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EFFECT OF REDUCING SUGAR (GLUCOSE) ON THE BIURET COLORIMETRIC REACTION

By C. E. O'HARA

A highly significant interaction between concentration of free reducing sugar (glucose) and total time of the biuret reaction has been found by following absorbence changes. However, concentrations of up to 12·5 mg glucose/25 ml biuret reagent subjected to a reaction period of 1½ hours caused no interference in absorbence value. Conditions for the specific application of the biuret colorimetric reaction for the determination of wheat flour proteins in the presence of free reducing sugars have been defined.

Introduction

It was observed during investigations of wheat proteins that the biuret colours produced by some flours faded and deposited cuprous oxide when left at room temperature for several hours. This effect was due to reducing sugars in the flour samples. The susceptibility of the cupric ions in the biuret reagent to reduction has been observed by Racusen & Johnstone.[1] These workers found it necessary to wash materials in which there were large concentrations of reducing sugars, before subjecting them to the biuret reaction. The presence of free reducing sugars could be a source of error in biuret determination of materials, where there is a high proportion of these present. Free glucose, fructose, sucrose, maltose, raffinose[2] and a series of glucofructans[3–5] have been detected in wheat flours. Immature and sprouting wheats contain variable amounts of free reducing sugars, glucose, fructose, maltose and maltodextrins (Clancy, M. J., Personal communication). The effect of different concentrations of reducing sugar (glucose) on the accuracy of the biuret method was studied by following the increase in cupric ions reduced and the decrease in optical densities of biuret colours.

Experimental

Reagents

All reagents were of analytical reagent grade. A biuret reagent having the following composition has been used in this laboratory: cupric sulphate 2g/l, sodium potassium tartrate 6g/l, potassium hydroxide 42·1g/l. This reagent was made up using CO_2-free water and stored in polythene bottles. Under these conditions, it was stable for at least a month.

Protein standard

Crystalline bovine serum albumin (Sigma Chemical Co.) was used as a protein standard to calibrate the biuret colour. All protein absorbences in this work are measured in serum albumin units. The serum albumin was dried *in vacuo* over phosphorus pentoxide at 60° for 18 hours.

Measurement of cupric ions in biuret solutions

Test solutions of biuret reagent were neutralised with 3N-H_2SO_4 and 5 ml 0·1N-KI was added. The solutions were titrated against 0·02N sodium thiosulphate, using starch glycollate as an indicator.

Results and Discussion

Effect of glucose on cupric ion content of the biuret reagent

Glucose (25, 50 and 100 mg) samples were accurately weighed in triplicate into 100 ml conical flasks, containing 0·05g serum albumin. The biuret reagent (25 ml) was added to the flasks, which were then shaken vigorously for 10 minutes on a Griffin & George wrist-action shaker, and left at room temperature for a suitable time. The amount of cuprous ions formed in each test solution was determined by the titre difference between the total cupric copper added and that remaining after reaction with glucose. The amounts of cuprous ion produced by 25 mg and 50 mg of glucose/25 ml biuret reagent up to 1½ hours exposure showed little difference (Fig. 1). Thereafter the differences became pronounced, and an experiment was carried out to determine the effect of different concentrations of glucose on the optical densities of test solutions. It was also observed that maltose, lactose, melibiose, cellibiose reduced the copper of the biuret reagent whereas, inulin raffinose, and sucrose did not.

Effect of glucose on the optical density of biuret colour

To a series of flasks containing serum albumin (0·05g) and varying amounts of glucose (12·5–125 mg), 25 ml biuret reagent were added. Each concentration of glucose was replicated three times for each point in time. The mixture was shaken to dissolve the solids and then set aside at room temperature for a suitable period of time. The optical densities of the biuret colour complexes were read at 540 nm against a reagent blank and expressed as percentages of the optical density produced by 0·05g serum albumin/25 ml biuret reagent without glucose. A concentration of 12·5 mg glucose/25 ml biuret reagent left at room temperature for 1½ hours (overall reaction time for biuret determination of flour proteins) caused no decrease in optical density (Fig. 2). With a concentration of 25 mg glucose/25 ml biuret reagent left at room temperature for the same period there was a decrease in optical density of 2·6%. Statistical analysis of the results demonstrates a highly significant interaction between the concentration of glucose and time of reaction

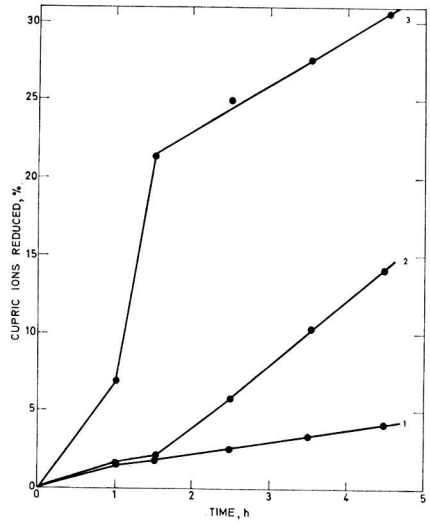

FIG. 1. *Effect of glucose on the cupric ion content of the biuret reagent with time*
1, 25; 2, 50; 3, 125 mg glucose/25 ml biuret reagent

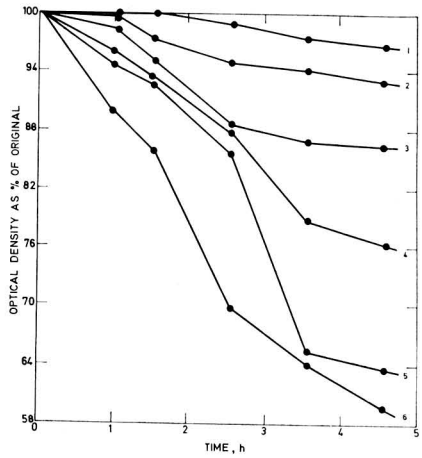

FIG. 2. *Effect of glucose on the optical density of the serum albumin biuret complex*
1, 12·5; 2, 25; 3, 50; 4, 75; 5, 100; 6, 125 mg glucose/25 ml biuret reagent

($P < 0{\cdot}001$). These results indicate the importance of strict standardisation of the time of reaction when the materials being analysed contain free reducing sugars.

The amount of free glucose, i.e. 25 mg reducing sugar/25 ml biuret reagent (which is equivalent to 5% apparent glucose in flour, since the concentration of flour/biuret reagent is 0·5 g flour/25 ml reagent), that causes interference with the biuret determination of protein when left for a total reaction period of 1½ hours would not be encountered even in flours of wheats harvested under adverse conditions. The range of maltose test values (1–5%)[6] for flours milled from wheats grown in Ireland is equivalent to a range of apparent glucose 0·55–2·75%.

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PESTICIDE RESIDUES IN FOODSTUFFS IN GREAT BRITAIN. V*.—Malathion in imported cereals**

By E. G. HILL and R. H. THOMPSON

Results are presented for the determination of malathion in Australian wheat, barley and oats and in Argentine wheat, maize and sorghum sampled on arrival in British ports. In Australian wheat the figures were mostly well below 8 ppm and the few instances exceeding this level are discussed. In Argentine wheat the maximum found was 6·2 ppm and most samples contained less than 2 ppm. Brief reference is made to the presence of other insecticides in Argentine wheat. The effect of treatment with insecticide on the freedom from infestation of the grain on arrival in Britain is discussed.

Introduction

With the increasing insistence in recent years by importing countries on food shipments being free from insect infestation, widespread use is being made of fumigants and contact insecticides in the countries of origin of the foodstuffs. One substance which is used extensively for the protection of grain against insect attack is the organophosphorus compound malathion (*S*-[1,2-di(ethoxycarbonyl)ethyl] dimethyl phosphorothiolothionate). The low mammalian toxicity of this compound permits it to be mixed with grain at a rate high enough to be effective over a prolonged period against a wide range of insect pests which attack stored products and much of the wheat exported to the United Kingdom from Australia and from the Argentine is treated in this way before shipment. This paper describes the results of tests that have been made to determine the malathion content of the grain on arrival at British ports. The work was done on behalf of the Panel on Residues of Pesticides in Foodstuffs, whose origin and terms of reference have been reported by Lee.[1]

Malathion in Australian wheat

The use of malathion in Australia for the treatment of wheat exported to the United Kingdom commenced in the latter half of 1961 and two shipments were sampled on arrival in the United Kingdom in November and December of that year. The insecticide had been applied to the grain in the form of an emulsion spray a week or two before shipment at a rate designed to give about 8 ppm on the grain. Samples taken from various depths in several holds contained between 1·1 and 6·8 ppm with a mean of 3·5 ppm. Following this preliminary test, more extended sampling was arranged with the co-operation of the Australian Federal and State authorities who identified the ships carrying malathion-treated wheat and gave details of the treatment given. At that time this usually consisted in spraying the grain on arrival at the port silo using an 80% premium grade emulsion concentrate diluted 1 part to 55 parts of water and applied at 1½ pints per ton (840 ml per 1000 kg) on the conveyor band just before the grain passed into the storage bins. The grain remained there a week or two and was transferred to the shipping bins shortly before the arrival of the ship for loading.

Sampling arrangements and method of analysis

The samples were taken by Insect Inspectors of the Ministry of Agriculture, Fisheries and Food and of the Department of Agriculture and Fisheries for Scotland, who regularly inspect food cargoes on arrival at British ports.[2] They were asked to take 1 lb samples, one for each 500 tons of wheat, during the discharging of the holds, the samples to be representative of the bulk, not skimmed from the surface when the hold was first opened up or taken from ledges. The samples were sent in polythene bags to the Ministry's laboratory in Liverpool for analysis, accompanied by separate samples in sealed tubes for determination of moisture content. The latter was done by an oven method, exposing the ground-up samples to a temperature of 120° for 4 hours. Each sample for malathion determination was transferred to a stoppered glass bottle and tumbled with a measured amount of analytical grade carbon tetrachloride for one hour. Samples which could not be extracted immediately were kept in a refrigerator before extraction. Tests with English and Canadian wheat to which known amounts of malathion had been added showed that this method of extraction gave 85% recovery of malathion. The figures in the tables given below have not been corrected for this recovery factor.

Aliquots of the extracts were examined by the method recommended by the Malathion Panel set up jointly by Government, Industry and the Society for Analytical Chemistry.[3] By this method, the malathion after extraction with carbon tetrachloride is decomposed to form sodium dimethyl phosphorothiolothionate which in turn is converted to the cupric salt. The cupric salt is extracted into carbon tetrachloride in which it gives an intense yellow colour. The colour absorption measurements were made on a Unicam SP 600 spectrophotometer using 4 cm cells. The standard curve was obtained from solutions of commercially pure malathion in carbon tetrachloride and corrected for 95% purity. Over the range 30–250 μg the graph was a straight line of slope 2·85 optical density units per mg of malathion.

Seven shipments were sampled on arrival in the United Kingdom between October 1962 and April 1963 and a further

* Part IV: J. Sci. Fd Agric., 1967, **18**, 579

three between February and May 1964. The results are shown in Table I.

In eight of the ten shipments sampled the maximum malathion content found was 7·8 ppm and the mean less than 5 ppm but in the other two, both loaded at Newcastle, New South Wales, much higher figures were obtained reaching a maximum of 17·7 ppm in one ship and 13·1 ppm in the other with mean values of 10·9 and 10·4 ppm respectively. These high figures might be explained by the fact that over the period in question heavy crops were harvested in New South Wales, which strained normal storage and collecting arrangements and resulted in some grain being treated with malathion on the farms as well as at the collecting centres. Further samples of the shipment showing the highest figures were taken after the grain had been discharged into two granaries, only one of which was equipped with a dust extraction system. Where no dust was separated from the grain, samples taken from just below the surface in a bin showed malathion contents ranging from 12·7 to 16·1 ppm (mean 14·3 ppm). In the other granary, where the dust was removed, the residual malathion in the grain samples lay between 4·0 and 6·2 ppm (mean 4·8 ppm).

The moisture content of the grain from New South Wales averaged just over 11% and of that from Western Australia, less than 10%. At these levels the rate of breakdown of malathion in grain is slow and little change would be expected during the voyage from Australia to Britain. The malathion content of the earlier consignments of grain at the time of shipment was not reported so no comparison with that on arrival in Britain is possible but for the shipments from Western Australia received in early 1964 the figures found in Britain tended to be slightly higher than those obtained in Australia.

Routine examination of Australian wheat

Following the surveys reported above, arrangements were made to sample as many shipments as possible of Australian wheat arriving in the United Kingdom. By this time the practice in Australia had largely changed to one in which the insecticide was applied immediately the wheat was received at the country storages, an additional amount sometimes being applied when the wheat eventually arrived at the port silos. The samples were taken by the Insect Inspectors as before but fewer samples were taken from each ship. From December 1964 up to the end of June 1967, 162 samples from 51 ships were examined. The results are given in Table II. Moisture content was not determined in every case and the figures are not included in the table but those tests that were made gave a mean of 10·2% for grain from New South Wales and 11·5% for that from South Australia.

Grain exported from ports in New South Wales in 1965 almost invariably had a higher malathion content than that from other States but from only two shipments were figures significantly higher than 8 ppm obtained. From one of these two shipments the samples analysed included a proportion of dust, which might be expected to contain any malathion rubbed off the surface of the wheat berries during handling and this could account for the finding of higher levels. (A sample from another shipment containing much dust and chaff had been found to contain as much as 104 ppm of malathion.) On the other hand it was reported that two samples analysed in Australia showed more than usual, namely 14·6 and 10·6 ppm, and the grain may have received an extra treatment. The other shipment with abnormally high residues was sampled again after discharge from the ship into dockside mill silos and also in the mill after conditioning and blending. The details are given in Table III. The ship had loaded part of its cargo in New South Wales and part in Victoria and it was the former that carried the high residues, in particular that loaded in the tween deck holds. Samples representative of the whole of the New South Wales cargo in this ship analysed in Australia by two independent laboratories showed 5 ppm and 7·2 ppm. High figures were also found in the 'Sydney' samples taken from the mill bins (range 7·4–13·7, mean 10·6 ppm). It was surprising that the 'Geelong' samples from the bins at this port (range 3·5–5·8, mean 4·7 ppm) were considerably higher than those taken from the ship at other ports of call (range 1·2–2·5, mean 1·9 ppm). A sample of the grain, exclusively from this ship, taken after the washing and drying of the mill's normal conditioning process showed 4·4 ppm. That this is not lower lends support to the conclusion of Acton & Parouchais[4] that malathion penetrates through the bran to

TABLE I

Malathion content of Australian wheat shipments on arrival in Britain
Experimental survey

Port of loading in Australia	Date of sampling in Britain	No. of samples	Malathion content in ppm range	mean	Mean moisture content %
Fremantle	Oct. 1962	12	2·2–4·9	3·8	8·4
Newcastle	Oct. 1962 / Nov. 1962	14	4·6–17·7	10·9	11·0
Newcastle	Nov. 1962	2	2·9–6·5	4·7	11·5
Newcastle	Dec. 1962	10	6·1–13·5	10·4	11·2
Geraldton & Fremantle	Jan. 1963	19	2·2–7·8	4·2	8·6
Fremantle	Jan. 1963	12	3·0–3·7	3·3	9·2
Albany	April 1963	7	0·3–2·1	1·3	10·8
Geraldton & Fremantle	Feb. 1964	12	0·9–4·3	2·9*	10·0
Fremantle	March 1964	27	1·7–6·5	3·5**	9·2
Fremantle	May 1964	15	3·6–6·2	4·8***	9·3

* Figures for malathion content on departure from Australia were 3·7 ppm on the 9,023 tons loaded at Geraldton and 1·7 ppm on the 4,777 tons loaded at Fremantle, equivalent to 3·0 ppm on the cargo as a whole
** Figure on departure from Australia 1·7 ppm
*** Figure on departure from Australia 3·8 ppm

TABLE II

Malathion content of Australian wheat shipments on arrival in Britain
Routine sampling

Port of loading in Australia	Date of sampling in Britain	No. of samples	Malathion content ppm	mean value
Sydney	Dec. 1964	1	8·1	8·1
Sydney	Dec. 1964	2	7·5, 8·3	7·9
Sydney	Jan. 1965	2	8·1, 8·5	8·3
Newcastle & Sydney	Jan. 1965	2	3·0, 5·8	4·4
Port Lincoln	Feb. 1965	2	1·9, 2·2	2·1
Thevenard	March 1965	3	Nil, 0·6, 5·3	2·0
Thevenard	March 1965	6	0·4, 0·4, 0·5, 0·6 1·8, 1·8	0·9
Ardrossan	April 1965	3	0·9, 0·9, 1·0	0·9
Wallaroo & Port Pirie	June 1965	3	Nil, 1·3, 1·4	0·9
Wallaroo	June 1965	3	0·9, 1·1, 1·8	1·3
Sydney	July 1965	2	10·0, 12·0	11·0
Sydney & Geelong	July 1965	15	1·2–34·6	(For details see Table III)
Port Pirie & Port Lincoln	July 1965	4	0·7, 0·7, 1·5, 1·8	1·2
Wallaroo & Port Adelaide	Aug. 1965	2	0·4, 0·6	0·5
Port Lincoln	Aug. 1965	1	0·3	0·3
Wallaroo & Port Adelaide	Sept. 1965	2	1·9, 2·3	2·1
Adelaide & Ardrossan	Oct. 1965	4	Nil, 0·5, 0·5, 0·7	0·4
Sydney	Nov. 1965	3	5·8, 6·4, 7·5	6·6
Sydney	Nov. 1965	2	5·9, 6·7	6·3*
Sydney	Nov. 1965	5	4·4, 6·0, 7·3 11·0, 26·7	11·1**
Sydney	Nov. 1965	10	Range 7·7–12·3	9·9
Fremantle	Dec. 1965	1	2·1	2·1
Geelong	Jan. 1966	2	1·6, 2·5	2·1
Thevenard	Jan. 1966	4	1·2, 1·2, 1·3, 3·3	1·8
Thevenard	Feb. 1966	6	Nil, 0·4, 1·8, 1·9, 1·9, 2·2	1·4
Fremantle	Feb. 1966	5	2·1, 2·2, 2·3, 2·3, 2·8	2·3
Port Lincoln	March 1966	3	0·9, 0·9, 1·5	1·1
Port Lincoln	March 1966	3	0·2, 0·5, 0·7	0·5
Port Lincoln	Apr. 1966	2	1·3, 1·7	1·5
Geraldton	May 1966	3	1·0, 1·1, 1·2	1·1
Geraldton & Fremantle	May 1966	6	2·3, 3·4, 3·4, 3·7, 4·1 4·3	3·5
Fremantle	June 1966	4	1·7, 2·0, 2·9, 4·0	2·7
Fremantle	July 1966	3	2·3, 2·5, 3·3	2·7
Fremantle	July 1966	1	1·5	1·5
Fremantle	July 1966	2	3·4, 3·4	3·4
Fremantle	Aug. 1966	3	2·6, 3·8, 4·4	3·6
Fremantle & Geraldton	Oct. 1966	3	2·9, 4·5, 5·0	4·1
Geraldton, Port Adelaide & Port Pirie	Dec. 1966	2	0·6, 0·8	0·7
Fremantle	Jan. 1967	2	2·0, 2·2	2·1***
Port Lincoln & Thevenard	Feb. 1967	3	0·4, 0·7, 1·0	0·7
Geraldton	Feb. 1967	1	2·3	2·3
Geraldton	Feb. 1967	1	5·9	5·9
Port Pirie	March 1967	5	1·5, 1·9, 2·0, 2·3, 3·0	2·1
Newcastle	April 1967	1	1·5	1·5
Newcastle	May 1967	8	3·7, 3·7, 4·3, 4·4, 4·5 4·7, 5·4, 6·0	4·6
Fremantle, transhipped at Amsterdam	May 1967	2	4·1, 4·9	4·5****
Fremantle, transhipped at Amsterdam	May 1967	1	8·3 66·4 (in sievings)	8·3*****
Newcastle	June 1967	2	1·7, 3·9	2·8
Fremantle, transhipped at Amsterdam	June 1967	1	2·4	2·4
Sydney, transhipped at Amsterdam	June 1967	4	0·7, 1·4, 1·9, 2·0	1·5
Fremantle, transhipped at Amsterdam	June 1967	1	4·1	4·1

* A sample of this shipment including much dust and chaff contained 104 ppm malathion

** The samples from this shipment were dusty. Samples taken in Australia also showed higher malathion contents than usual, however, namely 14·6 and 10·6 ppm on two samples and this is thought to be attributable to an extra treatment

*** This ship sailed from Fremantle in August 1966 and the grain was discharged into a dockside granary in London early in October. The samples were drawn from the granary 3½ months later. In addition to malathion they contained 7·2 and 8·6 ppm HCN

**** The inspector reported that the odour of malathion was quite strong when the grain was examined in the hold

***** The inspector commented that the grain seemed very dusty. A portion was sieved in the laboratory and the sievings tested separately

TABLE III

Malathion content of a cargo of wheat from New South Wales and Victoria, shipped in June 1965

Port of Loading	Sampling Point in ship or mill	Malathion content ppm
Sydney	1 Tween deck	9·9, 23·4
	3 Tween deck	14·9, 34·6
	4 Lower hold	8·4, 8·4, 5·0, 4·0, 3·7, 6·1, 8·9
Geelong	2 Lower hold	2·5, 2·2, 1·2, 1·5
Sydney	Wheat bins of flour mill	13·7, 11·7, 7·4, 9·7
Geelong	Wheat bins of flour mill	5·6, 5·8, 3·8, 3·5
	Screen room of mill after conditioning	4·4
	Grist containing 25% of wheat:	
	after first break	2·5
	after several breaks	1·9

the endosperm during the storage period following the spray treatment. Two samples taken at different stages during the milling of a grist containing 25% of wheat from this shipment showed 2·5 and 1·9 ppm, but as the malathion content, if any, of the remainder of the grist was not known, no conclusions can be drawn from these results.

In 1966 and the first half of 1967 no high figures were recorded. One sample from a very dusty consignment contained 8·3 ppm and dust sieved from a portion of this sample had 66·4 ppm but no other sample tested had more than 6 ppm. The dusty consignment, which was loaded in Western Australia, had been transhipped in Amsterdam for the last stage of its journey and this extra handling may have had some influence on the amount and distribution of the dust. It is noteworthy that another transhipment of the same original cargo also showed figures somewhat higher than usual for grain from this source and the inspector noted a smell of malathion when examining the grain in the hold.

Malathion in other Australian cereals

Two shipments of oats and one of barley were sampled in 1967. From the first shipment of oats only one sample was examined and this contained 3·1 ppm of malathion. From the other shipment six samples of oats were tested, each made up of small amounts drawn from several places in the holds sampled. The malathion contents were 1·9, 2·8, 2·8, 3·0, 3·8 and 4·5 ppm, mean 3·1 ppm. A sample of grain dust from one of the holds was also examined and this contained 35·2 ppm of malathion.

The single sample of barley tested contained 4·9 ppm of malathion.

Malathion in Argentine wheat

A marked reduction in the level of infestation in wheat arriving from the Argentine had been noted over the period 1962–4, and in 1965 it was learnt from the Argentine National Grain Board that most of the Board's silos were equipped to spray grain with malathion emulsion. Arrangements were therefore made in May 1965 to sample wheat shipments from Argentina in a similar fashion to those from Australia. Up to the end of June 1967, 161 samples were examined from 55 ships. The results are shown in Table IV, from which it will be seen that the levels found were considerably lower than those found in Australian grain. The moisture content of the grain tended to be slightly higher than that from Australia but not sufficiently high to cause rapid decomposition of the malathion. The results would therefore suggest that some of the cargoes sampled had not been treated with malathion and those that had, had received less than 10 ppm or had been stored for some time between treatment and shipment.

Thirty-eight samples from eleven shipments were examined by gas–liquid chromatography for other insecticides. In nine of these shipments traces of gamma-BHC (0·1 ppm or less) were found, in two, traces of DDT and in one a trace of carbaryl (1-naphthyl *N*-methylcarbamate).

Malathion in other Argentine cereals

A few samples of other cereals have been examined and the malathion content of these is shown in Table V. Apart from one figure of over 11 ppm on maize, the results do not differ markedly from those for wheat.

Effect of insecticidal treatment on infestation of grain on arrival in the United Kingdom

At the Infestation Control Laboratory records are kept of the insects found by the Insect Inspectors on imported foodstuffs and it is possible from these records to examine the variation in infestation between one commodity and another, between one country and another or from one year to the next. Table VI shows the variation in the degree of infestation of Australian and of Argentine wheat over the past 14 years. In each case, not only has the proportion of cargoes found to be infested fallen off progressively over the last five years, but the extent of the infestation has decreased dramatically as well.

A variety of causes is responsible for these effects besides the use of pesticides; for example, greater attention to hygiene, better organisation of the collection and storage of the grain before shipment and the introduction of regulations requiring Government inspection of the grain and freedom from infestation in the ships' holds before loading wheat for export. The pesticidal treatments used include fumigation with methyl bromide, aluminium phosphide and calcium cyanide, as well as the use of malathion but the prophylactic use of the last named is the most widely adopted pesticidal procedure in the two countries concerned, and it is fair to attribute the improvement of recent years largely to this insecticide.

Conclusions

Up to the end of 1965 grain shipped from New South Wales usually contained more malathion than that from other states in Australia and the few samples showing more than 8 ppm were all from this source, some of the grain concerned being more dusty than usual. Since the beginning of 1966 only one sample has been found to contain more than 8 ppm, again from a very dusty cargo, and the association of dust and high malathion content seems well established. The figures for the last 18 months showed greater uniformity than the earlier ones, and there is now little variation in malathion content between grain from one state and grain from another, most samples containing between 2 and 5 ppm, well below the figure of 8 ppm adopted as the tolerance on grain in U.S.A. and suggested by the Codex Alimentarius Commission

for Raw Cereals in International Trade.

The Argentine grain contained less malathion on arrival in the U.K. than that from Australia, most samples showing less than 2 ppm, but few details are available of the method of application employed or the stage at which it was made. A few samples were examined by gas liquid chromatography for other pesticides, and in some of these traces were detected, usually of gamma-BHC, sometimes of DDT and in one instance of carbaryl. The results of examining cereals for these and other pesticide residues will be the subject of a separate communication.

The treatments given in the exporting countries have kept the insect infestation to a low level but have not, except in very few instances—which have been drawn to the attention of the authorities concerned—left malathion residues in the grain exceeding the widely accepted limit of 8 ppm.

TABLE IV

Malathion content of Argentine wheat shipments on arrival in Britain

Date of sampling	No. of samples	Malathion content ppm	mean	Mean moisture content %
Feb. 1965	1	Nil	Nil	11·6
Feb. 1965	4	Nil, Nil, 0·6, 0·8	0·4	12·1
Feb. 1965	2	Nil, 0·5	0·3	11·5
Feb. 1965	2	0·8, 1·0	0·9	
Mar. 1965	2	Nil, Nil	Nil	11·9
Mar. 1965	1	0·3	0·3	11·6
Mar. 1965	2	0·6, 2·3	1·5	12·2
Apr. 1965	1	Trace	—	12·2
Apr. 1965	2	Nil, 4·2	2·1	11·6
Apr. 1965	1	0·5	0·5	
Apr. 1965	4	Nil, Nil, 1·1, 5·0	1·5	12·3
Apr. 1965	1	Nil	Nil	
Apr. 1965	2	Nil, 1·5	0·8	12·4
Apr. 1965	2	Nil, Nil	Nil	12·3
May 1965	2	Nil, Nil	Nil	12·7
May 1965	5	0·2, 0·5, 0·6, 0·6, 1·2	0·6	11·8
May 1965	5	Nil, Nil, Nil, Nil, Nil	Nil	12·4
June 1965	1	0·7	0·7	12·3
June 1965	4	Nil, Nil, Nil, Nil	Nil	12·9
June 1965	11	Range 0·2–2·5	0·7	12·4
June 1965	6	Nil, Nil, Nil, Trace, Trace, Trace	—	12·4
June 1965	6	Nil, Nil, 1·0, 1·1, 1·1, 1·7	0·8	
July 1965	5	0·3, 0·4, 0·4, 0·6, 4·7	1·3	
Aug. 1965	4	Nil, 0·6, 0·7, 1·4	0·7	12·1
Aug. 1965	4	0·8, 0·9, 0·9, 4·5	1·8	12·6
Aug. 1965	2	0·9, 1·8	1·4	
Sept. 1965	2	1·4, 1·6	1·5	13·5
Sept. 1965	3	3·3, 4·0, 4·4	3·9	
Sept. 1965	7	Nil, Nil, 0·3, 0·5, 0·5, 1·0, 4·3	0·9	14·2
Dec. 1965	2	5·2, 6·2	5·7	
Dec. 1965	3	0·2, 0·4, 0·7	0·4	12·9
Dec. 1965	3	0·4, 0·5, 0·6	0·5	12·9
Jan. 1966	3	Nil, 0·4, 1·4	0·6	
Jan. 1966	2	0·1, 0·6	0·4	12·3
Feb. 1966	1	1·0	1·0	
Mar. 1966	6	Nil, Nil, 0·4, 0·4, 0·5, 0·9	0·4	12·9
Mar. 1966	7	0·2, 0·3, 0·3, 0·3, 0·3, 0·4, 0·9	0·4	13·3
May 1966	3	Nil, Nil, Nil	Nil	
May 1966	3	1·2, 1·4, 1·8	1·5	
June 1966	2	0·1, 1·0	0·6	
June 1966	1	Nil	Nil	
June 1966	1	2·4	2·4	
June 1966	2	0·9, 1·1	1·0	12·2
July 1966	3	0·5, 1·1, 2·6	1·4	
Aug. 1966	3	0·5, 1·1, 1·1	0·9	
Dec. 1966	1	0·3	0·3	
Jan. 1967	1	0·9	0·9	
Jan. 1967	1	0·9	0·9	
Feb. 1967	2	0·6, 1·1	0·9	
Feb. 1967	2	0·2, 0·3	0·3	
Mar. 1967	3	0·2, 0·2, 0·3	0·2	
Mar. 1967	3	0·1, 0·1, 0·2	0·1	
Mar. 1967	2	0·2, 0·3	0·3	
April 1967	2	0·3, 1·1	0·7	
May 1967	3	0·6, 0·7, 0·7	0·7	
June 1967	3	0·1, 0·2, 1·4	0·6	

TABLE V

Malathion content of some Argentine maize and sorghum shipments on arrival in Britain

Commodity	Date of sampling	No. of samples	Malathion content ppm	Malathion content mean	Mean moisture content %
Maize	March 1965	2	Nil, 0·3	0·2	
,,	June 1965	1	1·1	1·1	13·9
,,	Nov. 1965	2	Nil, 1·0	0·5	
,,	Sept. 1966	3	1·0, 3·0, 11·7	5·2	14·5
,,	Feb. 1967	1	0·4	0·4	
,,	May 1967	1	1·0	1·0	
Sorghum	May 1965	2	2·6, 5·0	3·8	12·4
,,	June 1966	1	1·7	1·7	
,,	June 1966	2	1·5, 4·6	3·1	
,,	Dec. 1966	2	2·0, 2·0	2·0	

TABLE VI

Infestation of wheat cargoes from Australia and from Argentina on inspection in United Kingdom ports

Year	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966
Australia														
No. of cargoes inspected	48	25	49	66	94	30	48	68	69	54	79	68	90	59
% found to be infested	85	88	96	95	88	77	73	82	73	31	23	21	7	10
Infestation score	41	34	47	45	32	23	27	32	24	8	6	5	2	3
Argentina														
No. of cargoes inspected	37	66	52	47	89	91	71	63	38	85	34	37	78	33
% found to be infested	92	95	94	89	84	92	92	90	84	59	47	41	23	33
Infestation score	55	53	54	49	36	45	45	49	32	22	13	11	8	9

The infestation score takes account of the degree of infestation and is obtained according to the formula (25L + 50M + 100H)/N, where L, M & H are the number of cargoes, lightly, moderately and heavily infested respectively and N is the total number of cargoes inspected. The definitions of light, moderate and heavy are those of Freeman[5]

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CHEMICAL CHANGES AND LOSSES DURING THE ENSILAGE OF WILTED GRASS

By P. McDONALD, A. R. HENDERSON and A. W. MacGREGOR

Two experiments were carried out; in the first, wilted Italian ryegrass at two different dry matter (DM) levels (34% and 47%) was ensiled; in the second, fresh grass (15·9% DM) and similar herbage wilted to 30·3% DM were ensiled. Total edible DM losses from the wilted silages were low and ranged from 6·7 to 10·4%. Changes in individual sugars and organic acids were followed. The residual amounts of sugars in the wilted silages were directly related to the degree of wilting. All silages were well preserved, but little fermentation had occurred in the material wilted to 47% DM. From a knowledge of the sugars lost and amounts of mannitol and ethanol formed it has been possible to examine quantitatively the main chemical changes during the ensilage of the wilted materials. The results confirm the efficiency with which wilted grass is anaerobically conserved.

Introduction

The advantages of wilting crops, before ensiling them, to a dry matter (DM) content of 30% or above have been stressed by many workers.[1-5] Apart from reducing or eliminating effluent losses, the reduction in moisture content discourages clostridial activity and produces a silage which is more acceptable to ruminant animals.[6] It has been shown that these 'high DM' silages have higher pH values and contain more sugar than silages from unwilted herbage. Studies of losses during ensilage of wilted grass have given variable results although most workers agree that, provided the silo is adequately sealed, DM losses are low.

In spite of the well established advantages of wilting, there is still a lack of information about the detailed reactions which take place during the fermentation of such crops, and the purpose of this investigation was to carry out a comprehensive study of the chemical changes which occur during ensilage of partly wilted grass, and herbage wilted to a high DM content.

Experimental

Two separate experiments were carried out. In the first, wilted herbage, at two different DM levels (34% and 47%) was ensiled. In the second experiment, fresh grass (15·9% DM) and similar herbage wilted to 30·3% DM were examined.

Experiment 1

The silo unit used consisted of 4 metal silos,[8] each having a maximum capacity of approximately 1,000 kg fresh herbage. The silos were suspended from a weighing apparatus which was sufficiently sensitive to record a change in weight in the silo and contents of 0·1 kg. Conditions of filling were similar to those described in previous publications.[8,9] The ensiled herbage was covered with polythene sheeting and consolidated with stone blocks corresponding to a surface pressure of 37 g/cm^2. Assessment of true losses and surface waste measurements were made using a bag and marker technique already described,[9] and temperature changes were measured by means of thermocouples.

Italian ryegrass (*Lolium multiflorum*) obtained from one of the School farms was cut with a mower/crimper at 11 a.m. on 31 May, 1965 and wilted for 29 hours in the field before being lifted with a flail-type forage harvester and ensiled in silos A and B. During this period the grass was tedded 3 times. Similar material was tedded twice during a further 23 hour wilting period before being lifted with a forage harvester and ensiled in silos C and D. The weather was fine throughout the 52 h wilting period.

A total of 788 kg of wilted herbage was ensiled in each silo. The DM contents were 34·0% and 47·0% for the grasses wilted for 29 and 52 h respectively. The silos were opened 57 days after being filled. Weights (kg) of silage removed were: A, 779; B, 776; C, 778; D, 777. In order to obtain a measure of losses during wilting in the field, six areas per treatment, each 6 × 5 ft were weighed and sampled before and after wilting on 'terylene' netting placed at random in different parts of the field. During this period, field mechanical treatments were simulated by hand on each of the nets.

Laboratory silos

In addition to filling the 4 large silos, similar herbage was used to fill tube silos (32 mm × 200 mm) of 70 g capacity for detailed chemical studies in the laboratory. Prior to filling the grass was hand chopped to about 1 cm lengths; the material was well consolidated in the tubes which were fitted with sterile bungs carrying mercury valves. These tubes, which were kept at room temperature (20°) in the dark, were opened and the contents were examined after 30 h, 14 days and 58 days.

Analytical and digestibility techniques

Individual sugars were determined using paper chromatographic techniques, using ethyl acetate : pyridine : water (10 : 4 : 3), and ethyl acetate : acetic acid : formic acid : water (18 : 3 : 1 : 4) solvent systems, and ethanol by the method of Kent-Jones & Taylor.[10] Organic acids were determined by column chromatography,[11,12] and acetoin,

diacetyl and 2,3-butanediol by standard methods.[13] A toluene distillation procedure was used for estimation of dry matter.[14]

Mannitol was isolated by paper chromatography using the above solvent systems and converted by meta periodate oxidation to formic acid according to the method of Hirst & Jones;[15] the formic acid was then determined by the procedure of Kolthoff & Belcher.[16]

Digestibility trials were carried out in triplicate on grass and in duplicate on the four silages using Cheviot wether sheep. During these trials, the animals were fed to appetite. In all cases daily silage residues were never less than 15% of the ration consumed.

Experiment 2

In the second experiment carried out in May 1966, an attempt was made to reduce oxidative losses to a minimum by sealing the silos with heavy gauge polythene. In this case, consolidation weights were not applied and the plastic top was bonded with adhesive to the outer flange of the silos. Only two silos were used, the first (silo A) was filled with 1,045 kg of fresh Italian ryegrass cut with a flail-type forage harvester; the DM content of this herbage was 15·9%. The second (silo B) was filled with 1,045 kg of similar material wilted in the field for 52 hours (DM 30·3%). During the period of wilting, weather conditions were poor and showers fell at intermittent intervals. Thermocouples and marker sheets were not used in this experiment. The silos were opened after 141 days and weights of silage removed were A, 882 kg, and B, 1,034 kg.

Effluent (total 153 kg) was obtained only from silo A and this was collected daily or when it appeared, and stored at —18° until subsequently analysed. Field losses and digestibility measurements were not made in this experiment, but a number of laboratory tube silos were filled with fresh and wilted herbage and these were opened at various intervals of time during the period of the experiment.

Results

Experiment 1

Temperature changes

The temperatures in the four silos remained relatively low throughout the period of ensilage. Maximum values, which occurred on the 10th day, in the surface layers were: A, 23°; B, 21°; C, 24°; and D, 28°. This corresponded to a high ambient air temperature (20°) on the previous day.

Composition

The composition of the wilted grasses and silages is shown in Table I. The main changes which occurred during ensilage affected the nitrogen and soluble carbohydrate fractions. With regard to the former, considerable proteolysis had occurred, although the very small amounts of volatile N indicated that little de-amination had taken place. About 50% of the original water soluble carbohydrates (WSC) were recovered in the 34% DM silages whereas in the higher DM (47%) silages about 75% of the WSC in silage C and 95% in silage D were recovered. These recovery figures are, however, slightly misleading when based on total WSC figures because of the presence of pentoses which had been released from hemicelluloses during ensilage. Complete hydrolysis of fructosans and sucrose had taken place and this breakdown is reflected in the higher fructose values which were obtained for the silages.

A detailed organic acid analysis showed that the only volatile fatty acids present in the silages were acetic and propionic acids. Butyric, isobutyric, isovaleric and caproic acids, indicators of clostridial activity, were not detected in any of the silage samples.

Losses

The DM losses during wilting in the field, calculated from the 'terylene' net results and recorded to the nearest 0·1 kg, averaged 1·4% for the 29 h wilted grass and 4·0% for the 52 h wilted material. These findings are in agreement with the general conclusions of other workers.[17]

DM losses during ensilage have been calculated in two ways; the gross or 'edible' loss which includes the weight of waste, and the 'loss from the silo' which excludes the weight of waste and consists solely of gaseous loss. The 'losses from the silo' were extremely small, averaging 2·8% for silos A and B and 1·6% for silos C and D. The individual results are given in Table II. The weights of waste materials expressed in kg DM were: A, 13·0; B, 18·0; C, 14·5; and D, 20·5. In order to avoid complications resulting from the production of this waste material in the surface layers, the losses of DM and total hexoses and the weights of the two alcohols, mannitol and ethanol, were determined in herbage and silage below the marker sheets; these had been placed in the silos when they were three-quarters full of herbage. The figures are given in Table III.

Digestibility and intake studies

The results of the feeding trials with sheep are given in Table IV. There was little difference in digestibility between the grasses and silages although the 52 h wilted materials tended to be of slightly lower digestibility than those wilted for 29 h. These differences were also reflected in the intake figures obtained for the grasses.

Laboratory silos

Laboratory silos were opened after 30 h, 14 days and 58 days and analysed for soluble carbohydrates and organic acids. Results are shown in Table V. In addition to the two wilted grasses, the fresh herbage (DM 20·3%) was also ensiled. Rate of fermentation, assessed on the basis of sugar breakdown and acid formation, was most rapid in the fresh material. In the 52 h wilted material, very little change occurred after 14 days apart from some hemicellulose hydrolysis and some increase in acetic and lactic acids. The results indicate the facility with which fructosans and sucrose are hydrolysed. After 30 h, free fructose had increased in all silages, but whereas the fructose concentration was maintained in the wilted silages, the content of this sugar decreased in the fresh material during the ensiling period. The residual sugars in the silages after 58 days did not differ markedly from those found in similar material obtained from the large silos. The lactic acid content of the 29 h wilted silage was, however, noticeably higher than that obtained in silage from the large silos.

Experiment 2

Large silos

The method of sealing appeared to be successful in the case of silo B where no waste material was observed although

TABLE I

Composition of grass and silages

(% of true dry matter)

	Experiment 1						Experiment 2				
	Grass		Silages				Grass		Silages		Effluent
	AB	CD	A	B	C	D	A	B	A	B	A
Dry matter	33·98	46·99	34·07	33·64	46·94	47·55	15·90	30·34	15·91	28·35	7·00
Organic matter	91·7	91·2	91·4	91·5	90·8	91·0	90·7	90·9	89·8	89·9	84·4
Crude protein	11·8	12·4	12·1	12·6	13·0	12·8	18·1	18·2	18·0	18·4	26·6
Ether extract	2·0	2·1	3·1	3·0	3·0	2·9	2·7	2·5	3·1	3·5	—
Crude fibre	24·6	24·5	25·6	25·8	26·1	26·0	19·2	20·2	22·0	22·0	—
Total N	1·89	1·98	1·94	2·02	2·08	2·04	2·90	2·91	2·89	2·95	4·25
Protein N	1·50	1·51	0·72	0·76	1·03	0·79	2·39	2·26	0·92	0·99	—
Non-protein N	0·39	0·47	1·22	1·26	1·15	1·14	0·51	0·65	1·97	1·93	—
Volatile N	0·02	0·02	0·12	0·12	0·09	0·08	0·01	0·03	0·20	0·19	—
Water-soluble carbohydrates	21·4	21·1	10·6	11·7	16·4	20·3	23·8	24·8	1·7	9·3	17·5
Glucose	4·4	4·2	2·5	2·4	4·5	5·3	*8·8	*8·2	nil	2·2	2·8
Fructose	4·2	4·2	6·3	6·4	9·1	11·6	*15·0	*16·6	nil	5·3	5·3
Xylose	nil	nil	0·4	0·5	0·4	0·5	nil	—	nil	0·6	nil
Galactose	nil	nil	0·7	0·6	0·5	0·6	—	—	nil	0·6	0·14
Sucrose	3·2	2·0	nil	nil	nil	nil	—	—	nil	nil	nil
Fructosans	9·1	8·7	nil	nil	nil	nil	—	—	0·9	0·6	4·3
Mannitol	nil	nil	7·9	7·4	3·9	3·2	nil	nil	5·6	6·8	10·9
Cellulose	27·1	26·8	28·0	28·7	28·1	28·1	21·9	22·8	24·7	24·4	—
Lignin	4·2	4·2	4·3	4·4	4·6	4·8	2·8	3·3	4·1	4·3	—
Formic acid	nil	nil	nil	nil	nil	nil	nil	nil	0·05	nil	nil
Acetic acid	nil	nil	2·13	2·06	1·15	0·68	nil	nil	3·62	2·10	6·26
Propionic acid	nil	nil	0·15	0·15	0·11	0·10	nil	nil	0·13	nil	nil
Butyric acid	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil
Lactic acid	nil	nil	5·45	5·40	1·71	0·93	nil	nil	12·06	5·52	26·55
Succinic acid	nil	nil	0·18	0·19	0·13	0·08	tr.	nil	nil	nil	nil
Citric acid	0·53	0·51	nil	nil	nil	nil	0·65	0·77	nil	nil	—
Malic acid	1·15	1·18	nil	nil	nil	nil	1·75	1·71	nil	nil	—
Fumaric acid	nil	nil	nil	nil	nil	nil	0·19	0·09	hil	nil	—
Malonic acid	nil	nil	nil	nil	nil	nil	0·13	0·13	nil	nil	—
Ethanol	—	—	0·45	0·35	0·35	0·16	—	—	1·02	0·33	2·36
Acetoin	—	—	nil	nil	nil	nil	—	—	nil	nil	—
Diacetyl	—	—	nil	nil	nil	nil	—	—	nil	nil	—
2,3-butanediol	—	—	nil	nil	nil	nil	—	—	tr.	tr.	—
pH	5·88	6·09	4·16	4·12	4·88	4·90	6·10	6·10	3·67	4·18	3·91

* After hydrolysis of water-soluble carbohydrates

TABLE II

Percentage losses during ensilage

	Experiment 1				Experiment 2		
	A	B	C	D	A		B
					Total	Effluent	
Total 'edible loss' (including weight of waste) Dry matter	6·8	10·4	7·1	6·7	18·2	6·4	7·5
Loss from the silo (excluding weight of waste)							
Dry matter	1·9	3·7	2·1	1·1	16·0	6·4	7·5
Total N	+0·5	+2·7	+2·7	3·5	15·6	9·3	6·4
Water-soluble carbohydrates	51·4	47·4	23·7	4·8	93·9	4·8	66·4

the relatively high gaseous loss of DM (7·5%) suggested that some oxidation had occurred. In A, 28·4 kg of waste was produced, which indicated that some air had gained entry after filling. The composition of the fresh and wilted grasses and silages is shown in Table I. The WSC content of the original herbage was high (23·8%) and this was not markedly affected by wilting. In spite of this initially high level of sugars in the fresh crop, virtually all had disappeared during ensilage compared with a residual amount of 9·3% in the wilted silage. The lactic, acetic acid and pH values of the 28% DM silage were almost identical to the values obtained for the 34% DM silage in the first experiment, whereas the lactic acid content of the fresh silage was markedly higher (12·1%). Total 'edible loss' of DM during ensilage of the unwilted grass (18·2%) was more than double that from the wilted material (7·5%) and, of this relatively high loss, 6·4% was attributable to the effluent. The latter contained large amounts of WSC (17·5% in the DM) and lactic acid (26·6%).

Laboratory silos

In this experiment, mannitol was determined in addition to individual sugars, as is shown in Table VI. The total sugars in the samples of herbage taken from the tube silos

TABLE III

Recoveries of some constituents during ensilage in Experiment 1
(weights in kg based on material below marker sheet)

	A			B			C			D		
	In	Out	Loss	In	Out	Loss	In	Out	Loss	In	Out	Loss
Dry matter	250·0	249·7	0·3	243·5	240·0	3·5	349·4	348·6	0·8	342·7	345·6	+2·9
*Glucose	15·3	6·2	9·0	14·8	5·8	9·0	18·4	15·7	2·7	18·0	18·3	+0·3
†Fructose	39·8	15·7	24·1	39·0	15·4	23·6	51·8	31·7	20·1	50·8	40·1	10·7
Mannitol	—	19·7	—	—	17·8	—	—	13·6	—	—	11·1	—
Ethanol	—	1·1	—	—	0·8	—	—	1·2	—	—	0·6	—

* Including glucose present in sucrose
† Including fructose present in sucrose and fructosans

TABLE IV

Percentage digestibility (D), percentage of digestible nutrients (DN) and energy values of true dry matter (Experiment 1)

	Grass				Silages							
	AB		CD		A		B		C		D	
	D	DN	D	DN	D	DN	D	DN	D	DN	D	DN
Organic matter												
1	75·3	69·1	72·9	66·5	77·3	70·6	75·1	68·7	75·8	68·8	74·3	67·7
2	75·2	68·9	72·6	66·3	75·6	69·1	75·5	69·1	74·8	67·9	74·8	68·1
3	75·8	69·4	72·4	66·1	—	—	—	—	—	—	—	—
Mean	75·4	69·1	72·7	66·3	76·4	69·8	75·3	68·9	75·3	68·4	74·6	67·9
Crude protein												
1	65·7	7·8	60·9	7·5	67·4	8·2	62·8	7·9	65·5	8·50	63·6	7·7
2	66·7	7·9	59·0	7·3	65·8	8·0	66·5	8·4	60·8	7·90	63·8	7·7
3	67·6	8·0	59·2	7·3	—	—	—	—	—	—	—	—
Mean	66·7	7·9	59·7	7·4	66·6	8·1	64·6	8·2	63·2	8·2	63·7	7·7
Metabolisable energy (kcal/g)												
Mean	—	2·50	—	2·40	—	2·59	—	2·54	—	2·52	—	2·50
Starch equivalent												
Mean	—	62·4	—	59·5	—	64·0	—	62·7	—	62·1	—	61·5

Intakes (g dry matter/kg $W^{0.73}$)

	Grass		Silages			
	AB	CD	A	B	C	D
1	86·1	58·1	76·4	74·7	77·0	71·4
2	70·7	66·8	74·5	65·7	71·6	62·3
3	75·7	66·6	—	—	—	—
Mean	77·5	63·8	75·4	70·2	74·3	66·9

TABLE V

Changes in carbohydrates and organic acids in laboratory silos (Experiment 1)
(all figures given as % dry matter)

	Fresh grass (20·3% dry matter)				Wilted grass (34·7% dry matter)				Wilted grass (48·5% dry matter)			
	Original material	30h	14 days	58 days	Original material	30h	14 days	58 days	Original material	30h	14 days	58 days
Carbohydrates												
Glucose	5·7	5·5	3·2	2·3	4·5	4·8	3·8	2·6	4·2	5·0	4·9	4·2
Fructose	4·4	4·7	2·6	0·5	4·2	5·1	6·3	5·8	4·2	6·4	7·2	8·3
Sucrose	6·7	2·1	nil	nil	3·2	1·7	nil	nil	2·0	tr.	nil	nil
*Fructosans	7·1	3·5	tr.	nil	6·8	3·1	tr.	nil	5·2	2·2	tr.	nil
Xylose	nil	nil	tr.	tr.	nil	nil	tr.	0·6	nil	nil	tr.	0·5
Galactose	nil	nil	tr.	tr.	nil	nil	tr.	0·5	nil	nil	tr.	0·5
Acids												
Butyric	nil	nil	1·70	1·71	nil	nil	nil	0·63	nil	nil	nil	0·1
Propionic	nil	tr.	0·73	nil	nil	nil	nil	nil	nil	nil	nil	nil
Acetic	nil	0·71	0·83	1·17	nil	0·85	2·02	1·35	nil	tr.	0·32	1·17
Formic	nil	nil	0·10	nil	nil	nil	tr.	0·09	nil	nil	nil	nil
Succinic	nil	0·79	0·92	0·99	nil	0·21	0·11	0·14	nil	nil	nil	nil
Lactic	nil	4·15	11·16	13·86	nil	3·50	7·80	10·90	nil	0·30	1·73	3·72
Malic	1·17	nil	nil	nil	1·15	nil	nil	nil	1·18	0·30	nil	nil
Citric	0·53	nil	nil	nil	0·53	nil	nil	nil	0·51	tr.	nil	nil
pH	5·9	5·1	4·2	4·0	5·9	5·1	4·3	4·1	6·1	5·8	4·8	4·8

* Excluding oligosaccharides

TABLE VI

Changes in carbohydrates and organic acids in laboratory silos (Experiment 2)
(all figures given as % dry matter)

	Fresh grass (15·5% dry matter)					Wilted grass (30·5% dry matter)				
	Original material	24h	48h	7 days	36 days	Original material	24h	48h	7 days	36 days
Carbohydrates										
Glucose	7·0	5·2	3·9	1·1	1·1	6·3	5·7	5·7	3·5	2·9
Fructose	5·0	6·3	7·8	6·6	4·6	7·7	7·7	7·7	5·5	5·2
Sucrose	6·7	4·3	nil	nil	nil	5·4	1·2	0·3	nil	nil
*Oligosaccharides	2·9	3·0	3·3	1·2	1·2	1·9	1·6	0·9	0·9	0·8
Fructosans	6·5	5·7	5·2	4·5	3·9	5·9	5·6	5·4	3·3	2·4
Mannitol	nil	nil	2·2	5·6	6·3	nil	tr.	tr.	6·8	7·0
Xylose	nil	nil	nil	tr.	tr.	nil	nil	nil	nil	0·3
Galactose	nil	0·2	0·6	1·3	0·9	nil	tr.	tr.	tr.	0·4
Acids										
Butyric	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil
Propionic	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil
Acetic	tr.	0·11	0·22	0·83	2·21	nil	0·11	0·40	1·40	2·06
Formic	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil
Succinic	tr.	0·45	0·45	0·31	nil	nil	nil	nil	nil	nil
Lactic	nil	tr.	1·69	8·44	11·62	nil	tr.	1·00	2·78	6·91
Malic	1·75	0·80	nil	nil	nil	1·71	1·16	1·01	nil	nil
Citric	0·65	tr.	nil	nil	nil	0·77	0·50	0·48	nil	nil
Fumaric	0·19	nil	nil	nil	nil	0·09	nil	nil	nil	nil
Malonic	0·13	2·03	1·61	1·43	tr.	0·13	0·54	0·52	0·48	0·38
Glycollic	nil	nil	nil	nil	nil	0·14	nil	nil	nil	nil
pH	6·10	6·40	5·12	4·11	4·06	6·10	6·60	6·40	4·91	4·37

* Excluding sucrose, but including short-chain fructosans

were higher than those determined for the materials ensiled in the large silos. Mannitol was detected in measurable quantities after 48 h, and during this period of time sucrose had completely disappeared from the unwilted herbage. Galactose was found after 24 hours and this was presumed to have been formed from hemicellulase activity. In both silages lactic acid was present in only trace amounts after 24 h but after 48 h it was present in measurable amounts.

Discussion

It is well established that the extent of fermentation during ensilage is influenced by the DM content of the crop,[1,5,6,17] and this conclusion is confirmed in these studies. In unwilted silages, there is very little residual soluble carbohydrate and the composition of the wet silage A in the second experiment is typical of such material, in spite of an initial high WSC content (23·8%) in the DM. On the other hand the residual soluble carbohydrates in the wilted silages were directly related to the DM content of the ensiled material. Unfortunately the significance of this to the animal in terms of productive performance was not adequately measured and requires further investigation with regard to such factors as ruminal volatile fatty acid pattern and utilisation of metabolisable energy (ME). There is some evidence, however, from previous studies that the presence of soluble sugars in silage may result in a more efficient utilisation of the nitrogenous components.

The major nutrients fermented during ensilage of grass by lactic acid bacteria are glucose and fructose, and probably malic and citric acids. The fermentation products resulting from the breakdown of the major hexoses in grass vary depending upon whether the organisms responsible are of the homolactic or heterolactic type. The various pathways (excepting No. 5) have been reviewed in a recent publication[18] and these are summarised in Table VII. It can be seen that in the case of the homolactic organisms, lactic acid alone is normally formed, whereas with the heterolactic bacteria, mannitol is a major product of fructose fermentation and ethanol of glucose fermentation. Since accurate weights of herbage ensiled and silage removed were recorded, it has been possible to calculate the actual amounts of fructose and glucose which have disappeared during ensilage. This has been done in Experiment 1 where, because of the marker and bag technique used, it has been possible to calculate the losses of nutrients below the marker sheet, thereby eliminating any errors caused by oxidation in the surface layers. In this exercise the amounts of mannitol and ethanol formed were used as a basis for the calculations involving pathways 3 and 4 given in Table VII. It became clear from the results obtained that in order to account for the relatively large amount of mannitol formed, the quantity of fructose required was greatly in excess of that actually available, and an alternative pathway was indicated. Concomitant studies in the laboratory with isotopically (^{14}C) labelled hexoses indicated that a reaction involving a fermentation of glucose coupled with a reduction of fructose was possible according to pathway No. 5 in Table VII.[19] This pathway has been used in the calculations which are summarised in Table VIII giving the weights of lactic and acetic acids, together with carbon dioxide production. Also shown in the Table are the calculated weights of acids and CO_2 derived from the fermentation of citrate and malate according to the reactions outlined in Table VII. It is known that there are alternative pathways[18] for the dissimilation of these two acids, but as the end products include acetoin, diacetyl, 2–3, butanediol, and formic acid, and as none of these products was detected in the silages in Experiment 1 it has been concluded that the pathways shown in Table VII were the most probable.

In these calculations the activities of the plant enzymes have not been taken into account. In the case of the sugars, it is unlikely that any marked respiration would have occurred since the quantity of oxygen trapped in the mass has been calculated to be extremely small and would not account for a hexose loss of greater than 0·5 kg. This can be compared with the total hexose loss of 33 kg in silo A for example.

The presence of small amounts of xylose and galactose in the silages indicated that some hemicellulose breakdown had taken place. Previous studies[20] with unwilted herbage have shown that hemicelluloses disappear during ensilage to the extent of 11–55%, and that individual losses of polymers were, preferentially, araban > galactan > xylan. These studies also showed that pentoses were produced by both the action of plant hemicelluloses and acid hydrolysis. Both homo- and hetero-lactic bacteria ferment pentose according to the equation: 1 pentose → 1 lactic acid + 1 acetic acid.

It is perhaps relevant to note here that in silos A and B in Experiment 1, rather more lactic and acetic acids were produced than could be accounted for by the reactions given in Table VII. This indicates another source of these acids, which could have been free pentoses.

In the case of silos C and D of Experiment 1, the reverse is true for lactic acid where the calculated values were rather higher than the amounts actually found. The activities of bacteria in these dry silages were restricted, particularly in silo D, but it is possible that some yeast growth took place resulting in ethanol formation from hexoses. In wet herbage bacteria are more active than yeasts which are normally present in relatively small numbers, but on the other hand it has been established that in the ensilage of moist barley with DM contents as low as 60%, yeast organisms make the

TABLE VII

Fermentation pathways used in calculations

1. 1 Glucose ——→2 Lactic acid
2. 1 Fructose——→2 Lactic acid
3. 1 Glucose ——→1 Lactic acid + 1 Ethanol + 1 Carbon dioxide
4. 3 Fructose——→1 Lactic acid + 2 Mannitol + 1 Acetic acid + 1 Carbon dioxide
5. 2 Fructose + 1 Glucose——→1 Lactic acid + 2 Mannitol + 1 Acetic acid + 1 Carbon dioxide
6. 2 Citric acid——→1 Lactic acid + 3 Acetic acid + 3 Carbon dioxide
7. 1 Malic acid——→1 Lactic acid + 1 Carbon dioxide

TABLE VIII

Weights (kg) of sugars and fermentation products calculated from pathways in Table VII
(weights based on dry matter values given in Table III)

	Pathway	Glucose	Fructose	Lactic acid	Acetic acid	Ethanol	Mannitol	CO_2
Silo A								
	1	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—
	3	4·24	—	2·12	—	1·12	—	1·04
	4	—	14·96	2·49	1·16	—	10·08	1·22
	5	4·77	9·53	2·39	1·59	—	9·64	1·17
	6	—	—	0·31	0·62	—	—	0·46
	7	—	—	1·93	—	—	—	0·94
	Total	9·01	24·49	9·24	3·37	1·12	19·72	4·83
	Found	9·01	24·02	13·61	5·32	1·12	19·72	—
Silo B								
	1	—	—	—	—	—	—	—
	2	—	3·04	3·04	—	—	—	—
	3	3·28	—	1·64	—	0·84	—	0·80
	4	—	8·99	1·50	1·00	—	6·06	0·73
	5	5·79	11·57	2·90	1·93	—	11·70	1·42
	6	—	—	0·29	0·58	—	—	0·43
	7	—	—	1·93	—	—	—	0·94
	Total	9·07	23·60	11·30	3·51	0·84	17·76	4·32
	Found	9·07	23·60	12·96	4·94	0·84	17·76	—
Silo C								
	1	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—
	3	4·77	—	2·39	—	1·22	—	1·17
	4	—	20·17	3·36	2·24	—	13·59	1·64
	5	—	—	—	—	—	—	—
	6	—	—	0·42	0·83	—	—	0·61
	7	—	—	2·77	—	—	—	1·35
	Total	4·77	20·17	8·94	3·07	1·22	13·59	4·77
	Found	2·66	20·07	5·96	4·01	1·22	13·59	—
Silo D								
	1	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—
	3	2·15	—	1·08	—	0·55	—	0·53
	4	—	16·41	2·73	1·82	—	11·06	1·34
	5	—	—	—	—	—	—	—
	6	—	—	0·41	0·82	—	—	0·60
	7	—	—	2·71	—	—	—	1·33
	Total	2·15	16·41	6·93	2·64	0·55	11·06	3·80
	Found	+0·33	10·70	3·21	2·35	0·55	11·06	—

major contribution to fermentation (Whittenbury, R., unpublished results).

Although malic and citric acids in ryegrass are present in relatively small amounts compared with soluble carbohydrates, they nevertheless can make an important contribution to the lactic acid content of the silage. Malate can be degraded to a small extent by the action of plant enzymes, with the production of succinate, but it has been shown that the main breakdown of this acid is bacterial.[21] Succinate, although absent from the original grass, was found in small amounts in the silages in Experiment 1. It is difficult to conclude, however, whether the presence of the acid was at the expense of malate.

Carbon dioxide production in Experiment 1 has also been calculated from the reactions given in Table VII. Expressed as a percentage of DM ensiled (below the marker sheet), CO_2 production was: A, 1·9%; B, 1·8%; C, 1·4%; D, 1·1%. The actual DM losses during the ensilage of material below the marker sheet were: A, 0·1%; B, 1·4%; C, 0·2%; D, 0·8%. Since these figures are so low, it may be unwise to attach significance to the differences although the possibility of CO_2 fixation can be considered. That this can take place during ensilage has recently been shown in studies in this laboratory[22] using (^{14}C) labelled CO_2 where the main end-products of fixation were lactic and succinic acids.

In the second experiment, marker sheets were not used, since it was hoped to achieve completely air-tight conditions by sealing the silos with plastic. This, however, was not entirely successful, and consequently it is difficult to evaluate the fermentation changes adequately. Silage B was very similar in composition to the silages made from the 29 h wilted herbage in Experiment 1 and this suggests that a similar type of fermentation had occurred. The situation in silo A is complicated by the production of effluent, which contained high levels of both lactic acid and WSC. A further complication is indicated by the presence of formic acid and

2,3-butanediol which could have been formed from malate by phosphoroclastic cleavage. The presence of propionic acid in silage A, as well as in the silages of the first experiment, is worthy of comment. This acid could have resulted from either the coupled oxidation–reduction reaction (Stickland) of amino acids, de-amination of alanine, or the action of propionibacteria on sugars or lactate, reactions which all result in the formation of acetic acid.

In conclusion the results of these experiments show that the ensilage of wilted ryegrass under anaerobic conditions results in very little loss of nutrients. As stated previously, where high losses occur during ensilage of wilted grass, these losses arise from oxidation and not from fermentation. These results also suggest that there is little, if any, advantage to be gained in terms of reduced DM loss by wilting crops to DM contents of greater than 30–34%. Indeed, the higher DM losses in the field associated with prolonged wilting, may result in an higher overall loss. Whether the decreased extent of fermentation, with consequent saving in sugars, is of value to the animal, remains to be proven.

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MICROBIOLOGICAL BREAKDOWN OF CELLULOSE IN THE PRESENCE OF ALTERNATIVE CARBON SOURCES

By A. F. BRAVERY

Inhibition of cellulolytic activity by small quantities of two common ingredients of synthetic growth media, DL-asparagine and yeast extract, is demonstrated in a cellulose–agar medium. A modified medium is proposed which gives improved results when used for the detection of cellulase production.

Introduction

During assay of microfungi for cellulolytic activity and wood-destroying capability,[1] it was noted that some strains of fungi which were able to cause soft-rot of solid birch wood did not give cellulase reactions after 14 days' incubation on the medium proposed by Eggins & Pugh.[2] Further work was therefore undertaken to resolve this anomaly.

Experimental

In order to confirm the anomaly, the unidentified strains of microfungi noted by Greaves & Savory[1] (F.P.R.L. Culture No. S.896, S.897, S.898) were again inoculated on to the medium of Eggins & Pugh[2] (to be called E & P Medium), but the incubation period was extended to 12 weeks.

As a second test, the three isolates were inoculated according to the method of Savory *et al.*[3] on to each of a series of seven media modified from that proposed by Eggins & Pugh, i.e. Medium I in Table I. Loss of nitrogen through reduction or removal of DL-asparagine (as in Media IV, V, VI and VII) was compensated by proportionately increasing amounts of ammonium sulphate. Loss of vitamins through reduction or removal of yeast extract (as in Media III, IV, VI and VII) was partly compensated by addition of thiamine hydrochloride.

The hemicellulose preparation substituted in Medium II for the cellulose in E & P Medium, was extracted from birch holocellulose with potassium hydroxide–borate solution and contained a mixture of xylan and glucomannan. Culture plates incubated at 25°, were observed daily for the first 3 weeks and thereafter at weekly intervals.

Results

In the preliminary test, continued slow growth of the isolates was observed throughout the 12 week test period. At the end of this time, cellulolytic activity, as shown by the clearance zones around the inocula, was detected.

In the second test cellulolytic activities were recorded after 3, 7 and 14 days (Table II). Representative culture plates for isolate S.897 after 14 days incubation are shown in Fig. 1.

Discussion

The results of the first test after 2 weeks' incubation confirmed the anomaly observed by Greaves & Savory,[1] but the appearance of clearance zones after 11 to 12 weeks of incubation indicated the eventual production of cellulase. Possible reasons for this are: that the production of the requisite

TABLE I

Constituents of modified Eggins & Pugh media

	Constituents of media, g/l						
	I	II	III	IV	V	VI	VII
KH_2PO_4	1·0	1·0	1·0	1·0	1·0	1·0	1·0
KCl	0·5	0·5	0·5	0·5	0·5	0·5	0·5
$MgSO_4 \cdot 7H_2O$	0·2	0·2	0·2	0·2	0·2	0·2	0·2
$CaCl_2$	0·1	0·1	0·1	0·1	0·1	0·1	0·1
Agar	20·0	20·0	20·0	20·0	20·0	20·0	20·0
Cellulose	10·0	0	10·0	10·0	10·0	10·0	10·0
Hemicellulose	0	10·0	0	0	0	0	0
DL-asparagine	0·5	0·5	0·5	0·25	0	0	0
Yeast extract	0·5	0·5	0	0	0·5	0·25	0
$(NH_4)_2SO_4$	0·5	0·5	0·5	0·521	0·543	0·543	0·543
Thiamine hydrochloride	0	0	0·001	0·001	0	0	0·001

TABLE II

Cellulolytic activity on each medium

Isolate	Incubation time, days	Cellulolytic activity* on each medium						
		I	II	III	IV	V	VI	VII
S.896	3	—	—	—	—	—	?	+
	7	—	—	—	?	+	++	+++
	14	—	—	+	++	+++	+++	+++
S.897	3	—	—	—	—	—	—	+
	7	—	—	—	—	+	++	+++
	14	—	—	?	++	+++	+++	+++
S.898	3	—	—	—	—	—	?	+
	7	—	—	—	—	+	++	+++
	14	—	—	—	?	++	+++	+++

* +++ strong, ++ moderate, + weak, ? indefinite, — nil
N.B. Results for hemicellulose Medium II were still negative after 6 weeks

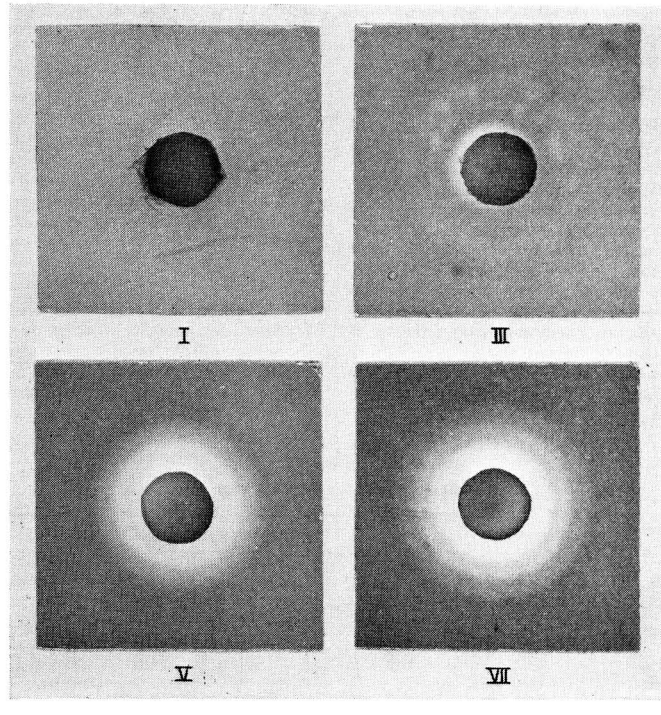

FIG. 1. *Central portions of upper surfaces of plates after 14 days incubation at 25°C*
Illuminated from below to show the clearance zone as a halo around the dark central inoculum

adaptive enzyme system by these particular isolates takes much longer than in the other isolates tested; or that the small quantities of alternative carbon sources present in the medium (DL-asparagine, yeast extract and hemicellulose) are more readily available and are adequate to sustain metabolism for 11 to 12 weeks.

Greaves & Savory[1] and Savory *et al.*[3] regarded the cellulose in the E & P Medium as 'the sole carbon source'. In fact the medium includes DL-asparagine (an amino acid containing approximately 32% carbon by weight), Difco yeast extract (a mixture of carbohydrates, amino acids and fats containing approximately 30% carbon by weight), and very small quantities of hemicellulose (approximately 2% as impurity in the cellulose).

Results obtained with Media I and II show that in the presence of DL-asparagine and yeast extract, enzyme activity is not detected more rapidly when hemicellulose is used as the major carbon source. In the absence of these alternative carbon sources, attack of cellulose itself was so rapid that any earlier utilisation of hemicellulose would have been relatively insignificant.

Comparison of the results with Medium I and Media III–VII shows that omission of DL-asparagine had a more marked effect than omission of yeast extract, but the most positive and rapid cellulase reactions are obtained when both are omitted but suitably compensated for. Since the carbon content of the two constituents is approximately equal, it is most likely that this is a qualitative effect. Presumably, because of the more complex nature of yeast extract, the availability of the carbon is variable and, while some is probably more readily available than that in DL-asparagine, a larger proportion is not.

That the presence of certain freely available carbon sources may inhibit utilisation of artificially prepared cellulose is already well established.[4–6] However, instances have also been recorded in which the presence of such sources leads to stimulation of cellulolytic activity *in vitro*, [4–6] and the same has been shown in experiments involving the ligno-cellulose of solid wood.[7,8] The significance of these observations and those reported in the present paper is that ancilliary carbon-containing growth substances which are frequently incorporated in synthetic media may affect enzyme activity on the primary substrate.

Conclusions

Destruction of cellulose by cellulolytic fungi may be inhibited by the presence of DL-asparagine and yeast extract acting as alternative carbon sources. For optimum detection and assay of cellulase using an agar culture technique, such carbon-containing growth stimulants should be omitted from the medium, and suitable compensations should be made.

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VARIATION IN CHEMICAL COMPOSITION OF APPLE LEAVES

By D. A. HOLLAND

A study was made of the variation in percentage N, P, K, Ca, and Mg in leaf dry matter, as observed in a three-year survey of forty commercial apple orchards. The contributions due to variation between years, orchards, trees, samples, and laboratory determinations were estimated, and the theory of random errors was found to apply. In general, results were not greatly affected by the methods of analysis employed by different laboratories, and 'haphazard'—as distinct from truly random—selection of sample trees was found to be an adequate practical procedure.

Introduction

Many authors have described the procedures they have used for taking samples of apple leaves for chemical analysis in their particular investigations. Much of this work has been summarised by Chapman[1] and by Bould.[2] The best sampling procedure, however, must depend on the purpose for which the sample is taken, i.e. to obtain a representative value for an orchard which will be generally applicable in any year, or a general value which was representative of a particular variety or rootstock, or a representative value for a particular tree in a particular year, when different sampling procedures would be needed.

In order to derive the best procedure for a given purpose it is first necessary to know the relative magnitudes of the various operative sources of variation. Despite the frequent use made of leaf analyses—whether for the purpose of elucidating treatment responses in an experiment or for providing a basis for advising on manurial requirements—studies of variation, as such, have been largely neglected.

Bould *et al.*[3] have considered the variance components associated with various schemes for sub-sampling a bulk sample and have concluded that a satisfactory compromise between speed and accuracy is attained by making duplicate determinations on a single finely ground sub-sample of the dried and crushed sample which should consist of 60 leaves. This work, however, did not consider the question of how the initial sample should be constituted.

Factors affecting variation between leaves within a tree have been studied by Moon & Hymas[4] who found significant differences in mineral composition between leaves on different main branches and at different heights on the tree.

More recently a study[5] of the results of a survey[6] of apple orchards in the south-eastern counties of England led to the estimation of the variance components corresponding to variation between seasons, orchards, trees within an orchard, and the seasonal inconsistencies of both trees and orchards. From such results it is possible to ascertain the degree of precision to be associated with an observation according to whether it was taken to represent a particular orchard in a particular season, the same orchard in any season, any orchard in a particular season, or any orchard in any season (i.e. a general value for a variety or rootstock for example). Conversely it is possible to determine the sampling procedure most appropriate for a particular purpose. It was not possible, however, to assess the variation within a tree or the errors of sub-sampling and laboratory determinations, as was done by Bould *et al.*[3]

In 1962 the N.A.A.S. 'Closed' Conference of Advisory Soil Scientists, Horticultural Committee, initiated an investigation into the best procedures to be used for soil or leaf sampling in an apple orchard for advisory purposes.[7] It was first necessary to make a more general study of variation *per se* in order to determine those factors likely to affect variability and to identify and estimate the operative sources of variation. The results of such a study, in addition to leading to the published conclusions,[7] are equally applicable to many other specific questions relating to variability and sampling and will be the concern of this paper.

Experimental

Source of data

Data were derived from a survey of forty grassed-down orchards of Cox's Orange Pippin on M II rootstock which was carried out over the period 1962 to 1964. A full description of this survey has already been given.[7] From each of these orchards samples were taken as follows:

Series I: duplicate samples of 25 leaves from each of two trees in 1962, 1963 and 1964, the same two trees being sampled on each occasion.

Series II: one sample made up of one leaf from each of 50 trees in 1962.

Series III: duplicate samples each comprising four leaves from each of 25 trees, an independent selection of trees being made for each sample.

Samplers were instructed to select trees in a haphazard fashion after eliminating any that were obviously abnormal. It was subsequently thought that a subjective element might have entered into the selection of trees as a consequence of this. Two further series of samples designed to investigate this possibility were taken, therefore, in 1964. Thus:

Series Ia: as for Series I but with trees formally selected at random using a table of random numbers (after eliminating the obviously abnormal).

Series IIIa: as for Series III but again formally selected at random.

Samples were taken as near the last week in August as possible from the middle third of current extension growth within reach from the ground. As far as possible the sample

was distributed evenly over all aspects (N, S, E and W) of the tree.

Determinations of the concentration of nitrogen, phosphorus, potassium, calcium and magnesium (as percentage dry matter) were made on these samples, duplicate determinations being made in 1962, by micro-methods which have been described in detail elsewhere.[8] All sub-samples were taken after the initial samples had been dried and ground.

In addition the 1962 Series I samples were analysed (usually by macro-methods) by the appropriate N.A.A.S. Regional Laboratory.

Method of interpretation

An observation on the concentration of N, P, K, Ca or Mg in a sample of apple leaves may be subject to any of a number of sources of variation related to seasons, orchards, trees and seasonal inconsistencies between orchards and trees (i.e. interactions between seasons and orchards and between seasons and trees within an orchard). In addition such an observation will be subject to sampling variation as a result of differences between the leaves on a tree; it will also be subject to errors of sub-sampling at the laboratory stage and the technical and human errors associated with the actual determination, which may be conveniently regarded as laboratory variation.

The following variance components may therefore be postulated:

S_y^2 — between years
S_o^2 — between orchards
S_t^2 — between trees within an orchard
S_{yo}^2 — interaction of years with orchards
S_{yt}^2 — interaction of years with trees within an orchard
S_w^2 — between samples of 25 leaves from within a tree
also, S_l^2 — between sub-samples from a sample in the laboratory, i.e. errors of determination etc. which form part of S_w^2.

Each of these components can be estimated from the Series I data with the exception that S_l^2 can only be estimated where duplicate determinations were made, i.e. in 1962.

It does not necessarily follow that every one of these theoretically possible sources of variation will make an effective contribution to the variation of every element. The practical importance of each source can be assessed by testing whether or not the corresponding variance component is greater than zero. Where it is not, then that source of variation may be ignored and the analysis of variance revised accordingly before other components are estimated.

An estimate of S_l^2 can also be formed from the Series II data. Here, however, it will refer to sub-sampling and determinations from a 50-leaf sample instead of a 25-leaf sample. The comparison of the two estimates of S_l^2 will, therefore, provide a measure of the possible effect of initial sample size on subsequent errors and an indication of the degree of homogeneity achieved by grinding and mixing.

The possible effect of subjectivity in selecting trees in an orchard can be made by the comparison of S_t^2 as estimated from the 1964 Series I data and as estimated from the Series Ia data and also by the comparison of within-orchard variances as observed in the 1964 Series III data and in the Series IIIa data.

The Series III data provide, in each year, and estimate of the total variance to be associated with a specific sampling procedure (i.e. 4 leaves from each of 25 trees within an orchard). In terms of the above components such a variance can be expected to have the value. $(S_t^2/25 + S_{yt}^2/25 + S_w^2/4 + 3S_l^2/4)$ in a particular year and orchard. Comparison of the observed value with this expected value gives an empirical check on the applicability of the laws of sampling theory to this practical situation.

Results

Effective variance components

Using all three years' data from the Series I samples estimates were made of each of the postulated variance components (excluding S_l^2, the sub-sampling variance component), and the significance of these departures from zero was tested. Where a source of variation was not found to be significantly distinguishable the assumed model was accordingly modified and the analysis repeated. The resulting estimates are set out in Table I; it will be noticed that no source of variation can be consistently regarded as unimportant with respect to all elements.

Effect of sample size on laboratory variation

Estimates of S_1, the standard error of a laboratory determination (including laboratory sub-sampling variation) were made from the 1962 Series I samples of 25 leaves and the Series II 50-leaf samples. These estimates, expressed as percentages of the mean (i.e. coefficients of variation) are given in Table II. In addition to being generally low in value there did not appear to be any marked, or consistent, difference between the estimates derived from the different

TABLE I

Estimated variance components corresponding to distinguishable sources of variation

Source	N	P	K	Ca	Mg
Years (S_y^2)	0·0114	0·0003	0·0025	†	0·0006
Orchards (S_o)	0·0162	0·0019	0·0310	0·0199	0·0008
Trees (S_t^2)	†	0·0001	0·0115	0·0018	0·0002
Years × Orchards (S_{yo}^2)	0·0239	†	0·0155	0·0172	0·0005
Years × Trees (S_{yt}^2)	0·0116	0·0016	0·0092	0·0059	0·0002
Samples (S_w^2)	0·0173	0·0002	0·0052	0·0046	0·0002
Mean level (% DM)	2·702	0·280	1·492	0·999	0·207

† Not a significantly distinguishable source of variation

TABLE II

Coefficients of laboratory variation for different bulk-sample sizes

Bulk-sample	N	P	K	Ca	Mg
25 leaves	2·4	4·1	2·3	2·7	5·0
50 leaves	3·2	4·4	2·7	2·0	3·8
Pooled estimate	2·9	4·2	2·4	2·4	4·3

sizes of bulked sample. Pooled estimates of laboratory variation were therefore made and these values are also given in Table II.

Effect of subjectivity in the selection of trees

Estimates of tree-to-tree variation (S_t^2) derived from both the 1964 Series I samples (based on a 'haphazard' selection of trees) and the 1964 Series Ia samples (based on a formally random selection of trees) are given in Table III. Also shown are the within-orchard standard errors of a 25 leaf × 4 tree sample as observed in the 1964 Series III ('haphazard' selection) and Series IIIa (random selection) samples. It appears from these results that no serious element of subjectivity had resulted from selecting trees in a 'haphazard' fashion instead of by formal randomisation procedures.

Validity of theory of random errors

Using the values of S_t^2, S_{yt}^2 and S_w^2 given in Table I and a pooled estimate of S_1^2 derived from the 1962 Series I and Series II samples (corresponding to the coefficient of variation given in Table II) the expected values of the standard errors of a 25 leaf × 4 tree bulked sample have been calculated to be

$$\left(\frac{S_t^2}{25} + \frac{S_{yt}^2}{25} + \frac{S_w^2}{4} + \frac{3S_1^2}{4}\right)^{\frac{1}{2}}$$

These values, expressed as percentages of the mean, are given in Table IV together with the corresponding observed values (Series III samples) for 1963 and 1964. The agreement between observed and expected values is sufficient that the applicability of the theory of random errors to sampling problems may be accepted.

Comparison of analytical methods

Since the 1962 Series I samples were also analysed by four N.A.A.S. Regional Laboratories (referred to here as A, B, C and D) using somewhat different methods from those used in this study,[8] it is possible to make an assessment of the extent to which the results of leaf analyses might be affected by the method of analysis.

TABLE IV

Observed and expected coefficients of 'within-orchard' variation

	N	P	K	Ca	Mg
Expected	3·7	5·4	3·7	4·3	5·6
Observed 1963	7·2	4·2	4·1	5·5	5·3
Observed 1964	3·0	3·5	4·3	4·2	5·8

If the values (x) resulting at a central laboratory are compared with the corresponding values (y) resulting at a regional laboratory by fitting the linear regression

$$y = a + bx$$

then $a \neq 0$ in this regression will represent a constant bias and $b \neq 1$ will represent a proportional bias (relative to the central laboratory).

These regressions of y on x were found to be significant at $P < 0{\cdot}001$ for each element and each region. The values of 't' resulting from testing the departure of the b-values from 1 are given in Table V together with the values resulting from testing the departure of the a-values from 0 where b was not found to differ from 1, and those for testing the difference between regional and central laboratory means for all orchards when b was found to differ from 1. It will be seen that, while there was no evidence of constant bias between the central laboratory and any regional laboratory, there was evidence of proportional bias in five of the twenty regressions. The practical importance of these biases can be judged from Fig. 1. Only in the case of extremely high (P) or low (K and Mg) levels would it appear that the method of analysis was likely to affect the result seriously. Although statistically significant, the bias in Ca determinations would not appear to be of great practical importance.

Discussion

For a given dried and ground leaf sample the standard error of a single determination in the laboratory was found, for each element, to be of the order of 4% of the observed value (Table II). This is much the same as that reported by Bould *et al.*[3] and by Moon & Hymas.[4] It may therefore be assumed that, relative to other sources of variation (Table I), variation arising at the laboratory stage is of minor importance and that modification of laboratory procedures is unlikely to lead to a worthwhile increase in overall precision. It was for this reason that no attempt was made to assess this source of variation after the first year but to include it in the within-tree sampling variation (S_w^2) as shown in Table I.

TABLE III

Effect of method of selecting trees

	N	P	K	Ca	Mg
Tree-to-tree variation (S_t^2)					
Haphazard selection	0·0074	0·0006	0·0169	0·0091	0·0004
Random selection	0·0175	0·0010	0·0185	0·0098	0·0002
Within-orchard S.E.					
Haphazard selection	±0·080	±0·009	±0·063	±0·043	±0·012
Random selection	±0·068	±0·008	±0·045	±0·046	±0·009

TABLE V

Values of 't' resulting from testing the significance of biases between analytical methods

		N	P	K	Ca	Mg
Proportional bias						
Laboratory	A	<1·0	3·75***	†	†	†
,,	B	<1·0	1·43	<1·0	1·90	1·39
,,	C	1·88	1·25	2·80**	2·90**	<1·0
,,	D	<1·0	1·81	1·19	2·17*	5·66***
Constant bias						
Laboratory	A	<1·0	<1·0	†	†	†
,,	B	<1·0	<1·0	2·00	<1·0	<1·0
,,	C	<1·0	<1·0	<1·0	<1·0	<1·0
,,	D	<1·0	<1·0	<1·0	<1·0	<1·0

† no data from regional laboratory
* significant at $P < 0{\cdot}05$
** significant at $P < 0{\cdot}01$
*** significant at $P < 0{\cdot}001$

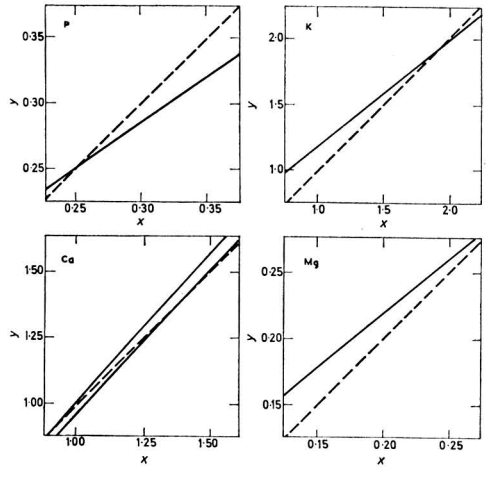

FIG. 1. *Values for leaf analysis of apples* (%DM) *assessed in regional laboratories* (y) *vs. those obtained at a central laboratory* (x) *where significant biases were found*
(a) P (b) K (c) Ca (d) Mg
— — — theoretical relation with no bias

Results given in Table I may be compared with the corresponding results[5] obtained from the earlier survey made in 1957–9. It must be remembered that in the earlier survey no assessment was made of within-tree variation, what was there referred to as tree-to-tree variation being equivalent to $(S_t^2 + S_s^2)$ in this paper. The two surveys also covered different periods in time, which were too short to give a really reliable estimate of random seasonal variation; it is not surprising therefore that there is a discrepancy between the two estimates. On the other hand, there is a close agreement between the two surveys with respect to the variances of a single sample from a random tree in a random orchard in a particular year $(S_o^2 + S_{yo}^2 + S_t^2 + S_{yt}^2 + S_w^2)$, and the proportions of this variance that can be ascribed to 'between orchards' and 'within orchards' $(S_o^2 + S_{yo}^2)$ and $(S_t^2 + S_{yt}^2 + S_w^2)$, respectively (see Table VI). The principal points of difference are greater variation in P and a greater emphasis on between-orchard variation in Ca in the 1962–4 survey. In comparing these results it should also be remembered that the samples were taken at different times within the season, mid-July and late August, respectively.

From the separate variance components presented in Table I it should be possible to predict the overall variances corresponding to any specific sampling procedure and purpose of sampling. It is conceivable, however, that certain physical factors could result in a discrepancy between these theoretical estimates and what is found in practice. For example, if drying and grinding did not result in a homogenous sample then sub-sampling errors could be affected by the size of the sample. This does not appear to have been the case, however, since the values of laboratory variation for 25- and 50-leaf samples did not appear to differ (see Table II). A further check for discrepancy between theory and practice is provided by Table IV. While it would appear that the predicted values slightly underestimate what will occur in practice, the discrepancy is small, and predictions based on the values given in Table I are not likely to be seriously in error.

As with any sampling scheme based on a theory of random errors a serious departure from expectation may result when a systematic element is allowed to enter into the sampling procedure. Unless some formal method of selection, e.g. the use of a table of random numbers, is employed, it is difficult to prevent this from happening. On the other hand it is desirable to avoid the use of such cumbersome practices in the field if possible. It would, for example, be an easy matter for samplers to select *typical* trees subconsciously, and if this were done the result would be to reduce tree-to-tree variation. From the results given in Table IV it would appear that any tendency towards subjectivity on the part of leaf samplers was negligible and that, with due care, it is possible to take a sample of leaves which is effectively random without resorting to the use of a table of random numbers.

TABLE VI

Comparison of 1957–9 and 1962–4 surveys

Element	Survey	Total variance	Percentage variance due to differences	
			between orchards	within orchards
Nitrogen	1957–9	0·0752	59	41
	1962–4	0·0690	58	42
Phosphorus	1957–9	0·0007	57	43
	1962–4	0·0038	50	50
Potassium	1957–9	0·0590	64	36
	1962–4	0·0724	64	36
Calcium	1957–9	0·0380	58	42
	1962–4	0·0494	75	25
Magnesium	1957–9	0·0023	69	31
	1962–4	0·0019	68	32

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VARIATION IN CHEMICAL COMPOSITION OF ORCHARD SOILS

By D. A. HOLLAND

Some factors likely to affect variation in pH, P, K, and Mg in soil samples from an apple orchard are examined using results from a survey of forty commercial apple orchards, and estimates are made of effective variance components. Variation within an orchard is found to comprise both systematic and random components, the latter being independent of the area sampled. Laboratory errors are found to be independent of sample size but to vary between different laboratories. All components are found to be equally applicable to samples from depths of both 0–6 in. and 6–12 in.

Introduction

Variation in the results of soil analyses arise from two main sources. Firstly there are those errors that arise in the field and are associated with the heterogeneity of the area sampled, and secondly there are those that arise at the laboratory stage after the sample has been taken, i.e. sub-sampling errors, differences between observers, technical errors of instruments, etc.

It is widely accepted[1–4] that field errors are of the greater practical importance, and a number of studies (reviewed by Cline,[2] Reed & Rigney,[4] Pritchett *et al.*,[5] Rigney,[6] and by Jacob & Klute,[7] who also give an account of the fundamental statistical principles involved) have been made of soil sampling errors.

From this work it is apparent that field sampling errors may be of considerable magnitude. Hemmingway[8], for example, found them to be too large to justify the practice of placing a soil into one of six categories according to its P or K content and advocated the use of only three categories for routine advisory purposes. Again, differences of up to 1·0 pH unit have been found between sample borings no more than one foot apart.[9–12]

With errors of this magnitude extensive sampling will be necessary to ensure a proper representation of the sampled area. Pritchett *et al.*[5] suggest 12 to 15 cores as being sufficient, Reed & Rigney[4] propose 30 cores for a 10% accuracy and Barker & Steyn[13] advocate samples of the order of 100 cores. It has also been pointed out that sample size may need to be varied according to purpose. Thus, Cline[2] suggests that while 20 cores may be sufficient for K determinations some 240 cores would be needed for the determination of Ca with similar accuracy and Hemmingway[8] proposes 24 cores for K, less for P and only 10–15 for pH determinations.

Davis[14] has advocated sampling at a rate of three cores per acre, thus implying that sampling errors increase with the area sampled; Youden & Mehlich[15] and Downes & Beckwith[9] have shown the variation of pH to increase with distance between samples. Other workers, however, have found to the contrary. Pritchett *et al.*[5] for example, found extreme values to be uniformly distributed over areas of up to eight acres, Hemmingway[8] also found sampling errors to be independent of the area sampled, and Hammond *et al.*[16] found the variance components of the third stage of a three-stage sampling scheme to be as great as those of the first stage. If, in fact, variation over small distances is as great as that over larger distances (i.e. due to fertility gradients, etc.) then there would be nothing to gain from taking a stratified random sample instead of a completely random sample.

While it may be that the laboratory variation is the less important source it does not follow that it is negligible. Little attention, however, has been paid to its reduction, nor has it been considered in relation to the size of the sample involved; sub-sampling errors could increase with sample size, for example, particularly following inadequate mixing of the sample.

Further, all of the studies cited above were made on arable or pasture soils, and very little attention has been paid to the conditions prevailing in a plantation of perennial trees, e.g. an orchard, where conditions may be very different. Apart from the work of Holland[17] who found the sampling error of pH in field trials with apples to be $\pm 0{\cdot}4$ units per plot, and that of Tinker[18] who found samples of the order of 60–100 cores necessary in an oil palm plantation, there appears to be little reference to the subject.

The most likely source of difference between an orchard soil and one carrying a succession of annual crops will be the limited amount of mechanical mixing taking place as a result of tillage, etc. In addition, the physical presence of the trees could induce systematic patterns of variation as a consequence of uneven fertiliser distribution between the rows and the alleyways and the effect of continual farm traffic along regular routes through the orchard, as suggested by Holland & Greenham.[19] Trees will also affect the procedure by which a soil sample is taken by presenting a physical barrier to the location of sampling positions. On the other hand they will provide a permanent grid of reference points whereby, for example, random positions can be selected by reference to row and tree numbers.

The study to be discussed in this paper was concerned with the identification and estimation of operative sources of variation, and the determination of those factors likely to affect them, in soil samples from an apple orchard. It was made as a necessary preliminary to an investigation of the best soil and leaf sampling procedures to be used when sampling an

apple orchard for advisory purposes. This investigation was instigated by the N.A.A.S. 'Closed' Conference of Soil Scientists, Horticultural Committee, and a full account of it has been published.[20]

Experimental

Source of data

Data for this study were collected in a survey[20] of forty grassed-down orchards of Cox's Orange Pippin on M II rootstocks distributed throughout four N.A.A.S. regions (designated A, B, C and D).

Soil samples from depths of 0–6 in. and 6–12 in. were taken in the winters 1962 and 1963 from each of these orchards, and analysed (for pH, P, K and Mg) at the appropriate N.A.A.S. regional laboratory. Three series of samples were taken, one in 1962 and two in 1963, as follows:

Series I. In each orchard duplicate three core samples were taken at positions half-way between each of two trees and their adjacent trees to the north and to the east. The duplicate samples were taken as close together as was practicably possible (i.e. about one foot apart) and the two trees in each orchard were selected at random from within an area that would normally be considered uniform by an experienced soil sampler. The average area involved was 1·3 acres. The eight samples (2 trees × 2 positions × 2 samples) from each depth in each orchard were kept separate, and duplicate determinations made on each.

Series II. Duplicate composite samples, each consisting of 25 cores taken from points mid-way between adjacent trees selected at random, but with the restriction that N–S alleys and E–W alleys contributed equally, were taken from the part of each orchard that was sampled previously. Duplicate determinations were made on each of the two composite samples per depth per orchard.

Series III. Samples were taken as in Series II but from an extended area of each orchard, though one which would still normally be taken as uniform for soil sampling purposes, up to a maximum area of 10 acres—the average was 4·5 acres. Again duplicate determinations were made on each sample.

These three series of samples were progressively designed to establish the important sources of variation and then to estimate their magnitudes. At the same time they were designed to assess the possible effects of gross sample size on subsequent variation at the laboratory level and of area sampled on variation at the field level.

Method of interpretation

The following possible sources of variation may be postulated:

S_o^2 = random field variation between orchards

S_t^2 = random field variation over distances corresponding to those between trees

S_c^2 = random field variation between three-core samples from the same sampling position

P^2 = systematic field variation corresponding to differences between sampling positions in the N–S and the E–W alleyways

S_l^2 = random laboratory variation between sub-samples.

By means of an analysis of variance on the Series I data it is possible to estimate each of the above components of random variation. At the same time the importance of the systematic element can be assessed by testing the mean difference between the two sides (north and east) of a tree—irrespective of which is the larger—for departure from zero.

From the Series II and III data it is possible to estimate S_f^2 appropriate to a 25 core sample (instead of a 3 core sample as in the case of Series I). Estimates of the separate components of field variation are not possible. Instead, however, these data lead to the estimation of a single field variation component, $S_f^2 = 1/25\,(S_t^2 + 3S_c^2 + P^2)$. Since the area sampled is unlikely to affect laboratory variation, the estimates of S_l^2 derived from each of Series I and Series II can be pooled to give a single more precise estimate. The two estimates of S_f^2, on the other hand, will refer to different areas sampled and their comparison will, therefore, indicate the extent to which field variation is related to the area sampled.

By comparing the values of S_l^2 derived from Series I with those derived from Series II and III it is possible to assess the effect of gross sample size on sub-sampling variation for gross samples of 3 and 25 cores.

Results

Preliminary examination of data

Since the samples from each region were analysed by the corresponding regional laboratory, data from each region were treated separately in the first instance. The resulting estimates of each postulated variance component for each region and each series of samples are given in Tables I, II and III, together with the mean value for all orchards in the region. Laboratory variation has not been estimated separately for Mg in one region since duplicate determinations were not made.

These results can be simplified and made more precise in many instances by combining regions and/or series to give a single pooled estimate based on a greater number of degrees of freedom.

Variation between orchards

It will be noted that variation between orchards (S_o^2) differed markedly from one region to another. However, it is the purpose of this study to estimate the general level of variation to be found between orchards wherever they might be. Further, orchard-to-orchard variation is a feature of the orchards sampled and not, therefore, affected by the method of sampling employed. Accordingly all regions and series have been combined to give single estimates of S_o^2 for each variate. The resulting pooled estimates, each based on 106 degrees of freedom, are shown in Table IV.

Systematic field variation

Expectations of the 'between-sides-of-a-tree-in-an-orchard' and the 'between-samples-from-the-same-side-of-a-tree' mean-squares in an analysis of variance on the Series I data are $(S_l^2 + 2S_c^2 + 4P^2)$ and $(S_l^2 + 2S_c^2)$, respectively. If pooled estimates over all regions are formed the significance of the mean |N side–E side| over all orchards can then be tested by an F-test based on 76 and 152 degrees of freedom. These mean values, and the significance of their departures from zero, are shown in Table V.

Random components of field variation

The total field variation per 3 core sample in the Series I data will be $(S_t^2 + S_c^2 + P^2)$. Series I data from all four

regions have again been pooled to give single estimates of S_t^2 and S_c^2, the two random components of field variation. In Table VI the resulting estimates are shown expressed as percentages of the total field variation as an indication of their relative importance.

Effect of area sampled on field variation

The total field variation per 25 core sample can be estimated from both the Series II and Series III data to give values corresponding to the original area sampled and the extended area, respectively. The results from all regions can again be pooled to give the single estimates shown in Table VII.

Effect of sample size on laboratory variation

The Series I data lead to the estimation of laboratory variation for samples of three cores while Series II and III lead to similar estimates appropriate to 25 core samples. Assuming that the area sampled will not affect laboratory variation, data from Series II and III can be pooled, as can data from the four regions, to give the estimates of S_l^2 shown in Table VIII.

TABLE I

Variance components from Series I data

	0–6 in.				6–12 in.			
	pH (units)	P (ppm)	K (ppm)	Mg (ppm)	pH (units)	P (ppm)	K (ppm)	Mg (ppm)
Region A S_0^2	0·932	82·2	3944·6	669·2	0·598	46·5	2875·2	447·0
S_t^2	0	7·3	0	173·1	0·036	22·6	740·0	337·2
S_c^2	0·022	7·0	1992·6	146·6	0·061	6·4	1326·7	102·0
S_F^2	0·057	5·6	198·6		0·035	11·0	90·2	
P^2	0·251	6·8	1911·5	224·8	0·127	10·8	349·7	69·3
Region B S_0^2	0·312	219·8	9818·3	84·7	0·364	115·1	5784·2	71·6
S_t^2	0	0	1359·7	0	0·002	2·5	518·9	15·9
S_c^2	0·015	15·7	2820·6	20·8	0·026	7·5	1041·2	13·1
S_l^2	0·006	8·0	1561·8	4·3	0·006	2·3	443·1	4·1
P^2	0·026	10·2	2991·8	23·6	0·025	4·2	2199·0	19·7
Region C S_0^2	0·426	384·7	6211·6	908·8	0·494	297·0	1758·0	914·6
S_t^2	0·259	49·5	0	486·4	0·140	248·4	0	319·9
S_c^2	0·020	6·2	693·2	107·1	0·022	17·0	159·8	35·6
S_l^2	0·003	1·3	28·7	24·3	0·002	1·7	8·9	20·5
P^2	0·035	3·9	2704·2	477·0	0·043	7·6	601·1	225·5
Region D S_0^2	0·499	300·6	15236·2	9610·3	0·560	422·7	10821·1	12408·7
S_t^2	0	28·4	0	0	0·043	0	0	0
S_c^2	0·055	43·5	1655·8	329·6	0·071	127·7	611·2	1191·3
S_l^2	0·002	1·1	17·1	48·1	0·007	1·1	14·9	46·4
P^2	0·254	40·9	2190·1	1152·8	0·069	0	1262·5	801·5
Mean level (all orchards)	6·50	19·5	227·2	132·4	6·73	15·8	164·9	118·6

TABLE II

Variance components from Series II data

	0–6 in.				6–12 in.			
	pH (units)	P (ppm)	K (ppm)	Mg (ppm)	pH (units)	P (ppm)	K (ppm)	Mg (ppm)
Region A S_0^2	0·745	34·1	4068·9	1586·0	0·519	53·1	3817·6	949·9
S_F^2	0·016	34·9	944·8	198·0	0·132	3·7	305·4	149·1
S_l^2	0·033	4·3	314·0		0·086	3·2	1179·0	
Region B S_0^2	0·365	371·2	2976·9	7396·2	0·240	190·3	3862·5	6975·4
S_F^2	0·006	0	220·0	31·5	0·025	1·5	105·0	120·8
S_l^2	0·004	6·3	132·5	29·7	0·008	7·0	130·0	68·8
Region C S_0^2	0·544	504·7	6853·2	1085·2	0·768	416·4	1509·7	760·3
S_F^2	0·020	2·8	357·4	76·7	0·029	9·9	140·6	29·6
S_l^2	0·002	0·9	28·1	15·3	0·003	0·6	7·5	19·8
Region D S_0^2	0·570	304·3	11394·4	7175·6	0·642	700·2	5969·0	8283·5
S_F^2	0·023	19·7	433·3	322·1	0·026	11·6	706·1	102·8
S_l^2	0·020	0·8	25·7	17·0	0·002	0·4	30·9	20·4
Mean level (all orchards)	6·24	20·6	224·7	134·5	6·48	15·9	152·3	116·7

TABLE III

Variance components from Series III data

	0–6 in.				6–12 in.			
	pH (units)	P (ppm)	K (ppm)	Mg (ppm)	pH (units)	P (ppm)	K (ppm)	Mg (ppm)
Region A S_0^2	0·718	39·9	18882·4	4390·8	0·460	44·9	11985·2	2044·5
S_2^2	0·080	19·9	230·0	} 88·1	0·104	45·7	0	} 120·8
S_1^2	0·031	5·5	327·4		0·043	3·3	260·3	
Region B S_0^2	0·238	319·5	3750·0	8094·6	0·244	181·1	3149·0	6716·4
S_2^2	0·008	11·4	40·0	50·8	0·006	3·4	182·5	81·7
S_1^2	0·006	0·9	160·0	25·8	0·008	2·4	122·5	85·6
Region C S_0^2	0·470	751·5	6378·7	1117·8	0·806	797·2	1516·9	676·1
S_2^2	0·003	15·1	255·0	4·2	0·023	24·7	46·2	7·3
S_1^2	0·003	1·2	26·2	9·8	0·004	0·8	9·3	8·2
Region D S_0^2	0·609	295·3	8046·6	5059·1	0·649	576·7	5492·6	6633·9
S_2^2	0·001	1·9	235·8	38·0	0·013	1·5	1082·1	101·1
S_1^2	0·005	0·3	29·7	25·0	0·003	1·6	33·4	15·9
Mean level (all orchards)	6·28	21·7	229·0	136·0	6·55	17·5	156·5	117·0

TABLE IV

'Between-orchards' variance components

Depth	pH (units)	P (ppm)	K (ppm)	Mg (ppm)
0–6 in.	0·561	268·1	7138·6	4169·4
6–12 in.	0·543	302·8	4537·3	5074·7

TABLE V

Mean differences between N-S alleyways and E-W alleyways

Depth	pH (units)	P (ppm)	K (ppm)	Mg (ppm)
0–6 in.	0·3***	3·4***	60***	20***
6–12 in.	0·2***	2·4	35***	20***

*** = significantly greater than zero at $P < 0·001$

TABLE VI

Components of random field variation as percentages of total field variation

Component	0–6 in.				6–12 in.			
	pH	P	K	Mg	pH	P	K	Mg
S_t^2	29	38	8	18	35	58	12	12
S_c^2	13	33	38	18	27	37	39	50

Laboratory variation at different laboratories

Although data from all regions have been pooled for the estimation of S_1^2 above it does not follow that this source of variation will be the same for all regional laboratories. Values of S_1^2 arising at each region are given in Table IX. These values resulted after pooling data from all three series on the assumption that sample size and area sampled had little or no effect on laboratory variation.

Discussion

Field variation

It would appear (Table V) that systematic patterns of variation, such as those which differentiate between the N–S and the E–W alleyways in an orchard, can make an important contribution to field variation. On average, differences between the two sides of a tree of the order of 15–20% were found in the orchards examined. This result could have been a consequence of very large differences in a minority of orchards; the fact that this occurred, and constituted a potential source of field variation, is sufficient to require that systematic variation be taken into account in the design of soil sampling procedures.

In so far as random components of field variation are concerned the evidence of Table VI supports Hammond's concept[16] of 'macro-uniformity and micro-heterogeneity' in that S_c^2 was, in general, as great as S_t^2, i.e. within that part of an orchard which would normally be regarded as uniform by an experienced soil sampler, variation was not typified by major trends over the area, with less variation occurring between nearby samples than between widely separated samples, but high and low values (of the variates examined) were distributed over the entire area sampled in an almost random fashion with little correlation between adjacent samples.

Random field variation can, therefore, be regarded as a single entity which together with systematic field variation accounts for the total variation encountered in the field. That similar values for this total field variation were found to be applicable to both the original and the extended areas of sampling (Table VII) further supports the concept of macro-uniformity and confirms the adequacy of a visual assessment of what constitutes a uniform area, the pH, P, K or Mg status of which might be described by a single value.

TABLE VII

Total field variation in original and extended areas (per 25 core sample)

Area	0–6 in.				6–12 in.			
	pH (units)	P (ppm)	K (ppm)	Mg (ppm)	pH (units)	P (ppm)	K (ppm)	Mg (ppm)
Original	0·016	14·0	476·0	149·2	0·050	6·9	324·3	85·0
Extended	0·021	11·6	190·3	31·2	0·034	17·7	354·8	64·6

TABLE VIII

Laboratory variation (all laboratories)

Sample	0–6 in.				6–12 in.			
	pH (units)	P (ppm)	K (ppm)	Mg (ppm)	pH (units)	P (ppm)	K (ppm)	Mg (ppm)
3 core	0·013	3·86	460·1	27·1	0·011	3·47	139·9	25·1
25 core	0·012	2·41	123·1	20·4	0·018	2·19	204·4	35·9

TABLE IX

Laboratory variation (for separate laboratories)

Laboratory	0–6 in.				6–12 in.			
	pH	P (ppm)	K (ppm)	Mg (ppm)	pH	P (ppm)	K (ppm)	Mg (ppm)
A	0·043	5·22	267·2	—	0·051	6·66	444·2	—
B	0·006	6·56	1090·0	16·7	0·007	3·14	337·0	28·4
C	0·003	1·27	28·3	20·4	0·003	1·21	8·7	18·4
D	0·006	0·96	20·7	39·1	0·006	1·11	20·7	37·0

Laboratory variation

If a soil sample is not completely homogenised before sub-sampling then a large sub-sample will be more representative of the whole than a proportionally smaller one. In consequence the variation between sub-samples will decrease as the sub-sample size is increased. If sub-sample size is constant then variation in sample size (i.e. the bulk of the initial sample) will have a similar effect in that a given size of sub-sample will be more representative of a small bulk than of a large bulk. In this study sub-sampling errors have been included with all subsequent sources of variation as laboratory variation. Table VIII shows the laboratory variation found when the initial sample consisted of three cores (Series I) or of 25 cores bulked together (Series II and III). It is apparent that initial bulk has not affected laboratory variation, from which it can be concluded that the procedures adopted for homogenising the sample before sub-sampling are adequate within the limits of sample size studied. This being so, it may be taken that both estimates of laboratory variation may be combined.

While it has been argued that any differences between regions in field variation are fortuitous and that a general value for the whole survey is the more appropriate value to consider, a similar argument cannot be applied to laboratory variation when different laboratories are concerned. Some laboratories produce a more precise determination than others (Table IX). Laboratories A and B gave considerably more variable determinations in respect of pH, P and K than laboratories C and D. In so far as Mg is concerned duplicate determinations were not made at laboratory A so no estimate of laboratory variation was possible while the other three laboratories all gave similar levels of laboratory variation. In general the values pertaining to laboratories C and D may be regarded as representing a degree of precision that might be expected, although such a standard might not be achieved by all laboratories.

Both field and laboratory errors are of a similar magnitude at depth of 0–6 in. and 6–12 in.

Estimates of the effective random components of variation are as follows:

	pH	P	K	Mg
S_0^2	0·552	285·450	5837·95	4622·05
S_f^2	0·030	12·540	336·30	82·50
S_1^2	0·004	1·135	19·60	26·60

where S_f^2 is the field variation of a 25 core sample distributed throughout the orchard with both N–S and E–W alleys contributing equally, and S_1^2 represents sub-sampling and

laboratory variation. It will be seen that the latter can be of minor importance relative to field variation except, possibly, in the case of Mg. At some laboratories, however, subsampling and laboratory errors may be considerably greater.

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BREAKDOWN OF ^{14}C-CHLORFENVINPHOS INSECTICIDE ON CROPS

By K. I. BEYNON and A. N. WRIGHT

The breakdown of chlorfenvinphos has been studied by application of ^{14}C-chlorfenvinphos, in the laboratory and in a glasshouse, to the foliage of potatoes, cabbage, and maize at initial dosage levels within the range 1–30 ppm. On cabbage there was evidence for a possible conversion of some of the *trans*(β) isomer of chlorfenvinphos to the *cis*(α) isomer. The initial half-life of chlorfenvinphos (*cis*- and *trans*-isomers together) was about 2–3 days, and after this initial period the rate of loss decreased. Some of the chlorfenvinphos was lost, presumably by volatilisation, and most of the remainder was converted gradually to breakdown products. A major breakdown product was a sugar conjugate of 1-(2′,4′-dichlorophenyl)ethan-1-ol, and traces of desethyl-chlorfenvinphos were also formed. There was no evidence of translocation of ^{14}C-activity from treated to untreated leaves.

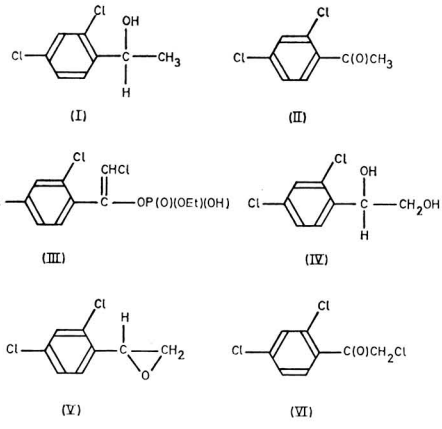
At harvest 80–112 days after application of chlorfenvinphos to foliage, no residues could be detected in the tubers of potatoes or in maize cobs, when the limit of detectability was 0·005 ppm. Residues (0·74 ppm–2·1 ppm, as equivalent ppm of chlorfenvinphos) were present on the foliage of potatoes and maize and were mainly of the conjugate of the ethan-1-ol. This ethan-1-ol has a low mammalian toxicity.

Cabbages at harvest 77 days after application contained 0·37 ppm of residues (expressed as chlorfenvinphos) which were mainly of the conjugated ethan-1-ol.

Introduction

Chlorfenvinphos [Birlane, 2-chloro-1-(2′,4′-dichlorophenyl)-vinyl diethyl phosphate] is a promising soil insecticide and its mode of breakdown in soils and in crops grown in treated soils has already been described.[1] In laboratory experiments the major decomposition products in soils were shown to be 1-(2′,4′-dichlorophenyl)ethan-1-ol (**I**), 2,4-dichloroacetophenone (**II**), and desethyl chlorfenvinphos (**III**). Also present in the soils, but in smaller amounts, were salts or conjugates of desethyl-chlorfenvinphos, (2′,4′-dichlorophenyl)ethan-1, 2-diol (**IV**), 2,4-dichlorophenyloxirane (**V**) and 2,4-dichlorophenacyl chloride (**VI**).

Cabbages, onions, and carrots which were grown in the glasshouse in soil treated with ^{14}C-chlorfenvinphos contained very low residues. None was detected in the edible parts of cabbage when the limit of detectability was 0·005 ppm. At harvest, 8–10 weeks after soil application of ^{14}C-chlorfenvinphos at 3–4 lb/ac, the edible roots of carrots contained 0·12



ppm of unchanged chlorfenvinphos and onion bulbs contained 0·07 ppm. There was evidence of trace amounts of a compound (0·024 ppm or less), which was considered to be a salt or conjugate of desethyl-chlorfenvinphos, in onions and carrots. Carrots also contained traces (near 0·005 ppm) of 2,4-dichloroacetophenone.

Chlorfenvinphos may also be used as an insecticide for direct foliar application, particularly for the control of Colorado beetle on potatoes. It is also possible that chlorfenvinphos could be useful in some locations for application to other crops such as brassicas and cereals. The breakdown of ^{14}C-chlorfenvinphos after direct application to the foliage of potatoes, cabbage, and maize has been studied and the results are described here.

Experimental

^{14}C-chlorfenvinphos and unlabelled marker compounds

A sample with a specific activity of 2·86 nc/μg labelled in the two vinyl carbons was used as described previously.[1]

The source of the unlabelled marker compounds has also been described.[1,2]

Plant growth

In most of the experiments the plants were grown in a cubicle of a Hartley glasshouse provided with good ventilation. Potatoes were grown in boxes 1 foot square and 8 inches deep. Cabbage plants were grown in 3 inch diameter polythene bags. Maize plants were grown in 3 inch diameter pots for short-duration experiments and in 1 foot square boxes for plants taken to maturity. The soil used was John Innes No. 2 compost.

In other experiments designed to detect any possible volatile breakdown products, cabbage and potato plants were grown in closed vessels. The soil surface around the plant stem and the polythene bag containing the soil were covered with aluminium foil, and the plants were placed in a 10 litre fermentation jar fitted with a 10 cm diameter flanged head. Air was passed through the vessel at a rate of ~ 50 ml/hour. The outgoing air was passed through a series of traps to collect any ^{14}C-components. The first trap contained methanol (50 ml) and was cooled in CO_2–acetone to −70°. The second trap contained a mixture of ethanolamine (17 ml) and methyl cellosolve (34 ml) to trap carbon dioxide.

Plant treatment

The upper surfaces of the leaves were treated with an aqueous solution (1 wt.-%) of the emulsifiers used in formulations, and were allowed to dry in air; they were then treated as evenly as possible with a known weight of ^{14}C-chlorfenvinphos in acetone using a microsyringe to give the required concentration (1, 6, 10 or 30 ppm) of chlorfenvinphos per plant. The doses quoted in ppm are approximations calculated from the amount (μg) of insecticide per g of fresh plant above ground level, the approximate fresh weight of the plant having been estimated by weighing typical plants from the batch being used and choosing similar-sized plants for treatment.

Details of the treatment times and levels are given in the Tables. All the plants developed normally and there was no evidence that treatment with ^{14}C-chlorfenvinphos affected their growth in any way.

Extraction procedures

Plants were cut at soil level and were extracted by thorough maceration with redistilled acetone followed by filtration through No. 3 porosity sintered glass. The plant material on the sinter was washed with more acetone and the filtrate was made up to a known volume (generally 10 ml for every 1 g of plant). The residuum was dried in a vacuum desiccator before further analysis.

Soils were extracted by treating 200 g portions twice with acetone (200 ml) and shaking the samples at 5–10 minute intervals for one hour, followed by filtration through No. 3 porosity sintered glass.

In the closed-vessel experiments the vessel and glass components used to contain the plant were washed with acetone and radiocounted. The methanol in the cold trap and the ethanolamine mixture were made up to known volume before radiocounting.

Before examination by chromatography all acetone extracts were concentrated by cautious evaporation on a Büchi rotary evaporator, and the watery residue was extracted twice with benzene. The separate phases were concentrated, and aliquots were subjected to radiocounting, which showed that there had been no loss of radioactive components during this procedure.

Aliquots of the aqueous phases of the extracts of the plants were concentrated and treated with 1% (w/v) aqueous β-glucosidase at pH 7 for 48 hours at 30° followed by extraction twice with benzene to isolate the aglycone liberated from any glycoside in the original extract. The unextractable radioactivity in the plant residuum was also subjected to this enzymic hydrolysis. With some residua, an acid hydrolysis procedure, involving treatment with 3M-HCl at 90° for 6 hours, was used in addition to enzymic hydrolysis.

Chromatographic, electrophoretic and radiocounting procedures

Thin-layer chromatography, electrophoresis, and radioactive gas–liquid chromatography were carried out as described previously.[1] Paper chromatography was carried out on Whatman No. 1 paper with ascending development to 15 cm.

Solid crop samples were ignited in an oxygen flask prior to liquid scintillation counting[1,3] and the soils remaining after extraction were subjected to a Van Slyke oxidation,[1,4] and $^{14}CO_2$ was absorbed and radiocounted.

The procedures for liquid scintillation counting and for the radio-scanning of the paper and thin-layer chromatograms were as described previously.[1]

Identification of radioactive components

Extracts were subjected to thin-layer chromatography and the separated components were desorbed from the gel layer using ethanol–benzene or ethanol–acetone. The individual components were then examined by t.l.c. using several other solvent systems with unlabelled standards run either on parallel tracks or mixed with the radioactive components. Further evidence for identification was based on gas–liquid chromatography, paper chromatography or electrophoresis, sometimes after a chemical reaction.

Results

Identification of the breakdown products

The retention values (R_f) of unlabelled standards in various thin-layer systems are shown in Table I. Typical radioscans of thin-layer chromatograms of the extracts are shown in Fig. 1–3. Two radioactive components (A and B) were detected in the acetone extracts.

Component A

Thin-layer chromatography indicated that component A was chlorfenvinphos because the R_f values with 20% (v/v) acetone in hexane, 5% (v/v) methanol in benzene, 10% (v/v) ethanol in benzene, and 30% (v/v) ethyl acetate in benzene were 0·23–0·31, 0·55–0·63, 0·65 and 0·25–0·35, which correspond to the values for chlorfenvinphos (300μ layers of Merck silica gel GF_{254}). The range of R_f values that is quoted corresponds to the R_f values of the upper and lower edges of the spot when the radio component and the standard were co-chromatographed. The *cis*(α) and *trans*(β) isomers could not be resolved by t.l.c. in any of the systems used. The identity of component A was confirmed by gas–liquid chromatography using electron-capture detection[5] which showed that it was a mixture of *cis*- and *trans*-chlorfenvinphos.

Component B

The R_f values of component B with 20% (v/v) acetone in hexane, 60% (v/v) ethanol in chloroform, and n-butanol (60 vol)-acetic acid (15 vol)-water (25 vol) agreed with those of desethyl-chlorfenvinphos (<0·1, 0·40, and 0·65, respectively); with 5% (v/v) ethanol in benzene, however, the respective values were <0·1 and 0·4. The R_f values of these two compounds also agreed in a paper chromatographic system (isopropanol 75 vol, water 24 vol, 0·88 ammonia 1 vol, R_f 0·37). However, on electrophoresis (pH 6·5, 5 kV, 37–42 mA, 30 minutes) desethyl-chlorfenvinphos ran to 10–11 cm whereas 98% of component B remained at the origin. About 1–2% of component B ran the same distance as desethyl-chlorfenvinphos.

On hydrolysis with β-glucosidase, component B was converted to another component (B1). This component was also formed when the plant residua, which still contained radioactivity after acetone extraction, were subjected to acid or enzymic hydrolysis. The thin-layer chromatographic properties of component B1 are summarised in Table II. Component B1 was also subjected to gas–liquid chromatography (4 ft × 0·095 in. Kunifer column packed with 3 wt.-% phenyldiethanolamine succinate on 100–120 mesh Gas Chrom Q at 118° with argon at 100 ml/min as carrier gas). A response was obtained at the same retention time for component B1 using both radiodetection and electron-capture detection and this retention time (8 min) was the same as that obtained for the unlabelled ethan-1-ol (**I**) using electron capture detection. The retention time of the benzyl alcohol (**IX**) was 12 minutes under similar conditions.

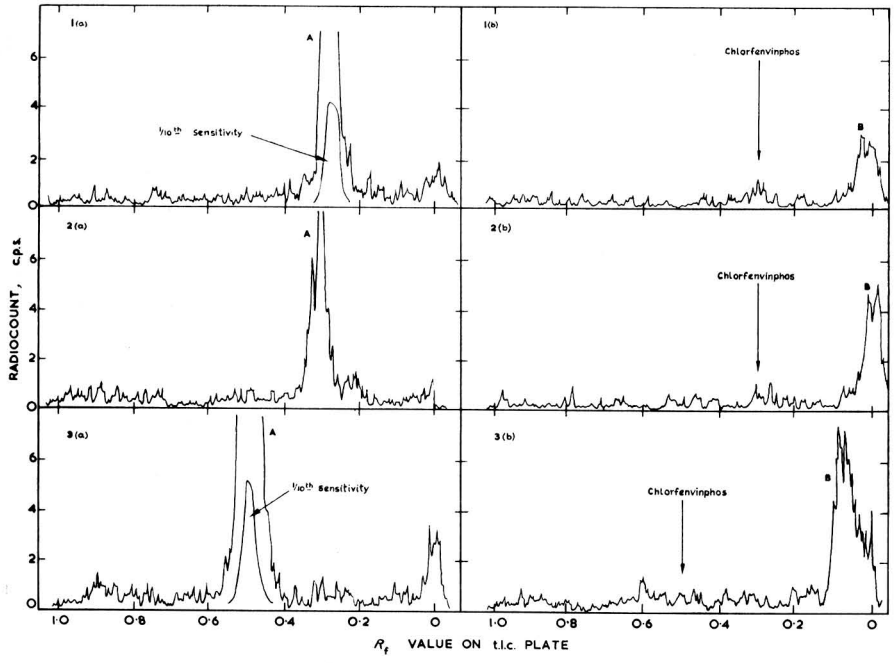
Only component B1 and unchanged component B could be detected on the enzymic hydrolysis of component B. When component B from treated cabbage leaves was subjected to acid hydrolysis six components were detected by thin-layer chromatography. The properties of these components are given in Table III.

These results indicate that component B1 is the ethan-1-ol (**I**). Compound B could contain 1–2% desethyl-chlorfenvinphos but the bulk of it appears to be a sugar conjugate.

TABLE I

Behaviour of unlabelled standards on thin-layer chromatography
Silica gel GF_{254} (300 μ layers)

	Unlabelled standard compound	R_f value in given system	
		20% (v/v) acetone in hexane	10% (v/v) ethanol in benzene
	Chlorfenvinphos	0·25	0·63
I	(ethan-1-ol)	0·36	0·56
II	(acetophenone)	0·50	0·70
III	(desethyl-chlorfenvinphos)	0·0	<0·1 streak
IV	(diol)	0·12	0·38
V	(oxirane)	—	~0·85
VI	(phenacyl chloride)	0·55	0·80
VII	2,4-Dichlorobenzoic acid	<0·1 streak	<0·45 streak
VIII	2,4-Dichlorobenzaldehyde	—	0·90
IX	2,4-Dichlorobenzyl alcohol	0·35	0·57
X	2,4-Dichloromandelic acid	0·1	—
XI	1-(2′,4′-Dichlorophenyl)-2-chloroethan-1-ol	0·35	0·70



FIGS 1–3. *Radioscans of thin layer chromatograms of extracts of plants treated with ^{14}C-chlorfenvinphos*

1 Potato foliage 7 days after treatment at 10 ppm. Acetone extracted and concentrated to give a watery layer which was shaken with benzene. Chromatography on Merck SG_{254} with 20% (v) acetone-hexane. (a) Benzene phase, (b) Aqueous phase.
2 Cabbage at 11 days from treatment at 6 ppm. Chromatography on Merck SG_{254} with 20% acetone–hexane. (a) Benzene phase, (b) Aqueous phase.
3 Maize at 7 days from treatment at 10 ppm. Chromatography on Merck SG_{254} with 5% (v) ethanol–benzene. (a) Benzene phase, (b) Aqueous phase.

(Arrows indicate R_f values of chlorfenvinphos although chlorfenvinphos itself is not necessarily present)

TABLE II

Thin-layer chromatography of component BI
300 μ layer of Merck silica gel GF_{254}

Eluant	R_f of component BI*	R_f of the ethan-1-ol* (I)	R_f of the benzyl alcohol* (IX)
15% (v/v) Acetone in hexane	0·44	0·44	—
2% (v/v) Ethanol in chloroform	0·45	0·45	—
15% (v/v) Ethanol in benzene	0·77	0·79	—
30% (v/v) Ethyl acetate in hexane	0·65–0·72	0·65–0·72	—
10% (v/v) Ethanol in benzene	0·43–0·53	0·43–0·53	—
20% (v/v) Acetone in hexane	0·45–0·58	0·45–0·58	—
Benzene	0·27–0·36	0·27–0·34	0·17–0·34
10% (v/v) Ethyl acetate in hexane	0·46–0·52	0·47–0·52	0·35–0·47

* When a range of values is quoted these correspond to the R_f values of the upper and lower edges of the spot when the radio component and standard were co-chromatographed.

The results show that at least 40% of B is a conjugate of the ethan-1-ol (**I**). This is the minimum amount of this conjugate that is present in B, and from the present results the actual amount could be higher.

Persistence of ^{14}C-chlorfenvinphos and its breakdown products on crops grown in the glasshouse

The persistence of chlorfenvinphos and its main breakdown product, the conjugate of the ethan-1-ol (**I**), on potatoes, cabbage and maize in the glasshouse is shown in Tables IV–VI. When potato leaves were treated with ^{14}C-chlorfenvinphos at 10 ppm in a manner similar to that described in Table IV the analysis of untreated leaves on the same plant indicated that no ^{14}C-activity was translocated from the treated to untreated leaves within 28 days of the application.

Persistence of ^{14}C-chlorfenvinphos and its breakdown products on crops grown in closed vessels

The results of the radioactivity balance studies with potatoes and cabbage are shown in Tables VII and VIII.

Discussion

Identification of breakdown products

On cabbage foliage the ratio of the residues of *cis*(α) and *trans*(β) isomers of chlorfenvinphos increased with time from 1 : 9 to 1 : 1. This could well occur on the other crops too but the effect was only investigated on cabbage. Whilst the effect could be due to greater persistence of the *cis*(α) isomer than the *trans*(β) isomer it may be due to isomerisation of the *trans*(β) isomer to *cis*(α), perhaps under the influence of sunlight.

The major breakdown products of chlorfenvinphos were hydrophilic, and of these the main component was a conjugate of the ethan-1-ol (**I**). Traces of desethyl-chlorfenvinphos

TABLE III

Radioactive components from acid hydrolysis of component B extracted from cabbage leaves

Products of acid hydrolysis of component B		Inference*
R_f value on silica gel GF_{254} 20% (v/v) acetone in hexane	% of total activity of component B	
0·0	30	Unchanged B
0·15	20	Reaction product of the ethan-1-ol (**I**) with acid in whole or in part
0·65	40	Ethan-1-ol (**I**)
0·75	3	Unknown
0·80	3	Unknown
0·95	4	Reaction product of the ethan-1-ol (**I**) with acid in whole or in part

* When a sample of ^{14}C-1-(2′,4′-dichlorophenyl)ethan-1-ol was subjected to the same acid hydrolysis conditions the thin-layer chromatogram of the extract of the reaction mixture showed ^{14}C-components at R_f 0·0, 0·2, 0·6 and 0·95

TABLE IV

Residues of ^{14}C-chlorfenvinphos and its breakdown products in potatoes grown in a glasshouse

Application : ^{14}C-chlorfenvinphos at 150 μg/shoot equivalent to about 10 ppm (except where stated).
Soil : Unsterilised John Innes No. 2 compost.
Temperature : 20–25°C.

Sample	Interval from treatment, days	Weight of sample, g	Residue* in acetone extract: Chlorfenvinphos ppm	Chlorfenvinphos %	Component B† ppm	Component B† %	Total ppm	Total %	Residue* (by combustion) not extractable by acetone ppm	not extractable by acetone %	Total residue* ppm	Total residue* %
Plant above ground	0	15	10	100	0	0	10	100	0	0	10	100
	2	20	4·4	59	0·25	3·3	4·7	62	0·34	4·5	5·0	67
	4	35	1·7	41	0·28	6·5	2·0	47	0·09	2·1	2·1	49
	7	55	0·84	31	0·34	11	1·2	41	0·10	3·7	1·3	45
	10	35	1·4	33	0·43	10	1·8	43	0·13	3·1	2·0	46
	16	55	0·71	26	0·39	9·3	1·1	35	0·13	4·8	1·2	40
	28	155	0·21	21	0·11	11	0·32	32	0·07	7·2	0·39	39
Leaves	57	—	—	—	—	—	0·22	—	0·06	—	0·28	—
Stump and root		—	—	—	—	—	<0·01	—	0·03	—	~0·04	—
Small tubers		—	—	—	—	—	<0·01	—	0·01	—	~0·02	—
Soil		—	—	—	—	—	<0·02	—	—	—	<0·02	—
Leaf	80	—	—	—	—	—	0·02	—	<0·01	—	0·03	—
Root		—	—	—	—	—	<0·01	—	<0·01	—	<0·02	—
Tubers		—	—	—	—	—	<0·005	—	<0·005	—	<0·005	—
Leaf**	80	—	—	—	—	—	0·62	—	0·12	—	0·74	—
Root**		—	—	—	—	—	<0·01	—	<0·01	—	<0·02	—
Tubers**		—	—	—	—	—	<0·005	—	<0·005	—	<0·005	—

* Expressed as chlorfenvinphos (ppm) equivalent to activity found or as % of ^{14}C-activity applied initially
** Initial treatment of leaf at 20 ppm
† About 1% of the activity assigned to component B could be desethyl-chlorfenvinphos

(**III**) were detected, but the residues of this compound were generally only 1% of the corresponding residues of the conjugated ethan-1-ol.

The breakdown of chlorfenvinphos is thus a much simpler process on foliage than in soils[1] in that fewer breakdown products are formed. The breakdown paths appear to be similar, however. The major breakdown product in soils and crops is the ethan-1-ol (**I**) although this is present in the free state in soils and as a conjugate in crops.

The mammalian toxicities (acute oral LD_{55} to rats) of the foliar breakdown products other than the ethan-1-ol conjugate have been determined by Tunstall Laboratory or by the Modesto Laboratory of Shell Development Company, U.S.A., and have been shown to be: Chlorfenvinphos *trans*(β), 39

TABLE V

Residues of ^{14}C-chlorfenvinphos and its breakdown products in cabbage grown in the glasshouse

Application : 1, 6, 10 and 30 ppm ^{14}C-chlorfenvinphos on cabbage leaves
Soil : John Innes No. 2 compost.
Temperature : 20–25°C.

Dosage applied (μg chlorfenvinphos) to plant of given weight	Time interval between application and sampling (days)	Weight of plant (above ground level) at sampling g	Residue* in acetone extract: Chlorfenvinphos ppm	Chlorfenvinphos %		Component B† ppm	Component B† %	Residue* (by combustion) not extractable by acetone ppm	Residue* (by combustion) not extractable by acetone %	Total residue* ppm	Total residue* %
72 μg to plants of near 12 g weight (6 ppm)	0 (1–2 hours)	12	5·7	95	(α:β 1:9)	0·30	5·0	0·10	1·6	6·1	102
	3	16	3·0	50	(α:β 1:3)	0·50	8·3	0·20	3·3	3·7	62
	7	20	1·4	23	(α:β 1:5)	0·80	13	0·30	5·0	2·5	41
	12	25	0·80	13	(α:β 1:2)	0·70	12	0·30	5·0	1·8	30
	24	33	0·40	6·7	(α:β 1:1)	0·40	6·7	0·40	6·7	1·2	20
	32	36	0·20	3·3	—	0·30	5·0	0·20	3·3	0·70	12
	77	96	0·025	0·40	—	0·14	2·3	0·20	3·3	0·37	6·0
14 μg to plant of near 14 g weight (1 ppm)	11	39	0·10	10	—	0·11	11	0·04	4·0	0·25	25
60 μg to plants of near 6 g weight (10 ppm)	0	6	9·5	95	(α:β 1:9)	0·3	3·0	0·05	0·5	9·9	99
	3	7	5·2	52	—	1·5	15	0·30	3·0	7·0	70
	6	7·5	3·4	34	—	1·7	17	0·80	8·0	5·9	59
	11	14	0·9	9·0	(α:β 1:2)	1·2	12	0·60	6·0	2·7	27
180 μg to plant of near 6 g weight (30 ppm)	11	21	3·7	12	(α:β1:2)	1·6	5·3	0·80	2·7	6·1	20

*, † See footnote to Table IV

TABLE VI

Residues of ^{14}C-chlorfenvinphos and its breakdown products in maize grown in the glasshouse

Application: ^{14}C-chlorfenvinphos, 100 μg to foliage of plants of about 10 g weight for plants sampled at 0–24 days and 1000 μg to plants of about 100 g weight to plants sampled 112 days after treatment.
Soil: John Innes No. 2 compost.
Temperature: 20–25°C.

Sample	Interval (days) from treatment	Weight of sample (g)	Residue* in acetone extract: Chlorfenvinphos ppm	Chlorfenvinphos %	Component B† ppm	Component B† %	Total ppm	Total %	Residue* (by combustion) not extractable by acetone ppm	Residue* (by combustion) not extractable by acetone %	Total ppm	Total %
Total plant above ground	0 (1–2 hours)	10	9·9	98	—	—	9·9	98	0·07	0·70	10	99
	2	10	4·5	45	0·30	3·0	4·8	48	0·23	2·3	5·0	50
	4	19	2·2	42	0·70	13	2·9	55	0·25	4·8	3·2	60
	7	18	1·6	28	0·65	12	2·3	40	0·52	9·4	2·8	49
	14	33	0·75	25	0·30	10	1·1	35	0·26	8·6	1·3	44
	24	40	0·66	26	0·30	12	0·96	38	0·39	16	1·4	54
Grain	112	—	—	—	—	—	<0·005	—	<0·005	—	<0·005	—
Cob core		—	—	—	—	—	<0·005	—	<0·005	—	<0·005	—
Leaf		Near 120	—	—	—	—	1·0	12	1·1	13	2·1	25**

*,† See footnote to Table IV
** Of this 25%, 1% was unchanged chlorfenvinphos and the remaining 24% was present as conjugate

TABLE VII

Distribution of residues from potato treated with chlorfenvinphos in a closed system

Application : About 10 ppm (153 μg) ^{14}C-chlorfenvinphos to 3 small potato shoots (near 15 g each) in cartons with John Innes No. 2 compost
Temperature: 20–23°C

Sample	Residue* at given time							
	Control		Day 0		Day 5		Day 9	
	ppm	%	ppm	%	ppm	%	ppm	%
On plant								
Extractable in acetone	<0·01	<0·1	10·3	103	8·4	84	7·9**	79
Not extractable in acetone	<0·01	<0·1	—	—	0·29	2·9	0·22	2·2
On vessel walls	—	—	—	—		10		11·0***
Cold methanol trap	—	—	—	—		≤0·1		0·3
Ethanolamine trap	—	—	—	—		1·3		5·1
Total	—	—	—	103		98·2		97·6

* As equivalent ppm of chlorfenvinphos or as % of ^{14}C-radioactivity applied initially
** Chlorfenvinphos 7·5 ppm, other component(s) 0·4 ppm
*** Mainly chlorfenvinphos with traces of a less polar compound, possibly the phenacyl chloride (**VI**)

TABLE VIII

Distribution of residues from a treated cabbage in a closed system

Application: About 8 ppm (128 μg) of ^{14}C-chlorfenvinphos to foliage of a plant (about 16 g) in John Innes No. 2 compost.
Temperature: 20–23°C.

Time interval to sampling: 4 days Weight of plant at sampling: 16 g		
Residue of applied dose at sampling at 4 days from treatment	% of ^{14}C-activity applied initially	ppm**
On plant (16 g):		
chlorfenvinphos	87·5	7·0
component B	6·2	0·5
not extractable in acetone	2·1	0·17
On vessel and in traps:		
chlorfenvinphos	3·0	–
Total	98·8*	

* Standard deviation of radiocounts was approximately ±3%
** As ppm of chlorfenvinphos equivalent to activity found

and *cis*(*α*), 33 mg/kg (Modesto); Desethyl-chlorfenvinphos (**III**) as Na salt, >1000 mg/kg (Tunstall)*; and 1-(2′,4′-dichlorophenyl)ethan-1-ol, >800 mg/kg (Tunstall)*.

Persistence of chlorfenvinphos and its breakdown products on glasshouse crops

The initial 'half-life' of the total chlorfenvinphos residues (i.e. *cis* plus *trans*) was 2–3 days on the foliage of potatoes, cabbage, and maize. After an initial period, in which a rapid loss of chlorfenvinphos occurred, the rate of loss decreased. However, the initial 'half-life' of the combined residues of chlorfenvinphos and of its breakdown products was 5–7 days. The residues of the breakdown products became greater than those of the remaining chlorfenvinphos 5 days after application to maize, at 12 days for cabbage and at about 30 days for potatoes.

* Higher doses not tested

At harvest 77–112 days after the application of chlorfenvinphos, detectable residues were still present on the leaves of the crops, and these residues were 0·74 ppm (expressed as equivalent chlorfenvinphos) on potato leaves, 0·37 ppm on cabbage leaves and 2·1 ppm on maize leaves. Most of these residues were present as the conjugate of the ethan-1-ol (**I**). There was no evidence for translocation of the activity from the treated foliage of potatoes to untreated parts of the plant. The residues could not be detected in the edible parts of maize (cob) or potatoes (tuber) at harvest and the limit of detectability was 0·005 ppm.

Although the dosage levels used in the present work were similar to those that would be used in commercial agriculture, in the field the residues at harvest would be expected to be much lower than in the present work, owing to the more severe weathering losses, especially by rain.

Persistence of ^{14}C-chlorfenvinphos and its breakdown products on crops grown in the glasshouse

A satisfactory recovery of the radioactivity was obtained when ^{14}C-chlorfenvinphos was applied to the foliage of potatoes and maize in closed glass vessels (Tables VII and VIII). The chlorfenvinphos was more persistent under these conditions than in the glasshouse. Some volatilisation of unchanged chlorfenvinphos occurred and it was recovered from the walls of the growth vessel and from the methanol and silica gel traps. With potatoes, radioactivity, presumably due to CO_2, was recovered in small amounts in the ethanolamine trap.

Conclusions

When chlorfenvinphos was applied to the foliage of potatoes, cabbage and maize in a glasshouse, half of the original compound was no longer present after 2–3 days. Part of this was lost, presumably by volatilisation, and part by conversion to breakdown products. A major breakdown product was a conjugate of 1-(2′,4′-dichlorophenyl)ethan-1-ol (**I**) which appears to be more persistent than the original chlorfenvinphos, but has a relatively low mammalian toxicity. There was no evidence for translocation of ^{14}C-activity from treated leaves to non-treated parts of the plant.

Chlorfenvinphos as a foliar insecticide is intended mainly to control Colorado beetles on potatoes. Residues could not be detected in the tubers. The small residues that can occur on the foliage are mainly of the conjugate of the ethan-1-ol (I) which is of relatively low toxicity to mammals and, moreover, the foliage of potatoes is only rarely used as animal fodder. The extent to which such residues can occur in the foliage of potatoes grown and treated in the field is, however, being investigated.

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PREPARATION OF GLIADIN BY UREA EXTRACTION

By J. W. LEE

A method in which, after a preliminary water extraction, all the gluten proteins in flour may be extracted by 2M urea is described. The protein so extracted is mainly gliadin, relatively free from high-molecular-weight glutenin. Gel filtration with Bio Gel P-150 polyacrylamide beads and electrophoresis in starch gel of varying porosity shows that the molecular weight distribution is relatively narrow. It is suggested that, under some conditions, an unidentified compound of low molecular weight is responsible for the intermolecular crosslinking of gliadin molecules to give high-molecular-weight glutenin.

Introduction

The solubility or extractability of gluten proteins have been the subject of many reports since the work of Osborne.[1] Both aqueous and organic solvents are capable of dissolving the protein from a gluten ball or extracting similar protein from flour or dough. In recent years, dilute organic acids have probably been used for the solution of these proteins more commonly than any other solvent. When dilute acetic acid solutions of gluten protein are examined they are found to incorporate two broad molecular weight classes: a fraction with high molecular weight up to $2–3 \times 10^6$, glutenin, and one with lower molecular weight $17–70 \times 10^3$, gliadin. Several reports suggest a relationship between these two classes based on the observation that on reduction in urea with mercaptoethanol both gliadin and glutenin give similar starch-gel electrophoresis patterns. Glutenin in its unreduced form is unable to penetrate the gel matrix during gel electrophoresis but after reduction by mercaptoethanol in urea, disaggregation is sufficient to permit penetration and resolution of components. Gliadin in either the reduced or unreduced state is capable of penetrating the gel matrix during electrophoresis and shows a series of bands. This paper reports a method for extracting, with aqueous 2M urea, all the gluten protein in a form that is capable of penetrating the gel without reduction. Comparisons are made with aqueous acetic acid extracts in terms of behaviour on starch-gel electrophoresis and gel filtration.

Experimental

Flour (N content 2·90%) was milled from Gabo wheat.

Extractions were conducted in centrifuge tubes containing 10 g flour and using successive 40 ml portions of solvent with agitation for 10 minutes prior to centrifugation. Protein concentrations in extracts and in fractions from gel filtration columns were measured on a Technicon Auto Analyser using the Lowry method.[2]

Starch-gel electrophoresis was carried out as described by Graham[3] with 0·017M aluminium lactate buffer, pH 3·1, with a voltage gradient of 15 volt/cm for 5 hours. Gels were stained on 0·02% nigrosine in 10% acetic acid overnight.

For gel filtration, columns (2·0 × 40 cm) of Bio Gel P-150 50–100 mesh were used (Bio Rad Laboratories, Richmond, California) and 0·01M acetate buffer, pH 4·1 with and without the addition of 2M urea. The column was loaded with 20 mg protein in 2 ml starting buffer. Flow rates were maintained at 1 ml/min and the column was jacketed at 30°.

Results and Discussion

The amounts of protein nitrogen extracted from flour by the conventional procedure of Coates & Simmonds[4] together with those for water extraction followed by 2M urea are given in Table I.

TABLE I

Comparison of the amounts of protein nitrogen extracted from flour by 0·05M acetic acid and by 2M urea

(Protein nitrogen expressed as a percentage of total nitrogen successively extracted from flour by various solvents)

Extractant			Extractant		
Sodium Pyrophosphate (0·01M, pH 7)	1	11·0	Water	1	10·9
,, ,,	2	4·9	,,	2	5·4
,, ,,	3	2·4	Urea (2M)	1	25·4
,, ,,	4	1·5	,, ,,	2	14·5
Acetic Acid (0·05M)	1	24·8	,, ,,	3	12·1
,, ,, ,,	2	18·2	,, ,,	4	6·5
,, ,, ,,	3	10·9	,, ,,	5	7·1
,, ,, ,,	4	8·9	,, ,,	6	5·1
			,, ,,	7	6·7
			,, ,,	8	4·0
Total N Recovery		82·6			97·7

These results show that acetic acid removes gluten protein at an initially greater rate than does urea; however, if a sufficient number of extractions are made with urea practically all of the protein can be extracted. Wrigley[5] using the method of Coates & Simmonds[4] found that 20% more protein could be removed by 0·1N sodium hydroxide after extraction with sodium pyrophosphate (pH 7) and 0·05M acetic acid.

The acetic acid extracts and the urea extracts were exhaustively dialysed against water, and the slurry of precipitated protein was freeze-dried. On solution in normal solvents for gluten (e.g. dilute organic acids), the urea-soluble protein gave a clear solution at 5% concentration while the acetic-acid-soluble protein gave a turbid solution even at 0·5%. Solutions of these proteins (1%) in aluminium lactate buffer (0·017M, pH 3·1, and containing 2M urea) were subjected to starch-gel electrophoresis, and the results obtained are shown in Fig. 1.

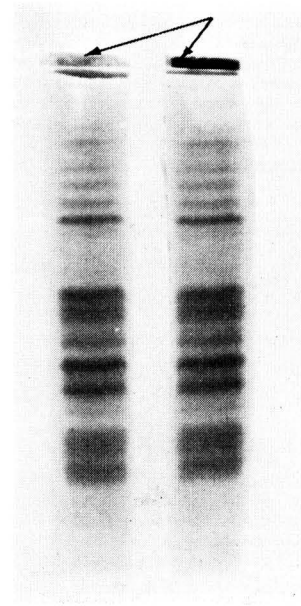

FIG. 1. *Starch-gel electrophoresis of flour proteins soluble in urea* (left) *and acetic acid*

Aluminium lactate buffer, pH 3·1

The starting slots at the ends of the gel (arrowed) have been cut off and turned at right angles to show the intensity of staining.

From this it can be seen that there is no obvious difference between the two methods of extraction, judged by the proteins which migrate into the gel. There is, however, a significant difference in the amount of stained material remaining in the starting slot. The acetic-acid-soluble protein left a considerable amount of stained material, which was completely absent with the urea-soluble protein. Examination of all successive urea extracts which had been dialysed against water, freeze-dried and dissolved showed absence of material in the starting slot. It would seem that extraction with urea after water brings protein into solution in a form of relatively low molecular weight and, from the gel electrophoresis pattern obtained, this appears to be gliadin. Acetic acid extracts on the other hand contain both gliadin and the high-molecular-weight glutenin.

To examine the distribution of molecular weight classes in the acetic acid and the urea extracts, they were subjected to zonal gel filtration on beads of polyacrylamide. Fig. 2 shows the elution profiles obtained.

Both the acetic-acid-soluble and the urea-soluble material show two peaks. The material that emerges first from the column appears at a volume corresponding to the volume of the column free space and represents material of relatively high molecular weight. The proportion of total protein represented by this high-molecular-weight material is much higher in the acetic-acid-soluble material; the urea-soluble material consists mainly of protein which is retarded by the gel column and thus is of relatively low molecular weight.

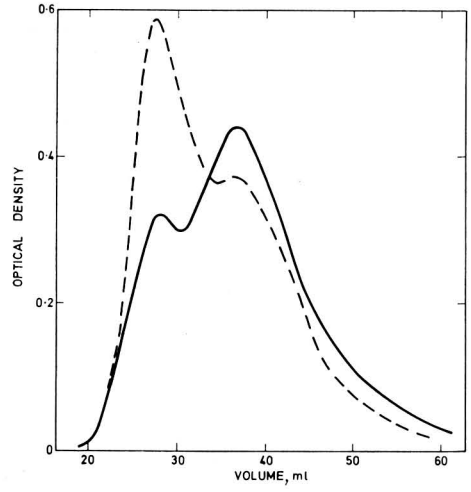

FIG. 2. *Gel filtration of urea extract (solid line) and acetic acid extract (broken line) on Bio Gel P-150 in acetate buffer, pH 4·1*

These results support those obtained with gel electrophoresis where it was found that virtually all of the urea-soluble protein was of sufficiently small molecular weight to penetrate starch gel. The results in Fig. 2 were obtained without the addition of urea to the buffer, but urea had no observable effect on the elution profiles.

Electrophoresis in gels of different starch or polyacrylamide concentrations[6,7] has been used to obtain a measure of protein molecular weight. The advantage of such a procedure is that individual components need not be isolated, whereas isolation would be necessary for the standard methods of molecular weight determination. In using the effect of the concentration of gelling agent on electrophoretic mobility there are, however, a number of problems. The method is not particularly sensitive, and extensive extrapolations with the consequent inaccuracies are unavoidable. With starch it is often difficult to reproduce exactly the conditions of gel preparation and in practice it is often found that slightly different electrophoretic mobilities are found for a particular protein in gels of the same starch concentration; for this reason starch concentration may not be a good practical measure of gel porosity. Ferguson[8] used two different reference proteins, albumin and prolactin, to 'calibrate' the gel and more recently (personal communication) has suggested bovine serum albumin and its polymers as alternatives.

A modification of Ferguson's method[8] was used to obtain an estimation of the order of the molecular weights of a number of the urea-soluble proteins resolved by starch-gel electrophoresis. Gels were prepared in the usual way except that the starch concentrations were varied by 1% increments from 9% to 18%.

Fig. 3 shows the bands to which this technique of molecular weight determination has been applied. Table II gives the estimation of molecular weights for these components assuming a value of 68,000 for the molecular weight of bovine serum albumin.

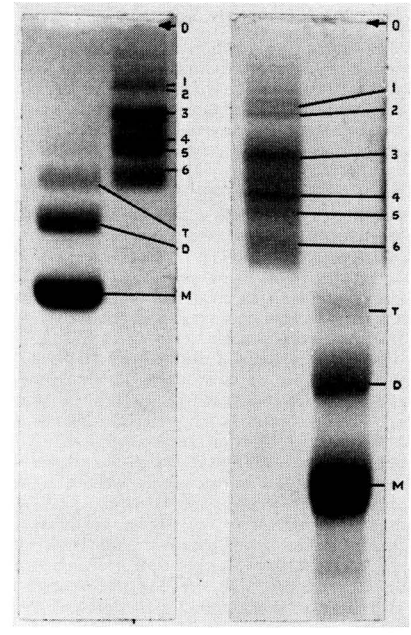

FIG. 3. *Starch-gel electrophoresis of urea-soluble flour protein and bovine serum albumin at starch concentrations of* 16% *(left) and* 11% *(right)*

Starting slot (O); flour proteins (1 to 6); bovine serum albumin monomer (M), dimer (D) and trimer (T)

TABLE II

Relative molecular weights of gliadin components obtained from starch gels of different porosities by electrophoresis

Component No. (from Fig. 3)	Relative molecular weight*
1	75,000
2	75,000
3	68,000
4	68,000
5	68,000
6	68,000

* Bovine serum albumin molecular weight assumed to be 68,000

It should be emphasised that the accuracy of these molecular weight values is not great not only for the reasons outlined here but also because of the uncertainty of the assumed value for bovine serum albumin. The results are, however, of sufficient accuracy to show that none of the components examined is a simple polymer of any other. The relative similarity of all the molecular weight values given in Table II would indicate that the separation achieved during starch-gel electrophoresis is largely due to differences in net charge, at least for the faster moving components.

Wu, Cluskey & Sexson[9] found molecular weights of approximately 45,000 and 81,000, respectively, for gliadin with and without the addition of 3M urea. The values obtained in this study for gliadin components in 2M urea fell between these two values. Wu *et al.*[9] showed that in aluminium lactate buffer both gluten and gliadin were aggregated, but the addition of 3M urea caused dissociation of such aggregates.

Conclusions

That portion of the protein, soluble in urea or acetic acid, which migrates in starch gel and is not completely excluded during gel filtration on Bio Gel P-150, is essentially gliadin. The fact that virtually all of the protein can be extracted from flour by aqueous urea after preliminary water extraction is of interest largely because this protein is relatively free from high-molecular-weight glutenin.

This method of extraction dissolves all of the gluten as gliadin, and it must be concluded that either the high-molecular-weight glutenin is being disaggregated by urea, or in other extraction methods the presence of glutenin is an artefact produced by the extraction method itself. The urea extraction procedure described in this paper involves exhaustive dialysis of the urea extract against water. Experiment has shown that this step is essential if material free from glutenin is to be obtained. Dialysis against dilute organic acids usually results in an increased proportion of high-molecular-weight material. A possible explanation of this behaviour is that a dialysable low-molecular-weight material is capable of crosslinking gliadin in the presence of dilute organic acids. If this material is removed or if the extract is kept free from organic acids then the crosslinking does not occur. This low-molecular-weight material may be a substance capable of promoting intermolecular disulphide bonding or it could be a peptide of the type described by Mauritzen & Stewart.[10] The formation of new intermolecular disulphide bonds by S-S, S-H interchange is, however, less likely to occur at the pH values obtained in aqueous organic acids than at neutral or alkaline pH. No attempt has yet been made to identify such components in the diffusates from urea extracts but this approach could prove profitable. It may be noted that extraction of a gluten ball by 2M urea followed by dialysis of the extract against water gives a starch gel pattern with considerable material in the starting slot. If dry flour is first extracted with absolute ethanol, an aqueous 2M urea extract shows no material in the starting slot on electrophoresis. It is not unlikely that dry ethanol may in fact have removed material responsible for associating gliadin into high-molecular-weight glutenin.

The molecular weight estimates of the gliadin proteins, while subject to considerable error, are still sufficiently accurate to establish that none of the components examined is a simple polymer of any other. This does not rule out the possibility that all components represent polymers of basic units of relatively low molecular weight, the polymers not being disaggregated by 2M urea.

Pomeranz[11] was able to correlate the dispersibility of flour proteins in 0·01M pyrophosphate buffer pH 7 containing urea at 4° with breadmaking potentiality. Such urea extracts contained the high-molecular-weight glutenin as well as gliadin and the water-soluble proteins.[12] The proteins in these extracts were found by Pomeranz[13] to retain their functional properties for breadmaking. Baking tests showed that the urea-soluble protein prepared by the method described in this paper did not. It would seem that to exhibit its functional properties for breadmaking a significant portion of the gluten protein needs to be in the aggregated state as glutenin.

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INTEGRITY OF GLUTEN COMPONENTS LABELLED WITH ^{14}C

By J. W. LEE

Wheat protein was labelled with ^{14}C by exposing immature wheat ears to uniformly labelled ^{14}C glycine. Labelled gluten proteins were fractionated on carboxymethyl cellulose, and the labelled fractions obtained were mixed with unlabelled and unfractionated protein. Study of the distribution of ^{14}C after electrophoresis on starch gel showed that each protein band examined retained its identity, and there was no evidence of *in vitro* protein hybridisation.

Introduction

Since the observation was made that wheat gluten consists of a large number of components, judged by ion-exchange chromatography[1,2] or gel electrophoresis,[3,4] doubts have been expressed concerning the authenticity of some of the 'peaks' in chromatography or the 'bands' in gel electrophoresis. There has always been the possibility of artefacts caused by various association phenomena,[5] although Ewart[6] found no evidence of protein–protein interactions in gliadin in sodium or aluminium lactate buffers. This paper reports some studies in which radioactive protein was produced by exposure of wheat ears to uniformly labelled ^{14}C glycine. The gluten protein extracted from this wheat was fractionated by ion-exchange chromatography and mixed with unlabelled protein, and the distribution of radioactivity in various fractions was determined by examining small sections excised from starch gels after electrophoresis.

Experimental

^{14}C labelling of wheat

The method followed was that described by Graham & Morton.[7] One hundred ears of wheat cv. Gamut were harvested 23 days after the onset of flowering. The stems were cut approximately 5 mm below the head and were immersed in 50 ml of a solution of uniformly labelled ^{14}C glycine (0·1 mc, specific activity 8·1 mc/mM, The Radiochemical Centre, Amersham, Bucks.) contained in a 2 l glass beaker. The beaker and its contents were placed in a glasshouse (day temperature approximately 27°) for 24 hours. The heads were dried over a fan heater, and the grain was threshed by hand. Another 100 ears were placed in unlabelled glycine as a control; these were exposed to light, dried and threshed in the same manner as the labelled wheat. Wheat samples were milled in a Quadrumat Junior mill (Brabender, Duisburg, W. Germany) to give a flour yield of approximately 60%.

Protein extraction and fractionation

Protein was extracted from flour either by the methods of Coates & Simmonds[8] as modified by Wrigley[9] or with 2M urea as described by Lee.[10] The acetic-acid-soluble proteins (gluten) were fractionated on carboxymethyl cellulose (CMC) by the method of Wrigley[11] and by starch-gel electrophoresis according to the method previously described.[10]

Scintillation counting

Protein solutions containing ^{14}C were counted in a Packard Model 3324 Tri-Carb liquid scintillation spectrometer at 5°. The scintillation fluid used, described by Bruno & Christian,[12] contained 1 : 4 dioxan (83% v/v)/2-ethoxyethanol (17% v/v) mixture containing PPO (2,5-diphenyloxazole) 1%, POPOP (1,4-bis-2-(5-phenyloxazolyl) benzene) 0·05% and naphthalene 5%. Protein in starch-gel bands was counted by removing 0·5 mm wide sections (approx. 0·1 g) from the centre of stained zones and macerating them in 9·9 ml scintillation fluid. Quenching due to the presence of dye (nigrosin) or starch in the scintillation fluid was apparently not a significant source of error, as between 95 and 105% of added isotope could consistently be recovered from stained starch-gel sections.

Results and Discussion

Fig. 1 shows the profile obtained when ^{14}C labelled acetic-acid-soluble protein (100 mg) was fractionated on a CMC column.

Fractions as indicated were dissolved in 0·05M acetic acid at concentrations of approximately 1 mg/ml. The protein concentration was measured by the Lowry method[13] and the ^{14}C activity was determined. The specific activities of these fractions after correction for background and counting efficiency are reported in Table I.

The specific activity of the unfractionated acetic-acid-soluble protein is of the same order as that reported for protein prepared in a similar manner by Graham & Morton.[7] There is some small variation between the specific activities reported in Table I for the different fractions from the CMC column, but the scatter between results from replicate experiments showed that these variations in specific activity could be accounted for by experimental error. From these results it must be concluded that, for the 24 hour period of exposure, the uptake of ^{14}C from glycine was essentially the same in all the CMC fractions. In addition, each of these fractions has a specific activity of the same order as that of the whole

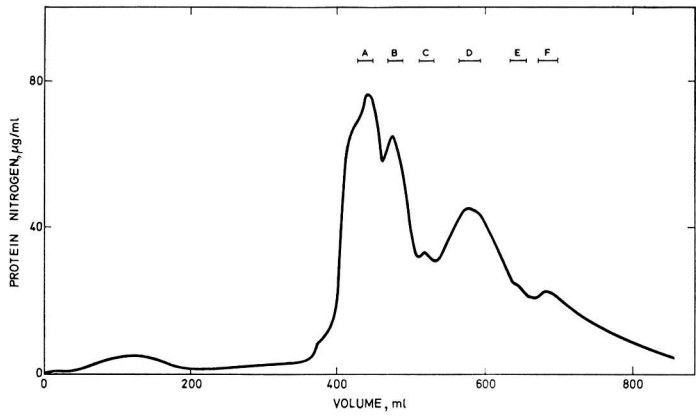

FIG. 1. *Fractionation of radioactive acetic-acid-soluble flour protein on carboxymethyl cellulose*[11]

Fractions (A–F) collected for subsequent study are indicated

TABLE I

Specific activity of ^{14}C labelled gluten fractions

Fraction	Specific activity* (counts/min/μg protein N)
Whole acetic-acid-soluble protein	253
A	265
B	234
C	272
D	249
E	245
F	272

* Corrected for background and counting efficiency.

acetic-acid-soluble protein (gluten). From this it could be inferred that glutenin, which is not fractionated on CMC, must also have a similar specific activity to each of the CMC fractions which collectively constitute gliadin.

Fig. 2 shows the starch-gel electrophoresis patterns for each of the gliadin fractions eluted from CMC. Mixtures of equal parts of unfractionated acetic-acid-soluble protein (gluten) and each of the CMC fractions (total protein concentration 10 mg/ml) were subjected to starch-gel electrophoresis and, after being stained, the bands and the starting slots were excised and counted for ^{14}C. In Table II results from these samples are compared with those obtained from the CMC fractions (loaded at 5 mg/ml). Counts are also given for unfractionated acetic-acid-soluble protein and urea-soluble protein.

For a variety of reasons the counts recorded in Table II are subject to considerable errors, but the results are adequate to arrive at several conclusions. With the first two samples mentioned, the quantity of protein remaining in the starting

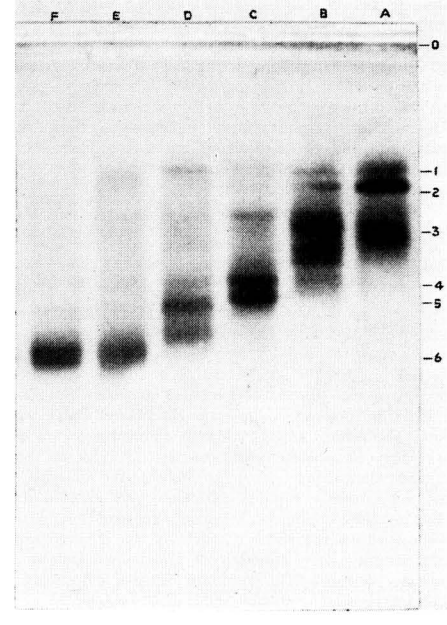

FIG. 2. *Starch-gel electrophoresis of protein fractions obtained from CMC fractionation (Fig. 1)*

The bands excised for scintillation counting (1–6) are indicated. The starting slots (O) are at the top of the picture

TABLE II

Distribution of ^{14}C in bands excised from starch gels

Sample	Total counts/minute*						
	Band						
	0 (slot)	1	2	3	4	5	6
Acetic acid-soluble protein**	932	251	495	1397	347	330	401
Urea-soluble protein**	72	417	523	1382	505	419	410
Fraction A	27	502	2070	917	—	—	—
Fraction A + gluten	13	399	1888	1006	—	—	—
Fraction B	17	242	500	1497	197	57	—
Fraction B + gluten	13	309	617	1291	250	78	—
Fraction C	0	90	103	188	541	699	—
Fraction C + gluten	3	132	127	212	617	621	—
Fraction D	26	156	42	67	194	389	—
Fraction D + gluten	18	119	78	29	286	381	—
Fraction E	21	60	47	29	50	47	981
Fraction E + gluten	19	85	31	50	51	81	1112
Fraction F	9	—	15	28	26	68	1287
Fraction F + gluten	11	—	14	41	15	90	1302

* Corrected for background and counting efficiency
** Radioactive, loaded on gel at 10 mg/ml

slot of the starch gel is considerably greater for the acetic-acid-soluble protein than for the urea-soluble material. This is in accord with the result reported in an earlier paper[10] in which gel filtration was used to show the proportion of high-molecular-weight material. The main conclusion is that all labelled proteins retained their identity and there is no evidence of transfer of activity to unlabelled bands. In other experiments, the same results were obtained when mixtures of labelled fractions and unlabelled whole protein extracts were precipitated by the addition of 0·2M sodium chloride and redissolved by the removal of the added salt by dialysis and then separated by electrophoresis.

Although the results in Table II show that much more material is retained in the starting slot on starch-gel electrophoresis of acetic acid extracts than is the case with urea extracts, the proportion of protein migrating and being retained cannot be estimated from these data. To determine this, ^{14}C labelled protein with known total activity was loaded on to starch gel and, after electrophoresis, the amount remaining in the slot was determined by scintillation counting. Results are shown in Table III.

It appears that approximately 80% of the protein in the acetic acid extract was capable of penetrating the gel matrix during electrophoresis. If gliadin is defined as the group of proteins, in an acetic acid extract, capable of entering the gel during electrophoresis then the gliadin/glutenin ratio is much higher than that normally reported for the gluten proteins. The fact that this ratio is even higher with a urea-extract emphasises the necessity of strictly defining the conditions of measurement if such ratios are to be meaningful.

TABLE III

Protein remaining at the starting slot during gel electrophoresis

Sample	Total counts loaded,* per min	Total counts in slot,* per min	Proportion retained %
Acetic-acid-soluble protein**	7570	1470	19·4
Urea-soluble protein**	7390	119	1·6

* Unsliced gels, counts corrected for background and counting efficiency.
** Protein solution (1%) centrifuged at 27,000 × g for 30 min prior to loading

Conclusions

Without methods for preparing and studying single components it is not possible to draw unequivocal conclusions concerning artefacts. The results do, however, strongly indicate that the proteins forming bands on starch gel are distinct entities (though not necessarily homogeneous proteins) which maintain their integrity under a variety of conditions. There is no evidence of *in vitro* protein hybridisation under the conditions studied. The possibility, reported by Cann,[14] of spurious band formation due to buffer–protein interaction has not been ruled out, although it has been observed that when single bands are cut from a gel after electrophoresis and inserted in the starting slot of a new gel, they move as single zones with the same electrophoretic mobility that they showed originally.[4] This would seem to make the existence of artefacts due to buffer–protein interaction unlikely.

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CHEMICAL TESTS FOR POTENTIALLY AVAILABLE NITROGEN IN SOIL

By D. S. JENKINSON

Previous work on the distribution of radioactivity in the organic matter of soils incubated with ^{14}C labelled ryegrass suggested that the amount of organic matter extracted by barium hydroxide might serve as an index of potentially available N in soil. This suggestion was tested on a set of soils for which the amounts of N mineralised during incubation, and the amounts of N taken up by ryegrass grown in the soils, were known. The amounts of organic C and non-nitrate N extracted by barium hydroxide were correlated fairly closely with the amounts of N released by the soils; polysaccharide (measured as the glucose equivalent) extracted by barium hydroxide was more closely correlated with release of N. The amount of 'glucose' extracted was significantly correlated with the yield of unfertilised barley in 36 field experiments ($r = 0{\cdot}83^{**}$); the correlation with response of barley to N was smaller ($r = -0{\cdot}54^{**}$).

The amount of 'glucose' extracted from soil by barium hydroxide increased during air-drying, but once air-dry no further change occurred, even on prolonged storage.

Barium hydroxide-extractable 'glucose' is proposed as an index of potentially available N in soil. The correlation coefficients between uptake of N by ryegrass grown in pots (14 soils) and soil measurements were $0{\cdot}70^{**}$, $0{\cdot}65^{*}$, $0{\cdot}70^{**}$, $0{\cdot}67^{**}$, and $0{\cdot}28$, respectively, for barium hydroxide-extractable 'glucose', N extractable by boiling water, or sodium bicarbonate, ammonia released by hot aqueous calcium hydroxide, and ammonia released by alkaline permanganate. None of the chemical tests were correlated as closely with uptake of N by grass, as the N mineralised when the re-wetted air-dried soil was incubated.

Introduction

As manuring specifications become more precise it is becoming increasingly important to allow for the amount of soil N released to a crop during the growing season. Although in advisory work a rough idea of a soil's capacity to mineralise N can often be had from the results of past cropping, it is likely that the accuracy of prediction could be usefully improved by a soil test for potentially available nitrogen that is quick and easy to do. Tests based on a single extraction ('chemical tests') have so many advantages in routine soil-testing over the lengthy incubation tests currently in use that it is not surprising that many different chemical tests have been proposed (see Bremner[1] for a review). The choice of reagents and conditions for these tests has usually been empirical.

During work on the decomposition of labelled ryegrass in this laboratory[2,3] some results were obtained which, it was reasoned, would be of guidance in selecting the best conditions for a chemical test. When labelled ryegrass had decomposed for a year in a calcareous soil, the soil contained a small organic fraction that was heavily labelled relative to the rest of the organic matter.[2] As decomposition continued, labelled C was lost faster from this fraction than from the soil organic matter as a whole. It was suggested that this heavily labelled fraction was located in the soil biomass. Other larger fractions, for example that part of the soil organic matter not

hydrolysed by 6N hydrochloric acid, were relatively lightly labelled.[3] None of the reagents tested extracted organic matter as heavily labelled as that known, from partial sterilisation experiments, to be present in the soil. Reagents such as boiling water, cold dilute hydrochloric acid, or cold dilute barium hydroxide extracted small amounts of organic matter that were more heavily labelled than the larger amounts extracted under more drastic conditions, for example by hot dilute acid. The organic matter extracted by barium hydroxide was more heavily labelled than that extracted by any other reagent tested. The amount of labelled C mineralised after partial sterilisation (considered to be a measure of the labelled soil biomass[2]) was highly significantly correlated with the amount of labelled C extracted by barium hydroxide.[3]

Soil organic matter contains fractions that differ greatly in stability[4] and resistance to mineralisation. A large but relatively inert fraction might conceivably maintain a microbial population comparable in size to that maintained by a smaller but more decomposable fraction. Thus, by taking the size of the soil biomass as a measure of the mineralising power of a soil, some allowance can be made for the N mineralised from slowly decomposing fractions and that from labile ones. If it is assumed, therefore, that a soil maintaining a large biomass mineralises more N per unit time than one maintaining a small biomass, and that barium hydroxide-extractable organic matter is correlated closely with the amount of biomass in a soil, then barium hydroxide-extractable organic matter should serve as a measure of the N mineralising power of soil.

Apart from its ability to extract heavily labelled organic matter, barium hydroxide has advantages as an extractant in soil testing. It does not disperse the soil nor does it have the disadvantage, common to all acid extractants, of reacting with soil carbonates.

A qualitative examination of the material in the barium hydroxide extract of soil 54 (Table I) showed that it contained material hydrolysable to α-amino acids, small amounts of free α-amino acids (see Paul & Schmidt[5]), ammonia, nitrate and much polysaccharide. Hexoses in the extract were determined by the anthrone method (the results being expressed as the 'glucose-equivalent'): about half of the sugars thus determined were retained when the extract was dialysed for 24 hours in Visking tubing, presumably because they were combined in polysaccharides of high molecular weight.

In the present work, three measurements were made on the barium hydroxide extracts; total non-nitrate N, total C (which was found to be very closely correlated with total non-nitrate N and so was not always measured) and 'glucose' as determined by the anthrone method. The objectives of the work were to evaluate three tests for potentially available N based on barium hydroxide extraction and to compare the results with those from some other chemical tests, using a set of soils for which laboratory, pot and field data on N release were available.[6,7] As these soils had been stored air-dry for periods of from three to ten years, a subsidiary study was made of the effects of storage on freshly sampled soils. A preliminary account of this work has already appeared.[8]

Experimental

Soils

Table I describes the soils used. Soils 1–14, sampled in 1957, and soils 15–28, sampled in 1958, were used by Gasser[6] in Part VI of his work on N mineralisation. Soils 29–42 were sampled in 1959, and used by Gasser & Jephcott[7] in Part VIII. Soil 43 was sampled in 1957, soils 44, 45 and 46 in 1958, soils 47, 48 and 49 in 1959. Soils 1–49 came mostly from commercial farms within a 25 mile radius of Harpenden, but a few were from Rothamsted or Woburn experimental farms; they were sampled, to plough depth (7–9 in.) in spring, after the seedbed had been prepared but before any fertilisers were applied or any seed was sown. Soils 50–53 were sampled in April 1963 from the 2–6 in. layer of soil, to minimise the effects of air-drying and re-wetting by excluding the surface soil. Soil 50 came from an inorganic fertiliser plot and soil 51 from a dunged plot of the Woburn market garden experiment; soils 52 and 53 from the unmanured and dunged plots, respectively, of the Broadbalk continuous wheat experiment. Soil 54 was also from the dunged plot of Broadbalk, sampled (0–6 in.) from the fallow section in March 1962.

Soil analysis

The procedures used in analysing soils 1–54 (Table I) for total N, organic C, pH, nitrate N, ammonium N, moisture, and carbonate C are given by Gasser,[6] except that with soils 50–54, nitrate- and ammonium-N were determined by d'Arifat & Warren's method.[9] Analyses were in duplicate or triplicate.

Incubation techniques

These have been fully described by Gasser;[6] briefly, sieved soil which had been stored air-dry for 12 weeks was moistened to 50% of its water-holding capacity and incubated for 21 days at 25°. The difference between the amounts of nitrate- and ammonium-N extracted from the soil before and after incubation was called 'Δ min(eral) N'. The amount of nitrate- and ammonium-N present in the fresh soil as sampled was called 'min N'.

Pot experiments

See Gasser[6,7] for details. Ryegrass was grown in pots containing 3000 g fresh soil, equivalent to 2000–2700 g dry soil. As the amount of dry soil per pot differed, the soil measurements were expressed as mg per pot, rather than mg per 100 g soil, in calculating correlation coefficients between soil measurements and plant measurements. A basal P and K dressing was given to all pots; one third received no N, one third 75 mg N (as ammonium sulphate) and one third 150 mg N per pot. The grass was cut three times, and the amount of N in each cut was determined. The results from the three sets of pot experiments are considered separately because the harvesting procedure in 1959 (soils 29–42) differed slightly from those used in 1957 (soils 1–14) and 1958 (soils 15–28), (see Gasser & Jephcott[7]); in addition glasshouse conditions differed slightly from year to year.

Field experiments

See Gasser[6] for details. All plots received a basal dressing of P and K; the yield of barley on the no-N plots was taken as the unfertilised yield. Two or three rates of N were applied and the response to a dressing of 0·5 cwt fertiliser N per acre was found by interpolation of the parabola of best fit calculated from the results. In 1957 seven sites were used (soils 3, 5, 8, 11, 12, 14 and 43: Table I) but the yields from one site (soil 8) were so anomalous that the field results from it were discarded. In 1958 thirteen sites were used (soils 16, 18, 19,

20, 22, 23, 24, 25, 27, 28, 44, 45 and 46). In 1959 seventeen sites were used (29–42 inclusive, 47, 48 and 49).

Chemical tests for 'potentially available' nitrogen

Extraction with barium hydroxide

Unless otherwise indicated, soil (10 g) was shaken for 30 minutes with 100 ml 0·1 N barium hydroxide on a wrist-action shaker. After standing for 2 minutes the supernatant solution was decanted through a Whatman No. 1 paper, the first few ml of filtrate being discarded.

Non-nitrate N in barium hydroxide extracts was determined by adding ferrous sulphate heptahydrate (250 mg) to an aliquot (usually 20 ml) of the extract, followed by sulphuric acid (4 ml 98% acid) and catalyst (1·3 g). The catalyst contained potassium sulphate (200 parts by weight), cupric

TABLE I

Description and analyses of soils

Soil No.	Type	pH	% N	% organic C	% carbonate C	△min N, mg/100 g soil	$Ba(OH)_2$ extracted 'glucose', mg/100 g soil	$Ba(OH)_2$ extracted non-NO_3 N, mg/100 g soil
1	Fine sandy clay loam	5·3	0·262	2·38	—	3·6	49	15·2
2	Calcareous clay loam	8·0	0·174	1·55	5·5	2·0	15	3·8
3	Silty clay loam	7·5	0·153	1·31	<0·1	2·9	17	5·0
4	Clay	7·0	0·165	1·42	—	2·3	14	5·1
5	Calcareous silt loam	8·0	0·267	2·21	0·6	3·7	25	5·4
6	Fine sandy clay loam	5·9	0·256	2·37	—	4·3	31	10·3
7	Calcareous clay	8·0	0·272	2·38	0·6	4·0	28	5·8
8	Silty clay loam	6·6	0·263	2·61	—	9·2	57	12·5
9	Sandy clay loam	6·5	0·231	2·22	—	4·0	40	12·1
10	Clay	8·0	0·187	1·54	0·6	2·4	16	3·9
11	Clay	8·0	0·263	2·28	0·4	4·2	26	5·4
12	Calcareous clay loam	7·9	0·312	2·29	2·6	3·0	22	5·1
13	Clay loam	7·2	0·155	1·40	<0·1	3·4	18	5·8
14	Silty clay loam	7·6	0·178	1·64	<0·1	2·6	16	5·2
15	Clay loam	7·8	0·161	1·56	<0·1	2·3	14	3·9
16	Calcareous silt loam	8·0	0·215	2·30	6·2	3·5	23	5·4
17	Clay loam	7·2	0·172	1·84	<0·1	3·0	14	5·2
18	Stony coarse sandy loam	7·9	0·156	1·90	0·5	1·3	13	3·4
19	Stony clay loam	7·9	0·248	2·60	0·5	3·4	22	5·2
20	Stony silt loam	7·9	0·168	1·90	0·2	2·7	18	4·4
21	Stony silt loam	7·9	0·178	1·66	0·2	2·8	21	4·4
22	Calcareous silty clay loam	8·0	0·242	2·32	5·2	4·0	28	5·8
23	Calcareous silty clay loam	7·9	0·236	2·33	2·0	3·5	23	4·7
24	Silt loam	6·4	0·143	1·62	—	2·6	21	7·1
25	Sandy loam	6·4	0·212	2·19	—	3·1	31	10·4
26	Clay loam	6·8	0·336	4·61	—	11·0	77	13·8
27	Clay loam	7·9	0·195	1·96	0·1	1·8	19	4·8
28	Sand	6·0	0·094	0·92	—	1·1	7	3·6
29	Calcareous loam	8·0	0·229	1·91	2·8	3·9	24	4·7
30	Loam	6·3	0·494	4·15	—	8·3	62	13·4
31	Chalky loam	8·0	0·177	1·90	3·2	2·6	20	4·4
32	Silt loam	7·6	0·177	1·95	<0·1	3·0	18	5·4
33	Clay loam	6·9	0·176	1·65	—	2·9	15	5·3
34	Loamy sand	7·0	0·162	1·80	—	3·1	26	6·5
35	Silt loam	7·4	0·157	1·62	<0·1	3·8	21	6·6
36	Sandy loam	7·9	0·139	1·98	0·5	2·6	13	3·1
37	Clay loam	8·0	0·191	1·80	0·2	2·3	14	4·2
38	Silt loam	7·6	0·205	2·10	<0·1	3·9	19	4·8
39	Silt loam	7·6	0·199	2·27	0·2	4·5	25	6·3
40	Calcareous clay loam	8·1	0·173	1·55	3·1	3·1	15	3·5
41	Calcareous silty clay loam	8·0	0·210	2·01	5·2	4·1	22	5·2
42	Clay loam	7·8	0·264	2·36	0·4	4·3	24	5·4
43	Very stony sandy loam	7·8	0·170	1·70	0·1	2·8	14	4·3
44	Sand	5·5	0·094	0·82	—	1·0	9	5·0
45	Clay loam	7·2	0·164	1·86	<0·1	2·8	13	4·6
46	Clay loam	7·1	0·179	1·81	<0·1	3·2	14	4·9
47	Sand	5·8	0·094	0·93	—	1·4	7	3·9
48	Silt loam	7·9	0·182	2·13	0·1	4·4	18	5·3
49	Chalky loam	8·0	0·192	1·81	8·6	4·6	28	5·2
50	Sandy loam	6·8	0·115	1·35	—	1·6	6	2·8
51	Sandy loam	6·6	0·209	2·45	—	3·9	15	4·5
52	Silt loam	8·1	0·107	0·91	0·1	2·0	8	2·0
53	Silt loam	7·6	0·274	2·88	0·1	5·4	18	3·9
54	Silt loam	7·6	0·262	2·80	0·1		17	3·5

sulphate pentahydrate (20 parts) and selenium (1 part). The mixture was digested for 1 hour after the organic matter disappeared, cooled, excess sodium hydroxide was added, and the ammonia was steam-distilled into boric acid indicator solution (5 ml). Forty-five ml of distillate were collected and the ammonia was titrated against 0·005 N sulphuric acid. The boric indicator solution was prepared by dissolving boric acid (40 g) in water, adding indicator solution (50 ml) and making up to 1 litre. The indicator solution was a mixture of 0·1% bromocresol green in ethanol (7 parts) and 0·1% methyl red in water (1 part). Ferrous sulphate almost completely eliminated interference from nitrate: when 200 ppm nitrate N were added to soil 7, 2% of the added nitrate N was recovered from the barium hydroxide extract in the presence of ferrous sulphate, 10% in its absence.

Barium hydroxide-extracted 'glucose' was determined by a modification of the anthrone method.[10] The anthrone reagent was prepared by dissolving purified anthrone (1 g) in a cooled mixture of concentrated sulphuric acid (475 ml) and water (37·5 ml). It was stored (for up to 7 days) at −15°. Anthrone was purified by treating Hopkin & Williams' G.P.R. grade anthrone with charcoal and recrystallising it from benzene/petroleum spirit (60–80°). The anthrone reagent (20 ml), cooled to 0°, was added to 4 ml aliquots of the extract, swirled to dissolve precipitated barium sulphate and the mixture was heated on a boiling water bath for 10 minutes. During cooling, the absorption was measured against a reagent blank on an EEL absorptiometer, using the 607 filter. The results were compared with those from standard solutions containing 20 and 60 γ glucose/ml. Glucose added to barium hydroxide extracts was recovered quantitatively. Although the soil extracts contained some nitrate, it was not present in sufficient concentration (even in soil 26, Table I, containing 2·1 mg nitrate N per 100 g soil) to interfere measurably with the anthrone colour reaction. Care is needed to avoid contaminating the extracts by cellulose particles from filter paper, dust, etc.

Barium hydroxide extracts were analysed for organic carbon by a modification of the Tinsley method.[11] Aliquots of the extract (20 ml), 0·2 N potassium dichromate (5 ml), sulphuric acid (25 ml 98% acid), and phosphoric acid (12·5 ml 88% acid) were boiled for 2 hours under reflux, and the unused dichromate was determined titrimetrically.

Extraction with boiling water

Soils were analysed for N extractable by boiling water as described by Keeney & Bremner.[12] 'Glucose' in boiling-water extracts was determined as in barium hydroxide extracts.

Extraction with sodium bicarbonate

Soils were analysed for bicarbonate-extractable N as described by MacLean,[13] except that ammonium in the Kjeldahl digest of the extract was determined by distillation and titration rather than by the Nesslerisation procedure used by MacLean. 'Glucose' in the extracts was determined as in barium hydroxide extracts.

Ammonia released by alkaline permanganate

Keeney & Bremner's modification[12] of Subbiah & Asija's method[14] was used to measure the ammonia released when soils were boiled with alkaline potassium permanganate.

Ammonia released by boiling with calcium hydroxide

The ammonia released when an aqueous suspension of soil and calcium hydroxide was boiled was measured as described by Prasad.[15]

Results

Extraction of soil with barium hydroxide

Fig. 1 shows the effects of time of extraction and soil/extractant ratio on the amounts of non-nitrate N and 'glucose' extracted by barium hydroxide from soil 54. The effects of barium hydroxide concentration on extraction of non-nitrate N are also shown. The extraction conditions (10 g soil shaken 30 minutes with 100 ml 0·1 N barium hydroxide) used throughout the remainder of this work were chosen mainly for convenience: the steepness of the slopes of graphs A, B, and C

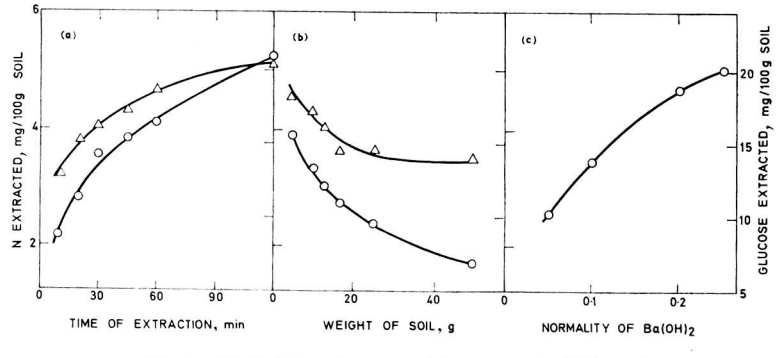

FIG. 1. *Effect of time of extraction (A), amount of soil (B), and $Ba(OH)_2$ concentration (C), on the extraction of non-NO_3 N (○) and 'glucose' (△) from soil 54 by $Ba(OH)_2$*

(Fig. 1) in the region of the chosen conditions indicates that there is little room for departure from these conditions, if results are to be reproducible.

Soil 54 was also used in an experiment to see whether grinding affected the extraction of organic matter by barium hydroxide. The amounts of non-nitrate N and 'glucose' extracted by 100 ml 0·1 N barium hydroxide in 30 minutes from 10 g air-dried soil that had passed a 2 mm sieve were 3·33 and 17·2 mg/100 g soil, respectively. When this soil was ground to pass a 100 mesh sieve and extracted under the same conditions, 3·65 mg non-nitrate N and 18·0 mg 'glucose' were extracted per 100 g soil. Thus fineness of grinding had little effect.

Effects of air-drying and subsequent air-dry storage

Most of the soils extracted with barium hydroxide had been stored for long periods after being air-dried. To assess the relevance of the results to fresh soils, or to soils stored for relatively short periods, an experiment was set up in which soils were air-dried, stored in sealed bottles for 3½ years and sampled at intervals. Changes in Δmin N and barium hydroxide-extractable organic matter were followed (Table II). Air-drying produced the usual increase in Δmin N; the non-nitrate N extracted by barium hydroxide also increased, as did barium hydroxide-extractable 'glucose'. Storage of the air-dried soil caused a progressive increase in Δmin N, but both barium hydroxide-extractable non-nitrate N and 'glucose' changed little, if at all, when the air-dried soils were stored for up to 3½ years. Experiments with soils 1–14 confirmed that the amounts of organic material extracted by barium hydroxide change little on storage of air-dry soil. In 1962, after 5½ years' storage, the mean amount of barium hydroxide-extractable 'glucose' for all 14 soils was 26·9 mg/100 g soil and the mean amount of non-nitrate N extracted was 7·19 mg/100 g soil. In 1967, after storage for a further 4½ years, the corresponding figures were 26·8 and 7·69 mg/100 g soil respectively. 'Glucose' extracted in 1962 was highly significantly correlated with that extracted in 1967 ($r = 0·995$): for non-nitrate N the corresponding correlation coefficient was 0·997.

Effects of straw on the extraction of organic matter from soil by barium hydroxide

Portions of a soil (soil 52, Table I) which had been stored air-dry for 185 weeks were moistened, alone, mixed with 0·6% straw (wheat stubble, containing 0·47% nitrogen) or mixed with 1·2% straw, and then air-dried. Barium hydroxide extracted 2·3 mg non-nitrate N/100 g soil from the soil alone, 2·6 mg from the soil mixed with 0·6% straw, and 3·0 mg from the soil with 1·2% straw. The corresponding amounts of barium hydroxide-extractable 'glucose' were 5·6, 7·3 and 8·4 mg/100 g soil. The soil without straw (initial mineral N content 1·6 mg/100 g) mineralised 2·6 mg mineral N per 100 g when moistened and incubated 21 days. The soil with 0·6% straw contained 0·9 mg *less* mineral N after incubation for 21 days than at the start; the soil mixed with 1·6% straw contained 0·8 mg *less* mineral nitrogen after incubation.

Barium hydroxide extracted slightly more non-nitrate N and 'glucose' from the soil with straw; this should indicate that the soil with straw will release slightly more mineral N on incubation than the unamended soil but in fact the soil with straw immobilised mineral N. This is a fundamental defect of chemical tests for potentially mineralisable N.

Heterogeneity of soil samples

Table III shows how Δmin N correlates with different soil measurements, taking soils 1–42 in 3 sets, 1–14, 15–28 and 29–42. Considering first the differences between the 3 sets, the correlation coefficients between Δmin N and either percentage N, barium hydroxide-extractable 'glucose' or barium hydroxide-extractable non-nitrate N for soils 15–28 did not differ significantly from the corresponding coefficients for soils 29–42, so that these soils can be treated as a single group. The correlation coefficient between Δmin N and percentage N was less for soils 1–14 than for soils 29–42; the difference between the two coefficients was highly significant. The correlation coefficient between Δmin N and barium hydroxide-extractable 'glucose' was significantly less for soils 1–14 than for soils 15–28; the coefficient between Δmin N and barium hydroxide-extractable non-nitrate N was also significantly less for soils 1–14 than for soils 29–42. As all soil

TABLE II

The effects of air-drying and storage on certain soil characteristics

Soil characteristics	Soil	Treatment					
		Moist	Air-dried				
		Unstored	Unstored	Stored 12 weeks	Stored 24 weeks	Stored 36 weeks	Stored 185 weeks
△min N, mg/100 g soil	50	1·1	1·5	1·6	1·9	2·3	2·8
	51	2·0	3·6	3·9	4·1	4·4	5·3
	52	0·8	2·2	2·0	2·4	2·3	2·6
	53	2·2	5·1	5·4	5·8	6·7	7·9
$Ba(OH)_2$ extracted non-NO_3 N, mg/100 g soil	50	2·3	2·3	2·7	2·7	2·5	2·8
	51	3·0	3·7	4·4	4·3	4·0	4·5
	52	1·5	2·0	2·1	2·2	2·0	2·0
	53	3·1	3·7	4·2	4·1	4·0	3·9
$Ba(OH)_2$ extracted 'glucose', mg/100 g soil	50	4·0	6·3	7·3	6·6	5·9	6·2
	51	9·2	13·9	14·9	14·6	14·1	14·9
	52	4·0	5·8	7·1	7·7	5·9	7·6
	53	10·9	17·5	20·0	18·8	18·6	18·2

TABLE III

Correlation coefficients between certain soil characteristics and release of N by soil, as measured either by uptake of N by ryegrass in pot experiments, or by △min N

Soils used	Soil characteristics	△min N	Uptake of N by ryegrass		
			No fertiliser N	75 mg fertiliser N per pot	150 mg fertiliser N per pot
1–14	% N	0·46	0·34	0·15	0·25
	△min N		0·84**	0·82**	0·83**
	$Ba(OH)_2$ extracted non-NO_3 N	0·59*	0·47	0·50	0·43
	$Ba(OH)_2$ extracted 'glucose'	0·81**	0·74**	0·69**	0·66**
15–28	% N	0·85**	0·86**	0·69**	0·71**
	△min N		0·85**	0·84**	0·89**
	$Ba(OH)_2$ extracted non-NO_3 N	0·83**	0·70**	0·73**	0·77**
	$Ba(OH)_2$ extracted 'glucose'	0·97**	0·84**	0·84**	0·88**
29–42	% N	0·93**	0·90**	0·93**	0·89**
	△min N		0·91**	0·91**	0·93**
	$Ba(OH)_2$ extracted non-NO_3 N	0·91**	0·86**	0·91**	0·92**
	$Ba(OH)_2$ extracted 'glucose'	0·95**	0·93**	0·97**	0·95**

* = value significant at P = 0·05
** = value highly significant at P = 0·01 in this and subsequent tables

measurements were done in the same way, soils 1–14 are probably a more heterogeneous group than are soils 15–42.

Correlations between soil characteristics and N release

Table III shows the correlations between four soil measurements and mineralisation of N, as measured either by uptake of N by ryegrass or by Δmin N; for correlations with other measurements on the same soils see Gasser,[6,7] and Hoyt.[16] Hoyt found that the amounts of chlorophyll extracted from soil by acetone were significantly correlated (r = 0·77**) with the uptake of N by ryegrass from soils 29–42, when not given fertiliser N.

The N mineralised (Δmin N) was, for all three sets of soils, highly significantly correlated with barium hydroxide-extractable 'glucose'. Total soil N (with one exception where the correlation was not significant) and barium hydroxide-extractable non-nitrate N (with one exception, where the correlation was significant) were also highly significantly correlated with Δmin N for each group of soils, but the correlation coefficients were less than those between Δmin N and barium hydroxide-extractable 'glucose'.

Of the four measurements considered in Table III, Δmin N gave highly significant correlations with the uptake of N by ryegrass in all three sets of soil, and was more consistent in this respect than any of the other measurements. All four measurements on soils 15–28 and 29–42 were highly significantly correlated with the uptake of N. With soils 1–14, the best correlation with N uptake was given by Δmin N, followed by barium hydroxide-extractable 'glucose' and then by barium hydroxide-extractable non-nitrate N; percentage N was not significantly correlated with uptake of N.

A feature of Table III is that in no case did the correlation coefficients between uptake of N and any one of the soil tests differ significantly, whether uptake was measured in pots not given fertiliser N, or in those given 75 or 150 mg fertiliser N. The effectiveness of the various soil tests in predicting N uptake from the soil is not measurably influenced by the presence or absence of fertiliser N.

The most informative results in Table III came from soils 1–14, for two reasons. Firstly, the non-significant correlation between percentage soil N and uptake of N by ryegrass is in accord with the suggestion made in the preceding section that this group of soils is more heterogeneous than soils 15–28 or 29–42. Correlation coefficients calculated for soils 1–14 thus provide a more critical test for a method of predicting N release than do correlation coefficients calculated for the other soils. Secondly, although the amounts of mineral N present in the soil at the beginning of the pot experiments were comparable (the mean for soils 1–14 was 32 mg nitrate- + ammonium- N per pot, 30 mg for soils 15–28 and 30 mg for soils 29–42), much more N was taken up by the ryegrass in 1957 (soils 1–14) than in 1958 (soils 15–28) or 1959 (soils 29–42); in 1957 the mean uptake of N from all 14 soils not given fertiliser N was 82 mg per pot; in 1958, it was 45 mg and in 1959, 68 mg. The N taken up by the ryegrass in these pots came partly from N mineralised by the soil during growth and partly from that initially present in the soil. The amount of mineral N originally in the soil is not necessarily correlated with the amount of N mineralised by the soil during growth of the ryegrass. Because a larger proportion of the N harvested was mineralised *during* the pot experiment in 1957 than in either 1958 or 1959, a correlation coefficient between N uptake and soil test for soils 1–14 is a better indication of the ability of a test to predict N release than is the corresponding coefficient for soils 15–28 or 29–42, where the correlation will be more influenced by the amount of mineral N initially present. For these reasons soils 1–14 were selected for use in the more detailed investigations of the next section.

Comparisons between some chemical tests for potentially mineralisable N

Table IV gives the correlations between a range of soil measurements and mineralisation of N as measured by either uptake of N by ryegrass or Δmin N. The first six measurements were done by Gasser within a few months of sampling the soils; measurements 7–15 were done in 1967, ten years

TABLE IV

Correlation coefficients between certain soil characteristics and release of N by soil, as measured either by uptake of N by ryegrass in pot experiments, or by △min N, using soils 1–14

Soil characteristics	△min N	Uptake of N by ryegrass				
		Fertiliser N applied, mg per pot				
		0			75	150
		N in 1st cut	N in all cuts except 1st	N in all cuts	N in all cuts	N in all cuts
% N[a]	0·46	0·29	0·34	0·34	0·15	0·25
% organic C[a]	0·66**	0·50	0·53*	0·54*	0·37	0·43
NH_4 N in fresh soil[a]	0·86**	0·76**	0·76**	0·79**	0·72**	0·77**
NO_3 N in fresh soil[a]	0·63*	0·25	0·33	0·31	0·38	0·34
(NH_4 + NO_3) N in fresh soil[a]	0·83**	0·59*	0·63*	0·64*	0·64*	0·65*
△min N[a]		0·75**	0·84**	0·84**	0·82**	0·83**
$Ba(OH)_2$ extracted C	0·63*	0·44	0·57*	0·54*	0·56*	0·49
$Ba(OH)_2$ extracted non-NO_3 N	0·57*	0·37	0·50	0·47	0·50	0·43
$Ba(OH)_2$ extracted 'glucose'	0·78**	0·60*	0·71**	0·70**	0·65*	0·61*
Boiling water extracted N	0·62*	0·53	0·67**	0·65*	0·51	0·53
Boiling water extracted 'glucose'	0·70**	0·60*	0·72**	0·70**	0·58*	0·60*
$NaHCO_3$ extracted N	0·79**	0·58*	0·72**	0·70**	0·67**	0·65*
$NaHCO_3$ extracted 'glucose'	0·84**	0·69**	0·77**	0·77**	0·70**	0·70**
NH_4 N released by $KMnO_4$	0·52	0·16	0·32	0·28	0·21	0·22
NH_4 N released by $Ca(OH)_2$	0·76**	0·50	0·71**	0·67**	0·58*	0·62*

[a] measured by Gasser[6]

after the samples were taken. Some of the correlation coefficients for barium hydroxide-extractable non-nitrate N and 'glucose' differ slightly from those given for the same soils in Table III because the barium hydroxide extractions for Table III were done in 1962, and those for Table IV in 1967. The uptake of N by ryegrass not given fertiliser N is shown in three ways, the N in the first cut, the N in the second and third cuts plus that in the stubble, and the N in all cuts plus stubble taken together. Of the three measurements, N in the first cut is always slightly less well correlated with the soil measurements.

The best correlation with plant uptake of N was given by Δmin N. The amount of ammonium N initially in the soil gave a correlation with uptake of N only slightly smaller than that given by Δmin N. However, this result is probably a unique feature of this particular group of soils, because, whereas Δmin N correlated well with plant uptake in soils 1–14, 15–28 and 29–42, ammonium initially present in the soil was not significantly correlated with the uptake of N (unfertilised pots, all cuts) in soils 15–28 ($r = 0·17$) and soils 29–42 ($r = 0·26$). These differences were almost certainly caused by differences in the storage of the soil samples. Pending measurement of ammonium N 'initially' present in the soil, soils 1–14 were stored at 2–5°, and soils 15–42 at −10°. As ammonification can proceed faster than nitrification at temperatures just above freezing,[17] some ammonium probably accumulated during storage of soils 1–14, in contrast to soils 15–42, where mineralisation of N was unlikely to be measurable.

Non-nitrate N and C extracted by barium hydroxide were very closely correlated with each other ($r = 0·992$), so it is not surprising that both were correlated to about the same extent with uptake of N by plants in the pots. Non-nitrate N extracted by barium hydroxide was less well correlated with uptake of N by plants than was the N extracted by boiling water, in agreement with Keeney & Bremner's results,[12] and the N extracted by boiling water was less well correlated with uptake of N than was that extracted by sodium bicarbonate. Likewise, N mineralisation, as measured by Δmin N, is better correlated with bicarbonate-extractable N ($r = 0·79$) and N extracted by boiling water ($r = 0·62$), than with N extracted by barium hydroxide ($r = 0·57$). This is largely because some of the soils used were slightly acid. When soils of pH 6·5 or less are excluded (leaving 11 soils), the correlation coefficient between Δmin N and barium hydroxide-extractable N increases to 0·97. Barium hydroxide extracts disproportionately more N from acid or slightly acid soils than from neutral or slightly alkaline soils; for example it extracted 15·2 mg non-nitrate N from 100 g soil 1 (pH 5·3), whereas it extracted only 5·4 mg from soil 5 (pH 8·0), although the two soils mineralised almost the same amount of N when incubated (Table I). Boiling water extracted 16·5 mg N from 100 g soil 1 and 13·9 mg from soil 5, and bicarbonate extracted 8·1 and 4·8 mg N, respectively, so that the results from these methods were more in accord with the incubation experiments than were the barium hydroxide results.

Barium hydroxide extracts, boiling water extracts, and bicarbonate extracts all contain polysaccharide. From 30% (soil 7) to 16% (soil 4) of the C extracted from soils 1–14 by barium hydroxide was calculated to occur in 'glucose' as determined by reaction with anthrone, and the mean for all 14 soils was 23%. Although the three procedures differ in their ability to extract 'glucose' (the mean amount of 'glucose' extracted from all 14 soils by barium hydroxide was 27 mg/100 g soil, that by boiling water was 75 mg/100 g soil and by sodium bicarbonate, 32 mg/100 g soil), the amounts dissolved

from different soils by the three methods are closely correlated. The correlation coefficient between 'glucose' extracted by barium hydroxide and that extracted by boiling water was 0·91; between 'glucose' extracted by barium hydroxide and by bicarbonate it was 0·98, and between 'glucose' extracted by boiling water and by bicarbonate 0·93. These results suggest that all three methods are extracting 'glucose' from the same source, but with differing effectiveness. For each of the three extractants, the amount of 'glucose' extracted was better correlated with uptake of N (or Δmin N) than was the total amount of non-nitrate N extracted.

Ammonia released by potassium permanganate[14] was not significantly correlated with either uptake of N by ryegrass in pots or with Δmin N. Ammonia released by calcium hydroxide distillation[15] was correlated with uptake of N to about the same extent as N extracted by boiling water.

Correlation between crop yields in the field and certain soil measurements

Table V gives the correlations and regressions between barley production in the field and some soil measurements. Results from all three years were combined. Treating the results in this way conceals differences: some statistically significant differences between the 1958 and 1959 results were demonstrated by Gasser & Jephcott.[7] For example the regression coefficient for response on min N was $-0{\cdot}61 \pm 0{\cdot}27$ in 1959, whereas it was $-1{\cdot}52 \pm 0{\cdot}21$ in 1958, a significant difference in slope. However, for the other soil measurements listed in Table V, differences are smaller, and too few results are available to justify treating each year separately.

The mean yield of unfertilised crop over all the experiments was 26·6 cwt grain/acre and the mean response to 0·5 cwt fertiliser N was 8·3 cwt grain/acre. Correlation coefficients between the soil measurements and unfertilised yield are consistently higher than those between response and soil measurements, almost certainly because the errors in measuring response are larger than those in measuring yield. 'Glucose' extracted by barium hydroxide is more closely correlated with yield than either percentage N, Δmin N, or min N, in that order. Correlated with response, 'glucose' takes second place to min N, but is better than either Δmin N or percentage N. The close correlation between yield and percentage N (0·72) suggests a homogeneous group of soils, which is reasonable because they all came from the same part of the country, all were growing barley and all had pH > 6·5.

As in the pot experiments, the N available to a crop comes presumably from two sources, the mineral N present in the soil when the crop is sown, and the N mineralised by the soil during crop growth. The N uptake (and yield) of a crop might therefore be expected to be more closely related to a combination of a measure of N in the seedbed (given by, say, min N) with a measure of potentially mineralisable N (given by, say, Δmin N, percentage N or barium hydroxide-extractable 'glucose'), than to either measure taken separately. When the multiple regression of yield on Δmin N and min N was calculated, the residual variance (i.e. variance not accounted for by the regression) was less than that calculated for the regression of yield on Δmin N alone, but the decrease did not quite reach significance ($P = 0{\cdot}05$). Likewise, the regression of yield on percentage N was improved by including min N, but the decrease in residual variance was not significant: with barium hydroxide-extracted 'glucose' there was no improvement when min N was included.

The relationship between yield and soil test is probably curvilinear. Unfortunately the present set of results is not very suitable for testing this, as there is only one soil in the upper range where the curves might be expected to flatten out. Equations of the type $y = A + Bx + Cx^2$ were fitted to the data. For the regression of response to N on barium hydroxide-extractable 'glucose', curvilinear regression was significantly better than linear regression; for the regression of yield on barium hydroxide-extractable 'glucose', curvilinear regression was not significantly better than linear regression.

The highly significant correlation between barium hydroxide-extractable 'glucose' and yield of barley in the field experiments must be interpreted with caution. Many factors, for example inorganic N residues below sampling depth or the prevalence of plant disease, can upset such correlations. The close correlation obtained indicates that such disturbing factors were not of great importance in these particular experiments.

Discussion

There can be little doubt that aerobic incubation remains the most reliable soil test for potentially mineralisable N. However it has several disadvantages in routine soil testing of which the most important are: the preparation of soil samples for incubation must be carefully standardised, as drying conditions, sieving and time of storage all influence the results; two extractions are needed, together with the associated analytical measurements, one before and one after incubation;

TABLE V

The relationship between certain soil characteristics and crop production at 36 different sites

Field measurement	Soil characteristics	Correlation coefficient	Regression equation of yield (or response), (y), on soil measurement (x)
Yield of barley without N fertiliser, cwt barley/acre	min N	0·63**	$y = 1{\cdot}17\,x + 13{\cdot}1$
	△min N	0·68**	$y = 0{\cdot}390\,x + 14{\cdot}1$
	% N	0·72**	$y = 75{\cdot}0\,x + 11{\cdot}8$
	$Ba(OH)_2$ extracted 'glucose'	0·83**	$y = 0{\cdot}661\,x + 13{\cdot}2$
Response of barley to 0·5 cwt N, cwt barley/acre	min N	−0·57**	$y = -0{\cdot}78\,x + 17{\cdot}3$
	△min N	−0·47**	$y = -0{\cdot}199\,x + 14{\cdot}6$
	% N	−0·45**	$y = -34{\cdot}7\,x + 15{\cdot}1$
	$Ba(OH)_2$ extracted 'glucose'	−0·54**	$y = -0{\cdot}317\,x + 14{\cdot}7$

and incubation must be done under controlled conditions for periods usually measured in weeks.

The most serious objection to chemical tests, as distinct from incubation tests, is that no single chemical measurement is likely to give due weight to both the processes leading to mineralisation of N and those leading to immobilisation. Mineralisation is accompanied by immobilisation of mineral N when organic materials low in N are decomposing in soil, so that a soil with a large mineralisation potential, as indicated by a soil test, may in fact mineralise no N if immobilisation is active. The highly significant correlations often obtained between the results of chemical tests and release of N are more likely to indicate that immobilisation is not very important in the soils used, rather than that the tests made allowance for the balance between mobilisation and immobilisation. The soils tested in this paper had been sampled in spring, so that fresh plant residues of wide C/N ratio would have had several months to decompose before the samples were taken.

Firm recommendations about the best chemical test cannot be made from the present work. The range of soils used was too narrow, and the effects of storage are too uncertain for all except the barium hydroxide tests. Of the tests proposed by other authors, boiling-water-extractable N and sodium bicarbonate-extractable N have most promise. Both involve an extraction, followed by a Kjeldahl digestion and ammonium determination, a more complex sequence of operations than is usual in routine soil testing laboratories. The very simple calcium hydroxide distillation method may also prove useful, even though it correlates a little less well with N release than some of the other methods. However, the most promising chemical tests to emerge from this work involve the measurement of 'glucose' extracted by either sodium bicarbonate, barium hydroxide, or boiling water. These, particularly the first two, are easy to do and merit testing on a wider range of soils. A disadvantage is that the anthrone reagent needs to be carefully purified to keep the blank low relative to the small readings obtained with barium hydroxide or sodium bicarbonate extracts of infertile soils. Boiling-water extracts have the advantage that the same results are obtained whether the soil sample is analysed moist, immediately after drying or after air-dry storage for 2 months.[12] Barium hydroxide extracts have the disadvantage that air-drying increases the amount of material extracted, although, once air-dry, prolonged storage does not further alter the results. Another disadvantage of barium hydroxide is that it extracts more organic C, N and polysaccharide from acid soils than would be expected from their mineralising power. It is not known how sample treatment and storage influence sodium bicarbonate extracts, although by analogy with barium hydroxide, air-drying probably influences the results. Barium hydroxide and hot water gave more reproducible results than bicarbonate.

The results in this paper suggest that the organic N dissolved by barium hydroxide, boiling water and sodium bicarbonate is fairly closely associated in some way with mineralisation processes in soil. The association between the 'glucose' content of the extracts and mineralisation is even closer. A possible explanation is that the polysaccharides in such extracts are associated with the soil biomass; soils with large microbial populations would mineralise much N and contain much polysaccharide; conversely soils with small populations would mineralise little N and contain little polysaccharide. This is consistent with the fact that barium hydroxide and boiling water are good reagents for extracting heavily labelled organic matter from soils containing a heavily labelled biomass.[3] However, this explanation must be qualified when applied to soils containing decomposable organic matter of wide C/N ratio, where any N mineralised will be promptly taken up by the xymogenic population. Acid soils are also exceptional, at least so far as barium hydroxide extracts are concerned. Perhaps the amount of material extracted by barium hydroxide is no longer proportional to the size of the biomass, if, for example, barium hydroxide extracted much non-biomass C from acid soils. Or perhaps the assumption that the amount of biomass in a soil is directly proportional to its power to mineralise N is invalid for acid soils: the biomasses in acid and neutral soils will differ qualitatively, in addition to the quantitative differences considered.

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COMPARISON OF WHEAT VARIETIES BY STARCH-GEL ELECTROPHORESIS OF THEIR GRAIN PROTEINS

By G. J. DOEKES

Single extraction of wheat flour with water proved to be necessary for obtaining clear electropherograms of the albumin/globulin fraction; triple extraction was required to obtain a clear distinction of the gliadin components. As the 'electrophoretic mobility' does not have a constant value, a different characteristic, the R_v value, analogous to the R_f value in chromatography, is suggested as a measure of the relative mobility of the components, a characteristic which is not affected by experimental factors.

From densitograms of gliadin fractions, 80 wheat varieties and selected lines from various parts of the world were arranged into five main groups, which formed a morphological series. Each main group comprised of a number of sub-groups which in certain cases were found to be based on genetic relationships. Densitograms of the albumin/globulin fractions could be used in conjunction with those of the gliadins for determination of wheat varieties.

Introduction

In the search for methods of assessing quality characteristics of wheat a study was made of the possibilities which electrophoresis offers.

Interest in the electrophoresis of wheat protein has grown steadily in the past few decades; Feillet[1] mentions most of the authors in this field. In a large number of cases starch gel was used as supporting material because it has very great resolving power in respect of wheat protein. The use of the starch gel method, however, usually requires the extraction, the preparation of gels, the pH and ionic strength of the buffer, and the age of the gels and the protein solutions to be rigidly controlled for reproducible results to be obtained.

Nevertheless, in the investigations under review this method was employed and applied to the protein composition of a large number of wheat varieties.

Experimental

Preparing the flour

After the grains had been moistened with water to 15%, the samples were ground to 70% flour extraction in a Brabender Quadrumat-Junior laboratory mill.

Extraction

The following solvents were examined: aluminium lactate–lactic acid buffer (pH = 3·1), lactic acid solutions, acetic acid (0·05 N), sodium pyrophosphate buffer (pH = 7), 70% alcohol, and water. These solvents were used with and without addition of urea (3M); the extracts were subjected to electrophoresis. It appeared that tailing occurred in the majority of cases, causing indistinct electropherograms. Dialysis of the extracts effected only little improvement. Tailing was consistently associated with the presence of glutenin at the place of application in the gel. Alcoholic and aqueous extracts, without urea, gave the best electropherograms.

Water was finally chosen as solvent because this eliminated the need for dialysis of the extracts, which was time-consuming and often unreliable. However, a disadvantage was the low concentration of gliadins in the extracts, compared with those of the albumins and globulins.

Moureaux[2] described the effect of gliadin solubility in water being reduced by all kinds of salts, especially phosphates (including phytate). Flour contains such salts in sufficient amounts to prevent the gliadins from dissolving.[3] The salts themselves are water-soluble, however, and if the residue from flour extraction with water is extracted further, the subsequent extracts contain more gliadins (see Fig. 1).

A single extraction was sufficient for electrophoresis of albumins and globulins; in order to obtain a good representation of the gliadin pattern, the flour had to be extracted three times, and the third extract used for assessment.

After the flour-to-water ratio and the extraction time that were the most favourable for yielding an ample amount of protein had been determined, the schedule shown in Table I was followed.

Fraction *S1* contains mainly albumins and globulins, fraction *S2* approximately equal concentrations of gliadins and albumins/globulins, and fraction *S3* mainly gliadins.

When an electropherogram of an *S2* fraction was made, either the gliadins, or the albumins and globulins were not separated distinctly enough, depending on the duration of the

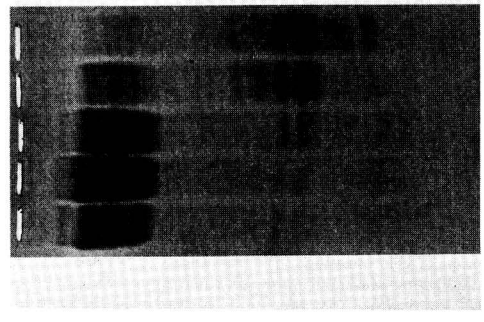
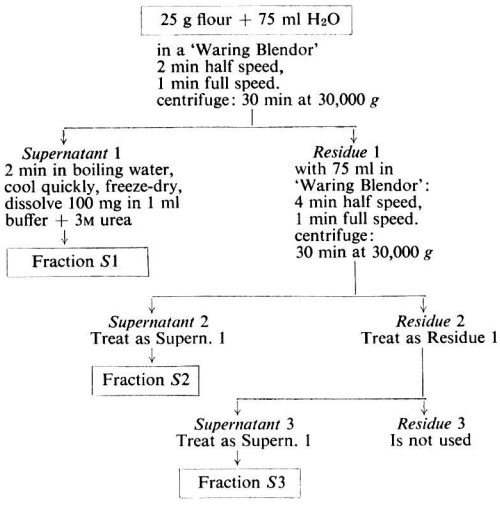


FIG. 1. *Extraction of flour with water*

Top to bottom, extractions 1 to 5. Decline of albumin/globulin concentration and simultaneous increase of gliadin concentration, until this declines after 4 extractions.

TABLE I



electrophoresis. As the gliadins apparently required a running time different from the other proteins, the *S3* and *S1* fractions were used instead of the *S2* fractions.

Freshly prepared protein solutions gave slightly indistinct electropherograms, and the solutions were therefore kept for at least one night (at 3°) before they were used.

Electropherograms of some high-protein wheats suffered from tailing, caused by too high gliadin concentration. In these cases it was necessary to dilute the protein solutions with buffer.

Buffer solution

The buffer used in the gel and electrode boxes was the aluminium lactate–lactic acid buffer (pH = 3·1) described by Jones *et al.*[4] Aluminium lactate was prepared according to the method of Jones & Cluskey.[5] The ionic strength of the buffer was kept at 0·06 as this value lies in the middle of the range (0·01–0·1) that offers optimum conditions both for obtaining sharp protein zones and for preventing undesirable heat development. To the stock solution of the buffer (5 l), 2 ml thymol (10% in 96% alcohol) were added to prevent mould growth, and the solution was kept in the dark at 3°.

Gels

As the electrophoresis results had been found to be largely dependent on the properties of the gel, the preparation of the latter was standardised as far as possible.

13 g 'starch hydrolysed' (Connaught Medical Research Laboratories, University of Toronto, Canada) were suspended in 100 ml buffer. The mixture was placed in a heating jacket and mechanically stirred for a fixed period of time. After complete gelatinisation, urea was added to a concn. of 3M, and heating was continued for some time. Dissolved gas was removed from the mixture by boiling it in the vacuum of a water aspirator. The time required for each operation was adhered to in subsequent preparations.

Preparing the gels in 'Perspex' troughs (in which layers of the walls can be removed for cutting the gels) takes unnecessary quantities of starch and too much time. The gels are difficult to manage and easily damaged in staining. These drawbacks are obviated if the gels are made on glass plates (in the present case 20 × 10 × 0·3 cm).*

One hour after the gelatinised starch solution was poured off and cooled to room temperature, the gels were covered with PVC film to prevent drying, and kept at 3° for at least 4 days before they were used, because in gels which were too fresh the electrophoresis results were not reproducible and the mobility of the proteins was much less than in older gels. This effect might be accounted for by the retrogradation of gelatinised starch, which seems to be completed after about 4 days.[6]

Electrophoresis

At one end of the gel plate a 4 cm strip of the PVC was cut off and, by suction through a small flattened piece of steel tubing, slots in to which a few drops of the fractions were introduced were cut into the gel. The gel was placed with its ends on the edges of the electrode compartments, and connected with the buffer solution by means of filter paper. Electrophoresis was consistently carried out at 13V/cm, and a standard current of 30 mA, for fractions *S1* for 80 minutes, for fractions *S3* for 160 minutes. The apparatus was installed in a room where the temperature was 3° and air was circulated, so that additional provisions for cooling the gels were not necessary.

Cutting, staining and scanning

The topmost layer of the gel was sliced off by means of a steel wire 0·1 mm thick. This was necessary to eliminate interfering surface effects. In order to have a uniformly thick layer for subsequent operations the device shown in Fig. 2 was designed.

The gel was then placed in a tray containing the following staining solution: 1100 ml methanol–acetic acid–water (5 : 5 : 1), with 0·5 g water-soluble nigrosine (Amsterdamse Chininefabriek) and 0·25 g 'Amidoschwartz 10B' (Merck, Darmstadt). In order to prevent irregular deposits of stain particles, the tray was placed on a platform which was slowly tilted up and down. This mixture of staining substances was chosen because the gluten proteins are stained better by nigrosine than by Amidoschwartz.[7] The latter stains the other proteins more intensively.

The duration of staining varied between 10 and 30 minutes, depending on the freshness of the staining solution. The gel was then rinsed several times with 5% acetic acid; after about 4 hours the background contrasted sufficiently with the protein bands. When a gel had to be kept overnight before it could be scanned, it appeared that the acetic acid caused the gliadin bands especially to run. Timely substitution of the acetic acid by a 5% sodium chloride solution prevented this.

After the gel was washed, densitograms were made of the protein tracks by means of a Vitatron Densitometer. Fig. 3 shows an example of the results obtained.

R_V values

A study of a number of densitograms led to the conclusion that all the samples of one variety examined had the same protein components, each holding its specific position among the others accurately. Consequently each component may be

* This method was developed at this Institute by A. Graveland

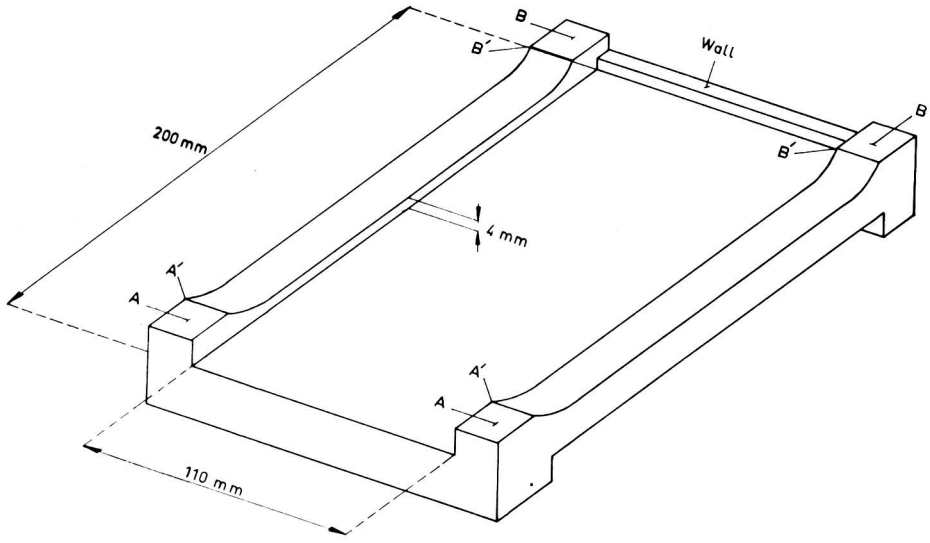


FIG. 2. *Device for cutting the gel sheets*

A taut wire is pulled along the edges from A to B. At A' it sinks into the gel and emerges at B'. The glass plates are 0·3 cm thick, so that there is always a gel 0·1 mm thick left on them. The raised edge ('wall') prevents the gel from sliding off when cut.

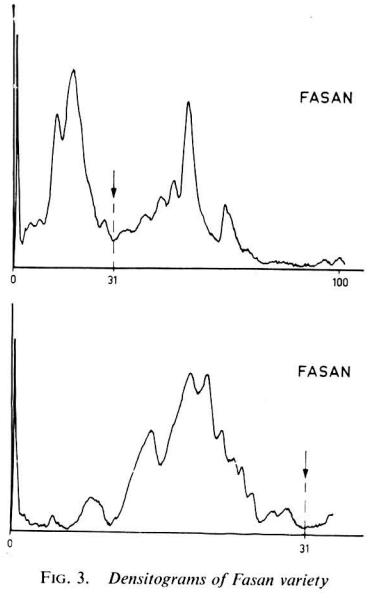


FIG. 3. *Densitograms of Fasan variety*

Top: fraction *S1*

Bottom: fraction *S3*; increased concentration and longer electrophoresis have made the sector to the left of the arrow better visible.

referred to by means of a number which represented its running speed. For this purpose the term 'electrophoretic mobility' is commonly used, expressed in cm/sec per V/cm, which indicates that the mobility depends solely on the voltage. There are other factors, however, which also have some effect, viz. the age, the viscosity and, perhaps, the temperature of the gel, as well as the age of the protein solution. The 'electrophoretic mobility' is therefore by no means constant. To eliminate these factors the relative mobilities of proteins were determined instead of the absolute ones, as follows:

The fastest component, clearly visible only in fraction *S1*, was allocated the value 100 (see Fig. 3), the starting point, 0.

Between these limits, all components were allocated proportional values, referred to as R_v. It is comparable with R_f in chromatography if the fastest component in an electropherogram can be compared with the eluent front in a chromatogram. Fig. 4 shows an example of the use of R_v values.

Calculating R_v values from a large number of densitograms is very time-consuming, and it is therefore convenient to use an interpolation graph. This diagram is placed under the densitogram and light is projected through both; the R_v values are read from the diagram on to the densitogram.

Definition of the wheat protein fractions

Osborne,[8] using solubility characteristics, distinguished the following fractions of wheat protein, which are still recognised by most authors: albumins, soluble in water; globulins, soluble in NaCl solutions; gliadins, specifically soluble in 70% alcohol; glutenin, soluble only in dilute acid or sodium hydroxide solution.

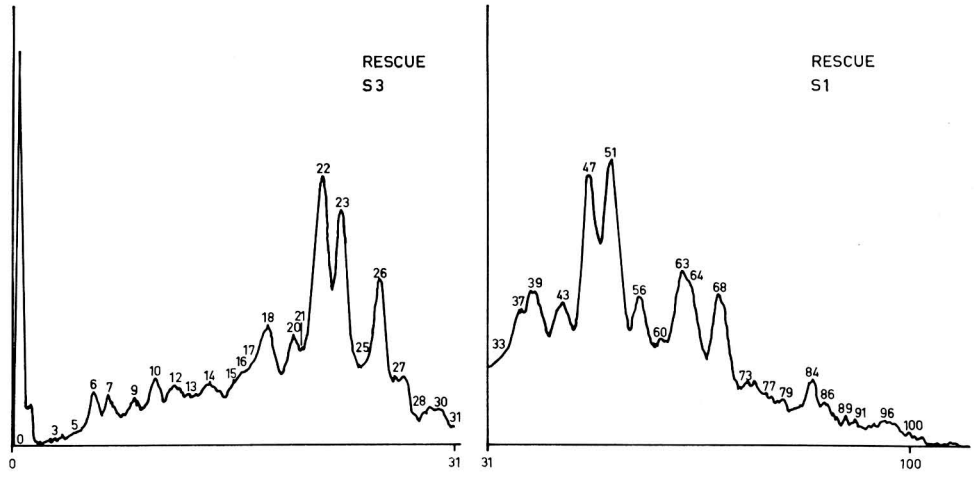


FIG. 4. *Densitograms of Rescue variety, with R_v values*

Right: *S1* with albumins and globulins only
Left: *S3* with gliadins

This characterisation, which has become traditional, is, however, open to objections from the results of electrophoresis. With regard to albumins and globulins electropherograms of aqueous extracts reveal the same protein components as those of extracts made with NaCl solutions. Any slight differences are within the range of errors inherent to the test. The differences found by Osborne were probably of a quantitative, and not of a qualitative, nature. In any case they have no practical significance for the study of wheat protein. With regard to gliadins, these dissolve well in water provided this contains no salts. Electrophoresis moreover shows that with 70% alcohol, albumins and globulins are extracted in addition to gliadins.

If the terms used by Osborne are to be maintained, new definitions will have to be drawn up which agree with the electrophoresis results. For starch gel electrophoresis (at pH = 3·1) a definition based on the following observations is suggested:

Fig. 1 reveals two separate protein groups: a fast-moving group, found in large amounts in fractions *S1* and *S2*, and a slow-moving group, contained especially in *S3* and subsequent fractions. A distinct absorption minimum between the two groups was observed in all densitograms (see arrow in Fig. 3). By reference to 44 densitograms (viz. those used in studying the effect of N fertilisation) the position of this point was determined and found to be at $R_v = 31$ (in 7 out of the 44 samples it was $R_v = 30$, in 29 cases $R_v = 31$, and in 8 cases $R_v = 32$).

In accordance with this, it is suggested that the gliadins should be defined as the protein fraction which in starch-gel electrophoresis in aluminium lactate buffer (pH = 3·1) is segregated between the starting point and a distinct absorption minimum at $R_v = 31$. The albumins/globulins are defined as the proteins having R_v values between 31 and 100. Glutenin is the fraction the molecular size of which causes it to escape electrophoresis; it remains at the starting point in the gel.

Wheat varieties examined

The Foundation for Agricultural Plant Breeding, the Institute of Research on Varieties of Field Crops and the Institute of Phytopathological Research, all at Wageningen, supplied 80 varieties and selected lines from West, Central and East Europe, India, and North and South America. The samples had been grown in Holland for quality testing. In addition there were 18 samples of 3 varieties, Orca, Fasan and Ring which, under otherwise identical conditions, had been dressed with different amounts of N at various stages of growth, as follows: 30 kg N (as ammonium nitrate limestone)/ha at tillering; 0 or 30 or 60 kg N/ha at stem extension; and 0 or 30 or 60 kg N/ha at the beginning of flowering.

As the use of N fertiliser has the effect of increasing the protein content of the grains, samples of the three varieties showed ranges of protein contents.

Results and Discussion

Effect of N fertilisation on the electrophoretic pattern

It seemed interesting to see if there were any changes in the electropherogram with increases in the protein content. Samples of flour made from Orca, Fasan and Ring were examined and Fig. 5 shows the results obtained with the Fasan variety. The other varieties showed similar results.

Fig. 5 shows that N dressing has no demonstrable influence on the electropherogram, irrespective of the amount added or the stage of growth at which it was added.

Some effect was discernible, however, on the extraction yields: the yield of *S1* protein from samples of the same variety remained virtually constant, but the yield of *S3* protein increased steadily. The reason this effect is not observed in

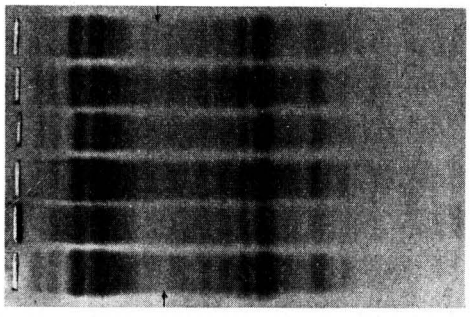


FIG. 5. *Samples of Fasan variety*
Top to bottom: increasing N fertiliser dressing, resulting in a protein content of the flour ranging from 9·2 to 12·1%

the total intensity of the pattern is that in preparing the protein solutions the same amount of dry matter was dissolved each time.

According to Coulson & Sim[9] conditions such as climate and soil properties do not change the electropherogram either. Lee & Ronalds[10] found the same although some slight variations were observed, which they ascribed to the test conditions.

On the strength of this information it is likely that the electropherogram of the protein of a wheat variety constitutes a genetic image which will not be changed by external conditions of growth.

Classification of wheat varieties according to gliadin pattern

After assessment of the effect of N fertilisation, the influence of variety was studied by reference to densitograms of 80 varieties and selected lines. In the first instance five main groups were observed in the densitograms (see Fig. 6) viz. one with a compact gliadin pattern, one with a well spread pattern clearly divided up into components, and a series of three consecutive intermediate types.

Each main group moreover consisted of sub-groups comprising varieties with very similar gliadin patterns. This arrangement (Table II) provided a morphological series consisting of a number of sub-groups in each main group.

From Fig. 6 it may be inferred that the very compact gliadin pattern is displayed mainly by winter wheat of main group I, whereas the well-spread pattern is found mainly in spring wheat of main group V. Between these extremes transitional types are found, among both spring and winter wheats. Generally, it might be said that: main group I contains soft-grain West-European winter wheats, having fairly poor baking qualities; main groups II, III and IV are varieties of varied origin and properties—winter wheats with large and small cold requirements, as well as true spring wheats; main group V contains predominantly spring wheats, characterised by hard vitreous grains and very good baking qualities. The scheme presents only a tentative classification, on purely morphological grounds. It is therefore open to modification. For some of the varieties it was difficult to find the appropriate place in this sub-group system with certainty.

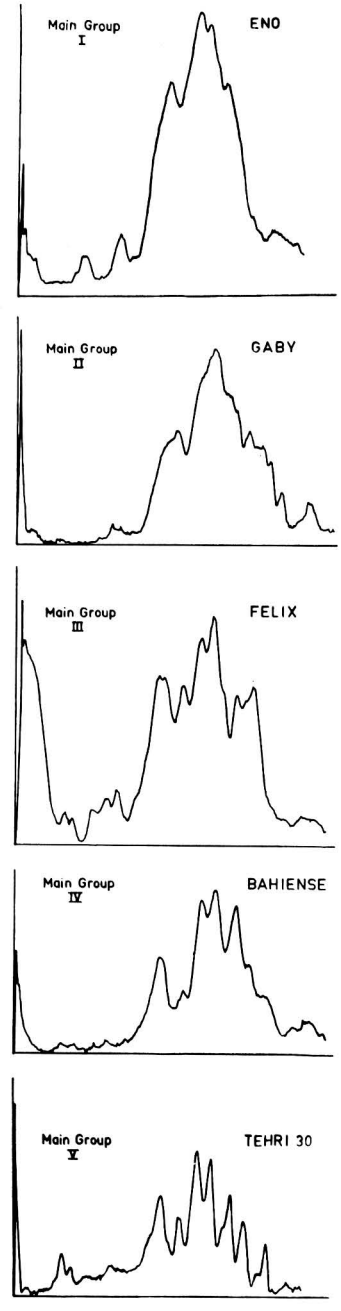


FIG. 6. *Morphological series of gliadin patterns*

TABLE II

Morphological series of gliadin patterns

Main Group	Sub-Group	Winter Wheat	Spring Wheat
I	a	Eno,* Flamingo	
	b	Vaillant, Cambier 13307–14	
	c	Heine VII, Nord, Capelle	
	d	Rembrandt, Heine 3950	
II	a	Carstens VI, Carstens 854, MGH 63–187, Doerfler W 16	Orca, MGH 60–293
	b	Sylvia, Tadorna	Gaby * Jufy I, Nepal 81
	c	Flevina, Novi Sad 58, Dippe's Triumph, Dippe's 2076, Cleo, CIV 7010	Peko, Fasan, Perso
III	a	Felix,* Manella	Carpo, CIV 228–5
IV	a	Hybride 46, Stella, Marchal, Prof. Delos, Minister	Caucho, Chili 3117–3
	b	Kharkov (C.I. 1442), Novi Sad, Banatka	Koga II, Cebeco 1009 Buck Bolivar, Bahiense,* Vilela Mar
V	a	Kasni K.B., Obilje B	Thatcher, Saunders, General Roca
	b		Opal, Doerfler 511
	c		Lee, Tehri 30,* Kalimpong 2, Rescue

* Example of densitogram shown in Fig. 6

Notes on the sub-group arrangement

More details can be given about the sub-groups listed in Table II, and a few examples are listed here.

IIb: Sylvia and Tadorna (Fig. 7) have very similar gliadin patterns.

These two varieties were selected from the descendants of the same cross, viz. (Chinese 165 × Panzer III) × Heine IV × (Teutonen × Hindukush 516) × Merlin.

IIc: Peko, Fasan and Perso (Fig. 8) also form a sub-group. They have the Peragis × Heines Kolben combination in common in their pedigree.

Va: Thatcher and Saunders are North American varieties, General Roca comes from Argentina (Fig. 9). Saunders and General Roca are direct descendants of Thatcher.

These three examples indicate that the sub-groups are based on genetic relationships.

Vc: Another example is shown in Fig. 10.

According to the official American list of varieties Lee would be Hope × Timstein. Hope is a cross between *Triticum timopheevi* (from Eurasia) and Marquis, which in turn is a combination of Red Fife (Russia) and Hard Red Calcutta (India). Timstein is a cross between *T. timopheevi* and Steinwedel (Australia), which is a combination of Harvest Queen (North America) and Goldendrop (England). There are indications, however, that the official description of Lee is not correct and that its descent is not known with certainty.

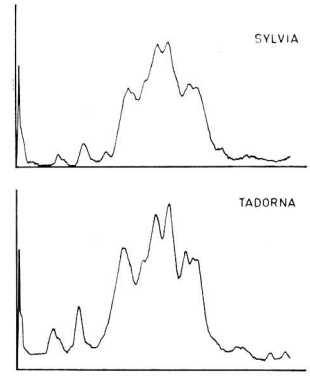


FIG. 7. S3 *densitograms of two IIb varieties*

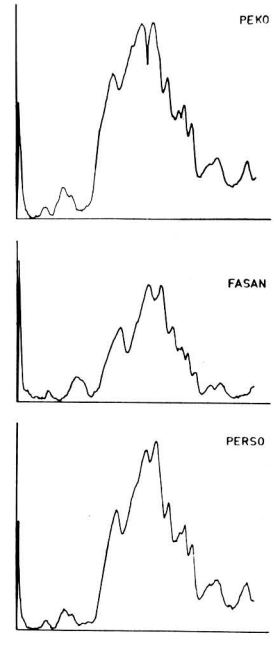


FIG. 8. S3 *densitograms of three IIc varieties*

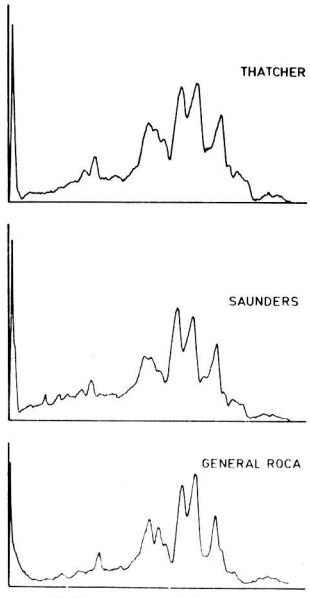


FIG. 9. S3 *densitograms of three Va varieties*

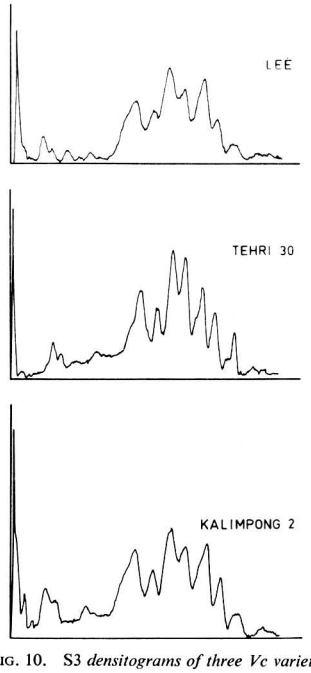


FIG. 10. S3 *densitograms of three Vc varieties*

A genetic relationship can be surmised between Tehri 30 and Kalimpong 2, both selected from Indian land populations, although the information available does not include a description of their descent.

The gliadin pattern of Lee corresponds to that of Tehri 30 and Kalimpong 2, which suggests a genetic relationship of Lee with Indian varieties.

The relevant literature has disclosed hardly any information regarding the grouping of wheat varieties on the basis of electropherograms. Graham,[11] when examining 7 Australian varieties, found varietal differences between the slow-moving fractions of acetic acid extracts, and similarity of protein patterns between three genetically related varieties.

Closer characterisation of wheat varieties by reference to the pattern of the albumins/globulins

The albumins/globulins likewise reveal differences in variety through the electropherograms, although these differences are less marked than those shown by the gliadins. It was therefore also attempted to group the varieties according to corresponding features in the *S1* patterns. In general,

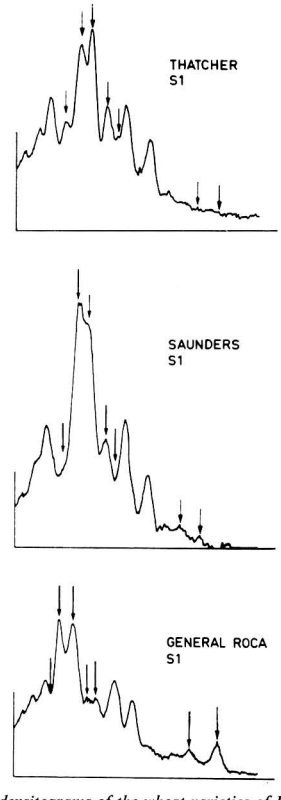


FIG. 11. S1 *densitograms of the wheat varieties of Fig.* 9 *for determination by combination of* S3 *and* S1 *densitograms*
The arrows indicate the differences between the members of the group

however, this produced groups which could not be based on genetic relationships. The provisional conclusion was therefore that there was little purpose in pursuing this approach.

On the other hand, the differences between the *S1* patterns of members of the sub-groups could be used for distinguishing between varieties (Fig. 11).

The Thatcher, Saunders and General Roca varieties are placed in main group V, sub-group a. Their gliadin patterns are identical, but they all have their individual characteristics in the albumin/globulin fractions, as indicated by the arrows.

It is suggested that a wheat variety can be identified through protein extraction of a sample of grains and electrophoresis. The theoretical possibility has already been discussed by Coulson & Sim.[9] The procedure could be as follows: place the densitogram of an *S3* fraction in one of the five main groups and then determine the appropriate sub-group. This greatly reduces the range from which to select the variety. The final definition of the variety can then be made by reference to the albumin/globulin pattern.

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ERRATUM

In the title and throughout the text of *J. Sci. Fd Agric.*, 1968, **19,** 60 (Pianka & Edwards) the term 's-alkyl' refers to 'α-branched alkyl'.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

MARCH, 1968

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Soils of the Andept suborder in Alaska. R. W. Simonson and S. Rieger (*Proc. Soil. Sci. Soc. Am.*, 1967, **31**, 692–699).—The morphology and composition of the profiles were studied. The soils were classified as Cryandepts (Inceptisols) in the 7th Approximation and seem closely related to other soils formed in volcanic ash. A. H. CORNFIELD.

Tundra soils formed over ice wedges in Northern Alaska. J. Brown (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 686–691).—Physical and chemical characteristics of the soils are presented. A. H. CORNFIELD.

Ash soils in Western Sudan. L. P. White (*J. Soil Sci.*, 1967, **18**, 309–317).—Characteristics of the soils are presented and discussed. A. H. CORNFIELD.

Properties and occurrence of acid Brown Earths in Switzerland; soil map of Landiswil-Ruderswil, Emmental BE. E. Frei and P. Juhasz (*Schweiz. landw. Forsch.*, 1967, **6**, 371–393).—Profiles of the Swiss Brown Earths studied were similar to those described in other European countries. The Swiss soils are characterised by a structure comprising gravel, sand, silt, org. matter (mull) and containing clay minerals having a non-contractile layer-lattice structure, amorphous reddish-yellow Fe^{3+} hydroxides and exchangeable, active Al. Soil maps are presented and the classification of these and associated soil types is discussed. A. G. POLLARD.

Properties and genesis of textural subsoil lamellae. J. C. Dijkerman, M. G. Cline and G. W. Olson (*Soil Sci.*, 1967, **104**, 7–16). An examination is recorded of thin subsoil layers occurring beneath some sandy soils and containing more clay than the layers beneath or above them. Such 'textural subsoil lamellae' may be formed as sedimentary layers derived from the original deposits or as the result of clay illuviation unrelated to prior sediments or may result from deposition of illuvial clay on prior sedimentary structures. Mechanisms of formation are discussed in the light of experimental data obtained by the use of sand columns. (28 references.) A. G. POLLARD.

Effect of reflective coatings on soil temperature and moisture and the establishment of peppers. C. J. Gerard and G. Chambers (*Agron. J.*, 1967, **59**, 293–296).—Petroleum resin emulsions were applied to form a coating on the soil surface. The coatings reduced soil temp. and conserved soil moisture, resulting in better germination of pepper seed and denser stands. The better treatments increased yields 5-fold over multi-irrigated non-coated plots. A. H. CORNFIELD.

Effect of farmyard manure on matric suctions prevailing in a sandy loam soil. P. J. Salter, G. Berry and J. B. Williams (*J. Soil Sci.*, 1967, **18**, 318–328).—The soil moisture characteristics of a sandy loam under ryegrass, either untreated or treated with farmyard manure were determined five times from March to Nov. For all plots on each occasion there was a linear relation between moisture content and log matric suction. A formula was derived to account for the seasonal changes in moisture characteristic and it was then possible to obtain matric suction values from the soil moisture contents obtained from twice-weekly sampling of each plot. Although differences between available-water capacity of the manured and unmanured plots were small throughout the 6 months period, the soil matric suctions of the manured plots were almost always lower than those of the unmanured plots. A. H. CORNFIELD.

Moisture regimes of soils developed on Keuper Marl. A. J. Thomasson and J. D. Robson (*J. Soil Sci.*, 1967, **18**, 329–340).—Physical properties and moisture characteristics of several representative profiles developed on Keuper Marl suggest that the textural B horizon of coarse blocky structure is the cause of waterlogging which occurs in the upper 61 cm of these soils. The C horizon, which has a fine blocky structure and a lower clay content, is more permeable and contained an appreciable vol. of air voids when the overlying horizons were fully saturated with water. After subsoiling to a depth of 76 cm at one site, the soil was waterlogged less often and then only for shorter periods than formerly. A. H. CORNFIELD.

Water retention by osmotic swelling of colloidal clays with varying ionic composition. S. A. El-Swaify and D. W. Henderson (*J. Soil Sci.*, 1967, **18**, 223–232).—Theoretical calculations, based on the diffuse double-layer model, predict that montmorillonite systems with a certain form of cation saturation and salt content should have greater swelling pressure or higher capability for water retention than do vermiculities, which have greater values than kaolinites under similar chemical conditions. This was confirmed by experimental data, although measured values were consistently higher than those predicted. Studies with mixed-ion (Na^+ and Ca^{2+}) montmorillonites showed that mutual existence of the two cation species in the clay-water system produced a near-linear relationship between exchangeable Na % and the moisture retention at a particular equilibrium pressure. A. H. CORNFIELD.

Comparison of pressure and suction methods for soil-water content—pressure-head determinations. L. de Backer and A. Klute (*Soil Sci.*, 1967, **104**, 46–55).—Comparison is made of the methods of Haines (*J. agric. Sci. Camb.*, 1930, **20**, 97) and of Richards (*Soil Sci.*, 1949, **68**, 95) using the same soil under alternate pressure and suction; changes in pressure-head and water content were noted. Appropriate apparatus is described. No differences were found between the pressure and suction curves of initially saturated soil; differences were considerable between the wet ranges of the two curves with initially unsaturated soil. Explanations of these effects are indicated. A. G. POLLARD.

Available water in soil as influenced by extraction of soil water by plants. D. E. Miller (*Agron. J.*, 1967, **59**, 420–423).—A 2-year study was made of the influence of extraction of soil water by plants on the downward flow of water in soil following irrigation and on the amount of water that may be considered as available to the plant. Drainage rates from soil cropped to lucerne were 20–25% less than those from uncropped soil 6–20 days after irrigation. Available water estimated by conventional methods in the upper 84 cm of soil was increased by 13–21% 6–10 days after irrigation when lucerne was removed at irrigation. The fact that soil water continued to move even 24 days after irrigation indicates that the field capacity method of estimating available water 2 days after irrigation is not sufficiently precise. A. H. CORNFIELD.

Effect of plant population and row spacing on evapotranspiration and water-use efficiency by soya-beans. D. R. Tommons, R. F. Holt and R. L. Thompson (*Agron. J.*, 1967, **59**, 262–265).—Evapotranspiration from soya-bean planting to the first killing frosts ranged from 33 to 46 cm over 2 years and was not affected by row spacing (20–102 cm) or plant population obtained from sowing 34 to 135 kg seed per hectare. The highest water-use efficiencies were obtained from the lowest plant population in the 20-cm rows. A. H. CORNFIELD.

Clay mineral formation in different rock types of a weathering boulder conglomerate. R. I. Barnhisel and C. I. Rich (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 627–631).—The K and Na feldspar crystals of granitic and gneissic rocks weathered to kaolin minerals, whereas the primary minerals of basic igneous rock (gabbro) weathered to montmorillonite. Significant differences in cation exchange capacity and exchangeable cations occurring for the various weathered boulders were related to original parent rock materials

and to the present clay mineralogy of the weathered boulders. The dominant cation in all systems was Al. A. H. CORNFIELD.

Discriminant function using zirconium and nickel, for parent rocks of strongly weathered Hawaiian soils. H. S. Kimura and L. D. Swindale (*Soil Sci.*, 1967, **104**, 69–76).—A function, based on Zr and Ni contents is established to distinguish between the parent rocks of certain strongly weathered soils. The total Zr in these soils increased with the degree of weathering. Although with advancing pedogenic development the eluviation of Zr and Ni showed different patterns the discriminant function differentiated andesite-derived from basalt-derived material in all horizons of several soils examined. A. G. POLLARD.

Method for investigating the chemical heterogeneity of soil material within natural soil aggregates. R. F. Holt and D. R. Timmons (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 704–705).—Soil from the surface of aggregates was separated from interior material by a freezing and thawing process. Results for a clay loam and a silt loam showed that exterior aggregate material was higher in water-sol. N, P, K, Ca, Mg, and Na than was interior aggregate material. In one soil interior material was higher in total N and exchangeable Na, and in both soils was higher in extractable P, than was exterior material. A. H. CORNFIELD.

Titration of hydrogen-clay suspensions with salt solutions. I. Shainberg and J. E. Dawson (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 619–626).—Neutralisation of H-montmorillonite with aq. $Na_2B_4O_7$ gives the permanent negative charge without decomposition of the clay or neutralisation of H^+ released from OH^- during titration. Neutralisation of the clay with CH_3COONa, HCOONa, or $NaNO_2$ also gives the permanent negative charge, providing exchangeable Al is absent. A. H. CORNFIELD.

Soil salinity changes with fallow and a straw mulch on fallow. L. C. Benz, F. M. Sandoval and W. O. Willis (*Soil Sci.*, 1967, **104**, 63–68).—On a saline silt loam with poor drainage the effects of a bare fallow and of a straw mulch (small-grain straw initially about 8 in. deep), maintained over 3 years, on soil salinity are examined. With the bare fallow, the necessity of maintaining a soil mulch to produce any reduction in salinity is confirmed. The straw mulch induced a considerable reduction in salinity. On soils normally growing wheat and barley, the reduced salinity following a straw mulch may result in notable crop increases and may be sufficient to permit the growth of less salt-tolerant crops. A. G. POLLARD.

Potassium–calcium exchange equilibria in soils: location of non-specific (Gapon) and specific exchange sites. P. H. T. Beckett and M. H. M. Nafady (*J. Soil Sci.*, 1967, **18**, 263–281).—A study was made of factors affecting the K : Ca exchange isotherm, which earlier work had shown consisted of a curved part at low values of $a_K/\sqrt{a_{Ca}}$, attributed to exchange at sites with a high specific affinity for K, and an upper linear part commonly described by the Gapon equation and attributed to non-specific sites. Na hexametaphosphate or changes in pH affected the curved but not the linear part, whilst cetyl Me_3NBr and changes in the amount or charge of exchangeable Al affected the linear but not the curved part of the isotherms. At low pH, the linear but not the curved part of the kaolinite isotherm obeyed Schofield's ratio law. Grinding had more effect on the curved than on the linear part. Specific sites are associated with the edges or peripheral interstices of stacks of clay plates, and non-specific sites with their planar surfaces. The specific sites took up K from solution more slowly than did the non-specific sites. The isotherms of completely dispersed bentonites had no curved part. The specific sites were attributed to the wedge-shaped interstices opened between clay plates by weathering, from which exchange is diffusion-controlled. Added org. cations reduced, whilst peroxide treatment increased the number of both kinds of sites. A. H. CORNFIELD.

Distribution of forms of nitrogen in a podzolic soil profile from Garpenburg, central Sweden. H. Nommik (*J. Soil Sci.*, 1967, **18**, 301–308).—In a podzolic soil developed under Norway spruce 17–27% of the total soil N was insol. in boiling 6N-HCl, the highest % being in the A_2 horizon. Amino-acid-N in the acid hydrolysate accounted for 50% of the total N in the humus layer (A_0 horizon) and decreased to 24% in the B horizon. Amino-acid composition varied little between horizons. Hexosamine-N accounted for 11–14% of the total N, tending to increase with depth. 15% of the total N was found in the soil hydrolysate as NH_4^+.
A. H. CORNFIELD.

Fractionation of nitrogen in three forest soils. J. R. Jorgensen (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 707–708).—In the 10 cm layer below the org. horizon of three forest soils total N was accounted for, on average, as NH_2-N 53%, hydrolysed NH_4^+-N + NH_2-sugar-N 20·6%, insol. humin-N 14·0%, and exchangeable NH_4^+-N + NO_3^--N 9%. A. H. CORNFIELD.

Predicting nitrogen availability to rice. I. Comparison of methods of determining nitrogen available to rice from field and reservoir soils. J. L. Sims, J. P. Wells and D. L. Tackett. **II. Assessing available nitrogen in silt loams with different previous year crop history.** J. L. Sims and B. G. Blackmon (*Proc. Soil Soc. Sci. Am.*, 1967, **31**, 672–675, 676–680).—I. Several chemical and incubation methods were tested for determining N availability in field and reservoir soils. For silt loams all methods of determining available N were poorly correlated with rice grain yields in pot tests. For clay soils the NH_4^+-N content after 6 days anaerobic (waterlogged) incubation at 35° gave the best correlation with grain yields.

II. NH_4^+-N produced during 6 days anaerobic incubation of silt loams was well correlated with rice grain yields where soils had previously been used for water storage or fish production, but was increasingly more poorly correlated where soya-bean, lespedeza, rice, and cotton had been grown previously. A. H. CORNFIELD.

Measurements of phosphorus availability in soils of Pennsylvania. D. E. Baker and J. K. Hall (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 662–667).—Comparison of a number of extractants showed that the Bray No. 1 extractant (0·025N-HCl—0·03N-NH_4F) was the most satisfactory for indicating the availability of P to maize in pot tests using 44 soils (pH 4·3–7·6). A. H. CORNFIELD.

Effect of soil clay content on phosphorus uptake. F. Baldovinos and G. W. Thomas (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 680–682).—The uptake of P by snap beans in pot tests with soils (pH 6·0) having 10, 23 and 64% clay and treated with different levels of sol. PO_4^{3-} increased with clay content at comparable soil solution P levels. The results obtained were similar to those previously reported for calcareous soils. A. H. CORNFIELD.

Evaluation of methods for determining available soil potassium. S. Feigenbaum and J. Hagin (*J. Soil Sci.*, 1967, **18**, 197–203).—The uptake of K by wheat in pot tests with six soils was correlated better with the change in free energy of exchange of K for Mg and Ca ($\triangle$ F) than with exchangeable K, %K saturation of the exchange complex or 0·01M-$CaCl_2$-extractable K. $\triangle$ F was also well correlated with log %K saturation. A. H. CORNFIELD.

Soil potassium. VI. Effect of potassium fixation and release on the form of the potassium: (calcium + magnesium) exchange isotherm. P. H. T. Beckett and M. H. M. Nafady (*J. Soil Sci.*, 1967, **18**, 244–262).—The immediate Q/I relation of K in a soil relates the amount of labile K present to its chemical potential, measured relative to the chemical potential of Ca + Mg in the same soil. The form of the Q/I relation was almost unchanged either by large additions of K (up to 3750 kg K_2O per hectare) and the fixation of up to 1900 kg K_2O, or by the depletion of the soil of both labile and some fixed K, equivalent to 5–20 years cropping. Release is shown to occur from a pool of non-labile K, at a rate which decreases as the non-labile pool is exhausted. The resulting increase in cation exchange capacity is greater than the amount of K released. A. H. CORNFIELD.

Availability of potassium in crop residues. D. W. Grimes and J. J. Hanway (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 705–706).—The availability of K in maize stalk and lucerne stem residues to ryegrass in pot tests was equal to that of the same amount of K applied as KCl. 90% of the K added was taken up in the above-ground portion of six cuttings of ryegrass. A. H. CORNFIELD.

Influence of ionic environment on the nature of iron oxides in soils. R. M. Taylor and A. M. Graley (*J. Soil Sci.*, 1967, **18**, 341–348).—The colour in a sequence of basaltic soils changed from red to red-brown near sea level to yellow-brown in more elevated areas. The colour change was correlated with decreasing goethite/haematite ratio and decreasing proportion of Ca + Mg on the exchange complex. A. H. CORNFIELD.

The ferric iron–hydroxide ion product in suspensions of acid soils. H. L. Bohn (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 641–644).—The (Fe) $(OH)^3$ ion production of seven acid soils in aq. $CaCl_2$ was const. after 139 days and averaged $1 + 0{\cdot}5 \times 10^{-39}$. Varying the soil: solution ratio from 1 : 10 to 1 : 1000 or the $CaCl_2$ concn. from 0·01 to 0·1M had no significant effect on the ion product.
A. H. CORNFIELD.

Aluminous chlorite origin of pH-dependent cation exchange capacity variations. J. M. de Villiers and M. L. Jackson (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 614–619).—The cation exchange

capacity (CEC) and K-fixing power of a micaceous vermiculite were markedly reduced following treatments with solutions of polymeric hydroxy-alumina. The partially chloritised product showed increasing CEC with pH (4 to 8·5) of the leaching solutions. The pH-dependent CEC appeared to arise from isomorphous substitutional charge (permanent negative charge), blocked by hydroxy-Al charged positively by $-AlOH_2$ groups, and restored by deprotonation of the latter on addition of base.

A. H. CORNFIELD.

Chemical changes in a Sarawak (Malaysia) soil after fertilisation and crop growth. J. M. Bailey (*Pl. Soil*, 1967, **27**, 33–52).—Chemical changes after fertilisation of a tropical red-yellow podzolic clay soil and in the growth of dryland rice are reported. Al^{3+} dominated the exchange complex and soil acidity reactions, whilst both Fe^{3+} and Al^{3+} played major rôles in phosphate transformations. Al-, Fe-, Ca- and organically-bound phosphates were available to plants, yet withstood leaching. The residual effects on regrowth were greater for $Ca(H_2PO_4)_2$ than for KCl, which in turn was greater than for $(NH_4)_2SO_4$. KCl reduced the concn. of exchangeable Al^{3+} and interacted with $Ca(H_2PO_4)_2$ to increase the amount of Fe- and Al- phosphates. A. H. CORNFIELD.

Field experimentation. J. R. Goldson (*E. Afr. agric. For. J.*, 1967, **33**, 100–118).—Methods of field experimentation developed at the Western Agricultural Research Station, Kakamega, Kenya are described. A. H. CORNFIELD.

Theory of the nuclear densimeter. D. Taylor and M. Kansara (*Soil Sci.*, 1967, **104**, 25–34).—The theoretical basis of two methods of measuring soil density is discussed with particular reference to the construction of calibration curves for the density-detector response. The back-scatter and the direct transmission techniques are considered and suggestions are made for the best operation of the instruments concerned. A. G. POLLARD.

Measurement of losses from fertiliser nitrogen during incubation in acid sandy soils and during subsequent growth of ryegrass, using ^{15}N-labelled fertilisers. J. K. R. Gasser, D. J. Greenland and R. A. G. Rawson (*J. Soil Sci.*, 1967, **18**, 289–300).—Four sandy soils (pH 5 and 6) were treated with ^{15}N-labelled $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ and incubated for 6 weeks at 21°. Added N was immobilised in the early stage, but re-mineralised during the later stage, of incubation. Part of the re-mineralised N came from native soil org. N. At the end of incubation $<5\%$ of the added NO_3^--N was lost, and about 5% of the added NH_4^+-N was lost from the two grassland soils studied. Addition of the nitrification inhibitor 2-chloro-6(trichloromethyl)pyridine before incubation prevented this loss. When the incubated soils were cropped with ryegrass more than 93% of the labelled N was accounted for in the plants and soil, except in the grassland soils treated with NO_3^-, where 87·4–91·4% of the added N was accounted for. It appears that more N may be lost during growth of grass when NO_3^- levels are high than when nitrification occurs in the absence of grass.

A. H. CORNFIELD.

Reversion of fertiliser nitrogen in soils. F. E. Broadbent and T. Nakashima (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 648–652).—The reversion of inorg. N to org. form in soils was studied by applying ^{15}N-labelled $(NH_4)_2SO_4$ to several soils and determining the uptake of native and applied N by prolonged cropping with Sudan-grass. N availability ratios (% of labelled N in the crop divided by % of labelled N in the soil at the onset of cropping) decreased with successive cuttings of the grass. The time required for the mineralisation of 1% of the residual fertiliser-N in soil was increased with successive cuttings. Where straw was added initially to promote immobilisation of added NH_4^+-N, about 66% of the added NH_4^+-N remained in the soil after 18 months of continuous cropping. N reversion cannot be accounted for solely on the basis of biological interchange; a non-biological mechanism of N stabilisation may also be involved in soils.

A. H. CORNFIELD.

Non-uniform distribution of phosphorus fertilisers: an analytical approach. D. Zaslavsky and R. S. Mokady (*Soil Sci.*, 1967, **104**, 1–6).—The response of crops to fertiliser treatment is discussed in relation to the pattern of (non-uniform) distribution e.g., band application. Factors influencing the choice of parameters on which mathematical interpretations of the problem can be based, are considered. A. G. POLLARD.

Plant Physiology, Nutrition and Biochemistry

Some aspects of competition for light in potatoes and sugar-beet. P. M. Bremner, E. A. K. El Saeed and R. K. Scott (*J. agric. Sci. Camb.*, 1967, **69**, 283–290).—The plants were grown in pots and arranged with two different spacings and with two different fertiliser treatments. Closer spacing depressed the relative growth rate of both crops; assimilation rates were lowered, leaf area ratio increased and mineral uptake diminished. With both plants the highest growth rates occurred when leaf area indices were considerably below normal max. The observed growth rates may have been near max. under the experimental conditions. A. G. POLLARD.

Effect of mineral element deficiencies on leaf temperature in tobacco. A. Wallace, E. Frolich and R. T. Ashcroft (*Agron. J.*, 1967, **59**, 386).—In the greenhouse the temp. of leaves of P-deficient tobacco plants averaged 5·4° more than those of normal plants. Leaves of K- and Mg-deficient plants also had higher temp. whilst leaves of N- and Fe-deficient plants had temp. which were little different from, control plants. A. H. CORNFIELD.

Salinity–fertility interaction study on maize and cotton. M. A. Khalil, F. Amer and M. M. Elgabaly (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 683–686).—Increasing soil salinity from 1·6 to 9·0 mmho per cm by addition of chlorides of Na, Ca, and Mg so as to maintain a const. Na absorption ratio decreased dry matter yields of maize and cotton in pot tests. Application of N and P did not counteract the adverse effects of salinity. Increasing salinity increased plant N%, reduced K%, but had no effect on P%.

A. H. CORNFIELD.

Effects of external salt concentrations on water relations in plants. III. Concentration dependence of the osmotic differential between xylem and external medium. J. J. Oertli (*Soil Sci.*, 1967, **104**, 56–61).—Earlier work (*Idem., ibid.*, 1966, **102**, 180, 258) is further developed and changes in osmotic adjustment of the xylem sap, as shown by the bleeding sap from decapitated plants, are examined in relation to changes in concn. in the external solution. This relationship, expressed as the osmotic differential ($\triangle\pi$) rises to a max. with increase in concn. of the external solution, thereafter declining and becoming negative. The dependence of $\triangle\pi$ on concn. and its response to changes in the capacity for salt transport and to transpiration rate support the author's earlier concepts. The bearing of these on the mechanism of plant injury by high salinity is noted. A. G. POLLARD.

Interrelation of carbohydrate metabolism, seedling development, and seedling growth rate of *Phalaris* species. R. D. B. Whalley and C. M. McKell (*Agron. J.*, 1967, **59**, 223–226).—The growth rate of harding-grass seedlings (*Phalaris tuberosa* var. *stenoptera*) is limited by the ability of the seedlings to use the sugars supplied by the endosperm. With perla-grass (*P. tuberosa* var. *hirtiglumis*) and *P. coerulescens*, the ability of the endosperm to supply sugars appears to be the factor limiting seedling growth.

A. H. CORNFIELD.

Nitrogen and sugar levels of pith tissue of maize as influenced by plant age and by chloride and potassium treatments. J. W. Martens and D. C. Arny (*Agron. J.*, 1967, **59**, 332–334).—Nitrogen levels in second internode pith tissue of maize were highest 11 weeks after planting. Total and reducing sugars increased up to 13–15 weeks after planting and then declined. Application of KCl (150 lb per acre) increased org. N levels in two of the three lines studied, but decreased reducing sugars in all lines. Application of NH_4Cl had effects similar to, but smaller than, those of KCl.

A. H. CORNFIELD.

Ion interactions in oats as affected by additions of nitrogen, phosphorus, potassium, chlorine and sulphur. K. F. Nielsen, R. L. Halstead and A. J. MacLean (*Soil Sci.*, 1967, **104**, 35–39).—Oats were grown in greenhouse pot experiments with soil treated with two different rates of each of N, P, K, Cl^-, and SO_4^{2-}. Grain yields increased with rise in applications of N, P and Cl^-, decreased with rise in applied K and were unaffected by rise in SO_4^{2-}. Straw yields were increased by increasing the amounts of K, P and N given. The concn. of the nutrients in the grain were not greatly affected by the different treatments; much more definite differences occurred in the straw. Mg, N, P and S taken up were largely deposited in the grain and K, Ca and Cl^- accumulated mainly in the straw. Interactions between nutrient effects within whole plant contents showed synergic effects of N on K and of Mg and K on S but antagonistic effects between P and S and also between Cl and S. A. G. POLLARD.

Factors in interstrain variation in zinc content of maize. H. F. Massey and F. A. Loeffel (*Agron. J.*, 1967, **59**, 214–217).—In 31 inbred lines of maize with widely varying kernel-Zn% the proportions of Zn decreased in the order germ + embryo, pericarp and endosperm. In greenhouse tests kernel-Zn% was not correlated

with Zn uptake by plants or Zn% in plants grown in either untreated or Zn-treated soils. In field-grown plants variations in kernel-Zn% among inbred lines was related to plant-Zn%, but this factor was modified by the extent to which the inbred was able to transfer Zn from the stalk and leaves to the ear.
A. H. CORNFIELD.

Sources of variation in leaf analysis in East Africa. J. B. D. Robinson and G. H. Freeman (*E. Afr. agric. For. J.*, 1967, **33**, 8–13).—Variations due to field sampling, laboratory handling + preparation, and chemical analysis stages in the content of major and trace elements in the leaves of *Pinus patula*, coffee, pineapple, cotton, and maize were studied. There were considerable differences in the extent of variation due to the different stages with different elements in the same crop and also with the same element in different crops. In most cases the largest source of variation was field sampling, but variation due to laboratory handling and prep. were also usually greater than that due to chemical analysis. The results indicate that duplicate analyses on each sample are not justified and that a single analysis on a subsample from a carefully prepared sample should be sufficient for routine work.
A. H. CORNFIELD.

Comparison of conventional procedures for determining nitrogen, phosphorus and potassium in plant material with automated procedures using a single digestion. R. L. Thomas, R. W. Sheard and J. R. Moyer (*Agron. J.*, 1967, **59**, 240–243).—The conventional procedures involved: for N, Kjeldhal digestion followed by distillation and titration of NH_3, for P, dry ashing followed by a molybdovanadate colorimetric method, and for K, dry ashing followed by atomic absorption spectroscopy. The automated procedure involved digestion of the plant material with conc. H_2SO_4 + 30% H_2O_2 and use of suitable aliquots of the digest for determining N by the alkaline phenol-hypochlorite method, P by the molybdate-ascorbic acid colorimetric method, and K by atomic absorption spectroscopy. The conventional and automated procedures gave very similar results for N, P and K in 10 samples of plant materials varying widely in content of the three elements.
A. H. CORNFIELD.

Effect of gibberellic acid on quality of early Italian prunes, *Prunus domestica*. E. L. Proebsting, jun. and H. H. Mills (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 135–139).—Spraying Italian prune trees before harvest with gibberellic acid (100 ppm) reduced internal browning of fruit during subsequent storage, increased shelf life and resulted in firmer fruit. A. H. CORNFIELD.

Combined method for determination of calcium, magnesium, phosphorus, sodium, and potassium. H. Zonnefeld (*Z. Lebensmittelunters. u. Forsch.*, 1967, **133**, 273–282).—The procedure described is applied to the ash of plant material obtained by method of Zonnefeld and Gersons (cf. *ibid.*, 1966, **131**, 205). For the determination of Ca the solution of the ash (previously freed from Fe, Al, and PO_4), buffered at pH 12·8, is titrated with standard EDTA (Na_2 salt) with glycoxal-bis(2-hydroxyanil) as indicator. For the determination of Ca + Mg the solution at pH 10 is titrated with standard EDTA with Eriochrome-black T as indicator. Phosphates are determined by extraction of the molybdophosphoric complex with BuOH – $CHCl_3$ and colorimetric measurement at 420 nm (cf. Collier, *Analyt. Abstr.*, 1954, **1**, 2387). The K and Na are determined by flame-photometry. Recoveries of added elements were quant. The mean errors were 2·4–5%. (16 references.)
P. S. ARUP.

Crops and Cropping

Varietal differences in growth parameters of wheat and their importance in determining yield. F. G. H. Lupton, M. A. M. Ali and S. Subramaniam (*J. agric. Sci. Camb.*, 1967, **69**, 111–123).—Observations of the growth and development of five varieties of winter wheat and of various hybrids between them, are recorded. Examination of successive samplings showed varietal differences in orthogonal polynomial regression coeff. fitted to data for tiller no., shoot wt. and leaf area. Correlation coeff. between these parameters are further examined from the view-point of the choice of varieties for breeding. A. G. POLLARD.

Influence of primary and/or adventitious root systems on wheat production and nutrient uptake. G. O. Boatwright and H. Ferguson (*Agron. J.*, 1967, **59**, 299–302).—Wheat was grown with the whole root system and also with only the primary or only the adventitious roots. Tillering was earlier with the whole root system than where only one type of root was present. Early tillering with P fertilisation was significantly greater and increased tillering due to P occurred only when the whole root system was present. Grain yields decreased in the order whole-root system, adventitious roots only, primary roots only. N and P translocation into the grain was greatest when the whole root system was present.
A. H. CORNFIELD.

Effect of nitrogen on leaf area, yield and nitrogen uptake of barley under moisture stress. R. E. Luebs and A. E. Lang (*Agron. J.*, 1967, **59**, 219–222).—Dry matter production during the vegetative growth period was greater at the high (68 kg) than at the low (17 kg per hectare) level of N application. High N also increased green leaf area between the late jointing and soft dough stages. Increasing moisture stress with higher N decreased green to necrotic leaf wt. ratios and increased straw to grain wt. ratios. N uptake increased with level of applied N, but at a given N level, N content was higher at the lower moisture level. Application of 3·7 cm water at heading increased grain yield by 560 kg per hectare.
A. H. CORNFIELD.

Effects of ammonium and nitrate fertilisers, with and without sodium and potassium, on spring barley. F. V. Widdowson, A. Penny and R. J. B. Williams (*J. agric. Sci., Camb.*, 1967, **69**, 197–207).—Thirteen trials with barley on soils overlying chalk indicate that N always increases grain yield. $Ca(NO_3)_2$ gives greater yields than does $(NH_4)_2SO_4$ except on light soils in wet conditions. Combine-drilled P or PK always increases yield, whilst Na and K alone do so only occasionally. Dry matter yields of green barley and grain are similar, although the former contains more N. Increasing N increases K and Na in green barley with only slight increases of Mg. K is slightly increased by K and Na, whilst Na is markedly increased by Na and decreased by K. Mg is decreased by both K and Na. Uptake of K varied between 61 and 120, of Na 2·1 and 10·8 and of Mg 2·7 and 5·1 lb/acre. M. LONG.

Effect of soil temperature, phosphorus, and plant age on growth analysis of barley. J. F. Power, W. O. Willis, D. L. Grunes and G. A. Reichman (*Agron. J.*, 1967, **59**, 231–234).—Growth chamber tests with barley showed that net assimilation rate increased with P supply (9–44 ppm, soil basis) but was not affected by soil temp. (9–22°). Leaf area was highly correlated with dry wt. at all temp., P levels, and stages of plant development.
A. H. CORNFIELD.

Aluminium tolerance of two barley varieties in nutrient solution, peat, and soil culture. L. B. MacLeod and L. P. Jackson (*Agron. J.*, 1967, **59**, 359–363).—Using top and root yields as criteria of tolerance, the barley variety Charlottetown 80 was more tolerant to increasing Al^{3+} in nutrient solution, peat or soil culture (all in acid conditions) than was Herta. Heavy fertilisation reduced root yields in unlimed and increased them in limed soils. K, Ca, and Mg% in tops and roots decreased whilst Al% increased with increasing Al^{3+} concn. in the nutrient solution. Uptake and translocation of P decreased with increasing Al^{3+} concn. of the nutrient. A. H. CORNFIELD.

Response of maize to a 'light rich' field environment. J. W. Pendleton, D. B. Egli and D. B. Peters (*Agron. J.*, 1967, **59**, 395–397).—Grain yields of maize were increased by inserting Al-covered reflectors between the rows so as to increase light supply to the middle and lower leaves. The greatest increases (35–41%) were obtained in border rows at the highest plant population. Yields from inside rows were increased by 2–7%. Light is the primary ecological factor limiting grain yields from maize when grown under highly productive conditions. A. H. CORNFIELD.

Effect of chloride on phosphorus uptake by maize roots. O. G. Carter and D. J. Lathwell (*Agron. J.*, 1967, **59**, 250–253).—The presence of up 0·1M-KCl in nutrient solutions had no effect on the short-term uptake of P from 2–260 μM-KH_2PO_4 solutions by maize seedlings or excised maize roots. A. H. CORNFIELD.

Effect of leaf removal on amylose content of maize endosperm. J. L. Helm, V. L. Fergason, J. P. Thomas and M. S. Zuber (*Agron. J.*, 1967, **59**, 257–258).—Defoliation of maize at pollinating time changed the amylose-amylopectin ratios of the endosperm only slightly. The removal of the six upper leaves at pollinating time reduced the amylose% in high-amylose inbred lines by 1–3%.
A. H. CORNFIELD.

Potassium nutrition and the distribution of carbohydrates in the immature endosperm of *Zea mays*. C. T. Dougherty (*Diss. Abstr.* B, 1967, **27**, 2207).—Maize was grown with different levels of K supply capable of producing yields of 70–160 bu/acre. Endosperm tissue (20 g) was excised 12 days after pollination and incubated with ^{14}C-glucose for 8 h. Various carbohydrates were determined in the product together with the distribution of ^{14}C between them.

The [K+] in the immature endosperm from plants grown with fertilisers which could give yields of 110–160 bu /acre, varied very little (0·043–0·046M). Lower concn. (0·035–0·038M) were found in maize yielding 70–80 bu/acre. The max. [K+] was 0·55 M, produced by very high K treatments. When endosperm-K was not changed by increasing the K treatment, the total carbohydrate content of the endosperm increased. The responses of maize to K feeding were related to the effect of K on plant metabolism, that of the endosperm being modified only when its K content was inadequate or excessive. Increases in endosperm-K up to 0·046M were associated with increase in % starch; higher K levels caused no further increase in starch and tended to disturb the carbohydrate metabolism. The water-sol. polysaccharide was a small but consistent % of the endosperm carbohydrate and of ^{14}C; it probably consisted of maltoligosaccharides. A. G. POLLARD.

Genetic and morphologic control of drying rate in 'mature' maize. (*Zea mays*, L.). J. L. B. Purdy (*Diss. Abstr.* B, 1967, **27**, 2208).—The drying rate of maize inbreds was estimated by the moisture loss of husked whole ears in a forced-air drier at 180°F for 18 h. The initial moisture content of the ears was 30–40% and that of the 'dried' ears 20–30%. Significant differences between the drying rates of each generation of each hybrid and between years and among entries, are recorded. Indications were found that the selection of hybrids for faster drying would result in selection for smaller ears, earlier silking date and lower moisture content at a given time after silking. Numerous relationships between phenotypic and genotypic factors are established. The different rates of drying of the hydrids result from the physical structure of the pericarp and not from any metabolic process. Faster drying is associated with thinner pericarp and with greater permeability. A. G. POLLARD.

Utilisation of nitrogen by rice in relation to time of application. S. Patnaik and F. E. Broadbent (*Agron. J.*, 1967, **59**, 287–288).—Pot tests with rice using ^{15}N-labelled $(NH_4)_2SO_4$ showed that applied N was utilised better when 66% of the N was applied at planting and 33% at the boot stage than when the whole of the N was applied at planting or at the tillering stage. With the best treatment 51% of the applied N was recovered by the plant by the boot stage. A. H. CORNFIELD.

Effect of continuous submergence *versus* alternate flooding and drying on the performance of rice. W. H. Patrick, jun., W. A. Quirk, F. J. Peterson and M. D. Faulkner (*Agron. J.*, 1967, **59**, 418–419).—In greenhouse tests N uptake by and yields of top growth of rice plants were higher under continuous submergence than under conditions of drying and reflooding once to three times during growth. The difference between the two methods of culture decreased with increasing application of N. In field tests grain yields were usually lower under drying and reflooding than under continuous submergence, except in soils which had previously been in pasture for a number of years, where the reverse was true. A. H. CORNFIELD.

Sources of nitrogen and methods of application for flooded rice. I. Comparison of two methods of applying slow release and standard fertiliser materials. G. V. Simsiman, S. K. De Datta and J. C. Moomaw (*J. agric. Sci., Camb.*, 1967, **69**, 189–196).—A series of slow-release N fertilisers were compared with $(NH_4)_2SO_4$ and urea. Two methods of application were compared for each fertiliser, broadcast and incorporated (**I**) and placement at 15 cm depth (**II**). The slow-release fertilisers released N too slowly during the early stages of growth so giving rise to lower yields than the medium release and standard fertilisers. **II** increased the N content at all stages of growth and significantly increased grain yield compared with **I**. Recovery of N with **I** was 38% and with **II** 68%. **II** maintained a higher level of mineral N at all stages of growth, suggesting that ammoniacal fertilisers persist for a longer time when placed in the reduced soil zone. M. LONG.

Accumulation of sodium and calcium by seedlings of cereal crops under saline conditions. L. Y. George (*Agron. J.*, 1967, **59**, 297–299).—Seedlings of barley, wheat and rice were treated with NaCl, $CaCl_2$ and mixtures of the two in iso-osmotic solutions so as to give osmotic concn. varying from 2 to 12 atm. The Na% and Ca% of both roots and shoots increased with osmotic strength of the solutions. Rice accumulated more Na and Ca in both roots and shoots than did barley or wheat. Wheat shoots accumulated more Na and less Ca than did barley shoots, but the reverse was true for roots. A. H. CORNFIELD.

Effects on yields of potatoes of three amounts of NPK fertiliser and the residual effects on following winter wheat. F. V. Widdowson, A. Penny and R. J. B. Williams (*J. agric. Sci., Camb.*, 1967, **69**, 247–257).—Use of 15 cwt/acre of 13-13-20 fertiliser on heavy soils is justified for potatoes, providing it is broadcast. Working the fertiliser into the soil is usually beneficial. At 5 and 10 cwt/acre placement of the fertiliser in bands 3 in. from the seed centre is better than broadcasting. Yields of green wheat, grain and straw are increased by previous fertiliser applications, but not so much as by fresh fertiliser. High winter rainfall reduces the residual effects most, regardless of method of application. Green wheat contains slightly more N, 1/5th less P and 2½ times more K than ripe grain and straw. M. LONG.

Yield and sugar production by sugar beet as affected by leaf area variations induced by stand density and nitrogen fertilisation. R. E. Campbell and F. G. Viets, jun. (*Agron. J.*, 1967, **59**, 349–354).—Higher beet sugar% and sugar yields per acre were obtained on a silty clay without application of N than with 224–336 kg N per hectare. Yields were higher with 46 cm than with 15 or 30 cm row spacing. The highest sugar yields were associated with the lowest leaf area index throughout the season and at harvest, with the lowest leaf area duration, and with the lowest top : root ratio (dry basis). A. H. CORNFIELD.

Effects of subsoiling and different levels of manuring on yields of cereals, lucerne and sugar beet. R. Hull and D. J. Webb (*J. agric. Sci., Camb.*, 1967, **69**, 183–187).—Subsoiling of an old arable clay loam soil increased the 3-yearly average of wheat by 0·6 cwt grain/acre, that of barley 0·4 cwt/acre, and of lucerne 0·6 cwt/dry matter/acre. The average increase of sugar beet over 4 years was 0·7 tons/acre of roots or 2·1 cwt sugar. Yield responses to fertiliser were unaffected by subsoiling, although previous cropping and fertiliser usage affected the optimum fertiliser N level for sugar beet. M. LONG.

Soil, plant and animal relationships in agricultural production. W. M. H. Saunders (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 101–108).—A review. Physical and some chemical characteristics of soils affecting growth of pastures and therefore of animals are noted, together with undesirable effects of unsuitable manurial practices and grazing systems. A. G. POLLARD.

Progress in pasture plant physiology. R. H. M. Langer (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 146–153).—A review of some recent experimental developments in pasture improvement resulting from a better understanding of physiological and genetic factors bearing on the pattern of growth of herbage plants in a mixed sward grazed by stock. The problem of the accumulation of dead material and its utilisation or removal is noted. A. G. POLLARD.

Increasing fodder production for the grazing animal. A. G. Campbell (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 126–138).—A review in which possible effects of irrigation, grazing management and the genetic improvement of pasture composition are discussed. (50 references.) A. G. POLLARD.

Breeding for improved quality and quantity of forage for the grazing animal. P. C. Barclay (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 139–145).—Methods of improving pasture by introduction of new species or hybrids, inducing polyploidy and by breeding varieties more suited to different environmental conditions, are discussed and illustrated by actual results of recent experimental work. A. G. POLLARD.

Effect of soil type on uptake of magnesium by pasture plants. K. J. McNaught and T. E. Ludecke (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 121–125).—Top-dressings of 2–4 cwt of dolomite or of serpentine superphosphate, providing 25–50 lb of Mg/acre increased the Mg content of pastures on pumice soils but had no such effect on other soils examined. Relationships of these observations to the occurrence of hypomagnesaemia are noted. A. G. POLLARD.

Potassium and sodium interrelations in growth and mineral content of Italian ryegrass. L. O. Hylton, A. Ulrich and D. R. Cornelius (*Agron. J.*, 1967, **59**, 311–314).—In nutrient solution studies K influenced growth of ryegrass more than did Na. Na substituted partly for K in top growth when the nutrient was low in K. Visual K deficiency symptoms were delayed and were less severe when Na was high in the nutrient. The youngest blade that was fully open and had a ligule was the best plant part for assessing the K status. The crit. K concn. was 0·8% K (dry basis) when more than 2·4% tissue-Na was present, and 3·5% when less than 0·3% Na was present. A. H. CORNFIELD.

Salinity tolerance of seven varieties of creeping bentgrass, *Agrostis palustris*. V. B. Younger, O. R. Lunt and F. Nudge (*Agron. J.*, 1967, **59**, 335–336).—Seven varieties of creeping-bentgrass showed

significant differences in tolerance to increasing salinity (20–120 mequiv. per l each of $CaCl_2$ and NaCl) in the nutrient solution, when yields of clippings were used as a criterion of tolerance. The variety 'Seaside' had highest tolerance under extreme salinity conditions and also showed the best recovery when transferred to non-saline solutions. There were indications of variation in salt tolerance even between individual plants of this variety.
A. H. CORNFIELD.

Legume-grass forage seeding mixtures. J. A. Jackobs (*Agron. J.*, 1967, **59**, 435–438).—The performance of forage mixtures consisting of three legumes (lucerne, red clover, and ladino clover) and three grasses (timothy, smooth brome, and orchard-grass) under hay production and grazing was studied. Lucerne and orchard-grass had the greatest influence on the performance of the mixtures, even when sown at fairly low rates. When lucerne was added to a mixture the total yield of forage was increased because the decrease in grass was not as great as the increase in the legume. When orchard-grass was added to a mixture the grass component was increased but there was a comparable decrease in the legume so the total yield remained the same.
A. H. CORNFIELD.

Lucerne survival in relation to effectiveness of drainage on sloping land. G. R. Benoit, K. D. Fisher and J. Bornstein (*Agron. J.*, 1967, **59**, 444–447).—Studies with a typical sloping poorly-drained silt loam with hardpan showed that the proportion of lucerne in the crop was increased by placing sub-drains at 102 cm depth. The % winterkilling was significantly correlated with soil water content in the upper 71 cm in spring. Winterkilling was negligible when soil moisture tension was >0·1 bar. A. H. CORNFIELD.

Comparison of the reaction of different grass species to fertiliser nitrogen and to growth in association with white clover. II. Yield of nitrogen. D. W. Cowling and D. R. Lockyer (*J. Br. Grassld Soc.*, 1967, **22**, 53–61).—Seven species of grasses differed in their uptake of N (yield of N) from soil, with S37 ryegrass and S48 timothy showing the highest N yields. The ability of each grass to take up fertiliser N was usually related to its uptake of native N. Perennial ryegrasses were the most, and *Agrostis tenuis* the least, efficient in using N taken up for the production of dry matter. When each grass was grown in association with white clover yields of N of the different mixtures were not significantly different. Grasses which gave high N yields with fertiliser N were also high yielding when grown with clover. Pure grass swards required more than 200 lb N per acre per year to yield the same amount of N as did grass-clover swards. The amount of N estimated to have been derived from clover (indirect effect of clover) increased each year and ranged from 23 to 46 lb N per acre per year.
A. H. CORNFIELD.

Forage and nitrogen production by subterranean clover–grass and nitrogen-fertilised California grassland. M. B. Jones (*Agron. J.*, 1967, **59**, 209–214).—In a year of low rainfall clover–grass swards produced forage yields equal to those from grass swards fertilised with 45–90 kg N per hectare. The clover yielded 90–179 kg N per hectare. In a year of adequate rainfall clover–grass swards produced more forage than did grass swards receiving 179 kg N per hectare, the clover yielding about double the amount of N compared with that in the grass. N fertilisation gave the greatest response during the winter period, whilst the clover–grass swards made the greatest gains in April-May. A. H. CORNFIELD.

Macro- and micro-nutrient distribution in ladino clover, *Trifolium repens*. S. R. Wilkinson and C. F. Gross (*Agron. J.*, 1967, **59**, 372–374).—Ladino clover plants grown in solution culture were analysed for P, K, Ca, Mg, Mn, Fe, Cu, B, Al, and Zn in various plant parts. P was concentrated in flowers, Al in leaflets, and Mg, Mn, and Fe in the roots. The P% and Zn% of leaves declined with increasing age, whereas Ca% increased. A. H. CORNFIELD.

Flooding tolerance of ladino white, intermediate white, Persian, and strawberry clovers. C. S. Hoveland and E. E. Mikkelson (*Agron. J.*, 1967, **59**, 307–308).—The herbage yields of both intermediate white and ladino clovers were reduced by flooding 3 days in 10. With longer duration of flooding intermediate white clover yields declined, but those of ladino were not reduced further. Yields of strawberry clover (*Trifolium fragiferum*) and Persian clover (*T. resupinatum*) were only slightly reduced by flooding. Herbage N% was reduced by flooding in intermediate white, but only slightly in the other clovers. Flooding resulted in increased Mn% in the herbage of all species but the extent of increase in Mn was not related to flooding tolerance. A. H. CORNFIELD.

Distribution and concentration of hydrocyanic acid in a sorghum–sudangrass hybrid. D. D. Wolf and W. W. Washko (*Agron. J.*, 1967, **59**, 381-382).—The HCN% in the whole plant (dry basis) decreased with age. The proportion of the total HCN in the leaf blade decreased whilst that in the stem and sheath increased with age. A. H. CORNFIELD.

Mineral and organic constituents of the leaves of eight *Prunus* species. A. Denney, D. R. Walker and R. A. Norton (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 140–149).—Free and total NH_2-acids, carbohydrate fractions, and major and trace elements in the leaves of peach, apricot, sweet and sour cherry, mahaleb cherry, myrobalan plum, prune and almond are reported. A. H. CORNFIELD.

Fruit firmness and pectic composition of cherries as influenced by differential nitrogen, phosphorus, and potassium applications. D. Curwen, F. J. McArdle and C. M. Ritter (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 72–79).—Increasing levels of applied N resulted in firmer cherries having a lower juice loss upon pitting and higher water-insol. pectic content. Increasing levels of K had effects opposite to those of N. Increasing levels of P had no consistent effect on any factor measured. High K levels reduced fruit Ca%.
A. H. CORNFIELD.

Changes in starch and soluble sugar content of peach tissue during the dormant period. W. M. Dowler and F. D. King (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 80–84).—Starch% (dry wt. basis) of twigs, scaffold limbs, trunk, and bark of peach trees decreased rapidly from Oct. to Jan., and then remained relatively const. until March. Sol. sugars% showed the reverse trend. Starch% in the various tissues was significantly correlated (positively), whilst sugar% was significantly correlated (negatively), with mean max. temp. during the preceding 15 days. Total carbohydrate% in twigs, but not in other tissues, was significantly (positively) correlated with max., min., and mean temp. during the preceding 15 days.
A. H. CORNFIELD.

Effect of gamma irradiation on ripening and quality of nectarines and peaches. E. C. Maxie, C. F. Johnson, C. Boyd, H. L. Rae and N. F. Sommer (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 91–99).—Irradiation (100–600 krad) initiated the climacteric and ripening sequence in nectarines and peaches, apparently by inducing the unripe fruit to produce stimulatory amounts of C_2H_4. Irradiated fruit ripened 4–5 days earlier, were noticeably redder, and their skin and flesh were higher in anthocyanin than control fruit. Irradiated nectarines were acceptable for flavour and appearance, but aroma and texture were poor. The treatment also reduced flesh firmness. A. H. CORNFIELD.

Free amino-acids in various parts of *Vitis vinifera* at different stages of development. A. R. Nassar and W. M. Kliewer (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 281–294).—Twenty-two free NH_2-acids were identified quant. in several parts of grapevines at four stages of growth. Eight others were tentatively identified. The total free NH_2-acid content of fruit, peduncle and shoot wood increased 3–8 fold during the growing season. Arginine accounted for 37% of the total free NH_2-acids in the fruit at maturity, 41% in the peduncle, and 51% in the shoot wood. Proline in the fruit increased 30-fold during ripening. Roots were the richest part of the vine in free NH_2-acids content. Concn. of proline, threonine, pipecolic acid, glutamine, asparagine, glutamic and aspartic acids decreased rapidly in the leaf blades from 4 to 36 days after budburst and then remained about const. until sensecence. Several of these NH_2-acids increased in concn. in the fruit, peduncle, wood and roots during senescence. A. H. CORNFIELD.

Organic acid synthesis and accumulation in sweet lemon, *Citrus limmettiodes*, and sour lemon, *C. limon*, fruits. E. Bogin and A. Wallace (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 182–194).—Studies were made on the mechanism of citrate and malate synthesis in sweet lemon, which is low, and sour lemon, which is relatively high, in org. acids. Sweet lemon mitochondrial prep. were more active in oxidation and phosphorylation than were those of sour lemon. Sweet lemon showed a higher activity than sour lemon for pyruvate amination to give alanine. Higher values of CO_2 fixation with isocitric dehydrogenase were obtained with prep. from sour than with those from sweet lemon. Sour lemon produced larger quantities of citramalate than did sweet lemon. An hypothesis is presented to explain the greater amount of org. acids in sour lemon. A. H. CORNFIELD.

Effects of Alar, Cycocel and BTOA on flower bud induction of lemon trees. S. P. Monselise, R. Goren and A. H. Halevy (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 195–200).—Spray application, during the summer, of 1,000 ppm Cycocel (2-chloroethyl trimethyl ammonium chloride), 2,500 ppm Alar (*N*-dimethylaminosuccinamic acid) and 25 ppm BTOA (benzothiazole-2-oxyacetate) considerably

increased flower and fruit production in lemons. BTOA was relatively more effective on older branches, whilst the two other materials were relatively more effective on the younger branches. Growth of younger branches was reduced by BTOA but not by the two other materials. A. H. CORNFIELD.

Pigmentation and viscosity of juice and sauce of cranberry varieties. B. M. Zuckerman, I. E. Demoranville, F. J. Francis, K. Hayes, R. L. Norgren, S. Regling, C. W. Miller and S. M. Paracer (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 248–254).—When grown in the same environment there were significant differences in juice pigmentation and viscosity between different cranberry varieties. Both characteristics were also affected by location of growth. There was an inverse relationship between pigmentation and viscosity. A. H. CORNFIELD.

Determination of hexuronic acids in fruit by gas chromatography. R. C. Wiley, M. Tavakoli and M. D. Moore (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 34–39).—α-D-Galacturonic acid, β-D-glucuronic acid, α-D-glucose and α-L-arabinose were separated simultaneously by gas liquid chromatography as trimethylsilyl derivatives made by direct addition of pyridine, hexamethyldisilazane and trimethylchlorosilane at room temp. The method is suitable for the rapid analysis of fruit cell-wall constituents. A. H. CORNFIELD.

Fertiliser experiments on carrots in 1941 and 1942. H. V. Garner (*J. agric. Sci., Camb.*, 1967, **69**, 209–215).—$(NH_4)_2SO_4$ tended to depress yields or to have little effect. P as superphosphate gave slight yield increases and KCl and NaCl both increased the yield of sound roots. N, K and NaCl usually increased both yield and number of split roots, but only NaCl appreciably increased the proportion of splits. M. LONG.

Response of lettuce to soil application of zinc. F. W. Zink (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 406–414).—Application of $ZnSO_4$ (10–34 lb Zn per acre) increased the growth rate and yields and accelerated the maturity of lettuce grown on soils (pH 7·8–8·1) containing 0·55–0·65 ppm dithizone-extractable Zn, even though control plants showed no foliar symptoms of Zn deficiency. The treatments increased Zn% in the above-ground parts of the plants, had little effect on % N, P, K, Ca, or Na, and reduced Mg% in early growth. Foliar applications of 0·5% $ZnSO_4$ or Zn-EDTA had no effect on lettuce yields. A. H. CORNFIELD.

Growth and development of soya-beans as related to nutrient composition and environmental factors. R. D. Frazier (*Diss. Abstr.* B, 1967, **27**, 2225).—Pairs of similar soils, one highly productive of soya-beans and the other of low productivity, were compared in relation to differences of management, environmental conditions, leaf nutrient composition, soil conditions and plant performance. Among factors influencing plant performance, increased foliar contents of Cu, Mn and Zn were associated with increased no. of pods/plant. Leaf-Mn and -Zn were inversely related to soil pH. Possible antagonism between Mn and Zn in some enzymic reactions is discussed. During critical periods of growth of soya-beans increasing soil moisture was associated with increasing no. of pods. Increases in leaf-K and -Mn were associated with increased seed wt. Leaf-Zn and seed wt. were inversely related. Soil pH affected seed wt. indirectly through its influence on soil nutrients. Grain yield, bu/acre, increased with increases in leaf-K, -Mg, -Fe and -Cu. A. G. POLLARD.

Influence of plant population on yield and other characteristics of soya-beans. B. J. Johnson and H. B. Harris (*Agron. J.*, 1967, **59**, 447–449).—The variety Bragg produced max. yields with two plants per ft of row (36 in. apart) whilst three other varieties required eight plants per ft for max. yields. Plant height increased with no. of plants per ft of row up to eight plants. Lodging was serious with only one variety in one of the 3 years. Seed size decreased slightly in only one variety with increasing number of plants (1–16) per ft of row. A. H CORNFIELD.

Effect of root temperature variation on growth and transpiration of cotton seedlings. L. E. Nelson (*Agron. J.*, 1967, **59**, 391–395).—Fresh and dry wt., leaf area, and water use of cotton seedlings grown in nutrient solution decreased with decreasing root temp. from 24° to 12°, at which growth was virtually nil. When root temp. of seedlings grown initially at 12° was changed to 24° growth thereafter was similar to that of seedlings whose roots were maintained at 24° for the whole period. A. H. CORNFIELD.

Differential tolerance of cotton varieties to an acid soil high in exchangeable aluminium. C. D. Foy, W. H. Armiger, A. L. Fleming and C. F. Lewis (*Agron. J.*, 1967, **59**, 415–418).—Fourteen varieties of cotton, adapted to various regions of the cotton belt of the U.S.A., differed significantly in top growth when grown on a clay loam (pH 4·4) high in exchangeable Al. Addition of $CaCO_3$ at increasing levels increased top growth. Max. yields of all varieties were attained when sufficient $CaCO_3$ was added to increase soil pH to about 5·4. Varieties showing the greatest tolerance to the acid soil were western in origin, whilst those showing the least tolerance were of eastern, delta, and western origins. Results are discussed in relation to breeding of acid-tolerant varieties. A. H. CORNFIELD.

Effect of relative humidity, temperature, and light intensity during boll opening on cottonseed quality. J. M. Woodruff, F. S. McCain and C. S. Hoveland (*Agron. J.*, 1967, **59**, 441–444).—The % germination of cottonseed from bolls subjected to increasing R.H. (60–100%) for 21 days decreased with increasing R.H. Free fatty acid in the seed increased considerably with increasing boll R.H. Varying boll temp. from 25° to 40° and light intensity from 50 to 2,000 ft candles had little or no effect on seed quality. A. H. CORNFIELD.

Soil factors influencing the growth of cotton following peach orchards. C. R. Lee and N. R. Page (*Agron. J.*, 1967, **59**, 237–240).—Cotton grown in pots using a sandy soil (pH 5·3) from a peach orchard site showed stunted growth and leaf chlorosis and contained high concn. of Zn and Mn. Raising soil pH to 6·0 or higher by treatment with $CaCO_3 + MgCO_3$, peach tree ash or Na_2CO_3 produced normal growth of cotton. The poor growth of cotton on the untreated soil was traced to residues of Zn sprays applied to peach trees in the past. A. H. CORNFIELD.

Relative effects of acid subsoils on cotton yields in field experiments and on cotton roots in growth-chamber experiments. F. Adams, R. W. Pearson and B. D. Doss (*Agron. J.*, 1967, **59**, 453–456).—In growth-chamber tests there was a considerable increase in the rate of penetration of primary roots of cotton with increasing pH (4·2–6·4) of the subsoils used. In field experiments yields of seed cotton on three soils (with surface layers limed to pH 6·0–6·5) were 5–30% lower where the subsoils (15–30 cm depth) had a pH $\geq$4·9 than where the subsoils had pH of $\leq$5·2. A. H. CORNFIELD.

Fertiliser experiments with rape. L. Gisiger and R. Bonjour (*Schweiz. landw. Forsch.*, 1967, **6**, 286–300).—In a soil containing K sol. in saturated aq. CO_2, >2 and P sol. in the same >8 mg/100 g, rape showed no response to K and P fertilisers. The N requirement in absence of farmyard manure was 135 kg/ha, this being best applied as 90 kg early and 45 kg just before flowering. Inclusion of S sources [gypsum, K_2SO_4, $(NH_4)_2SO_4$] in the manurial treatment produced no definite increase in yield or wt./vol. of grain or yield of straw. Late (pre-blossom) top-dressings of N as urea, NH_4NO_3 and foliar applications of urea produced similar increases in yield. A. G. POLLARD.

Sugarcane yields as related to acidity of a humid tropic ultisol. F. Abruna-Rodriguez and J. Vicente-Chandler (*Agron. J.*, 1967, **59**, 330–332).—Sugarcane yields on a clay ultisol increased very greatly as pH was increased from 3·8 to 4·8 and as the exchangeable base content increased from 2 to 8 mequiv. per 100 g. Increasing pH was accompanied by decreasing exchangeable Al. A. H. CORNFIELD.

Nutrition of coffee: deriving reliable data for advisory purposes. J. B. D. Robinson (*E. Afr. agric. For. J.*, 1967, **33**, 95–99).—Problems associated with soil and leaf analysis for indicating the nutritional status of coffee with a view to efficient use of fertilisers are presented and discussed. A. H. CORNFIELD.

Effect of soil moisture on nodulation in inoculated groundnuts. D. Shimshi, J. Schiffman, Y. Kost, H. Bielorai and Y. Alper (*Agron. J.*, 1967, **59**, 397–400).—The placement of inoculum at planting time at a 3–4 cm soil depth resulted in better nodulation in groundnuts than did placement at 12 cm depth, in spite of the drying of the upper soil between repeated irrigation treatments. Yields and quality of nuts were higher with inoculation than with application of $(NH_4)_2SO_4$ (85 kg per 1000 sq. m). A. H. CORNFIELD.

Castor-bean production as related to length of growing season. I. Effect of date of plant desiccation. D. L. Kittock and J. H. Williams (*Agron. J.*, 1967, **59**, 438–440).—Castor-bean yields increased as desiccation treatment (2·75 kg diquat per hectare) was advanced from early Sept. to late Oct. The increases due to delay in desiccation were accounted for mainly in the secondary and tertiary racemes. The highest germination% of seeds was obtained with late-Sept.–early-Oct. desiccant treatments. Drying of seed following plant desiccation did not reduce germination. A. H. CORNFIELD.

Effect of soil fertility and plant competition on grain sorghum panicle morphology and panicle weight components. A. Blum (*Agron. J.*, 1967, **59**, 400–405).—High soil fertility (N, P and K applied at high levels during the previous 4 years) increased the 1000-grain wt. and no. of grains per panicle compared with no fertiliser treatment. Increasing the distance between rows (35–100 cm) also increased these components. Higher soil fertility and wider spacing promoted a longer panicle through increase in rachis internode length and no. of internodes respectively.
A. H. CORNFIELD.

Leaf area indices and nitrogen uptake of flue-cured tobacco as affected by plant density and nitrogen rate. R. J. Miller, G. W. Langdale and D. L. Myrhe (*Agron. J.*, 1967, **59**, 409–412).—Leaf area index values of tobacco increased with rate of application of N (44–134 kg per hectare) and decreased with increasing spacing (25–76 cm) between plants in rows 122 cm apart. In both years leaf area was highly correlated with leaf wt., particularly at the high N rate. Varying spacing had little effect on the relationships. Although yields of flue-cured tobacco increased with closer spacing and increasing N rate, quality of leaf decreased with increasing N. The most profitable conditions were a 76 cm spacing at the low N rate. A. H. CORNFIELD.

Factors affecting the ascorbic acid content of Acerola, *Malpighia glabra*. H. Y. Nakasone, R. K. Miyashita and G. M. Yamane (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 161–166).—The ascorbic acid content of acerola fruit was max. 16–18 days after floral anthesis, with concn. in the region of 4 g per 100 g of fruit flesh. Fruit from plants grown on their own roots were only slightly lower in ascorbic acid than that of grafted plants. Picked fruit exposed to sunlight lost 25% of their ascorbic acid in 8 h. Fruit ascorbic acid content decreased with increasing amount of shading of the plants. A. H. CORNFIELD.

Pest Control

Phytotoxic pesticide interactions in soil. R. G. Nash (*Agron. J.*, 1967, **59**, 227–230).—When dalapon was applied to soil together with Di-Systox, phorate, or carbaryl, phytotoxic effects were additive, whilst synergistic effects were produced when diuron was added with either of the three materials. The effect of captan was independent of the presence of dalapon. Chloranil was antagonistic towards the herbicidal effect of diuron.
A. H. CORNFIELD.

Movement and persistence of DDT and lindane in soil columns. W. D. Guenzi and W. E. Beard (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 644–647).—There was virtually no movement of DDT under the influence of leaching water but downward movement of lindane was considerable. The movement of lindane increased with the amount of water applied and was greater in a coarse- than in a fine-textured soil. When soils were subjected to alternate wetting and drying lindane was more susceptible to decomposition than was DDT. A. H. CORNFIELD.

Differential effects of mercuric chloride on growth of certain fungi associated with maize seed. H. M. Leon-Gallegos (*Proc. Indiana Acad. Sci.*, 1967, **76**, 217–220).—Maize kernels were placed in 0·1% solution of $HgCl_2$ for various periods (1–6 min.), dried and placed on agar plates inoculated with various pathogenic fungi. Growth of *Cephalosporium acremonium*, *Penicillium cyclopium*, *P. frequentans*, *P. funiculosum*, *P. herquei*, *P. multicolor*, *P. regulosum*, *P. variable* and *Pythium ultimum* was inhibited. *A. flavus*, *D. maydis*, *F. monilleforme*, *Gibberella zeae* and *Nigrospora oryzae* were unaffected. Residual $HgCl_2$ on the seed could be almost completely removed by rinsing with aq. NaOCl.
A. G. POLLARD.

Aphid transmission of beet yellows virus inhibited by mineral oil. J. Vanderveken and J. Semal (*Phytopathology*, 1966, **56**, 1210–1211).—Virus-infected aphids (*Myzus persicae*) were transferred to sugar-beet seedlings previously sprayed with a 2% emulsion of light, white paraffin oil. Transmission of the virus from the aphids to the seedlings was markedly inhibited. A. G. POLLARD.

Chemotaxis of zoospores of *Aphanomyces cochlioides* to sugar-beet seedlings. P. V. Rai and G. A. Strobel (*Phytopathology*, 1966, **56**, 1365–1369).—Root exudates of sugar-beet seedlings had a chemotaxic action on zoospores of *A. cochlioides* as well as stimulating their germination and the growth of germ tubes. Detailed analysis of the crude exudate showed the presence of 14 ninhydrin-positive compounds, three org. acids and nine sugars. The amino-acid fraction stimulated the germination of the zoospores and the growth of germ tubes but did not attract the zoospores. The org. acids and the neutral fractions attracted the zoospores but had no effect on their germination. Of the org. acids gluconic acid showed the greatest attraction for zoospores. Among the sugars, glucose and fructose showed strong and maltose and sucrose a very weak attractive effect; melibiose had no definite effect and raffinose and ribose appeared to repel the zoospores. A. G. POLLARD.

Amino-acid composition of a crystalline host-specific toxin. R. B. Pringle and R. P. Scheffer (*Phytopathology*, 1966, **56**, 1149–1151).—The isolation of a host specific toxin from culture filtrates of *Periconia circinata* (the causal agent of a disease of sorghum) is described. The purified material inhibited the growth of susceptible but not that of resistant sorghum plants. The cryst. toxin on hydrolysis with HCl yielded four amino-acids: alanine, aspartic acid, glutamic acid and serine in the mol. ratio 6 : 4 : 2 : 2.
A. G. POLLARD.

Chemical and genetic basis for insect resistance in cucurbits. O. L. Chambliss and C. M. Jones (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 394–405).—*In vivo* and *in vitro* studies of cucurbitacins, the bitter principles of the *Cucurbitaceae*, indicated a quant. relationship between levels of these compounds and degree of insect feeding. *In vitro* studies showed the attractiveness of some curcurbitacins apart from the influences of other possible host plant differences. Cucurbitacins of different structure varied in attractiveness to insects. A. H. CORNFIELD.

***Pythium* species still viable after twelve years in an air-dried muck soil.** P. E. Hoppe (*Phytopathology*, 1966, **56**, 1411).—In a naturally infected muck soil which had remained in an air-dry condition for 6 years, *Pythium* spp. remained active, probably as oospores, as shown by incubation of the wetted soil with maize grain. A. G. POLLARD.

Effect of fentin acetate, maneb and copper oxychloride on the population density of the Colorado beetle (*Leptinotarsa decemlineata*, Say.) in field trials. R. Murbach (*Schweiz. landw. Forsch.*, 1967, **6**, 345–357).—Experimental data recorded confirmed earlier work (Murbach & Corbaz., *Phytopath. Z.*, 1963, **49**, 182). Unlike copper oxide, fentin acetate has no repellent action on the adult beetle but restricts or inhibits feeding thus lowering the fertility of the females without diminishing their numbers. Egg production was lowered; larvae consumed only small amounts of sprayed foliage and their mortality rate was high. Maneb had no apparent effect on the population density of the beetles.
A. G. POLLARD.

Invasion by fungi of rice stored at moisture contents of 13·5 to 15·5%. H. A. Fanse and C. M. Christensen (*Phytopathology*, 1966, **56**, 1161–1164).—Invasion of Texas-grown, seed-grade rice by storage fungi was similar at 13·5–15·5% H_2O/20–25°, for 4 months. A moisture content of 14% is probably the lowest permitting fungal invasion of sound rice. A. G. POLLARD.

Competitive effects of quackgrass, *Agropyron repens*, upon maize as modified by fertilisation. J. D. Bandeen and K. P. Buchholtz (*Weeds*, 1967, **15**, 220–224).—The presence of quackgrass delayed maturity and greatly reduced the yields and height of maize plants in comparison with plots where quackgrass was controlled by pre-emergence treatment with linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], (1·7 lb per acre) and post-emergent treatment with 2,4-D (2,4-dichlorophenoxyacetic acid), 8 oz/acre. Fertilisation with N (100–200 lb), P_2O_5 (50–100 lb) and K_2O (100–200 lb per acre) did not overcome the effects of quackgrass competition. Average annual uptake over two seasons by quackgrass was 105, 15 ,and 60 lb per acre for N, P and K respectively and accounted for approx. half of the total nutrient uptake.
A. H. CORNFIELD.

Heat pasteurisation for control of post-harvest decay in fresh strawberries. H. M. Couey and M. N. Follstad (*Phytopathology*, 1966, **56**, 1345–1347).—Post-harvest decay of the fruit by *Botrytis cinerea* and *Rhizopus stolonifer* was controlled by pasteurisation using moist air at 44° for 40 or 60 min. No effect on flavour or texture was observed. A. G. POLLARD.

Low-oxygen atmospheres for control of post-harvest decay of fresh strawberries. H. M. Couey, M. N. Follstad and M. Uota (*Phytopathology*, 1966, **56**, 1339–1341).—Fresh strawberries were stored at 3° in atm. of different [O_2], the [CO_2] being kept at 1·5%. The fruit was examined after 5 days and again after an additional 2 days at 15°. In all cases the gas concn. was maintained by a continuous flow of the requisite mixture. *Botrytis* decay of the fruit was diminished by lowering the [O_2] to $\geq$0·50%. Off-flavours developed in fruit stored in [O_2] $\geq$0·25%. Small varietal differences in response to low [O_2] were noted. A. G. POLLARD.

Bitter pit as related to calcium level in Baldwin apple fruit and leaves. M. Drake, W. D. Weeks, J. H. Baker, D. L. Field and G. W. Olanyk (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 23–29).—Six spray application of 0·5% $Ca(NO_3)_2$ from July 20 to October 6 increased leaf-Ca but had no effect on leaf-N. High incidence of bitter pit in fruit at picking and after 5 months' storage was related to low leaf-Ca, but was even better related to low Ca% in the fruit skin; skin of sound fruit contained twice the concn. of Ca as that from pitted fruit. A. H. CORNFIELD.

Phytotoxicity to blackcurrants of sprays containing sulphur. B. D. Smith and G. M. Clarke (*Ann. appl. Biol.*, 1967, **59**, 101–109).—Studies over 3 years of the phytotoxic effects on blackcurrants of sprays containing S, particularly CaO–S, showed that there was considerable variation in the degree of effect between years and between experiments in the same year. It was difficult to damage flowers except at low temp., but if fruit set was affected then yield was depressed. Leaf scorching needed to be very severe before yields were depressed. Application of 0·25–4% CaO–S reduced the rate of shoot growth for short periods, dependent on concn., but total bud numbers, flower/vegetative bud ratios and yields were not affected. The adverse effects of the treatments were not carried over into the following year. A. H. CORNFIELD.

Effects of fungicides on germination of pollen and fruit set of cranberry. A. Y. Shawa, C. C. Doughty and F. Johnson (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 255–258).—Captan, ferbam, maneb and phaltan applied to the surface of agar plates, completely inhibited germination of cranberry pollen. Pollen collected from sprayed plants showed significantly reduced germination where maneb and phaltan, but not where captan and ferbam, had been used. All materials and daconil 2787 significantly reduced cranberry yields when the plants were sprayed during the blossoming period. Botran and zineb did not reduce yields.
A. H. CORNFIELD.

Persistence of picloram, 4-amino-3,5,6-trichloropicolinic acid, in soils. M. G. Merkle, R. W. Bovey and F. S. Davis (*Agron. J.*, 1967, **59**, 413–415).—When the herbicide picloram was added to soils (clay, sandy loam and sand) at 0·25 ppm (0·5 lb per acre) and incubated at moisture contents of field capacity and 10% of field capacity at 4–38°, residues were detected even after one year. Picloram was leached downwards more readily in light- than in heavy-textured soils. Picloram was quickly degraded by the action of sunlight. A. H. CORNFIELD.

Microbial versus chemical degradation of atrazine in soils. H. D. Skipper, C. M. Gilmour and W. R. Furtick (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 653–657).—The chemical and microbial degradation of ^{14}C-labelled atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) was determined in greenhouse incubation studies. Less than 4% of the ^{14}C in atrazine was evolved as CO_2 during 3 weeks incubation of untreated soils or soils treated with various bacterial isolates (from atrazine-treated field soils) and *Aspergillus fumigatus*. During the same period there was a 73% loss of atrazine toxicity to oats. The results support chemical hydrolysis of chloratrazine to hydroxyatrazine as the major pathway of degradation in soils with microbial attack being of minor importance.
A. H. CORNFIELD.

Movement of herbicides in soil. C. I. Harris (*Weeds*, 1967, **15**, 214–216).—A soil column method is described for determining the relative movement of herbicides in soils using oats as the test crop and monuron [3-(*p*-chlorophenyl)-1,1-dimethylurea] as a standard. Thus movement was expressed as a single number (the 'relative mobility factor'). Results with 28 herbicides using a silty clay loam and a sandy loam are presented. The aromatic acid herbicides were the most, and the toluidines the least, mobile.
A. H. CORNFIELD.

Soil incorporation and site of uptake of pre-emergence herbicides. E. L. Knake, A. P. Appleby and W. R. Furtick (*Weeds*, 1967, **15**, 228–232).—As depth of incorporation in the soil of most of the herbicides tested increased (surface application to 3 in. depth) so the extent of control of green foxtail decreased. The effects of trifluralin (α, α, α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) and CP 3193 (*N*-chloro-*N*-isopropylacetanilide) in controlling foxtail were similar at low, moderate or high soil moisture contents whether the material were surface-applied or incorporated at 1 in. depth. Amiben (3-amino-2,5-dichlorobenzoic acid) was more effective at high and moderate than at low moisture contents with both methods of application. Linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] and atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) were effective at all moisture contents when surface applied, but were less effective at high and moderate than at low moisture contents when incorporated. The reverse was true for EPTC (ethyl *N*,*N*-dipropylthiolcarbamate).
A. H. CORNFIELD.

Influence of DCPA, dimethyl 2,3,5,6-tetrachloroterephthalate, on soil micro-organisms. M. L. Fields, R. Der and D. D. Hemphill (*Weeds*, 1967, **15**, 195–197).—Addition of DCPA (5–50 ppm) to soil had no effect on the numbers or activity of *Thiobacillus*, *Azotobacter*, *Rhizobium*, *Aspergillus*, *Paramecium*, *Colpidium* and mixed soil ciliates over 84 days. DCPA stimulated the growth of a mixed culture of soil algae and fungi. A field soil treated annually for 7 years with DCPA at 10 lb per acre showed no accumulation of the herbicide. A. H. CORNFIELD.

Herbicidal residues in pond water and hydrosoil. P. A. Frank and R. D. Comes (*Weeds*, 1967, **15**, 210–213).—When dichlobenil (2,6-dichlorobenzonitrile, applied to give 0·4–0·6 ppm on pond water basis) was applied to control pond weeds, residues persisted in both water and hydrosoil for more than 160 days. Fenac (2,3,6-trichlorophenylacetic acid), (1·0–1·6 ppm) residues also persisted for 160 days, whilst residues of 2,4-D (1·33 ppm) were low in water after 24 days and in the hydrosoil after 55 days. Endothal-amine salt [mono-*N*,*N*-dimethylcocoamine-7-oxabicyclo (2.2.1)-heptane-2,3-dicarboxylic acid], (1 ppm), paraquat, (1,1′-dimethyl-4,4′-bipyridinium salt) (1·14 ppm), and diquat [6,7-dihydrodipyrido(1,2-*a*:2′,1′-*c*)-pyrazidinium salt] (0·62 ppm), were relatively less persistent in water and were no longer found after 24, 8, and 4 days respectively. Paraquat and diquat persisted in the hydrosoil for more than 85 and 160 days respectively.
A. H. CORNFIELD.

Factors in paraquat-induced chlorosis with *Phaseolus* foliar tissue. D. L. Barnes and J. Q. Lynd (*Agron. J.*, 1967, **59**, 364–366).—Paraquat (applied at 0·025–1 ppm) did not affect chlorosis in *Phaseolus* leaf discs in the dark, or in the light at 1°. When the discs were exposed to light the extent of chlorosis (measured by determining leaf chlorophyll) in discs increased with the level of paraquat applied, with light intensity (200–750 ft candles) and with temp. (15–45°). A. H. CORNFIELD.

Interaction of paraquat, 1,1′-dimethyl 4,4′-bipyridylium dichloride, with mineral soils. B. A. G. Knight and T. E. Tomlinson (*J. Soil Sci.*, 1967, **18**, 233–243).—Solution-equilbration studies with paraquat and a range of mineral soils showed that up to a limiting value (strong adsorption capacity, SAC) the concn. of paraquat in solution was reduced below chemical detection; this strongly held paraquat was held against extraction with 0·1–2·0 N-NH_4^+. The greater part of the strongly adsorbed material was de-activated herbicidally. Removal of soil org. matter usually did not greatly change the SAC. Where amounts of paraquat above the SAC were present, this was more weakly adsorbed, but the total adsorption capacities of soils at saturation were less than their exchange capacities for inorg. cations. The adsorption of paraquat by soils is probably strongly influenced by factors other than simple electrostatic interaction. A. H. CORNFIELD.

Improvement of pastures by treatment with paraquat, 1,1′-dimethyl-4,4′-bipyridylium salt. G. G. Bowes and G. Friesen (*Weeds*, 1967, **15**, 241–243).—Spring applications of paraquat (1 lb per acre) on native grasslands were effective in suppressing existing grasses and forbs, thereby enhancing the establishment of lucerne. Autumn application of paraquat was ineffective in suppressing native vegetation, so that lucerne sown in the following spring failed to establish an adequate stand. A mixture of 2,4-D and 2,4,5-T was ineffective in controlling woody species in grassland. A. H. CORNFIELD.

Use of herbicides in white clover seed crops. A. Zaleski (*J. Br. Grassld Soc.*, 1967, **22**, 62–65).—Application of paraquat (1·15 lb per acre) at the early stages of clover growth in the spring increased the dominance of clover over grass and also increased clover seed yields. Dalapon (2–8 lb per acre) also increased the proportion of clover in the sward but tended to retard flower and seed formation and did not increase seed yields.
A. H. CORNFIELD.

Effects of trifluralin, α, α, α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine, on cotton seedlings. W. P. Anderson, A. B. Richards and J. W. Whitworth (*Weeds*, 1967, **15**, 224–227).—Greenhouse tests on the effects of placement and rate of trifluralin on cotton seedlings showed that the herbicide should be placed at depths of 1 to 3 in. at rates not exceeding 1 lb per acre. Although temporary stunting sometimes occurred, the seedlings soon recovered and subsequent growth was normal. A. H. CORNFIELD.

Chemotherapeutic activity of symmetrical dichlorotetrafluoro-

acetone. C. L. Keswani (*Diss. Abstr.* B, 1967, **27,** 2223).—Dichlorotetrafluoroacetone hydrate, (DCTFA), was not active against various fungi *in vitro*, but showed notable activity against some phytopathogenic bacteria; *Agrobacterium tumefaciens*, *Xanthomonas malvacearum* and *Erwinia carnegieana*. It inhibited the development of established bean rust when applied as a spray or *via* roots, but uredospores, matured before application of DCTFA, germinated well when collected from treated bean plants. DCTFA at concn. 500 ppm caused marginal leaf burning, but at 25 ppm was phytotoxic when absorbed by the roots. It had no effect on respiration or net photosynthesis but increased polyphenol-oxidase activity in host plants when absorbed *via* roots. Trichlorotrifluoroacetone in comparable concn. was completely inactive. A. G. POLLARD.

Control of huisache, *Acacia farnesiana*, with picloram, 4-amino-3,5,6-trichloropicolinic acid. R. W. Bovey, F. S. Davis and M. G. Merkle (*Weeds*, 1967, **15,** 245–249).—Soil or foliar applications of picloram (K salt, 0·5 lb per acre) caused complete defoliation of huisache plants, with little leaf regrowth afterwards. When plants were defoliated before treatment there was considerable regrowth afterwards. Most of the picloram applied to foliage was found in and on the leaves 30 days after treatment. The concn. of picloram in roots was similar whether the herbicide was applied to the soil or as a foliar spray. A. H. CORNFIELD.

Breakdown products of ^{14}C-labelled *N*-dimethylamino-succinamic acid (alar) in the apple tree. G. C. Martin (*Proc. Am. Soc. hort. Sci.*, 1966, **89,** 1–9).—Alar labelled in the dimazine and succinic acid parts was applied by trunk injection and root dips to apple trees and seedlings in the field and greenhouse. Radioactivity in various plant parts and chemical fractions of the apple tree showed similar decomposition rates for both labelled parts. Alar moved freely into all parts of the tree as well as into the soil *via* the roots. The major part of the extractable radioactivity in various plant parts appeared as unchanged alar, although there was slow decomp. of alar throughout the growing period. A. H. CORNFIELD.

Effect of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on transpiration and water content of apricot seedlings and trees. N. Marei, J. C. Crane and M. V. Bradley (*Proc. Am. Soc. hort. Sci.*, 1966, **89,** 61–71).—Application of sprays of 2,4,5-T (50–100 ppm) to apricot seedlings reduced transpiration within 24 h; this persisted for 3 weeks until the plants began to die. When 3-year-old orchard trees were sprayed in May with 2,4,5-T (50–200 ppm) leaf water content decreased, whilst water content of current season's and 1-year-old wood, increased. Histological changes due to the treatments are also reported. A. H. CORNFIELD.

Post-emergence action of CIPC, isopropyl *N*-(3-chlorophenyl) carbamate. Y. Eshel and G. F. Warren (*Weeds*, 1967, **15,** 237–241).—Post-emergence applications of CIPC (3–6 lb per acre) rapidly killed seedlings of redroot pigweed, *Amaranthus retroflexus*, and large crabgrass, *Digitaria sanguinalis*. These species showed injury symptoms within 1–2 days of treatment and these symptoms were accompanied by reduced photosynthesis, decreased chlorophyll content, and increased respiration. Pale smartweed, *Polygonum lapathifolium*, did not show injury symptoms or physiological changes until about two weeks after treatment with CIPC. A. H. CORNFIELD.

Dalapon for control of quackgrass, *Agropyron repens*. A. C. Carder (*Weeds*, 1967, **15,** 201–203).—The best treatment for eliminating quackgrass was to rejuvenate the quackgrass in the spring by thorough tillage, to apply dalapon (2,2-dichloropropionic acid) (20 lb per acre) when the sprouts were 4–6 in. high, to till again two weeks later and then at monthly intervals until frosts. There were no residual effects of dalapon on cereal crops grown the year after treatment. A. H. CORNFIELD.

Subsurface placement of EPTC, ethyl *N*,*N*-dipropylthiocarbamate for weed control in seedling legumes. D. L. Linscott, R. R. Seaney and R. D. Hagin (*Weeds*, 1967, **15,** 259–264).—Subsurface applications of liquid or granular formulations of EPTC (1·0–3·0 lb per acre) at sowing time effectively controlled annual grass weed species in lucerne and birdsfoot trefoil, but control of annual broadleaf weeds was not very good. Post-emergence application of 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid], (1–3 lb per acre) in addition to EPTC resulted in excellent overall weed control. A. H. CORNFIELD.

Pyrazon for control of weeds in sugar beet. R. Frank (*Weeds*, 1967, **15,** 197–201).—Pre-emergence application of pyrazon (5-amino-4-chloro-2-phenyl-3-(2*H*)-pyridazinone), 4–5 lb per acre), controlled broadleaf weed species but was marginal in controlling foxtail. When DCPA was applied at 6–7·5 lb per acre beet stands were reduced, but final stands were satisfactory because of the normal practice of overseeding sugar beet. A. H. CORNFIELD.

Factors affecting response of prickly pear, *Opuntia* sp., to 2,4,5-trichlorophenoxyacetic acid. R. E. Meyer and H. L. Morton (*Weeds*, 1967, **15,** 207–209).—Prickly pear pads were killed when ester and ether-ester formulations of 2,4,5-T were applied to one side only. Rate of killing increased with temp. (21–43°). At temp. above 21° 2,4,5-T at 4 lb acid-equiv. per 100 g solution had little effect, whilst 8 lb acid equiv. usually killed the pads. The toxicity of 2,4,5-T was similar whether applied in water or diesel oil. 2,4,5-T was more toxic than 2,4,-D. A. H. CORNFIELD.

Seasonal susceptibility of guava to herbicides. F. H. Tschirley, R. T. Hernandez and C. C. Dowler (*Weeds*, 1967, **15,** 217–219).—Guava, a serious weed in tropical grazing areas, was more susceptible to control by 2,4-D : 2,4,5-T (1 : 1 ratio of butoxyethanol esters of 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acids) when treated during the wet than during the dry season. The extent of defoliation following treatment was significantly correlated with rainfall during the 3 weeks preceding treatment. Similar results were obtained for dicamba (2-methoxy-3,6-dichlorobenzoic acid) and picloram (2,3,6-trichloropicolinic acid), but the period of max. susceptibility occurred later in the season than for 2,4-D: 2,4,5-T. A. H. CORNFIELD.

Herbicide–crop rotation for control of witchweed, *Striga* spp. E. L. Robinson, J. E. Dale and W. C. Shaw (*Weeds*, 1967, **15,** 243–245).—Land heavily infested with witchweed required 3–4 years of pre- and post-emergent herbicide treatments with continuous maize or maize–cotton–groundnuts–soya-beans rotation before competition of witchweed was eliminated. The most effective herbicide treatment was post-emergent application of 2,4-dichlorophenoxyacetic acid (0·5 lb per acre) when needed in continuous maize. A. H. CORNFIELD.

Problems in woody plant control evaluation in the tropics. F. H. Tschirley (*Weeds*, 1967, **15,** 233–237).—Because of the high diversity of species present in wet tropical forests problems arise in sampling and analysis of data regarding herbicidal effectiveness. Three simplified sampling procedures were tested to determine their reliability in providing an estimate of whole-plot defoliation from broadcast applications. A procedure using a 50-tree sample including all species provided the most reliable data. A. H. CORNFIELD.

Animal Husbandry

Agricultural development and research in New Zealand. J. B. Hulton (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27,** 1–16).—A presidential address largely concerned with the future expansion of animal production in New Zealand and likely means of achieving this. A. G. POLLARD.

Principles and practices used in livestock improvement programmes in the U.S.A. A. B. Chapman (*Proc. N.Z. Soc. Anim Prod.*, 1967, **27,** 17–28).—Practical applications of the principles of genetics to the improvement of livestock in the U.S.A. are illustrated. On-the-farm recording programmes and the establishment of central testing stations for recording data of value in the selection of breeding stock are described. Some general effects of mating systems in the development of better grades of stock are discussed. A. G. POLLARD.

Use of a steady-state feeding system in nutrition experiments with ruminants. M. J. Ulyatt (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27,** 181–192).—A moving belt device is described by which pelletted feed is added at a steady rate to the feeding box and adjusted to produce a steady continuous fermentation in the rumen of sheep. Examples of use of the device in studies of ruminant nutrition are given. A. G. POLLARD.

Symposium on atomic energy in animal science. I. The Illinois animal science counter: performance characteristics and animal radioactivity measurement procedures. A. R. Twardock, T. G. Lohman, G. S. Smith and B. C. Breidenstein. **II. Estimation of carcass lean muscle mass in steers by ^{40}K measurement.** T. G. Lohman, B. C. Breidenstein, A. R. Twardock, G. S. Smith and H. W. Norton (*J. Anim. Sci.*, 1966, **25,** 1209–1217, 1218–1226).—I. A large-vol. liquid scintillation detector is described, for the measurement of radioactivity in human beings and animals up to the size of cattle. Operational details are included.

II. The ^{40}K content of 42 steers was determined by the large-vol.

counter and relationships with the lean muscle mass of the carcasses as determined by chemical analysis are examined. The precision of the detector measurements was affected by variations in the amount of radioactivity in the gastrointestinal tract, by the efficiency of the whole-body counter and by the positioning of the animal with respect to the scintillating detector. With improved procedures the whole-body count was repeatable from day to day within 2–3%, and the predicted carcass lean was obtained with standard errors $< 3\%$, within two narrow wt. ranges. Body-K can be measured with sufficient accuracy to afford a practical prediction of carcass lean muscle in live steers or in carcasses.
A. G. POLLARD.

An airflow calorimeter for the measurement of sensible heat loss of animals. F. Cockrem and R. M. Clarke (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 69–70).—The apparatus is based on passing a continuous air current through an animal calorimeter and measuring in-going and out-going air temp. By varying the rate of flow of air, temp. differences can be adjusted to appropriate ranges. In repeatability trials with sheep differences between two sheep were detectable (e.g. $12{\cdot}2 \pm 0{\cdot}30$ and $10{\cdot}3 \pm 0{\cdot}38$ cal/sec), ($P < 0{\cdot}001$).
A. G. POLLARD.

Efficiency of feed utilisation. I. E. Coop (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 154–165).—Factors influencing the efficiency of pasture utilisation, e.g., stocking rate, adjustment of pasture production to stock requirements, and of stock management to seasonal growth of pasture, are discussed. The importance of digestibility of pasture plants and of the selection of livestock having high efficiency of conversion is stressed.
A. G. POLLARD.

Use of ^{198}gold for measuring changes in rate of ingesta passage in the ruminant as influenced by diazapam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one). E. J. Bris, I. A. Dyer and I. D. Teare (*Agron. J.*, 1967, **59**, 225–227).—A method is described for measuring the rate of ingesta passage through the ruminant by administering ^{198}Au (20–90μ dia.) orally and following the time course of excreted Au. Au has a reproducible excretion pattern and is easily measured in the faeces of ruminants. The method showed that diazapam, a muscle relaxant, reduced the ingesta passage in steers and lambs.
A. H. CORNFIELD.

Collaborative *in vivo* studies on lucerne hay. E. Donefer (*J. Anim. Sci.*, 1966, **25**, 1227–1231).—In a collaborative series of tests using sheep and cattle involving 15 laboratories the chemical composition, digestibility and voluntary intake of lucerne hay were examined. Variability of chemical and *in vivo* data between laboratories is attributed to lack of uniformity of the test material and to differences in chemical and *in vivo* methods used in the different laboratories.
A. G. POLLARD.

Nutritive value of maize silages containing chemical additives, as measured by growth and milk production of dairy animals. W. G. Schmutz (*Diss. Abstr.* B, 1967, **27**, 212).—Silages containing urea, $(NH_4)_2HPO_4$, $CaCO_3$ and/or $CaHPO_4$ are compared. There was a small increase in the initial and in the final pH of the silages for each additive. The crude protein equiv. of the silages was increased by addition of urea or $(NH_4)_2HPO_4$. The % of the theoretical crude protein recovered ranged from 85 to 89%; the % of the non-protein lost was 32–45%. Use of the additives increased org. acid (acetate, lactate) production in the silage. Treated silages produced adequate gains in live wt. of heifers. Addition of $CaCO_3$ to urea-treated compared with untreated silages tended to lower the gain in wt. In general addition of urea increased the gain in wt. produced by the silage; further addition of $CaCO_3$ lowered this increase. Animals receiving silage containing 1% of $(NH_4)_2HPO_4$ consumed less silage dry matter/day, less dry matter, (*DM*)/100 lb body wt., total *DM* and total digestible nutrients, and produced less 4%-fat-corrected milk/day. With addition of 0·5% $CaCO_3$ alone or in combination with $(NH_4)_2HPO_4$ the depression of intake did not occur. The apparent digestibility of *DM*, ash and protein in the silage was slightly lowered by inclusion of urea.
A. G. POLLARD.

Influence of grain processing factors on the *in vitro* fermentation rate by a mixed suspension of rumen micro-organisms. J. E. Trei (*Diss. Abstr.* B, 1967, **27**, 2212–2213).—Effects of various methods of processing grains on their utilisation by rumen organisms are determined by an *in vitro* fermentation test in which the vol. of gas produced is the factor measured. Grain samples are ground to pass a 20-mesh screen and after incubation for 3 h with a suspension of mixed rumen organisms, the vol. of gas formed per unit dry matter incubated (ml/g) is highly correlated with the loss of dry matter taking place. Gas production varied with species and variety of grain. High-amylose maize fermented significantly more slowly than did 'regular' maize. Gas production from steamed milo, without flaking, was less than, and that from steamed and flaked milo exceeded that from unprocessed milo. Increasing the flatness of milo flakes increased the production of gas. Pressure-cooked but non-flaked milo gave the same results as did unprocessed grain. Barley acted similarly. Progressively increased gas production followed increased cooking pressure, without flaking; flaking the cooked material further increased gas yields. Steam processing (min. pressure/moist heat) was as effective as pressure-cooking at 4·2 kg/sq. cm and then flaking. Autoclaving without flaking, lowered the gas production rate. In general, satisfactory flaking after moist heat treatment of the whole grain gives best results and ensures close correlation with the gas production test.
A. G. POLLARD.

Grazing natural grassland in Western Kenya. A. V. Bogdan and E. M. Kidner (*E. Afr. agric. For. J.*, 1967, **33**, 31–34).—Live-wt. gains of steers over 5 years on natural grassland were no different between continuous grazing and 4-paddock rotational grazing (each paddock receiving 2 weeks grazing followed by 6 weeks of rest). Both these systems usually produced higher wt. gains than did a 3-paddock deferred grazing system (each paddock receiving a rest for one-third of the season), although the differences were just below the level of significance. There were significant differences in live-wt. gains between years, the differences being related to amount and distribution of rainfall. Changes in the botanical composition of the sward after 5 years of grazing are reported.
A. H. CORNFIELD.

Digestible energy content of East African grasses. B. Marshall and M. I. E. Long (*E. Afr. agric. For. J.*, 1967, **33**, 64–66).—The digestible energy content, calculated in five different ways from digestibility coeff. of dry matter, of *Cynodon dactylon*, *Chloris gayana*, *Panicum maximum*, and *Themeda triandra* hays, and *Pennisetum purpureum* are presented.
A. H. CORNFIELD.

Chemical composition, palatability, and digestibility of tall fescue, a ryegrass × tall fescue amphiploid hybrid and a ryegrass × tall fescue backcross hybrid. R. C. Buckner, J. R. Todd, P. B. Burrus, and R. F. Barnes (*Agron. J.*, 1967, **59**, 345–349).—The amphiploid hybrid was higher in crude protein, total sugars, moisture and *in vitro* digestibility and lower in SiO_2 and crude fibre than were the backcross and tall fescue varieties. All chemical constituents were closely associated and influenced digestibility and palatability. Silica was positively associated with crude fibre and negatively related to protein, sugar, moisture and digestibility. In breeding work sugar may be a valuable constituent for evaluating the grasses.
A. H. CORNFIELD.

Estimation of herbage intake from nitrogen, copper, magnesium and silicon concentration in faeces. W. R. McManus, M. L. Dudzinski and G. W. Arnold (*J. agric. Sci., Camb.*, 1967, **69**, 263–268).—Multiple regressions involving N, Cu, Mg and Si gave slightly better prediction than N by itself. Data for 11 forages investigated could not be brought into one expression. Cu, Mg and Si when used in the ratio technique gave a generally poor prediction of dry matter intake.
M. LONG.

Effect of breed, sex, type of cereal and supplementary hay on the performance of beef cattle given high-concentrate diets. E. Owen and G. M. Davies (*J. agric. Sci., Camb.*, 1967, **69**, 79–94).—Three breed types (Friesian (i), Charolais × Friesian (ii) and Welsh Black × Friesian (iii), both females and castrates, were fed individually and *ad lib* on one of three rations; (*a*) ground barley (87%) + protein, vitamins and mineral supplement, (13%); (*b*) as (*a*) with 1 lb hay/day, or (*c*) ground maize 87%, + supplement 13%, from 200 lb live-wt. until slaughter at 800–900 lb live-wt. Growth rates and feed efficiency varied considerably between breed types, the general order being (ii) > (i) > (iii). Carcass characteristics are recorded, comparisons being made between breed types and between females and castrates. The hay supplement to the barley ration significantly increased growth rates (by 10·3%); feed conversion was also increased though not significantly. Hay increased the appetite, notably in animals of < 600 lb live-wt. and reduced the incidence of bloat. Substitution of maize for barley had little effect on growth rates but affected feed conversion and fat deposition in some cases. Breeding type (ii), (castrates and females) appeared the most suitable for high-concentrate rations.
A. G. POLLARD.

Wheat *vs* maize in all-concentrate cattle rations. R. R. Oltjen, P. A. Putnam, E. E. Williams jun. and R. E. Davis (*J. Anim. Sci.*, 1966, **25**, 1000–1004).—Rations containing (*a*) 90% maize, (*b*) 60% maize + 30% wheat, (*c*) 60% wheat + 30% maize, or (*d*)

90% wheat were fed *ad lib* to groups of yearling steers. Growth was faster with (*a*) and (*b*) than with (*c*) or (*d*). All groups showed similar performances during the first 70 days but during the next 28 days less of (*d*) was consumed. Steers given (*c*) and (*d*) showed relatively greater concn. of ruminal volatile fatty acids and NH_3 but lower pH. Carcass characteristics were not greatly affected. Abscessed livers were more frequent in steers given wheat-containing rations. A. G. POLLARD.

Use of oxytocin for estimating milk production of beef cows. F. J. Schwulst, L. J. Sumption, L. A. Swiger and V. H. Arthaud (*J. Anim. Sci.*, 1966, **25**, 1045).—Three groups of cows were used to evaluate the use of intramuscular injections (2 ml) of oxytocin in estimating milk production. Treatments given were (i), control, (ii) oxytocin given after calf-nursing or (iii) given before calf nursing. Oxytocin had no significant effect on milk consumption by the calf or on the total milk production. Data suggest that more milk was obtained than was available to the calf under treatment (iii) than under (i) or (ii). The value of oxytocin in facilitating the 'let-down' of milk is discussed. A. G. POLLARD.

Ingestion of soil by sheep. W. B. Healey (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 109–120).—Ingestion of soil and consequent excessive wear of incisor teeth of sheep becomes intensified during winter when pasture is scarce and appetite is high. Supplementary feeding at this time of year limits the wear of teeth. Soils of strong structure are less readily ingested than are those of weak structure. Other effects considered are the intake of micro-elements and insecticides by the sheep. A. G. POLLARD.

Performance recording of sheep. E. A. Clarke (*Proc. N.Z. Soc. Anim. Sci.*, 1967, **27**, 29–45).—A system of recording adopted in New Zealand is described. Data collected include fertility records of sheep, character and wt. of fleece, weaning and shearing details of young sheep. Methods of making available appropriate data for breeders and methods of predicting the outcome of flock management are discussed. A. G. POLLARD.

Residual effects of prenatal nutrition on the postnatal performance of merino sheep. G. C. Everitt (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 52–68).—Data presented show the adverse effects of undernutrition in early pregnancy on the growth of lambs during the first 7 months of their post-natal life. Severe undernutrition of ewes in late pregnancy reduced the birth wt. and growth rate of single lambs throughout the 3-year experimental period and also lowered the wool follicle population and clean wool yield. Cumulative effects of these periods of undernutrition are established. Wether lambs were always heavier than ewe lambs, differences in body wt. being related to pasture productivity and climatic conditions. A. G. POLLARD.

Plasma lipids of newborn and adult ruminants and of lambs from birth to weaning. W. M. F. Leat (*J. agric. Sci., Camb.*, 1967, **69**, 241–246).—Plasma-lipid concn. of lambs are 20–40% of those found in their dams at parturition. Plasma-free fatty acids rise to max. about 45 days post partum and then decline. Plasma esterified lipids, mainly phospholipids and cholesterol esters, are low at birth and reach a max. 20 days later. The phospholipid composition of red blood cells of newborn lambs is generally similar to that of the adult and so is considered to be genetically rather than nutritionally controlled. M. LONG.

Areas of the stomach of sheep that are sensitive to formic acid and histamine. H. Neumark (*J. agric. Sci., Camb.*, 1967, **69**, 297–303).—The infusion of formic acid (**I**) and histamine (**II**) into the rumen *via* a cannula has no effect on time taken to eat food, although a mixture of **I** and **II** infused *via* a tube has variable effects on appetite, depending on the length of tube introduced. **II** but not **I**, depresses the rate of eating when introduced into the caudal part of the abomasum. **I** and **II** introduced into the oral part of the abomasum results in refusal of food, sometimes for several days. Sodium formate at the level of the cardia passes rapidly to the abomasum, having no effect on time devoted to eating. **I** given intravenously has no effect on eating, but **II** inhibits it for a short time. A mixture of **I** and **II** introduced at the level of the cardia through a stomach tube causes a marked increase of **I** in the blood. The introduction of the mixture into the rumen *via* a cannula resulted in a smaller temporary rise. M. LONG.

Effects of temperature on body growth and other traits of open- and woolly-faced Romney lambs. F. Cockrem (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 193–209).—Wether lambs were kept at 45° or 65°F for the period between 7 and 12 months of age and fed *ad lib* on pellets containing lucerne, barley meal and wheat husk. They were sheared at age 11 months. Among control animals in the field open-faced lambs showed the higher growth rate, but no differences in growth were apparent among those kept at const. temp. Body growth, wool growth and feed consumption were greater at 45° than at 60°F. At 45°F open-faced lambs grew more wool on the sides and less on the face than did the woolly-faced lambs and had a lower feed intake. At 65°F wool production was similar in both types. Body temp. of woolly-faced lambs kept at 65° were higher than those of open-faced sheep but were similar or lower after shearing; the fall in temp. following shearing was greater in lambs kept at 65°F than in those kept at 45°. (27 references.) A. G. POLLARD.

Winter nutrition of Romney hoggets. K. R. Drew (*Proc. N.Z. Soc. Anim. Sci.*, 1967, **27**, 210–222).—Eight-months old wethers were used in a comparison of five winter feeding systems; (i) swedes, (ii) swedes + hay, (iii) hay, (iv) unrestricted grass, (v) restricted grass. Rations (i) and (ii) produced high growth rates; with (ii) the sheep consumed 5% more digestible energy than with (i) but the gain in wt. was 50% greater. Consumption of swedes available *ad lib* in the field was 110–140% higher than in pens. Unrestricted autumn-saved pasture as (iv) did not yield gains in body-wt. as high as did rations (i) (ii) or (iii). A. G. POLLARD.

Influence of various levels of readily available carbohydrates in purified rations on cellulose digestibility by sheep. G. L. M. Chappell (*Diss. Abstr.* B, 1967, **27**, 2209).—In metabolism trials wethers were fed purified rations, the basal ration containing cellulose 77·8, protein 11·1, minerals 7·2 and maize oil 3·9%. Readily available carbohydrates (*RAC*), supplied as a 1 : 1 mixture of cerelose and starch, were used to replace cellulose in other rations to give 2, 4, 6 or 8% *RAC*. The level of *RAC* in the ration did not affect the digestibility of the dietary components or the N balance; cellulose digestibility tended to be high for the 8% *RAC* ration. In a further trial replacements of 32, 40 or 48% *RAC* were made in the basal ration, thereby lowering the digestibility of total carbohydrates (cellulose + N-free extract) and of cellulose. The butyric acid content of the rumen fluid increased when the substituted rations were fed. When the basal ration was adjusted to contain 8 or 32% of *RAC* and fed after preliminary periods of 10–50 days, the duration of the preliminary periods did not affect the digestibility or N utilisation of the test ration. It was calculated that for each 1% increase in *RAC* the digestibility of the cellulose of the ration was lowered by 0·5%. A. G. POLLARD.

Factors influencing the utilisation of urea in purified diets for lambs. T. A. McDonald jun. (*Diss. Abstr.* B, 1967, **27**, 2210).—In a feeding trial the isolated soya-bean protein in a lamb ration was replaced, partly or completely, by urea. With a 66% replacement of the protein, gains in body wt. were unchanged. A 33% replacement of the protein resulted in higher dry matter intake, blood-urea balance, % absorbed N retained, digestible energy, digestibility of cellulose and dry matter, and lower blood-NH_3 levels than when 0, 66 or 100% of the protein was replaced by urea. Serum-Mg levels were higher on the 100% than on the 0 or 33% urea replacement diets. Addition of 0·55% DL-methionine, 0·75% lysine or 0·19% additional S to the all-urea diet (basal diet contained 0·19% S) did not affect the N balance, % absorbed N retained, blood-urea, or the digestibility of glucose, cellulose, total carbohydrate or dry matter. The addition of S to the diet increased blood-NH_3; starch digestibility diminished when dietary S was increased from 0·19 to 0·38%. Increase in the cellulose content of the all-urea-N diet from 0 to 75% caused a linear increase in faecal N and a linear decrease in N balance; glucose and starch digestibility was lowered both linearly and quadratically and the mol. % of valerate in the rumen fluid increased. Utilisation of urea was best when in a 25% cellulose diet whereas energy utilisation was most efficient with no dietary cellulose. Addition of 5% of dehydrated lucerne or cane molasses or 50% extra trace minerals to the basal all-urea purified diet had no significant effect on the N balance, digestion or blood or rumen fluid criteria. A. G. POLLARD.

Energy metabolism in sheep as influenced by interactions among the ration's energy content, physical form and buffers. R. P. Kromann and J. H. Meyer (*J. Anim. Sci.*, 1966, **25**, 1096–1101).—Young wether sheep were used in determining the effects of the source of energy and of the physical form (milled or pelletted) of the ration and the use of buffers on the energy metabolism. The concentrate energy source (barley) had higher digestible, metabolic and net energy values than did the roughage energy source (animal fat). Nutrient digestibility and digestible energy decreased when the roughage energy source was pelletted, but the values did not change appreciably when the concentrate energy source was

pelletted. Pelletting the roughage increased feed intake, growth and gain in energy. Addition of $NaHCO_3$ (5 or 12%) to the ration decreased feed intake and growth especially when the roughage energy source ration was fed; it also lowered the % carcass fat to a greater extent than would be expected from the decreased feed intake. Neither pelletting nor the addition of $NaHCO_3$ to the ration affected the metabolisable energy nor the net energy (maintenance and production). A. G. POLLARD.

Influence of frequency and source of protein supplementation on energy metabolism in feeder lambs. R. P. Kromann, E. E. Ray and A. B. Nelson (*J. Anim. Sci.*, 1966, **25**, 1040–1044).—The response of cross-bred lambs in respect of growth, carcass characteristics and the net energy of the ration (*NE*), to the source and frequency of dietary protein supplementation is examined. Growth and carcass data were similar whether dehydrated lucerne or cottonseed meal was used in the supplement. Among lambs given the protein supplement there was no significant difference in gain in wt. or carcass data when the total supplement was divided proportionally and given at 1-, 7-, 14- or 21-day intervals. Wethers gained significantly more wt. than did ewe lambs and utilised the energy of the ration more efficiently. Rations supplemented with cottonseed meal had greater *NE* (maintenance and production) than did those supplemented with dehydrated lucerne but there was no difference in *NE* due to frequency of protein supplementation. A. G. POLLARD.

Use of the comparative slaughter technique to estimate the nutritive value of pasture for hoggets. J. P. Joyce and R. P. Newth (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 166–180).—Wether sheep were fed, either *ad lib* or on a restricted basis, on fresh white clover or perennial ryegrass, both having similar digestibility, for 100-day periods. Net energy values were determined by the comparative slaughter method. White clover had higher contents of crude protein and of water-sol. sugars and lower cellulose and hemicellulose contents than did the ryegrass. With *ad lib* feeding 34% more crude protein and gross energy were consumed as clover than as ryegrass. Live-wt. gains were greater with clover feeding but this effect was only partially related to the level of digestible org. matter consumed. Retention of energy and N by sheep fed clover at high levels of intake were greater than when ryegrass was fed; at maintenance levels there was little difference between the two feeds in this respect. A. G. POLLARD.

[A]. Rumen volume as a factor involved in individual sheep differences. [B]. Variations in rumen volume and associated effects as factors influencing metabolism and protozoa concentration in the rumen of sheep. D. B. Purser and R. J. Moir (*J. Anim. Sci.*, 1966, **25**, 509–515, 516–520).—[A] The apparent rumen vol. of 3-year wether sheep fed semi-purified rations containing different proportions of N (10–20 g daily) was determined by the Cr_2O_3 method and compared with the 'physiological vol.', after slaughter and the 'physical capacity' as measured by filling the emptied rumen with water. Sheep wt. in the range 61·8–75·5 kg were associated with physiological vol. of rumen ranging from 2·5 to 7·6 l. Correlations between these factors are established and discussed.

[B] Sheep were fed a basal ration supplemented with casein and urea to provide various N intakes (10–20 g/day). Rumen holotrich protozoa concn. were negatively associated with water consumption, propensity to eat and with large rumen vol. Fast-eating sheep showed an above-average concn. of NH_3 in the rumen. Sheep having relatively small rumen vol. utilised N more effectively, whereas those having large rumen vol. digested the larger proportion of the ingested dry matter. A. G. POLLARD.

Relationship of rumen volatile acids, blood-glucose and plasma-nonesterified fatty acids in sheep. A. Trenkel and K. V. Kuhlemeier (*J. Anim. Sci.*, 1966, **25**, 1111–1115).—Wether lambs (36–41 kg body wt.) were fed a ration based on maize, ground maize cobs, lucerne hay and soya-bean meal. Blood and rumen samples were taken periodically after each meal. The concn. of non-esterified fatty acids (**I**) in the plasma were 0·11, 0·60 and 1·32 mequiv./l, 4, 24 and 48 h after feeding. Blood-glucose showed little change during this period. The increase in **I** after feeding coincided with the decrease in concn. of rumen volatile fatty acids. Intravenous injection of glucose, Na propionate or butyrate into fasting sheep decreased plasma-**I** within 1 h. Injection of NaOAc caused no change. Blood-glucose was increased by injection of propionate or glucose, but was unchanged by that of acetate and was diminished by that of butyrate. A. G. POLLARD.

Influence of rumen protozoa on volatile acid production and ration digestibility in lambs. R. Luther, A. Trenkle and W. Burroughs (*J. Anim. Sci.*, 1966, **25**, 1116–1122).—A suspension of rumen protozoa and certain bacterial inocula obtained from a fistulated steer were used in *in vitro* studies of the digestibility of high-roughage and high-concentrate rations. Addition of protozoa to bacterial fermentations increased the production of volatile fatty acids (*VFA*) and NH_3. The presence of protozoa in the rumen of lambs fed rations containing 80% roughage, as compared with defaunated lambs, also increased ruminal *VFA* and NH_3. Faunated lambs given the 80%-roughage ration had higher concn. of ruminal propionic acid but no higher concn. of total *VFA* than did defaunated lambs. In faunated lambs receiving high- or low-concentrate rations, the ratio ruminal acetate/propionate was narrower than in protozoa-free lambs. No differences in digestibility which could be ascribed to protozoa were apparent. A. G. POLLARD.

Effects of sulphur and nitrogen sources and copper levels on the metabolism of certain minerals by sheep. R. D. Goodrich and A. D. Tillman (*J. Anim. Sci.*, 1966, **25**, 484–491).—Effects of S sources (elementary S, SO_4^{2-}) and of N sources [urea, purified soya-bean protein (**I**)] and of dietary Cu (as carbonate) on the growth, liver-Cu and balances of N, S, Cu, P and Ca in lambs fed individually, are examined. With rations containing **I**, the lambs retained less liver-Cu than did those given urea. Sulphate also diminished the retention and liver storage of Cu. The digestion and retention of N by lambs fed the **I** ration were diminished by addition of Cu (100 ppm) to the diet. The digestibility of S was greatest when SO_4^{2-} was given but because of large urinary losses, S retention was highest when elementary S was used. Retention of Ca was diminished by feeding urea or sulphate. Retention of P was lowered by feeding Cu (100 ppm) with the sulphate ration. (30 references.) A. G. POLLARD.

Fate of a physiological dose of selenate in the lactating ewe; effect of sulphate. G. D. Paulson, C. A. Baumann and A. L. Pope (*J. Anim. Sci.*, 1966, **25**, 1054–1058).—Lactating ewes receiving a physiological dose of ^{75}Se, as SeO_4^{2-}, by rumen puncture excreted 69% of the dose in the faeces within 7 days; the urine contained <5%. The amounts excreted were greatest on the second day after administration. The ^{75}Se contents of blood and milk increased until 68 h after administration and then diminished slightly; the concn. in whole blood was four times that in milk. Seven days after dosing the SeO_4^{2-} contents of kidneys, small intestines, pancreas and spleen were relatively high and those of skeletal muscle and fat were low. In general supplementary SO_4^{2-} (0·5% S) dosage had no appreciable effect on the fate of SeO_4^{2-} in the ewe. This does not explain the apparent antagonistic effects of S on Se in relation to nutritional muscular dystrophy. A. G. POLLARD.

Techniques for estimating lamb carcass composition. S. D. Lathum, W. G. Moody and J. D. Kemp (*J. Anim. Sci.*, 1966, **25**, 492–496).—Carcass characteristics of 120 cross-bred lambs of different genetic background but with similar ranges of wt. and grading were slaughtered at ~40 kg live-wt. From statistical analyses of the data, relationships suitable for the prediction of total carcass lean, fat and bone are established. A. G. POLLARD.

Method for estimating ether extract in the boneless portion of lamb carcasses. A. W. Munson, J. V. Whiteman and L. E. Walters (*J. Anim. Sci.*, 1966, **25**, 967–971).—Data for samples from 60 (1963) and 63 (1964) carcasses of lambs of average slaughter wt. 46–47 kg and ether extract in the range 20–40% are presented. The boneless portion of each carcass was ground twice and mixed. Eight random 50 g 'grab' samples were combined at random into two composite samples, each of which was homogenised in a high-speed blender and mixed after blending. Duplicate samples were drawn from each composite sample and the % ether extract was determined. The standard estimates of error were 1·35 and 0·85% respectively for the 1963 and 1964 material. Using the above procedure the estimate of ether extract ±0·85% would contain the true value in 68% of the cases. Estimates of % ether extract may be improved by increasing both the no. of 'grab' samples per composite sample and the no. of composite samples per lamb. A. G. POLLARD.

Effect of sodium or potassium on ovine urinary calculi. H. R. Crookshank (*J. Anim. Sci.*, 1966, **25**, 1005–1009).—Comparison is made of the influence of Na and K salts on the occurrence of the calculi in weanling wether lambs. K salts were more effective than the corresponding Na salts (except carbonates which were equally effective) in controlling or diminishing the incidence of the calculi. Data are presented for phosphates, chlorides and bicarbonates, used either at the same % in the ration or in isomolar proportions. In all cases the development of clinical cases was

more rapid when the Na than when the corresponding K salts were given. A. G. POLLARD.

Utilisation of lactose by the growing pig. I. J. Shearer and A. C. Dunkin (*Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 72).—In feeding trials with pigs wheat starch was replaced by lactose at levels of 0, 15, 30 and 45% in the ration, the rations being fed as wet mash. With 30 and 45% lactose the pigs grew (10% and 39% respectively) more slowly than did the controls; with 45% lactose growth was slower than with 15 or 30% lactose. The reduced growth was partially the outcome of lowered food consumption. In digestibility trials ~95% of the reducing sugar voided appeared in the urine, but the total urinary sugar represented < 1% of the lactose ingested. The major faecal sugar was tentatively identified as xylose; that from a badly scouring pig also contained glucose and galactose but no lactose. The urinary sugars from pigs receiving lactose were mainly galactose and lactose. A. G. POLLARD.

High level cereal diets for the growing-finishing pig. II. The effect of cereal preparation on the performance of pigs fed diets containing high levels of maize, sorghum and barley. T. L. J. Lawrence (*J. agric. Sci., Camb.*, 1967, **69**, 271–281; cf. *Idem, ibid.*, **68**, 269).—Grinding gives better digestibility than crimping and digestible energy decreases in the order maize, sorghum and barley. N retention is the same for all three grains, although grinding gives a higher retention than crimping at 107 lb live-wt. From start to slaughter at 200 lb growth rates and food conversion efficiencies are higher for maize, sorghum and ground diets than for the barley diets. Grinding gives slightly fatter carcasses. M. LONG.

Influence of aged maize and supplemental vitamin A on growing-finishing swine. T. A. McDonald, W. H. Smith, R. A. Pickett and W. M. Beeson (*J. Anim. Sci.*, 1966, **25**, 1024–1028).—The age of stored maize used in pig rations had no effect on rates of gain in wt. or on serum-vitamin A values. Liver-vitamin A levels were higher after feeding new maize than after feeding that stored for 4 to 8 years. Addition of vitamin A to the feed increased both serum- and liver-vitamin A, but the rate of gain in wt. was increased only when vitamin A was fed in drylot during the summer. A. G. POLLARD.

Effects of environmental temperature and thiouracil feeding on growing-fattening pigs. A. M. Pearson, E. P. Reineke, J. A. Hoefer and R. E. Morrow (*J. Anim. Sci.*, 1966, **25**, 994–999).—Groups of pigs kept in temp.-controlled pens at 4 or 27° were fed individually with free access to water. Half the pigs in each group were given a basal ration; others received the same ration with 0·15% of thiouracil. Use of thiouracil for > 30 days lowered the activity of the animals considerably and, after 40 days, appetites decreased, rates of gain diminished, blood-lipid levels increased, liver and thyroid glands increased in wt. and carcasses were shorter. With ambient temp. at 4° blood-lipid levels and the wt. and activity of thyroids and liver increased whereas feed efficiency, back-fat thickness and the % fat trim diminished. A. G. POLLARD.

Relationships among some physical and chemical parameters of full- *vs* limited-fed Yorkshire pigs slaughtered at different live-weights. G. M, Babatunde, W. G. Pond, L. D. Van Vleck, G. H. Kroening, J. T. Reid, J. R. Stouffer and G. H. Wellington (*J. Anim. Sci.*, 1966, **25**, 526–531).—Comparison is made of carcass characteristics of pigs, initially ~45 kg live-wt., given 1·82 kg of feed daily with corresponding pigs fed *ad lib* throughout the experimental period. Relationships between various relevant parameters are examined. A. G. POLLARD.

Hypervitaminosis A in the young pig. M. D. Anderson, V. C. Speer, J. T. McCall and V. W. Hays (*J. Anim. Sci.*, 1966, **25**, 1123–1127).—Symptoms of hypervitaminosis A in the pig are described. Vitamin-A toxicity restricted growth, feed consumption and efficiency and also produced significant changes in some blood and bone characteristics. Neither bone ash nor its Ca content was affected. Dietary levels of 440,000–1,100,000 i.u. of vitamin A/kg caused symptoms of hypervitaminosis A to appear in about 16–43 days. No symptoms appeared in 8 weeks with diets containing 220,000 i.u./kg. A. G. POLLARD.

Lack of response by growing-finishing pigs to germicidal ultra-violet radiation. S. R. Morrison, H. F. Hintz and R. L. Givens (*J. agric. Sci., Camb.*, 1967, **69**, 131–132).—Pigs of initial wt. ~25–36 kg were exposed to u.v. radiation for periods of 54–70 days during winter or summer. The radiation was of germicidal wave-length (~2537 Å) and the animals received no direct sunlight. Rations, which included supplementary vitamin D_2 were available *ad lib*. No significant improvement in growth rate or feed efficiency was apparent. A. G. POLLARD.

Effect of dietary chemotherapeutics on the performance and faecal flora of baby pigs. T. F. Kellogg, V. W. Hays, D. V. Catron, L. Y. Quinn and V. C. Speer (*J. Anim. Sci.*, 1966, **25**, 1102–1106).—Effects of eight chemotherapeutics on gain in wt., feed efficiency and faecal flora of 120 pigs averaging 15 days of age and 4·2 kg body wt. are recorded. Additions to the diet of chlortetracycline, oxytetracycline, penicillin or streptomycin increased the rate of gain in wt. with less feed per unit gain; counts of lactobacilli, **(I)** total aerobes **(II)**, total anaerobes **(III)** and streptococci **(IV)**, in the faecal flora were lower than in controls. Dietary supplements of $CuSO_4$ increased rates of gain in wt. and lowered faecal counts of **I, II,** and **III**. Supplements of bacitracin or 3-nitro-4-hydroxy-phenylarsonic acid lowered growth rates and caused higher faecal counts of **I, III** and **IV**. A. G. POLLARD.

Influence of various nutritional factors and physical forms of feed on oesophagogastric ulcers in swine. D. C. Mahan, R. A. Pickett, T. W. Perry, T. M. Curtin, W. R. Featherston and W. M. Beeson (*J. Anim. Sci.*, 1966, **25**, 1019–1023).—The occurrence of the ulcers in pigs was not lowered by increasing the dietary ascorbic acid or B-vitamins. Feeding of finely ground maize was associated with the occurrence of more ulcers than when coarsely ground maize was used. Expanded maize produced more ulcers than did ground unprocessed maize. Although fineness of dietary particles is a factor favouring the occurrence of ulcers it is not the sole cause. Contents of stomachs with oesophagogastric ulcers were more fluid and the pH was lower than in normal stomachs. The incidence of ulcers was unrelated to sex. A. G. POLLARD.

Effect of pelletting on the nutritive value of horse rations. H. F. Hintz and R. G. Loy (*J. Anim. Sci.*, 1966, **25**, 1059–1062).—Over a 63-day experimental period pairs of horses were fed the same ration, one in pelletted form and one in normal condition. Pelletting did not affect rates of gain in wt. or the efficiency of feed conversion. There were no differences in digestibility between the two forms of feed except that of ether extract which was greater in the pelletted feed. The rate of passage through the digestive tract was faster with the pelletted ration which was also consumed in less time. Fresh faeces of horses fed the non-pelletted ration contained the the higher % of dry matter. A. G. POLLARD.

Determination of the faecal excretion rate of horses with chromic oxide. G. F. W. Haenlein, R. C. Smith and Y. M. Yoon (*J. Anim. Sci.*, 1966, **25**, 1091–1095).—Diurnal variations in the excretion of Cr_2O_3 by six horses in digestion trials are examined, lucerne hay being supplied loose-chopped, pelletted or wafered. The average recovery of Cr_2O_3 was 98·4%. Faecal excretion of Cr_2O_3 showed wider diurnal variations than did other faecal components and were best described by a sinusoidal periodic function; the phase of the curves moved when the physical form of the lucerne was changed. Estimates of faecal excretion rates by the 4-day rectal grab-sample method with determination of Cr_2O_3 content were similar to those by the 10-day total collection procedure provided the recovery rate of the Cr_2O_3 was considered. Faecal Cr_2O_3 samples taken at random for 10 days yielded estimates of faecal excretion rates differing from that obtained by the total collection method. A. G. POLLARD.

Protein and energy value of peanut hull and wood shaving poultry litters. A. N. Bhattacharya and J. P. Fontenot (*J. Anim. Sci.*, 1966, **25**, 367–371).—In metabolism trials with yearling wethers the protein and energy value of the litters from broiler houses were incorporated (25 or 50%) in a ration based on maize and hay. The digestibility of the crude fibre of the litter-containing (*L*) rations was higher and those of the dry matter, N-free extract and energy were lower than were the corresponding values of the control ration. Crude fibre digestibility in *L* was depressed by increasing the proportion of litter from 25 to 50%. The apparent digestibility of the crude protein of the litter averaged 72·5%. No significant differences in digestible protein, digestible energy, metabolisable energy or total digestible nutrients (dry basis) between kinds or levels of litter in the rations were found; average values were 22·7%, 2440 kcal/kg, 2181 kcal/kg, and 59·8% respectively. A. G. POLLARD.

Nutritional value of 'opaque-2' maize for the chick. G. L. Cromwell, J. C. Rogler, W. R. Featherston and R. A. Pickett (*Poultry Sci.*, 1967, **46**, 705–712).—Chicks fed 'opaque-2' maize (which is higher in lysine and has a more favourable NH_2-acid balance than normal hybrid maize) showed very similar wt. gains and feed conversion compared with those fed normal maize in diets containing 15–21% protein. When extra methionine was added to diets sub-optimal in protein, chicks receiving opaque-2 maize performed better than those receiving ordinary maize. The

advantage of opaque-2 maize over ordinary maize was shown to be due entirely to the higher lysine content of the former.
A. H. CORNFIELD.

Calcium requirement of the turkey breeder hen. L. G. Arends, D. L. Miller and S. L. Balloun (*Poultry Sci.*, 1967, **46**, 727–730).—Egg production by turkey breeder hens increased with Ca level of the diet up to 2·25%, but was not improved further by 3·0% Ca in the diet. Hens receiving 0·75% dietary Ca ate a large proportion of the eggs laid. Max. hatchability and skeletal mineralisation also occurred with 2·25% Ca in the diet.
A. H. CORNFIELD.

Relationship of protein level of sorghum grain to its nutritive value as measured by chick performance and amino-acid composition. D. H. Waggle, C. W. Deyoe and P. E. Sanford (*Poultry Sci.*, 1967, **46**, 655–659).—The NH_2-acid composition of the grain of low-, medium-, and high-protein varieties of sorghum was very similar. When soya-bean meal was used to bring all diets to 18% total protein, the performance of chicks was the same irrespective of the protein content of the sorghum grain (8·3–12·1% protein). When diets were formulated to contain the same amounts of sorghum grain and soya-bean meal wt. gains increased with level of grain sorghum protein.
A. H. CORNFIELD.

Cassava root meal for chicks. F. Q. Enriquez and E. Ross (*Poultry Sci.*, 1967, **46**, 622–626).—Methionine was the major limiting nutrient in the meal of cassava (*Manihot esculenta*) root. Providing adequate protein and methionine were supplied satisfactory chick growth was obtained with even 50% cassava root meal in the ration.
A. H. CORNFIELD.

Keratins as sources of protein for the chick. III. Metabolisable energy and amino-acid composition of raw and processed hog hair meal. E. T. Moran, jun., H. S. Bayley and J. D. Summers (*Poultry Sci.*, 1967, **46**, 548–553).—Autoclaving hog hair at 50 lb per sq. in. for 30 min. (148°) increased the metabolisable energy from 0·58 to 2·14 kcal per g. Glycine, particularly, and phenylalanine, serine and isoleucine in the hog hair protein were increased by autoclaving, whilst cystine, particularly, and glutamic acid, lysine and arginine were decreased.
A. H. CORNFIELD.

Lipid composition of chick embryo and yolk as affected by stage of incubation and maternal diet. W. E. Donaldson (*Poultry Sci.*, 1967, **46**, 693–697).—Yolk lipid composition of chick embryos changed little during the first 12 days of development. By 20 days phospholipid and triglyceride levels were lower whilst diglyceride, cholesterol, free fatty acids, and cholesterol ester were higher. The 12-day embryos contained high levels of phospholipid and low levels of triglyceride, but the reverse was true at 20 days. When the hen's diet was supplemented with 10% oleic acid, yolk and embryo fatty acid distributions were more unsaturated compared with controls, except at 20 days, where the fatty acid composition of embryo phospholipid was identical for both diets.
A. H. CORNFIELD.

Magnesium deficiency in the laying hen. A. C. Cox and J. L. Sell (*Poultry Sci.*, 1967, **46**, 675–680).—When a low level of Mg (95 ppm in the feed compared with 345 ppm) was supplied to hens egg production, feed consumption, egg wt., and Mg% in the eggshell and yolk were reduced. Reduction in egg production occurred within 7 days of putting the hens on the low-Mg diet. A high Ca level (4·0%), compared with a lower level (2·5%), hastened the onset of Mg deficiency symptoms.
A. H. CORNFIELD.

Effectiveness of plant and inorganic phosphorus in supporting egg production in hens and hatchability and bone development in chick embryos. P. W. Waldroup, C. F. Simpson, B. L. Damron and R. H. Harms (*Poultry Sci.*, 1967, **46**, 659–664).—Egg production and hatchability were not affected by addition to the control diet (0·34% total P) of hominy to raise the level of P to 0·54%, whilst addition of 0·2% inorg. P (defluorinated phosphate) to the control diet increased egg production and hatchability. The extra org. P aided in preventing the development of embryonic rickets.
A. H. CORNFIELD.

Effect of feeding tung oil on the performance of laying hens. H. M. Edwards, jun., F. A. Suso and J. Mason (*Poultry Sci.*, 1967, **46**, 564–569).—The addition of tung oil (**I**) to the ration of laying hens caused an abrupt decrease in egg production and egg size. The effect increased with level of **I** supplied (2–8% of the ration). The highest level caused a sharp drop in body wt. of hens and other symptoms of toxicity also occurred. Hatchability of fertile eggs, and survival and growth of progeny from the hens was not influenced by feeding **I**. It was shown that the use of both polar and non-polar liquid phases for GLC analysis along with hydrogenation strengthened evidence for identification of elaeostearic acid in **I**.
A. H. CORNFIELD.

Factors affecting the discoloration of hard-cooked egg yolks. R. C. Baker, J. Darfler and A. Lifshitz (*Poultry Sci.*, 1967, **46**, 664–672).—The greenish-black discoloration often formed at the surface of the yolk of hard-boiled eggs was due to FeS caused by combination of Fe^{2+} released from the yolk and H_2S released from the albumin. The extent of yolk surface discoloration increased with time of cooking, pH of egg yolk, length of storage before cooking and time taken for cooling after cooking and decreased with time of storage after cooking.
A. H. CORNFIELD.

Influence of dietary calcium, vitamin D_3 and fibre on the availability of phosphorus to turkey poults. M. Griffith and R. J. Young (*Poultry Sci.*, 1967, **46**, 553–560).—Soya-bean meal (**I**), soya-bean hulls, oat hulls, and alkali-treated soya-bean hulls were highly effective in improving P availability (as indicated by wt. gains and bone ash%) from anhyd. $CaHPO_4$ when added to purified diets. The improvement in bone ash observed when **I** was added to a P-deficient purified diet could not be duplicated by adding high levels of vitamin D_3 to the diet. **I** increased bone ash when inadequate, but not when adequate, levels of vitamin D_3 or available P were supplied. Increasing dietary Ca above 1·3% depressed bone ash when the diet was inadequate in P, more so with purified diets than when **I** was added.
A. H. CORNFIELD.

Survey of chemical compounds tested *in vitro* against rumen protozoa for possible control of bloat. F. L. Willard and R. Kodras (*Appl. Microbiol.*, 1967, **15**, 1014–1019).—Chemical agents (170) were screened in 0·1–0·05% concn., inorg., antibiotics, biocides, neuromuscular agents, arsenicals, plant and animal hormones, antimalarials, anthelmintics, surface active agents etc. being included. The most active were $CuSO_4$, $NiSO_4$, H_2O_2, nitrofurazone, dodecyl sodium sulphate, pelargonic acid, iodoacetic acid, 1-diethylaminoethylamino-4-methylthioxanthone, Na-arsanilate, Na-arsenate, Bi-glycolyl arsanilate, 1-β-hydroxyethyl-2-methyl-5-nitro-imidazol and *p*-nitroaniline. The Cu ion is not particularly effective against entodinia and Ni ion had no effect upon holotrichs. H_2O_2 and iodoacetic acid were effective at concn. 0·005%. Anionic surface active agents, specially the long chain sulphates and phosphates were very effective.
C.V.

[A] **Toxicosis and residues in bromophos-dipped sheep.** D. E. Clark, R. L. Younger and C. H. Ayala. [B] **Determination of bromophos residues.** P. Bracha and J. P. Bonard (*J. agric. Fd Chem.*, 1966, **14**, 608–609, 609–612).—[A] Nine weekly dippings in 0·5% aq. bromophos caused no poisoning in lambs. The content of bromophos in the omental fat, determined by microcoulometric gas chromatography, declined from 9·75 to 0·33 ppm during 3 weeks after the final dipping. Recoveries of bromophos added to control fat were 79%.

[B] Bromophos *O,O*-dimethyl *O*-(2,5-dichloro-4-bromophenyl) phosphorothioate was extracted with $COMe_2$ from mud surfaces and determined by its colour reaction with 4-aminophenazone as described by Smith and Thiegs for the determination of fenchlorophos. For the determination of bromophos and the metabolite 2,5-dichloro-4-bromophenol in urine, a thin-layer chromatographic method is described in which the spot area-to-weight relationship is used (*cf.* Purdy and Truter, *Analyt. Abstr.*, 1963, **10**, 2071).
P. S. ARUP.

Bone zinc concentration in range cattle. C. Blincoe and V. R. Bohman (*J. agric. Fd chem.*, 1966, **14**, 645–646).—The concn. ranged from a min. of 139 ± 25 ppm of Zn in the frontal bone to a max. of 271 ± 50 ppm in the caudal vertebrae; intermediate concn. were found in the other vertebrae and bones. Bone-Zn decreased markedly with age. (21 references.)
P. S. ARUP.

Developments in control of ectoparasites of livestock. J. C. Wood (*Chemy Ind.*, 1967, 1731–1736).—Recent advances in the more intensive and effective use of insecticides based on chlorinated hydrocarbon, org.-P and carbamate groups for controlling maggot fly, sheep lice, sheep ked, sheep scab, cattle ticks, and mange (caused by psoroptic, sarcoptic and chorioptic mites) in other animals are reviewed extensively. Systemic insecticides have proved invaluable in treating cattle, although as yet persistence of effect is not combined with non-persistence of toxic residues in tissues. New compounds must be developed to combat the increasing resistance of the parasites to some present insecticides. (22 references.)
W. J. BAKER.

Effect of sodium sulphate on blood levels of oxytetracycline in chicks, turkey poults, and laying hens. J. G. Panissett and L. G. Mathieu (*Poultry Sci.*, 1967, **46**, 560–563).—The addition of

1·25% Na_2SO_4 to chick and poult diets containing 200 g oxytetracycline (**I**) per ton of feed produced blood-**I** levels 1·6 times as high as those in birds receiving **I** alone. The Na_2SO_4 treatment did not affect blood-**I** levels in laying hens. A. H. CORNFIELD.

Meticlorpindol as a coccidiostat. W. M. Reid and R. N. Brewer (*Poultry Sci.*, 1967, **46**, 638–642).—In two battery trials addition of 0·0125% Meticlorpindol two days before inoculation of chicks with coccidial oocysts gave complete protection against a mixture of six species of *Eimeria*, whereas control birds showed 60% mortality. A. H. CORNFIELD.

Efficacy of buquinolate against six species of coccidia. R. N. Brewer and W. M. Reid (*Poultry Sci.*, 1967, **46**, 642–646).—Birds receiving 0·00825% buquinolate in their feed showed no mortality and gained 93% as much wt. as did uninoculated controls, whilst birds not receiving the drug and inoculated with six species of *Eimeria* showed 60–88% mortality. The new drug was more effective than the three reference coccidiostatic drugs with respect to feed conversion and signs of morbidity or abnormal droppings.
A. H. CORNFIELD.

Effect of chemicals on the incorporation of iodine-131 in the chicken egg. H. G. Pena, K. M. Barth, W. V. Kessler, M. P. Plumlee and J. E. Christian (*Poultry Sci.*, 1967, **46**, 426–429).—Daily oral dosage with $KClO_4$ (0·2 g per bird) for 16 days reduced ^{131}I deposition in the egg by 91%. KI (0·08 g) reduced deposition by 68%, whilst pilocarpine nitrate (0·0025 g injected intramuscularly every 4 days) had no effect on deposition.
A. H. CORNFIELD.

Effect of restricted caloric intake upon the egg weight response to dietary maize oil. D. J. Bray (*Poultry Sci.*, 1967, **46**, 476–484).—When 8 parts maize oil were substituted for 20 parts of starch in a maize–soya-bean meal diet there was an increase in egg wt. and body wt. of pullets. Egg wt. was increased to a smaller extent when the diet was diluted with cellulose to the extent that voluntary available calorie intake did not support an increase in body wt. However, when the gain in body wt. was prevented by limiting the time that feed was supplied, there was no effect on egg wt.
A. H. CORNFIELD.

Studies of dietary piperazine, phenothiazine, and dibutyltin dilaurate. II. Yolk mottling and other egg quality characteristics. J. L. Fry and H. R. Wilson (*Poultry Sci.*, 1967, **46**, 319–322).—Dietary piperazine and phenothiazine, added at the manufacturers' recommended levels, had no significant effect on egg wt., Haugh units, shell thickness or yolk mottling. Dibutyltin dilaurate added alone or in combination with the two other materials increased yolk mottling, particularly 7 days after treatment began, but had no effect on the other egg quality characteristics. A. H. CORNFIELD.

Compounds in cottonseed oil that cause pink-white discoloration in stored eggs. R. J. Evans, S. L. Bandemer and J. A. Davidson (*Poultry Sci.*, 1967, **46**, 345–365).—A fraction of crude cottonseed oil fatty acids, sol. in acetone and light petroleum at −60° gave a strong Halphen reaction and contained 3·4% sterculic acid. When fed to laying hens the eggs produced developed very pink whites and enlarged thick brown yolks during storage and contained increased levels of stearic acid. When the above fraction was separated into two parts by pptn. with urea, the fatty acids from the urea-inclusion compounds gave a strong Halphen reaction but only sometimes produced pink whites, whilst the sol. fraction gave a negative Halphen reaction, but consistently produced pink whites. When crude cottonseed oil, refined cottonseed oil and a mixture of *Sterculia foetida* seed oil were heated at temp. ranging from 100° to 250° for 1–4 h, heat destroyed the Halphen activity of crude cottonseed oil before it destroyed the pink-white activity; in refined cottonseed oil pink-white activity was destroyed before Halphen activity, whilst in the *Sterculia foetida* seed oil–maize oil mixture both were destroyed at the same time. All three unheated oils increased the deposition of stearic acid in eggs, whereas oils heated so that they no longer gave positive Halphen reactions or produced pink-white did not increase egg stearic acid levels.
A. H. CORNFIELD.

Deposition of the anthelmintic Tetramisole in eggs and poultry tissues. F. Allewijn and R. Marsboom (*Poultry Sci.*, 1967, **46**, 388–391).—Tetramisole was administered orally at 0·04 g per kg of body wt. and in 4% aq. solution once to four times from the second to the tenth week of age. No gross pathological lesions were observed on autopsy and the drug could not be detected in the organs or tissues one week after the final treatment. A single high oral dose (0·07 g) gave traces of the drug in eggs during 6 days, with a max. level of 0·76 ppm. A. H. CORNFIELD.

Effects of 'Tetramisole' (R 8299) on the growing chicken. R. Marsboom and D. Thienpont (*Poultry Sci.*, 1967, **46**, 365–367).—Tetramisole (an orally and parenterally effective broad spectrum anthelmintic) was administered orally by tubage to broiler chickens once, twice or four times during the growing period at 0·04 g per kg of body wt. The treatment had no significant effect on mortality, growth rate or feed efficiency.
A. H. CORNFIELD.

Sulphadimethoxine therapy of avian coccidiosis. M. Mitrovic and J. C. Bauernfeind (*Poultry Sci.*, 1967, **46**, 402–411).—Sulphadimethoxine showed high chemotherapeutic activity against all pathogenic species of *Eimeria* in chicks when supplied at 0·05% in the drinking water for 6 consecutive days, for 3 days after infection. The drug was also effective for turkeys when supplied at 0·025% in the drinking water. A. H. CORNFIELD.

Insecticidal control of ear tick in the ears of cattle. R. O. Drummond, T. M. Whetstone and S. E. Ernst (*J. econ. Ent.*, 1967, **60**, 1021–1025).—Many insecticides were tested for control of *Otobius menini* by application to the ears. The length of time before re-infestation was variable; >90% control after 1 month was given by 5% coumaphos dust or 4% solution, by 0·5% trichlorfon as an aerosol and 0·1% clursban emulsion. C. M. HARDWICK.

Potential of animal systemic insecticides for eradicating cattle grubs, *Hypoderma* spp. O. H. Graham and R. O. Drummond (*J. econ. Ent.*, 1967, **60**, 1050–1053).—A review of the history and present position. (40 references.) C. M. HARDWICK.

Degradation and elimination of Temik in rats. N. R. Andrawes, H. W. Dorough and D. A. Lindquist (*J. econ. Ent.*, 1967, **60**, 979–987).—The metabolism of Temik [2-methyl-2-(methythio) propionaldehyde *O*-(methylcarbamoyl)oxime] was first studied in a rat-liver enzyme system. It was then administered orally to female rats and its metabolism studied with a liquid scintillation spectrometer and chromatography; 80% was eliminated within 24 h. The major metabolite was Temik sulfoxide (**I**). When administered directly to rats, **I** was excreted more slowly. (12 references.) C. M. HARDWICK.

Quinoxaline derivatives. C. Pfizer and Co., Inc. (B.P. 1,058,047, 20.8.65. U.S., 16.9.64 and 9.7.65).—Compounds, useful as urinary tract antiseptics, systemic anti-infectives, animal growth promotants, and agents for controlling respiratory disease in poultry and improving feed efficiency in animals, are prepared by condensing 2-COR^I-3-R^{II}-quinoxaline-1,4-dioxides with NH_2R wherein R^I and R^{II} are H or alkyl of 1–6 C; R is $NH{\cdot}CONH_2$, $NH{\cdot}CS{\cdot}NH_2$, $NH{\cdot}C(:NH)NH_2$, NHR^{III}, 2-oxo-tetrahydro-oxazol-3-yl, $NH{\cdot}CO_2R^{IV}$, $NHCOR^V$, OR^I or 4-(2′-hydroxyethyl)-piperazino (R^{III} is alkyl of 1–6 C, Ph, CH_2OH, or hydroxyalkyl of 2–4C; R^{IV} is alkyl of 1–6 C or C_{2-4}-hydroxy- or halogeno-alkyl; R^V is C_{1-6}-alkyl or Ph). In an example, a solution of Me carbazate (48) in MeOH 250 is added quickly to MeOH 2500 c.c. containing dissolved 2-formylquinoxaline-1,4-dioxide (**I**) (100), then after adding 2 drops of conc. aq. HCl the mixture is stirred during 3 h. Ppt. (121·8 g) is filtered off, washed with MeOH, and purified by boiling with $CHCl_3$, to give **I**-*carbomethoxyhydrazone*, m.p. 239·5–240° (decomp.) F. R. BASFORD.

Foot-and-mouth disease vaccines. Wellcome Foundation Ltd. (Inventor: A. D. Kanarek) (B.P. 1,058,081, 22.8. and 6.11.62).—A conc. vaccine is produced by adding to an aq. suspension of living attenuated foot-and-mouth disease particles a water-sol. polysaccharide (or an ionic deriv. thereof); a water-sol. cellulose deriv. or a water-sol. polyalkylene or polyoxyalkylene alcohol or glycol (alkylene of 2–4 C), then adjusting the ionic concn. e.g., by addition of NaCl, to form an aq. 2-phase system comprising a phase in which the virus preferentially collects and is smaller in vol. than the original virus suspension and which is collected.
F. R. BASFORD.

Derivatives of adamantane. E. I. du Pont de Nemours & Co. (B.P. 1,063,365–6, 19.3.64. U.S., 22.10.63).—[A] Compounds claimed comprise 1-NRR^I-adamantanes and salts thereof with non-toxic acids wherein R and R^I are H (R^I only), alkenyl of 3–12 C (with ⩽1 CH_2 between the N and the first unsaturation of the alkenyl group), alkynyl of 3–12 C (with ⩽1 CH_2 between N and the triple-bonded C), cycloalkyl of 3–8 C (optionally containing 1–2 Me and/or 1 Et), cycloalkylmethyl of 3–9 C, or cycloalkylmethyl substituted by Me and/or Et; and NRR^I contains ⩾12 C. In an example, allyl bromide (24·2) is added to a mixture of 1-aminoadamantane hydrochloride (37·5), EtOH 500 cc, and Na_2CO_3 (50·4), then after heating to 65° the reaction is allowed to

proceed until no more gas is evolved. After cooling solid is filtered off, the filtrate is evaporated, and residue is extracted with ether in presence of 10% aq. NaOH. Distillation of the extract affords 1-*allylaminoadamantane* (14·6 g), b.p. 55–58°/0·1 mm in 38% yield. [B] Compounds claimed comprise the salicylates, pamoates, caprochlorone, and 1-(adamantyl)-3-*p*-toluene sulphonyl-urea salts of 1-NRR^{I}-adamantanes wherein R and R^{I} are H, alkyl of 1–12 C, $C_{<5}$-alkyl substituted by halogen, OH, alkoxy of <4 C, NH_2, NHR^{II}, $NR_2{}^{II}$ (R^{II} is Me or Et), hydroxy-$C_{<4}$-alkoxy-$C_{<5}$-alkyl, Ph, CH_2Ph, $[CH_2]_2Ph$, or $[CH_2]_3Ph$, or R^{I} is Cl, Br, CH_2CO_2H, OMe, or Ac (NRR^{I} containing <12 C), or NRR^{I} is cycloalkyleneimino of 2–6 C or morpholino. The compounds of [A] and [B] are antiviral agents in animal medicine—especially useful against swine influenza. F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Determination of ethylene chlorohydrin, ethylene dibromide and other volatile fumigant residues in flour and whole wheat. S. G. Heuser and K. A. Scudamore (*Chemy Ind.*, 1967, 1557–1560).—Alternative methods, other than direct steam distillation, for recovery of ethylene chlorohydrin (**I**) are studied and results are discussed in respect of possible conversion of absorbed $(CH_2)_2O$ into **I** during the analysis. Recoveries of **I** from flour are similar for the four methods used, whether **I** is derived as a reaction product of $(CH_2)_2O$ or is introduced as adsorbed vapour. Extraction with cold acetone–water (5 : 1) is far more effective than steam distillation (direct or after extraction with cold or hot EtOH), **I** passing unchanged into the solvent for determination by GLC at 85° on a long column of 15% polypropylene glycol on Chromosorb-W with He as carrier. The determination of ethylene dibromide (**II**) is made similarly, with recoveries of 98 ± 2% (98 ± 3% from whole-wheat grains); the sensitivity for **I** and **II** is $\sim 5 \times 10^{-9}$ g. More than 95% of any free $(CH_2)_2O$ and MeBr in flour or whole-wheat are also determined by the acetone–water extraction and chromatographic procedure, which should provide a general method for simultaneous determination of several relatively volatile residues in cereals, etc. treated with liquid fumigants. W. J. BAKER.

Determination of iron in cereal flour. K. Lauber and H. Aebi (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1966, **57**, 363–373).—Samples are mineralised by heating with H_2SO_4 and H_2O_2, and aliquots of the acid solution, buffered with a solution prepared from AcONa, $MgSO_4$, and NaOH, are tested with a reagent containing ascorbic acid (to reduce Fe^{III} to Fe^{II}) and bathophenanthrolin disulphonate. The extinction due to the colour developed is measured at 550 nm in comparison with a standard. P. S. ARUP.

Utilisation of cottonseed flour. N. Subramanian (*J. Fd Sci. Technol.*, 1966, **3**, 71).—The composition of processed cottonseed flour and its use as a source of dietary protein are discussed. The problems of removing free gossypol pigments, which are toxic to monogastric animals, are considered. Attempts to prepare edible quality cottonseed flour are described. S. A. BROOKS.

Protein concentrates by dry milling of wheat millfeeds. D. A. Fellers, A. D. Shepherd, N. J. Bellard and A. P. Mossman (*Cereal Chem.*, 1966, **43**, 715–725).—Laboratory milling and sieving studies aimed at upgrading wheat millfeeds to produce high protein flours suitable for use in food products are reported. Coarse and fine bran and shorts were milled three times on the reduction rolls of a Brabender Quadrumat Senior flour mill and the flour fractions sieved out after each milling were analysed for protein, oil, starch, total sugar, ash, pentosans and fibre. Flour yields were highest from shorts and lowest from coarse bran. Highest levels of protein and starch, and lowest levels of pentosan, fibre and ash were obtained by milling at 9–11% moisture, highest yields by milling at 3–7% moisture. Possible yields of high protein flour (>20% protein, mainly derived from the aleurone layer of the grain) with a fibre content <3·8% from the wheatfeeds studied are estimated at 20, 30 and 40–50% respectively. (14 references.) E. C. APLING.

Review of wheat flour proteins and their functional properties. J. Holme (*Baker's Dig.*, 1966, **40**, 38–42 and 78).—The present knowledge of wheat proteins is reviewed; a clear-cut relationship between their properties and the baking properties of flour has not yet been revealed. (38 references.) I. DICKINSON.

Review of recent studies of wheat flour lipids in breadmaking. Y. Pomeranz (*Baker's Dig.*, 1966, **40**, 44–48 and 77).—The article includes methods of lipid study, extraction of wheat flour lipids, effects of vegetable shortening and flour lipids on loaf vol., lipids *vs.* proteins as loaf additives and effect of wheat classes and varieties on baking. (38 references.) I. DICKINSON.

Determination of ash in starch and starch candying products. G. Graefe (*Stärke*, 1966, **18**, 374–376).—Standard methods are proposed based on those used in the Corn Industries Research Foundation, U.S.A. For starch, a 5 g sample is placed in a pre-baked dish and heated to complete carbonisation before being placed in a muffle furnace at 525° for 2 h. Error is ~2%. Syrups and dextrose contain small amounts of inorg. salts; H_2SO_4 is added initially to facilitate destruction of org. matter and to give uniform ash values. Results are expressed as sulphated ash or if multiplied by a factor of 0·85, as chloride ash. J. B. WOOF.

Coacervation of starch. III. Complex coacervation of starch with gelatin. H. Yau Chung and M. M. MacMasters (*Stärke*, 1966, **18**, 377–382). (In English.)—An instance of starch–chloral hydrate (**I**) dispersion undergoing coacervation in presence of isoelectric gelatin sol (**II**) is described. With a I : II ratio of 1 : 1, coacervate forms at pH levels from 3 to 4·5 but with ratios of 1 : 2 and 1 : 3 it only forms in presence of neutral salt. Outside these limits no coacervation was observed. Starch paste in water gives only a ppt. under these conditions. Coacervate droplets show typical characteristics of reversibility, vacuolation and adsorption. (14 references.) J. B. WOOF.

Maize drying and wet milling. II. Colloidal properties of starch from artificially dried maize. G. Wahl (*Stärke*, 1966, **18**, 383–390).—Maize samples from different varieties were dried in the laboratory at 40, 60, 80, 100 and 120° and the properties of the extracted starches compared. The consistency, swelling range of the grains, swelling capacity in aq. pyridine, gel elasticity, elution analysis with $HClO_4$ and limiting viscosity were used to assess the influence of artificial drying on these properties. Artificial drying resulted in the same changes observed when free starch is heated: swelling temp. and gel elasticity increase whilst consistency deteriorates. If the initial moisture content of the grain is high or the grain is unripe, changes at elevated drying temp. are particularly marked. Over extended drying periods at high temp. the final moisture level is very low and swelling temp. and consistency are reduced with an increase in the max. swelling values in aq. pyridine. Although colloidal properties are changed, chemical degradation is very small and affects only the amylopectin. (28 references.) J. B. WOOF.

Rheology of doughs. III. Effect of ingredients on the tension relaxation of wheat doughs. L. T. Kováts and R. Lásztity (*Periodica polytech.*, 1966, **10**, 237–247).—A previously described instrument was used to measure the tension relaxation of 10 samples of typical wheat flours and the influence of NaCl, saccharose, fats, surface-active substances, $KBrO_3$, ascorbic acid and *N*-ethylmaleinimide on the values. It was found that comparative measurements were only possible if the experimental conditions such as temp., intermediate storage time and deformation were kept the same. (14 references.) (In German.) M. SULZBACHER.

Shortening as a component of continuous process bread. R. J. Baeuerlen (*Baker's Dig.*, 1966, **40**, 56–59 and 76).—The use of complete shortenings and of individual shortening components is discussed. Most shortenings can be interchanged with good results, but in some instances changes in bread formulation or operating procedures may be required. Knowledge of optional ingredients can be a contributing factor to the success of a bread operation. I. DICKINSON.

Weakening action of thioctic acid in unyeasted and yeasted doughs. L. K. Dahle and R. S. Hinz (*Cereal Chem.*, 1966, **43**, 682–688).—In unyeasted doughs Farinograph curves demonstrate a weakening effect from additions of either reduced thioctic acid or dithiothreitol, but not from additions of (cyclic) thioctic acid or cyclic (oxidised) dithiothreitol. In yeasted systems (additive pre-incubated with yeast suspension before Farinography) a weakening effect resulted from additions of thioctic acid but not of cyclic dithiothreitol, thus demonstrating the specificity of the thioctic-reducing enzyme system in yeast. Baking results showed significant reduction in bread vol. by additions of thioctic acid as low as 0·025 μ mole/g (5 ppm). Additions of thioctic acid to flour slurries resulted in increasing yields of water-sol. and acetic acid-sol. proteins on incubation. A connection between flour strength and endogenous thioctic acid levels is suggested. E. C. APLING.

Studies on the flavour fraction of bread crust adsorbed by cation exchange resin. T. Morimoto and J. A. Johnson (*Cereal Chem.*, 1966, **43**, 627–637).—Column, paper and gas chromatographic studies of the aroma components from bread crust and from the reaction of proline (**I**) and glucose (**II**) are reported. Aroma components extracted from bread crust with 70% EtOH, and from reaction of **I** and **II** at pH 7, were adsorbed on Amberlite IR-120 or CG-120 and eluted with 0·2 N NaOH after washing the columns with citrate buffer (pH 5·28 to remove free amino-acids and peptides from bread crust extract, and pH 3·35 to remove unreacted **I** respectively). The eluted fractions from each source were resolved by paper chromatography into two fractions giving blue and yellow colours with ninhydrin and showing u.v. absorption max. at 280–290 nm; the chromatograms and spectra of these fractions from bread crust and from the **I**–**II** reaction were similar but not identical and gas chromatography of the fractions made alkaline with NaOH separated a peak of strong biscuit-like aroma from the products of the **I**–**II** reaction only. (23 references.)

E. C. APLING.

Studies with radioactive tracers. IX. The fate of sucrose-^{14}C during breadmaking. C. C. Lee and Ching-Hong Chen (*Cereal Chem.*, 1966, **43**, 695–705).—Bread was made with 5 g of active sucrose per 100 g of flour and the distribution of activity in fermentation gases, oven condensate and crust and crumb of the bread was studied. Recovery was incomplete (66–69%) but ~42% of the original activity remained in the finished bread and 20% was recovered from the condensate. Most of the activity in the crumb and crust consisted of water-sol. neutral compounds (including glucose and fructose in the ratio 1 : 3·5), but water-insol. active material accounted for 18% of the activity of crumb and 24% of crust and small amounts of water-sol. basic and acidic components were also present. Insignificant activity was associated with maltose, the major component of the bread extracts. Nearly all the activity in the oven vapour condensate and crumb distillate was in the alcoholic fraction, but small amounts of active carbonyl and acid compounds were also present; activity of the carbonyl fraction of the oven condensate was almost entirely due to hydroxymethylfurfural. Fermentation and oven CO_2 accounted for about 6% of the total activity. (17 references.) E. C. APLING.

Studies with radioactive tracers. X. The fate of glycine-1-^{14}C during breadmaking. Y. H. Liau and C. C. Lee (*Cereal Chem.*, 1966, **43**, 706–715).—Bread was made with 200 mg of active glycine (**I**) per 100 g of flour. Some conversion to active CO_2 occurred but none of the volatile condensates showed appreciable activity. About 20% of the original activity was found in the crust and 40% in the crumb (total recoveries 66·5 and 70%). Activity of the aq. extracts was mainly in the basic fraction which included besides unchanged **I**, 10–20 other active components (less in crust than in crumb), some of which were hydrolysable to give new products or regenerated **I**. The results demonstrate the occurrence of condensation reactions involving **I** during breadmaking and give strong evidence for the occurrence of Maillard type browning reactions, involving condensations between amino-acids and reducing sugars. E. C. APLING.

Sorbic acid, its use in yeast-raised bakery products. C. DeSa (*Baker's Dig.*, 1966, **40**, 50–52).—Current literature and new experimental data are reviewed. The effectiveness of the sorbic preservatives is demonstrated in comparison with the propionates. Procedures are suggested for incorporating the sorbic preservatives into yeast-based products to maintain normal proof volume and organoleptic acceptability. (11 references.) I. DICKINSON.

Cyclic anhydrides of acidic lipids. Procter & Gamble Co. (B.P. 1,051,058, 4.11.64. U.S., 4.11.63).—Title products have the formula $O{\cdot}CO{\cdot}CHX{\cdot}CH{\cdot}O{\cdot}COR$ (X is H or $O{\cdot}COR$ and R is alkyl or alkenyl of 11–21 C) and are additives in bakery products (doughs, batters, etc., to provide superior foam stability and air incorporation). A typical product is malic stearate anhydride (**I**), m.p. 70·7°. It is prepared in 76% yield by stirring a mixture of malic stearate, Ac_2O, toluene, and 70% aq. $HClO_4$ at room temp., then diluting with hexane, cooling at 0–5° for 15 min., adding xylene slurry containing NaOMe, and recrystallising **I** from hexane.

F. R. BASFORD.

Prefried cereal breading. Griffith Laboratories Ltd. (Inventor: J. A. Ziegler) (B.P. 1,053,700, 25.10.65).—The product has a deep-fat fried flavour and colour and is produced by cooking lengths of dough of predetermined cross section in a deep fat cooking medium at a predetermined temp., to achieve in the lengths a fat content of 15–25% and a moisture content of 3–15%; then cooling the removed lengths; and grinding to crumbs. F. R. BASFORD.

Sugars and confectionery

Application of radio-isotopes to the study of continuous diffusion in sugar factories. P. M. Hoffmann, W. Gawłowska, S. Cieślik, A. Poczna̜ło and S. Gawrych (*Rapp. Inst. Badań Jadrowych*, 1966, 759/XXII, 20 pp.).—Using a ^{24}Na isotope the movement of the sugar beet mass through the diffuser is followed. The degree of extraction, as a function of movement, is studied. Further work was carried out with ^{140}La-versenate to show the path followed by the juice through the diffuser. (In French.)

T. M. BARZYKOWSKI.

Gluco-amylase. A. E. Staley Mfg Co. (B.P. 1,051,565, 30.3.65. U.S., 21.9.64).—Gluco-amylase (**I**) is produced by fermenting, in a nutrient medium (pH 3–7) containing assimilable sources of N, C and nutrient minerals (e.g. ground yellow dent corn, urea, $MgSO_4$ and K_2HPO_4), *Aspergillus phoenicis* Staley 298–150 (ATCC 15,555) or *A. phoenicis* Staley 298–155 (ATCC 15,556). Production of dextrose by digesting starch with **I** (obtained as prep. of high potency) is also claimed. E. ENOS JONES.

Production of sugar syrups. Miles Laboratories Inc. (B.P. 1,059,544, 10.2.64. U.S., 25.2.63).—A starch syrup, suitable for enzymatic conversion into sugar-containing syrup is produced by treating an aq. starch slurry with a bacterial amylase composition having dextrinising and saccharifying properties at the optimum dextrinising temp. to liquefy all of the starch (e.g., during 2–20 min. at 80–90°), then cooling to the optimum saccharifying temp., e.g., 50–70° and keeping thereat (48–96 h) to produce a syrup of dextrose equiv. 30–42. A heat stabilising agent (gypsum or NaCl) is present. F. R. BASFORD.

Candy product. Richardson-Merrell Inc. (B.P. 1,058,621, 12.1.65. U.S., 16.1.64).—A hard candy free from graining tendencies which remains clear under normal temp. and humidity is prepared by cooking a high maltose corn syrup (free from sucrose) containing 35–55 wt.-% of maltose and 27–65 wt.-% of higher saccharides, to <4 wt.-% of water. F. R. BASFORD.

Candy sweetmeats. M. M. Schwartz and A. E. Kotlier (B.P. 1,058,879, 24.12.65).—The construction of a holder for a rotating lollipop is described. F. R. BASFORD.

Chocolate manufacture. R. Beetz (B.P. 1,060,795, 8.4.64. Ger., 8.4.63).—The kneading time may be adjusted in the claimed process for the production of masses of viscous material, such as chocolate, from individual components by selecting predetermined quantities of the components under the control of a programme switching device, mixing (in a predetermined sequence) the components in a pre-mixing stage, continuing the mixing for an adjustable time, transferring the pre-mixed charge to a main kneading stage, kneading and then continuously discharging the material to form a strip.

S. D. HUGGINS.

Fermentation and Alcoholic Beverages

Enzymatic production of glucose syrup from grains and its use in fermentations. M. C. Cadmus, L. G. Jayko, D. E. Hensley, H. Gasdorf and K. L. Smiley (*Cereal Chem.*, 1966, **43**, 658–669).—Laboratory studies of the composition and possible use as fermentation media of glucose syrups produced from ground maize, wheat or sorghum with a combination of α-amylase (from barley malt, or unrefined prep. from *Bacillus subtilis*) and unrefined glucoamylase prep. from *Aspergillus niger* (NRRL 3122) or *A. awamori* (NRRL 3112) are reported; 90–95% conversion to D-glucose, with maltose and iso-maltose amounting to <10% of the total sugars, was obtained within 24–48 h. Solubilisation of N during the enzymolysis was about 25% of the total grain N of maize and sorghum and 41% of wheat. The residue on filtration was 20–25% of the original grain solids, had N content approx. three times that of the grain, and might have potential value as an animal feed supplement. Fermentation yields of several microbial polysaccharides, citric acid, fumaric acid and 2-ketogluconic acid from the experimental syrups were generally equal to, or better than, those obtained from commercial D-glucose, although the syrup from wheat gave poor yields of citric and 2-ketogluconic acids, probably due to excessive amounts of sol. N. (18 references.)

E. C. APLING.

Utilisation of gasoil by a yeast culture. T. L. Miller and M. J. Johnson (*Biotechnol. Bioengng*, 1966, **8**, 567–580).—A culture of *Candida intermedia* and *C. lypolytica* utilised n-paraffin hydrocarbons quant. but other oil fractions were not affected. With various fractions generation times of 4·0–9·0 h were obtained and 70–90% yields on the basis of paraffin utilisation. 19–24 C compounds were most efficiently utilised. Partial utilisation of paraffin wax when dissolved in pristane was observed.
J.A.C. Abstract.

Utilisation of normal alkanes by yeasts. T. L. Miller and M. J. Johnson (*Biotechnol. Bioengng*, 1966, **8**, 549–565).—A stable mixed culture of *Candida intermedia* (**I**) and *C. lipolytica* (**II**) was used to study alkanes especially those solid at fermentation temp. The yeasts were grown on full salt media with alkane added as C source; CO_2 production was used as a measure of growth rate and yield and content of N and lipid were also measured. The mixed culture grew more rapidly than **I** whilst **II** did not grow at all on unsupplemented salt–alkane medium. When alkanes of 15–28 C were dissolved in 2,6,10,14-tetramethylpentadecane cell yields were 74–89% and mean generation times 3·0–8·0 h, a max. in each case being obtained with docosane. Cells contained 6·75–8·81 % N, 34·4–47·6% crude protein and 1·9–13·4% lipid. Manometric study of resting whole cells of **I** showed that oxidation is constitutive and not substrate specific. J.A.C. Abstract.

Oxidative assimilation of ethyl alcohol by bakers' yeast. B. Drews and K. Hessler (*Mschr. Brau.*, 1967, **20**, 224–241).—Ethanol metabolism of a bakers' strain of *Saccharomyces cerevisiae* has been studied manometrically and by the use of ^{14}C labelled substrate. The intermediate radioactive products were traced by two dimensional TLC and autoradiography and then by scintillation counting of the eluted components; detection and counting were 10–20 times more sensitive than with paper chromatography. After growth on glucose medium, with ethanol as the sole substrate, the yeast respires with an initial R.Q. of 0·66. Respiration is *via* the citric acid cycle. As ethanol concn. increases, catalytic oxidation to MeCHO occurs. Functional metabolism is inhibited by 8% ethanol but MeCHO is produced up to 17–20%. If a period of adaptation is permitted the yeast develops anabolism and isotopic labelling shows that the glyoxalate cycle is operative; before this starts, continuous respiration and assimilation by the tricarboxylic acid cycle (TCC) is already occurring. An increase in the portion of respired ethanol and more even utilisation of C atoms can be achieved by adding excess amino-acids; use of radioactivity permits deductions concerning rate and direction of ethanol incorporation and equilibria between amino-acid pool and intermediates in the TCC cycle. (43 references.) J. B. Woof.

Simplified photometric procedure for the determination of wort colour in selection of hybrid barley varieties suitable for brewing. L. Reiner (*Brauwissenschaft*, 1967, **20**, 269–275).—The colour of wort products produced from trial samples is a characteristic of the variety and can be used for selection purposes. A method, suitable for routine determinations, is described, in which interference by turbidity in the sample is largely overcome by the use of colour filters. The differences in blue light extinction (436 mμ) and yellow light extinction (578 mμ) are measured in the EBC-Komparator. Colour values thus determined agree well with those found by the photometric procedure. The correlation coeff. r is 0·929 for a series of 91 different worts. (33 references.) I. Dickinson.

Barley gums and their behaviour during malting and brewing. III. Significance and variation of gums from first wort to the finished beer. K. Schuster, L. Narziss and J. Kumada (*Brauwissenschaft*, 1967, **20**, 280–289).—Gum content does not depend a great deal on mashing conditions, procedure or concn. of the mash, except when high temp. are used. The relative η of the gums represents 20% to 25% in the first wort, 30% to 35% in the finished wort and 40% to 45% in the finished beer of the total η. During fermentation the gums affect clarification by their pptn. and the stability on account of their high η which hinders the settling of cloudy material. Unstable gum is present in the finished beer when undermodified or withered malt is mashed, or high temp. are used; this impairs the chill stability of the beer. Gums have a favourable effect on foam stability and taste. I. Dickinson.

Evaluation of barley and malt quality by computer. O. J. Banasik, K. A. Gilles, M. O. Holoien and D. E. Peterson (*Brauwissenschaft*, 1967, **20**, 324–329).—An attempt is made to define in proper machine language comparisons and interpretations that were previously accomplished by visual inspection. The most important data for the evaluation are for barley: kernel thickness, protein content, extract content and diastatic power, for malt: kernel thickness, protein content, ratio of wort N to total N, extract, diastatic power and α-amylase activity. Method of calculation, a code and examples of IBM data cards are given.
I. Dickinson.

Comparative trials on Turkish barleys by use of micro- and small-scale malting. T. Yazicioglu (*Brauwissenschaft*, 1967, **20**, 365–368).—Results of trials on eight samples of Turkish barley are described. Average 1000 corn weight was 41·7 g, husk content 10·4, water 7·9%. A steeping time of 50 to 57 h was sufficient to obtain a moisture content of 43%. Germination was normal, percentage of rootlets fluctuated between 2·6 and 4·0 and the malting loss varied from 7·3 to 9·7%. Wort colours were normal, the hot water extract which amounted to 74·2 to 76·8% of the dry wt. was low, while extract differences ranged from 2·2 to 3·7%. The N modification was low (Kolbach index 26·2 to 31·8), the formol N was 133 to 156 mg/100 g and the extract proportion at 45° ranged from 28·8 to 33·6. The diastatic power was very good at 253 to 413 and the Brabender index varied between 77,000 and 85,000. Hot water extracts from the micro-malting trials were determined refractometrically and agreed very well with corresponding values obtained in the small-scale malting trials. The max. difference was ±0·4 and the correlation coeff. r was $0·950 \pm 0·136$ ($n = 7$). I. Dickinson.

Barley variety, cultivation conditions, wort composition and fermentation pattern. IX. Dependence of carbohydrate composition of malt worts on barley variety and growing region. F. Wullinger and A. Piendl (*Brauwissenschaft*, 1967, **20**, 443–451).—The carbohydrate composition of worts from light barley varieties grown in 17 different regions was determined. Results are given. The Bido variety was superior to all other varieties tested, it combined high hot water extracts with very high fermentability, average hexose and very high maltose content. (11 references.)
I. Dickinson.

Rapid procedure for alpha-amylase determination in malt. D. G. Medcalf, E. E. Tombetta, O. J. Banasik and K. A. Gilles (*Cereal Chem.*, 1966, **43**, 675–682).—The method depends on determining the Hagberg falling no. for a mixture of corn or wheat starch and the malt or malt extract under test. The method is simple, rapid and reproducible, and correlations between 20° α-amylase units determined directly by the ASBC standard method and calculated from falling no. values were good (semi-log relationship), but standard curves must be redetermined for each batch of starch used as diluent. E. C. Apling.

The anthocyanogens and their importance in malting and brewing—a literature survey. F. Kraffczyk (*Mschr. Brau.*, 1967, **20**, 249–253).—A review of the nomenclature, structure, isolation techniques and estimation methods of the anthocyanogens occurring in beer, barley, malt and hops. The behaviour of these compounds during brewing and their ultimate effect on chill haze is discussed. (58 references.) J. B. Woof.

Action of a diaphragm on brewery effluents. II. Separation of colloidal disperse systems in brewery effluents. F. Knorr (*Brauwissenschaft*, 1967, **20**, 289–291).—The use of a diaphragm together with ozone is described; CH_2O, MeCHO, Et_2CO and MeEtCO were detected in steep liquors. A determination of the size of the colloid particles was attempted to assess the biologically active state of the effluents. I. Dickinson.

Tables for the refractometric–pyknometric determination of alcohol and extract content of spirits. E. Bohm (*Dt. Lebensmitt-Rdsch.*, 1967, **63**, 333–336).—Tables are given with examples explaining their use. J. B. Woof.

Simplified method for the colorimetric determination of citric acid in wine and grape must. H. Rebelein (*Dt. LebensmittRdsch.*, 1967, **63**, 337–340).—A rapid method (~1 h) for determination of ~67–1000 mg/l of citric acid (**I**) in 5 ml samples of wine and grape juice is compared with other methods. **I** is separated as the Ba salt, and determined colorimetrically by reaction with a diazo solution (prepared from $NaNO_2$ and Griess reagent) and addition of Pb acetate. Recovery of added acid is almost quant. and citramalic acid does not interfere. J. B. Woof.

Substances present in yeast head. [I] Determination of humulone content in yeast head. [II] Determination of fatty acid constituents. W. Riedl and M. Kellner (*Brauwissenschaft*, 1967, **20**, 312–316; 362–365).—[I] Humulone was determined in yeast head after acid hydrolysis according to the method described by E. Schild and W. Riedl (*ibid.*, 1952, **5**, 81). An average of 2–4 wt.-% of humulone was found in the dry matter of the yeast head, which indicates that 0·5 wt.-% of the humulone provided by the hops passes into the

yeast head in an unaltered form during the fermentation of the wort. A dark green pigment, not identical to the chlorophyll of hops, was found in a concn. of 2 wt.-% of dry matter of the yeast head. (16 references.)

(II) Capric (**I**) and lauric acid (**II**) in yeast heads are detected by gas chromatography; **I** and **II** are isolated by countercurrent distribution between n-heptane and 0·5 M phosphate buffer and are identified as their *p*-bromophenacyl esters. A simple method is also described by which **I** and **II** can be determined together with the humulones. The lead salts of **I** and **II** are obtained and decomposed with dil. H_2SO_4; the m.p. composition of the binary system then allows quant. determination of the acids. The presence of an average of 0·15% by wt. of **I** and 0·4% by wt. of **II** suggests that it is unwise to re-use the yeast head and thus economise on hops, because the acids have a detrimental effect on foam stability and impart a soapy, pungent flavour to the finished beer. (18 references.) I. DICKINSON.

Protein modification and its effect on the finished beer. I. Malting. A. Kaiser (*Brauwissenschaft*, 1967, **20,** 350–361).—A close correlation exists between the quality of barley, malt and beer. Impaired foam stability, increased tendency to yeast degeneration and the risk of 'turbidity' flavours are due to direct and indirect influence of the protein composition in barley, malt and beer. A scheme for steeping, germination and kilning is proposed which provides suitable and normal protein modification even in barley of the 1966 harvest which was found difficult to modify. Extract differences used for the evaluation of the cytolytic modification of malt are not always reliable but suggestions are made on how extract differences can be favourably influenced by suitable malting procedures. I. DICKINSON.

Detection of quaternary ammonium compounds in beer. K. Raible and U. H. Mohr (*Brauwissenschaft*, 1967, **20,** 429–433).—Quaternary ammonium compounds (**I**) are used as disinfectant in the brewery; although they are non-toxic, residues can cause cloudiness in beer and diminish foam stability. A method is described which can detect 1 ml of a 0·2% solution in 1 l of beer. Thus, shake 500 ml of beer with 1 g of kieselgel for 24 h, remove kieselgel by centrifuging and suspend in 10 ml of aq. 10% Na_2CO_3 for 6 to 8 h. Add 6 ml of a solution of 40 mg of bromophenol blue, 1 ml of 0·1 N Na_2CO_3 and water to 100 ml, then 20 ml of $CHCl_3$, shake overnight and centrifuge next day. A blue coloration of the $CHCl_3$ layer indicates the presence of **I**. I. DICKINSON.

Conditioning and the carbon dioxide content of beer. H. E. Raupach and G. Glauninger (*Brauwissenschaft*, 1967, **20,** 433–438).—Importance of CO_2 content depends mainly on the amount of yeast in suspension and the time of stoppering the barrel but also on the pressure due to stoppering and the temp. When the main fermentation is accelerated, the amount of yeast used for conditioning must be carefully standardised. If the pattern of increase of CO_2 in green beer in storage vessels is studied, a prediction of final content of CO_2 at the end of the conditioning period can be made, thus avoiding an excess of CO_2 (and the resulting deterioration of the foam volume) by choice of the right time to commence conditioning. I. DICKINSON.

Relation between cold sensitivity and viscosity of beer. W. Kleber and J. Kloepfer (*Brauwissenschaft*, 1967, **20,** 475–477).—The cloudiness due to a fall in temp. and the theory of viscosity are discussed. Experiments are described in which pale beer and a standard solution containing of 81·5% water, 4·5% sugar and 4% ethyl alcohol, are used. These experiments revealed that a prediction of cold stability cannot be made by measuring the viscosity and that a direct relation between viscosity and stability cannot be drawn. I. DICKINSON.

Shortening of fermentation and lagering time in beer production. G. Krauss and G. Sommer (*Mschr. Brau.*, 1967, **20,** 49–77).—The first part of the paper reviews factors affecting fermentation and maturation rate. Since 1900 the lager production process has been reduced from 12–14 weeks to 9 days fermentation followed by 6–9 weeks lagering. Primary fermentation rate depends on wort composition, yeast strain and concn., temp., agitation and surface area. Removal of cold sediment increases fermentation rate without affecting flavour. Greater likelihood of infection and autolysis and of formation of diacetyl and yeasty flavours with increased yeast pitching rates are reported. At 10–13° wort ferments quite rapidly and the product is clear, the head good and the flavour unaffected. Lagering has been carried out at 10° to produce good carbonation and clarity and this is helped by stirring. The second part of the paper describes experiments out carried on miniature (23–27 kg of malt) brewery decoction wort to test the effects of changing the parameters discussed. Analyses of diacetyl, acetoin, higher alcohols, esters, aldehydes, dissolved O_2 and yeast concn. were carried out on the beers, normal variations having first been assessed. Increasing yeast propagation raised pH, increased higher alcohols, and reduced bitter substances and assimilable N. Raising the inoculum from 0·5 to 20 l/hl lowered the fermentation time from 7–12 to 4–5 days; loss of bittering substances rose from 38 to 46% without affecting the beer quality. Primary fermentation at 20° with top and bottom fermenting yeasts gave unobjectionable beers with diacetyl levels of 0·2 mg/l after only 72 h; bitterness losses rose to 63% and higher alcohols increased. Flavour stability was comparable with normal beer. Lagering was carried out at 20° for 4–5 days, 15° for 9 days and at 7° for 14 days. The longest period was found to be necessary for protein stability and adequate carbonation. (100 references.) J. B. WOOF.

Influence of nitrate content in natural water on the brewing of beer worts. K. Vogl, G. Schumann and W. Pröpsting (*Mschr. Brau.*, 1967, **20,** 116–120).—Tap water with a total hardness of 16·2° dH and total salt content 477 mg/l and well water with a total hardness of 43·7° dH and total salt content of 1288 mg/l were used for the experiments. The water from each source was adjusted with $NaNO_3$ to 50, 75 and 100 mg/l nitrate content; distilled water was used for comparison. The fermentation process (at a temp. of ~12°) was observed for 18 days. A toxic, inhibiting influence is shown after the first day, which increases from the third to the sixth day and reaches a max. on the 12th and 14th day. The toxic effect is greatest when $NaNO_3$ is added to distilled water; in hard water compensation of the toxic influence of up to 15% seems to take place. This may explain the differences in results reported by various breweries when water with a similar $NaNO_3$ content but different degrees of hardness is used. (42 references.) I. DICKINSON.

Gas-chromatographic determination of CO_2 and alcohol in beer. K. Silbereisen, E. Krüger, B. Schubert and F. Anthon (*Mschr. Brau.*, 1967, **20,** 121–124).—A method in which 3 μl of beer is injected directly into the chromatograph is described. The use of Poropak Q as column packing made a good separation of CO_2, water and alcohol possible; nine beers were analysed. The CO_2 standard curve was obtained by injecting aq. ammonium carbamate into the chromatograph, the standard alcohol curve by determining alcohol–water mixtures at various concn. with a pyknometer. Highest deviation when compared with the Blom method was 0·008% for CO_2 and 0·06% for alcohol. I. DICKINSON.

Determination of minute solvent residues in hop extracts and their retention in the manufacture of beer. K. Vogl and G. Schumann (*Mschr. Brau.*, 1967, **20,** 124–126).—Beer made with hop extracts obtained by the use of CH_2Cl_2 as extractant, were analysed systematically for 4 years. The solvent residue content was less than the permitted limit in each case; doubts about the use of hop extracts for this reason are therefore unfounded. A gas-chromatographic method for the determination of CH_2Cl_2 in hop extracts is described. I. DICKINSON.

Effect of some fermentation factors on the higher aliphatic alcohol- and isoamyl acetate-content of beers. B. Drews and J. Riemann (*Mschr. Brau.*, 1967, **20,** 254–268).—Volatiles were removed from beer (200 ml) containing NaCl (20 g) by distillation and extraction of the distillate with ether–pentane. Isoamyl acetate (**I**), Pr^nOH, Bu^iOH, Bu^nOH, 2-methyl- and 3-methyl-butanol were identified and determined quant. by GLC on a 1,2,3-butantriol column. High O_2 levels in the wort and aeration after pitching favoured the formation of 2-methyl-butanol, Bu^iOH and **I**. These components were also increased to the greatest extent when fermentation temp. was raised from 8° to 20°. High pressure combined with high temp. produced lower amounts of higher alcohols. Pitching rate does not affect the formation of volatiles at the same attenuation levels but in stirred fermentations in closed vessels more higher alcohols and less **I** were produced. Relative amounts of each product varied with the yeast strain, but flocculence characteristics were not important. Barley variety and malt modification caused changes in the relative but not the total concn. of the products. Cold break and autolysis were without effect. Addition of sucrose, assimilable N and pyruvic acid was also studied. (86 references.) J. B. WOOF.

Attenuation and beer quality. H. Schilfarth and G. Sommer (*Mschr. Brau.*, 1967, **20,** 325–332).—The attenuation of beers produced in the last few years ranges from <75 to >85% with most in the 77–83% range. Miniature brewery beers have now been produced from the same raw materials but under different mashing

conditions so their final attenuations were 73, 80 and 86% respectively. After primary and secondary fermentation and 8 weeks lagering, the beers had almost reached their attenuation limit and were bottled and analysed. No significant differences were apparent in colour, protein, isohumulone or chill stability but increase in attenuation was found to increase pH, total N and the concn. of esters and higher alcohols whilst reducing η and head retention. Taste tests indicated a slight tendency to associate fuller body with the 80% attenuation. It is concluded that % attenuation has less effect than previously thought and that the value of 80% used in German light beers should be maintained as it can be achieved without difficulty in a double decoction mash. (21 references.) J. B. WOOF.

Effect of biflavonoid proanthocyanidins (anthocyanogens) on the chill stability of beer. K. Silbereisen and F. Kraffczyk (*Mschr. Brau.*, 1967, **20**, 332–337).—The method of Harris and Ricketts as modified by McFarlane has been used to measure proanthocyanogens. Barley biflavonoid procyanidin (**I**) gives the same calibration curve as cyanidandiol. When 20–40 mg/l of **I** was added to several Pilsner beers, the chill stability could be reduced by half, but some beers were found to take up to 50 mg/l and still exhibit remarkable stability. These compounds are therefore not the only factors in controlling the stability. Their haze forming action is enhanced by aerial oxidation and the same effect is observed with catechin. The increase in tanning power is accompanied by a decrease in the colour yield in the analytical method used and it is concluded that proanthocyanidin level is no measure of chill stability. (14 references.) J. B. WOOF.

[A] **Hop treatment.** [B] **Water-soluble salts of hop acids.** S. S. Steiner Inc. (B.P. 1,058,975–6, 6.8.63 U.S., 15.8.62).—[A] A high level of flavour control in beer manufacture is achieved by extracting α-acids from the α- and β-acids (obtained by treating hops with a water-immiscible org. solvent, e.g. hexane) by stoicheiometric reaction with aq. NaOH at low temp. to prevent isomeric conversion. Water is then removed by freeze drying. If the hexane solution is further treated with aq. NaOH, water-sol. salts of the β-acids are obtained. In an example, total recoveries of α- and β-acids were 91·4 and 89·0%, respectively.

[B] Useful as an additive in the brewing of beer, the title compounds are a dry powdered mixture of water-sol. salts of α-acids and isohumulone. Thus, a hexane extract of ground, dried hops is distilled in a vacuum to give a solution that contains 45·6% α-acids and 29·17% β-acids. The extract is divided in 4 pt.; the first pt. is neutralised with NaOH at 25°, the second at 45°, the third pt. is evaporated to dryness, the residue suspended in naphtha and extracted with NaOH, while the fourth is extracted with NaOH at 65°. The % α-acids obtained by these treatments is 83·4, 87·07, 45·6 and 75·5 respectively, the first and second samples showing little or no isomerism into isohumulones. S. D. HUGGINS.

Stabilisation of beverages. Société Viscose Suisse (B.P. 1,059,895, 5.1.66. Switz., 23.2.65).—Undesirable changes in beverages (beer) due to presence of anthocyanogens are prevented by contacting the drink with polyamide fibres having fine hook-like filaments extending from the main stem (preferably polycaprolactam or nylon 66). F. R. BASFORD.

Alcoholic beverages. Kyowa Hakko Kogyo Co. Ltd. (B.P. 1,060,681, 28.2.64. Japan, 4.3.63).—The beverages (beer, ale, etc.) are prepared by subjecting a mash produced from cereal grain and plant protein to decomposition and saccharification by addition of a proteolytic enzyme and a mould culture (e.g. *Rhizopus delemar* or an *Aspergillus*) capable of saccharifying starch (or one or more enzymes obtained by purifying the mould culture), and, optionally, some malt, preparing a wort from the solution and subjecting this wort to fermentation and filtration. E. ENOS JONES.

Fermentation of brewers wort. Arthur Guinness Son and Co. and Courage Barclay and Simonds Ltd. (Inventors: J. R. A. Pollock and H. F. P. Webber) (B.P. 1,060,722, 27.11.62 and 24.1.63).—A concn. of yeast, higher than is used in conventional methods, is fermented at 40–75°F, in the faster process claimed. Thus, barrels of wort, at 60°F, suitable for making stout, are treated with O_2 (5% by vol.) and pitched with a strain of *Saccharomyces cerevisiae* (0·6 g/l) for a stirred fermentation of 28 h. The yeast is allowed to settle for 4 h and beer drawn off, leaving a slurry of yeast (for subsequent pitching of the next batch) for centrifuging. After a repeated 6 cycles, the collected beers are then prepared in the conventional manner as naturally conditioned stout, and are indistinguishable from conventionally batch-prepared stouts. S. D. HUGGINS.

Fruits, Vegetables, etc.

Application of gas–liquid chromatography to determination of potato chip staleness. W. O. Miller (*Diss. Abstr.* B, 1967, **27**, 3554).—Correlation of organoleptic evaluation of potato chips and statistical analysis of GLC data from headspace analysis was investigated in an attempt to predict staleness of potato chips or % fresh chips in mixtures of fresh and off-flavoured chips. Measurement of the height of a single peak could be used to predict % fresh chips in mixtures, but was not successful in prediction of chip age and composition of mixtures. F. C. SUTTON.

Production of instant mash potato. K. E. Eapen and P. K. Ramanathan (*J. Fd Sci. Technol.*, 1966, **3**, 66–68).—Potato mash is prepared in the usual way and then extruded, conditioned, subjected to sudden vacuum for a short period and dehydrated to produce porous beads of peanut size. The product can be reconstituted instantaneously with hot water or it can be fried and consumed as crispies. S. A. BROOKS.

Determination of propham and chloropropham in potatoes. C. Reinhard (*Dt. LebensmittRdsch.*, 1967, **63**, 340–342).—A TLC method for determination of $\leq 0{\cdot}2$ μg of the herbicides is described. A slurry of peeled, homogenised potatoes was mixed with anhyd. Na_2SO_4 and kieselguhr; when dry the mass was extracted with CH_2Cl_2 and adsorbed on a column of a mixture of active C, Florisil and Celite (1 : 2 : 2). After washing with aq. Na_2SO_4, the sample was eluted with CH_2Cl_2, the eluate taken to dryness and redissolved in CH_2Cl_2. This solution was separated by TLC on silica gel G–alumina (1 : 1) with n-hexane–CH_2Cl_2 (1 : 4) as solvent. The plates were sprayed with a solution of dimethylaminobenzaldehyde in EtOH–HCl (19 : 1). For propham, recovery was $96 \pm 9{\cdot}1$% and for chloropropham $104 \pm 12{\cdot}4$%. (17 references.) J. B. WOOF.

Manufacture of preserved olives. D. F. Marsico (*Revta argent. Grasas Aceites*, 1966, **8**, 27–34).—The various classes of product, olive varieties used, the fermentation process, defects and specifications are reviewed. (19 references.) L. A. O'NEILL.

Dried fruit flakes or powder. J. Aquirre, R. H. Bundus and P. P. Noznick (B.P. 1,059,609, 22.10.65).—An edible flake or powder from fruit, having (on a liquid basis) >7% of sugar is produced by macerating fruit; homogenising the resulting purée; heating this with stirring; feeding it to a rotating drum dryer; removing the dried film product in presence of a blast of air which is cooler and drier than the surface of the drum and the surrounding air; suspending the film in the blast while reducing its temp.; then cooling to form a brittle mass. F. R. BASFORD.

Non-alcoholic beverages

Passion fruit juices. E. Benk (*Riechstoffe Arom. Körperpflegemittel*, 1967, **17**, 185–186).—Analyses are presented of the juices of *Passiflora edulis* subgen. *Granadilla* and *P. edulis* var. *flavicarpa* of various origins, including pasteurised and deep-frozen samples. The juices are characterised by the presence of starch, their low content of pectins and tannins, and their high content of K, vitamin C, and β-carotene. They are suitable for admixture with other fruit juices. P. S. ARUP.

Tea, coffee, cocoa

Incorporation of trichloroethylene into constituents of coffee during its decaffeination. H. Brandenberger and H. Bader (*Helv. chim. Acta*, 1967, **50**, 463–465).—Following commercial practice, green Santos coffee beans were swollen with steam to a moisture content of 24% and 43% and decaffeinated in 22 stages with C_2HCl_3 labelled with ^{14}C. Solvent residues were removed by alternate treatment with steam and vacuum. The beans showed radioactivity, even after being roasted at 240°. It is estimated that 0·25-1 g of C_2HCl_3 is incorporated per kg of beans. The nature of the compounds formed is still unknown but attention is drawn to the possible formation of highly toxic *S*-dichlorovinyl deriv. such as those encountered in the extraction of soya beans with C_2HCl_3. (18 references.) M. SULZBACHER.

Milk, Dairy Products, Eggs

Interpretation of the milk alkaline phosphatase reactivation process. G. C. Kresheck and W. J. Harper (*Milchwissenschaft*, 1967, **22**, 72–75).—Zn, Cu, Ca, etc. act as natural inhibitors in milk which

affect the sulphydryl group which is liberated from alkaline phosphatase by heat treatment. Other sulphydryl groups however can act as effective chelating agents and if present at an optimum level can enhance reactivation. The experimentation relating to this phenomenon is reviewed and the possible mechanism is discussed. (28 references.) C.V.

Effect of detergents and disinfectant sprays on milk tanks. I. F. Kiermeier, K. von Grundberr and G. Wildbrett (*Milchwissenschaft*, 1967, **22**, 76–82).—Tanks constructed of 18/8 chrome-nickel steel, 99·5% aluminium and glass fibre reinforced polyester resins were studied, being repeatedly cleaned and sprayed with various preparations under pressure and at high temp. Even at 80° no damage is recorded by spraying techniques but to avoid deposits acid cleaning has on occasion to be resorted to. (16 references.) C.V.

Gas chromatographic examination of sheep milk fat. A. Lotito and A. Cucurachi (*Riv. ital. Sostanze grasse*, 1967, **44**, 341–348).—The fatty acid composition of 81 samples of sheep milk fat has been determined by gas chromatography on a column of ethylene glycol succinate–Chromosorb P at 208°; numerous minor component acids were identified. No general relationship between fatty acid composition and breed of sheep was observed. Comparative analysis of cow milk fat showed significant quant. differences. The contents and ratios of contents of the following fatty acids were most useful for differentiation, the range of values respectively for sheep and cow milk fat being: C_{10} 3·33–11·56, 1·48–3·38; C_{12}/C_{10} 0·49–0·86, 1·04–1·54; $C_{14:1}$ 0·25–0·92, 1·13–1·59; $C_{15}/C_{14:1}$ 2·00–5·88, 0·93–1·47 (where subscript colon followed by numeral indicates no. of double bonds). L. A. O'NEILL.

Thin-layer chromatographic analysis of 2,4-dinitrophenylhydrazones of carbonyl compounds present in butter fat. O. Abousteit (*Fette Seifen AnstrMittel*, 1967, **69**, 1–5).—After separation by mol. distillation, the carbonyl compounds from fresh butter, mouldy butter, butter kept at 50° for 10 h and at 100° for 9 h, are converted to their 2,4-dinitrophenylhydrazones (**I**) and these are separated by TLC. The main fraction from the mouldy butter was subjected to partition chromatography and eleven fractions were detected. The most pronounced were **I** derived from pentan-2-one, heptan-2-one and nonan-2-one. The amount of **I** that can be prepared from butter heated at 100° increases from 16 to 53 mg/kg with heating time (0–9 h). Heated butter gave nine fractions of methyl ketones (C_3 to C_{15}). Thin-layer chromatograms of **I** derived from rapidly heated (15–20 sec) fresh butter contain many fractions including **I** deriv. of diacetyl and acetoin. W. E. ALLSEBROOK.

Puff spray-dried milk products. F. P. Hanrahan, Riggs National Bank (executors of R. W. Bell), and B. H. Webb (B.P. 1,055,424, 3.7.63. U.S., 9.7.62).—A dry milk product of good stability and rapid dissolving properties is made by heating a fluid lacteal material at least long enough to effect pasteurisation, then concentrating; injecting into the concentrate an innocuous, relatively insol. inert compressed gas, and immediately spray-drying. F. R. BASFORD.

Sterilisation of milk. Alfa-Laval Co. Ltd. (Inventor: R. T. Clark) (B.P. 1,057,286, 21.9.64).—The raw milk is subjected to centrifugal clarification to remove >70% of the leucocytes, prior to the milk being sterilised by heating at 280°F, thus reducing deposit formation and frequency of shut-down. S. D. HUGGINS.

Edible Oils and Fats

Alimentary and nutritional aspects of animal fats. L. Travia (*Riv. ital. Sostanze grasse*, 1967, **44**, 306–311).—Fat metabolism and the relation between alimentary fats and depot and reserve fats in the body are discussed. L. A. O'NEILL.

Gas chromatographic analysis of lard derived from melted pig fat. B. Doro (*Riv. ital. Sostanze grasse*, 1967, **44**, 349).—Gas chromatographic analysis of the fatty acids indicates the following criteria for a genuine lard: $C_{14}/C_{16}<0·065$; $C_{18:2}/C_{18:1}<0·02$; $(C_{14:1}+C_{15r})/C_{14}<0·045$; $(C_{14t}+C_{15t})/C_{16}<0·075$; C_{14r} and $C_{14:1}$ absent, and C_{15r}, C_{15} and $C_{16r}<0·1\%$ (where r = branched acid, t = *trans* isomer; and subscript followed by numeral indicates no. of double bonds, isomer unspecified). L. A. O'NEILL

Margarine manufacture. Evog, Etablissement für Verwaltung und Organisation (B.P. 1,059,156, 18.3.65. Ger., 20.3.64).—Milk containing 2–3% souring bacterial culture of a human intestine variant of e.g. *Lactobacillus acidophilus*, *Streptococcus laetis*, is added to suitable fatty materials; the culture having been stabilised in its characteristics by preliminary culturing in milk. Thus, a fat phase prepared from sunflower, coconut and palm oils is melted and emulsified with an aq. phase containing additives (e.g. corn starch, glucose, NaCl and citric acid) and skim milk to which a bacterial culture of *L. acidophilus* and *S. laetis* from freshly drawn, unpasteurised milk has been added. The emulsion is then cooled to convert it to a water-in-oil emulsion. S. D. HUGGINS.

Meat and Poultry

Detection of oestrogens in meat. E. Schaal and I. Kleikamp (*Dt. LebensmittRdsch.*, 1967, **63**, 325–328).—Using the mouse uterus test, the occurrence of as little as 10^{-9} g of oestrogen in 100 g of meat could be detected. The sample was homogenised with light petroleum and the supernatant filtered off and clarified by centrifugation. Solvent was removed and the residue made up to 3 ml with sesame oil. This was injected into the test mice using as controls (*i*) mice which had had no injection, (*ii*) ones which had been injected with sesame oil alone and (*iii*) ones which had received 1 mg of Cyren B. J. B. WOOF.

Transformation of fats during the curing of sausage products. C. Cantoni, M. R. Molnar, P. Renon and G Giolitti (*Riv. ital. Sostanze grasse*, 1967, **44**, 399–401) —The development of individual fatty acids, volatile acids and carbonyl compounds during the curing of salami sausage, both *in vitro* using a culture of *Micrococcus* C13, and under natural conditions has been followed. L. A. O'NEILL.

Edible extrudable collagen from hides. Johnson & Johnson (B.P. 1,052,872, 1.10.63. U.S., 1.10.62).—Fresh, undehaired hides are soaked in a dil. aq. solution of an acid (**I**) of dissociation constant 1×10^{-5}–1×10^{-3} (e.g. AcOH) until swollen and softened; then removed from the acid bath and scraped on both sides to remove all hair, etc. The resulting stripped corium is treated with dil. aq. alkali to neutralise the acid and de-swell the corium; and, after washing and comminution, the product is swollen in a soln. of **I** to form a mass of pure collagen fibrils useful for extruding to collagen casings (sausage skins, etc.). H. L. WHITEHEAD.

Spices, Flavours, etc.

Preservatives

Chromanols. Merck & Co. Inc. (B.P. 1,057,345, 1.6.65. U.S., 5.6.64).—A 1,4-quinone, substituted at position 2 by Me, position 3 by $CH_2 \cdot CH : CHMe \cdot CH_2R$ and at positions 5 and 6 by aryl or two Me or OMe groups, where R is H, $(CH_2 \cdot CH_2 \cdot CHMe \cdot CH_2)_nH$ or $(CH_2CH : CMe \cdot CH_2)_nH$ and n is 1–9, is reacted with a compound containing an -enediol group (ascorbic or iso-ascorbic acid) in presence of Cu or Fe ions to produce the corresponding chromanol. E.g., co-enzyme Q_{10}, hexahydroco-enzyme Q_4, 2,3,6-trimethyl-5-phytol-1,4-benzoquinone, or vitamin $K_{1(20)}$, is reacted with L-ascorbic acid in the presence of $CuCl_2$ or $FeCl_3$. The products are useful as anti-oxidants for oils, fats and other foodstuffs. J. M. JACOBS.

α-Tocopheryl quinone. Sun Oil Co. (B.P. 1,059,155, 15.3.65. U.S., 27.3.64).—α-Tocopheryl quinone (**I**) is made by contacting α-tocopherol (**II**) with activated MnO_2 at a temp. below the decomp. temp. (preferably at 60–225°) in a solvent ($CHCl_3$), and fractionating on Al_2O_3 to obtain **I** and a keto-ether dimer of **II**. **I** is a food anti-oxidant precursor. E. ENOS JONES.

Food Processing, Refrigeration

Dehydration of animal and vegetable substances. H. Griffon (B.P. 1,058,821, 29.7.63. Fr., 30.7.62 and 28.1.63).—In the dehydration of e.g. potatoes, the material to be treated is conveyed continuously through apparatus (figured) wherein it is first cooked, then plunged into a cold bath (with rapid cooling), and finally reduced to a purée or pulp. F. R. BASFORD.

Methods of thawing and subsequently warming frozen foods. Burger Eisenwerke A.-G. (B.P. 1,059,577, 31.8.65. Ger., 5.9.64).—

Artificially circulated warm air is supplied to the food to be thawed in such a way that more heat energy is supplied during an initial period than towards the end of the operation.
F. R. BASFORD.

Reconstituting frozen food. Foster Refrigerator Corp. (B.P. 1,059,841, 10.4.64. U.S., 11.4.63).—The frozen food is subjected to pulsating heat produced by cyclically energising and de-energising heating means for predetermined periods in an apparatus which is figured and claimed. F. R. BASFORD.

Packaging

Studies on the storage of packed rations. III. Cashewnuts. K. Vidyasagar, T. M. Aswathnarayana, S. Ramanujam and G. Kameswara Rao (*J. Fd Sci. Technol.*, 1966, **3**, 59–61).—Storage studies under ambient and accelerated storage conditions on raw, fried and fried-spiced-and-salted cashewnuts in flexible packs are reported. Cellophane–polyethylene laminate did not provide enough protection under accelerated storage conditions, while raw and heat-treated cashewnuts in paper–foil–polyethylene laminate remained in satisfactory condition for at least 6 months. No significant difference was found for nuts packaged under N_2.
S. A. BROOKS.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Dependence on protein quality of the protein to calorie ratio in a freely selected diet and the usefulness of giving protein and calories separately in protein evaluation experiments. G. Pol and C. den Hartog (*Br. J. Nutr.*, 1966, **20**, 649–661).—Conc. protein diets (60%) prepared with potato protein (*PP*) and wheat gluten (*WG*) were fed at two levels separately to six groups of rats. Relationship between body wt. and total calorie intake was confirmed and the regression equation was calculated. The rat possesses a regulating mechanism controlling the intake of non-protein calories needed for any limited or free protein intake. The intake of non-protein calories is completely determined by N-retention and hence by quality and quantity of protein, this holding true for any dietary protein level. On a high quality *PP* and a low quality *WG*, rats can reach the same optimal wt. gain when allowed to choose the calorie protein ratio spontaneously. Under these conditions, utilisation of calories for growth is also optimal and almost identical for both groups. To attain this far more *WG* is needed than *PP* whereas the no. of total calories consumed is similar. (11 references.) C.V.

Microbiological assay of protein quality with *Tetrahymena pyriformis* W. IV. Measurement of available lysine, methionine, arginine and histidine. J. A. Stott and H. Smith (*Br. J. Nutr.*, 1966, **20**, 663–673).—The values found were compared with those reported by other workers using the protozoan and also with the results obtained using fluorodinitrobenzene over a range of concn. *Tetrahymena* assays of available lysine (**I**) in groundnut meals (**II**) and soya-bean meals (**III**) showed little variation between samples of one type of oilseed meal and the values obtained suggest that modern commercial processing has little or no effect on **I** availability in **II** and **III**. There was considerable variation between the available **I** content of cottonseed meals this possibly being due to the binding of **I** by the gossypol present in these meals. There was a considerable variation in the available **I** and methionine content of different samples of the same type of cereal. (20 references.)
C.V.

Interaction of proteins during gel electrophoresis. J. A. D. Ewart (*J. Sci. Fd Agric.*, 1966, **17**, 526–532).—Applications of starch-gel electrophoresis to the study of wheat-proteins are discussed particularly in regard to the detection of protein–protein interactions (A+B⇌C type). Tailing, extra bands and mobility changes may appear with the milder type of interaction. No definite evidence was obtained for protein–protein interactions among the slow-moving gliadins of varieties Bison and Capelle and the total no. of bands probably represents the min. no. of mol. species present. A wheat protein concn. needs to be $> \sim 0{\cdot}1$ mg/ml to be observable on a starch gel. (16 references.) E.M.J.

Spectrophotometric determination of vitamin D in freshwater fish liver oils. R. K. Barua and M. V. K. Rao (*Analyst*, 1966, **91**, 567–570).—In the method described, interference by vitamins A_1 and A_2 is eliminated by conversion of these into their anhydro-products by saponification and then separating them from vitamin D by chromatography on a 9-cm column of Al_2O_3 (8% H_2O) using light petroleum for development. The two anhydro-vitamins flow into the first 75 ml of eluate, while all the vitamin D remains in the colourless band of the column. This band is extended and eluted with ethyl ether for determination of vitamin D at 500 mμ by the Zimmerli-Nield-Russel $SbCl_3$ method. Recoveries are usually <90%. W. J. BAKER.

Assessment of relative nutritive value of proteins using *Streptococcus zymogenes*. J. Saunders and M. H. McFadyen (*Chemy Ind.*, 1968, 56).—To make the R.N.V. scale (35–100) used in the original method (*Idem., Nature, Lond.*, 1964, **202**, 4932) comparative with other methods of evaluating proteins in foodstuffs it is proposed that this scale be extended to the range 0–100 by introducing the term R.N.V. equivalent (R.N.V.E.). The value of R.N.V.E. would be 1·54 R.N.V.–54; results for many foods of varying protein content and quality show that correlation between R.N.V. (using *S. zymogenes* whole egg = 100) and N.P.U. (net protein utilisation) is given by N.P.U. = 2·19 R.N.V.–96.
W. J. BAKER

Rendering effluent harmless by improved utilisation of raw materials in the production of potato starch. II. J. Malcher, St. Zelenka, A. Břečka and K. Medal (*Stärke*, 1966, **18**, 390–392).—Superheated steam and an immersion gas heater have been used to coagulate high mol. wt. N compounds from potato water. After removal of the sludge by centrifugation, the supernatant and solid were analysed. There was no change in pH, little destruction of components by the elevated temp. involved and no evidence for the formation of toxic gas combustion products. Coagulable substances were low in amino-acids and considered most suitable for fodder. The supernatant contains most of the amino-acid and trace materials which make it useful as a fermentation medium.
J. B. WOOF.

Neutralised proteinates. Griffith Laboratories Ltd. (B.P. 1,050,867, 4.3.65. U.S., 5.3.64).—Water-sol. alkali, e.g., NaOH or $Ca(OH)_2$, is reacted with a solid form of hydrophilic protein (casein) in presence of sufficient free water to provide during and after the reaction a moist, crumbly to free-flowing mass and effect reaction of all the alkali with the protein prior to decomposition of the latter. The reacted mass is subjected to mechanical pressure, then dried below the denaturing temp. and, if desired, is finally denatured. The proteins are used as food supplements and emulsifiers. J. M. JACOBS.

Foodstuff manufacture. Irish Sugar Co. Ltd. and W. J. S. Peschardt (Inventors: W. J. S. Peschardt, L. M.-N. O'Concubhair and D. J. Conlon) (B.P. 1,057,567, 12.8.64).—The foodstuff, containing protein in a starch base, is obtained by mixing the protein and base with water to form a stiff paste, gelatinising the starch and homogenising the protein–starch combination by pressure cooking, air-drying the resulting mass until it is solid but flexible and then cutting into pieces and air-drying to bring the moisture content to 8–12% by wt. Thus, a foodstuff containing, in pt. by wt., 78 wheat starch, 30 wheat flour, 6 protein (prawn powder), 6 NaCl, 0·5 pepper and 1·0 onion powder, is processed to slices of e.g. 2·5 cm dia. and 3–4 mm thickness.
S. D. HUGGINS.

Unclassified

Mycotoxin problems in the United Kingdom and the tropics. W. D. Raymond (*Fd Technol.*, 1966, **20**, No. 7, 54–60).—A review of the development of methods for the assay of aflatoxin in foods and feeding stuffs and research on the incidence of aflatoxin and possible occurrence of other mycotoxins. The results of two international collaborative studies of the assay of aflatoxin-containing meals are reported, and the need for development of standard procedures for the assessment of mould contamination is emphasised. (31 references.) E. C. APLING.

Effect of freezing on standard plate and coliform counts of soft-serve ice-cream mixes. J. Foley and J. J. Shewing (*Ir. J. agric. Res.*, 1965, **4**, 215–221).—In soft-serve mixes standard plate counts showed initial increases during the pre-crystn. period in a conventional soft-serve freezer; ice crystal formation was accompanied by reduced counts. Destruction of organisms was most marked during the first 10–15 min. of freezing but continued slowly for 4–5 h. On freezing soft mixes inoculated with *E. coli* (approx. 1×10^6 cells per g), *coli* counts decreased considerably.
A. G. POLLARD.

Latest developments in research on botulism. E. M. Foster and H. Sugiyama (*J. Milk Fd Technol.*, 1966, **29**, 342–347).—A general review of the literature. (40 references.) C.V.

Examination of faeces from food handlers for salmonellae, shigellae, enteropathogenic *Escherichia coli* and *Clostridum perfergens*.

H. E. Hall and G. H. Hauser (*Appl. Microbiol.* 1966, **14,** 928–933).—A total of 219 specimens was examined by two laboratories. None showed the presence of salmonellae or shigellae. *C. perfergens* (**I**) was found in 171 (78·1%), *E. coli* 178 (79·9%) and enteropathogenic *E. coli* 14 (6·4%). The occurrence of haemolytic and non-haemolytic strains of **I** is also discussed. (15 references.) C.V.

Hygienic and analytical aspects of the presence of fluorine in foods. J. Zábranský and A. Rippel (*Prům. potravin*, 1966, **17,** 251–253).—General data on the presence of F^- in foods and food materials are reviewed. The various determination methods are discussed, and the preferred colorimetric methods with Zr-alizarine and Zr-eriochromcyanine R are described. The influence of some accompanying ions, such as Al^{3+}, PO_4^{3-}, SO_4^{2-}, can be eliminated by a suitable adjustment of pH, and in view of the temp. sensitivity room temp. of 18–20° are recommended. The range of the colorimetric determination varies within 0–100 μg F^- in the measured solution quantity. (64 references.) J.S.B.

Problems and methods in food analysis. K. G. Berger (*Dt. Apoth. Ztg*, 1967, **107,** 215–220).—The general methodology and problems encountered in chemical analysis of foods is briefly discussed, with particular reference to the determination of the constituents of confectionery, egg products, the enzymic differentiation between fresh and frozen meats, the amino-acid constituents of wine, and the inclusion of dyes and preservatives. The effect of radioactivity upon foodstuffs is discussed, and the content of certain radioactive species in washed and unwashed salads is compared, as well as the radioactive constituents in fresh milk. G. R. WHALLEY.

Prevention of non-enzymic browning. D. L. Ingles (*C.S.I.R.O. Fd Preserv. Q.*, 1966, **26,** 39–44).—A review dealing with the chemical mechanisms of inhibition of browning by SO_2. (17 references). P. S. ARUP.

Disulphide bridges and soluble tryptic peptides of calf rennin. B. Foltmann and B. S. Hartley (*Biochem. J.*, 1967, **104,** 1064–1074).—The cysteic acid peptides from various digests of calf rennin are purified by diagonal paper electrophoresis. The amino-acid sequences of these peptides account for 38 amino-acids around the three unique disulphide bridges in the protein. One bridge connects two acidic regions of the chain, one forms a loop of five residues, and the other a loop of six residues. These bridges are homologous with those of pig pepsin. Tryptic peptides from the C-terminus of rennin account for 22 residues, 18 of which are homologous with the C-terminus of pepsin. Sequences that account for 94 of the 270 residues in rennin are identified and the degree of homology with pepsin is ~70%. J. N. ASHLEY.

Microbial oxidation of methanol. Purification and properties of alcohol dehydrogenase of *Pseudomonas* sp. M27. C. Anthony and L. J. Zatman (*Biochem. J.*, 1967, **104,** 953–959).—Purification of the nicotinamide nucleotide-independent enzyme is described. The purified dehydrogenase shows a single main component of mol. wt. 146,000 in the ultracentrifuge. Electrophoresis in polyacrylamide gels between pH 5·0 and 9·55 reveals only one protein band; the isoelectric point is between pH 7 and 8. The enzyme is not a flavoprotein and it contains no significant metal content. An unusually small no. of cysteine/cystine residues per mol. are present, together with ~4·1% of glucoamine. NH_3 or NH_2Me is required as an activator. J. N. ASHLEY.

Microbial oxidation of methanol. Prosthetic group of alcohol dehydrogenase of *Pseudomonas* sp. M27; new oxidoreductase prosthetic group. C. Anthony and L. J. Zatman (*Biochem. J.*, 1967, **104,** 960–969).—The purified dehydrogenase has absorption peaks at 280 and 350 mμ, and little or no absorption at or above 450 mμ. The enzyme does not fluoresce, but green fluorescent material that diffuses on dialysis is formed when the solution of the dehydrogenase is boiled or treated with acid or alkali. This green fluorescent material is partially purified; it is probably the prosthetic group and may be a pteridine deriv. J. N. ASHLEY.

Relationship of 4-hydroxybenzoic acid to lysine and methionine formation in *Escherichia coli*. R. G. W. Jones and J. Lascelles (*Biochem. J.*, 1967, **103,** 709–713).—A multiple aromatic mutant, *Escherichia coli* 156 : 53D2 requires *p*-hydroxybenzoic acid for rapid aerobic growth on several sources of C, but in absence of this acid aerobic growth is stimulated by a mixture of lysine and methionine, and by succinate. The effect of the amino-acids is attributed to sparing of succinyl CoA. Organisms grown aerobically in absence of *p*-hydroxybenzoic acid have low activities of both α-oxoglutarate dehydrogenase and fumarate reductase, with consequent impairment of both mechanisms known for formation of succinate. Low fumarate reductase activity is due to repression of enzyme synthesis by aeration and not to inactivation. Lactate and ethanol dehydrogenases are induced, which indicates other routes of NADH oxidation when electron transfer to O is impaired. Other Kreb's cycle enzymes are influenced little by deficiency of *p*-hydroxybenzoic acid, but anaerobiosis causes a fall in activity. J. N. ASHLEY.

Spectrophotofluorometric determination of Buquinolate in poultry tissue and eggs. P. L. Cox, R. D. Hollifield and J. P. Heotis (*Poultry Sci.*, 1967, **46,** 680–686).—The method is sensitive to 0·1 ppm Buquinolate (**I**) in poultry tissue and eggs. After feeding birds for periods ranging from 10 weeks to 18 months with 100 g of **I** per ton of feed there was <0·1 to 0·39 ppm **I** in liver, kidney, fat and skin and less than 0·1 ppm in muscle. All tissues contained less than 0·1 ppm of the drug 1–2 days after withdrawal. Eggs from layers supplied with 100 g of **I** per ton of feed for 18 months contained 0·11 to 0·23 ppm of the drug. A. H. CORNFIELD.

Gas–liquid chromatographic analysis of piperazine as diacetylpiperazine in animal feed. F. L. Fricke and S. M. Walters (*J. Ass. off. analyt. Chem.*, 1966, **49,** 1230–1232).—Piperazine in a filtered aq. extract of the feed is mixed with Celite and Na_2CO_3 and the column is eluted with $CHCl_3$ after addition of acetic anhydride. The gas chromatographic column is packed with 7·5% OF-1 on Gas Chrom Q. and detection is by argon ionisation or hydrogen flame. Phenothiazine is used as internal standard. Recoveries of piperazine adipate were 97·6 to 100·2%. A. A. ELDRIDGE.

Determination of antimony, cadmium, cerium, indium, and silver in biological material by radioactivation. H. J. M. Bowen (*Analyst, Lond.*, 1967, **92,** 118–123).—The samples and standards are ashed at 450° in SiO_2-crucibles in a SiO_2-lined furnace and are then irradiated for 28 days in a flux of thermal neutrons to form five long-lived radionuclides. These are then separated by an 18-stage radiochemical procedure to minimise contamination from ^{32}P, etc. The ppt are weighed for chemical yields (from 93% for Cd to 57% for Ir) and their activities determined by β- or γ-counting. Results for standard kale powder (Sb 0·09, Cd 0·35, Ce 0·38, Ir 0·01, Ag 0·03 ppm) are regarded as satisfactory for Ag and Sb, but less so for Cd, Ir and Ce. W. J. BAKER.

Determination of calcium in biological samples by *X*-ray fluorescence. K. P. Champion and R. N. Whittem (*Analyst, Lond.*, 1967, **92,** 112–114).—The sample (*e.g.* ash of milk, oyster-shell, flesh) is digested in hot conc. HNO_3, the solution is taken to dryness and the Ca in the aq. extract of the residue is determined in an *X*-ray fluorescence spectrometer using the CaK_a line at 3·36Å. Results agree with those obtained by the usual chemical methods; sample prep. takes ~4 h for 10–20 samples and instrument time is ~2 min. per sample. W. J. BAKER.

Increasing the water solubility of 3,4-benzpyrene by addition of 1,3,7-trimethylxanthin (caffein). II. Effects of adding organic acids to the caffein solution. J. Eisenbrand and G. Becker (*Dt. Lebensmitt Rdsch.*, 1967, **63,** 342–343).—The addition of caffein has been shown to increase the solubility of benzpyrene (**I**) but this is limited by the solubility of the caffein (2%). By adding 17·6% ascorbic acid, the level of caffein can be raised to 7·76% and in this solution up to 440 mg/l of **I** can be dissolved. Citric and tartaric acids may also be used. The additives do not, in absence of caffein, significantly affect the solubility of **I**. J. B. WOOF.

Definition of disease in food regulations. H. Demme (*Dt. Lebensmitt Rdsch.*, 1967, **63,** 328–331).—A discussion of the interpretation of the terms disease and infection in the German food regulations. (20 references.) J. B. WOOF.

3.—SANITATION, WATER, etc.

Water wastes and sewage

First biological evaluation of the efficiency of a single-stage sewage fermentation plant. M. Gillar and P. Marvan (*Prům. potravin*, 1966, **17,** 328–335).—The single-stage fermentation is a relatively new method utilisable for purification of dairy sewage. The results of the first biological evaluation of the purification process observed at two sewage purification plants are described and evaluated. The quant. biological analyses of the sewage and of fermented water portions were made by microscopical inspection of the residues of centrifuging in count chambers. According to the species of organisms present in major proportions, the fermented aq. sewage could be differentiated into that with yeast cells, and that with flagellates, occurring according to the operational conditions of the purification plant. J.S.B.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

MARCH, 1968

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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