3% of **III** or 400 mg./kg. doses of **II** showed some pathological changes of the testes. G. R. WHALLEY.

Oral toxicity and pathology of polyoxyethylene derivatives in rats and hamsters. E. Eagle and C. E. Poling (*Food Res.*, 1956, **21**, 348—361).—In rats fed polyoxyethylene-20-sorbitan monolaurate there was 100% incidence of enlarged kidneys, renal calculi, etc., 75% incidence of stones in the urinary bladder, and microscopically, 100% incidence of atrophy of the testicular tubules, etc. and decreased spermatogenesis (86%). Similarly in hamsters fed two polyoxyethylene monostearates and polyoxyethylene-20-sorbitan monolaurate there was incidence (25—100%) of fatalities, chronic diarrhœa, small testes, etc. Stones 53—94 mg. were found in urinary bladders. E. M. J.

Trace elements in food. II. Fluorine. C. F. M. Fryd (*Food Manuf.*, 1956, **31**, 236—238).—The official procedures, based on the Willard and Winter process of distillation from $HClO_4$, for estimation of F in foodstuffs are described. (11 references.) J. S. C.

Paper disc method for subculturing acidified food products. H. N. Miller (*Food Res.*, 1956, **21**, 372—374).—A modified auxanographic method was used successfully by subculturing the confirmatory broths on agar plates. Sterile filter paper discs were dipped into the confirmatory broths and aseptically transferred to the surface of uninoculated nutrient agar plates. Positive broths were indicated when growth occurred round the edge of the paper disc. E. M. J.

Continuous recording of gel formation in creep and recovery. C. O. Chichester and C. Sterling (*Food Res.*, 1956, **21**, 397—401).—An apparatus which records the continuous deformation with time is described including the optical and electrical systems involved. E. M. J.

Potentiometric titrations in food analysis. K. Woidich, L. Schmid and H. Gnauer (*Z. LebensmittUntersuch.*, 1956, **104**, 97—104).—The suitability of electrometric titration based on the analytical process of Cruse (*Angew. Chem.*, 1953, **65**, 232) and graphical method of Tubbs (*Analyt. Chem.*, 1954, **26**, 1670) for determination of the equivalence point and pH range from the titration curve, is discussed in a series of test analyses, specially useful results being obtained for the determination of acid in jam, flour, sugar and vanillin. Adaptations of the method to improve accuracy and save time are described. (31 references.) E. M. J.

War-time food processing problems in retrospect. J. H. Bushill (*Chem. & Ind.*, 1956, 446—452).—A review with particular reference to supplies of sugar, eggs, meat, food yeast and baking powder, transport and refrigeration. J. S. C.

Canning experiments with non-spore-forming bacteria. O. B. Williams (*Food Res.*, 1956, **21**, 502—504).—One unidentified coccus and a known entero-toxin producing *Staphylococcus aureus* were grown in oyster brine; large no. of each were inoculated into oysters in 211 × 400 (No. 1) cans. No can processed for 10 min. at 212°F. had living cocci and only two such cans had surviving spores. E. M. J.

Knowledge of the stimulus variable as an aid in discrimination tests. E. L. Raffensperger and F. J. Pilgrim (*Food Technol.*, 1956, **10**, 254—257)—The relative effectiveness of seven different types of instructions imparting knowledge of the variable in the triangular test is discussed. E. M. J.

Packagaing of cereal products. A. V. Grundy (*Cereal Sci.*, 1956, **1**, 97—99).—Innovations in commercial packaging are reviewed. E. M. J.

Calculating device for use during the preparation of mix batch recipes or formulæ for comestibles. E. L. E. Humphriss (B.P. 741,890, 19.5.53).—The slide rule device described enables the production of a final mix (especially ice cream) of composition very close to that of the required specification. F. R. BASFORD.

Cutter and slicer for onions and the like. O. Cupper (B.P. 741,222, 30.5.51. Ger., 28.6.50).—The device described can be adapted to give slices, strips or cubes of the vegetable. K. RIDGWAY.

Manufacture of alginic products. Alginate Industries, Ltd. (Inventor: R. R. Merton) (B.P. 741,990, 31.5.52. Addn. to B.P. 716,112; J.S.F.A. Abstr., 1955, i, 386).—A paste prepared by admixture of Ca alginate with M_3PO_4 (M is alkali metal) in presence of water is added (optionally after drying) to aq. medium containing acid (gelling agent) and sequestering agent for Ca^{II}, e.g., $Na_4P_2O_7$, to give a gel (suitable for use in confectionery). F. R. BASFORD.

Compositions for prevention of clouding of wines. E. Griffin, V. A. Strom, John M. Evans, Daniel G. White, J. H. Fessler, trading as Cuvinex Co. (B.P. 741,663, 15.8.52. U.S., 16.8 and 23.10.51).—Clouding of wine (due to presence of Cu) is overcome by treating with an aq. solution containing K ferrocyanide (**I**), enough Fe^{II} compound to form an insol. complex with **I**, alkali (to give pH $\not<7$, viz., 9—10), and optionally a Fe^{III} salt (Fe^{II} : Fe^{III} ratio $\sim$2 : 1). F. R. BASFORD.

Improving the adhesion of synthetic sausage casings to the filling. Wolff & Co. Kommandit-Ges. auf Aktien (B.P. 741,163, 15.5.53. Ger., 4.7.52).—Casings produced from an alginate basis have their charge reversed by treatment with a solution containing 10% glycerol and either 1% Al or Zr oxychloride, or by adding 1% of $Zr(NO_3)_4$ to the initial $CaCl_2$ precipitation bath. The treatment time is approx. 1 min. The casing then adheres as well as a natural casing. K. RIDGWAY.

Heat treatment of chocolate and chocolate couverture. Lesme Ltd. (Inventor: E. H. Harbard) (B.P. 741,357, 8.2.52).—Molten chocolate is passed between two horizontal rotating cylinders provided with teeth: the cylinders are heated by means of a water jacket at the desired tempering temp. The material is entrained between the teeth and is removed when the latter are re-engaged. J. A. BARNARD.

Method and means for forming chocolate pastes. Soc. Anon. Française pour la Separation, l'Emulsion et le Melange (Procédés S.E.M.) (B.P. 741,412, 2.6.50. Fr., 2.6.49).—The homogenised mixture of powdered chocolate and cocoa butter is circulated around a closed circuit by means of conveyor worms. Fresh components are added at one point in the circuit and an equivalent portion of the mixture removed at another. The rate of introduction of fresh components is about one-sixth the rate of circulation of the whole mixture. The kneading of the mass is performed by pairs of screw-conveyors rotating side by side in opposite directions.
J. A. BARNARD.

3.—SANITATION

New grain pest (Khapra beetle, *Trogoderma granarium*) in Rhodesia. J. A. Whellan (*Rhod. agric. J.*, 1956, **53**, 41—50).—Characteristics of the pest are described. Fumigation of stored grain with MeBr (2 lb. per 1000 cu. ft.) is recommended for control of the larvae. γ-$C_6H_6Cl_6$ was effective, whilst DDT was ineffective, in controlling the beetle. A. H. CORNFIELD.

Peet–Grady method. Anon. (*Soap, N.Y.*, 1956 *Blue Book*, 243—244, 267).—Details are given of the official method of the Chemical Specialties Manufacturers' Association (N.Y.) for determining the relative efficiency of contact insecticides dissolved in fly spray base oils suitable for household and industrial use. The method is a standardised procedure for determining the mortality of *Musca domestica* of a standard strain. J. S. C.

Aërosol test method for flying insects. Anon. (*Soap, N.Y.*, 1956 *Blue Book*, 247—278, 266—267).—Details are given of the official method of the Chemical Specialties Manufacturers' Association (N.Y.) for assaying aërosols for flying insects. It follows the official Peet—Grady test procedure, so far as is practical (cf. preceding abstract). J. S. C.

Aërosol insecticides storage test. Anon. (*Soap, N.Y.*, 1956 *Blue Book*, 245—246).—Details are given of the tentative official method of the Chemical Specialties Manufacturers' Association (N.Y.) for ascertaining the shelf-life of a package of aërosol insecticide and the suitability of the valve and container compounds. J. S. C.

Cockroach spray test method. Anon. (*Soap, N.Y.*, 1956 *Blue Book*, 249—250).—Details are given of the official method of the Chemical Specialties Manufactiurers' Association (N.Y.) using the German cockroach, *Blattella germanica*, as test insect. J. S. C.

Cockroach aërosol test method. Anon. (*Soap, N.Y.*, 1956 *Blue Book*, 251—252, 265—267).—Details are given of the tentative method of the Chemical Specialties Manufacturers' Association (N.Y.) using *Blattella germanica* as test insect. J. S. C.

Fly control in Wyoming barns. G. R. DeFoliart (*J. econ. Ent.*, 1956, **49**, 341—344).—In the control of flies in dairy barns residual sprays of Diazinon (1%) were most effective, followed by methoxychlor (2) and malathion (1·25%), in descending order.
A. A. MARSDEN.

Effects of different temperatures and piperonyl butoxide on the action of malathion on susceptible and DDT-resistant strains of house flies. L. Rai, S. E. D. Afifi, H. C. Fryer and C. C. Roan (*J. econ. Ent.* 1956 **49**, 307—310).—The lethal effects of topical applications of malathion in acetone, alone and with piperonyl butoxide, at −17, 21, 24 and 28°, on resistant and non-resistant house flies were studied. There was pronounced antagonism to malathion by piperonyl butoxide. A. A. MARSDEN.

Effectiveness against house flies of some 3 : 4-methylenedioxyphenoxy compounds as synergists for pyrethrins and allethrin. J. H. Fales, O. F. Bodenstein and M. Beroza (*J. econ. Ent.*, 1956, **49**, 419—420).—Of seven synthetic compounds containing the 3 : 4-methylenedioxyphenoxy-group, four showed a high degree of synergism to both pyrethrins and allethrin. The 2-(2-ethoxyethoxy)ethyl 3 : 4-methylenedioxyphenyl acetal of acetaldehyde was the most active material tested with both insecticides. A. A. MARSDEN.

The "benthal" and aërobic decomposition of cellulosic materials. N. J. Lardieri (*Dissert. Abstr.*, 1955, **15**, 2508—2509).—The "benthal" oxidation of cellulosic sludges is probably more rapid in swiftly moving streams than in stagnant water. The highly acid conditions in sludges retard linear oxidation rates and cause lack of gasification. Benthal oxidation of acidic sludges is primarily a surface phenomenon; it is modified in rate and character by buffering. Under buffered conditions cellulosic stream sludges seed the decomposition of α-cellulose; aërobic decomposition of α-cellulose is affected by pH, temp., the presence of growth factors and the development of seed. O. M. WHITTON.

Comparative analyses of sewage sludges. M. S. Anderson (*Sewage industr. Wastes*, 1956, **28**, 132—135).—Tables show (1) N and P contents of activated and digested sludges for 1931—35 and 1951—1955, (2) min., average, and max. Cu, Zn, B, Mn and Mo contents of activated and digested sludges, and (3) origin and % composition of the sludges studied. The influence of such factors as synthetic detergents, industrial wastes and the increased use of home garbage grinders is discussed. J. S. C.

The relative stability test [for sewage]. W. L. Tidwell and J. H. Sorrels (*Sewage industr. Wastes*, 1956, **28**, 136—139).—The mechanism of the methylene blue test is examined. It is shown experimentally that relative stability varies indirectly with the amount of org. matter and no. of bacteria and directly with concn. of nitrates; its relation with dissolved O_2 is shown by a curve which rises to a max. at ~4—5 p.p.m. dissolved O_2. J. S. C.

Effects of high concentrations of nitrogen on activated sludge. R. L. Taylor, E. R. Mathews and C. W. Christenson (*Sewage industr. Wastes*, 1956, **28**, 177—182).—Experimental activated sludges were used to study the effects of various concn. of NO_3' and $NH_4^{\cdot}$. N in the form of NO_3' was found to have no deleterious effect in concn. up to 4800 p.p.m., provided adequate time is allowed for acclimation. In the form of $NH_4^{\cdot}$, however, a deterioration appears with sewage-fed sludge at a concn. of 480 p.p.m. and a B.O.D. to N ratio of 0·2 to 1. J. S. C.

Slime formation in sewage. III. Nature and composition of slimes. H. Heukelekian and E. S. Crosby (*Sewage industr. Wastes*, 1956, **28**, 206—210).—The physical, chemical and biological composition of submerged slime growths is described. Moisture, ash and N content correspond to those of solids settling from raw sewage. The microfauna present in slimes developing in anaërobic and aërobic conditions at different stages were examined microscopically and are characterised. J. S. C.

Determination of low chemical oxygen demand of surface waters by dichromate oxidation. W. A. Moore and W. W. Walker (*Analyt. Chem.*, 1956, **28**, 164—167).—The use was studied of 0·025 and 0·05N-$K_2Cr_2O_7$ as oxidant in the estimation of the org. content of surface waters by the wet combustion method. Results compared favourably with the method using 0·25N-$K_2Cr_2O_7$ as oxidant.
G. P. COOK.

Dishwashing machines. General Electric Co. (B.P. 741,332, 28.5.53. U.S., 31.5.52).— J. A. BARNARD.

Continuous vaporisation of volatile substances. T. Yamaguchi (B.P. 751,522, 25.9.53. Jap., 27.2 and 9.4.53).—A solution of the substance, e.g., insecticide, in a volatile org. solvent capable of catalytic exothermic oxidation (e.g., petrol, ethanol, methanol, acetone) is fed continuously to a preheated oxidation catalyst, e.g., Pt, Ag, Cu and/or V (or the oxide thereof) from a container packed with cotton wool, and the substance is there vaporised by the heat of the exothermic reaction. F. R. BASFORD.

Organic derivatives of thiophos ric acid. Fisons Pest Control, Ltd. (Inventors: D. W. J. Lane and D. F. Heath) (B.P. 742,796, 31.12.52).—Compounds $OR(OR')\cdot PO\cdot S\cdot[CH_2]_2\cdot SOR''$, useful as pest-control agents, are made by oxidising $OR(OR')\cdot PO\cdot S\cdot[CH_2]_2\cdot SR''$ with aq. H_2O_2 (R—R'' are alkyl of $\geq$4C). Thus, a 0·212% aq. solution of $(OEt)_2PO\cdot S\cdot[CH_2]_2\cdot SEt$ is treated with 2·5% aq. H_2O_2 at 25·4°, with formation of OO-*diethyl* S-(*ethylsulphinylethyl*) *thiophosphate*. F. R. BASFORD.

4.—APPARATUS AND UNCLASSIFIED

Determination of volatile C_1—C_6 aliphatic acids in biological liquids by paper chromatography. J. Guillaume and R. Osteux (*C. R. Acad. Sci., Paris*, 1955, **241**, 501—502).—A quantity of sample estimated to contain ~0·01 g.-mol. of mixed volatile acids (C_1—C_6) is steam-distilled into excess NaOH, the distillate concentrated to ~1 ml. and Na removed by addition of a calculated amount of Permutit 50; 5—10 ml. of a pure solution of the acids results and this is neutralised and chromatographed on Whatman No. 3 filter paper, developing with a spray of *o*-cresolphthalein buffered with Na veronal. Of a large no. of solvents tested, only allyl alcohol and propylene glycol gave satisfactory separation of formic and acetic acids. Three solvent mixtures are specified and R_F values for the various acids are tabulated in respect of each. A method of separating *n*- and *iso*-acids is also briefly indicated. J. S. C.

SOCIETY OF CHEMICAL INDUSTRY

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Conclusions.

Acknowledgments.

References.

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