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THE OXIDATION OF CYSTEINE, GLUTATHIONE AND THIOGLYCOLLATE BY IODATE, BROMATE, PERSULPHATE AND AIR

By F. J. R. HIRD and J. R. YATES

The oxidation of cysteine, glutathione and thioglycollate by bromate, iodate, persulphate and air has been studied at 28° and at near-neutral pH values. The oxidations have been followed by a combination of methods—by reaction with *p*-chloromercuribenzoate (spectrophotometric), methylmercuric iodide (polarographic) and, in the case of cysteine, chromatographically and manometrically.

The oxidation is rapid by iodate, slow with bromate and persulphate and slower still with air. The major product in the case of cysteine is the disulphide cystine, but up to 10% of the higher oxidation products, cysteinesulphinic and cysteinesulphonic acids, are formed.

The oxidation of thiol groups is discussed in relationship with the action of the improvers used in the bread industry.

Introduction

This investigation was made as the first part of an attempt to define in chemical terms the underlying differences responsible for the varying baking qualities of different wheaten flours.

An important contribution towards the final baking quality of a flour is known to be made by the protein fraction but there is little precise chemical knowledge about the importance of this fraction in determining differences in quality. The chemical evidence available concerns the action of the commercial improvers which, with some exceptions, are oxidising agents. These substances are known to bring about rheological changes in doughs that are consistent with oxidation of thiol groups.[1, 2]

It has commonly been assumed that the action of improvers is to oxidise thiol (labile) groups to produce a three-dimensional protein network of disulphide (non-labile) bonds.[1, 2] Evidence for the oxidation of thiol groups of flour by improvers has been presented[3, 4] and is consistent with this view.

Information concerning well-defined chemical oxidation of thiol groups, in aqueous medium, at about neutral pH values is meagre. Early work by Ziegler[5] showed that bromate and persulphate brought about the disappearance of thiol groups of glutathione and Holme & Spencer[6] later confirmed the oxidation by persulphate.

To obtain more precise information an investigation has been made of the action of certain appropriate oxidising agents on some simple thiols at concentrations, pH values and a temperature appropriate to dough preparation.

Experimental

Materials

Glutathione, cystine and cysteine hydrochloride were obtained from Hoffman-La Roche (cysteine hydrochloride was adjusted to the appropriate pH before use) and cysteinesulphinic acid from Mann Research Laboratories. Cystine disulphoxide was a gift from Dr. J. McLaren. *p*-Chloromercuribenzoate was a commercial sample recrystallised according to the method suggested by Boyer.[7] Methylmercuric iodide was prepared by the method of Maynard.[8]

Estimation of thiol groups

The method of Boyer[7] for measuring thiol groups was used to follow their disappearance after exposure to the oxidising agents used.

Procedure

Spectrophotometric method.—The oxidations were carried out at 28° under commercial oxygen-free ' nitrogen in the following system: 14 ml. of 0·1M-phosphate buffer containing oxidising agent of the required concentration and 1 ml. of 10^{-2}M-thiol compound, so that the

initial concentration of thiol compound in the system was $6{\cdot}67 \times 10^{-4}$M. The concentration of oxidising agent in the system was varied to give a molar ratio of thiol/oxidising agent of 12, 6, 3 and 1. After the appropriate reaction time, 1·5 ml. of this solution was pipetted into a solution containing 5 ml. of 5×10^{-4}M-*p*-chloromercuribenzoate, 5 ml. of 0·1M-phosphate buffer and 8·5 ml. of water. The change in optical density was read against a *p*-chloromercuribenzoate blank with water in place of reactants.

Glass-distilled water was used throughout to prepare reagents to reduce as far as possible oxidation catalysed by metallic ions. To allow for optical absorption by products of the reaction or of the oxidising agent itself, a blank was carried out in which the *p*-chloromercuribenzoate was replaced by buffer, and measured against a buffer blank. The optical densities obtained have been corrected for this value. In the case of iodate the iodide formed increases the difference in optical density and a correction has therefore to be applied.

Chromatography

Phenol saturated with water was used to follow the appearance of oxidation products, collidine saturated with water for the disappearance of cysteine and appearance of products, and n-propanol/water (80 : 20 v/v) for the oxidation of glutathione. After being dried, the chromatograms were sprayed in the usual way with 0·2% ninhydrin in water-saturated butanol. Residual cysteine and glutathione were reacted with an excess of *N*-ethylmaliemide to overcome oxidation during the chromatographic runs (Hanes *et al.*[9]). The concentration of cysteine and glutathione used was 30 mM. The oxidising agents were added at a range of concentrations (2·5–100 mM) and the pH of the solution was 6·0, established with phosphate buffer. Preliminary experiments showed that a standard reaction time of 1 h. was satisfactory. After this time 0·5 ml. of the oxidation system was removed and added to 0·15 ml. of *N*-ethylmaleimide solution (0·1 M) and 1–2 μl. of the solution applied to the Whatman No. 1 paper with a micropipette.

Polarography

Polarography was used as an independent method to follow the disappearance of thiol groups and, in the case of cysteine and glutathione, to identify the products of oxidation. Methylmercuric iodide, recommended by Leach[10] as a rapidly reacting monofunctional reagent with suitable current–voltage curves for quantitative analysis, was used as the thiol reagent. The first curve with a half-wave potential of −0·46 and −0·55 V in absence and presence of sulphite respectively has been found suitable to estimate thiol groups. The measurements were made on a Metrohm Polarecord Type E with ammonia–ammonium chloride buffer (0·5M) at pH 9·0. The reference electrode was silver/silver chloride.

Cystine was measured as cysteine after reduction with sulphite as recommended by Leach.[11] Cysteine was identified as a reduction product by a fall in the concentration of added methylmercuric iodide and by the appearance of the characteristic current–voltage curve for the cysteine mercaptide (half-wave potential at −0·85 V).

(1) *Oxidation of cysteine and glutathione.*—The oxidising system was the same as described above for the spectrophotometric method at pH 6·0. After the appropriate time interval, 5 ml. of the reaction system were added to 10 ml. of methylmercuric iodide (5×10^{-4}M), 5 ml. of NH_3/NH_4Cl buffer (0·5M, pH 9·0) and 0·4 ml. of gelatine (1·0%). The percentage oxidation was calculated from the amount of methylmercuric iodide consumed by the residual thiol groups.

(2) *Estimation of disulphide formed.*—After the appropriate reaction time 5 ml. of the system were added to 5 ml. of the NH_3/NH_4Cl buffer containing sodium sulphite (0·2M). The rest of the procedure was as before. The amount of cystine sulphur present was calculated from the difference in wave heights in the presence and absence of sulphite ; allowance being made for the difference in wave heights of methylmercuric iodide in the sulphite system and for the appearance of one thiol group per disulphide reduced.

(3) *Identification of the solid oxidation product obtained in the chromatographic system.*—In order to investigate the precipitate formed in the oxidising systems used, a quantity was prepared by carrying out the reaction in the following system. Cysteine hydrochloride (30 mM) was oxidised with iodate, bromate and persulphate (final concentration 100 mM) in 41·6 ml.

of phosphate buffer (0·5M) at pH 6·0. After a reaction period of 1 h. the precipitate was filtered off, washed with water and dried with ethanol and ether. The white powder so prepared was then compared in the polarograph with an equal weight of cystine made up to give a final concentration of 2×10^{-4}M. The solids were dissolved in 15 ml. of methylmercuric iodide (5×10^{-4}M), 5 ml. of sodium sulphite (0·5M) and 5 ml. of NH_3/NH_4Cl buffer (0·2M) at pH 9·0 by vigorous shaking for a few minutes. The disappearance of methylmercuric iodide and the appearance of its mercaptide with cysteine was then followed polarographically. It was found necessary to add methylmercuric iodide at the same time as the sulphite. If this were not done, there was a substantial disappearance of thiol groups at this alkaline pH, depending on the time interval involved.

Manometry.—Acidic groups formed during the oxidations were followed manometrically by liberation of carbon dioxide from a bicarbonate buffer as described by Umbreit *et al.*[12] The system used was as follows:

Main compartment $NaHCO_3$ solution (18·2 mM) 2 ml., cysteine solution (30 mM) 1 ml. and water 3 ml.

Side arm $NaHCO_3$ solution (6·07 mM) 0·3 ml. containing oxidising agent 100 mM.

The reaction was followed in large rectangular flasks at 28° with a gas phase of 95% N_2, 5% CO_2. The pH of the flask contents was calculated to be 6·0. The release of carbon dioxide on addition of acid was found to be linear over the range used to follow the oxidations.

Results

Effect of various oxidising agents on thiol groups

Figs. 1–3 show the rate of reaction of cysteine and glutathione with the various oxidising agents. Both thiol compounds react similarly and immediately after addition of iodate at a thiol/iodate ratio of 3 : 1, both are completely oxidised. The negative nitroprusside test which is obtained immediately after addition of iodate to cysteine provides further evidence for the rapidity of the reaction. Persulphate and bromate oxidise the thio compounds less rapidly, while chlorate has no effect under these reaction conditions.

Oxidation by atmospheric oxygen proceeds steadily with time (Table I), glutathione having the slowest rate of oxidation at pH 6 but the fastest at pH 8, but cupric ions markedly affect the rate of oxidation by air, the reaction being complete in 15 min. at 10^{-4} Cu^{2+}. Thus the % oxidation of $6{\cdot}67 \times 10^{-4}$M-cysteine in air at pH 6·0 and 38° in 1 h. was 14 with no Cu^{2+}; 10^{-7}M-Cu^{2+} 16; 10^{-6}M-Cu^{2+} 31; 10^{-5}M-Cu^{2+} 71; and 10^{-4}M-Cu^{2+} 100.

Rate of oxidation in relation to concentration of oxidising agent

Fig. 1 shows that at all the *iodate* concentrations used, oxidation of both cysteine and glutathione is very rapid and is complete with a molar ratio thiol/oxidising agent between 3 : 1 and 6 : 1—figures in agreement with the more accurate figure of 4 : 1 obtained polarographically.

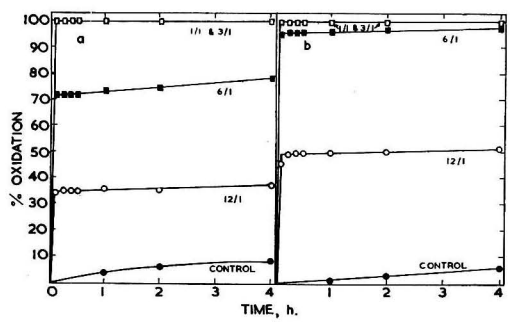

FIG. 1.—*Oxidation of (a) cysteine and (b) glutathione by different concentrations of iodate with time at pH 6·0 and 28°*

Concentration of thiol compound $6{\cdot}67 \times 10^{-4}$M; molar ratios thiol/iodate as indicated. For other conditions see text

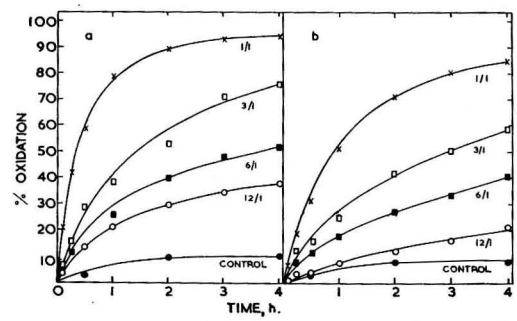

FIG. 2.—*Oxidation of cysteine (a) and glutathione (b) by different concentrations of bromate*
Conditions as for Fig. 1; molar ratios thiol/bromate as indicated

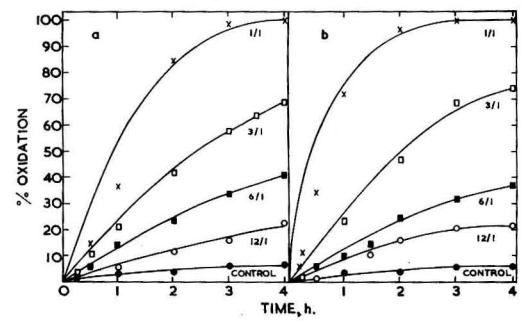

FIG. 3.—*Oxidation of cysteine (a) and glutathione (b) by different concentrations of persulphate*
Conditions as for Fig. 1; molar ratios thiol/persulphate as indicated

The oxidation of glutathione appears to be more complete with the lower concentration of oxidising agent. The rate curves for *bromate* (Fig. 2) and *persulphate* (Fig. 3) are very similar, the extent of oxidation increasing with concentration of oxidising agent, but persulphate oxidises cysteine more slowly and glutathione more rapidly than does bromate.

Thioglycollate behaves similarly to the other thiol compounds with these oxidising agents.

In view of the fact that the relative rates of oxidation of cysteine and glutathione by each oxidising agent differ, it is possible that the various thiol groups of flour-proteins may also exhibit different susceptibilities to oxidation.

Effect of pH on the oxidation of thiol groups

Oxidation of cysteine and glutathione by iodate and bromate was determined at pH 6, 7 and 8 but there was no significant difference in rates of oxidation at the concentrations of oxidising agent used, i.e., molar ratios for thiol/oxidising agent of 3 for bromate and 1 for iodate.

The differences in rates of oxidation by air at different pH values (Table I) cannot be

Table I

% Oxidation of thioglycollate, cysteine and glutathione in air
Concn. of thiol compound $6{\cdot}67 \times 10^{-4}$M; temperature 28°

Time	Thioglycollate		Cysteine		Glutathione	
	pH 6·0	pH 6·0	pH 6·0	pH 8·0	pH 6·0	pH 8·0
30 min.	11	1·5	13	3·0	1·6	7·0
1 h.	23	3	24	10	3·0	16
2 h.	40	8	47	22	6·1	37
3 h.	69	12	70	32	13·0	55

attributed to pH alone as the change in composition of the buffer components with the concomitant change of metallic ion impurities is probably a major factor in determining the rate of oxidation. In addition the impurities in the samples of thiols can also be a factor (Anson[13]).

Oxidation of thiol groups followed polarographically

The results by this method for iodate and bromate acting on cysteine and glutathione were identical with those obtained by the spectrophotometric method. As the ratio of cysteine to iodate was lowered from 7 : 1 to 2 : 1, thiol groups disappeared (as shown by the full recovery of methylmercuric iodide) when the ratio 4 : 1 was reached and thereafter remained constant.

The oxidation of cysteine to cystine by the iodate ion :

$$6CySH + IO_3^- \rightarrow 3CySSCy + 3H_2O + I^-$$

would give a value of 6 at the equivalence point. The experimental figure of 4 is consistent with the presence of the higher oxidation products cysteinesulphinic acid and cysteinesulphonic acid found on chromatograms of reaction products.

Oxidation of cysteine and glutathione followed chromatographically: appearance of products of oxidation

In addition to cystine the chromatograms showed two other distinct products with R_F values less than that of cystine. The slower-running of these compounds was identified as cysteinesulphonic acid and the faster as cysteinesulphinic acid by running authentic samples with the unknown. These two oxidation products after reaction with ninhydrin also give colours similar to those with authentic compounds.

Cystine disulphoxide, another possible oxidation product, would not be detected by chromatography as it is known to decompose on chromatograms to give cystine and cysteinesulphinic acid. Cystine disulphoxide was not found to be reduced by sodium sulphite to produce a thiol compound. The solid oxidation product obtained experimentally thus behaved differently. However, the results do not exclude the possible formation of cystine disulphoxide as an intermediate product of oxidation.

It has also been observed that iodate, bromate and persulphate will oxidise cysteinesulphinic acid to cysteinesulphonic acid. In the presence of excess oxidising agent therefore the composition of the mixture will change even on the chromatogram. Nevertheless in the case of iodate and bromate the chromatograms show that cysteinesulphinic acid exceeds cysteinesulphonic acid in amount at all concentrations of oxidising agents in the reaction time used.

Oxidised glutathione has been shown to be a product of the oxidation of glutathione by iodate, bromate and persulphate.

The insoluble product resulting from the oxidation of cysteine by iodate, bromate and persulphate has also been investigated polarographically in the presence of sulphite and under these conditions has been shown to combine with the same amount of methylmercuric iodide as does cystine.

Microanalyses of this material gave the following percentages of oxygen : oxidation product with iodate 26·7% ; with bromate 28·0% ; with persulphate 26·8%, while the theoretical value for cystine is 26·7%.

It is concluded that the major product of oxidation from cysteine is the disulphide, cystine.

Production of acid groups in the oxidation of cysteine and glutathione by oxidising agents

Fig. 4 shows that the acid groups produced by iodate and bromate amounted to approximately 10% of the initial cysteine. With these two oxidising agents the results for acid production are in agreement with those from chromatography. Further the rate of acid production by these compounds resembles the overall rate of oxidation followed spectrophotometrically and polarographically, i.e., iodate reacting most rapidly.

In the case of persulphate there are only traces of cysteinesulphinic acid and small amounts of cysteinesulphonic acid on chromatograms. However, persulphate is a very vigorous acid

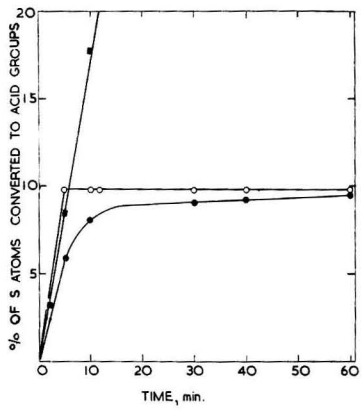

FIG. 4.—*Oxidation of cysteine by* (○——○) *iodate,* (●——●) *bromate and* (■——■) *persulphate to show percentage conversion to acid groups*

Concentration of cysteine and oxidising agents $4{\cdot}76 \times 10^{-3}$M, pH 6·0 28°. For manometric conditions see text

producer when the reaction is followed manometrically (Fig. 4) and the reaction proceeds steadily with time. Cystine under the same conditions is not oxidised significantly over a 2-h. period. There is thus no agreement between the chromatographic and manometric evidence as regards $-SO_2H$ and $-SO_3H$ group as products of oxidation. This has not been investigated further, but it may be relevant that persulphate produces two ninhydrin-reacting substances that are not produced by either iodate or bromate.

Discussion

The present work shows that the thiol group of cysteine, glutathione and thioglycollate is oxidised by iodate, bromate, persulphate and air but not by chlorate. The results thus confirm earlier work with bromate and chlorate[5] and persulphate[5, 6] but cannot be compared quantitatively because of different experimental conditions.

The rate of oxidation by the various oxidising agents tested is in agreement with the generally held rates of action as improvers, i.e., rapid with iodate, slower with bromate and persulphate, slower still with air and negligible with chlorate.[14, 15] It is of interest that the action of cupric ion as an improver is also correlated with its catalytic rôle in oxidising thiol groups.

The product by chemical oxidation, in the case of cysteine, has been shown to be the disulphide cystine—in 90% yield. With iodate and bromate there is evidence of the higher oxidation products, cysteinesulphinic acid and cysteinesulphonic acid, with a combined total of about 10% of the reaction products. It is of interest in this respect that Moran *et al.*[16] have obtained cysteinesulphonic acid from hydrolysates of extracts of flour treated with chlorine dioxide. In the case of glutathione a major product of oxidation is the disulphide, but due to lack of authentic compounds to use as markers, the range of products has not been investigated in the same detail as has that for cysteine.

The thiol groups are oxidised with molar ratios of thiol/oxidising agent as high as 12 : 1 at absolute concentrations of oxidising agent of $5{\cdot}5 \times 10^{-5}$M, which are of a similar order as those effective as improvers in the baking industry.

The presence of thiol groups in flours or flour proteins has been shown by a variety of methods[3, 4, 6, 17–21] and there is evidence for the disappearance of thiol groups in flour on the addition of improvers[3, 4] and on mixing in oxygen.[17] Experiments, to be reported later, on gluten reduced by sodium borohydride and on thiolated gelatin, show that the thiol groups on these proteins are oxidised by iodate, bromate and persulphate.

There is thus a good correlation between the oxidation of thiol groups and the action of improvers. In this connexion also the well-known oxidation of thiol groups by oxygen confirmed in present work is also consistent with its action as an improver. Cunningham & Hlynka[22]

have further shown that oxygen and bromate when acting as improvers act on a common site. From the evidence presented in the present paper it is likely that thiol groups would be such a site.

No attempt has been made here to determine the reaction mechanism for the oxidation of cysteine, but the presence of cysteinesulphinic acid and cysteinesulphonic acid as well as cystine suggest that there may be competing reactions perhaps with a reactive intermediate (cysteinesulphenic acid ?). The subsequent course of the reactions may vary depending on the concentration, availability or accessibility of other groups reacting with this intermediate, e.g., thiol groups and oxidising agent. Thus the course of the reaction may be considerably different in the case of accessible thiols of low molecular weight such as cysteine and glutathione, than in the case of perhaps less accessible thiols associated with proteins, i.e., the proportions of -S-S-, and $-SO_2H$ and $-SO_3H$ may be different in the case of protein. It is therefore conceivable that improvers which are oxidising agents may act in several ways to produce rheological changes in doughs undergoing mixing or resting :

(1) Thiol groups on adjacent protein molecules may be oxidised to produce an intermolecular disulphide bond, so increasing the strength of dough.

(2) Thiol groups on the same molecule may be oxidised to produce an intramolecular disulphide bond.

(3) Thiol groups may be oxidised to the sulphinic or the sulphonic acid and so prevent the possible formation of intermolecular disulphide bond by oxidation or by disulphide–thiol exchange. Oxidation to the disulphide stage both intra- and intermolecular would also reduce the concentration of thiol groups necessary for disulphide–thiol exchange.

Evidence for the existence of such interchanges in dough has recently been obtained by Mecham,[23] Goldstein[24] and Bloksma[25] and is the subject of a communication, from this laboratory, submitted elsewhere.

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University of Melbourne
Victoria
Australia

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FUMIGATION OF AGRICULTURAL PRODUCTS. XVII.*—Control of *Ascochyta* Blight of Peas by Fumigation

By JULIET KENNEDY

Ethylene oxide, methyl bromide, mercury and organo-mercury compounds were ineffective as fumigants for the purpose. Chloropicrin proved more satisfactory, fumigation at saturation concentrations killing the three pathogens, when diseased Zelka seed with a moisture content of 16% was exposed for 72 h. at 20°. This unusually long treatment was necessitated by slowness in penetration. Zelka showed little fumigation damage in the laboratory or in the field. A ' Blue ' variety showed little damage in field trials, in which the emergence of Feltham Advance was reduced by 29%. All seeds were so heavily infected that some interaction between fungal and fumigation damage was anticipated. A commercial sample of the variety Onward was very susceptible to chloropicrin. In all experiments the vigour of plants grown from fumigated seeds was normal. Cultures of the pathogens were killed by dilute aqueous solutions of chloropicrin, the responses of the three species being markedly different.

Introduction

Ascochyta blight of peas, a disease known for the past 100 years, is caused by *Ascochyta pisi*, *Ascochyta pinodella* and *Mycosphaerella pinodes*, with its perfect stage, *Ascochyta pinodes*. These three fungi form a symptom complex, one or more of them infecting a single plant. The disease is exclusively seed-borne and infected seeds may be macroscopically symptomless. During cool, wet summers, which often prevail in the pea-growing areas of Great Britain, disease lesions may appear on any parts of the plants. Damage includes a decreased stand, foot-rot and uneven maturity of the peas in infected pods ; the last is a matter of serious concern to the rapidly expanding frozen pea industry. Considerable research has as yet yielded no satisfactory control method which is commercially practicable, so considerable sums are expended each year on the importation of seed from dry, disease-free areas. A brief account of the pathogens, the symptoms produced and certain attempted control methods other than fumigation will appear elsewhere. This paper is concerned with fumigation techniques which, although in current use against a considerable number of seed-infesting insects, have not so far been used commercially in this country for the control of a seed-borne disease.

In preliminary experiments, fumigants were selected for trial on the basis either of their successful use against insect pests, or of their known fungitoxicity. Of the compounds tested, ethylene oxide was phytotoxic at fungicidal concentrations and methyl bromide affected neither the fungi nor the seeds at concentration–time products as high as 2000 mg.h./l. Fumigations in which saturation concentrations of mercury were used for periods up to 3 weeks, at temperatures of 20° and 28°, had no effect on the fungi or the seeds. Similar treatments with volatile organo-mercurials resulted in only slight surface disinfestation.

Chloropicrin is well known as an effective soil fumigant, toxic to many micro-organisms, as well as to insects and nematodes. Furthermore, in 1948 Stark[1] showed its promise for the elimination of seed-borne fungi. Accordingly, preliminary experiments were made with infected seeds, and the results were sufficiently encouraging to justify the more thorough investigations subsequently described.

Experimental

Seed samples and methods of conditioning

Infected seeds of the marrowfat variety, Zelka, were obtained for fumigation. Some of these were quite blackened by the disease ; others were apparently healthy. In most of the experiments a random sample was used, but in one, only those seeds which were free from lesions. All seeds with broken testas were discarded and a healthy commercial sample of Onward seed was also fumigated for comparison.

The seeds were adjusted to different moisture contents, either by allowing them to take up moisture from a saturated atmosphere, or by drying them in an air-stream. The values

* Part XVI : *J. Sci. Fd Agric.*, 1958, **9**, 360

obtained straddled the critical range commonly encountered in commercial samples. Before each fumigation, moisture contents were determined by the two-stage air-oven method specified in the Handbook of the Official Grain Standards of the U.S. Dept. of Agriculture.[2] Twenty-g. portions of seeds were used for the first stage of this procedure, and four 5-g. portions for the final stage. Conditioned batches of seed were stored in air-tight containers in a constant-temperature room at 20°.

Fumigation of seed

The fumigation vessel consisted of a cylindrical mild steel chamber and lid (130 l. capacity) with a vitreous enamel lining, resistant to chloropicrin. The fumigant was circulated by a fan at the side of the chamber, mounted on a brass spindle running in an air-tight bearing, and the lid was sealed with a rubber gasket. Two 12-in. lengths of 15 s.w.g. stainless steel tubing were joined by a sleeve of 12 s.w.g. tubing and fixed by a Luer attachment to a 50-c.c. burette which was fitted with an 'Exelo' tap requiring no grease. The tubing passed into the chamber through a central hole in the lid. The bung was protected with polythene sheeting to reduce sorption, and the tip of the tubing was bent so as to dip into a thin layer of absorbent cotton wool in a Pyrex dish at the front of the chamber, directly beneath the fan. Batches of 325 or 500 seeds were spread over a shallow enamel tray immediately behind this dish. The seeds were placed in the chamber at least 24 h. before each fumigation, to allow the humidity of the air in the chamber and the moisture content of the seeds to reach equilibrium. Chloropicrin (approximately 97% pure) was then introduced from the burette. In the absence of any information on the sorption of the fumigant either by the peas or by the components of the chamber, arbitrary doses of 27·5 c.c. were used in all experiments. From the results of preliminary experiments, it was believed that this dose would maintain a saturated atmosphere for several days. The theoretical amount for saturation in the absence of any sorptive material is 9·8 c.c. The temperature during and after fumigation was 20°, the seeds being aired for at least 3 h. at the same temperature before being replaced in air-tight jars.

Gas sampling and determination of chloropicrin

Feinsilver & Oberst[3] state that combustion methods for the determination of chloropicrin which rely on the volumetric determination of the chloride ion require relatively large samples for accurate results. Accordingly, their colorimetric method was at first adopted. In this method the chloropicrin, dissolved in isopropyl alcohol, is refluxed with sodium peroxide to give sodium nitrite. The nitrite ion is then determined colorimetrically with sulphanilic acid and the Bratton–Marshall coupling reagent (*N*-α-naphthylethylenediamine dihydrochloride), the colour produced being measured with a photoelectric absorption meter. A large number of experiments, using standard concentrations of chloropicrin, failed to yield consistent results, and after numerous modifications had been tried without success, the method was abandoned on account of its evident sensitivity to contaminating materials.

Next, chloropicrin was determined by a modification of the catalytic combustion method first devised by Lubatti & Harrison[4] for the determination of methyl bromide, and later improved by Lubatti & Blackith.[5] Known amounts of chloropicrin were introduced into ampoules which were sealed and placed in glass 'Turtle' chambers of the type illustrated by Lubatti.[6] The taps were covered with graphite, since chloropicrin is known to dissolve in grease. The ampoules were fractured after evacuation of the chambers, which were then connected to the decomposition apparatus. A stream of purified air was drawn through the apparatus for 1 h., at a rate of 100 c.c. per min. for the first 10 min. and 250 c.c. per min. for the remainder of the period. The combustion train consisted of two furnaces and two bubblers, containing a mixture of 30 c.c. of 0·2N-NaOH and 2 c.c. of 100-volume hydrogen peroxide. Ten c.c. of this mixture were put in the first bubbler, and 5 c.c. in the second. The chloropicrin was decomposed on the surface of electrically heated platinum spirals, and the chlorine liberated was absorbed by the bubbler solutions. These solutions were evaporated to dryness, and the residues dissolved in 17 c.c. of approximately 0·2N-H_2SO_4 and neutralised with $NaHCO_3$. The sodium chloride formed was determined volumetrically by Mohr's method, with 0·05N-silver nitrate and a carbon filament lamp to detect the end point. Since the recovery of chloropicrin by this

method was considered satisfactory (see Table I) it was used, with the modifications mentioned below, to determine concentrations in all the experiments in which infected seeds were fumigated.

The coefficient of variation, calculated from the above results, is 1·17%.

Duplicate samples of chloropicrin-laden air were taken in evacuated vessels of known volume (~75 c.c.). At first, samples were taken throughout the fumigations, in order to find the length of time required to saturate the atmosphere in the chamber. The results are shown in Table II.

Later, only concentrations at the beginning and end of the fumigations were determined. Only one bubbler, containing 8 c.c. of the mixture described above, was required to absorb the small amounts of fumigant in the samples. A purified air stream was drawn through the apparatus at a rate of 100 c.c. per min. for the first 5 min. and 250 c.c. per min. for the final 10 min. After evaporation of the solutions, the residues were dissolved in 2 c.c. of distilled water, 1 drop of phenolphthalein was added and 0·5N-H_2SO_4, until the pink colour just disappeared. The solutions thus obtained were too dilute for chloride determination by Mohr's method with 0·05N-silver nitrate, so electrometric indication of the equivalence points was adopted, using the ' bottled end point ' principle, as adapted by Wade,[7] and 0·05N-silver nitrate. A mains-operated valve voltmeter of the type described by Scroggie[8] was used to determine the potential at equivalence point. The change was about 30 mV and was unaffected by excess acid and by changes of temperature in the range 5° to 20°.

Laboratory assessment of disease, germination and emergence

At the end of every fumigation 100 fumigated and 100 unfumigated seeds were subjected to each of the treatments described below. The unfumigated control seeds were surface-sterilised by immersion in a sodium hypochlorite solution containing 2% available chlorine for at least 30 min., and then washed for 15 min. This procedure substantially reduced the number of surface contaminants, which would otherwise have made results difficult to interpret. Fumigated peas were not surface sterilised because it was feared that soaking in hypochlorite might interact with fumigation.

(1) *Disease and germination.*—Ten seeds were put in each of a number of Petri dishes of 9 cm. diameter. These contained approximately 15 c.c. of pea extract agar, acidified to pH 4·2–4·4 with lactic acid. The medium was prepared by steaming 400 g. of peas in 1 litre of water for 1 h., when the testas remained intact. The supernatant liquor was poured off, made up to 1 litre, mixed with 20 g. of agar and sterilised. The dishes were kept at 25° and the number of seeds associated with colonies of fungi and bacteria was recorded at intervals for 10 days. The number of seeds which germinated was also recorded, for comparison with the results obtained in the following test.

(2) *Emergence.*—Pyrex dishes, 9 in. in diameter and 1·5 in. deep, were filled with sand to a depth of 1 in. and sterilised at 20 lb./sq. in. for 30 min. Twenty c.c. of distilled water were added to each, then 25 seeds were sown, 0·5 in. deep. Emergence counts were made until the number of plants remained constant. Unfortunately, some of the experiments were set up during the winter when, owing to the low temperature of the greenhouse available, the dishes had to be kept in a well-lit constant-temperature room at 23°. Under these conditions the plants were etiolated, so their heights and disease symptoms could not be determined.

Table I

Recovery of chloropicrin by the catalytic combustion method

Initial dose, mg.	Recovery, %
29·9	95·8
49·4	94·0
59·0	97·1
59·1	94·8
59·5	94·4
61·0	95·1

Table II

Rise in concentration during chloropicrin fumigations

Time from start of fumigation, h.	Concentration of chloropicrin, mg./l.
0·5	58·6 : 58·9
1·0	113·7 : 119·5
2·0	129·5 : 128·9*
4·0	128·5 : 132·0*
7·0	129·5 : 128·9*
72·0	128·8 : 129·1*

* Saturation concentration

Penetration of chloropicrin into quartered seeds

Infected seeds having a moisture content of 14·2% were cut into quarters. Lots of 110 quarters were placed in ' Turtle ' chambers for 24 h. at 20°, and fumigated for 3, 6, 9, 12, 24, 48 and 72 h. at the same temperature. For each fumigation, 1·5 c.c. of chloropicrin were introduced into the chambers through straight-bore taps, by means of a 12-in. stainless steel cannula (20 s.w.g.) attached to a micrometer head from an Agla syringe. After treatment, the quartered seeds were aired for 3 h. at the same temperature. The amount of disease was then assessed as previously described. Unfumigated quartered peas formed the controls, which were surface-sterilised for 10 min. and then washed for a further 10 min.

Treatment of cultures of the pathogens with aqueous solutions of chloropicrin

Fifty-c.c. quantities of pea extract agar were introduced into 10-oz. medical flasks (capacity 300 c.c.) fitted with air-tight aluminium caps. The flats were inoculated with single pycnidia of *Ascochyta pisi*, *Ascochyta pinodella* or *Mycosphaerella pinodes* and incubated at 25° for 12 days when the fungi were well grown and abundant chlamydospores and pycnidia could be seen. From a stock solution of 2 c.c. of chloropicrin in 2 litres of distilled water, dilutions were prepared by the method of Stark,[1] the final solutions containing 500, 334, 250, 200 or 167 p.p.m. of the fumigant. This range of concentrations was chosen on the basis of results of preliminary experiments. Treatments with 25 c.c. of the solutions at each concentration, or 25 c.c. of distilled water in the case of the controls, were carried out in triplicate with each of the three pathogens. When the well-stoppered flats had been incubated at 25° for 24 h., the solutions were drained off and two 9-mm. discs were cut from each colony and placed, mycelium downwards, in Petri dishes containing pea extract agar. These were incubated at 25°, and the colony diameters measured after appropriate intervals.

Field trials

In order to substantiate the results of laboratory experiments, trials were made both at the Imperial College Field Station, Sunninghill (light, sandy loam), and at Rothamsted Experimental Station (clay with flints). A large number of seeds were sown in March and April, 1958, but dry conditions after sowing and subsequent infestation by the pea weevil (*Sitona* spp.) ruined the trials. The results of a second sowing in August, 1958, were more informative and details are given below.

Three different types of seeds were used : peas of the marrowfat variety, Zelka, and a variety of ' Blue ' peas, with moisture contents of 14·3 and 14·4% respectively, as well as seed of the marrowfat variety, Feltham Advance, with a moisture content of 14·3%. All seeds were from the 1955 harvest and were heavily diseased rejects from electronic sorting machines. As before, those with damaged testas were discarded. Routine tests of the kind previously described were carried out to assess % disease and % germination of the seeds. Samples were fumigated for 3 days at 20° in an atmosphere saturated with chloropicrin and aired for 5 h. at the same temperature, after which small samples were used for redeterminations of % disease and % germination. The remainder, together with unfumigated control peas, were sown in a randomised block experiment. Each treatment was replicated twice in each area, and the results obtained from the four blocks were treated as a single experiment, for the purpose of statistical analysis.

The blocks were 6 ft. square and each contained six rows. Thirty-six seeds were sown in each row, in drills 2 in. deep and at intervals of 2 in. The rate of sowing was thus approximately equivalent to the agricultural rate of 3 bushels per acre. To reduce cross infection from rain splash, the blocks were separated by 8 ft. in all directions and the use of guard rows reduced losses from birds and mice.

Emergence of shoots was counted after 2, 3 and 5 weeks, and the plants were then harvested. No estimate of yield was possible since it was too late in the season for pods to develop, but the height of plants, taken between the base of the hypocotyls and the tip of the growing plants, was measured. The plants were assigned to one of four grades on the basis of disease severity, as follows :

Grade 1: No foot-rot, slight stem or leaf lesions.
Grade 2: No foot-rot, severe stem or leaf lesions.
Grade 3: Foot-rot, slight stem or leaf lesions.
Grade 4: Foot-rot, severe stem or leaf lesions.

To determine the significance of the difference in stand, an analysis of variance was performed on the figures obtained for emergence 3 weeks after sowing. In a second analysis, differences between the height of plants in the various plots were examined more closely by subjecting the results obtained on harvesting first to an analysis of variance and then to an analysis of covariance, so as to remove the effect of unequal emergence.

Results

Fumigation of infected seeds with chloropicrin: laboratory trials

Different moisture contents of the seeds and different fumigation treatments markedly affected % disease, % germination and % emergence from sand, as shown in Table III. The figures given were obtained at the final recordings. Those recorded earlier were difficult to interpret, probably because surface sterilisation of the control samples reduced the number of superficial micro-organisms and stimulated the rate of germination, thus obscuring any retarding effect of the treatments.

Two important factors affect the interpretation of results. The diseased Zelka samples were very heavily infected compared with commercial seed, in which a 50% infection is considered heavy. Although complete elimination of pathogens in the samples used was not obtained and may have been impossible to achieve, it does not follow that complete elimination could

Table III

Effect of fumigation with saturation concentration of chloropicrin on % disease, % germination and % emergence of infected peas

(100 seeds used for each test)

Moisture content, %	Duration of fumigation, h.	No. of seeds which germinated	No. of seeds which emerged from sand	No. of seeds associated with colonies of		
				Fungi	Pathogens	Bacteria
Heavily diseased Zelka seeds						
14·2	20	56	41	96	*	21
14·2	control	56	45	89	*	10
14·2	24	56	42	75	29	18
14·2	control	54	46	79	57	13
14·2	48	57	38	55	27	31
14·2	control	53	48	79	52	13
14·2	74	37	39	32	11	29
14·2	control	43	45	72	46	6
14·2	96	6	4	8	3	67
14·2	control	57	42	68	37	10
10·8	73	62	43	81	21	21
10·8	control	64	55	58	36	10
14·1	74	56	48	25	12	28
14·1	control	71	59	60	37	7
15·8	72	62	45	8	6	30
15·8	control	75	66	37	26	8
18·4	72	34	27	11	6	45
18·4	control	58	52	47	28	7
16·4	61	32	34	13	8	47
16·4	control	58	62	49	29	15
Less diseased Zelka seeds						
14·1	74	64	63	37	17	15
14·1	control	86	82	63	28	2
Onward seeds						
14·4	73	38	31	6	0	49
14·4	control	94	88	14	1	5

* The large number of fungi present made accurate identification impossible.

not be effected with commercial samples. The considerable phytotoxic effect of some of the treatments is probably less serious than would at first appear, since it is well known that heavily diseased seeds are the most easily damaged.

When batches of Zelka seeds, all at a moisture content of 14·2%, were treated and the duration of fumigation varied, an exposure period of 96 h. was the only one which effectively controlled the *Ascochyta* blight, and this treatment also killed the seeds. The results obtained when seeds at different moisture contents were fumigated for 72 h. were more promising. As expected, an increase in the moisture content of the seeds resulted in an increased kill of both the pathogens and the seeds, but at the 16% level there was little phytotoxicity and almost complete control of the pathogens. This result remained unchanged when the duration of fumigation was reduced to 61 h. The ' less diseased Zelka seeds ' were selected from the heavily diseased bulk by eye. Some increase in germination and emergence was secured but little reduction in the number of seeds diseased internally. The results of treatments using these ' less diseased Zelka seeds ' and commercially clean Onward seed did not yield information on the toxicity of chloropicrin to normal, healthy seeds. Most of the Onward peas were killed, presumably because the variety is susceptible to the fumigant, whilst the ' less diseased ' Zelka sample was in fact heavily infected internally and must, therefore, have contained a large number of macroscopically symptomless peas. Table III also shows the striking stimulatory effect of fumigation on bacteria. These micro-organisms are known to be more resistant than fungi, and although no theory can so far be put forward to explain the stimulation, it is well known that low doses of poisons frequently stimulate growth.

Although the seedlings grown in sand were normal, their emergence was often delayed, which suggests that the primary plumules and radicles may have been damaged, and the tissues later replaced. The remarkable regenerative power possessed by legumes has previously been reported by Lubatti & Blackith.[9]

Throughout, results show uneven germination of the controls which is explained by the varying storage times prior to fumigation. It is known that storage of seeds at a moisture content of 14% and above will result in some loss of viability.

Penetration of chloropicrin into quartered seeds

Fumigation of quartered seeds with a moisture content of 14·2%, for periods of 24 h. and above, resulted in almost complete fungal kill. The bacteria were killed only after the most severe treatment. The results of fumigation for 9 h. were similar to those obtained when whole seeds were fumigated for 96 h.

Treatment of cultures of the pathogens with aqueous solutions of chloropicrin

The effect of the various concentrations of chloropicrin on the three fungi can be seen in Table IV.

Field trials

Emergence in all the blocks was low, due presumably to the poor quality of the seed used. This made the results difficult to interpret, and the trials must therefore be regarded as preliminary.

The analysis of variance showed that different areas, varieties and treatments affected

Table IV

Effect of dilute aqueous solutions of chloropicrin on cultures of Ascochyta *spp.*

Concentration of chloropicrin, p.p.m.	Average colony diameters (mm.) after 13 days		
	Ascochyta pisi	*Ascochyta pinodella*	*Mycosphaerella pinodes*
500	0	90	0
334	0	90	0
250	0	90	69
200	60	90	74
167	76	90	82
0	73	90	80

the stand at all three levels of significance. The different response of the varieties to chloropicrin was very marked. In both areas, emergence of the marrowfat Zelka and Feltham Advance peas was reduced by about 8% and 29% respectively, and the ' Blue ' variety by about 6%. The results of laboratory tests carried out after the treatments were markedly different and showed that germination of the ' Blue ' seed was quite unaffected, whilst that of the marrowfat varieties was reduced by about 30%. This discrepancy probably arose because a large number of the seedlings produced on filter papers showed severe bacterial infection, favoured by the moist filter paper. This stimulation of bacteria with chloropicrin has been noted above. The co-variance analysis of the results of the field trials showed conclusively that fumigation of all three varieties of seeds did not affect the heights of the plants produced. Furthermore, their vigour was equal to, or greater than, that of plants grown from untreated seeds. Grading of the plants was inconclusive due to the considerable amount of cross infection which occurred during the wet growing season.

Discussion

The fumigation of certain varieties of peas with chloropicrin for the control of *Ascochyta* blight may be commercially practicable. Fumigation on a laboratory scale showed that heavily diseased Zelka seeds, with moisture contents in the range commonly encountered in commerce, were remarkably resistant to fumigation at saturation concentration for a duration long enough to kill the pathogens. A series of fumigations at approximately 20°, in which the moisture contents of the seeds and the duration of treatment were varied, showed that fumigation of seeds with a moisture content of approximately 14–16% for 72 h. was most satisfactory, the treatment resulting in slight phytotoxicity and almost complete fungitoxicity. Complete fungal kill in such a heavily diseased sample may well be impracticable, and since the level of infection allowed commercially is at present 10%, it seems that elimination, though desirable, is not an essential feature of any otherwise satisfactory control method. Furthermore, since infected seed is more severely damaged by fumigation than is healthy seed, it may be that peas which had already been severely damaged by the fungi accounted for most of those killed. This differentiation would presumably be advantageous in practice, as poor seed will produce an unhealthy crop.

The fumigations of quartered seeds showed that the reason for whole seeds requiring a 72-h. exposure period was the slowness in penetration of the fumigant, and not the resistance of the fungi. If more rapid penetration could be secured, either by fumigation under reduced pressure or in a current of air and fumigant, this duration of treatment might be reduced. Increased penetration by raising the moisture content of the peas beyond 16% appears to be ruled out by the probability of increasing phytotoxicity. Since toxic action at a given concentration–time product is not strictly independent of the individual values of concentration and time, it is possible that some improvement in differential toxicity to peas and pathogens might be obtained by fumigating at lower concentration for a longer time.

Further work is needed to determine the susceptibility to chloropicrin of various commercial varieties of peas and the pathogens within these peas. Indications from the present work suggest that different varieties and the three pathogens differ widely in their responses to the fumigant. Diseased Zelka peas appeared to be resistant to the fumigant, both in the laboratory and in the field. A variety of ' Blue ' peas was also resistant in the field, whereas the emergence of diseased Feltham Advance was reduced by as much as 29%. A commercial sample of Onward peas, the fourth variety tested, were shown, as a result of laboratory experiments, to be extremely susceptible.

A single preliminary experiment carried out on Zelka peas suggests that peas sorb a great deal of chloropicrin. If this suggestion proves correct, then penetration of the fumigant into bags of peas as well as into individual seeds may be slow. The rate of penetration will then be an important factor requiring determination.

The combustion method for the determination of chloropicrin is perfectly satisfactory in the laboratory, but commercially, the thermal conductivity method would be preferable, provided the components of the apparatus used were non-corrodible. Chloropicrin rapidly corrodes steel, the material commonly used in the construction of fumigation chambers. This difficulty

might be overcome by the use of chambers of polyester-fibreglass laminates, or by coating steel chambers with epoxy-resin paints.

Control of the pathogens on plant debris in the soil will also be essential if plants grown from healthy seeds are to remain uninfected in seasons favourable for the development of *Ascochyta* blight. For this purpose, chloropicrin may again prove satisfactory. Although the cost of chloropicrin and its rapid evaporation from the soil surface has precluded its use as a surface fungicide, the disinfection of debris which has been ploughed in to a depth of several inches may become a practical proposition.

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Imperial College Field Station
Sunninghill
Ascot, Berks.

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RESIDUES IN WHEAT AND WHEAT PRODUCTS AFTER FUMIGATION WITH ETHYLENE DIBROMIDE

By S. G. HEUSER

Possible methods for determining bromide remaining as reaction product and as unchanged fumigant in wheat and flour after fumigation with ethylene dibromide (EDB) have been reviewed and tested, and new analytical methods developed. The effects of duration of treatment, temperature, moisture content and milling of wheat on the sorption and desorption of fumigant and on the size of the water-soluble bromide fraction produced during fumigation were determined and the practical implications of the results are discussed. Recommended procedures for the determination of bromide residues in cereal products after fumigation with EDB are described.

Introduction

Ethylene dibromide (1,2-dibromoethane, EDB) has been used in a variety of ways as a fumigant for disinfesting stored foodstuffs. In certain areas, notably Israel[1] and India,[2] it has been used alone in the treatment of bagged grain and other commodities. Elsewhere it has been used in admixture with certain aliphatic chlorohydrocarbons such as ethylene dichloride and carbon tetrachloride for the treatment of bulks of grain on floors or in bins. Since 1952

these mixtures have been used on a trial basis for certain treatments of grain in Great Britain,[3] and a programme of experimental work has been undertaken in this laboratory to determine the nature and the amount of the residues remaining after fumigation.

It became clear in the course of this work that EDB is very strongly sorbed by wheat and wheat products; that there is very little decomposition of the EDB or reaction with these materials at ordinary temperatures and that loss of unreacted fumigant during the airing period is very slow. This residue of unreacted fumigant appeared to be the chief toxic hazard in treated foodstuffs. Bridges[4] has reported part of the investigation in which the nature of the residue was studied by the use of EDB labelled with ^{82}Br. He particularly studied the effect on the residue of heating as in baking and found that, whereas at ordinary temperatures a very small amount of reaction takes place between EDB and the wheat protein, on heating, a substantial proportion of the sorbed EDB undergoes decomposition to ethylene glycol and inorganic bromide, the remainder being lost by volatilisation.

In studying the nature and amount of the residues which may be present in foodstuffs after commercial-scale fumigation with ethylene dibromide, analytical procedures are required which will differentiate between unreacted EDB physically sorbed on the material and the water-soluble inorganic bromide which may result from the decomposition of the EDB or its reaction with the material. These analytical methods cannot be based upon the use of labelled fumigant. Similar studies with other fumigants, for example the work of Lubatti & Harrison[5] on residual ethylene oxide in wheat, and of Lewis & Eccleston[6] on residues of methyl bromide in wheat flour, have shown the types of procedure which are likely to be useful. The methods must be tested to ensure that they provide a complete recovery of each kind of fumigant residue substantially as it exists in the foodstuff before the application of the analytical procedure.

One method of developing and testing these analytical methods is to arrange controlled fumigations of the food material on a small scale in glass vessels with known amounts of the fumigant, and to attempt to account for the whole of these amounts by recovery of unreacted fumigant and by determination of any reaction products. In other types of test, indirect techniques are used to check the value of the analytical methods. In the account which follows Expts. 1–8, designed in the former manner, were made to verify the methods of procedure subsequently used and recommended for the estimation of physically sorbed unreacted fumigant and of reacted bromide, after fumigation of wheat and wheat products on the large scale.

Experimental

Preliminary tests (Expts. 1–8)

For initial tests on the total recovery of EDB from ground wheat a cylindrical 1-l. glass chamber of a type designed by Turtle[7] was used. This has a standard B24 joint to facilitate loading with sorbent and a 2-mm. stopcock at each end enabling air to be passed over the sorbent. The chamber is so shaped that an ampoule of fumigant may be broken inside without wetting the sorbent. EDB in samples of the atmosphere is absorbed in a 1 : 1 mixture of redistilled monoethanolamine and dioxan, and after 48 h. at 25° (when decomposition is complete[8]) the bromide is determined by electrometric titration. Samples of laboratory grade EDB gave an assay of 99·3% by this method. Tests were made (Expts. 1 and 2) to determine the amount of EDB recoverable from the chamber without sorbent, by allowing small quantities of fumigant introduced in ampoules to vaporise (with gentle heating) and to remain in the apparatus for 2 h. The free vapour was then aspirated through a train of three absorption bubblers containing the monoethanolamine/dioxan reagent. Approximately 96% of the weight of EDB in the ampoule was recovered by aeration for 2 h.; the balance was assumed to be lost by solution in the minimal amount of grease used in assembling the apparatus.

In the first test with sorbent in the chamber (Expt. 3) 20 g. of ground wheat of 13·0% moisture content were exposed for 2 h. to a known weight of EDB contained in an ampoule. The free gas was aspirated through absorption bubblers for 5 h. at 100–200 ml./min. and, after being kept overnight, for 1 h. the following day. The distribution in the bubblers of EDB removed by aspiration was as follows: 70·0, 8·6 and 0·5% in three bubblers in series and 3·1 and 0·2% in two further bubblers after keeping the samples overnight (% based on original weight of EDB used). The total recovery was 82·4% (see Table I).

After this thorough aeration, the ground wheat could be handled in still air without loss of adsorbed EDB, and no odour of fumigant was detectable. When allowance has been made for the weight of fumigant lost on the apparatus itself the balance of the EDB applied remains associated with the wheat either as strongly adsorbed EDB or as reaction products. Material prepared in this way formed the basis for checking the percentage recovery of total residual bromide from the wheat by various methods.

Sinclair & Crandall[9] obtained 100% recovery of EDB from citrus fruit by heating with monoethanolamine for 1 h. at 100° in a tightly stoppered bottle and determining as inorganic bromide. Heseltine *et al.*[10] reported 50% recovery of EDB gas samples by absorption and reaction with 5% alcoholic potash. Olomucki & Bondi[11] also obtained 50% recovery of EDB added in the liquid form to cereals, by treatment with 2% alcoholic potash. As 100% recovery of inorganic bromide from wheat is obtained by digestion of material with alcoholic potash,[12] it appeared that two determinations on samples of the same material, one with monoethanolamine to obtain total recovery of EDB and inorganic bromide, and the other with alcoholic potash to recover 50% of the EDB and 100% inorganic bromide, would enable differentiation of the reacted and unreacted fumigant residues.

Five-g. samples of ground wheat of known total bromide content from the exposure described above were treated : (*a*) for 1 h. at 100° with monoethanolamine ; (*b*) overnight at room temperature with 5% alcoholic potash. Each sample was then washed into a silica dish with 2% alcoholic potash and the bromide content determined by an ashing method as described by Lewis & Eccleston.[6] Blank determinations on unfumigated wheat were also carried out.

In a similar test (Expt. 4) ground wheat was exposed for 24 h. to EDB vapour and volatile fumigant aspirated into absorption bubblers. These were changed each hour to obtain an estimate of the rate of removal of EDB from the sorbent. Samples of the aired ground wheat were then analysed as before, but digested with monoethanolamine at 50° only.

For Expt. 5 a larger quantity of ground wheat (40 g.) was exposed to EDB vapour in the Turtle chamber for 24 h. ; the amount of total bromide remaining associated with the wheat was estimated after 2 h. aeration at 200 ml./min. After prolonged aeration the distribution of the EDB on the sorbent and in the bubblers was determined as a check on its total recovery by the analytical procedures used.

The treatment of ground wheat with monoethanolamine at 50° although giving good recovery of added EDB (see Table I) reduces it to a toffee-like mass rather difficult to extract completely from the stoppered bottle. When ground wheat after fumigation with EDB was treated with monoethanolamine and dioxan at 30° for 72 h., a jelly-like mass was obtained and recovery of EDB was over 95% (Expt. 6*a*). With whole wheat, the grains swelled and the seed coats split, and very good recovery of EDB was obtained (Expt. 6*b*). When EDB was added as liquid to whole grains and digested with the reagent, recovery of the EDB was complete in 72 h. (Expt. 6*c*). Results are shown in Table I.

Improved aeration chambers

For determination of fixed bromide residue remaining after complete aeration, it was considered that the strongly adsorbed EDB would be more effectively removed from wheat products if air were able to flow more freely over the grains than was possible in the Turtle chamber, in which the sorbent tends to aggregate. An experimental chamber, in which air flowed upwards through a thin layer of sorbent on a coarse, sintered glass platform, was discarded since the EDB was so strongly adsorbed by the sintered glass itself that it could not be adequately removed with 24 h. aeration.

A later apparatus (Fig. 1) has an aeration chamber A approximately 2 in. diameter and 8 in. long closed at one end by a 2-mm. stopcock and carrying a B50 socket at the other. Just below the axis of the tube two glass rods are sealed in position to support a gauze tray which carries the sorbent in the air stream. Chamber B is a 500-ml. bulb with 2-mm. and $\frac{1}{2}$-in. bore stopcocks at opposite ends and B50 cone for connexion to part A. The weighed ampoule of EDB is broken in bulb B by shaking, the gas allowed to vaporise and the two parts of the apparatus then assembled with a minimal amount of grease. When the $\frac{1}{2}$-in. bore stopcock is opened, the gas diffuses into chamber A, and remains in contact with sorbent for the desired

Table I

Preliminary experiments with ground and whole wheat in aeration chambers

Expt.	Wt. of wheat, g.	Added EDB, mg.	Exposure, h.	Dry aeration Time, h.	Dry aeration Recovery, %	Additional recovery % after treatment of aerated wheat	Total recovery, %	Method of treatment of aerated wheat	Notes
3	20 (ground)	119·4	2	5	79·1				
				5 + 1	82·4	14·2	96·6	Monoethanolamine 1 h., 100°	Residual Br^- after 21 days' aeration 5 p.p.m.
						16·1*		5% alcoholic KOH overnight	* Recovery based on 50% reaction
4	20 (ground)	157·6	24	1	29·4				† Total aeration
				2†	44·9				
				4†	60·6	34·1	94·7	Monoethanolamine 18 h., 50°;	Residual Br^- after 21 days' aeration 25 p.p.m.
				4†	60·6	41·3*		5% alcoholic KOH overnight	
5	40 (ground)	150·9	24	2	41·6	55·0‡	96·6	Monoethanolamine 18 h., 50°	‡ Calc. for whole weight of wheat
				2 + 2	56·7	39·5‡	96·2		
6*a*	30 (ground)	153·8	24	4	62·5	32·8	95·3	Monoethanolamine/dioxan 72 h., 30°	
6*b*	10 (whole)	44·4	24	24	73·2	23·1	96·3	Monoethanolamine/dioxan 72 h., 30°	
6*c*	1 (whole)	137·0					99·6	Monoethanolamine/dioxan 72 h., 30°	EDB added to reagent + whole wheat

period. The sorbent is then aired by drawing a stream of air through the 2-mm. bore stopcocks, the EDB being absorbed in bubblers as before. In a test without sorbent, recovery of EDB by aeration into bubblers after 24 h. in the apparatus (Expt. 7) was close to that obtained with the original simple chamber (95·7%). A test (Expt. 8) on 10 g. of ground wheat exposed to EDB in the improved apparatus for 24 h. gave a total recovery of bromide (96·3%) comparable with that in Expts. 3 and 4.

Differentiation between strongly adsorbed residual fumigant and fixed bromide residue (Expts. 9–13)

In the foregoing tests the aeration period was not sufficiently long to allow removal of the whole of the adsorbed EDB. Whilst with a sufficiently long aeration period the whole of the adsorbed EDB may be removed eventually, this is tedious and the possibility of a continuing reaction cannot be excluded. This total aeration is, however, useful as a check on the maximum amount of reacted bromide which can be found by a selective method. The possibility of distinguishing between the residual adsorbed EDB and inorganic bromide present by their different percentage recoveries in monoethanolamine/dioxan and in 5% alcoholic potash respectively was rejected on the results of Expts. 3 and 4 (Table I). It was shown that the reaction of

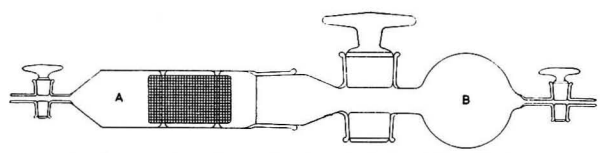

FIG. 1.—*Apparatus for determination of rate of airing of ethylene dibromide from wheat after fumigation*

the unchanged EDB with 5% alcoholic potash when adsorbed on ground wheat gave indeterminate recoveries in excess of 50%, so that the very small amounts of reacted bromide present could not be determined with any accuracy by differential analysis.

Bridges,[4] working with ^{82}Br-labelled EDB, found that both ether and normal EDB failed to extract the adsorbed labelled fumigant from wheat. Olomucki & Bondi,[11] however, following the method of Shrader *et al.*[13] for methyl bromide, claimed to remove adsorbed EDB from grain by chloroform extraction, and stated that bromide remaining associated with the grain after this treatment was non-volatile and water-soluble.

In view of the failure of Bridges to remove adsorbed EDB with powerful solvents, the chloroform extraction method was examined (Expt. 9). Forty g. of ground wheat were exposed for 24 h. at 25° to approx. 0·25 g. of EDB in a Turtle chamber,[7] and then spread on a muslin-covered frame to air for 24 h. The ground wheat would then be expected to contain a considerable amount of adsorbed EDB plus a small amount of reacted bromide. Aliquot weights of the exposed and partially aired material were treated separately in the following manner:

(1) total bromide content determined by immediate treatment with monoethanolamine/dioxan, subsequent evaporation with alcoholic potash followed by Kolthoff–Yutzy[14] oxidation of an extract from the ash;
(2) aliquot aired for 21 days to remove all unreacted EDB and total bromide content then determined as above;
(3) aliquot immediately extracted with water and bromide determined on the extract;
(4) following the method of Shrader *et al.*[13] as adapted by Olomucki & Bondi,[11] part of the wheat was extracted with 3 × 15-ml. portions of chloroform at room temperature for 1, 5 and 15 min. respectively, each intermediate residue being washed with 3 × 5-ml. quantities of chloroform between extractions. Hot extraction was avoided because of the likely decomposition of EDB sorbed on the wheat.[4] Half of the residue after extraction (*a*) was treated with monoethanolamine/dioxan as in (1) for total bromide content. The other half (*b*) was treated as in (3).

The results obtained were as follows (all calc. as p.p.m. EDB):

(1)	total bromide content of wheat	1030
(2)	total bromide content of wheat after complete aeration	35
(3)	immediate water-soluble bromide	30
(4)	(*a*) bromide content after chloroform extraction	900
	(*b*) water-soluble bromide after chloroform extraction	45

These figures show that after airing for 24 h., of the large amount of bromide present in the wheat, almost all was unchanged EDB eventually removable by prolonged aeration. The very small amount of non-volatile reaction product remaining was in no way related to the high bromide content of the chloroform-extracted material 4 (*a*). Therefore the assertion that bromide remaining after repeated chloroform extraction represents the non-volatile water-soluble fraction is invalid. It is clear that only about 10% of the free EDB was removed by cold chloroform extraction and of the bromide remaining only 5% was water-soluble. The finding of Olomucki & Bondi[11] that 90–95% of the chloroform-insoluble residual bromide in fumigated meals was water-soluble suggests that the final warm solvent extraction used by Shrader *et al.*[13] for methyl bromide, when applied to EDB sorbed on cereals, causes its decomposition to give water-soluble bromide.

Because of the heterogenous nature of ground wheat the taking of small representative samples as in these tests is difficult. To reduce sampling errors, later tests on finely divided material were made with National Flour (80% extraction). It was also thought that with this material complete removal of volatile bromide by aeration would be easier and the preparation of material containing only a fixed residue would be achieved more quickly.

Lewis & Eccleston[6] showed that 94–98% of bromide residue in flour fumigated with methyl bromide and thoroughly aired could be recovered from a water extract of the flour after repeated centrifuging. They also found that when the extraction procedure was applied to imperfectly aired flour, some unchanged methyl bromide was recovered in the water extract. If, after extraction with water, however, the soluble fraction of protein from the flour were precipitated with tannic acid, the supernatant liquor no longer contained free methyl bromide.

These procedures were applied to flour which had been exposed to EDB (Expt. 10) and partially aired. Ten-g. quantities of flour were exposed for 48 h. at 20° in separate flasks to

similar weights of EDB vapour drawn from a reservoir. After exposure the flour from one flask was spread on muslin and allowed to air partially in room conditions for 5 h. The other flask was placed in a water-bath at 100°, dry air was passed over the flour at 300 ml./min. for 3 h., and the desorbed gas was collected in a bubbler train to check total recovery of EDB. After aeration, samples were drawn from both lots of flour for determination of total bromide content, and water extraction with and without precipitation of soluble protein was carried out (Table II).

Although removal of unchanged EDB was more rapid at 100°, even at this temperature a large part of the remaining bromide had been broken down to a water-soluble form. Unlike methyl bromide it appeared that from the cold-aired flour little or no unchanged fumigant was removed by water extraction, although the solubility of EDB in water at 30° is approximately 5 times that of methyl bromide.[15] Since unchanged EDB was not removed by water-extraction it follows that the reduction in bromide content of the water extract from the hot-aired flour, after precipitation of soluble protein with tannic acid, was due to removal of reacted bromide.

To see whether reacted bromide could in fact be removed from fumigated material by water extraction without interference from unchanged fumigant, further tests were carried out in which the maximum possible amount of reacted bromide present in prepared samples was determined after complete aeration of volatile fumigant (Expt. 11). Thirty g. of National flour were exposed to the contents of a weighed ampoule of EDB in a Turtle chamber for 7 days at 15°. Aeration of the flour was then commenced, initially by drawing air over the flour in the chamber at 200 ml./min. and collecting desorbed gas in bubblers. At the end of 1 h. and 3 h. the bubblers were changed and 6-g. samples of the partially aired flour were divided for analysis of total and water-soluble bromide content, the amount of EDB found in the bubblers at each stage being used as a check on the assay for total bromide. After 24 h. aeration the flour was removed from the chamber, further samples were drawn for total and water-soluble bromide determination and the remainder of the flour spread on a muslin-covered frame to air completely, i.e., until the total bromide content remained constant. After a total of 14 days' aeration the total bromide content very closely approached the water-soluble bromide content of the flour, and a further 7 days' aeration reduced the total bromide content by 2 p.p.m. only, so that aeration was substantially complete after 21 days (see Table III).

Next, the effect on the size of fixed water-soluble residue produced in flour by varying lengths of exposure to a nominally constant concentration of EDB vapour was investigated (Expt. 12). National flour (100 g.) was placed in an open-ended glass cylinder inserted through a port in a large fumigation chamber (1700 l., maintained at 15°), in such a way that samples of the flour could be withdrawn from the tube during the exposure without altering the gas concentration materially. A measured quantity of EDB was distilled into the chamber with

Table II

Trial recovery of bromide from EDB-fumigated flour (Expt. 10)

	Flour aired 5 h. at 20°	Flour aired 3 h. at 100°
Total Br^- calc. as EDB	800 p.p.m.	370 p.p.m.*
Water-sol. Br^- calc. as EDB	25 ,,	275 ,,
Water-sol. Br^- after precipitation of soluble protein	0 ,,	85 ,,

* Together with content of bubblers, 100% of applied EDB accounted for

Table III

Aqueous extraction of reacted bromide from flour before and after complete aeration (Expt. 11)

After aeration for	Bromide, p.p.m.	
	Total	Water-soluble
1 h.	1310	—
3 h.	1140	29
24 h.	1010	28
14 days	30	29
21 days	28	27

stirring, so establishing a concentration of approx. 40 mg. per litre. Samples of flour were withdrawn from the tube after 3, 7 and 12 days' exposure for determination, after preliminary airing for 48 h., of the water-soluble bromide content, and also, after prolonged airing (21 days), of the final total bromide content (see Table IV). Gas samples were also taken from the chamber at intervals to estimate the concentration–time product to which each sample of flour had been exposed.

To test the validity of the water extraction method for determining fixed bromide in whole wheat treated with EDB, a 500-g. sample of English wheat containing 13·8% of moisture was exposed in a thin layer for 48 h. to approximately 33 mg. of EDB vapour per l. in a large fumigation chamber maintained at 20° (Expt. 13). At the end of the exposure the remaining gas was swept from the chamber and the wheat was aired in smaller quantities on muslin-covered frames. Samples were removed from the frames after 1 h., 7, 14, 28 and 56 days and again divided in two, one portion being used for determination of water-soluble bromide content after grinding and further aeration for 48 h. or 21 days. The other portion was placed in a stoppered bottle with monoethanolamine/dioxan reagent for subsequent determination of the total bromide content at each stage of airing. From the rate of removal of EDB from flour during aeration (Table III) it would be expected that the ground wheat would contain free EDB after only 48 h. aeration after grinding, but that aeration would be complete after 21 days. It is thus possible to compare the amount of water-soluble bromide extracted from wheat containing free EDB and from completely aired material (Table V).

Table IV

Increase of residual bromide content of flour, not removable by aeration, with length of exposure period (Expt. 12)

Exposure	Concn.–time product, mg. h./l.	Water-sol. bromide after 48 h. airing, p.p.m.	Total bromide after 21 days' airing, p.p.m.
3 days	2530	29	32
7 days	5780	39	44
12 days	9620	60	70

Table V

Free EDB and fixed bromide residue found in fumigated wheat during airing period (Expt. 13)

Airing period (whole grains)	Total bromide in whole grains, p.p.m.	Water-sol. bromide in wheat ground and aired for 48 h., p.p.m.	Bromide in wheat ground and aired for 21 days, p.p.m.	
			Water-sol.	Total
1 h.	1380	46		
7 days	430	48	49	53
14 days	280	48	48	52
28 days	210	47	48	53
56 days	150			

Effect of temperature and moisture content of wheat on sorption, extent of reaction and rate of removal of EDB (Expts. 14–16)

In Expt. 14 the effect of temperature and moisture content on the amount of fumigant remaining associated with wheat after exposure, both as free EDB and as reacted bromide, was determined. Batches of whole and ground unfumigated English wheat were conditioned to R.H. of 30% and 70% at 15°, when moisture contents of 9·5% and 14·5%, respectively, were obtained. The state of division of the grains did not affect the moisture content once equilibrium had been attained. Samples from each batch of material were exposed to a concentration of approximately 20 mg. of EDB/l. for 24 h. in chambers maintained at 15° and 25°. At the end of this period the whole wheat was ground and aired for 72 h. on muslin-covered frames in flowing air, when small quantities of each sample were taken for analysis of total bromide content. Assuming similar rates of airing of fumigant from samples of similar particle size, the relative amounts of free EDB remaining associated with the material should be roughly proportional to the amount of fumigant taken up during the exposure period. The remainder of each sample was allowed to air for a total of 28 days when, after further aeration, no drop in total bromide content could be detected (Table VI).

Expt. 15.—The total amount of fumigant taken up by a sorbent (wheat) may be measured indirectly by the fall in gas concentration in the free space above the sorbent, provided there is homogeneity of the gas in the flask and that a correction is made for loss of gas by adsorption

Table VI

Effect of temperature and moisture content on bromide residues produced in whole and ground wheat during fumigation (Expt. 14)

Temperature of exposure	15°		25°	
Mean concentration of EDB	20·6 mg./l.		21·5 mg./l.	
Aeration period	72 h.	28 days	72 h.	28 days
*Whole wheat 9·5% moisture	20	4	12	1
* ,, ,, 14·5% ,,	13	5	15	3
Ground wheat 9·5% moisture	88	14	110	49
,, ,, 14·5% ,,	100	22	140	77

* Whole wheat ground after exposure for purpose of aeration

on the surfaces of the vessel. Since this loss is purely physical it may, for small concentration changes, be assumed to be proportional to the pressure of the gas, and is determined in control experiments with flasks containing no wheat. Twenty-five-g. quantities of English whole wheat containing 14·5% of moisture were placed in 5-l. flasks at 15°, 20° and 25°. Each flask was fitted with a glass capillary tube for withdrawal of gas samples from above the surface of the wheat and a glass stirrer plate was freely suspended in the flask. An external mechanical device was used to keep the flask and stirrer in constant motion, thus avoiding the use of a stirrer gland. Similar control flasks without sorbent were set up at each temperature. Known amounts of EDB were introduced into each flask from a 50-l. reservoir, the fall in concentration in the reservoir being used to calculate the weight of EDB entering each exposure flask. At intervals during the following 168 h. 20-ml. gas samples were taken from each of the 5-l. flasks for determination of the fall in concentration due to sorption by the wheat. These samples were considered small enough to avoid disturbance of the equilibrium between sorbent and sorbate to any extent, provided allowance is made for the very slight lowering in gas concentration caused.

In order to compare directly rates of sorption from gas concentrations which vary in rate of fall, for example due to different temperature conditions in the flasks, the mean gas concentration over the period of exposure under consideration in each case was determined. The amount of EDB sorbed, expressed as p.p.m. on the weight of sorbent, per mg./l. mean concentration was then plotted against time (Fig. 2). All the tests were carried out in the mean concentration range 22–27 mg./l.

In *Expt. 16* comparison was made between rates of removal of EDB from exposed whole and ground wheat under similar conditions. A 10-g. sample of English wheat containing 14·5% moisture was exposed for 24 h. to the vaporised contents of an ampoule of EDB in the two-chamber apparatus described under Expt. 7 (see Fig. 1). After this period dry air was drawn over the sorbent at 200 ml./min. and the EDB swept from the free space and desorbing from the wheat was collected in bubblers containing monoethanolamine/dioxan. After aeration for 1, 2, 4 and 6 h. the bubblers were replaced by fresh sets to determine the rate of removal of fumigant. The final set of bubblers remained connected for 18 h., so that positive rate of removal of EDB up to 24 h. was determined by analysis of the bubbler contents. After the initial 24 h. aeration in the apparatus the wheat was transferred to a muslin-covered frame for further airing for 6 days. The temperature was maintained at 20° throughout the test. Rate of removal of fumigant in this latter airing period was calculated from the fall in total residual bromide content of the wheat determined on successive 2-g. samples. The experiment was repeated under the same conditions with ground wheat as sorbent. Curves showing the higher initial bromide content of the ground wheat after exposure and relative rates of removal of fumigant are reproduced in Fig. 3.

Field test

To investigate the persistence of EDB when applied to grain under practical conditions for insect control a large-scale trial was arranged on 17½ tons of English wheat. This was stored in a covered farm silo bin to a uniform depth of 9 ft. 6 in. and treated with a mixture of 47·5% by volume of carbon tetrachloride, 47·5% of ethylene dichloride and 5% EDB at the rate of 1 gal. of mixture per 5 tons by spraying over the grain surface with a watering can. Ventilating

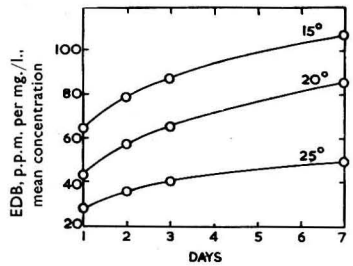

FIG. 2.—*Total sorption of ethylene dibromide by wheat at 15°, 20° and 25°* (Expt. 15)

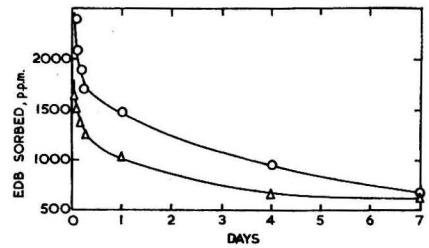

FIG. 3.—*Retention of ethylene dibromide by whole and ground wheat after fumigation* (Expt. 16)
○ ground wheat △ whole wheat

gaps at the eaves were sealed with paper and after application of fumigant the access hatch was sealed with adhesive tape. The temperature of the grain was approximately 12°. During the fumigation period (7 days) gas samples for measurement of gas concentrations were drawn into evacuated flasks through capillary tubing inserted into the grain at depths of 6, 18 and 36 in. at the centre of the bin and near the periphery. Gas samples were also taken in the free space above the grain. From previous experience it was anticipated that the EDB component of the mixture would not sink much below 3 ft. in the grain.

At the end of the nominal fumigation period, the sealing was removed from the bin and samples of grain were taken with a sampling spear at points as close as possible to those for which gas concentration-time products for the exposure had been determined, including surface samples. The samples were transferred quickly to sealed bottles for subsequent analysis for total and water-soluble bromide content. At intervals of 14, 28 and 56 days after the start of fumigation further grain samples were drawn from the same sites to determine the rate of airing of free EDB and any increase in water-soluble bromide, the bulk of grain remaining undisturbed meanwhile. The results of analyses which are shown in Table VII refer to increases over the original naturally occurring bromide content of the wheat. At the end of the airing period, i.e., 49 days after removal of sealing from the bin, a quantity of wheat was taken from the surface layers for milling. Free EDB and water-soluble bromide were determined on the whole wheat and on the flour (see Table VIII).

Table VII

Bromide content of wheat after silo fumigation with halogenated hydrocarbon mixture containing 5% *EDB*

	Concn.-time product (EDB only), mg. h./l.	Bromide, p.p.m. Total				Water-sol.		
Time after start of fumigation, days		7	14	28	56	7	28	56
Sampling position								
Free space	50							
Centre: Surface		277	288	144	51	9	15	14
Centre: 6 in. deep	545	133	208	139	124	9	12	15
Centre: 18 in. deep	170	38	61	94	64	0	18	11
Centre: 36 in. deep	26	51	124	64	30	0	9	10
Periphery: Surface		304	352	108	76	9	16	9
Periphery: 6 in. deep	803	384	471	150	105	11	27	13
Periphery: 18 in. deep	134	181	60	48	26	2	8	7
Periphery: 36 in. deep	30	14	9	6	16	0	3	2
Bottom of bin	0							

Temperature of wheat at fumigation 12°
Moisture content ,, ,, ,, 14·5%

Table VIII

Bromide residues in milled fractions of wheat aired for 49 days after silo fumigation

Fraction	Wt., g.	Total bromide		Water-sol. bromide	
		mg.	p.p.m.	mg.	p.p.m.
Flour	2576	155	58	18	7
Bran	653	71	109	14	21
Fine offal	161	10	62	8	50
Total weight	3390	236	70	40	12
Whole wheat before milling			80		12

Discussion

It has been shown that if vaporised EDB is allowed into contact for some time with an empty glass vessel having joints with minimal lubrication, approximately 95–96% of the weight of vapour applied is recoverable by aeration. The assay method used to determine this result is capable of accounting for 99·6% or more of EDB actually absorbed in the reagent (Heseltine,[8] also Expt. 6*c*). If, therefore, the sum of the weights of EDB or equivalent bromide recovered from a sorbent such as wheat or flour by aeration in the chamber and by chemical extraction from the residue amounts to approximately 96% of the weight applied, it may be said that complete recovery of bromine forms associated with the sorbent has been accomplished. This assumes that the free space concentration of sorbate and consequent loss on the vessel is not materially lowered by the presence of the sorbent. For this reason the amount of sorbent used in each exposure was kept as small as practicable.

Applying the above criteria it is shown that digestion of ground wheat with monoethanolamine for 1 h. at 100° or 18 h. at 50° or with monoethanolamine/dioxan 1 : 1 mixture for 72 h. at 30°, in each case followed by evaporation to dryness with 2% alcoholic potash, effects complete breakdown and recovery of sorbed EDB as potassium bromide. The mixed reagent also effects breakdown and recovery of EDB from whole wheat without preliminary grinding (Expt. 6*b*).

If however the sorbent containing free EDB is immediately treated with 5% alcoholic potash as in Expts. 3 and 4 and the assumption is made that 50% recovery of the bromine in the EDB molecule is obtained according to the reaction

$$CH_2Br{\cdot}CH_2Br + KOH \rightarrow \overset{\uparrow}{CH_2{:}CHBr} + KBr + H_2O$$

then the calculated amount of EDB recovered is higher than the possible maximum. Olomucki & Bondi[11] and workers at this laboratory have however obtained a true 50% recovery of liquid EDB alone or added to cereal grains, by using alcoholic potash as reagent. It therefore seems that when EDB is strongly held by adsorption, as is the case after prolonged exposure to the vapour phase, the reaction is modified. It is probable that solution and subsequent reaction of the strongly sorbed fumigant are slow enough for part of the vinyl bromide produced to be attacked by the reagent instead of immediately escaping as normally postulated. That the increased recovery of bromide above the theoretical 50% is not alone due to the presence of an inorganic reaction product already in the sorbent is shown by the low final bromide content of completely aired material (Expts. 3 and 4). These results preclude the use of the differential recovery of EDB by the reagents mentioned to detect a reaction product in the presence of EDB.

The results of Expt. 9 indicate that cold solvent extraction is quite ineffective in removing adsorbed EDB from ground wheat. The heating of milled cereal containing adsorbed EDB to only 100° as in Expt. 10 results in a high percentage breakdown of the EDB to a fixed water-soluble form so that hot solvent extraction would almost certainly cause some similar decomposition. Adequate separation of the two forms of bromine present by non-aqueous extraction of unchanged fumigant therefore seems unattainable.

Only a very small fraction of the total bromide content, however, was found in a water extract from flour which after exposure had been partially aired at room temperature (Expt. 10). This water-soluble fraction consists solely of fixed bromide residue which is not removed from

the sorbent by very prolonged aeration (Expt. 11), of which about 90% is extracted by the present technique. After a short preliminary aeration to remove loosely held EDB which might possibly dissolve in the extraction liquid, it appears that even large amounts of firmly adsorbed EDB do not interfere in the extraction despite the nominal limited solubility of EDB in water (1 in 250 at 30°).[15] This result is perhaps not so surprising when even liquids with which EDB is completely miscible fail to remove much of it from milled cereals. Aqueous extraction of the partially aired sorbent therefore represents a fairly rapid method of determining the amount of reaction which has taken place between the commodity and the fumigant, and in Expt. 12 (Table IV) it is shown that the reaction proceeds slowly but continuously during the exposure period. On removal of sorbent from the gas however the reaction appears to cease, the amount of reacted bromide remaining relatively constant during aeration (Expt. 13, Table V).

The decomposition of fumigant in ground wheat (Expt. 14, Table VI) was more marked at 14·5% than at 9·5% moisture content and at both an increase in temperature of treatment from 15° to 25° led to a considerably higher permanent bromide residue. These effects were not produced with whole wheat, little difference in behaviour being noted with the varying conditions of test, and the amounts of residual bromide produced were very much smaller than with the milled material.

Fig. 2 (Expt. 15) shows a marked decrease in the total sorption of EDB by whole wheat for a given mean concentration, with increase of temperature. This up-take is almost entirely reversible and is therefore predominantly physical in nature. Because the heat of adsorption is positive this is the normal behaviour where adsorption phenomena in general are observed. These results are however the reverse of those reported by Olomucki & Bondi[11] for sorption of EDB by barley and sorghum, and the up-take of fumigant which they measured was only a fraction of that reported here for wheat. Their technique, involving removal of the sorbent from the gas and weighing before covering with reagent is thought to result in large losses of fumigant by instantaneous desorption, giving very different values from those by the static method used here. This loss, which would be more rapid from milled cereal, would also account for the apparent paradox of higher adsorption on whole grain than on milled grain which they found at high gas concentrations, despite the very much larger surface area available with milled grain.

These authors reported results for space concentrations of EDB of 100, 400 and 1000 mg./l. at 25° and 45°, whereas the saturated vapour pressure of EDB at 25° is 10·83 mm. Hg[16] equivalent to 126 mg./l. (calculated molar volume 21·2 l.[16]). With an excess of liquid sorbate in the vessel the actual free space concentration would be the saturation concentration, under which condition condensation of liquid fumigant on the sorbent would be likely. It is contended that the increased total sorption found by Olomucki & Bondi at higher temperature was the effect of a higher saturation concentration in the air space at that temperature.

The curves comparing retention of EDB by whole and ground wheat (Fig. 3, Expt. 16) show that after the initial rapid loss of loosely held fumigant the rate of airing became relatively low in both cases, but somewhat higher from the sorbent having the larger surface area exposed. After about 8 days the bromine content of the ground material fell below that of whole wheat despite the much higher initial value. The rate of airing from whole wheat had by then become extremely slow and this behaviour represents the main hazard in the use of EDB for grain fumigation, as is shown most clearly in the results of the farm silo fumigation (Tables VII and VIII).

Here, in a typical grain bulk, maximum EDB content of samples from 1 to 3 ft. below the surface was not reached until 14 days after application of the fumigant, because of slow downward movement of the vapour, although sealing was removed from the bin after 7 days. Even 56 days after application of fumigant the EDB content of grain samples from this area was still considerable although increases in inorganic bromide residue were minute. As would be expected the grain retaining the highest EDB content was obtained from the area which had received the highest gas concentration–time product during exposure, i.e., approx. 6 in. below the grain surface. Little EDB vapour penetrated below 3 ft. in depth and none reached the bottom of the bin. Table VIII indicates that of the 80 p.p.m. total bromide content of the wheat taken from the upper layers for milling, a large proportion was unchanged EDB and most of this appeared in

the milled fractions, particularly in the bran. The significance of these EDB residues may be lessened by three considerations:

(1) they would appear only in the upper part of a treated bulk of grain, unless forced circulation were used;
(2) at the time of milling the fumigated grain would normally be diluted with grain from other sources;
(3) unchanged EDB is broken down to relatively harmless components or vaporised in the baking process.[14]

There remains the hazard of insufficiently aired grain being fed to animals. For example Bondi *et al.*[17] showed that feeding hens with grain containing free EDB caused diminution in the size of their eggs and in extreme cases complete cessation of laying. Based on the results of the foregoing tests, it is recommended that not less than 10 weeks should elapse after fumigation, before such grain is used, and that where possible fresh air should be allowed to circulate freely over it during this period. It is advantageous to fumigate grain with EDB when warm (up to 30°) since far less fumigant is sorbed and the rate of airing is greater. The small increase in the amount of reaction with the grain which may occur is of no significance.[4]

Recommended procedures for determination of total bromide, reacted bromide and hence free EDB in fumigated cereal products

(A) *Total bromide*

Cover 5 g. of the fumigated material with 10 ml. of redistilled monoethanolamine and 10 ml. of dioxan in a stoppered bottle and set aside for 72 h. at 30°. Transfer the jelly-like mass to a 120-ml. silica dish with 40 ml. of 2% alcoholic KOH, add 3 ml. of saturated sodium chloride solution and heat on a boiling water-bath until the residue appears dry. Heat the dish in an air oven at 120° for 1 h., then heat in a muffle furnace at 250° for 30 min., 400° for 30 min. and at 500° for 1 h. (Do not allow the temperature to exceed 500°.) Allow to cool, then carefully break up the ashed residue and extract with two 25-ml. portions of 0·5N-HCl, filter through a fine paper on a Buchner funnel, and wash residue on the funnel with three successive 20-ml. lots of distilled water. Make the filtrate very slightly alkaline with 5% NaOH (1 drop of methyl red as indicator), add a piece of porcelain and boil down to a volume of approx. 10 ml. The bromide in the concentrate is then oxidised to bromate by the method of Kolthoff & Yutzy[14] and iodine released by the bromate from acidified potassium iodide solution is titrated with 0·02N-sodium thiosulphate. A blank experiment must be carried out using all the above reagents, which should be of bromine-free standard.

1 ml. 0·02N-sodium thiosulphate ≡ 0·266 mg. Br^- ≡ 53 p.p.m. Br^- on wet weight.

(B) *Inorganic bromide*

Spread a small quantity of the finely ground fumigated material in a thin layer on a muslin-covered frame to allow loosely held EDB to air off for 6 h. Then take 2 g. in a beaker, cover with 30 ml. of distilled water and stir for 15 min. Transfer to centrifuge tubes with the washings and centrifuge down. Pour off the clear supernatant liquor into a silica dish. Stir residue in tubes with sufficient distilled water and again centrifuge. Add the supernatant liquor to the first extract and then wash the residue once more on centrifuge. To the bulked liquors add 3 ml. of saturated sodium chloride solution and one pellet of KOH and evaporate to dryness on a water-bath. Dry for 1 h. at 120° and then place the dish in muffle furnace and complete exactly as in (A). A blank experiment starting from the addition of sodium chloride solution and KOH to 90 ml. of distilled water should be carried out.

1 ml. 0·02N-sodium thiosulphate ≡ 148 p.p.m. Br^- on wet weight (based on 90% extraction).

Then free EDB content of fumigated material
= 62·6 [Titration A* − (2·78 × Titration B*)] p.p.m. EDB on dry weight.

N.B. As a check on a result obtained by method B, it should correspond approximately

* Recalculated to correct for % moisture in respective samples.

with the result obtained by the application of method A to the finely ground material which has been aired for a further 21 days after taking the sample for aqueous extraction.

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Agricultural Research Council
Pest Infestation Laboratory
London Road
Slough, Bucks.

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VEGETABLE OILS. IX.*—Application of Reversed-phase Chromatography to the Analysis of Seed Oils

By F. D. GUNSTONE and P. J. SYKES

A method of determining the composition of mixtures of saturated, unsaturated and oxygenated acids by application of reversed-phase chromatography has been applied to four seed oils: *Gmelina asiatica* (Linn.), *Cephalocroton peuschelli* (Pax.), *Vernonia camporum* (A. Cheval), and *Jatropha curcas* (Linn.).

Introduction

The possibilities of reversed-phase chromatography as a means of separating fatty acids were first demonstrated by Howard & Martin[1] and the technique has since been exploited by a number of investigators.[2–10] As most commonly used, the mobile phase is aqueous acetone and the stationary phase is liquid paraffin contained on a column with kieselguhr which has been made non-wetting by treatment with dichlorodimethylsilane. The saturated acids usually encountered in triglyceride studies (C_{12}–C_{24}) are readily eluted from the column by various aqueous acetone solutions. A double bond has roughly the same effect on column behaviour as a reduction in chain length of two carbon atoms so that oleic acid is eluted with palmitic

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acid, and linoleic and hexadecenoic acids with myristic acid, etc. Various methods have been used to determine the amount of unsaturated acid(s) accompanying the saturated acid in any eluate.

Boldingh,[2] with a system rather different from that generally employed, chromatographed the mixed acids only after complete hydrogenation or after removal of unsaturated acids by oxidation with alkaline permanganate. The results so obtained do not give a completely unambiguous solution with complex mixtures and this method suffers from the further practical difficulty that the oxidation procedure is known to degrade saturated acids to some extent, particularly those below C_{14}. Crombie and her colleagues[5] chromatographed the mixed acids alone and after oxidation with alkaline permanganate. Popják & Tietz[3] and Lough & Garton[8] tried to overcome these difficulties by collecting the eluate, combining appropriate fractions, recovering the mixture of saturated and unsaturated acids, and re-chromatographing after complete hydrogenation. A mixture of myristic, hexadecenoic and linoleic acids which are eluted together would then be separated as myristic, palmitic and stearic acids. The oxidation procedure was thus avoided and additional values, if required, were obtained by alkali isomerisation. Savary & Desnuelle[10] hydroxylated the unsaturated acids and subsequently separated the di- and tetra-hydroxystearic acids from oleic and linoleic acid respectively on another column using castor oil as stationary phase.

A method has now been developed whereby from chromatograms of the mixed acids (i) alone, (ii) after hydrogenation and (iii) after ozonolysis, sufficient data can be derived to determine even complex acid mixtures. This procedure has several advantages: (*a*) the results follow from chromatographic data alone although other determinations can be used to check the accuracy of the results, (*b*) the unsatisfactory permanganate oxidation is replaced by ozonolysis, (*c*) certain difficulties associated with the quantitative recovery of eluted acids as required in the method of Popják & Tietz[3] and Lough & Garton[8] are avoided. Further, by the use of acetylated castor oil the procedure has been satisfactorily extended to a wide range of oxygenated acids.

Experimental

The chromatographic methods used are based on those described by Howard & Martin[1] and Silk & Hahn[4] to which reference should be made for full details.

Materials

Kieselguhr (Hyflo Super-Cel) is treated with dichlorodimethylsilane. Medicinal liquid paraffin is neutralised by percolation through a column of alumina. Castor oil is extracted with light petroleum (b.p. 40–60),[11] acetylated by refluxing with acetic anhydride, and neutralised by percolation through a column of alumina. Aqueous acetone (x%) is made by diluting acetone (x ml.) to 100 ml. with boiled distilled water; it is saturated with a little of the appropriate stationary phase and contains bromothymol blue (0·001%, w/v) as indicator. The eluting solvents should be kept at the temperature at which they are to be used and should be freshly made each day.

The columns are made from a mixture of kieselguhr with paraffin (1 : 1·4) or acetylated castor oil (1 : 1·3). Twenty-five g. of these mixtures make columns 1·3 cm. in diameter and 35 cm. in length which can generally be used four or five times.

Chromatography

The columns are prepared and loaded as previously described.[1, 4] Paraffin columns are run at 35° being jacketed with isopropyl chloride vapour; acetylated castor oil columns are run at 20°. The eluate is collected in a 2-ml. siphon which empties into a specially constructed titration cell where it is titrated with 0·01N-methanolic alkali with an Agla micrometer syringe as a micro-burette. Development is carried out with a range of aqueous acetone solutions each of which elutes a particular acid or group of acids. Paraffin columns are used for the usual range of saturated and unsaturated acids and give some information about oxygenated acids which are, however, more adequately separated on acetylated castor oil columns. The appropriate solvents are listed in Table I: details of a simple method of finding the eluting solvent have been given elsewhere.[12]

Table I

Eluting solvents (aqueous acetone)

Kieselguhr + paraffin column	Acid	Kieselguhr + acetylated castor oil column	Kieselguhr + paraffin column	Acid	Kieselguhr + acetylated castor oil column
43%	Dihydroxystearic	61%			
35%	Hydroxyoleic	68%	62%	Myristic, Hexadecenoic, Linoleic	80%
43%	Diacetoxystearic				
53%	Hydroxystearic, Epoxyoleic*		67%	Palmitic, Oleic	
?	Diacetoxyoleic*	74%			
			73%	Stearic, Eicosenoic, Docosadienoic	85%
58%	Epoxystearic, Ketostearic				
	Acetoxystearic	80%	78%	Arachidic, Docosenoic	—
53%	Lauric, Tetradecenoic, Hexadecadienoic, Linolenic	74%	83%	Behenic	—

* These results were not obtained from the study of partition coefficients but from the investigation of a seed oil containing epoxyoleic acid.

The mixed acids alone, after hydrogenation and after ozonolysis, are chromatographed on paraffin columns using about 20–30 mg. of acid, though this may have to be varied to suit a particular case. For practical reasons it is often better to investigate the hydrogenated acids before the mixed acids. In the presence of epoxy acids the mixed acids must be acetylated prior to hydrogenation. If oxygenated acids are present it may also be necessary to chromatograph the mixed acids before and after acetylation on an acetylated castor oil column.

Mixed acids

Fatty material is extracted from crushed seeds by extraction with light petroleum (b.p. 40–60°). The triglycerides are hydrolysed with N-alcoholic KOH at room temperature for 24 h. and the unsaponifiable material is extracted.[13] The soap solution and washings are acidified with an ion-exchange resin (Zeo-Karb 225) and a trace of sodium acetate. This last facilitates the subsequent ether extraction of the liberated acids. The cold hydrolysis[14, 15] and unusual method of acidification are used to prevent hydrolysis of any epoxides which may be present. The iodine value[13] and saponification equivalent,[13] and if necessary the epoxide value[16] and glycol value,[17] of the mixed acids free of unsaponifiable material are measured.

Hydrogenation

Complete hydrogenation is effected by shaking an ethanol solution of the mixed acids (~100 mg.) with 20% palladium/charcoal in an atmosphere of hydrogen for 24 h. The catalyst is removed by centrifuging and the hydrogenated acids recovered by evaporation of the solvent.

Ozonolysis

Ozonolysis is carried out in methyl acetate solution at −40° with 100% excess of ozone. With 50–100 mg. of material the reaction takes approximately 5 min. in a 3·5% stream of ozonised oxygen. The reaction mixture is allowed to warm to 20° and after being boiled with water (5 ml.) for 2–3 h. all the solvent is removed to leave the ozonised product which is loaded quantitatively on to the column.

Acetylation

The mixed acids are boiled with (*a*) acetic acid, (*b*) acetic anhydride/pyridine and (*c*) water to effect complete acetylation of hydroxy and/or epoxy groups.

Qualitative identification of acids

Appropriate eluate fractions may be collected and combined for identification of the acids contained therein. Monoethenoid acids are hydroxylated with alkaline permanganate[18] and polyethenoid acids are brominated in ether solution.

Results and discussion

Synthetic mixtures

Each step in the analytical process was carefully checked by the investigation of single acids and of synthetic mixtures prepared from pure acids. A selection of results is given in Table II. In all cases recoveries were 100 ± 3%. The agreement between observed and calculated results is satisfactory and has probably been improved with further experience.

Table II

Analysis of some synthetic mixtures

1. [a]Saturated acids, chromatography of mixed acids only

Acid*	12 : 0	14 : 0	16 : 0	18 : 0	20 : 0[b]	22 : 0
Calc. (%)	15·2	17·3	10·5	21·8	25·2	0
Obs. (%)	14·9	16·8	20·6	21·8	21·3	4·6

2. Saturated and unsaturated acids, chromatography of mixed acids only

Acid*	12 : 0	18 : 0	18 : 1	18 : 2
Calc. (%)	10·6	14·3	39·9	35·2
Obs. (%)	10·4	14·4	39·5	35·7

3. Saturated and unsaturated acids, chromatography before and after hydrogenation[c]

Acid*	12 : 0	14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	?[d]
Calc. (%)	4·4	8·4	23·9	6·2	15·9	22·6	18·6	0
Obs. (%)	4·5	8·4	25·1	6·2	15·8	21·8	17·1	1·1

4. Saturated and unsaturated acids, chromatography before and after ozonolysis

Acid*	12 : 0	14 : 0	16 : 0	18 : 0	18 : 1	18 : 2	
Calc. (%)	—	18·8	—	19·3	61·9	—	Mixture A
Obs. (%)	—	18·4	—	19·0	62·6	—	Mixture A
Calc. (%)	21·8	—	18·0	—	—	60·2	Mixture B
Obs. (%)	21·4	—	18·4	—	—	60·2	Mixture B

5. Oxygenated acids, chromatography of mixed acids only

Acid	Dihydroxystearic	Hydroxyoleic	Diacetoxystearic	Acetoxystearic
Calc. (%)	22·6	22·2	25·1	30·1
Obs. (%)	22·9	21·7	25·2	30·2

* The symbols used indicate the number of carbon atoms and the number of unsaturated centres per molecule.

[a] Paraffin columns used for (1)–(4) and acetylated castor oil for (5).
[b] The arachidic acid was known to be crude and apparently contained behenic acid.
[c] These results were obtained by hydrogenation of combined eluates from the chromatography of the mixed acids, a procedure not now recommended. The poor results for palmitic and oleic acids are probably related to a poor recovery of the acids in this fraction.
[d] Some product possibly formed by deterioration of linoleic acid.

Seed oils

The acids derived from four seed oils were investigated by the general methods described and the results are summarised in Tables III and IV. The octadecenoic and octadecadienoic acids were shown in all cases to be oleic and linoleic acid by characterisation as *erythro*-9,10-dihydroxystearic acid (m.p. 132°) and 9,10,12,13-tetrabromostearic acid (m.p. 114°) respectively. The iodine value and saponification equivalent calculated from the results agree well with those measured experimentally.

Gmelina asiatica (*Linn.*) *seed oil*

This investigation was carried out on a sample of seed obtained from Singapore. A second sample from India contained the same acids in very similar proportions.

The mixed acids, free from unsaponifiable matter, were chromatographed (*a*) after hydrogenation, (*b*) alone and (*c*) after ozonolysis. The eluate curves are shown in Fig. 1. By summing the titres under each peak, correcting for the small acidity of the developing solvent, and comparing with the total titre it is possible to derive the results given in Table IV.

Table III

Extraction data and characteristics of mixed acids free from unsaponifiable matter

	Gmelina asiatica (Verbenaceae)	*Cephalocroton peuschelii* (Euphorbiaceae)	*Vernonia camporum* (Compositae)	*Jatropha curcas* (Euphorbiaceae)
Oil in seeds (%)	6·4	29·6	8·4	32·2
Oil in kernel (%)	59·8	—	—	65·6
Unsaponifiable (%)	2·8	1·3	2·5	0·5
Iodine val.[a] (obs.)	104·2	93·9	118·7	102·9
(calc.)	102·0	94·2	119·8	102·6
Sap. equiv.[a] (obs.)	287·0	291·8	277·6	277·0
(calc.)	285·2	292·2	277·8	277·3
Palmitic (wt.-%)	7·9[b]	2·9[c]	13·6	16·4
Stearic	8·2	2·5	8·5	6·6
Oleic	30·6	6·6	22·1	40·3
Linoleic	35·7	14·5	55·2	36·7
Arachidic	2·9	0·5	0·6	—

[a] Values measured on mixed acids free of unsaponifiable matter and calculated from the composition determined in this investigation.
[b] Also 20 : 1 (10·1%), 20 : 2 (0·8%), 22 : 0 (2·4%), 22 : 1 (0·8%) and 24 : 0 (0·6%) (see footnote to Table I)
[c] Also 12 : 0 (0·2%), 14 : 0 (0·4%) and epoxyoleic (72·4%) (see footnote to Table I)

Table IV

Chromatography results (mole-%) on paraffin columns

Treatment of mixed acids	Load, mg.	Recovery, %	Eluting solvent (% acetone)* 53	62	67	73	78	Other solvents
Gmelina asiatica								
Hydrogenation	24·4	99·3	—	—	8·8	75·7	12·6	2·4, 0·5 (83%)
None	28·5	99·6	—	36·3	40·4	17·5	3·3	2·0, 0·5 (83%)
Ozonolysis	51·9	—	—	—	8·8	8·2	2·6	1·9, 0·6 (83%)
Cephalocroton peuschelii								
Acetylation and hydrogenation	21·8	97·8	0·5	0·5	3·6	23·2	0·6	71·6 (43%)
None	23·6	98·7	71·2	15·5	10·1	2·8	0·4	—
Ozonolysis	102·4	—	0·3	0·5	3·3	2·6	0·5	—
Vernonia camporum								
Hydrogenation	17·8	98·8	—	—	14·8	84·7	0·5	—
None	22·5	98·6	0·1	54·6	36·4	8·3	0·6	—
Ozonolysis	34·8	—	—	—	14·7	8·8	0·6	—
Jatropha curcas								
Hydrogenation	21·2	99·2	—	—	17·8	82·2	—	—
None	32·2	98·7	—	36·3	57·3	6·4	—	—
Ozonolysis	49·0	—	—	—	17·7	6·4	—	—

* Each chromatogram is developed with the full range of solvents, viz.: 35, 43, 53, 62, 67, 73, 78 and 83% aq. acetone

The composition of the saturated acids may be obtained directly from the chromatographic results after ozonolysis; the results for the hydrogenated acids indicate the content of all acids of the same chain length; for the mixed acids it is assumed that one double bond has the same effect as two less carbon atoms so that, for example, eicosadienoic, oleic and palmitic acids will run together. The figures in Table IV (mole-%) thus provide a number of simultaneous equations from which the results in Table III (wt.-%) are computed. It may sometimes be necessary to assume that an acid which is unlikely to be present is in fact absent. There are frequently more equations than are strictly necessary and these provide an additional check on the correctness of the results. The agreement is good but not exact and some minor modification of the result may be made to give the best possible agreement.

The C_{20} monoethenoid acid was shown to be eicos-*cis*-11-enoic acid by conversion to *erythro*-11,12-dihydroxyarachidic acid (m.p. 129–130°) identical with an authentic specimen.

The only detailed report on this oil is by Aggarwal & Soni[19] who found it to contain palmitic (9·6%), stearic (19·7%), oleic (33·6%), linoleic (25·8%) and ricinoleic acids (11·3%). The

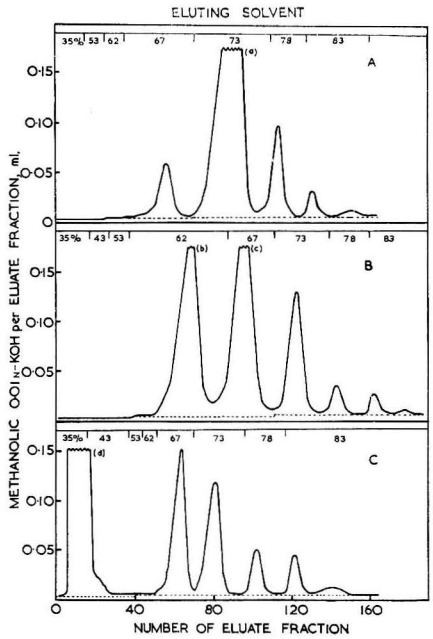

FIG. 1.—Gmelina asiatica *seed oil*

The dotted line shows the correction to be made for the slight acidity of the eluting solvent. The peaks (*a*), (*b*) and (*c*) rise to 0·30, 0·19 and 0·21 respectively; peak (*d*) is due to a mixture of acidic degradation products and is neglected.

A mixed hydrogenated acids
B ,, acids
C ,, ,, after ozonolysis

present study gives very different results; there is no evidence of any hydroxy acid and in addition to the usual C_{16} and C_{18} acids there are several C_{20} and C_{22} acids (total 17%). In this respect it also differs from teak nut oil,[20] the only other Verbenaceae seed fat to be examined in detail, for this contains only the usual range of C_{16} and C_{18} acids.

Cephalocroton peuschelii (*Pax.*) *seed oil*

The mixed acids, free of unsaponifiable material, derived from seeds obtained from Pretoria, in addition to the values given in Table III, had an epoxide value of 73·3 (% weight as epoxyoleic acid, cf. 72·4% calculated from the composition of the mixed acids given in Table III). This value is so close to the value measured on the original oil (74·1% as epoxyoleic glyceride) as to indicate that there is no degradation of epoxide during hydrolysis of the oil. The infra-red spectrum of the acids showed peaks characteristic of the *cis*-epoxide group at 11·85 and 12·17 mμ.[15, 21] It is apparent from the agreement between observed and calculated values (Table III) that epoxy-acids, unlike some hydroxy-acids,[22] do not interfere with the iodine value determination (Wijs): as further confirmation of this the iodine value of epoxystearic acid was shown to be nil.

The standard chromatographic procedure was unsatisfactory in this case in that hydrogenation appeared to convert some of the oxygenated acid to stearic acid. In a separate experiment epoxystearic was reduced in part (14%) to stearic acid, although no mention is made of this in the work of Mack & Bickford.[23] This difficulty was overcome, however, by acetylating the mixed acids by reaction first with acetic acid and then with acetic anhydride/pyridine, before hydrogenation. The diacetoxy-acid was preferred to the dihydroxy-acid since the latter does not chromatograph satisfactorily,[5] probably because of its low solubility in both the mobile and stationary phase.

The results from the three paraffin chromatograms give the amount of the usual saturated and unsaturated acids and indicate the presence (71 mole-%) of one or more oxygenated acids. The agreement between this and the measured epoxide value and the eluting solvent required

for the acid originally (53% aq. acetone) and after acetylation and hydrogenation (43% aq. acetone) suggest one or more monoepoxy-acids. Since *threo*-12,13-dihydroxyoleic acid (m.p. 54–55°)[24] could readily be isolated in good yield from the seed oil after acetolysis and hydrolysis it appears that the acid present is entirely or mainly *cis*-12,13-epoxyoleic acid. The calculated iodine value and saponification equivalent provide further evidence of this although they do not preclude the presence of small amounts of epoxy-acids differing in chain length and/or degree of unsaturation. The chromatographic behaviour of the acid on paraffin and acetylated castor oil columns both before and after acetylation provides further evidence that it is entirely epoxyoleic acid. The oxygenated acid is eluted from the acetylated castor oil columns with 75% aqueous acetone both before and after acetylation. In all cases a symmetrical eluate peak is obtained giving the same value (71–72 mole-%) and although there is no evidence at the moment of how the other possible epoxy-acids would behave it is likely that a mixture of two or more such acids differing in unsaturation and/or chain length would lead to some distortion of the eluate curve. Chromatography results are given in Table IV.

Previous studies[25] have shown that *Cephalocroton cordofanus* seed oil contains 12,13-epoxyoleic acid (62·0 wt.-%) and a small amount of 12,13-dihydroxyoleic acid (3·7%). It is possible that the latter may have been formed from the epoxy acid during storage of the seeds or during investigation of the seed oil, although it is relevant that Tulloch[26] considers that 9,10-dihydroxystearic acid and 9,10-epoxystearic acid are both present in the oil of wheat stem rust. It was thus of interest to examine other seed oils of the *Cephalocroton* genus and it is now apparent that *C. peuschelii* seed oil is very similar to that of *C. cordofanus*. The major acid in both is 12,13-epoxyoleic acid (72·4, 62·0 wt.-%, respectively) accompanied by decreasing amounts of linoleic (14·5, 17·1%), oleic (6·6, 9·8%), palmitic (2·9, 3·9%) and stearic acids (2·5, 2·8%, respectively), and by very minor amounts of other acids. The main difference between the two is that *C. cordofanus* seed oil may contain 12,13-dihydroxyoleic acid which is definitely absent from *C. peuschelii* seed oil.

Vernonia camporum (*A. Cheval*) *seed oil*

Two *Vernonia* seed oils have previously been examined[24, 27] and both contain high proportions of epoxyoleic acid. It is desirable to investigate other seed oils of this genus to see how general is the occurrence of this acid and also as a possible source of other epoxy-acids. *V. camporum* seeds were obtained from Sierra Leone and investigated by the methods described in the Experimental section. The results (Tables III and IV) show the mixed acids to be a relatively simple mixture of linoleic, oleic, palmitic and stearic acids with a minor amount of arachidic acid. The iodine value and saponification equivalent calculated on this composition agree with those observed experimentally and the absence of epoxy-acid was shown chromatographically and confirmed by the infra-red spectrum of the mixed acids and by the epoxide value[16] (nil). This oil is clearly different from those derived from *V. anthelmintica* and *V. colorata* seeds and plans are being made to continue the investigation of seed oils of this genus.

Jatropha curcas (*Linn.*) *seed oil*

This seed oil has been the subject of several investigations. Two reports[28, 29] suggest that a hydroxy-acid is present, whilst several others claim that acids of this type are absent.[30–36] Investigation by the reversed-phase chromatographic procedure has also shown hydroxy-acids to be absent from the sample under study which was obtained from Ibadan. The agreement between the observed and calculated iodine value and saponification equivalent is remarkably good. The present results are compared with those previously reported in Table V.

Conclusions

Although reversed-phase partition chromatography is being more widely applied as paper chromatography, its use in columns as first suggested by Howard & Martin[1] provides a convenient and accurate method for the quantitative study of mixtures of saturated acids. This has been extended to include unsaturated acids by the use of oxidation and hydrogenation procedures and also oxygenated acids by the use of other stationary phases. With ozone instead of permanganate as oxidising agent and by hydrogenating the mixed acids rather than acids

Table V

Jatropha curcas seed oil

Reference	3	31	33	34	35	Present work
Myristic		—	1	1	—	—
Palmitic	14	14	12	16	17	16
Stearic		10	5	10	6	7
Oleic	36	53	63	41	37	40
Linoleic	50	23	19	32	40	37

recovered from other chromatograms the disadvantages of earlier methods of using this technique are overcome. By suitable attention to details and careful choice of the correct eluting solvent it is possible to get good resolution between subsequent fractions (Fig. 1) and consistently high recoveries of acids from the columns (Table IV). The satisfactory results obtained with synthetic mixtures and the excellent agreement between calculated and observed iodine value and saponification equivalent also indicate the general accuracy of the results. The agreement of the iodine value is considerably better than some recently reported for analysis by gas–liquid chromatography.[37–39] The advantages of acetylated castor oil columns along with the paraffin columns are not fully developed in these analyses but will become apparent with more complex mixtures of oxygenated acids.

Though not as simple as gas–liquid chromatography to operate, the techniques here described may have some advantages in particular circumstances: the analysis is effected under very mild conditions, it is easily carried out on quantities sufficient for further investigation, it requires no expensive or elaborate equipment, and it may be more easily adapted to the less volatile oxygenated acids.

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Chemistry Dept.
The University
St. Andrews

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IMPROVED METHOD FOR DETECTION AND APPROXIMATE ESTIMATION OF FUNGAL AMYLASE SUPPLEMENTATION OF WHEAT FLOUR

By K. J. HAYDEN

A simple method is described for the detection and semi-quantitative estimation of fungal α-amylase activity of wheat flour. It depends on the selective inactivation of cereal amylases on bentonite in presence of sodium acetate and detection of the fungal amylase by a cup-plate technique with a starch-agar substrate.

Introduction

It has long been recognised that a deficiency of diastatic enzymes during dough fermentation in bread making results in poor gas production, which yields bread of low volume and insufficient crust colour. While most bread flours are considered to be adequate with regard to β-amylase activity,[1] a deficiency of the α- or dextrinogenic component is of frequent occurrence and it is for this reason that malt flour supplementation has become common practice at the mill or in the bakery.

The treatment of flour with malt enzymes has its limitations since in certain circumstances an accumulation of dextrins or sugars in the bread may lead to poor crumb texture or dark crust colour. Large amounts of malt flour often result in an undesirable softening of the dough. These factors cannot be controlled independently and it is not a simple matter to obtain the correct balance of enzyme activity in the finished flour. In addition, malt flour varies considerably in its enzyme activity and keeping properties and cannot be regarded as an ideal flour additive.

The recently introduced fungal enzyme preparations are virtually free from these defects and are largely replacing malt flour as a diastatic corrective both in this country and in America.[2, 3] The fungal amylases are stable, accurately standardised and highly active preparations derived from selected strains of the mould *Aspergillus oryzae*.[4] They are, for all practical purposes, free from enzymes that are undesirable in the bread-making process. Their lower thermal stability permits a wider range of treatment levels to be used without risk of over-treatment and the products of enzyme activity cannot accumulate excessively in the crumb since the fungal preparations are inactivated at an earlier stage in the baking process than is the case with malt flour addition.[5–7]

Hitherto, methods of differentiating between fungal and cereal amylase activity in wheat flour have been based upon this difference in thermal stability. A procedure suitable for the

detection and semi-quantitative determination of fungal amylase in flour has been described by Knight.[8] The Hagberg test responds to cereal amylase activity only, since fungal enzymes are inactivated under the conditions of starch gelatinisation prescribed.[9] No simple test has been reported by means of which fungal amylase activity in wheat flour may be assessed directly without the necessity for a double determination involving thermal inactivation, a procedure that leads inevitably to some destruction of the fungal enzyme.

It has been found that, by treating flour extracts with bentonite under suitable conditions of pH and in the presence of acetate ions, both cereal α- and β-amylases are inactivated almost completely with little or no action on the fungal enzymes. This property is made use of in a simple technique for the direct identification and approximate measurement of fungal α-amylase activity in bread flours.

Experimental and results

Materials

Samples of commercial fungal amylase preparations from three different sources were used. These samples, when assayed according to the method of Sandstedt *et al.*,[10] showed α-amylase activities from 200 to 500 SKB units per g. Flours were treated with each of the samples at rates equivalent to 1 oz. per sack of 280 lb. on the basis of 400 SKB units per g., so that all treatments were equal in terms of fungal α-amylase activity.

Freshly milled malted wheat flour was used as a source of cereal amylases. α- and β-amylase activities were determined by the methods of Sandstedt *et al.*[10] and Kneen & Sandstedt,[11] respectively.

Wheat flour samples were drawn from commercial bread flours known to be unsupplemented with diastatic aids. They were treated with fungal amylase or malt flour, with or without the addition of improvers and bleachers, as required. Lower levels of treatment were obtained by suitably diluting the prepared samples with untreated flour. Each sample was thoroughly mixed before use to ensure an even distribution of additives.

Samples of bentonite from three different suppliers were examined for their ability to remove α- and β-amylase activity from buffered malt flour extracts. No differences were observed between purified and technical grades and the material employed in the experiments described was a technical grade obtained from British Drug Houses Ltd.

Development of method and results

The action of bentonite on the cereal and fungal amylases at different pH values in solutions approximately 0·2N with respect to acetate concentration is shown in Fig. 1. The solutions contained initially 12 units of enzyme activity per ml. and were treated with bentonite in the proportion of 1 g. per 100 ml. of fungal enzyme or malt flour extract.

Fig. 2 shows the separation of cereal from fungal α-amylase in a solution containing initially 3·3 units per ml. of each enzyme under the same conditions of acetate concentration and bentonite treatment. These results show clearly the efficiency with which bentonite removes cereal amylases from solution at pH 5 with no measurable effect on fungal enzyme activity. A pH value of 5 was chosen for the experiments on flour extracts because this is in the region of optimum pH for amylolytic activity[4] and for separation of the cereal and fungal enzymes.

Unfortunately, these methods cannot be applied at the low levels of activity encountered in flour extracts and a more sensitive procedure was sought. Dingle *et al.*[12] applied the cup-plate technique to the determination of various enzymes including amylases. This is an extension of the procedure employed in certain antibiotic assays and reveals the presence of the enzyme after suitable incubation and development as contrasting zones surrounding the cups containing the sample extract. In this instance the zones of converted starch appear as light areas against a blue-purple ground when stained with a weak solution of iodine.

Experiment showed that this technique, with certain modifications, was capable of responding sufficiently well to the low levels of enzyme activity expected in bread flours treated with fungal amylase. Incorporation of 8% of bentonite into the starch–agar substrate was as effective as in buffered liquid media in the inhibition of cereal amylase activity. The zone

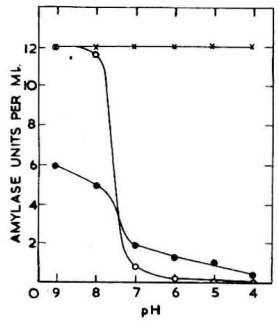

FIG. 1.—*Effect of pH on bentonite adsorption of fungal and cereal amylases in the presence of* 0·2N-*acetate*

×——× Fungal α-amylase
●——● Cereal α-amylase
○——○ Cereal β-amylase

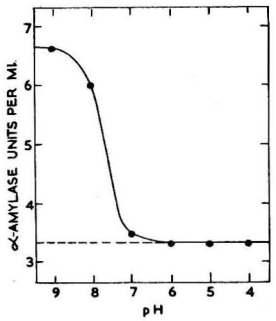

FIG. 2.—*Effect of pH on separation of cereal from fungal α-amylase by bentonite in* 0·2N-*acetate*

Initially 3·3 units per ml. of each enzyme

diameters were small but distinct even down to one-tenth of the normal level of treatment with fungal enzyme preparations. Table I shows the average zone diameters obtained in replicate tests made on different flours and using three fungal amylase preparations.

While flour samples supplemented with malt flour showed no diffuse zones characteristic of fungal amylase treatment, a colourless narrow band frequently appeared due to diffusion of cereal amylases into the substrate. Limited diffusion of the cereal enzymes is inevitable since adsorption occurs only when the enzymes have passed through a sufficient layer of bentonite particles. There is no difficulty, however, in distinguishing between these bands and fungal amylase zones, the diameter of the bands being relatively immeasurable even when malt flour is present to the extent of 16 oz. per sack.

Similar tests were run on flours treated at the normal levels with improvers and bleachers, with and without the addition of fungal α-amylase. Negative results were shown by all the flours containing no fungal amylase and those flours containing both added amylase and other additives showed equally positive results by the method described.

The results obtained with six commercial flour samples and four reference flours at different levels of treatment with fungal α-amylase are shown in Fig. 3. An untreated flour with and without added malt flour is included in this plate. The results indicate that, although the cups are fairly well randomised, duplicate tests show satisfactory agreement with regard to zone diameters.

Details of method

The bentonite–starch–agar substrate is prepared as follows. Bentonite (20 g. technical grade) is floated on the surface of a mixture of 50 ml. of N-sodium acetate solution, 15 ml. of

Table I

Average zone diameters given by different levels of fungal amylase treatment

Treatment of sample (equiv. oz. per sack of 280 lb.)	Average zone diameters, mm.		
	A	B	C
1 oz. fungal α-amylase (400 SKB units per g.)	16·3	16·1	16·5
½ oz. ,, ,, ,, ,,	14·5	14·3	14·5
¼ oz. ,, ,, ,, ,,	13·5	13·0	13·2
⅛ oz. ,, ,, ,, ,,	11·0	11·5	11·5
Untreated flour	0	0	0
16 oz. malted wheat flour	0	0	0
16 oz. malted wheat flour + ½ oz. fungal amylase	14·0	14·5	14·5

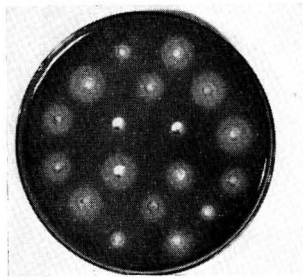

FIG. 3.—*Typical cup-plates prepared from flour samples with and without fungal amylase*

Treatments reading from the left (oz. per 280 lb. sack)

Top row— *a* 16 oz. malt flour
b ½ oz. fungal amylase
2nd row— *a* 1 oz. ,, ,,
b ½ oz. ,, ,,
c Commercial flour (positive test for fungal amylase)
3rd row— *a* ½ oz. fungal amylase
b Untreated flour
c ,, ,,
d Commercial flour (positive test for fungal amylase)
4th row— *a* ,, ,, ,, ,, ,, ,, ,,
b 1 oz. fungal amylase
c ½ oz. ,, ,,
d ¼ oz. ,, ,,
5th row— *a* Commercial flour (positive test for fungal amylase)
b ,, ,, ,, ,, ,, ,, ,,
c ,, ,, negative ,, ,, ,, ,,
Bottom row—*a* 16 oz. malt flour
b ½ oz. fungal amylase

N-HCl and 35 ml. of water. When thoroughly wetted, the adsorbent is stirred in until a homogeneous suspension is obtained. Powdered agar (5 g.) and soluble starch (2 g.) are dispersed in 150 ml. of water by autoclaving or by digesting on a boiling water-bath, water lost by evaporation being replaced. The buffered bentonite suspension is added to the hot starch–agar and again stirred until a uniform suspension is obtained. The pH of the substrate is 4·9–5·0. (If stored in the refrigerator it remains in satisfactory condition for 7 days.)

Plates are prepared by filling suitable glass dishes to a depth of about 4 mm. with the melted substrate and placing on a level surface until set. Cups are cut from the agar with a 7-mm. (No. 4) cork borer and the plugs removed with a fine knife point. The cups are conveniently placed approximately 1 in. apart. A 3½-in. Petri dish requires about 20 ml. of substrate and provides seven cups, which is an adequate number for the examination of four flour samples.

Samples are prepared by mixing 6 g. of flour with 10 ml. of water until a smooth cream is obtained, avoiding as far as possible the incorporation of air into the mixture. After being kept for 5 min. the suspensions are remixed and transferred to the cups by means of a wide-bore dropping pipette, the volumes taken being just sufficient to fill the cups without risk of overrunning the surface of the substrate. The plates are then incubated overnight at 37°. The incubation temperature is not critical and a temperature within the range 35–40° is satisfactory.

After incubation, the plates are flooded with very dilute iodine solution (0·001–0·005N in 0·5% potassium iodide solution is suitable) and developed by a rocking motion to ensure an even blue-purple background intensity. When the zones are sufficiently well defined the plates are thoroughly washed in running water. The zones appear more prominent if the plates are examined 10–15 min. after development and washing. Although the background colour fades within an hour or two, if desired the plates may be redeveloped by repeating the treatment with iodine solution.

For qualitative purposes it is necessary only to run one test extract and an untreated control flour extract for comparison. For an approximate estimation of the amount of fungal amylase present at least one reference sample should be included in the same plate. Four test extracts may be run against an untreated flour and reference flours at two levels of fungal amylase treatment on a 3½-in. Petri dish with seven cups. By direct comparison of the zone diameters it is possible to obtain a reasonable estimate of the enzyme activity of the test extracts. For example, it is a simple matter to ascertain whether a particular flour sample has received a fungal amylase treatment at ¼, ½ or 1 oz. per sack, and a distinctly positive test is given by a fungal amylase addition corresponding to $\frac{1}{16}$ oz. per sack.

Conclusions

By the simple test described it is possible to detect and assess approximately the extent of treatment of bread flours with fungal α-amylase. As overnight incubation is necessary, the procedure cannot be regarded as rapid, but this disadvantage can be offset against the low working time involved, the need for no specialised materials or equipment and the applicability of the test to large numbers of samples at one time.

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DETERMINATION OF TRACES OF COPPER IN RUBBER LATEX ASH BY NEUTRON ACTIVATION ANALYSIS*

By J. A. W. DALZIEL and R. C. H. HSIA

Neutron activation is used to determine copper in latex ash. Purification of the copper activity by homogeneous sulphide precipitation in the presence of carriers is sufficient for the resolution of the 0·51-MeV γ-activity of ^{64}Cu by scintillation spectrometry. The further purification, particularly from ^{32}P, which is needed for β-counting is achieved by electrodeposition. Chemical yields are determined by EDTA titration. A typical ash was found to contain 370 ± 20 p.p.m. of copper.

Introduction

Copper is found mainly in ionic form in the aqueous serum of the latex colloid; some occurs in the lutoid particles in association with polyphenoloxidase,[1] and a little is probably bound directly to the rubber particles.[2] Its presence in natural rubber is of economic importance because it catalyses the aerial oxidation of the rubber.[3] An upper limit of 8 p.p.m. has been specified as an upper limit for the copper content of trade grades of natural rubber.[2]

This paper describes neutron activation methods, utilising β- or γ-counting, for the determination of copper in small samples of latex ash by the reaction ^{63}Cu(n γ)^{66}Cu, $t_{\frac{1}{2}}$ = 12·5 hours.[4] Bowen has already described comparable methods for the determination of copper, zinc and other trace elements[5, 6] in plant materials, by β-counting. γ-Counting is effected by scintillation spectrometry which has been used in other connexions (e.g., metals) for determination of copper, but its application to plant materials is relatively new and the problems encountered are rather different from those in other fields. These chiefly comprise difficulties arising from the relatively large amounts of ^{32}P present and careful purification of the copper is necessary.

Neutron activation methods, because of their high sensitivity, should be useful for research work in which the collection of large samples presents difficulties, e.g., in the correlation of the copper distribution with the metabolism in rubber trees. The methods have the additional advantages that chance contamination of the samples is impossible after irradiation and, if necessary, plant materials can be irradiated before they are ashed.

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

Experimental

The ash was prepared from seedling and clonal latices collected at the Experimental Station, Rubber Research Institute of Malaya, in the spring of 1957. The bulked latices were blended and then dry-ashed in platinum, in an air muffle at 550°. The hygroscopic ash was stored in glass under vacuum. Weighed amounts of cupric acetate diluted with calcium carbonate (AnalaR) were used as standards. Samples of the ash and standard, sealed in polythene tubes, were irradiated in B.E.P.O., Harwell, for 7 days. The main activities after the irradiation were estimated approximately to be $^{24}Na = 27$, $^{12}K = 10$ and $^{32}P = 3$ μc per mg. of ash.

Purification of copper

The irradiated samples were dissolved in HCl (40 ml. of 0·5N) and a 'spike' of copper (10 mg. as sulphate solution) together with sodium and potassium chlorides (0·5 g. each) were added. The copper was precipitated as the sulphide by hydrolysis of thioacetamide[7] (5 mg. per mg. of copper) in the presence of ammonium acetate (0·1 g.). The centrifuged precipitate was washed with water. Recoveries were more than 90%. For additional purification the sulphide was dissolved in bromine and HNO_3 and the copper deposited electrolytically on to a platinum gauze cathode.[8]

Counting

An end-window Geiger–Müller counter was used for solid β-counting, the reprecipitated cupric sulphide being mounted for counting on filter paper discs by filtration. Calibrated scintillation equipment (Isotope Developments Ltd., Type 653A and 652) with a small phosphor and single channel pulse height analyser (Type 672) was used for liquid γ-counting the 0·51 MeV energies of ^{64}Cu, formed in pairs by β^+ annihilation.[6] The chemical yields of copper in the counted sources were determined by direct complexometric titration with EDTA and the metal-ion indicator P.A.N.[9] The EDTA solution was standardised against the copper sulphate solution used for the 'spike'; reproducibility of titres was better than $\pm$0·5%.

Results

The radiochemical purification needed for the β- and γ-assay of ^{64}Cu was first examined. Fig. 1 shows the β-decay of samples which had been subjected to different purification cycles. Purification by precipitation, electrodeposition and reprecipitation of the sulphide (Curve 1c) gave the linear decay of ^{64}Cu over more than five half-lives. The small residual activity was observed over 20 days (Curve 1d), and identified as most probably ^{32}P. Back-extrapolation showed that at zero time the ^{32}P activity was negligible (less than 0·1%) compared with the ^{64}Cu activity. The standard material showed pure ^{64}Cu decay after a single sulphide precipitation. Fig. 2 shows the changes in the γ-spectra of standard and latex ash at different times after irradiation. It is clear that a single sulphide precipitation from latex ash does not give a pure source of ^{64}Cu. However, when the decay of the 0·51 MeV photo-peak was measured with the pulse-height analyser set for 1- and 5-V channel widths (1 V = 68·6 KeV) as in Fig. 3, it was found that the narrower channel minimised the need for further purification.

When the extent of purification had been decided, the two methods of assay were compared. A series of analyses were made on samples of between 10 and 20 mg. of ash. The results, which are summarised in Table I, showed general agreement between the two methods but the reproducibilities were rather unsatisfactory. Seven of the ten results were within a mean value of 370 $\pm$ 20 p.p.m. for the copper content of the ash. To test the reproducibilities of the techniques alone a single large ash sample (83 mg.) and two large samples of the standard (100 mg. each) were irradiated and made into known volumes of solution. Aliquots equivalent to 10–20 mg. of sample were analysed and the results (Table II) showed good reproducibility and agreement between the two methods. It is probable that the three higher results obtained earlier arose from heterogeneity in the samples, or possibly from their chance contamination prior to irradiation, rather than from errors in the actual techniques used.

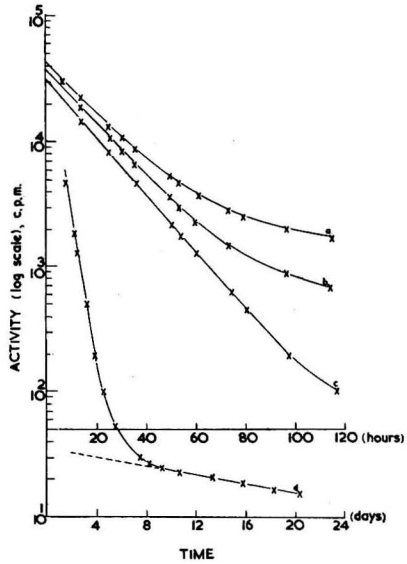

FIG. 1.—*β-Decay of copper sources separated from latex ash by:*

(*a*) single sulphide precipitation, (*b*) double precipitation, (*c* & *d*) precipitation, electrodeposition and reprecipitation of the sulphide (two time scales)

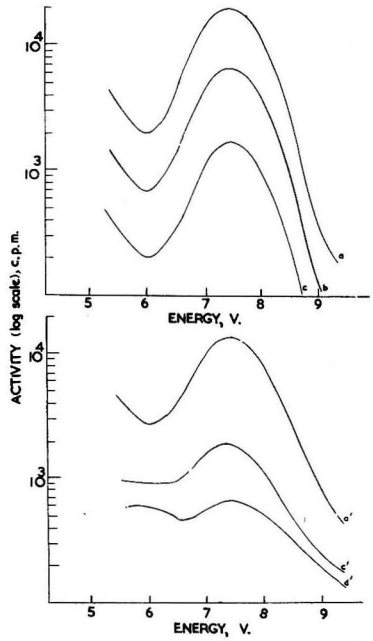

FIG. 2.—*γ-Spectra of sources separated by single sulphide precipitation from standards and latex ash*

(standards in top figure ; latex ash in bottom figure)

(*a* & *a'*) 12 hours, (*b*) 30 hours, (*c* & *c'*) 50 hours, (*d'*) 75 hours after irradiation

FIG. 3.—*γ-Decay of copper sources from latex ash by:*

(*a*) single sulphide precipitation and counted with 5-V channel width, (*b*) single sulphide precipitation and counted with 1-V channel width, (*c*) precipitation, electrodeposition and re-precipitation and counted with 1-V channel width

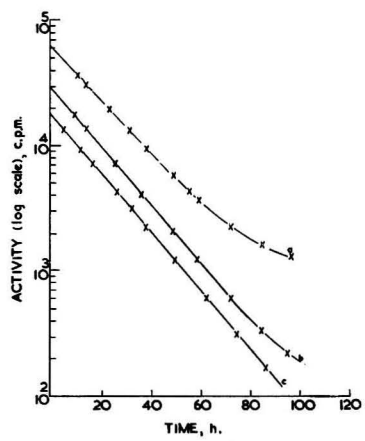

Table I

Copper content of latex ash determined by β- and γ-assay on small samples

β-Assay :	Sample weight (mg.) :	10·3	10·0	10·0	12·2	8·3
	Copper found (p.p.m.) :	463	450	388	376	370
γ-Assay :	Sample weight (mg.) :	20·2	20·2	20·1	20·7	20·1
	Copper found (p.p.m.) :	360	367	382	368	462

Table II

Copper content of latex ash determined by β- and γ-assay, using aliquots from solutions of large samples of the ash and standard materials

	Sample weight per aliquot, mg.		Activity of sources at zero time, c.p.m.	Chemical yield, %	Net activity per mg. at zero time, c.p.m./mg.*	Copper found, p.p.m.
γ-Assay						
Samples	20·7		28,500	98·(1)	1407	369
			27,400	98·(3)	1407	369
			28,500	98·(4)	1405	369
Standards	20·1	A	37,000	95·(2)	1894	500
		B	38,000	96·(4)	1916	
β-Assay						
Samples	8·3		24,000	93·(8)	3099	370
			24,200	94·(9)	3089	369
Standards	10·1	A	42,000	97·(0)	4203	500
		B	41,300	95·(8)	4175	

* All standard activities were corrected for the blank activity found in AnalaR calcium carbonate (105 ± 10 c.p.m./mg. and 40 ± 5 c.p.m./mg. for β- and γ-activities, respectively).

The β-method of assay is about twice as sensitive as the γ-method (Table I) but the latter is more simple and is therefore more likely to be useful for analyses of a semi-routine nature.

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

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EFFECTS OF FERTILISERS ON THE YIELD OF POTATOES IN S.E. SCOTLAND

By K. SIMPSON and P. CROOKS

A series of field experiments was made on maincrop potatoes grown for seed with farmyard manure. The optimum rates of application of ammonium sulphate, superphosphate and potassium chloride were considerably lower than those at present practised in the area.

Use of 120 lb. of N per acre, consistently depressed yield of both ware and seed. Moderate applications of superphosphate (60 lb. of P_2O_5 per acre) increased seed yield generally but had little effect with soils high in easily soluble phosphorus. Potassium chloride increased the ware/seed ratio considerably. Interactions between the effects of the fertilisers on yield were very small.

Introduction

Previous work done in this Department[1] suggested that considerable reductions might be made in the level of phosphate fertilisers applied to potatoes on many soils in S.E. Scotland, especially where farmyard manure is used at rates between 10 and 15 tons per acre. Surveys of fertiliser practice[2] show that in East Lothian and Angus, respectively, 86 and 61% of maincrop potatoes receive farmyard manure at average rates of 13·8 and 11·2 tons per acre.

In a further series of experiments, in which rates of N and K (as ammonium sulphate and potassium chloride) were varied,[3] on potatoes grown for seed, the yield of seed was reduced and that of ware increased when more than 56 lb. of N and 70 lb. of K_2O per acre were used. (Ware potatoes are here defined as $>2\frac{1}{4}$ in. and seed $1\frac{1}{4}$–$2\frac{1}{4}$ in. in diameter.)

These two series of experiments may be criticised because in one the level of P was varied and the applications of N and K were constant while in the second only N and K were varied. It was, therefore, not possible to assess fully the interactions between the three fertilisers.

The present series of experiments was devised to investigate the main effects and interactions of N, P and K (as ammonium sulphate, superphosphate and potassium chloride, respectively) on the ware, seed and total yield of potatoes (variety Majestic) grown with 10–15 tons per acre of farmyard manure.

Experimental

Seventeen field experiments were made during the four seasons 1956–9 on a range of soil types of varying fertility, mostly derived from glacial till and having pH values between 5·5 and 6·5. The potato crops, with one exception, followed cereals and 10–15 tons per acre of farmyard manure was applied to the stubble and ploughed in. The site for experiment No. 5 was ploughed out of 6-year-old grass for potatoes and no farmyard manure was used.

Soil samples from each plot of every experiment were taken before the application of fertilisers and pH values, and ' easily soluble ' phosphorus and potassium were determined. The method used to estimate ' easily soluble ' nutrients was an adaptation of that of Peech & English[4] with an ammonium acetate–acetic acid buffer, pH 4·5, as extractant.

Fertilisers were applied in the split drills immediately before hand planting of the tubers with a space of 1 ft. from centre to centre of adjacent tubers. Stock seed potatoes (variety Majestic) were used in all experiments, all tubers used in a particular year coming from the same source. Where possible the tubers were chitted before planting.

Each plot consisted of six drills, 27 in. wide and 20 ft. long of which four drills × 14 ft. were harvested. (The crop from 56 parent tubers was lifted, plot yields being 60–100 lb. Harvesting was by hand with forks.) The crop from each plot was bagged and set aside for 2–3 weeks before being passed over $2\frac{1}{4}$ in. and $1\frac{1}{4}$ in. riddles to separate ware, seed and chats. The yield of chats ($<1\frac{1}{4}$ in.) was usually small and is not recorded separately below, but is included in the total yield.

Designs

Fourteen of the experiments were uniform in design and had 27 treatments consisting of all possible combinations of the following:

	N, lb./acre		P_2O_5, lb./acre		K_2O, lb./acre
N_0	0	P_0	0	K_0	0
N_1	60	P_1	60	K_1	90
N_2	120	P_2	120	K_2	180

These treatments were randomised in two blocks, each of three sub-blocks containing nine plots. Two degrees of freedom (d.f.) of the second-order interactions are completely confounded with blocks, but in the statistical calculations the other 6 degrees of freedom available for assessing this interaction have been bulked with those for error (24) making a total of 30 d.f. for error.

The remaining three experiments (Nos. 15–17) were designed to test four rates of each fertiliser—0, 60, 120 and 180 lb. of N ; 0, 40, 80 and 120 lb. of P_2O_5 and 0, 80, 160 and 240 lb. of K_2O per acre. There were 64 plots in 8 blocks. The NP, NK and PK interactions were partially confounded with blocks. The second-order interactions (23 d.f.) were used as an estimate of experimental error.

Results

In many of the experiments the high level of a particular fertiliser gave smaller yields than lower rates and it was decided to present the effects $(f_1 - f_0)$ and $(f_2 - f_1)$, where f = N, P or K, instead of the linear and quadratic components of the main effects.

The mean ware, seed and total yields for all plots in each experiment are shown in Tables I, II and III together with the mean soil analyses for easily soluble P and K and the effects $(f_1 - f_0)$ and $(f_2 - f_1)$.

In the experiments 15–17 the effect of the highest level of N, P or K on yield was always negative compared with that of lower rates and, for simplicity, only the effects $(f_1 - f_0)$ and $(f_2 - f_1)$ are shown.

The mean responses for all centres to the two levels of N, P and K are shown at the bottom of the tables and a rough estimate of the overall significance of the effects has been obtained by calculating the square root of the sum of squares of the least significant difference (L.S.D.) values for all experiments divided by the number of centres. To assess the accuracy of predicting responses from soil analysis data, the centres have been split into groups—' high ' and ' low ' with respect to both easily soluble P and K. In the following text ' high-P ' and ' low-P ' soils refer to those with more or less than 0·35 mg. of P/100 g. of soil with a similar arbitrary division for easily soluble K at 13 mg. of K/100 g. of soil estimated by the method used.[4]

The first-order interactions and their components were calculated and were non-significant (P = 0·05) in 145 out of 153 cases. Apart from the very few significant ones the interactions were very small and approximately evenly distributed between positive and negative effects. Therefore the main effects presented below are regarded as valid for all levels of the other nutrients.

The mean yields of ware potatoes (Table I) show a very wide range, from nil to 7·6 tons per acre. The application of 60 lb. of nitrogen per acre increased the yield of ware in eleven experiments (significantly in four), the mean effect being +0·41 tons per acre. A further 60 lb. of N increased the yield significantly at only one centre and reduced the yield at several, one significantly. The mean effect of this second addition was very small and non-significant.

The first level of superphosphate (60 lb. P_2O_5/acre) had small effects, significant at only three centres. The addition of a further 60 lb. did not give significant effects at any centre. The mean responses for low- and high-P soils show that most of the positive effects of superphosphate application on ware yield were on low-P soils and that even on these soils only the first 60 lb. P_2O_5 per acre level of application increased yield.

The first level of application of potassium chloride (90 lb. of K_2O/acre) had considerable positive effects on ware yields in almost all experiments, the effect being significant at 10 centres and highly significant for the mean of all experiments. The yield of ware was generally further increased by the higher level of application, but to a lesser extent, the mean effect being +0·31 tons per acre as compared with +0·77 tons for the first level.

The effects of K applications on high- and low-K soils (foot of Table I) show that ware yield was increased considerably on the low-K soils up to the level of 180 lb. of K_2O per acre but only to the level of 90 lb. on the high-K soils.

Table I

Main effects of fertilisers on yield of ware potatoes (tons per acre)

Centre	'Easily soluble' nutrients (mg./100 g. soil) P	K	Mean yield	N_1-N_0	N_2-N_1	P_1-P_0	P_2-P_1	K_1-K_0	K_2-K_1	L.S.D. 5%
1	0·20	5	0·80	+0·33	+0·08	+0·73**	+0·07	+0·70**	+0·07	0·34
2	0·30	10	5·10	+1·10**	−0·22	+0·59	−0·37	+0·69*	+0·01	0·66
3	0·60	14	5·91	−0·18	+0·04	+0·56	+0·10	+0·63*	+0·13	0·57
4	0·13	13	3·54	−0·22	+0·24	+0·83*	+0·38	+1·84**	+0·32	0·73
5	0·13	14	4·61	−0·35	+0·40	+0·16	−0·04	+0·60	−0·11	0·75
6	0·35	20	2·92	+0·13	−0·48*	−0·41	−0·06	+0·23	+0·01	0·45
7	0·25	11	3·59	+0·16	+0·12	+0·36	−0·13	+0·92**	+0·64	0·57
8	0·35	8	1·48	−0·06	+0·15	−0·09	+0·30	+0·53*	+0·44	0·53
9	1·50	16	7·46	+1·03	+0·32	+0·19	+0·16	+0·43	+0·19	1·18
10	0·60	20	4·24	+0·39	−0·29	−0·12	+0·07	+0·57	−0·02	0·63
11	0·10	15			No ware					
12	0·20	10	5·88	+0·65	+0·06	+0·05	−0·03	+0·93*	+1·18**	0·80
13	0·60	8	7·54	+1·42**	+0·14	+0·24	−0·63	+1·73**	+0·02	0·86
14	0·15	12	4·50	+0·80*	+0·09	+0·82**	+0·20	+1·65**	+0·77*	0·61
15	0·13	10	3·76	+0·61	−0·53	+0·20	+0·32	+0·44	+0 58	0·79
16	0·90	9	5·19	+1·21**	+0·96*	−0·33	−0·38	+0·24	+0·51	0·76
17	0·50	18	7·63	−0·09	−0·13	−0·59	+0·14	+0·92*	+0·54	0·85
Mean of all experiments				+0·41**	+0·06	+0·19*	+0·01	+0·77**	+0·31**	0·17
Low-P soils				—	—	+0·37**	+0·07	—	—	0·20
High-P soils				—	—	−0·07	−0·09	—	—	0·30
Low-K soils				—	—	—	—	+0·97**	+0·45**	0·29
High-K soils				—	—	—	—	+0·48**	+0·11	0·27

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

As would be expected from the fairly close spacing of the crop, the yield of seed at most centres (Table II) was much greater than that of ware, varying from 5·5 to 10·7 tons per acre.

The first level of ammonium sulphate application increased seed yield at 12 centres (6 significantly) and the overall effect was +0·48 tons per acre. Increasing the rate to 120 lb. of N per acre, however, reduced seed yield in almost all experiments as compared with the 60 lb. rate. The average yield from 120 lb. N per acre was, in fact, very similar to that where no nitrogen was applied.

The beneficial effect of superphosphate, at 60 lb. of P_2O_5 per acre, on seed yield (Table II) was significant at eight centres. The overall effect of increasing the rate to 120 lb. per acre was negligible and no significant positive effects were found although at centres 3 and 4 there were appreciable increases in yield. At centre 5 there was a significant negative effect.

On the low-P soils the overall effect of 60 lb. of P_2O_5 per acre was very satisfactory (+1·19 tons per acre) but was much less on high-P soils. Even on the low-P soils the 120 lb. rate did not increase yield of seed significantly above that with 60 lb.

While potassium increased ware yields (Table I), the seed yield was decreased at 10 centres by the application of 90 lb. of K_2O per acre. Also, compared with the lower level of application, 180 lb. reduced the yield at 14 centres. While few of these effects were significant at a particular centre the mean effect was significant.

The total yield (Table III) was generally increased by 60 lb. of N per acre, giving a highly significant mean effect of +0·89 tons per acre, but increasing the rate to 120 lb. reduced yield at 14 centres.

An application of 60 lb. P_2O_5 per acre was beneficial in most experiments and gave an overall increase of +1·01 tons, but increasing the rate to 120 lb. gave no further significant rise in yield. Much of the mean increase in yield was contributed by experiments on low-P soils (mean +1·61 tons per acre for 60 lb. of P_2O_5) with a further non-significant increase of 0·26 tons from the higher rate of application.

The contrasting effects of potassium chloride on ware and seed yield are reflected in Table III, where, despite the increase in ware yield from potassium dressings, the positive effect of 90 lb. of K_2O per acre on total yield was significant at only four centres. At centre 12 only,

Table II

Main effects of fertiliser on yield of seed potatoes (tons per acre)

Centre	Mean yield	N_1-N_0	N_2-N_1	P_1-P_0	P_2-P_1	K_1-K_0	K_2-K_1	L.S.D. 5%
1	7·00	+1·75**	−1·11**	+3·05**	+0·16	−1·55**	−0·35	0·56
2	7·21	+0·26	−0·30	+0·13	+0·52	−0·19	0·00	0·59
3	9·56	−0·03	−0·98*	−0·01	+0·61	+0·26	−0·68	0·85
4	10·74	+1·35**	−1·47**	+0·73	+0·63	−0·58	−0·57	0·80
5	4·56	+0·47	+0·06	+1·31**	−0·73*	−0·42	+0·17	0·57
6	7·04	+1·17**	−0·44	+0·42	+0·07	+0·04	−0·46	0·49
7	5·39	−0·39	−0·20	+1·01*	+0·43	−0·87	−0·23	0·91
8	7·28	−0·22	−0·19	+1·89**	+0·17	+0·14	−0·65	0·71
9	5·53	+0·92*	−0·37	+0·19	−0·19	−0·47	−0·32	0·83
10	8·40	−0·50	−0·01	+0·01	+0·50	−0·18	−0·23	1·17
11	5·99	+0·84	−0·36	+0·52	+0·50	+0·19	−0·19	1·08
12	8·18	−0·27	−0·08	+1·24**	+0·34	−0·80*	−0·38	0·70
13	5·77	+0·05	+0·13	−0·06	+0·04	−1·00*	+0·07	0·83
14	6·81	+1·65**	−0·39	+0·53*	+0·08	−0·21	−0·22	0·53
15	8·82	+0·12	−0·67	+1·48**	−0·09	−0·76*	−0·98*	0·68
16	8·34	+0·22	−0·42	+1·47**	−0·52	+0·20	−0·02	0·77
17	6·33	+0·85*	−0·31	+0·14	0·00	−0·68*	−0·18	0·63
Mean of all experiments		+0·48**	−0·42**	+0·83**	+0·15	−0·15	−0·31**	0·19
Low-P soils		—	—	+1·19**	−0·20	—	—	0·23
High-P soils		—	—	+0·33*	+0·07	—	—	0·31
Low-K soils		—	—	—	—	−0·14	−0·33**	0·23
High-K soils		—	—	—	—	−0·18	−0·27	0·32

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

Table III

Main effects of fertiliser on total yield of potatoes (tons per acre)

Centre	Mean yield	N_1-N_0	N_2-N_1	P_1-P_0	P_2-P_1	K_1-K_0	K_2-K_1	L.S.D. 5%
1	8·22	+2·12**	−1·08**	+3·85**	+0·26	+2·17**	−0·28	0·58
2	12·63	+1·24**	−0·45	+0·62*	+0·19	+0·54	+0·09	0·61
3	15·91	−0·23	−0·89	+0·57	+0·65	+0·90	−0·51	0·91
4	14·66	+1·14*	−1·25*	+1·65**	+1·03	+2·35**	−0·22	1·07
5	9·35	+0·16	+0·44	+1·51**	−0·73*	+0·14	0·00	0·70
6	10·27	+1·20**	−0·85**	+0·21	−0·04	+0·37	−0·60*	0·51
7	9·10	−0·19	−0·13	+1·39*	+0·37	0·00	+0·42	1·12
8	9·08	−0·29	−0·04	+1·88**	+0·44	+0·53	−0·24	0·67
9	13·07	+1·97**	−0·02	+0·39	−0·05	−0·12	−0·16	0·82
10	13·02	−0·17	−0·07	−0·22	+0·60	+0·50	−0·33	1·23
11	6·65	+0·86	−0·36	+0·70	+0·41	+0·12	−0·02	1·17
12	14·33	+0·38	−0·03	+1·36**	+0·05	+0·09	+0·76*	0·71
13	13·44	+1·44**	+0·30	+0·34	−0·59	+0·70*	+0·10	0·65
14	11·53	+2·49**	−0·27	+1·37**	+0·25	+1·38**	+0·54	0·79
15	12·81	+0·78*	−1·20**	+1·75**	+0·28	−0·42	−0·38	0·75
16	13·79	+1·47**	+0·49	+0·17	−0·86*	+0·45	+0·46	0·74
17	14·13	+0·72	−0·45	−0·39	+0·03	+0·16	+0·26	0·89
Mean of all experiments		+0·89**	−0·34**	+1·01**	+0·13	+0·58**	−0·01	0·21
Low-P soils		—	—	+1·61**	+0·26	—	—	0·27
High-P soils		—	—	+0·15	−0·04	—	—	0·32
Low-K soils		—	—	—	—	+0·78**	+0·13	0·25
High-K soils		—	—	—	—	+0·30	−0·19	0·35

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

the increase in yield between the lower and higher level of application was significant, but this result appears anomalous as the lower rate gave practically no increase in yield. Potassium applications were more effective on low-K soils, the mean effect of applying 90 lb. of K_2O per acre being +0·78 tons per acre (sig. P = 0·01) compared with +0·30 tons on high-K soils. The higher rate of application had no further effect on low-K soils.

Discussion

The consistent depressing effect on yield, particularly of seed, of more than 60 lb. of N per acre suggests that it would be unwise to apply more than this amount in the presence of 10–15 tons per acre of farmyard manure. This agrees fairly well with the findings of Reith & Inkson[5] in the North of Scotland who found that 60–80 lb. of N per acre was optimal in the absence of farmyard manure and that this level could not be reduced greatly in the presence of dung. Singh[6] also found 56 lb. of N per acre to be optimum in S.E. Scotland and 112 lb. to be a 'luxury' dressing. These results also confirm the findings in previous work[3] where only N and K were varied and maximum seed production was achieved by applying 56 lb. of N per acre.

The mean response of 0·89 tons for 60 lb. of N (approximately half ware and half seed) agrees very well with that of Reith & Inkson[5] but is considerably lower than that of workers in England.[7–9]

Although the effects varied from centre to centre, increasing the application from 60 to 120 lb. of N per acre consistently reduced yield. Visual observations at most centres and measurements of shoot yield at one centre (not reported here) suggest that the reduction in tuber yield is associated with considerable stimulation of shoot and leaf growth. The depressions in yield may, therefore, be associated with a delay in the time at which the optimum leaf area index is reached or with excessive leaf area cutting down the effectiveness of photosynthesis. Excessive nitrogen applications may cause new leaf formation quite late in the season and prevent efficient transfer of carbohydrates to tubers. Work is being initiated to investigate these hypotheses. Fertiliser surveys[2, 10] made out recently in three potato-growing areas of Scotland show that the present rate of application of nitrogen for potatoes, usually along with farmyard manure, is between 0·8 and 1·1 cwt. per acre for maincrop potatoes and may be as high as 1·8 cwt. for early crops. Similar surveys in England[11] show an average application of 0·9 cwt. These amounts appear to be excessive in S.E. Scotland.

The mean response of 1·01 tons of tubers from 60 lb. of P_2O_5 per acre is of the same order as that found by other authors,[5, 7, 8, 12] but contributing largely to the mean response were several centres where the soil had very low easily-soluble P. For example, at centres 1, 4, 5 and 8 the response was 3·85, 1·65, 1·51 and 1·88 tons respectively and the mean effect at all low-P centres was +1·63 tons. This effect was accounted for mainly in the seed fraction and, as the value of seed is usually higher than that of ware, would be very profitable. On the other hand there was little effect of phosphate on any fraction of the yield on high-P soils. The effect of increasing the rate of application to 120 lb. of P_2O_5 per acre was not usually significant and at two centres gave a significant decrease in total yield.

These results strongly support previous work[1a] which showed a lack of response in superphosphate on high-P soils, and the more recent publications[1b–d] where on soils already well supplied with readily soluble phosphate, large applications of fertiliser-phosphorus may delay tuber development and reduce final yield.

The results indicate that it may be profitable to apply slightly more than 60 lb. of P_2O_5 per acre on some low-P soils and definitely less than 60 lb. on high-P soils. This agrees with the results of Walker,[13] who in ten experiments found a response to applied superphosphate at only one centre where dung was applied. Reith & Inkson[5] recommended the application of rather more (80–100 lb.) where dung was used. Crowther & Yates[12] estimated that where dung was applied the optimal dressing in Scotland was about 150 lb. of P_2O_5 per acre, but this is much above the optimal value found in the present experiments. The present fertiliser practice in Scotland,[2, 10] where dung is applied, is 100–140 lb. of P_2O_5 per acre, and Church[11] reported an average rate of about 100 lb. P_2O_5 per acre in England. It is felt that, particularly on high-P soils, applications of this order will not only have no beneficial effects on yield but may be detrimental.

The general increases in ware and reductions in seed yield due to the application of potassium chloride, particularly at the higher level, indicate that not more than 90 lb. of K_2O per acre is needed for optimum seed production. This fairly low rate is probably associated with the presence of farmyard manure. Other authors also found that dung reduced the optimum level of potassium application.[5, 7, 9, 12]

The mean response in total yield (+0·58 tons) to 90 lb. of K_2O per acre is very satisfactory and on low-K soils the response was even greater (+0·78 tons) but it is hardly necessary on either low- or high-K soils to exceed this level. This is further demonstrated by Table IV where the ware/seed ratio is increased considerably by the application of potassium.

A similar effect was quoted by Garner[14] who, in over 100 experiments, found an average increase in ware of 7% from potassium applications. Nitrogen applications increased the ware/seed ratio only in the absence of P and K and applications of superphosphate reduced this ratio, but this effect was not so marked with high levels of N and K.

The final assessment of the value of fertiliser applications lies in the cash return from the crop. Table V shows the mean effect of treatments on the net extra profit from fertilisers calculated by the formula

$$(W_t + S_t) - (W_0 + S_0) - F_t$$

where W_t and S_t represent the value of the ware and seed produced by treatment, W_0 and S_0 the value of ware and seed from control treatment and F_t the cost of fertiliser treatment. (The values of ware and seed potatoes respectively were taken as £12 and £15 per ton.) Table V shows the inadvisability of applying N, P or K alone and also demonstrates the overall superiority of $N_1P_1K_1$. While it is realised that the use of means in a Table such as this may be misleading it strongly supports the conclusions made above from Tables I, II and III.

These results suggest strongly that considerably lower rates of application of N, P and K than those at present in use in Scotland may be used for potatoes grown for seed in the presence of farmyard manure.

Suggested rates of application (lb./acre) for seed crops are:

	N	P_2O_5	K_2O
Low-P soils	60	80	90
High-P soils	60	40	90

Table IV

Effect of nutrient level on ware/seed ratio

Nutrient	Level of other two nutrients		
	00	11	22
N_0	0·49	0·58	0·65
N_1	0·54	0·61	0·65
N_2	0·60	0·58	0·67
P_0	0·49	0·65	0·74
P_1	0·48	0·61	0·77
P_2	0·41	0·57	0·67
K_0	0·49	0·42	0·48
K_1	0·60	0·61	0·70
K_2	0·65	0·63	0·67

Table V

Effect of nutrient level on extra profit (£/acre) as compared with control

Value of (ware and seed) — cost of fertiliser — value of (ware and seed) from control

Nutrient	Level of other two nutrients		
	00	11	22
N_0	—	+5·2	+7·6
N_1	+1·7	+21·0	+17·8
N_2	−10·9	+13·5	+11·2
P_0	—	+6·2	−8·4
P_1	−5·7	+21·0	+9·5
P_2	−4·0	+14·7	+11·2
K_0	—	+11·9	+1·1
K_1	−8·4	+21·0	+21·0
K_2	−6·6	+16·1	+11·2

Conclusions

Field experiments were carried out in South-East Scotland on potatoes to measure the responses to, and the two factor interactions between, nitrogen, phosphorus and potassium applied in the presence of 10–15 tons of farmyard manure, with the following results:

(1) Ware and seed were generally increased by 60 lb. of N per acre but increasing the rate to 120 lb. did not affect ware yield and consistently reduced that of seed.

(2) Application of 60 lb. of P_2O_5 per acre increased seed yield considerably especially on soils low in easily soluble phosphorus. Ware yield was increased on low-P soils only. Increasing the rate to 120 lb. per acre had negligible effects on ware yield but increased the yield of seed at some centres with very low soil phosphorus content.

(3) Ware yield was increased considerably on all soils by the application of 90 lb. of K_2O per acre, but seed yield was generally decreased. Increasing the rate to 180 lb. per acre further stimulated ware yield but reduced seed yield by approximately the same amount.

(4) Interactions between any pair of nitrogen, phosphorus and potassium were usually not significant for ware, seed and total yield.

(5) The ware/seed ratio was generally increased greatly by potassium, slightly increased by nitrogen and slightly decreased by phosphorus.

(6) The suggested rate of application given at the end of the Discussion are shown by comparison with Fertiliser Survey data to be well below the present level of fertiliser usage for the crop.

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Edinburgh School of Agriculture
West Mains Road
Edinburgh, 9

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CHEMICAL AND BIOLOGICAL EXAMINATION OF COMMERCIAL PYRETHRUM EXTRACTS FOR INSECTICIDAL CONSTITUENTS*

By R. M. SAWICKI† and E. M. THAIN‡

Samples of three commercial pyrethrum extracts were examined chemically and biologically to determine the number of insecticidal constituents they contain. These constituents, which were completely removed from commercial pyrethrum extract by exhaustive extraction with nitromethane, were separated by displacement chromatography. The insecticidal activity of the eluate was restricted to fractions identified chemically as cinerin I, pyrethrin I, cinerin II and pyrethrin II. Solutions containing the four pure active constituents in the same ratios as in the three commercial extracts had the same activity as the corresponding extracts. Pyrethrin II was more active than pyrethrin I but the cinerins were much less active. If any other insecticidal constituent occurs in commercial pyrethrum extract its contribution to the total activity is negligible.

* Read at meeting of Pesticides Group, 16 November, 1959
† Rothamsted Experimental Station ‡ Tropical Products Institute

Introduction

The exact number of insecticidal constituents in commercial pyrethrum extracts is uncertain, although it has been generally assumed, since the work of La Forge & Barthel,[1] that there are only four, i.e., pyrethrin I and II and cinerin I and II.

The biological activity of extracts with similar contents of ' pyrethrins I ' and ' pyrethrins II ' (i.e., mixtures of cinerin I + pyrethrin I and cinerin II + pyrethrin II, respectively) varies (cf. Harper[2]); Roy & Ghosh[3] attributed such variations to the presence or absence of non-pyrethroid insecticides, whereas Parkin[4] attributed them to different ratios of pyrethrins to cinerins in the two ' pyrethrins '. These workers could determine the amounts of only the two ' pyrethrins ' in pyrethrum extracts. The amounts of the four constituents in commercial pyrethrum extracts was first estimated by Brown *et al.*,[5] who used Ward's[6] chromatographic technique to separate the constituents in a pure state.

The work described below was done to find whether the insecticidal activity of pyrethrum extracts resides only in these four constituents.

Experimental

Biological methods

The test insects were adult (5–6 days old) female houseflies, *Musca domestica* L., of a normal susceptible strain. The larvae were bred at 25–26°, and 50–60% R.H., on a milk powder, yeast and agar medium. The average pupal weight on the first day after pupation was 20·5 mg.

The flies, immobilised by cooling, were sexed and the females were transferred into Petri dishes (15–20 females per dish) in a cooling cabinet, by the technique of Lord *et al.*[7] After being sorted, they were given a milk paste and sucrose solution on cotton wool pads and were kept in darkness in a cabinet for 24 h. at 20° and 70–80% R.H. On the next day the flies were immobilised by chilling for a short period, and a 1 μl. drop of the solution under test was applied to the thoracic sternites of each insect, using a measured-drop technique. The flies were not fed after being dosed and were returned to the cabinet until the number killed was counted, usually not earlier than 25–27 h. after dosing. If control mortality did not exceed 10% 48 h. after dosing, a further count was made. The end point, i.e., the time after which no more flies were either killed or recovered from the action of the poison, was usually reached within 25–27 h. of dosing.

In experiments where the LD_{50} values were required, five concentrations were used to obtain a regression line, which was first fitted by eye and later subjected to probit analysis. Three or four Petri dishes, i.e., 45–80 flies, were used per concentration, depending on the number of insects available. The dilution factor was $\sqrt{2}$. Only those experiments in which χ^2 with a probability of 0·05 was less than that shown in Table VI (Finney,[8] p. 307) were considered.

In experiments with chromatographic fractions (all the fractions were made up to the same concentration), the percentage kill obtained with each fraction was plotted against the fraction number, to obtain a general picture of the toxicity of all the fractions from the chromatograph column. The insects were sorted and dosed in the manner already described. The results obtained when three replicates of 15 flies per dish were used varied widely; these variations were decreased, but not eliminated, when the number of insects was increased to eighty.

All solutions for biological testing were made in ' AnalaR ' acetone.

Nitromethane extraction of insecticidal constituents from commercial pyrethrum extract

As a preliminary to column chromatography, the insecticidal constituents were extracted from commercial pyrethrum extract with nitromethane. The nitromethane-soluble portion and the residue were both tested biologically to determine the activity of each, and to find whether, when the fractions are recombined, the activity increases, decreases or corresponds to that of the commercial product.

(i) *Belgian Congo pyrethrum extract.*—This extract containing 25% pyrethrins (6·56 g.) was extracted as shown in Table I with successive portions of redistilled nitromethane, in a stout-walled separating funnel which fitted in a centrifuge sleeve. The emulsion which formed during each extraction was completely broken by centrifuging for 5 min. at 2000 r.p.m.

Table I

Extraction of Belgian Congo pyrethrum extract with nitromethane

Extraction no.	Volume of nitromethane, ml.	Weight of extracted material, g.
1	5	1·778
2	5	0·549
3	5	0·180
4	5	0·005
5	10	0·119
6	10	0·062
7	10	0·043
Weight of extracted material		2·736 g. = 41·7%
Weight of insoluble residue		3·391 g. = 51·7%
Total recovery		93·4%

Four solutions were prepared in acetone for biological tests:

A. A 0·5% solution of the original Belgian Congo pyrethrum extract.
B. Fractions 1–7, recombined in the proportions in which they had been extracted, and made up to a total concentration of 0·208%.
C. As B with addition of the due proportion of the insoluble fraction, made up to a total concentration of 0·466%.
D. A 0·5% solution of the insoluble fraction in nitromethane.

The results of the biological tests on these fractions are presented in Table II.

Table II

Efficiency of extraction by nitromethane and biological activity of the insecticidal constituents of Belgian Congo 25% pyrethrum extract

A. Original commercial 25% Belgian Congo extract
B. Nitromethane-soluble fraction
C. Combination of nitromethane-soluble and -insoluble fractions
D. Nitromethane-insoluble fraction

Solution under test	LD_{50} w/v of solutions	LD_{50} w/v of active constituents	Log ($LD_{50} \times 10^3$) of solutions ± S.E.	Slope of probit line ± S.E.	χ^2	Degrees of freedom	Relative toxicity
A	0·178	0·045	2·25 ± 0·029	3·85 ± 0·65	1·06	3	100
B	0·173	0·043	2·24 ± 0·024	4·56 ± 0·70	0·94	3	103
C	0·168	0·042	2·23 ± 0·023	4·93 ± 0·73	3·54	3	106
D	No kill registered 24 h. after dosing						

Three replicates of 20 insects in each Petri dish were used for each concentration.
There is no significant departure from parallelism among the three lines. Treatment differences are not significant.

Solution B was biologically identical with the crude commercial product; addition of the fraction insoluble in nitromethane did not increase the biological activity, and the insoluble fraction was biologically inert both as an insecticide and as a synergist.

(ii) *Kenya pyrethrum extract.*—Extract of Kenya pyrethrum (25% pyrethrins, 9·720 g.) was extracted eight times with redistilled nitromethane (10-ml. portions), the separation being facilitated as before by centrifuging. The combined nitromethane extracts were evaporated under reduced pressure to yield a viscous green oil (3·780 g.) equal to 38·9% of the original weight. A part of this oil (1·869 g.) was dissolved in redistilled nitromethane (10 ml.) and passed through a column (10 cm. × 1 cm.) of charcoal (May & Baker, decolorising grade). The column was washed with pure solvent until a drop of the eluate evaporated on a filter paper gave no reaction when immersed in a dilute solution of potassium permanganate (i.e., contained no ' pyrethrins '). The eluate was evaporated under reduced pressure to give a nearly colourless residue (1·715 g., i.e., 35·2% of the original extract).

Table III shows the results of biological tests with solutions in acetone of the varying fractions. The nitromethane extract and the same extract decolorised were biologically identical with the commercial pyrethrum. The insoluble residue was biologically inert.

Table III

Efficiency of extraction by nitromethane of the insecticidal constituents present in Kenya 25% pyrethrum extract and biological activities of the fractions

A.	Original Kenya 25% pyrethrum extract	1% in acetone
B.	Nitromethane-soluble fraction	0·389% ,, ,,
C.	Nitromethane extract decolorised by charcoal	0·352% ,, ,,
D.	Residue insoluble in nitromethane	5·0% ,, ,,

Solution	LD_{50} w/v of active constituents	Log ($LD_{50} \times 10^3$) ± S.E.	Slope of probit line ± S.E.	χ^2	Degrees of freedom	Relative toxicity
A	0·043	1·63 ± 0·023	3·97 ± 0·66	0·53	2	100
B	0·041	1·61 ± 0·021	4·25 ± 0·67	2·17	2	105
C	0·040	1·60 ± 0·019	4·24 ± 0·50	4·19	3	106
D	No kill registered 24 h. after dosing					

Three replicates of 20 insects in each Petri dish were used for each concentration.
There is no significant departure from parallelism among the three lines. Treatment differences are not significant.

Chromatographic separation of the material extracted with nitromethane

To separate the biologically active constituents, the nitromethane-soluble material (which contained all the biological activity of the crude pyrethrum extract) was passed through an alumina chromatography column. The eluate was fractionated and the biologically active fractions were examined and identified chemically.

The biologically active constituents were separated by displacement chromatography as used by Ward[6] and Brown *et al.*[5] The column, which consisted of four sections (0·6 cm. diam. × 8 cm., 0·9 cm. × 9 cm., 1·1 cm. × 10 cm. and 1·5 cm. × 10 cm.) connected by B14 standard joints, was charged with chromatographic alumina (Woelm neutral grade, activity 3). The sample to be separated (~250 mg.) was dissolved in light petroleum (2 ml., b.p. 80–100°, aromatic-free) and run on the column, which was then developed with a solution of myristic acid (0·75%) in the same solvent. The flow-rate of the column under gravity was adjusted to about 10 ml./h. and fractions were collected automatically at 15-min. intervals.

The absorbance of the eluate in the ultra-violet region was determined for each fraction by diluting a sample (10 μl.) in absolute ethanol (5 ml.) and measuring the optical density at 226 mμ on a Unicam SP500 spectrophotometer. Fractions containing cinerins and pyrethrins were differentiated by a modification of the phosphoric acid colour test of Williams *et al.*[9] An aliquot (100 μl.) of each fraction was evaporated to dryness in a test tube under reduced pressure at a temperature below 40°. The residue was dissolved in ethyl acetate (1 ml.), syrupy phosphoric acid (4 ml., 85%) was added, the mixture shaken, and immediately heated in a boiling water-bath for 5 min. The optical density of the solution was read at 530 mμ, the wavelength at which the red colour produced by pyrethrins has maximum absorption.

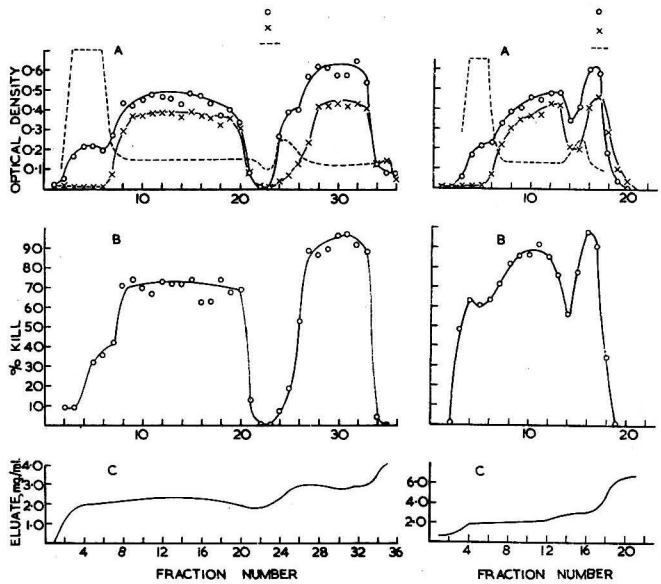
Preparation of solutions for biological testing

The amount of material in each fraction from the column was determined by evaporation under reduced pressure of an aliquot (0·5 ml.) in a platinum dish the residue being weighed on a microbalance. After measurement of the volume of the remainder of the fraction to calculate the weight of the non-volatile material, the solvent was completely removed under reduced pressure and sufficient acetone was added to give a 0·075% solution.

Biological activities of chromatographic fractions

With the method used to determine the biological activity of the chromatographic fractions, it was impossible to obtain exact toxicological data or discern small differences in the toxicity of individual fractions. The method was used primarily to detect whether the general pattern of biological activity corresponded with the chemical composition.

Figs. 1 and 2 show the results of the tests on two commercial extracts (from Kenya and Belgian Congo pyrethrum). The biological activity of the Kenya extract was in two humps (Fig. 1B, fractions 4–21 and 24–34) which were identified chemically as ' pyrethrins I ' and



Chromatographic separation of the material extracted with nitromethane from (FIG. 1, *left*) *Kenya pyrethrum extract,* (FIG. 2, *right*) *Belgian Congo pyrethrum extract*

A u.v. absorption, red colour test, and ratio of u.v. absorption to red colour test, of an alumina displacement chromatogram
B mortality results for the chromatogram fractions on 5–6 days old female house-flies, each dosed with a 1 μl. drop of a 0·075% solution
C weight of eluate from column

○ u.v. absorption at 226 mμ × phosphoric acid red colour
----- ratio of u.v. absorption to red colour

pyrethrins II '. They were separated by a few biologically inert fractions. In the first hump, two biologically active materials were detected by their different relative toxicities. The first, identified chemically as cinerin I, forms the slowly rising part of the curve (fractions 4–7). The plateau which follows (fractions 8–20) is due to pyrethrin I. A similar picture was obtained for cinerin II and pyrethrin II. The former occurred in fractions 24–26, the latter in the short plateau (fractions 27–33). Cinerin II was not clearly shown in the biological results because its content in this extract was small. Some of the biologically inert material was probably still in fraction 24, so reducing the kill, whereas pyrethrin II, present in some amount in fraction 26, tended to increase its toxicity. Therefore, instead of a short plateau, a steeply rising curve was obtained.

The biological activities of the fractions from the Belgian Congo extract (Fig. 2) are similar to those of the Kenya extract. The main difference is the incomplete separation of the two ' pyrethrins '—presumably less inert material occurs in this position than in the Kenya extract.

The relative toxicity of three of the four constituents at 0·075% concentration in acetone was: pyrethrin II 130, pyrethrin I 100, cinerin I 60. The relative toxicity of cinerin II is probably similar to that of cinerin I. (Myristic acid used as displacing agent in the chromatographic column was biologically inactive.)

Preparation of solutions for measurement of the infra-red absorption spectra

To check whether the four constituents obtained from the chromatographic columns were pure, the infra-red spectra of the fractions were determined. After removal of aliquots to determine the ultra-violet absorption and for the phosphoric acid colour test, the remainder of the fraction was evaporated to dryness *in vacuo* and the residue dissolved in carbon disulphide (2 ml., A.R. grade) to give an approximately 0·25% solution. The absorption spectra were

taken over the range 800–1800 cm.$^{-1}$ in a rocksalt cell of path-length 1·5 mm., in a Hilger 800 spectrophotometer. The infra-red spectra of the four constituents (Fig. 3) from the columns were identical with those of the pure materials.

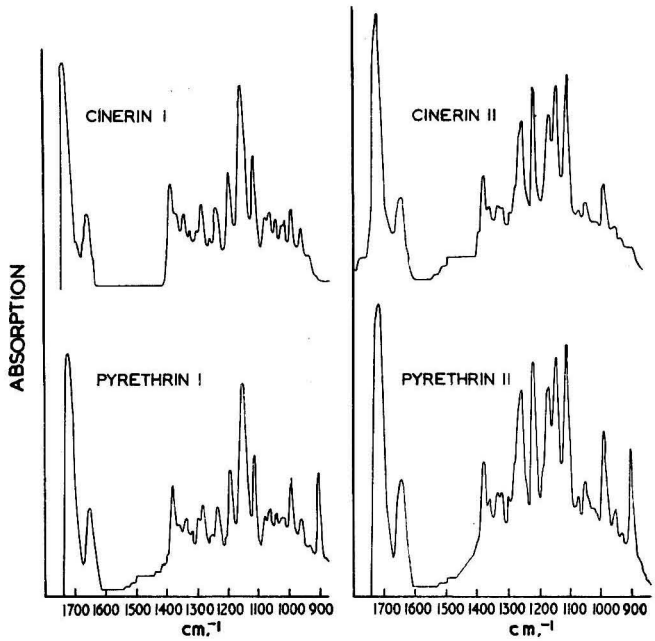


FIG. 3.—*Infra-red absorption spectra of the four constituents of pyrethrum taken over the range* 800 *to* 1800 *cm.*$^{-1}$
(rocksalt cell of path-length 1·5 mm.; Hilger 800 spectrophotometer)

Quantitative estimation of the four constituents in commercial pyrethrum extract from results of chromatographic separations

Figs. 1A and 2A show typical separations of the cinerins and pyrethrins present in decolorised nitromethane extracts, prepared quantitatively from commercial 25% Belgian Congo and Kenya pyrethrum extracts as described above. The fractions containing ' pyrethrins ' are indicated by the graph, and as the concentrations (w/v) and the total volumes of the individual fractions are also known, the total recovery of the ' pyrethrins ' from the extract can readily be calculated. This recovery is not considered to assess the total pyrethrins in the original extract reliably because, when known weights of pure pyrethrin I and pyrethrin II are run on this type of column only 85–90% of the original weight is recovered. The recovery figures shown in Table IV are therefore lower than the results obtained with the official method of analysis of the Pyrethrum Board of Kenya.

The proportions of the individual constituents can also be estimated from the results of the chromatographic analysis, if it is assumed that only the pyrethrins give a positive reaction with the phosphoric acid reagent. Cinerins are present in fractions that absorb strongly at 226 mμ, and give little colour with the phosphoric acid reagent. Such fractions are indicated by peaks in the dotted lines in Figs. 1A and 2A. Cinerin I in Fig. 1 is assumed to be the only constituent present in fractions 4 and 5, whereas fractions 6 and 7 contain increasing amounts of pyrethrin I. The actual amount of pyrethrin I in these fractions was estimated from the intensity of the red colour compared with that of pure pyrethrin I in fractions 10 or 12. The

Table IV

Chromatographic analysis of commercial 25% pyrethrum extracts

Source of extract	% of ' pyrethrins ' recovered from the commercial extract	% Composition of ' pyrethrin ' fraction			
		Cinerin I	Pyrethrin I	Cinerin II	Pyrethrin II
Belgian Congo	22·0	22·9	45·5	10·9	20·7
	—	25·1	46·9	7·7	20·3
Kenya I	21·9	16·5	42·3	11·8	29·4
Kenya II	21·0	12·9	42·0	13·6	31·5

weights of cinerin I and pyrethrin I present in the extract were then obtained by adding the weights present in individual fractions (Table IV).

Similar considerations apply to the estimation of cinerin II and pyrethrin II. However, because the extracts examined contained so little cinerin II, the corrections applied are relatively larger and, as a consequence, the estimation of cinerin II is less accurate. This method is similar to that used by Brown *et al.*,[5] but is simplified because the amount of material in each fraction is weighed directly (see graph C in Figs. 1 and 2) and so calculation from the ultra-violet absorbance is not necessary.

Reconstruction of pyrethrum solutions from the pure constituents

To determine whether the total biological activity of commercial pyrethrum extract could be accounted for by mixture of the pyrethrins and cinerins, the following experiments were made.

Pure samples of pyrethrin I and II and of cinerin I and II were prepared chromatographically. Each compound was obtained only in milligram amounts, so the purity was checked by measuring, in n-hexane, the wavelength of maximum absorption in the ultra-violet and the molecular extinction coefficient. The values obtained are shown below, and agree well with those given by Ward,[6] and by Elliott.[10]

	Cinerin I	Cinerin II	Pyrethrin I	Pyrethrin II
$\lambda_{max.}$, mμ	221	229	222·5	228
$\epsilon_{max.}$	21,100	27,900	38,800	47,500

Solutions of reconstructed pyrethrum (1% w/v total ' pyrethrins ' in acetone) corresponding to the three commercial extracts shown in Table IV were prepared from the four pure compounds, assuming the proportions of cinerins and pyrethrins to be those given in Table IV. For comparison, solutions (0·4% w/v in acetone) of each of the commercial 25% extracts were prepared.

The biological activity of these six solutions were determined on two occasions, the six solutions being tested on the same day and on the same fly population. The results (Table V) show that all the toxicity of the commercial extracts is accounted for by the four compounds present in them.

Discussion

The extraction of the insecticidal constituents from commercial pyrethrum extracts with nitromethane, used by Ward[6] and Spickett[11] to concentrate and purify the insecticidal constituents as a preliminary to column chromatography, was introduced by Barthel and his colleagues[12] to produce concentrates suitable for aerosols. They claimed that over 90% of the original activity was recovered by this method of extraction.

In a preliminary investigation a careful repetition of their experiments gave an extract only 60–70% as toxic as the commercial extract. This difference could have been due either to the incomplete extraction of the active constituents or to the presence of an insecticidal substance insoluble in nitromethane. As seven successive extractions of commercial Belgian Congo pyrethrum extract with nitromethane gave a product as toxic as the commercial product, the first alternative seems correct. The residue, insoluble in nitromethane, had no insecticidal activity and did not increase the activity of the nitromethane-soluble portion (Table II). This

Table V

Comparison of biological activities of crude and reconstructed pyrethrum extracts

(concentrations of the four pure constituents in the reconstructed extracts were the same as those in the crude extracts, as determined by chromatographic analysis)

Origin of extract	Extract	LD_{50} w/v	Log ($LD_{50} \times 10^3$) ± S.E.	Slope of probit line ± S.E.	Common slope of the two lines	χ^2	Degrees of freedom	Relative toxicity
Belgian Congo	Original	0·054	1·73 ± 0·015	4·15 ± 0·38	4·12 ± 0·27	3·71	3	100
	Reconstituted	0·050	1·70 ± 0·024	4·09 ± 0·59		7·16	3	108
Kenya I	Original	0·053	1·73 ± 0·013	4·89 ± 0·40	4·64 ± 0·92	1·03	3	100
	Reconstituted	0·055	1·74 ± 0·015	4·37 ± 0·42		1·10	3	97
Kenya II	Original	0·056	1·75 ± 0·012	5·66 ± 0·43	4·97 ± 0·29	0·54	3	100
	Reconstituted	0·055	1·74 ± 0·014	4·41 ± 0·39		2·07	3	102

Five replicates of 20 flies in each Petri dish were used for each concentration
There is no significant departure from parallelism between the individual pairs of lines
Treatment differences were not significant

was also true of a Kenya pyrethrum extract treated in the same manner (Table III) but also decolorised with charcoal.

All samples of commercial pyrethrum extract used in later experiments on the chromatographic separation of the biologically active constituents were first concentrated and decolorised. The decolorised nitromethane extracts contained all the biologically active constituents of commercial extract.

The chromatographic separation of the nitromethane extract showed that the biological activity was confined to fractions containing the cinerins and pyrethrins. Moreover, the biological activity and the ultra-violet absorbance at 226 mμ of the eluate were closely correlated (Figs. 1 and 2). Three of the four active constituents could be identified biologically by differences in their relative toxicities because all the fractions were made up to the same strength. Cinerin II, present only in small quantities, could not be identified as a separate constituent. Further, because some fractions contain 'pyrethrins I' (a mixture of pyrethrin I and cinerin I) (fractions 6–8 in Figs. 1 and 2) and there is neither break nor discontinuity in the toxicity graph, synergism or antagonism between these compounds seems unlikely.

The sweet-smelling compounds emerging from the chromatographic column before cinerin I, and between pyrethrin I and cinerin II, and the solid waxes emerging after pyrethrin II, were all devoid of insecticidal activity; so was the compound present in fraction 36 (Fig. 1) which, from its colour reaction and ultra-violet absorption, is presumed to be a pyrethroid.

Determination of the infra-red spectra and the wavelength of maximum absorption in the ultra-violet region, indicated that the four constituents were coming off the column in a pure state. On occasions when the separation of the constituents was not clearly defined, the infra-red spectra showed that the irregularities were because the pyrethrins and cinerins were incompletely separated and not because other compounds were present.

Despite the great similarity in the infra-red spectra of pyrethrin I and cinerin I on the one hand, and of pyrethrin II and cinerin II on the other (Fig. 3), the presence of a peak at 905 $cm.^{-1}$ in the two pyrethrins could be used to distinguish them from the respective cinerins, and this characteristic can be used instead of the phosphoric acid colour test. The peak at 905 $cm.^{-1}$ had already been noticed by Freeman[13] in the infra-red spectra of pyrethrum extracts and in allethrin, and attributed to the terminal methylene group in the pyrethrolone side chain (cf. Bellamy[14]). This assignment is confirmed by our observation that this peak does not occur in the spectra of the cinerins.

The results of the chromatographic separation indicate that pyrethrum extract owes its insecticidal activity solely to the presence of the pyrethrins and cinerins. This conclusion was confirmed by showing that the 'reconstituted' pyrethrum solutions were biologically identical with solutions of commercial extracts containing the same quantities of pyrethrins and cinerins (Table V).

The proportions and amounts of pyrethrins and cinerins in the commercial extracts were determined from the chromatographic data; the three extracts contained about 22% of total 'pyrethrins' (Table IV). When analysed by the method advocated by the Pyrethrum Board of Kenya,[15] the total 'pyrethrin' contents of these extracts was estimated at 25%. The two methods give different results because commercial extracts contain false pyrethrins (up to 12% of total pyrethrins according to Brown *et al.*[5]), and because only 85–90% of pure pyrethrins I and II are recoverable from the type of column used. Therefore, it seems likely that the total concentration of 'pyrethrins' in the original extracts was between 23 and 24%.

All the present results imply that the biological activity of commercial pyrethrum extracts lies in pyrethrins I and II and cinerins I and II. However, the limitations in the precision of bio-assays mean that the possibility of other active constituents occurring cannot be excluded even though the bio-assays gave very consistent results, and a full statistical analysis shows that there is no significant difference between the commercial extracts and the reconstituted solutions. In all the tests where the commercial product was compared with the pyrethrum solutions the treatment differences were not significant, well within the 5% fiducial limits and in terms of relative toxicities always less than 10%.

It is concluded, therefore, that the insecticidal activity of commercial pyrethrum extracts resides exclusively in the four known constituents. If there is any other active substance, it does not contribute enough to the total activity of commercial pyrethrum extracts to be detectable by the methods of analysis used.

Acknowledgments

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Tropical Products Institute
56/62 Gray's Inn Rd.
London, W.C.1
and
Rothamsted Experimental Station
Harpenden
Herts.

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NITRATE AND NITRITE METABOLISM IN A BACON-CURING BRINE AND THEIR RELATION TO THE BACTERIAL POPULATION

By B. P. EDDY and A. G. KITCHELL*

The effects of salt concentration, temperature and bacterial population density on the rates of metabolism of nitrate and nitrite have been investigated in a bacon-curing brine initially devoid of both. Increasing salt concentration and decreasing temperature diminish the rates of metabolism. The rates could not be related satisfactorily to either total microscope or viable counts of bacteria, but a relationship with total counts seems more probable.

Introduction

In the autumn of 1958 a request for advice on the prevention of 'glaziness' was received from a bacon factory and at the same time a sample of the cover brine, in which it had been found very difficult to control the nitrite content, was sent since the manufacturer thought that this might be partly the cause of the trouble. This pickle was reputed to contain 2000 p.p.m. sodium nitrite and to have pH 6·8. It was brownish in colour, very cloudy and had an unusually high total count of bacteria—$2{\cdot}5 \times 10^8$/ml.: analysis showed that the protein content was abnormally high (0·99%), the salt content rather low (21·9%) and that both nitrate and nitrite were absent. The high bacterial count and protein content probably related to the low salt content. It was subsequently learned that the value of 2000 p.p.m. nitrite had been obtained some weeks previously and that since then, because this value was thought to be too high, no solid nitrate had been added to the sides in the tank. Nitrate was still present in the pump pickle, therefore the maturing sides could not be presumed to be devoid of nitrite. Nevertheless, the matured bacon seemed somewhat deficient in colour and nitrite analyses gave values of the order of only 8–10 p.p.m.

Tests in which nitrate and nitrite were added separately to the brine showed that, on incubation, the former was rapidly reduced and the latter rapidly destroyed. It seemed possible that, in spite of the abnormally high bacterial count and protein content, experiments might be carried out with this brine which would help to elucidate some of the as yet unsolved problems concerning the activity of bacteria in bacon brines towards nitrate and nitrite. Such experiments are described in this paper.

Both nitrate and nitrite are metabolised in bacon-curing brines, but the absolute rates at which the two processes take place are difficult to determine because both are proceeding simultaneously and methods available for nitrate estimation are insufficiently accurate to permit balance sheets to be drawn up. However, some relevant observations have been made. For example, Callow[1] observed that 5° is the curing temperature at which most nitrite accumulates and Ingram *et al.*[2] showed that, during the practical manipulation of bacon-curing brine, the nitrite concentration falls if the salt concentration is slightly raised, and vice versa. The precise physiological effect of these changes in the conditions is still unknown.[3]

Experimental

Methods

All the experiments were carried out on the same sample of cover brine, which was stored at −3°. They were all made on a laboratory scale and no attempt was made to simulate factory conditions either with regard to the volume of brine used or the presence of meat. Between the taking of the sample mentioned above and the taking of the bulk sample for experimental use, the salt content had been raised to 25·4% and the pH had risen to 6·9: the total count was still $2{\cdot}5 \times 10^8$/ml. Before use, the total volume required for an experiment was diluted to 80% strength with water to give a salt concentration of 20% and solid sodium chloride was added when it was desired to bring the salt concentration back to 25%. In this way the

* Present address: British Joint Services Mission, Washington, U.S.A.

concentrations of all the other constituents of the brine were kept at the same level in the different experimental brines. The brine mixtures were incubated in 50-ml. quantities in loosely capped 100-ml. bottles. The bottles were gently shaken before the removal of samples but were otherwise left undisturbed.

Nitrite determinations were carried out as described by Eddy[4] after the protein had been removed by adding dialysed iron and filtering.

The bacterial populations in the experimental brines were determined during the course of the experiment in three ways:

(1) by total microscope count under phase contrast;[5]
(2) by surface count on nutrient agar containing 10% NaCl, incubated at 25° for 4 days;
(3) by surface count on pork-extract salt agar, prepared as described by Jespersen & Riemann[6] but with the nitrate omitted, incubated at 25° for 15 days.

Results

A preliminary experiment, in which both the rate of destruction of added nitrite and the accumulation and disappearance of nitrite produced from added nitrate (1% KNO_3) were followed, indicated that the rates of the reactions were roughly doubled by raising the temperature from 5° to 10° and halved by increasing the salt concentration from 20% to 25%.

These results were confirmed by later experiments (Series 1) in which nitrate (1%) or nitrite (500 p.p.m. of $NaNO_2$ = 100 p.p.m. of N) was added to the brine, which was analysed at intervals to determine nitrite accumulation (Fig. 1) and disappearance (Table I) respectively. The results of this experiment demonstrate clearly the effects of salt concentration and temperature on the metabolism of nitrate and nitrite in the brine. The most rapid changes take place at 10° in 20% salt and the least rapid at 5° in 25% salt. Increasing salt concentration and decreasing temperature both lower the metabolic rate and vice versa. In the experiment with added nitrate total microscope counts of the bacterial populations made simultaneously with the nitrite determinations showed an increase in population density during the course of the experiment. The multiple increases in population density under the four experimental conditions were ×2·0 at 5°/25% salt, ×3·2 at 5°/20% salt, ×3·5 at 10°/25% salt, and ×3·9 at 10°/20% salt, but there is no indication from this experiment (Fig. 1) that comparable increases in the rates of metabolism occurred.

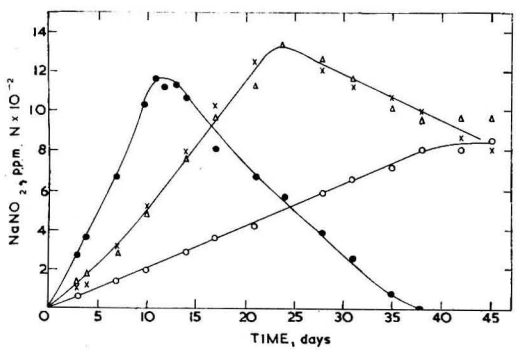

FIG. 1.—*Accumulation and disappearance of nitrite produced from KNO_3 (1%) in a bacon-curing brine at two temperatures and two salt concentrations*

●——● 10°/20% NaCl ×——× 5°/20% NaCl
△——△ 10°/25% NaCl ○——○ 5°/25% NaCl

However, the results in Table I indicated that, when nitrate was added to the brine, the rate of destruction of nitrite following accumulation was greater than the rate of destruction of nitrite added alone. Hence, in another experiment (Series 2) the effect of 'adapting' the brine population to nitrate and nitrite was investigated. A sample of brine containing 0·1%

of KNO_3 was incubated at +5° and the accumulation and disappearance of nitrite followed. When all the nitrite had disappeared (i.e., after 22 days) the experiment described above was repeated with the adapted brine. The corresponding results are shown in Fig. 2 and Table II: a comparison of Figs. 1 and 2 will show the general similarity of the results. The most striking difference from the Series 1 experiment was the diminution, following adaptation, of the rate of destruction of nitrite both when added as such and when produced from nitrate (cf. Tables I and II).

The apparent lack of correlation between increases in bacterial numbers and rate of metabolism was investigated in a further experiment (Series 3) with brines having different initial population densities. The brine was diluted to 20% salt and some of it Seitz-filtered. This sterile brine was then used to dilute the unfiltered brine (brine A) so as to give samples containing one-third (brine B) and one-tenth (brine C) of the bacterial population in the unfiltered brine. Potassium nitrate (1%) was dissolved in both the diluted samples and in unfiltered brine and these experimental mixtures were incubated at 10°. Samples were taken at intervals for nitrite determinations and bacterial counts were also made as already described. The nitrite accumulation curves for the three treatments are shown in Fig. 3 and the mean daily increases in nitrite content are given in Fig. 4. The bacterial counts by the three methods employed are given in Fig. 5.

Table I

Rates of accumulation and destruction of nitrite in a bacon-curing brine at two temperatures and two salt concentrations

(results given as p.p.m. N/day)

Temp., °C	Salt concn., %	1% KNO_3 added to brine		500 p.p.m. $NaNO_2$ added to brine
		Mean rate of accumulation of $NaNO_2$	Mean rate of destruction of $NaNO_2$	Mean rate of destruction of $NaNO_2$
5	25	18·3	—	7·4
10	25	56·7	25	12·8
5	20	56·7	25	14·4
10	20	110	44	31·0

Table II

Rates of accumulation and destruction of nitrite in an 'adapted' bacon-curing brine at two temperatures and two salt concentrations

(results given as p.p.m. N/day)

Temp., °C	Salt concn., %	1% KNO_3 added to brine		500 p.p.m. $NaNO_2$ added to brine
		Mean rate of accumulation of $NaNO_2$	Mean rate of destruction of $NaNO_2$	Mean rate of destruction of $NaNO_2$
5	25	13·2	—	4·4
10	25	37·2	11·5	6·4
5	20	57·7	11·5	9·8
10	20	133	33·3	13·4

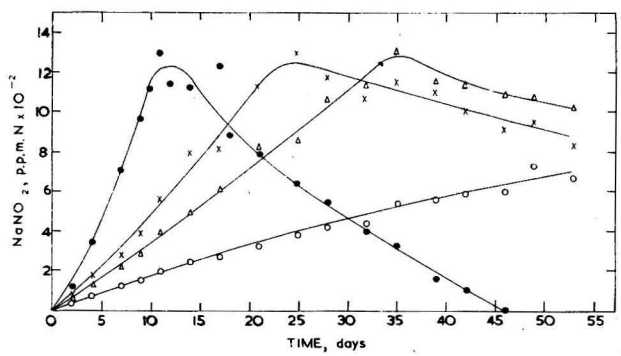

FIG. 2.—*Accumulation and disappearance of nitrite produced from KNO_3 (1%) in an 'adapted' bacon-curing brine at two temperatures and two salt concentrations*

●——● 10°/20% NaCl
△——△ 10°/25% NaCl
×——× 5°/20% NaCl
○——○ 5°/25% NaCl

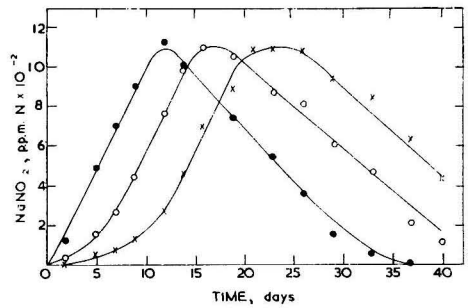

FIG. 3.—*Accumulation and disappearance of nitrite produced from KNO_3 (1%) in a bacon-curing brine at three population densities (10°/20% NaCl)*

●——● Brine A—full brine population
○——○ Brine B—1/3 brine population
×——× Brine C—1/10 brine population

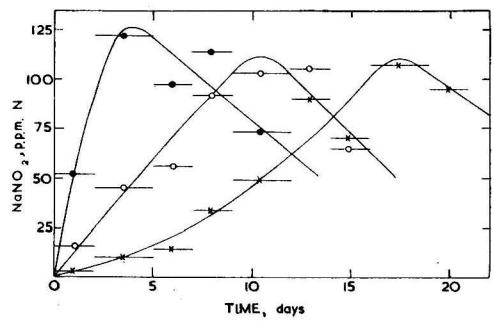

FIG. 4.—*Mean daily increase in nitrite content of brines with different initial population densities (10°/20% NaCl)*

●——● Brine A—full brine population
○——○ Brine B—1/3 brine population
×——× Brine C—1/10 brine population

Discussion

It is of obvious, practical importance to know whether the chemical changes in a brine are caused by all the cells, or by only a special group of bacteria detected by a viable count on some particular medium. This is especially so when there is a large discrepancy between total and viable counts as in bacon brines.[5] Unfortunately, in addition to the problem of the nature of the micro-organisms concerned, interpretation of the metabolic changes here discussed is difficult because of uncertainty as to the actual rates involved.

As mentioned earlier, when nitrate and nitrite are both being reduced simultaneously, it is difficult to determine the absolute rate of each process. If nitrite reduction takes place more rapidly than nitrate reduction, nitrite does not accumulate, and if the reduction of nitrate is the more rapid process, then the nitrite which accumulates is the difference between that produced and that destroyed. In the Series 1 and 2 experiments in which nitrate was added, stoicheiometric recovery of nitrate as accumulated nitrite would have corresponded to 1400 p.p.m. of N and it is therefore clear that at 5°/20% salt and 10°/25% salt (Figs. 1 and 2) the recovery of nitrate approaches so nearly the theoretical as to suggest that very little destruction of nitrite took place while any nitrate remained unreduced. Hence the more closely the peak value for nitrite approaches the theoretical, the more nearly the rate of nitrite accumulation approximates to the rate of nitrate reduction. At 10°/20% salt in the Series 1 experiment,

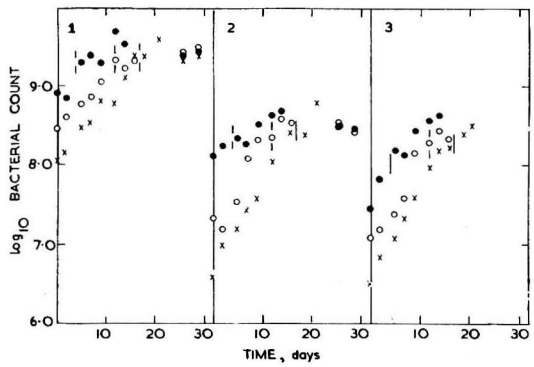

FIG. 5.—*Growth of bacteria, estimated by three methods of counting, in brines, with added KNO_3 (1%), having different initial population densities* (10°/20% NaCl)

1—total microscope count ; 2—count on pork extract salt agar ; 3—count on nutrient agar + 10% salt
Vertical lines show the points at which the maximum rate of nitrite accumulation was reached in each brine

● Brine A—full brine population
○ Brine B—1/3 brine population
× Brine C—1/10 brine population

however, the nitrite does not approach the theoretical and it must therefore be assumed that some destruction of nitrite occurred simultaneously with production. Under the same conditions in the Series 2 experiment, however, the nitrite approached more closely to the theoretical, which may be a reflection of the reduced ability of the adapted brine to destroy nitrite.

To investigate in greater detail the finding that increases in population density did not seem to be accompanied by increases in activity, the experiment (Series 3) illustrated in Figs. 3–5 was made on brines with reduced initial bacterial populations so as to permit more multiplication during the experiment. Comparison of Figs. 3 and 4 suggests that a short period of 'adaptation' was necessary before the attainment of the full rate of nitrite accumulation of which the initial population was capable. After this period of adaptation, although there was an increase in the total count of the system with the undiluted population (A), there was no further increase in the metabolic rate. In the systems with reduced populations (B and C) the period of adaptation was followed by a period during which the rate of nitrite accumulation increased exponentially until it reached the rate in system A (day 12 in B, day 17 in C). There was then no further increase in rate although, as in A, there were subsequent increases in population.

If the assumption is made that a short period of adaptation is necessary (the initial brine had been devoid of nitrate for some time), and the first two points of each graph in Fig. 4 are accordingly ignored, it is possible to estimate the 'initial' rate of accumulation of nitrite by plotting the increases logarithmically and extrapolating to zero. The values found for B and C are 22 and 4·5 p.p.m. of N/day, giving 5-fold and 24-fold increases, respectively, in the rate of nitrite accumulation during the course of the experiment. These increases occurred in 12 days in B and 17 days in C. For comparison with these increases the increases in population, by the three methods of counting used, are shown in Table III. It will be seen that the increases in metabolic activity are more closely related to the total than to the viable counts, and that there is little difference in this respect between the viable counts on media with 10% or 20% salt. This interpretation is confirmed in another way. If the estimated rates of nitrate reduction per cell, computed from the numbers of cells determined by the three methods of counting (Table IV), are compared with those quoted for pure cultures,[5] it appears that the rate of nitrite production is better related to the total than to the much smaller viable counts. Even with the special media used in the present work, only about 10% of the total visible bacteria were

Table III

Bacterial populations at the start of the experiment (1) and when the maximum rate of nitrite accumulation is reached (2) together with the multiple increases in population density

Method of counting	Bacterial population ($\times 10^7$/ml.) (1)			Bacterial population ($\times 10^7$/ml.) (2)				Multiple increase in population		
	Brine A	Brine B	Brine C	Brine A	Brine B	Brine C	Mean	Brine A/o–4 days	Brine B/o–12 days	Brine C/o–17 days
Total microscope count	90	29	12	160	200	250	200	×1·8	×6·9	×21
Pork extract salt agar	13	2·3	0·41	20	32	26	26	×1·5	×14	×63
Digest agar +10% salt	2·9	1·2	0·34	11	20	21	17	×3·7	×17	×50

Table IV

Rate of nitrite accumulation in terms of the numbers of cells present, as determined by the three methods of counting, compared with data obtained using washed suspensions of pure cultures[5]

(R = μmoles of nitrite accumulating/10^{11} cells/h.)

Method of counting	Data from Series 1 experiments: Initial population density ($\times 10^7$/ml.)	R at 5°/25% salt	R at 10°/20% salt	R at 15°/20% salt	R at 10°/20% salt	Data from Series 3 experiments: Mean population density when maximum rate of accumulation reached ($\times 10^7$/ml.)	R at 10°/20% salt	Data from Ingram[5]: R
								Micrococcus
Total microscope count	73	8·3	25	25	45	200	19	5°/3% salt 2·5 25°/3% salt 17
Pork extract salt agar	16	38	113	113	203	26	160	Gram-positive rod 3/3 5°/10% salt 0·6 25°/10% salt 5
Digest agar +10% salt	9·6	63	189	189	340	17	240	*Bact. coli* 37°/10% salt 110

recovered as viable. The rates, on the basis of the viable counts, seem high even when compared with that for *Bact. coli* which is well known to have a high metabolic rate ; and unreasonably high compared with those observed for brine organisms even under favourable conditions. A similar situation has been reported for the reduction of sulphate in fermenting sewage sludge, where the viable count was only 10^{-3}–10^{-4} of that expected from the rate of sulphate reduction by pure cultures.[7] The results of the Series 3 experiments suggest that here the nitrate–nitrite conversion was related to a group of bacteria representing at least the majority of the total number of cells present. If this relationship exists, it is closest at that point in time when the rate of accumulation of nitrite reaches its maximum : after this, in both the Series 1 and Series 3 experiments, there were increases in the counts with no accompanying increase in metabolic activity.

It may not always be the case that the metabolic activity is most closely related to the total count. In a normal brine, Kitchell (unpublished) observed that during 9 weeks the concentration of nitrite fell and there was a parallel decrease in the viable counts on the medium with 20% salt ; while, during the same period, the total counts and those made on a medium with 10% salt remained unchanged.

The brine here studied was abnormal for a bacon tank brine : it resembled the beef-curing brines described by Hornsey & Mallows,[8] and the ham-curing brines of Sulzbacher[9] in that all

have exhibited accumulation of nitrite to a very high peak value, followed by a fall; with a correspondingly rapid utilisation of nitrate. Such behaviour is unsuitable for the cyclic operations of bacon curing, where the brine is usually fairly stable at a moderate nitrite concentration. It was hoped that this investigation might throw some light on the reasons for this important difference between the two types of brine. It is at least probable from the experiments that such differences are not simply a function of salt content since the same general pattern of behaviour was repeated at different salt concentrations and temperatures. Nevertheless, it is possible on the basis of the results obtained in these experiments to put forward a hypothesis to explain the effect of small changes in salt concentration on the nitrite content of bacon brines. While there remains any nitrate unreduced, bacterial destruction of nitrite is negligible—hence disappearance of nitrite is very largely due to absorption by the sides of bacon. The nitrite content of the brine is therefore the resultant of that produced from nitrate less that absorbed and there is a salt concentration at which these two processes are balanced. This concentration will depend on temperature and on the size and nature of the bacterial population. If the salt content falls, more nitrite will be produced than can be absorbed and the nitrite concentration will tend to rise: if the salt content rises, less nitrite will be produced than can be absorbed and the nitrite concentration will tend to fall. This hypothesis is only tentative and develops in one direction the suggestions made by Ingram *et al.*[2] Factory experiments on a pilot scale would be needed to test its validity.

Low Temperature Research Station
Cambridge

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ROUTINE ANALYSIS OF CARBOHYDRATES AND LIGNIN IN HERBAGE

By R. E. DERIAZ

An approximate analytical method is described for the rapid routine determination of carbohydrates and lignin in herbage. Dried milled samples are extracted successively with diethyl ether, 0·5% ammonium oxalate solution, N-H_2SO_4, and 72% H_2SO_4. Soluble carbohydrates in the ammonium oxalate extract are determined with anthrone, and hydrolysed pentosans and hexosans in the combined N-H_2SO_4 and 72% H_2SO_4 extracts are determined with aniline acetate and chromotropic acid respectively. The method gives a maximum variation of 4% from the mean of duplicates with each carbohydrate component. The residual material is ashed to obtain crude lignin, and a nitrogen correction applied.

Introduction

Measurements of the nutritive value of animal feeds have long been made by the system of proximate analysis. The arbitrary nature of this system is well known,[1] particularly in the division of the carbohydrates into crude fibre and nitrogen-free extractives. In 1930, Waksman & Stevens[2] attempted to fractionate the carbohydrates of plants by graded extraction with alcohol and mineral acids. Norman & Jenkins[3] introduced a method of measuring the cellulosic framework of forages, based on the chlorination method of Cross & Bevan[3a] for wood. The determination of ' cellulose ', lignin, and ' other carbohydrates ' in nutritional studies was advocated by Crampton & Maynard.[4] Quantitative paper chromatography was used by Laidlaw & Reid[5] to examine the alcohol-soluble fraction of the carbohydrates of grass, and the technique has been further developed to determine the water-soluble and structural carbohydrates of grasses and lucerne.[6-11] As chromatographic methods are lengthy, the present work has been carried out to devise a shorter method suitable for routine use.

Soluble carbohydrates

It is convenient for purposes of analysis to group together as ' soluble carbohydrates ' those carbohydrates of herbage which in the plant are in solution, together with those which function as undissolved reserves for plant growth, as is the case with starch in legumes. This group, which includes sugars, oligosaccharides, and the fructosans of grasses, provides a source of nutrients readily available to livestock.

The soluble carbohydrates of grass[12] and of lucerne[9] have been determined by successive extractions with aqueous alcohol and with hot water, and a method for the determination of glucose, fructose, sucrose and fructosans in the two extracts from grass has been worked out by de Man & de Heus.[13] In the present method the soluble carbohydrates are extracted and determined as a single fraction.

Structural carbohydrates

The remaining carbohydrates, which comprise the plant cell-wall, are the β-1,4-glucan cellulose, the hemicelluloses, which include other polymeric sugars and possible combinations of these with glucuronic acid, and pectin (polygalacturonic acid). The structural carbohydrates contain glucose, xylose, arabinose and galactose, together with mannose in lucerne.

The determination of both ' hemicellulose ' and ' cellulose ' is hampered by lack of a precise definition of these compounds. For example, short chains of cellulose[9] may be included in such arbitrary definitions of hemicellulose as solubility in 17·5% NaOH or 24% KOH.[14] Similarly ' cellulose ' may have different meanings according to whether it is defined solely as a glucose polymer, or defined in terms of insolubility in alkali or in other media, as in the determination of ' α-cellulose ' and in the methods of Norman & Jenkins[3] or of Crampton & Maynard.[4] Determination of the reducing power of acidic extracts of the structural carbohydrates has also been used, further characterisation[15] of the fractions being carried out by determinations of yield of furfural, or by changes in the reducing power after fermentation with yeast.

The structural carbohydrates need not be divided into fractions based on arbitrary solubility properties if they are expressed as pentosan and hexosan, and this mode of expression is used in the present work. Pectin and the glucuronic acid of the hemicelluloses are not determined in the present analytical scheme.

Few direct methods have been developed for the estimation of pentosans and hexosans in mixtures. Anthrone may be used to determine pentoses[16] or hexoses[17] separately, but there is interference when mixtures of these sugars are determined.[18] An orcinol reagent was used[19] for the simultaneous determination of pentose and hexose in bacterial cell hydrolysates, but the method required careful control of time and temperature of heating. Tracey[20] described a method for determining pentoses in the presence of hexoses and uronic acids at room temperature, and Klein & Weissman[21] introduced a method to estimate hexoses in blood serum. In the present analytical scheme the last two methods have been used for the specific determination of pentoses and hexoses in acid hydrolysates of the total structural carbohydrates of herbage.

Lignin

The determination of lignin is necessarily empirical in view of the variation in composition of lignin in plants,[22] and also because of the difficulty of deciding if contaminants are retained when lignin is separated from plant material, or if the whole of the lignin has been isolated.

Experimental

Development of method

(1) *Preparation of herbage samples*

The oven-drying of herbage requires careful control if losses of dry matter or enzymic changes are to be minimised. Waite & Boyd[12] showed that herbage samples dried in a forced-draught oven at 95° suffered little change in chemical composition from fresh herbage. Raymond & Harris[23] found that with the forced-draught Unitherm oven at 100°, dry-matter losses of the order of 1% only can be achieved if the oven is carefully loaded.

To test the possible effects of oven-drying, the soluble carbohydrate contents of herbage samples were determined in two ways, (*a*) by extraction of fresh herbage with boiling water and (*b*) by boiling-water extraction of similar herbage dried in the Unitherm oven at 100°. The soluble carbohydrate contents of three herbages examined by these methods, respectively, were: 28·0 and 27·5%; 11·7 and 12·3%; 15·2 and 14·6%. These results indicate little difference between analyses based on fresh or oven-dried herbage. The preparation of herbage by oven-drying and milling before analysis has therefore been adopted because it is simpler and because sampling errors are smaller.

(2) *Solvent extraction*

Removal of fats, waxes and pigments is carried out with diethyl ether rather than with alcohol–benzene as the latter extracts some of the sugars. A 72-h. period is used to achieve maximum extraction.

(3) *Extraction of soluble carbohydrates*

The time required for complete extraction of the water-soluble carbohydrates in herbages was investigated by extracting ether-extracted samples with 150 ml. of water under reflux conditions for periods varying from 1 min. to 3 h. Although removal of soluble carbohydrates from grasses can be carried out with cold water,[6] hot water removes in addition some araban and galactan.[6] Comparison of cold- and hot-water extracts in the present experiments showed a slightly greater content of soluble carbohydrates in the latter. Thus cold-water extraction of ether-extracted ryegrass gave 18·7% of soluble carbohydrates compared with 19·5% by a 2-h. hot-water extraction.

The removal of starch from lucerne requires hot-water extraction.[9] Conditions for complete removal of starch were not investigated in the present experiments, but very little increase in apparent soluble carbohydrate content could be obtained by prolonging the period of hot-water extraction of lucerne beyond 75 min. (Table I) and the small increase that was noted may have been partly due to progressive extraction of galactan. In the later stages of this study, soluble carbohydrates were extracted by hot 0·5% ammonium oxalate solution for 2 h., in place of hot water. Pectin is completely extracted by this solution, so that possible interference by pectins with the colorimetric reagents for pentoses and hexoses (see below) is avoided. While small amounts of polysaccharides associated with pectin are also extracted by oxalate,[24] these produce only a slight augmentation of the apparent soluble-carbohydrate content, and are not determined separately in the present method.

(4) *Determination of soluble carbohydrates*

The method of Fairbairn[25a] was followed, except that the anthrone reagent was stabilised with thiourea[25b] and heating on the boiling water-bath was carried out for 20 min., instead of the 12 min. recommended by Fairbairn, in order to obtain equivalent colour intensities from glucose and fructose.

Table I

Soluble carbohydrate content of ether-extracted herbage determined after extraction with hot water for varying times

(% of herbage dry matter)

Time of extraction (min.)	1	10	20	30	45	60	75	120	180
Ryegrass	19·0	—	—	19·4	—	19·6	—	19·5	19·8
Lucerne	3·37	3·49	3·58	—	3·66	—	3·79	3·80	—

Although Yemm & Willis[17] reported that galacturonic acid gave 10% of the colour yield of glucose with anthrone reagent, it was found that under the different analytical conditions of the present work, galacturonic acid gave only 4% of the colour yield of glucose. Thus in a sample of lucerne containing 10% uronic acid (of which 75% is extractable by ammonium oxalate[9]), the colour developed from this source would be equivalent to 0·3% of glucose, and can usually be neglected.

Grasses have a lower pectin content than lucerne (2–4%)[11b] and errors due to uronic acid from pectin in the determination of the soluble carbohydrates with anthrone are negligible.

(5) *Extraction and determination of structural carbohydrates*

Removal of the structural carbohydrates from 0·5% ammonium oxalate-extracted herbage was carried out by modifying Harwood's two-stage extraction method.[7] Extraction with N-H_2SO_4 was followed by extraction with 72% H_2SO_4, but secondary hydrolysis after dilution of the latter was carried out after the addition of water and combining the solution with the N-H_2SO_4 extract to make the acid concentration of the resultant solution N. The effect of time of treatment with 72% H_2SO_4 on the apparent hexosan and pentosan contents of ryegrass and lucerne is shown in Table II, from which it is seen that a period of 4 h. is desirable, as was found by Harwood. The time of secondary hydrolysis could be varied from 2 to 4 h. without any change in the results obtained for pentosan and hexosan. Pentoses were determined in the filtered extract (after further dilution of the solution) with Tracey's reagent,[20] modified slightly by reduction of the amount of oxalic acid to avoid crystallisation of aniline oxalate from the reagent during storage.

To determine hexoses in the filtered extract, the method of Klein & Weissman[21] was used, except that, after heating the test solutions on the water-bath, they were not diluted with 9M-H_2SO_4. This modification increased the sensitivity, without affecting either the linearity of response up to 0·3 g. of hexose per litre or the colour intensities given by galactose and mannose relative to glucose.

Errors involved in the determination of the pentosan and hexosan contents.—Pentoses are determined in the structural carbohydrate hydrolysate against a pentose standard of 70% xylose and 30% arabinose, although the proportions of xylose to arabinose in herbage show some variation. Examination of the data of MacKenzie & Wylam[8] for ryegrass first cuts and aftermath showed that for the combined leaf and stem, polyxylose accounted for 73–82% of the pentosans. In the case of lucerne, Hirst *et al.*[9] showed that polyxylose accounted for 45–82% of the pentosans. There were only four values with less than 60% polyxylose and these were for young lucerne whose total pentosan content was also the lowest. Since arabinose gives only 50% of the colour produced by an equal weight of xylose under the conditions of determination used, some error can arise from the use of the above standard. This will lead to an under-estimate of the pentosan content in young lucerne up to a maximum of 0·64% of the sample dry weight, and to an over-estimate of the pentosan content in more mature lucerne and ryegrass up to a maximum of 0·93% (Table III, col. 3).

An additional source of error arises from the interference of hexoses with Tracey's reagent. To assess the size of this error reference was made to the data of MacKenzie & Wylam[8] and of Hirst *et al.*[9] on the content of structural hexosans in ryegrass and lucerne (as measured by paper chromatography), and also to the relative colour intensities given by hexose and pentose sugars (Tracey[20]). It was calculated that interference by hexoses would result in an over-estimate of the pentosan content by 0·26–0·86%, depending on the age of the herbage (Table II, col. 4).

Table II

Pentosan and hexosan contents of herbage determined after different times of hydrolysis with 72% H_2SO_4

(% of herbage dry matter)

Time of hydrolysis after dilution, 2 h.

Herbage	Time in contact with 72% H_2SO_4, h.	Pentosan	Hexosan
Ryegrass	2	14·3	26·3
	4	14·7	26·8
Lucerne	2	8·3	24·9
	4	8·6	24·6

Table III

Errors in the determination of structural carbohydrates of herbage by specific colorimetric reagents

(% of herbage dry matter)

	(1) Pentosan, %	(2) Hexosan, %	(3) Error due to standard	(4) Error due to interference from hexose or pentose	(5) Error due to hydrolysis losses	(6) Error due to uronic acids	(7) Total error
Young ryegrass[8]	3·8		+0·02	+0·30	−0·19	Nil	+0·13
		8·2	+0·20	+0·19	−0·41	+0·29	+0·27
Mature ryegrass[8]	11·46		+0·83	+0·68	−0·57	Nil	+0·94
		23·29	+0·55	+0·57	−1·16	+0·71	+0·67
Young lucerne[9]	4·22		−0·64	+0·26	−0·21	Nil	−0·59
		9·56	−0·44	+0·21	−0·48	+0·51	−0·20
Mature lucerne[9]	12·72		+0·93	+0·86	−0·64	Nil	+1·15
		32·03	−0·75	+0·64	−1·60	+0·51	−1·20

The error on a dry-matter basis is greatest with more mature herbages, which contain the greater percentage of cellulose : they also contain a larger percentage of pentosans, so that the relative error is decreased.

The structural hexosan content was determined with the chromotropic acid reagent of Klein & Weissman.[21] In the present studies the addition of pentose standard solution to the hexose standard caused an augmentation of colour produced, and some colour was produced by pentose alone. This indicated that the chromotropic acid reagent is not entirely specific for hexoses. The maximum error which arises from this source is 0·64% for the samples investigated (Table III, col. 4). The standard hexose solution used contained 90% glucose and 10% galactose. Inspection of analytical figures for the structural carbohydrates of whole ryegrass[8] showed that polyglucose (cellulose) accounted for 95·5–98·3% of the structural hexosans. In lucerne structural carbohydrates,[9] where mannose is also present, polyglucose (cellulose) accounted for 83–91% of the structural hexosans. In the present work galactose and mannose gave 69·4% and 51·3% respectively of the colour yield of an equal weight of glucose with the chromotropic acid reagent. Calculation of the error introduced by use of the hexose standard described, showed that the ryegrass hexosans were liable to be over-estimated by up to 0·55% of herbage dry matter and lucerne hexosans to be under-estimated by up to 0·75%, the errors in each case being greatest with the more mature herbages (Table III, col. 3). Harwood[7] reported losses of the order of 5% in the two-stage hydrolysis procedure, and, on the assumption of similar losses, the errors arising from this source were calculated for ryegrass and lucerne (Table III, col. 5).

Interference from non-pectin uronic acids in the colorimetric determination of structural pentosan and hexosan was also investigated as a possible source of error. Glucuronic acid (the main uronic acid present) probably occurs in the hydrolysate as aldobiuronic acid. Tracey[20] has shown that colour formation from galacturonic acid is low and structural uronic acids are likely to yield little colour with the aniline acetate reagent. Xylose forming the non-uronic acid moiety of aldobiuronic acid will not be estimated by Tracey's reagent, since substituted pentoses give little colour.[20]

The possible interference of non-pectin uronic acids (as glucuronic acid) with the chromotropic acid reagent has been found in the present studies to be 17% of the hexose standard. Referring to the previous example, in a sample of lucerne containing 10% of uronic acid of which 25% is non-pectin uronic acid, there is an over-estimate of 0·57% in hexosan content from this interference. In the absence of other information, the same values for uronic acid interference in hexosan determinations have been assumed for young and for mature lucerne (Table III, col. 6). In ryegrass, interference by structural uronic acids in hexosan determinations has been assessed on the basis of a content of 1·7% and 4·2% of ' hemicellulose uronic anhydrides ' given by Waite & Gorrod[11b] for young and mature ryegrass (Table III, col 6).

The total errors which may be expected in the colorimetric determination of the structural carbohydrates have been calculated as the sum of the contributory errors discussed above (Table III, col. 7).

(6) *Determination of lignin*

The residue from the two-stage hydrolysis contains lignin and any unremoved nitrogenous material. Thomas & Armstrong[26] showed that similar values for ' true ' lignin were obtained when the residual N, calculated as crude protein, was subtracted from crude lignin obtained either by the methods of Norman & Jenkins[27] or of Ellis *et al.*[28] In the present analytical method, a value for lignin was obtained by determining the loss in weight on ignition of the residue, and a correction applied for N × 6·25 after Kjeldahl analysis of the residue of another portion of the same herbage.

Proposed analytical method

Reagents

(1) *Anthrone reagent.*[25]—Add 760 ml. of conc. H_2SO_4 (AnalaR) to 330 ml. of water, cool to room temp. and make up to 1 l. at 20° with water. Dissolve 1 g. of thiourea and 1 g. of anthrone successively in this solution, and store the reagent in the refrigerator.

(2) *Tracey's reagent*[20] *(modified).*—Add 16 ml. of colourless aniline to 100 ml. of glacial acetic acid and stir. Add 24 ml. of water and 5 ml. of 5% aqueous oxalic acid. (The reagent is most satisfactory if prepared immediately before use.)

(3) *Chromotropic acid.*[21]—Add 395 ml. of conc. H_2SO_4 (AnalaR) to 130 ml. of water, cool and dilute to 500 ml. with water (this gives 15M-H_2SO_4). Dissolve 0·5 g. of chromotropic acid sodium salt (analytical reagent grade for formaldehyde determinations) in 5 ml. of water, and add 250 ml. of 15M-H_2SO_4. (Although the reagent is most satisfactory when fresh, it may be stored in a refrigerator for one or two weeks.)

(4) N-H_2SO_4.

(5) 72% w/w H_2SO_4.—Add 326 ml. of conc. H_2SO_4 to water to make 500 ml. of solution.

(6) *Silicone solution.*—Dissolve 20 g. of Silicone Fluid Antifoam ' A ' (Hopkin & Williams, Ltd.) in 1 l. of carbon tetrachloride.

Standard solutions

(1) Glucose solution containing 0·200 g. of glucose per litre.

(2) Pentose solution (of 0·100 g. per litre) containing 0·070 g. of xylose and 0·030 g. of arabinose per litre.

(3) Hexose solution (of 0·200 g. per litre) containing 0·180 g. of glucose and 0·020 g. of galactose per litre.

The standards are prepared weekly and are stored in a refrigerator.

Procedure

Dry a sample of the cut herbage in a forced-draught oven at 100° for 8–12 h. and grind in a laboratory mill to pass a 0·8-mm. sieve. Dry approx. 1 g. of ground herbage in a 20 × 50 mm. glass tube at 100° for 3 h. Weigh to the nearest mg. and transfer the sample to a folded 12·5-cm. Whatman No. 541 (hardened) filter paper and reweigh the tube. Extract the sample with diethyl ether in a Soxhlet extractor for 72 h., remove it from the extractor, and let it air-dry. Brush the sample from the filter paper into a 500-ml. flask (flask F). Add 150 ml. of 0·5% ammonium oxalate solution and 6 drops (more if necessary) of silicone solution to suppress frothing, and boil under reflux for 2 h. Allow the solution to cool. Add approx. 1 g. of ashed Celite 545 (Johns-Manville) filter-aid and transfer the solution to a 250-ml. centrifuge tube. Rinse flask F with about 50 ml. of water and transfer the washings to the centrifuge tube. Centrifuge the solution for 5–10 min. at 2200 r.p.m. (1925 *g*) and carefully decant the supernatant into a 1-l. standard flask. Add about 100 ml. of water to the residue, stir and centrifuge again. Add the supernatant to the standard flask and make the solution up to volume with water and filter about 30 ml. through a 15-cm. Whatman No. 44 filter paper. Use the filtrate (solution A) for determination of soluble carbohydrates.

Transfer the residue from the centrifuge tube into flask F with 67 ml. of N-H_2SO_4 used in three or four portions. Add approx. 3 drops of silicone solution and boil under reflux for 1 h. Allow to cool, transfer the solution to a 250-ml. centrifuge tube with 67 ml. of water in several portions, and centrifuge. Transfer the supernatant to flask F. Wash the residue with 105 ml. of water, centrifuge, and add the supernatant to flask F. Set aside flask F and contents. Wash the residue with about 100 ml. of acetone, centrifuge and discard the acetone layer.

Allow the residue to dry overnight at about 40° in an oven, with the centrifuge tube placed on its side. Break the residue into a powder with a spatula and remove the last traces of solvent at 100° for 15 min. Allow to cool to room temperature. Add 12 ml. of 72% H_2SO_4, mix thoroughly with a glass rod until the mixture is entirely free from lumps, and set aside for 4 h. at room temperature (~20°) with occasional stirring. With 105 ml. of water, used in three or four portions, wash the material in the centrifuge tube into flask F. Add approx. 6 drops of silicone solution and boil the liquid under reflux for 2 h. Allow to cool and filter the solution through a sintered glass crucible (porosity 1), with the minimum suction necessary in order to obtain a clear filtrate. Wash the residue with about 150 ml. of water. Transfer the combined filtrate and washings to a 1-l. standard flask, make up to volume and use this solution (solution B) for determination of the structural carbohydrates. Wash the residue with about 50 ml. each of acetone and diethyl ether. Dry the crucible to constant weight at 100°, ignite at 450° and reweigh.

Run a separate portion of the original herbage through the stages described to the point of filtration of the acid-insoluble residue. Then isolate the residue by centrifugation, wash successively with water and acetone, dry, and determine the nitrogen content by the Kjeldahl method.

Colorimetric determinations

(1) *Soluble carbohydrates.*—Pipette 2 ml. of the test solution A in duplicate, 2 ml. of the glucose standard in triplicate, and 2 ml. of water (as blank) into 1 × 6 in. boiling-tubes. Add 10 ml. of anthrone reagent slowly to each tube, with cooling in a beaker of tap water. Cover the boiling-tubes and heat on the water-bath at 100° for 20 min. Cool in tap water for 10 min. and measure the colours (which are stable) at 625 mμ against the reagent blank in a 1-cm. cell with a Unicam SP.600 spectrophotometer.

(2) *Structural carbohydrates—pentosan.*—Dilute 5 ml. of the test solution B with an equal volume of water. Pipette 2 ml. of the diluted test solution in duplicate, 2 ml. of the pentose standard in triplicate and 2 ml. of water (as blank) into 1 × 6 in. boiling-tubes. Add 6 ml. of Tracey's reagent to each boiling-tube. Ignore any coloration which may appear immediately. Keep the tubes overnight at room temperature. Measure the colours (which are stable) at 472 mμ against the reagent blank as above.

(3) *Structural carbohydrates—hexosan.*—Measure 2 ml. of the test solution B in duplicate, 2 ml. of the hexose standard in triplicate and 2 ml. of water (as blank) into 1 × 6 in. boiling-

tubes. Add 10 ml. of chromotropic acid reagent to each boiling-tube and cover them. Heat on the water-bath at 100° for 30 min. Cool in tap water and measure the colours (which are stable) at 570 mμ.

Calculations

(1) *Soluble carbohydrates.*—Colour intensities for glucose solutions up to 0·3 g. per litre are linearly related to concentration, so that the concentration of a test solution is calculated by relating its optical density directly to that of the standard. The results are expressed as % glucose of the sample dry matter; no allowance is made for the presence in the original herbage of some glucose and fructose partially in the anhydroform (cf. determination of the 'total available carbohydrates', Weinmann[29]).

(2) *Pentosan.*—The concentration of pentose in the diluted test solution B is calculated by directly relating the optical density of the developed coloration to that of the standard, since there is a linear relationship between optical density and pentose concentration up to 0·120 g. per litre. The pentosan content is obtained by multiplying the (apparent) pentose content of the herbage dry matter by 0·88.

(3) *Hexosan.*—Klein & Weissman[21] showed that optical density is directly proportional to glucose concentration up to 0·3 g. per litre. The calculated (apparent) hexose content of the herbage is multiplied by 0·90 to obtain the hexosan content.

Application to herbage samples

Table IV gives analytical results by the method described, from 12 samples of first cuts of S.24 perennial ryegrass. Summation of soluble carbohydrates, pentosan, hexosan, N-free lignin,

Table IV

Soluble carbohydrate, structural carbohydrate and lignin contents of first cuts of S.24 perennial ryegrass

(% of herbage dry matter)

Date of cutting (1958)	Soluble carbo-hydrates	Pentosan	Hexosan	N-free lignin
22 April	18·0	8·3	16·7	2·81
30 April	16·3	9·4	18·4	2·49
2 May	16·4	11·2	21·1	3·08
8 May	18·7	13·3	20·4	3·22
13 May	19·2	14·4	24·2	4·15
15 May	17·0	14·6	24·0	4·40
19 May	18·5	16·2	23·6	4·84
23 May	20·3	14·5	21·8	4·64
27 May	21·0	18·0	25·8	5·36
2 June	19·6	17·9	24·8	6·75
9 June	19·0	20·1	28·0	7·66
16 June	20·9	20·6	29·2	7·99

ether-soluble material, crude protein and ash accounted for 87·7% of the dry matter as mean value, with extremes of 82·5% and 91·3%. Undetermined fractions included pectin, organic acids, water-soluble pigments, structural uronic acids, and xylose attached to uronic acid. Agreement between duplicates for each carbohydrate fraction was better than ±4% of the mean.

Application to other materials

In the analysis of roughages such as straws and hays containing a higher proportion of structural carbohydrates[24] than pasture herbage, it is more convenient to start with approx. 0·5 g. of material instead of 1 g.

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Grassland Research Institute
Hurley
Berks.

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VALUE OF BUSH, GRASS OR LEGUME FALLOW IN GHANA

By KHAZAN SINGH

(1) Field studies compare the effects of different periods of bush or grass fallow and of different species of grass and legume in fallows, on the nutrient status of fallow plots and on the yields of subsequent crops.

(2) Bush fallowing for 2 years was better than for 1 year, while a third year gave no advantage in respect of yield, and a 5- or 6-year period gave yields no better than did a continuous cropping system.

(3) Among grasses and legumes used as fallow cover crops, elephant grass followed, in order, by Guinea grass and pigeon pea were the most effective.

(4) At two southerly stations maize yields were increased after fallow but this was not so in three more northerly stations. The difference may depend on the type of fallow, whether grass or legume, and on the availability of nitrogen in the soil. Andropogon grass fallow did not prove so successful in the nitrogen-deficient soils of the north as did the elephant or Guinea grass in the rich soils of the southern area of forest. The pigeon pea which is less demanding on soil-nitrogen than are the grasses gave good results at one of the stations in the Guinea Savannah.

(5) The final cut of the grass burnt *in situ* and the burning of the surface soil affected the nutrient status of the soil, increasing chiefly the P, K and Mg ; there was no significant difference between the three grass fallow treatments in this respect.

Probably, the residues of grass in the 2- and 3-year fallows incorporated in the soil improved the physical condition of soil, thus contributing to the superiority of these grass fallows over the 1-year fallow in regard to yields of succeeding crops.

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Introduction

Of the three types of fallows forming the subject of this paper *bush fallow* has been prevalent in Ghana since land was first cultivated for growing food crops, while grass and legume fallows are of comparatively recent introduction. In the bush fallow system of farming the farmer fells the bush trees usually by burning (leaving the stumps of trees to send fresh shoots again) and cultivates the land for some years. The area is then abandoned for re-growth of the bush thicket, and the process repeated, the resting period of the land under bush varying from 2 to 5 or 6 years or even up to 9 to 15 years.

By burning the vegetation not only are minerals returned to the soil but the water-holding capacity and porosity of the soil are improved considerably.[1, 2] The ash of dicotyledonous shrubs and herbs composing bush thicket is high in calcium and magnesium (see Nye[3]) and the importance of these elements (especially calcium) in the acid soils of Ghana cannot be exaggerated. In addition the fallen leaves and stems after decomposition help to restore the humus of the soil depleted by arable cultivation. The weeds of cultivation, especially rhizotomous grasses such as *Imperator cylindrical*, are smothered and soil structure is restored by the root action of shrubs and herbs and by the organic matter added to the soil during the fallow. On visits to the farms the writer found the soil dark with humus and friable after the land was cleared of bush growth. Hoeing the land to a depth of only 2–3 in. left the soil in good tilth for planting. The bush fallow system of farming is simple, beneficial for maintaining soil fertility and economical in crop production. Mechanised methods of clearing of bush thicket and of cultivation suggested by some workers (Charter[4]) would only make this system complex and uneconomical.

However, two points require consideration if the system of bush fallow farming is to continue in Ghana. Firstly, long bush fallow is extravagant in the use of land when compared with other means of maintaining soil fertility and controlling erosion (Stamp[5]). Secondly, the long-term bush fallow would lead to exhaustion of soil fertility rather than its maintenance over a number of years of fallow. Nye & Bertheux[6] in their studies on Ghana soils showed that only a very small proportion (1–2%) of the total nutrients (phosphorus) was held in the lower horizons of soil (9–80 in. depth) and so, contrary to the common belief, the beneficial effect of deep-rooted bush fallows does not lie in replenishing the nutrients in the upper layers from lower depths. Thus, the longer is the bush fallow the greater the exhaustion of upper layers of soil is likely to be. Hence, a most important point in bush fallows is to reduce their length consistent with best results from the point of view of both reducing their extravagance in the use of land and maintaining the fertility of the soil.

Grass and legume fallows.—These are alternatives to bush fallows in areas where mechanisation of cultivation is necessary or in soils deficient in nitrogen and potassium (the ash of grass is higher in potassium and that of legumes in nitrogen as compared with the ash of dicotyledonous shrubs and herbs of the bush fallow). The value of grass and legume fallows in restoring depleted humus and degraded structure of soil after arable cultivation and in replenishing available nutrients is well known.[7–12] However, in Ghana soils as in other tropical soils the effects of fallows on soil and succeeding crops are uncertain and several problems including their effects on nutrient status of soil need investigation.

Information is scanty as to the optimum period of bush, grass and legume fallows in Ghana, apart from the indirect inferences drawn from other sources, quoted above. The present studies were made to ascertain the length of bush fallow required for maximum yields and to determine the effects of grass or legume fallow on yield and the nutrient status of the soil.

In 1949 and 1950 a number of field experiments were set up at various centres by the Dept. of Agriculture, Gold Coast, to deal with problems of cropping, fallowing, manuring and general soil management. These have been described by Nye and his colleagues,[6, 13] and among much other information afford evidence that crop responses to the various systems of fallowing are likely to be influenced largely by location (soil type and climate), the nature of the crops during fallow and production and by supplementary manuring, notably with nitrogen and phosphate.

The present investigations are to be regarded as extensions of these experiments. They have included (i) a sample survey of crop yields after 5–6, 3, 2 and 0 years of bush fallow at 30 farms on the Lower Densu basin (Southern Ghana), (ii) rotation experiments with grass or

legume fallow treatments alone or with N and P fertilisers in all combinations at two centres, Kwadaso and Pokoase, in southern Ghana (Forest region), and at three centres, Ejura, Wenchi and Nyankpala, in Northern Ghana (Guinea Savannah) (see Fig. 1).

FIG. 1.—*Location of experimental sites including their soils and vegetation*
(Adapted from 'Vegetation and Geological Maps of the Gold Coast')

Soils and climate

(a) *Soil characteristics*[14] *and rainfall*

*Non-sedimentary—Granite (Forest)**

Kwadaso

Cape Coast Granite (Forest Ochrosol[15]). Upper slope, 58 in. annual rainfall.
0–6 in. depth.—Yellowish brown (10YR 4/4), gravelly coarse sand.
Sub-soil.—Reddish brown, mottled sandy clay.

Pokoase

Cape Coast Granite. Upper slope, 36 in. rainfall.
0–6 in. depth.—Light brownish grey (10YR 6/2), slightly humus, loamy sand.
Sub-soil.—Very pale coarse sand with ironstone concretions at about 3 ft. depth.

Adaiso and Odupongkpehe

Cape Coast Granite. Upper slopes, variable in depth, 30–40 in. annual rainfall.
0–9 in. depth.—Sandy to light loam.
Sub-soil.—Sandy clay.

Sedimentary—Sandstone (Guinea Savannah)†

Wenchi

Upper Voltain Sedimentary formation. Medium slope, 50 in. annual rainfall.
0–6 in. depth.—Brown (7·5YR 5/4) fine sand.
Sub-soil.—Clayey sand with an ironstone concretionary pan at about 2 ft. depth.

Ejura

Upper Voltain Sedimentary formation (Savannah Ochrosol). Medium slope, 58 in. rainfall.
0–6 in. depth.—Brown (7·5YR 5/4) medium sand with no gravel.
Sub-soil.—Clayey sand with an ironstone concretionary pan at about 3 ft. depth.

Nyankpala

Lower Voltain Sedimentary formation. Lower slope, 43 in. annual rainfall.
0–6 in. depth.—Brown (7·5YR 5/4) silty sand, slightly humus.
Sub-soil.—Sandy and silty clay with an ironstone pan at about 2 ft. depth.

* There are two wet seasons, (i) major, from May to July, and (ii) minor, from September to November, in the forest with a dry season from December to April.

† In the southern area of Guinea Savannah (Wenchi and Ejura) the seasons are the same as in Forest, whereas in the more northerly part of Guinea Savannah (Nyankpala), there is one rainy season only, from May to October, and a very dry season from November to April.

(b) *Soil analyses*

Soil samples of upper horizon (6 in. depth) were examined for pH (colorimetric method[16]), phosphorus (Bray[17]) and organic carbon content (Walkley & Black[18]), exchange capacity (Piper[16]), exchangeable K and Ca (flame photometric method) and exchangeable Mg (Titan Yellow method). The results are given in Table I.

Table I

Soil analyses at 0–6 in. depth

Station	pH	Acid soluble + adsorbed P (p.p.m.)	Organic carbon, %	Nitrogen, %	Exchangeable cations, mequiv.-%			
					Capacity	K	Ca	Mg
Kwadaso	6·2	4·6	1·46	0·133	9·4	0·11	4·7	0·67
Pokoase	6·6	8·6	1·11	0·078	5·8	0·21	3·6	0·98
Wenchi	5·5	3·0	0·71	0·049	5·0	0·04	3·0	—
Ejura	6·3	3·2	0·46	0·034	4·1	0·25	2·5	—
Nyankpala	5·8	3·0	0·31	0·032	3·4	0·23	1·5	0·49

All the soils are slightly acid or acid and low in available phosphorus (see also [6, 19, 20]). Except for the Forest soils they are also low in organic carbon and nitrogen but on the average have high C/N ratios. De Endredy[19] and Greenwood[21] also found high C/N ratios in West African soils. The soils in general do not differ from each other in respect of exchangeable K content which is apparently fairly high in these sandy soils (except Wenchi soil which has low exchangeable K).

Experimental and results

(a) *Effects of different lengths of bush fallow on yields*

The data obtained from the survey of maize yields following 5–6, 3, 2 and 0 years of bush fallow on 30 sites (farmers' fields) in the two areas, Adaiso and Odupongkpehe, of the Lower Densu basin are given in Table II.

Table II

Effects of continuous cropping and bush fallow on the yield of maize in the Adaiso and Odupongkpehe areas of the Lower Densu basin

Cropping season		Mean yield† of cobs (with sheaths) per acre	
		Adaiso	Odupongkpehe
5–6-year fallow	(A)	483 ± 77·7	696 ± 53·9
3 ,, ,,	(B)	563 ± 37·2	871 ± 106·9
2 ,, ,,	(C)	708 ± 94·1	1015 ± 86·2
Continuous cropping	(D)	442 ± 33·9	679 ± 56·3

Significance: B, C > D* C > D*, A*

* = Significant at 5% level

† = $\frac{\text{Weight of cobs}}{\text{Dry grain weight}} = 1{\cdot}6$

The 2- or 3-year fallow gave greater yields of maize than did continuous cropping in both the Adaiso and Odupongkpehe areas, there being no significant difference between the 5–6-year fallow and continuous cropping in either area.

There is a fall in yield from the 3- to the 5–6-year fallow. This may be due to the poor sub-soil, the deep-rooted bushes drawing mostly on the upper horizon of soil for their nutrients[6] and returning only part of the nutrients assimilated to the soil. The bush fallows provide much of the fuelwood used for domestic purposes in the production of charcoal in Ghana;[22] in this way much of the nutrients are lost to the soil.

(b) *Effects of different lengths of grass fallow on fertiliser response and yield of crops*

Four rotation experiments were set up on lands cleared from secondary scrub bush; the first two were started at Pokoase and Nyankpala respectively in 1949 and the other two at

Kwadaso and Ejura in 1950. There were four main treatments: 3-year grass fallow (A); 2-year grass fallow (B); 1-year grass fallow (C); 0-year grass fallow (D); and 9 sub-units comprising N and P fertiliser combinations at three levels, N_0, N_1, N_2, and P_0, P_1, P_2 (Kwadaso, Ejura and Nyankpala only), respectively. The three levels of fertilisers included applications of ammonium sulphate (21% N) and single superphosphate (18% P_2O_5) at 0, 120, 240 lb./acre respectively at Kwadaso and Nyankpala and half these quantities at Ejura. The fertiliser treatments were not applied to grass in fallow. Where there were two cropping seasons a year the application of fertiliser was made to the first season crops, the second season crops having only the residual effects of the first season application. The grass in the fallow periods was cut once a year and the cut grass was left on the plots to decompose, but at the end of the fallow period the grass was cut and burnt on the plots a few weeks before ridging and planting, except at Pokoase where instead of burning the cut grass was ploughed in the soil. The fertilisers were broadcast over the sub-plots before ridging and planting.

The whole experiment was laid out in five blocks in a split plot design with complete randomisation of the main treatments and sub-units in the blocks, which also provided for the analysis of seasonal effects, five seasons in this case. Table III gives the 5-year crop rotation and the sequence of the three grass fallow treatments at the four centres.

Table III

Five-year crop rotation and sequence of the three grass-fallow treatments

Station		1st year	2nd year	3rd year	4th year	5th year
Pokoase	1st season	Maize	Groundnut	Cassava	Maize	Groundnut
	2nd ,,	Soya-bean	Maize	,,	Soya-bean	Maize
Kwadaso	1st ,,	Maize	,,	,,	Maize	Cassava
and	2nd ,,	Groundnut	Cassava	,,	Groundnut	,,
Ejura						
Nyankpala	One ,,	,,	Maize	Guineacorn	Maize	Guineacorn
Fallow* (all	1-year	Crops	Crops	Crops	Crops	Grass
stations)	2- ,,	,,	,,	,,	Grass	,,
	3- ,,	,,	,,	Grass	,,	,,

* Pokoase—Guinea grass (*Penicum maximum*); Kwadaso—elephant grass (*Pennisetum purpureum*); Ejura and Nyankpala—mixture of *Andropogon gavanus* and *Pennisetum polyotachvon*

Varieties of crops (all stations).—Maize (local); groundnut (Kumawu); cassava (local); soya-bean (Malayan).

The results for one complete 5-year cycle ending 1956/7 for Pokoase and Nyankpala and 1957/8 for Kwadaso and Ejura are given in Table IV.

Kwadaso.—The results on first-year maize were not significant in 1953 but in the four years 1954–57 yields were greatly improved by the preceding grass fallow. Maize showed no response to ammonium sulphate but was much improved by application of single superphosphate at 120 lb./acre, no further improvement resulting from double this application. The following crop of groundnuts showed no marked response to the preceding fallow treatments or to any residual effect of the fertilisers. The following maize crop again showed good response to grass fallow and to superphosphate at 120 lb./acre and a slight further improvement from double this dose.

Ejura.—On average, the first-year maize crop did considerably better without a preceding grass fallow. There was a good response to ammonium sulphate at 120 lb./acre but only a small response to superphosphate. The following groundnut crop was, on the average, better after 2 or 3 years' grass fallow than after 1 or none. There was no apparent residual effect of fertilisers applied to maize. The average second-year maize crop was better after 2 or 3 years' grass fallow than after 1 or none. There was good response to ammonium sulphate and to superphosphate, both at 60 lb./acre, with no further benefit from double these rates.

Nyankpala.—The first-year maize crops did better without a preceding grass fallow. They responded well to ammonium sulphate and to superphosphate, the 240-lb. dressings giving rather higher yields than the 120-lb. dressings. The following crop of Guineacorn showed no response to the preceding grass fallow but there was appreciable response to both fertilisers. Both crops showed a positive N × P interaction which was not observed at Kwadaso or at Ejura.

Table IV

Effects of different lengths of grass fallow on fertiliser response and yield of crops

(Yield totals in lb. per 9/10 acre)

1. *Main treatment* (*cropping* = *S*)

Station	Crop	A	B	C	D	S.E.	Significance†
Kwadaso	Maize[1]	895	973	878	588	51·1	A, B, C > D* (1954–57); (1953)—insig.
	Groundnut	329	437	395	375	43·8	B > A*, C*, D* (1954, 1956); C > A*, B*, D* (1955); A, B, C > D* (1957)
	Maize[2]	886	910	792	528	53·6	A, B, C > D*
Ejura	Maize[1]	655	514	684	841	57·2	A > B*, C*, D* (1956); D > A*, B*, C* (1953–55)
	Groundnut	592	594	513	510	31·2	B > A*, C*, D* (1954); A, D > B* (1953); A, B > C*, D* (1955, 1957)
	Maize[2]	1219	1073	891	922	107·0	A, B > C*, D* (1955–56, 1958); A, D > B* (1954)
Nyankpala	Maize	391	351	398	472	39·9	A, D > B*, C* (1956); D > A*, B*, C* (1955); C > A*, B* (1952)
	Guineacorn	253	272	280	248	21·8	C, D > A*, B* (1956); A, B, C > D* (1955)
Pokoase	Maize[1]	746	752	458	454	(*a*)	B, C > A*, D* (1955–56); A, B > C*, D* (1956–57); (1952/3–1954/5)—insig.
	Soya-bean	685	569	569	547	267·7	
	Groundnut	543	759	721	673	(*b*)	
	Maize[2]	785	755	712	529	86·5	

2. *Sub-treatment* (*fertiliser* = *N*, *P*)

Station	Crop	N_0	N_1	N_2	P_0	P_1	P_2	S.E.	Significance ‡
Kwadaso	Maize[1]	1119	1076	1139	960	1212	1162	29·4	$P_1, P_2 > P_0$ (1954–57); N; N × P—insig.
	Groundnut	483	508	495	485	484	517	16·9	N; P; N × P—insig.
	Maize[2]	1026	1069	1021	796	1144	1176	29·1	$P_1, P_2 > P_0$**; N; N × P—insig.
Ejura	Maize[1]	767	881	1046	873	883	938	21·7	$P_2 > P_0$*; $N_1, N_2 > N_0$**; N × P—insig.
	Groundnut	730	750	729	738	739	732	15·4	N; P; N × P—insig.
	Maize[2]	1227	1458	1420	1198	1430	1477	38·7	$N_1, N_2 > N_0$**; $P_1, P_2 > P_0$**; N × P—insig.
Nyankpala	Maize	320	592	700	271	624	717	27·7	$N_1, N_2 > N_0$**; $P_1, P_2 > P_0$**; NP > N*, P*
	Guineacorn	261	356	436	262	388	403	15·6	$N_1, N_2 > N_0$*; $P_1, P_2 > P_0$*; NP > N*, P*

* Significant at 5% level. ** Significant at 1% level.

† Cropping treatment (S) also significant at 1% level on average of 5 years only for maize[1, 2] at Kwadaso and for maize[1] at Ejura only.

‡ Interactions S × N or S × P or S × N × P insignificant for all test crops in all seasons and at all the three centres.

(for A–D, N_0–N_2 and P_0–P_2 see text)

Pokoase.—Yields of both first- and second-year maize were improved by the preceding grass fallow but the benefit was less clear in the soya-beans and groundnuts that came between two crops of maize.

(c) *Relative effects of grass and legume fallows on yield of following crops*

Two rotation experiments with 3- and 0-years' grass or legume fallow were started in 1956 at Kwadaso, in the Forest zone. A similar experiment but with 2- and 0-years' fallow began at Wenchi in the same year. The layout of the experiments was in five randomised blocks and the treatments were as follows:

	Legumes	Grasses
Kwadaso	(A) *Tephorosia vogelli*	(D) Guinea grass
	(B) Pigeon pea	(E) Giant ,,
	(C) *C. juncea*	(F) Elephant grass
	(G) Nil	

	Legume	Grass
Wenchi	(A) Pigeon pea	(B) Guinea grass
	(C) FYM (farmyard manure)	
	(D) Complete inorganic fertiliser (N, P, K)	
	(E) Nil	

The plot size at each centre was 1/40 acre and after exclusion of discarded border strips it was 1/51 acre. At Wenchi manurial treatments were: FYM at 30 cwt. per acre, and a

dressing of 120 lb. of each of sulphate of ammonia (21% N) and single superphosphate (18% P_2O_5) and 60 lb. of muriate of potash (60% K_2O) per acre. No manure or fertiliser was applied to grass or legume fallow or to the following crop. The results are given in Table V for Kwadaso and Wenchi.

Table V

Effects of grass and legume fallows on yield of maize

(Mean yield, lb. per acre)

Kwadaso, 1959

Fallow		Expt. 1	Expt. 2	Average
T. vogelli	(A)	224	306	265
Pigeon pea	(B)	245	632	439
C. juncea	(C)	214	408	311
Guinea grass	(D)	398	479	439
Giant ,, Elephant grass	(E)	122	316	219
	(F)	377	724	551
Nil	(G)	122	367	245
S.E.		±39·8	±88·7	±553·0
Significance		Fallow	Fallow	Soil*; Fallow**; Soil × Fallow—Insig.

Wenchi, 1958

Treatment		Mean yield
Pigeon pea	(A)	267
Guinea grass	(B)	215
FYM	(C)	587
Fertiliser	(D)	382
Nil	(E)	257
S.E.		± 78·4
Significance		C > A*, B*, E*

D, F > A*, B*, C*, E*, G* B, F > A*, C*, D*, E*, G* B, D, F > A*, C*, E*, G*

* Significant at 5% level; ** Significant at 1% level

At Kwadaso on the average the pigeon pea fallow among the legumes and elephant or Guinea grass among the grasses gave greater yields of maize than did any other treatment or continuous cropping. No significant difference existed in the yield of maize between the other two legume fallows, *T. vogelli* and *C. juncea*, and continuous cropping or between the latter and giant grass. Elephant grass fallow was slightly better than the Guinea grass fallow.

On the other hand at Wenchi the maize crop showed no response to the preceding fallow with pigeon pea or Guinea grass; no significant difference between pigeon pea fallow and continuous cropping or between the latter and Guinea grass fallow existed in respect of yield. In this soil the crop showed a large response to FYM and to complete inorganic fertiliser, the former being slightly better than the latter. Lynn[23] obtained substantial responses from small dressings of Kraal manure (FYM) in the Northern Territories of the Gold Coast in 1937 and similar results were obtained later at Babile (Northern Ghana).[24]

(d) *Fallow, yield and nutrient status of soil*

At two southerly stations (Kwadaso, Pokoase) maize yields were increased after fallow but this was not so in three more northerly stations (Ejura, Wenchi, Nyankpala). The difference may depend on the type of fallow and availability of nitrogen.

It is seen in Table V that of the three grass fallows at Kwadaso only elephant or Guinea grass increased yields of maize and that pigeon pea was the only one of three legume fallows that gave results better than did continuous cropping. At Wenchi the maize crop did not respond to Guinea grass or to pigeon pea fallow. Elephant grass fallow was used at Kwadaso and Guinea grass at Pokoase and these were the centres where fallow was most effective. Andropogon grass fallow was used at the other two centres, Ejura and Nyankpala in Northern Ghana. Some observations on yields of elephant and Andropogon grasses are given in Table VI.

It is evident that the mean yield of elephant grass at Kwadaso was double and treble that of Andropogon at Ejura and Nyankpala, respectively, at which locations the Andropogon grass fallow was not successful. This fallow was substituted by the pigeon pea fallow at Ejura in the second 5-year cycle beginning in 1955. The results for 4 years ending 1959 indicate that large increases in yield of maize crops were obtained after pigeon pea fallow.[25]

The results for fertiliser response of crops (discussed above) afford little evidence of a shortage

Table VI

Yearly yield of elephant and Andropogon grasses

(means of 3 years, lb./acre)

Fallow	Elephant grass	Andropogon grass	
	Kwadaso	Ejura	Nyankpala
3 years	10·2	6·6	4·4
2 years	14·3	6·2	3·7
1 year	13·7	6·4	3·9
Average	12·7	6·4	4·0

of nitrogen in the southern Forest area whereas, in the more northern Savannah zone, sites recently cleared after bush fallow are seriously short of available nitrogen (see also Nye[13a]). The grasses, which have a higher nitrogen demand than the legumes, did not give good results in the soils of Ejura and Nyankpala which are deficient in available nitrogen; the reverse was true in the rich Kwadaso soil.

(e) *Nutrient deficiency in soil and its amelioration by grass fallow*

The nutrient deficiencies in the Forest zone and Guinea Savannah have been described by Nye.[13] The results of the present field experiments at one site in the Forest zone and two sites in the Guinea Savannah agree with Nye's observation[13a, b] that there is a widespread deficiency of phosphorus in the soils of Ghana, whereas deficiency of nitrogen occurs only in the more northerly areas of Guinea Savannah, the southern area of forest showing little sign of a shortage of available nitrogen. The important point brought out by the results discussed in Section 3 (b) is that the deficiencies of nitrogen and phosphorus occurred equally in all plots whether under grass or continuously cropped. (See Table IV.)

Some analyses of soil, and corresponding yields of grass and of succeeding crops at Kwadaso, are tabulated in Table VII to show the effects of different lengths of grass fallow on the nutrient status of soil and crop yields. The proportions of acid-soluble phosphate, exchangeable K and Mg in the plots with the three grass fallow treatments were greater than those in the continually cropped plots; no significant differences between the three grass fallow treatments existed in this respect, or between the four cropping treatments in regard to organic carbon, nitrogen, exchangeable Ca or exchange capacity. The average annual yield of grass was greater in the plots under the 1- or 2-year grass fallow than in the plots with the 3-year fallow; there was no significant difference between the 1- and 2-year fallow treatments. On the other hand

Table VII

Analysis of soil, yield of grass incorporated in soil or burnt, and crop yield at Kwadaso

	3-year fallow	2-year fallow	1-year fallow	Continuously cropped
A. *Soil analysis*				
Organic carbon (% of dry soil)	1·42	1·42	1·45	1·46
Nitrogen (% of dry soil)	0·128	0·125	0·131	0·133
Acid-soluble + adsorbed phosphate (p.p.m. of P)	5·9	6·3	5·7	4·6
Exchange capacity (mequiv. per 100 g.)	8·7	8·8	9·9	9·4
Exchangeable K (mequiv. per 100 g.)	0·22	0·23	0·22	0·11
Exchangeable Ca (mequiv. per 100 g.)	4·0	4·4	5·7	4·7
Exchangeable Mg	1·02	0·94	1·00	0·67
B. *Yield of grass (tons per acre)*				
Annual-cut at the end of each year	10·2	14·3	13·7	—
Total amount of grass added to soil prior to planting	30·6	28·6	13·7	—
C. *Yield of succeeding crop, lb. per acre*				
1st-year maize	895	973	878	588
,, groundnuts (second season)	329	437	395	325
2nd-year maize	886	910	792	528

the total amount of grass added to the soil prior to planting the crop was greater in the 3- or 2-year fallow plots than in those under 1-year fallow; results on crop yields show a similar trend.

It appears that the final annual-cut of grass of the 3-, 2- and 1-year fallows, respectively, burnt on the plots affected the nutrient content of soil rather than the total amount of grass added to soil prior to planting the crop. Probably, the nutrients contributed by the other cuts of the 3- and 2-year grass fallows left on the plots to decompose were used up by the successive grass ratoons and the organic matter oxidised rapidly in these soils (see Nye[13c]). The grass residue of the 3- and 2-year fallow plots prior to the last cut benefited the crop indirectly, by improving the physical condition of the soil, especially its structure and water regime. The literature is large on this subject and only a few references may be mentioned here. Martin[11] in East Africa found that grasses improved soil aggregation. Vorolieva[12] found a positive influence of perennial grasses in forming the structural stability of soils.

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Ministry of Food and Agriculture
Division of Agriculture
P.O. Box 299, Accra, Ghana

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

FEBRUARY, 1961

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

INDEX OF AUTHORS' NAMES

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

FEBRUARY, 1961

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Weathering and aluminium interlayers in a soil catena. B. L. Sawhney (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 221—226).—Characteristics of the catena are presented and discussed with particular reference to the effect of weathering on the removal of Al interlayers. A. H. CORNFIELD.

Ordination of soil profiles. F. D. Hole and M. Hironaka (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 309—312).—Laboratory and soil profile description data were processed by means of a formula to yield indices of similarity between soil profiles. Results are reported for members of soil catenas and for 25 profiles representing the Great Soil Groups. A. H. CORNFIELD.

Rôle of microclimate in soil genesis. A. W. Cooper (*Soil Sci.*, 1960, **90**, 109—120).—North- and south-facing slopes in the same locality have been compared with regard to microclimate and soil analysis. South-facing slopes had a warm dry (xeric) micro environment whereas the north slopes were cooler and more moist and approached the mesic conditions. Average A horizon temp. were higher on the south slopes throughout the entire growing season. During winter, snow cover was short-lived on south slopes but on north it was of 3 months' duration. Moisture contents were generally higher on north slopes. The total depth of solum and the depths of the various horizons were less on south slopes. Colour varied also; the B horizon was redder in hue and contained significantly more silt and clay on south slopes. T. G. MORRIS.

Gilgaied soils in South Dakota. E. M. White and R. G. Bonestell (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 305—309).—Gilgaied soils in the region are described and their possible modes of origin are discussed. A. H. CORNFIELD.

Characteristics of the multiple yellowish-red bands common to certain soils in the southeastern United States. G. H. Robinson and C. I. Rich (*Proc. Soil Sci. Soil Amer.*, 1960, **24**, 226—230).—Coloured bands or strata of fine-textured material occurring in certain sandy soils were higher in org. matter and cation-exchange capacity, but not in % of free Fe in the clay fraction, than was material above or below the bands. The bands are probably the result of geologic deposition. A. H. CORNFIELD.

Crystalline and amorphous soil minerals of the Mississippi coastal terrace. L. E. DeMumbrum (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 185—189).—Vermiculite and allophane were the predominate clays of the poorly-drained and vermiculite and gibbsite of the better-drained soils of the area. Vermiculite was interlayered with Al_2O_3 in both situations. An amorphous aluminous mineral was also present in the soils. A. H. CORNFIELD.

Saline and sodic soils of Spain. A. D. Ayers, A. Vazquez, J. de la Rubia, F. Blasco and S. Samplon (*Soil Sci.*, 1960, **90**, 133—138).—A general picture is given of the extent and severity of the saline soil problem in Spain. T. G. MORRIS.

Relationships of Atterberg limits to some other properties of Illinois soils. R. T. Odell, T. H. Thornburn and L. J. McKenzie (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 297—300).—Atterberg-limit tests were correlated with other properties for 70 samples representing the horizons of 23 soil series of widely differing characteristics. Liquid limit, plastic limit and plasticity index were all particularly highly correlated with % org. C, % clay, and % montmorillonite in the clay. A. H. CORNFIELD.

Thermodynamic properties of soil water. S. A. Taylor and G. L. Stewart (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 243—247).—The temp. dependence of the soil water potential as measured by tensiometers under conditions of const. soil moisture content, solute content and pressure was used to calculate the soil water enthalpies in the range $-0{\cdot}1$ to $-1{\cdot}0$ joules per g. of soil water, and the soil water entropies in the range $(-4$ to $-20) \times 10^3$ ergs per g. per degree for soil water. A. H. CORNFIELD.

Soil capillary conductivity: comparison of measured and calculated values. D. R. Nielsen, D. Kirkham and E. R. Perrier (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 157—160).—Capillary conductivity calculated by the Marshall method (*J. Soil Sci.*, 1958, **9**, 1—8) for two loess and two glacial till soils at four depths and at moisture tensions up to 100 cm. water were considerably less than measured values for all soils. Values calculated by the Childs–Collis–George method (*Proc. roy. Soc.*, [A], 1950, **201**, 392—405) agreed satisfactorily with measured values for the loess, but not for the glacial till, soils. A. H. CORNFIELD.

Water movement into soil from idealised vertical mulch channels. D. Swartzendruber (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 152—156).—Water movement through an idealised vertically-mulched soil is explained mathematically. A. H. CORNFIELD.

Non-steady-state moisture, temperature and soil air pressure approximation with an electric simulator. R. J. Hanks and S. A. Bowers (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 247—252).—An electric simulator capable of approximating the solution of many problems in soil moisture, temp. and air pressure is described. A. H. CORNFIELD.

Evaporation from layered soils in the presence of a water table. W. O. Willis (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 239—242).—Evaporation from two-layered soil systems with varying depths to water table is described for steady-state conditions. A. H. CORNFIELD.

Utilising drought-days in evaluating irrigation and fertility response studies. W. L. Parks and J. L. Knetsch (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 289—293).—Drought days were used in evaluating experiments containing moisture and fertility variables. The resultant production accounted for 87·5% of the variation among treatment means, and was used to determine the optimum fertilisation rate as well as returns for irrigating at a certain stage of plant development. A. H. CORNFIELD.

Analysis of the vertical downward flow of water through a two-layered soil. S. Takagi (*Soil Sci.*, 1960, **90**, 98—103).—The problem of the flow of water downwards through two layers of soil, the upper one of which is less permeable than the lower, is considered theoretically. T. G. MORRIS.

Cropping-management factor evaluations for a universal soil-loss equation. W. H. Wischmeier (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 322—326).—Soil-loss data from 37 locations in 21 states over 30 years were analysed to determine the influence of crop type and sequence, and fertility and management practices on soil loss due to rainfall. The data was grouped for different periods prior to and during cropping and soil losses are presented as % of losses from fallow under identical rainfall. The application of the data in conjunction with rainfall erosion index maps and monthly distribution curves computed from local rainfall records as a guide to conservation farm planning is discussed. A. H. CORNFIELD.

Relation of soil moisture to ion absorption by maize plants. H. J. Mederski and J. H. Wilson (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 149—152).—Maize plants were grown from seed for 25 days in pots using a split root technique in which the upper portion of the roots grew in wet sand and the lower portion (separated from the upper portion by a wax membrane) in soil of varying moisture content. When the plants were grown at low atm. R.H. top and root dry matter yields and K% and P% in the tops increased with moisture content from wilting % to field capacity. At high R.H. top and root dry matter yields and Mg % in the tops increased with soil moisture content. Mg % and Ca % in the tops showed no consistent relationship with soil moisture at low R.H. and K% and P % in the tops showed no consistent relationship with soil moisture at high R.H. A. H. CORNFIELD.

Activity measurements in water and clay–water systems with tertiary electrodes. G. Uehara and M. M. Mortland (*Proc. Soil Sci. Soc., Amer.*, 1960, **24**, 261—264).—A tertiary amalgam electrode was used for measuring $CaCl_2$ and Ca^{2+} activities. Activity coeff. from e.m.f. data compared favourably with those in the literature. The relative influence of metal ion salts on the $CaCl_2$ and Ca^{2+} activities was in the order $Rb > Mg > K > N > Li$ for both kaolinite and bentonite. The activities of $CaCl_2$ and Ca^{2+} in bentonite–kaolinite mixtures differed from predicted activity values based upon pure clay systems. A. H. CORNFIELD.

Oxidation-reduction studies on the mechanism of B horizon formation in podsols. L. J. McKenzie, E. P. Whiteside and A. E. Erickson (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 300—305).—Oxidation–reduction studies in all seasons on a hydrosequence of sandy soils in the podsol region indicate that soil redox potentials vary

with soils horizons and position in the hydrosequence. Seasonal variations are related to leaf fall, temp., rainfall and activity of micro-organisms. A. H. CORNFIELD.

Preparation of clay suspensions with specified ionic composition by means of exchange resins. G. H. Bolt and M. J. Frissel (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 172—177).—Factors influencing the efficiency of the prep. of homo-ionic clays by means of exchange resins were studied. Application of the method for preparing homo-ionic clays or clays with a specified mixed ionic composition is described. A. H. CORNFIELD.

Determination of exchangeable sodium, potassium, calcium and magnesium in soils by atomic-absorption spectrophotometry. D. J. David (*Analyst*, 1960, **85**, 495—503).—The apparatus used is a modification of that previously described (*ibid.*, 1958, **83**, 655). Interferences by Al, PO_4^{3-}, silicate and SO_4^{2-} in the determination of Na, K, Ca and Mg in NH_4Cl-extracts of soils are investigated. Sr is a more efficient suppressor of the combined interferences of these ions than is La. The method is applicable to 200-ml. extracts of 10-g. portions of soil with preliminary concentration, and with the apparatus described 0·1 p.p.m. of Na, 0·05 p.p.m. of Mg, 0·1 p.p.m. of K and 0·2 p.p.m. of Ca can be determined. (12 references.) A. O. JONES.

Modified Hittorf method of measuring transference numbers of ions in soil and clay pastes. J. Letey and A. Klute (*Soil Sci.*, 1960, **90**, 121—128).—The transference numbers of K^+ and Cl^- have been measured by a modified Hittorf method in plugs of bentonite, kaolinite and a subsoil. The errors inherent in the method are discussed. In all materials tested the K^+ transference number through the plugs decreased as the external concn. increased and with a given external concn. increased with the cation-exchange capacity of the soil. T. G. MORRIS.

Use of high-salt waters as a flocculant and source of divalent cations for reclaiming sodic soils. R. C. Reeve and C. A. Bower (*Soil Sci.*, 1960, **90**, 139—144).—The so-called " valence dilution " effect is briefly discussed, and from it it is concluded that the Na adsorption ratio (SAR) is inversely proportional to the square root of the dilution factor and that equilibration of a soil with successive dilutions of sea water, or similar high-salt water, results in corresponding reductions in exchangeable Na (%). Sieved soil was leached with sea water diluted to different degrees with river water. In all treatments the exchangeable Na was greatly reduced. Time of reclamation varied markedly, e.g., 12—20 days, whereas with river water alone it required 120 days. T. G. MORRIS.

Significance of the suspension effect in the uptake of cations by plants from soil–water systems. R. A. Olsen and M. Peech (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 257—261).—Rates of uptake of Rb^+ and Ca^{2+} from clay and resin suspensions and their corresponding equilibrium dialysates (ED) were compared. Although the cation concn. of the clay or resin suspension greatly exceeded that of the corresponding ED, the rate of uptake of the two cations by excised roots of barley and mung beans from the suspension and ED was exactly the same. A. H. CORNFIELD.

Nitrification of fixed ammonium in clay minerals as affected by added potassium. L. F. Welch and A. D. Scott (*Soil Sci.*, 1960, **90**, 79—85).—Samples from a high- and low-exchange capacity vermiculite were saturated with NH_4^+ by leaching with NH_4 acetate and washed free of NH_4^+ with 70% methanol. Amounts of the material were added to a low-K nutrient and inoculated with low-K cultures of nitrifying bacteria (prep. described). The systems were aerated and incubated for up to 30 days. In all vermiculites examined all the available NH_4^+ was nitrified in 21 days when there was no added K (0·5 p.p.m. present). The amount of NH_4^+ nitrified decreased with increasing additions of K. Using $(NH_4)_2SO_4$ without vermiculite nitrification was also complete in 21 days, the ammonium in the clay being as available as that in the salt, and the low amount of K having no blocking effect on the clay. Increasing the added K with $(NH_4)_2SO_4$ did not lower nitrification ; probably the lowered rates with clay were due to the blocking effect of K. The magnitude of the K effect was different in different clays. T. G. MORRIS.

Volatile losses of nitrogen from acid or neutral soils or solutions containing nitrite and ammonium ions. D. H. Smith and F. E. Clark (*Soil Sci.*, 1960, **90**, 86—92).—The soils were placed in containers in atm. of either NO or a 4 : 1 He–O_2 mixture and sealed with serum caps. Water, buffer, etc., were injected into the containers after the atm. had been established. In NO elemental N resulting from the reaction between NH_4^+ and NO_2^- was evolved from neutral or alkaline solutions only in trace amounts. At pH 4, the amount of N evolved was dependent on the reactant concn. In He–O_2 only about one-third as much NO_2^- reacted with NH_4^+ as it did under NO. The oxidation of NO_2^- to NO_3^- was 5 times as rapid under O_2 as under NO. In He–O_2 the amount of N_2 evolved increased in presence of soil. In one soil having no added NH_4^+ the N_2 evolved was as much as when NH_4^+ was added, indicating that NO_2 was reduced by some other agent. There was no evidence that N was lost as NO or NO_2. T. G. MORRIS.

Recovery of ammonium nitrogen from soils. B. J. Stojanovic and F. E. Broadbent (*Soil Sci.*, 1960, **90**, 93—97).—Four acid soils and one calcareous soil were treated with either water or an NH_4^+ salt solution (50 p.p.m. of N per 25 g. soil) and then used either wet or air-dried at 95°, left in contact for 30 min. with Na acetate–acetic acid at pH 4·8, or N-KCl–HCl at pH 1·0. The NH_3 in the leachate was determined. In untreated soils, air- or oven-drying generally increased the NH_3 recovery and the two extracting solutions removed similar amounts. With the treated acid soils recovery of NH_3 from NH_4Cl- and aq. NH_3-treated samples was consistently lower than that from $(NH_4)_2PO_4$- or NH_4NO_3-treated samples. Recovery from dry soil was higher than from wet. Much NH_4^+ was fixed by the wet calcareous soil ; on drying some of this was lost. Both extractants behaved similarly. In some tests, ^{15}N-enriched $(NH_4)_2SO_4$ was used as a source of NH_4^+. The recovery of tracer N was invariably less than that of total N, particularly in the case of the calcareous soil. T. G. MORRIS.

Nitrogen fixation in extracts of *Azotobacter vinelandii*. D. J. D. Nicholas and D. J. Fisher (*J. Sci. Fd Agric.*, 1960, **11**, 603—608).—Cultures of *A. vinelandii*, O strain, were grown for 18 h. at 30°. When the cells were disrupted in the culture medium by lysozyme treatment or ultrasonic probe, ^{15}N was incorporated in amounts comparable with those assimilated by whole cells. Even after centrifuging the lysed cells in the culture medium at 25,000 *g* for 30 min., there was still a substantial fixation of ^{15}N in the cell-free extracts, viz., between 0·22 and 0·54 atom-%. (12 references.) E. M. J.

Nitrate production in the field by incubating the soil in polyethylene bags. C. F. Eno (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 277—279).—The nitrification rate of NH_3 added to soil was similar whether the soil was incubated in closed polyethylene bags or in ventilated bottles. Polyethylene is permeable to both O_2 and CO_2, has a low permeability to water vapour, and does not lose NO_3^- by diffusion even over long periods. The method was particularly useful for studying changes in nitrification rate with temp. during winter months when bags of soil were buried in the field. A. H. CORNFIELD.

Effect of low temperature on nitrification of ammonia in Cecil sandy loam. O. E. Anderson (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 286—289).—Nitrification rate in a sandy loam treated with anhyd. NH_3 (150 p.p.m.) was low at 2·7° and increased with temp. to 11·1°. NO_3^- accumulation during 12 weeks expressed as % of that occurring at 11·1° was 72, 35 and 4% at 8·3°, 5·6° and 2·7° respectively. The rate of nitrification of added NH_4NO_3 showed a similar trend with increasing temp., but the rate declined at all temp. with increasing rate of application of NH_4NO_3 (50—400 p.p.m. N). A. H. CORNFIELD.

Nitrite transformations in California soils. K. B. Tyler and F. E. Broadbent (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 279—282).—The rate of conversion of added NO_3^- (200 p.p.m.) to NO_3^- during incubation of alkaline soils (pH 8) was much lower at 7·2° than at 23·9°. Disappearance of NO_2^- was accompanied by increased NO_3^-, but there was a small loss as N_2. In acid soils (pH 5·3—5·4) there were considerable losses as N_2, although these losses were less when the soils were limed prior to incubation. Addition of NO_2^- inhibited the respiration rate of soils, acid soils being more sensitive in this respect than alkaline soils. A. H. CORNFIELD.

Correlation of nitrogen soil tests with nitrogen uptake by the tobacco plant. L. A. Peterson, O. J. Attoes and W. B. Ogden (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 205—209).—The relationship between total N content of the above-ground portion of tobacco grown in pots in 37 soils (15 Gray-Brown Podsolic and 22 Prairie) and " available N " as determined by chemical extraction and biological tests was studied. Total N uptake was highly correlated with both initial soil NO_3^- and the *Aspergillus niger* soil test for the first, but not for the second, crop of tobacco. Total N uptake by the second crop was significantly correlated with nitrification rate, total soil N, soil org. matter, NH_3 extracted or released by alkaline permanganate, 0·1N-, N-, 4·5N- and 18N-H_2SO_4 and 4N-HCl, and NH_3 + org. N extracted by 0·1N- and N-H_2SO_4 and 4N-HCl. Total soil N was particularly highly correlated with NH_3 released on treatment with alkaline permanganate, soil org. matter, and NH_3 extracted by 4·5N-H_2SO_4. A. H. CORNFIELD.

Effect of partial pressure of oxygen on denitrification in soil. F. E. Allison, J. N. Carter and L. D. Sterling (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 283—285).—There was virtually no loss of added NO_3^- through denitrification when a sandy loam was incubated (moisture added to 0·33 atm. tension) in air either with or without

1% of added glucose or 2% of wheat straw. When aerated with N_2 containing 2·27% O_2 1—8% of the NO_3^--N was lost as N_2. When aerated with N_2 containing 0·46% O_2 10% of the NO_3^--N was lost as N_2 in the absence, and 50% in the presence, of 0·5% glucose. A. H. CORNFIELD.

Phosphate phase equilibria in soils. W. L. Lindsay and E. C. Moreno (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 177—182).—Solubility criteria were used as a basis for explaining inorg. PO_4^{3-} reactions in soils. Consideration was given to phosphate known to form in soils and to soil phases contributing ions for their pptn. Through simple derivations the activity isotherms for various Ca, Fe and Al phosphates were represented on a single solubility diagram. The relative stability of these compounds in soil and changes expected to occur on fertilisation and liming are readily predicted from the graph. A. H. CORNFIELD.

Behaviour of slightly soluble calcium phosphates as revealed by phase-equilibrium calculations. W. E. Brown (*Soil Sci.*, 1960, **90**, 51—57).—A discussion. T. G. MORRIS.

Reactions of dicalcium phosphate dihydrate in soils. E. C. Moreno, W. L. Lindsay and G. Osborn (*Soil Sci.*, 1960, **90**, 58—68).—An acid fine sandy loam was equilibrated for periods of up to 30 days with $Ca(H_2PO_4)_2$ (**I**) and water or $CaHPO_4,2H_2O$ (**II**) and water, acid-washed charcoal being added in some cases. The systems soil–**II**–H-resin and soil–**II**–Fe or Al oxides were also examined. The Ca and P contents and the pH of the supernatant liquid were determined. Results obtained with **I** show that the solution became saturated with **II** in less than an hour of equilibration and remained so. In the case of **II** the pH rose steadily during equilibration up to 60 days. Complete dissolution of **II**, added in small amounts, occurred during the first 10 days of equilibration; larger amounts (1·2—5 g./50 g. of soil and 100 ml. of water) remained throughout the experiment. Org. matter complexed Ca at pH levels >6 causing an apparent supersaturation in respect of **II**. Addition of Fe and Al oxides to the equilibrating mixtures removed P from solution. T. G. MORRIS.

Soil properties affecting the solubility of calcium phosphates. S. R. Olsen, F. S. Watananbe and C. V. Cole (*Soil Sci.*, 1960, **90**, 44—50).—The chemical properties of systems in which $CaHPO_4,2H_2O$ (DCPD) and hydroxyapatite (HA) were mixed with calcite and various salts and soil are reported. Suspensions of DCPD, HA or soil were equilibrated with water or salt solution in an atm. of constant $[CO_2]$. Ca, Mg, bicarbonate and P were determined in the supernatant liquid. The solubility product $pCaHPO_4$ was calculated for all results and in water, KCl or $CaCl_2$ it was 6·53—6·56, with the pH varying between 6·05 and 6·42. A rise in pH or the presence of calcite in the system caused the DCPD to change to a less sol. phase, the conversion being due to pH and not to calcite. The solubility of P from DCPD and HA was greater in aq. $CaSO_4$ and K_2SO_4 than in equal concn. of chlorides, but after correcting for undissolved $CaSO_4$ the $pCaHPO_4$ and pHA values were constant. The same was found to some extent in calcareous soils. The solubility of P in calcareous soils increases as the soil/water ratio rises. T. G. MORRIS.

Characterisation of phosphate reaction products in acid soils by the application of solubility criteria. B. C. Wright and M. Peech (*Soil Sci.*, 1960, **90**, 32—43).—Samples (34) of different soils which had received P fertiliser >5 years previously were extracted with 0·01M-$CaCl_2$ for two periods of 24 and 72 h. and then with 0·01M-$CaCl_2$ which was 0·001M with respect to HCl for a further 72 h. Repeated extraction with 0·01M-$CaCl_2$ established the constancy of the variscite solubility product. In the soils studied the average pK_{sp} value for variscite was almost the same for fertilised and unfertilised soils. The native P in most of the soils was present as Fe phosphate; P fertilisers increased this fraction primarily in most soils but in some the fertiliser P was found to be converted into Al phosphate. A variscite–strengite type of mineral is probably the ultimate reaction product of applied P in these acid soils. T. G. MORRIS.

Inorganic phosphate transformations by chemical weathering in soils as influenced by pH. P. H. Hsu and M. L. Jackson (*Soil Sci.*, 1960, **90**, 16—24).—The solubilities of hydroxyapatite, variscite and strengite are plotted as functions of pH, since it is shown that transformations of one phosphate form to another are probably also a function of pH. The effect of the presence of $CaCO_3$ is discussed and the general picture of some calcareous soils from Wisconsin is considered. T. G. MORRIS.

Rates and mechanisms of dissolution of some ferric phosphates. E. O. Huffman, W. E. Cate and M. E. Deming (*Soil Sci.*, 1960, **90**, 8—15).—The dissolution of three possible soil phosphates, $CaFe^{3+}$ phosphate (**I**), strengite ($FePO_4,2H_2O$) (**II**) and colloidal Fe^{3+} phosphate (**III**) by 0·5, 0·1 or 0·01M- aq. NH_3, 0·0011 or 0·0001M-H_3PO_4 or water is examined. Comparisons of rates were made during the first 30 min. since after this time changes in surface occurred with the dissolution. In aq. NH_3 the initial rate of solution of **I** was high, but this decreased rapidly with time. **II** dissolved at a constant rate, due to the formation of colloidal $Fe(OH)_3$ which coated the particles of the Ca salt. The atomic ratio Ca/P in solutions of **I** in H_3PO_4 and water ranged from 0·84 in H_3PO_4 at pH 3 to 0·44 in water at pH 4·2. The net chemical reaction was equivalent to the dissolution of Ca phosphate and precipitation of ferric phosphate. **II** and probably **III** dissolve readily in the stable triple point solution of the system $CaO–P_2O_5–H_2O$, the probable immediate environment of the $CaHPO_4$ in soil. In greenhouse tests P uptake from ferric phosphate was related to the rates of dissolution in water. T. G. MORRIS.

Evaluation of some iron aluminium phosphates as sources of phosphate for plants. A. W. Taylor, E. L. Gurney and W. L. Lindsay (*Soil Sci.*, 1960, **90**, 25—31).—Seven different Fe and Al phosphate materials were used as sources of P in greenhouse experiments, with a P-deficient fine sandy loam of pH 4·8. In some tests the soil was limed with sufficient $CaCO_3$–$MgCO_3$ to raise the pH to 6·3. In all tests liming increased the total uptake of P. $Ca(H_2PO_4)_2$ gave the highest yield and that from Ca ferric phosphate was almost the same; KFe leucophosphite caused little difference in yield from that in the unfertilised soil. Ca ferric phosphate, taranakite and colloidal Al and Fe phosphates were efficient whereas KFe phosphate and Al and Fe leucophosphites were inefficient P sources. T. G. MORRIS.

Phosphate-sorption reactions that involve exchangeable aluminium. N. T. Coleman, J. T. Thorup and W. A. Jackson (*Soil Sci.*, 1960, **90**, 1—7).—Samples (60) of subsoils covering a wide variety of clay, mineral oxide and exchangeable Al contents were equilibrated with aq. KH_2PO_4 and the sorbed PO_4^{3-} and exchangeable Al determined. The PO_4^{3-} sorbed was correlated with the exchangeable Al. Leaching with $CaCl_2$ to remove exchangeable Al diminished PO_4^{3-} sorption in soils containing much exchangeable Al. When Al montmorillonite (**I**) was leached with NaCl or $CaCl_2$ to displace all the Al, PO_4^{3-} sorption was reduced to very small proportions. When titrated with $Ca(OH)_2$ to pH 6 **I** absorbed all the PO_4^{3-} added; with **I** at pH 3·5, PO_4^{3-} equivalent to 24% of the exchangeable Al was bound; at pH 5 and 7 it was 70 and 97% respectively. In salt-free Al–clay systems PO_4^{3-} binding was max. at pH 7 but in the presence of chlorides, which displace Al from exchange sites, the max. occurred at pH 4. T. G. MORRIS.

Effect of lime on residual phosphorus in soil. D. F. Paton and J. F. Loneragan (*Aust. J. agric. Res.*, 1960, **2**, 524—529).—On acid sandy soils in Tasmania, poor growth of clover, characterised by symptoms of acute P deficiency, is remedied by liming. Experiments with superphosphate containing ^{32}P showed that, when $CaCO_3$ was applied, 62% of the original P was recovered from the top inch of soil; without $CaCO_3$, only 25% of the P remained in the top inch. The effect of lime in increasing uptake of P by clover is attributed in part to prevention of leaching of P. (12 references.) M. D. ANDERSON.

Soil–plant relations in phosphorus uptake. M. Friend and R. E. Shapiro (*Soil Sci.*, 1960, **90**, 69—76).—A review T. G. MORRIS.

Effect of soil water movement *vs.* phosphate diffusion on growth and phosphorus content of maize and soya-beans. R. E. Shapiro, W. H. Armiger and M. Fried (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 161—164).—Plant growth in media containing varying proportions of sand and soil and a comparison of static and recirculated soil solution indicated that P diffusion alone was too slow to account for the P uptake rate by young plants. Continuous movement of soil solution increased both plant growth and P concn. in the plant. Soil water movement probably accounts for a greater transfer of P to the roots than does diffusion. A. H. CORNFIELD.

Simultaneous determination of phosphate and sulphate ions in presence of metal contaminations. L. Szekeres and E. B. Polgár (*J. agric. Fd Chem.*, 1960, **8**, 417—419).—The PO_4^{3-} and SO_4^{2-} in P fertilisers are determined in presence of Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+} and F^- by dissolving the sample in 15% HCl, bringing to pH 2 to 3 with aq. NH_3 and masking Fe and Al by 0·1M-1,2-diaminocyclohexane-tetra-acetic acid (**I**). Ca and Mg are removed as stable complexes by adding 0·1M-EDTA (**II**) and adjusting to pH 10·0—10·5 with aq. NH_3. Eriochrome black T indicator is added. After eliminating excess **I** and **II** by titrating with $MgCl_2$ to transition colour and adding ethanol, phosphate is determined by titrating with $MgCl_2$ and sulphate with $BaCl_2$ after adding EDTA. Results agree with those of gravimetric procedures. M. D. ANDERSON.

Soil-potassium reactions as related to clay mineralogy of Kentucky soils. M. G. Cook and T. B. Hutcheson, jun. (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 252—256).—The clay fractions of Kentucky soils of high K-supplying power contained more illite than did those of low

K-supplying power. The former group also contained more exchangeable K. Drying the soils usually decreased exchangeable K when the initial value was high (>0·5 mequiv. per 100 g.) and increased it when initial value was low. Implications concerning the magnitude and origin of the interlayer charge in 14 Å components as well as degree of weathering are noted. A. E. CORNFIELD.

Chemical extraction of potassium from soils and micaceous minerals with solutions containing sodium tetraphenylboron. I. Preliminary experiments. A. D. Scott, R. R. Hunziker and J. J. Hanway (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 191—194).—Addition of Na tetraphenylboron (**I**) to salt solutions increased the amounts of K extracted from micaceous minerals and soils. After prolonged contact most of the K in vermiculite, 47% of that in illite and 12% of that in muscovite was removed by salt solutions containing **I**, even though little K was removed from these minerals when salt solutions alone were used. Increased extraction of K due to the presence of **I** is due to the effect of **I** in eliminating the " blocking " effect of extracted K. The NH_4-adsorption capacity of illite was increased by removing K with salt solutions containing **I**, but the increase was less than the amount of K removed. The extra K extracted from soils by salt solutions containing **I** in comparison with salt solutions alone may be indicative of the ability of soils to supply K over a long period. A. H. CORNFIELD.

Build-up of available potassium under subterranean clover pastures on a podsolic soil. C. H. Williams and J. Lipsett (*Aust. J. agric. Res.*, 1960, **11**, 473—484).—In podsolic soils with virgin pasture or pasture improved by clover, close relations were found between exchangeable K in the soil, and the K uptake and content of the crops, but K uptake was greater than decrease in exchangeable K. The plants probably contribute to the conversion of non-exchangeable to exchangeable K. Build-up of exchangeable K under clover is important in eliminating the potential K deficiency of these soils in the virgin state. A level of 0·25 to 0·30 mequiv. of exchangeable K per 100 g. is apparently required in these soils for normal plant growth. Removal of hay would seriously deplete the reserve of available K. (12 references.) M. D. ANDERSON.

Determination of reserve sulphur and soluble sulphates in soils. C. E. Bardsley and J. D. Lancaster (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 265—268).—" Total " soil S was determined by ignition with $NaHCO_3$ (500°), followed by extraction with 2N-AcOH–0·1N-NaH_2PO_4 and turbidimetric determination of the $BaSO_4$ formed on addition of $BaCl_2$. Soil SO_4^{2-} was extracted with 0·5N-NH_4OAc–0·25N-AcOH + activated C and determined turbidimetrically. " Reserve " S is " total " S minus SO_4^{2-}-S. Reserve S was highly correlated with total N in 23 surface soils. There was a significant correlation between reserve S and uptake of S by white clover in greenhouse tests with 17 soils. The SO_4^{2-} content of the soils was not correlated with uptake of S. A. H. CORNFIELD.

Effectiveness of manganese materials in supplying manganese to crops. L. Shepherd, K. Lawton and J. F. Davis (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 218—221).—Yields of onions on a muck soil (pH 7) were increased to about the same extent by application of Mn (50 lb. per acre) and $MnSO_4$, " NuManese " (48% Mn oxide), or " Mangasoil " (68% Mn oxide). Spray applications to the foliage (Mn 3·4 lb. per acre spread over five sprayings) were not quite as effective in increasing yields. There was little difference in response between broadcasting and band-placement of the materials. Yields of maize on alkaline soils in greenhouse tests were increased to about the same extent by application of the above materials as well as by Mn frits and Mn-EDTA. $MnSO_4$ and a Mn frit were the only materials which increased bean yields on alkaline soils. A. H. CORNFIELD.

Manganese content and its distribution in some East Pakistan soils. A. Q. M. B. Karim, M. Hussain and S. Choudhury (*Soil Sci.*, 1960, **90**, 129—132).—All soils except the coastal saline group were acidic and of high org. matter content. The exchangeable Mn (NH_4 acetate extract), content of alluvial, coastal saline and hill tract soils was almost the same despite textural differences ; that of lateritic soils was higher. In all soils the Mn levels were highest in the surface layers and decreased with depth. Org. matter also decreased with depth and appeared to be the dominant factor controlling Mn levels. T. G. MORRIS.

Extractable soil cobalt in soils of the southeastern United States. L. A. Alban and J. Kubota (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 183—185).—Cobalt extracted from the A_1 horizon of poorly-drained soils (Humic Gley, Low-Humic Gley and Ground-Water Podsols) ranged from 0·008 to 0·394 p.p.m. and from well-drained soils (Regosols and Red-Yellow Podsolics) from 0·06 to 3·74 p.p.m. with 2·5% AcOH-dithizone as extracting agent. For each group of soils extractable Co was highly correlated with the Co content of the leaves of black gum, *Nyassa sylvatica*, growing on the sites sampled. A. H. CORNFIELD.

Cobalt reactions with montmorillonite. J. F. Hodgson (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 165—168).—In addition to the normal cation-exchange reaction Co was bound to montmorillonite in two other sp. forms. One of these forms was slowly dissociable and the Co^{2+} could exchange with Cu^{2+}, Zn^{2+} and other Co^{2+} ions, but not with Ca^{2+}, Mg^{2+} or NH_4^+. The other form was not dissociable and could not be extracted with 2·5% AcOH and probably represents Co^{2+} which has entered the crystal lattice. A. H. CORNFIELD.

Phosphorus and bicarbonate effects on ^{85}Sr accumulation by bush beans. A. Wallace (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 327—328).—Culture tests on the effect of varying levels of HCO_3^- and PO_4^{3-} on the uptake of ^{85}Sr by bush bean seedlings were carried out. Without added HCO_3^-, the content of Sr in the roots, but not in the tops, increased with P concn. in the culture solution (0·00—0·01M). With 0·01M-HCO_3^- in the medium the Sr content of both tops and roots decreased with increasing P supply. Without added P the Sr content of roots increased markedly with HCO_3^- supply (0·00—0·01M). Sr content of the tops increased with HCO_3^- supply up to 0·005M and then decreased. A. H. CORNFIELD.

Extraction of soil organic matter with anhydrous formic acid. J. W. Parsons and J. Tinsley (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 198—201).—Anhyd. HCOOH, particularly when hot, was an effective extractant of org. matter from soils and composts. The efficiency of extraction was increased by the addition of inorg. cations. The dissolved material was recovered by pptn. with di-isopropyl ether containing 1% acetyl chloride, which held inorg. cations in solution. The recovered org. material had a good physical texture and was low in ash. The N extracted from five widely different soil types ranged from 27% to 43% and from two composts from 62% to 87% of the total N present in the materials. A. H. CORNFIELD.

Nitric acid oxidation of the organic matter of a podsol. M. Schnitzer and J. R. Wright (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 273—276).—Succinic, glutaric and adipic acids were found in the boiling 1 : 1 HNO_3 digest of the A_0, but not in the digest of the B_h horizon of a podsol. Benzene-tetra- and -penta-carboxylic acids occurred to a somewhat greater extent in the digest of the B_h than that of the A_0 horizon. Picric acid was present to about the same extent in both digests. The acids identified accounted for 5% of the original org. matter of both materials. A. H. CORNFIELD.

Physical and chemical properties of peats used as soil amendments. R. S. Dyal (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 268—271).—Moisture, org. matter, ash, pH and moisture retained at 0·1, 0·33 and 15-atm. tension are reported for peats derived from different vegetation in various stages of decomposition from a no. of localities. A. H. CORNFIELD.

Extraction of free amino-acids from soil. E. A. Paul and E. L. Schmidt (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 195—198).—Aq. $Ba(OH)_2$ was the most effective of a no. of reagents tested for extracting amino-acids from soils. From 73% to 121% of acidic and neutral and from 35% to 41% of basic amino-acids added to soil were recovered by leaching with 0·1N-$Ba(OH)_2$. Leaching with 0·5N-NH_4OAc (pH 6·8) recovered 31—83% of 18 amino-acids added to a soil. Although this represented a lower recovery range than that obtained with $Ba(OH)_2$ leaching, NH_4OAc was considered the more satisfactory reagent because of the possibility of amino-acids produced by hydrolysis when $Ba(OH)_2$ was used. A. H. CORNFIELD.

Colorimetric determination of soil organic matter. E. R. Perrier and M. Kellogg (*Soil Sci.*, 1960, **90**, 104—106).—Soil (0·5 g.) of 10-mesh size is mixed carefully with 10 ml. of N-$K_2Cr_2O_7$ and 5·5 ml. of conc. H_2SO_4 in a tube which is then heated in a boiling water bath for 5 min. and cooled. The mixture is then diluted to 1 l. After thorough shaking, 1 ml. is treated with 3·3 ml. of 6N-H_2SO_4 and 1 ml. of *s*-diphenylcarbazide reagent (saturated solution in 95% EtOH) is added and diluted to 100 ml. with water. After shaking, the optical density at 540 mμ is determined within 10 min. of mixing. The violet-coloured complex obeyed Beer's Law and there was a significant correlation between % org. matter by dry combustion and the log % transmittance. T. G. MORRIS.

Statistical evaluation of the rhizosphere effect. J. W. Rouatt, H. Katznelson and T. M. B. Payne (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 271—273).—Counts of soil organisms in soil on the surface of plant roots were compared (paired t-test) with those in soil sampled 6—8 in. from the roots. The rhizosphere soil of 5-weeks-old spring wheat was significantly higher in total bacteria, actinomycetes, fungi and protozoa and significantly lower in algae than was adjacent control soil. Within the bacteria group ammonifiers, anaerobes, denitrifiers, aerobic cellulose decomposers and radiobacter types were also higher in the rhizosphere soil. Similar results were obtained with barley and soya-bean soils. Rhizosphere soils were also higher in bacteria requiring amino-acids for optimum growth

and lower in bacteria requiring yeast and soil extracts for optimum growth than were control soils. A. H. CORNFIELD.

Liquid fertilisers—manufacture and application. V. A. Klevke and Ya. I. Kil'man (*Zh. prikl. Khim.*, 1959, **32**, 1649—1658).—Manufacture and use of liquid fertilisers in the U.S.A. and U.S.S.R. are compared. Results of experimental work are given in the use of liquid N fertilisers especially on cotton and sugar beet from 1956 to date. A. L. B.

Rock phosphate and superphosphate compared as pasture fertilisers on acid soils. K. D. McLachlan (*Aust. J. agric. Res.*, 1960, **11**, 513—523).—At equivalent levels of applied P, superphosphate was at first a better fertiliser than rock phosphate for acid soils, as judged by yield of pasture plants. Both sources of P had good residual values, and gave the same yield of pasture over 7 years. Mixtures of rock phosphate and superphosphate, either physical or obtained by underacidulation of rock phosphate, give the quick response characteristic of superphosphate alone. Gypsum has a low residual value as a source of S. Where plant growth is limited by S deficiency, or by climatic conditions, P from rock phosphate remains available in the soil until it is taken up by the plants. Org. P in the soil is much less available to plants than is P in rock phosphate, and conversion of inorg. to org. P is more important in limiting the residual value of fertilisers than is inorg. " fixation " by soil. (18 references.) M. D. ANDERSON.

Methods of analysis and efficiency of phosphate manures. R. Cadet and L. Soubiès (*C. R. Acad. Agric. Fr.*, 1960, **46**, 658—661).—Comparisons of the results of pot experiments with wheat for determining fertilising efficiency (according to the Burgevin technique) with analytical results, show that neutral (or slightly alkaline) aq. citrate (as used in some official methods) fail to extract the available P from fertilisers as satisfactorily as does the (Joulie) alkaline citrate used in the French official method. The extraction of Phosphal (Al phosphate + calcined chalk) with neutral aq. citrate leads to highly erratic results. P. S. ARUP.

Calcium sulphate transitions in superphosphate. S.-E. Dahlgren (*J. agric. Fd Chem.*, 1960, **8**, 411—412).—Thermodynamic equilibrium curves are given for the system $CaSO_4/H_3PO_4$/water, showing the transitions of $CaSO_4$ dihydrate to α-semihydrate, and to anhydrite II, in phosphoric acid solutions. The information agrees with observations on the transitions of $CaSO_4$ in superphosphate during acidulation and maturing, and on methods that prevent caking. M. D. ANDERSON.

Solubility and availability of micronutrients in relation to phosphorus fertilisation. F. T. Bingham and M. J. Garber (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 209—213).—Application of a very heavy dressing of $Ca(H_2PO_4)_2$ (P 900 lb. per acre), in comparison with a moderate dressing (P 76 lb. per acre), to 19 different soils in pots growing sour orange seedlings consistently reduced Cu % in the leaf and caused visual Cu deficiency symptoms. The high-P treatment usually increased leaf-Mo % in acid and decreased it in alkaline (pH 7·1—8·2) soils. The treatment usually increased leaf-Mn % in acid-neutral soils, but not in alkaline soils and reduced leaf-B % and -Zn % usually in acid soils only. In another test there were significant differences in trace element content of leaves due to rate and source of P [$Ca(H_2PO_4)_2$, H_3PO_4, KH_2PO_4 and $NH_4H_2PO_4$], but these varied with soil type used and were consistent only in the reduced leaf-Cu % due to high dressings of P. Soil water-sol. Cu and Zn were increased by excessive P fertilisation. Water-sol. Mn, B and Mo paralleled uptake data. A. H. CORNFIELD.

Monitoring fluoride content of air, water and vegetation. C. R. McHenry and H. Charles (*Farm Chem.*, 1960, **123**, No. 8, 58—62).—Sampling equipment and analytical procedures (distillation followed by thorium acetate titration) described were used by the phosphate industry in studying the area affected and the efficiency of methods for preventing air pollution by HF. A. G. POLLARD.

Diagnosis of fluorine injury [to trees]. II. Detection of fluorine-containing fumes by the storage of fluorine in the cortex of trees. F. Lampadius (*Phytopath. Z.*, 1960, **38**, 384—393).—The Th titration method is applicable to the determination of F in tree bark (mean error, 0·65—3·39%). The average F content of barks from trees not exposed to F was <0·06 mg. per 5 g. of air-dried sample. A relationship was established between the F content of bark and distance of tree from source of F. A. G. POLLARD.

Germination of small grain and maize as influenced by urea and other nitrogenous fertilisers. B. L. Brage, W. R. Zich and L. O. Fine (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 294—296).—Stands of barley were reduced with increasing rate of application of urea applied with the seed. Stands were reduced even further when the urea contained biuret. Urea containing 2·5% of biuret applied with the seed at 20 lb. of N per acre reduced winter wheat stands by 30%. Urea containing 10% of biuret broadcast at 160 lb. of N per acre and disced in did not affect the stand of barley or maize. $(NH_4)_2CO_3$ was even more toxic than urea in affecting emergence. NH_4NO_3 and $(NH_4)_2SO_4$ reduced emergence to some extent. The toxic effect of urea on seed is due to the NH_3 formed from it as well as to any biuret present. A. H. CORNFIELD.

Maintenance of soil fertility in Uganda. I. Soil fertility experiment at Serere. J. D. Jameson and R. K. Kerkham (*Empire J. exp. Agric.*, 1960, **28**, 179—192).—Under a 5-year rotational system of cultivation the area could be cropped 3 years in 5 : application of farmyard manure (5 tons/acre every fifth year) made possible four crops in 5 years. Cover crops in the resting years had no differential effects on soil fertility. A. G. POLLARD.

System for injecting measured volumes of liquid into soil. E. F. Wallihan and A. P. Vanselow (*Soil Sci.*, 1960, **90**, 107—108).—Appropriate apparatus is described. T. G. MORRIS.

Application of direct photometry to agricultural analysis. R. O. Scott (*J. Sci. Fd Agric.*, 1960, **11**, 584—592).—The uses of two direct reading spectrometers (porous-cup spark method) are described : (i) a two-channel instrument for determining 0·3—24 p.p.m. of Mg in AcOH and $AcONH_4$ extracts of soils and HCl extracts of plants, a coeff. of variation of ~±2·0% being found ; (ii) the 11-channel Hilger Medium Direct Reader for determination in soil extracts of 0·1—24 p.p.m. of Cu, 1—400 p.p.m. of Mn and 1—50 p.p.m. of Zn, the coeff. of variation being ±1·10, ±1·64 and ±7·01%, respectively. A tentative rotating disk technique for determining Zn, B, Fe, Mn and Cu in plant ash is described, coeff. of variation being ±1·9, ±2·8, ±17·9, ±3·3 and ±5·4%, respectively. E. M. J.

Contamination problems in soil and plant analysis. R. L. Mitchell (*J. Sci. Fd Agric.*, 1960, **11**, 553—560).—A survey of problems arising especially in trace element analysis, transport of soil samples, etc., and the need for stringent laboratory precautions is presented. A means of assessing the extent of soil contamination of plants by determining the apparent content of an element with a high soil/plant ratio, viz., Ti (ratio 10,000 : 1) is described. (14 references.) E. M. J.

Rapid polarographic method for determining extractable zinc in mineral soils. H. L. Barrows and M. Drosdoff (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 169—171).—The soil is extracted with 0·1N-HCl, the filtrate treated with a NH_4Cl–KCl–Na_2SO_3–gelatin–aq. NH_3 buffer (pH 11) and the Zn determined polarographically. Available Zn determined in this way in a no. of soils treated with varying levels of Zn was highly correlated with the Zn content of the leaves of tung seedlings and trees growing on these soils. A. H. CORNFIELD.

Determination of the precipitated potassium in sodium tetraphenylboron–micaceous mineral systems. A. D. Scott and M. G. Reed (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 326—327).—A modified method for determining K in the Na tetraphenylboron–salt extracts of soils and micaceous minerals (*ibid.*, 1960, **24**, 191—194) is described. A. H. CORNFIELD.

Fertilisers. A. James (B.P. 824,844, 9.11.55).—There is claimed a fertiliser in the form of soluble cubes, containing 80—90 wt.-% of plant food (bone meal, treated excrement, dried blood, superphosphate, pulped vegetable matter, lime, hoof and horn mixture, soot, KNO_3, K_2SO_4, $FeSO_4$ and $MgSO_4$), 5—6 wt.-% of pest-deterrent (naphthalene, ammonium sulphate, liver of S, $KMnO_4$ and DDT), solid inert diluent (sand, plaster, whiting, Portland cement or china clay), and optionally traces of $MnSO_4$, $CuSO_4$, $ZnSO_4$, Na borate and Al molybdate. F. R. BASFORD.

Phosphate fertilisers. Iowa State College Research Foundation (B.P. 826,234, 2.3.56. U.S., 23.8.55).—A phosphate fertiliser containing a substantial proportion of $CaHPO_4$ in admixture with $Ca(H_2PO_4)_2$ comprises heating the latter with water (at 50—120°) to hydrolyse at least a portion thereof of $CaHPO_4$, with liberation of H_3PO_4 (as an aq. solution) ; then heating the resulting mixture with phosphate rock at 125—280 (150—260°)/1 atm. to remove water and cause formation of more $Ca(H_2PO_4)_2$ by interaction of the rock with the H_3PO_4. F. R. BASFORD.

Fluid fertilisers. Scottish Agricultural Industries Ltd. (Inventor : I. A. Brownlie) (B.P. 823,449, 22.3.57).—A readily handled fluid fertiliser composition contains NH_4NO_3, urea and water, has a solid phase consisting of urea and a liquid phase of *d* not less than that of the solid phase. Possible compositions are represented on a phase diagram. F. R. BASFORD.

Compositions of nutrients for plant growth. Ferro Enamels Ltd. (Inventors : C. A. Vana and G. E. de Geronimo) (B.P. 823,216, 16.11.55).—A composition for use as plant nutrient comprises a

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glass matrix (SiO_2 26—85%) formed by fusing a mixture of SiO_2 (26—85), CaO (1—10), K_2O (1—15), MgO (1–5) and Na_2O (1—20%) with 2—50 wt.-% of an inorg. compound of Cu, B, Zn, Co, Mo or V (calculated as oxide) at 1800°—2800° F, then fritting the fused mass (to give material of 90% of 20—200 in.-mesh). The amount of Co and/or Mo present is preferably less than 10% of the amount of Mn, Zn, Co or B. The resulting frit may be admixed with a N-releasing material, e.g., urea, if desired. F. R. BASFORD.

Trace element fertilisers. W. Jost (B.P. 825,612, 20.3.57. Ger., 22.3.56).—A Cu-containing waste comprising particles of large superficial area and 44—85% of Cu, e.g., Cu hammer scale, is admixed with at least one other trace element-containing factory waste of high metal content and large surface area (excluding material containing a high proportion of Fe), e.g., material containing Zn (such as brass grinding dust or waste from a galvanising unit or a Zn smelting works), to provide a trace element fertiliser.
F. R. BASFORD.

Plant Physiology, Nutrition and Biochemistry

Photosynthesis by sea plants in relation to salt concentration. F. Gessner and L. Hammer (*Planta*, 1960, **55,** 306—312).—Photosynthesis in the leaves of *Posidonia oceanica* is entirely inhibited on transference to fresh water; in *Ulva lactuca* the process is reduced by ~77%. Responses to alternation between fresh and salt water are reversible and prompt. A curve is given showing the decrease in photosynthesis by *P. oceanica* on progressive dilution of sea water with tap water. The significance of these results is considered. P. S. ARUP.

Nitrate reductase activity in maize seedlings as affected by light and nitrate content of nutrient media. R. H. Hageman and D. Flesher (*Plant Physiol.*, 1960, **35,** 700—708).—Both light and NO_3 are necessary for the formation of NO_3^- reductase in quantities required by the plant for normal growth and there is a positive correlation between reductase activity and growth and protein content. E. G. BRICKELL.

Co-operation of visible and infra-red radiation in induction of ear formation in *Hordeum vulgare*. M. Muschik (*Planta*, 1960, **55,** 292—305).—Experiments with various radiation filters indicate that i.r. radiation is not without effect. The intensity of visible radiation is also a factor. (19 references.) P. S. ARUP.

Metabolism of isolated leaves. I. Changes in the protein, soluble nitrogenous compounds, sugars and organic acids in tobacco leaves in light and dark. S. Ranjan and M. M. Laloraya (*Plant Physiol.*, 1960, **35,** 714—725).—Although the total amino-N content increases both in light and darkness in detached leaves, the behaviour of individual amino-acids varies considerably due possibly to interconversions regulated by certain growth factors. S-20 variety of tobacco leaves showed no malic-citric conversion when cultured in the dark, Cheroot and Connecticut did so, but not in antibiotic solution. Culture of S-20 in antibiotic solutions did not affect the behaviour of org. acids. The phenomenon is ascribed to the operation of enzyme systems other than those encountered in the Krebs citric acid cycle, probably malic-enzyme, and this is blocked in the presence of antibiotics. E. G. BRICKELL.

A carotene precursor: its proposed structure and place in biosynthetic sequence. G. A. Thompson, jun., A. E. Purcell and J. Bonner (*Plant Physiol.*, 1960, **35,** 678—682).—A substance, isolated from tomatoes, appears to be an intermediate in the pathway from mevalonic acid to the carotenes. Infra-red spectroscopy and degradative studies indicate a probable structure of 3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene. E. G. BRICKELL.

Anthocyanin synthesis in maize endosperm tissue cultures. II. Effect of certain inhibitory and stimulatory agents. J. Straus (*Plant Physiol.*, 1960, **35,** 645—650).—The strongest inhibitory agents are riboflavin, methionine, asparagine, glutamine and valine. The strongest promoters of pigment synthesis are aspartic acid and cystine. Adenylic and cytidulic acids promote anthocyanin synthesis to some degree but experiments with purine and pyrimidine analogues indicate that the nucleotides do not participate directly in pigment formation. E. G. BRICKELL.

Constitution of the hemicullulose of lucerne (*Medicago sativa*). Hydrolysis of hemicellulose and identification of neutral and acidic components. D. V. Myhre and F. Smith (*J. agric. Fd Chem.*, 1960, **8,** 359—364).—The hemicellulose of lucerne yielded, on acid hydrolysis: L-arabinose (12·0%), D-xylose (67·3%), D-galactose (8·1%), D-glucose (8·1%) and L-rhamnose (4·5%), also the five acids oxalic, D-galacturonic, 4-*O*-methyl-D-glycuronic, 2-*O*-(4-*O*-methyl-α-D-glucosyluronic acid)-D-xylose and *O*-4-*O*-methyl-α-D-glycosyluronic acid-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylose, and two other acids, which are probably D-galactosyluronic acid D-xylose, and D-galactosyluronic acid D-galactose. The relatively high content of L-rhamnose appears to be characteristic of legumes; little or none has been found in the hemicelluloses of grasses. (28 references.) M. D. ANDERSON.

Nature of the variations in flower colour in the genus *Lathyrus*. R. C. Peckett (*New Phytol.*, 1960, **59,** 138—144).—In the flowers of each of nineteen species of *Lathyrus* one or more of the anthocyanidins, delphinidin, malvidin and petunidin were present as monoglucosides (except in *L. sativus* which contained free malvidin). L. G. G. WARNE.

Fatty-acid composition of lipids of pasture grasses. G. A. Garton (*Nature, Lond.*, 1960, **187,** 511—512).—A complete analysis is reported of total fatty acids (**I**) in artificially dried milled grass (perennial rye 50, cocksfoot 25, timothy 10, Italian rye 10%). **I** were 2·3 wt.-% of dry grass; 88% were in lipids of EtOH extracts (**II**) and 12% in those of $CHCl_3$–MeOH extracts (**III**). Composition of **I** closely resembles that of leaf lipids of maize and of clover-rich pasture, with unsaturated C_{18} acids >75% (linolenic 61%) and palmitic acid (16%) predominant in the saturates. Lipids of **III** contained more palmitic acid (27%) and less linolenic acid than did those of **II**, with arachidic and behenic acids (~5%) (**IV**) in addition. A neutral lipid (triglyceride, ≡~3% of **I**) and galactosyl glyceryl esters (≡~80% linolenic acid) were isolated from **II** by chromatography. About 30% of fatty acids, inclusive of **IV**, in lipids of **III** are insol. in $COMe_2$. W. J. BAKER.

Electrolytic procedure for differentiating between frost injury of roots and shoots in woody plants. J. Wilner (*Canad. J. Plant Sci.*, 1959, **39,** 512—513).—Frost damage to the roots of apple trees in pots is shown by a decrease of ~70% in the electrolytes extractable by water at 100°, in comparison with those obtained from undamaged roots. Similar material extracted at room temp. from the damaged roots represents ~76% of that extracted at 100°; for normal roots, the ratio is ~30%. P. S. ARUP.

Free amino-acids in lucerne as related to cold hardiness. M. D. Wilding, M. A. Stahmann and D. Smith (*Plant Physiol.*, 1960, **35,** 726—732).—Amino-acids increased (20%) from Aug. to Dec. in the roots of most hardy variety and this was associated with a 31% increase in non-amino-N over the same period. Non-hardy lucerne showed little change in either N fractions as hardiness developed. E. G. BRICKELL.

Chilling injury in citrus fruits. I. L. Eaks (*Plant Physiol.*, 1960, **35,** 632—636).—A pronounced increase in rate of CO_2 evolution was associated with chilling and there was a cumulative time–temp. influence of chilling on the degree of stimulation of CO_2 evolution. An increase in the rate of O_2 uptake at 20° by immature orange, lemon and lime fruits was observed after exposure to 0° and 10°. The stimulated respiratory rate of chilled fruit may arise from changes in the relative amounts of certain metabolities which are rapidly oxidised at the non-chilling temp. E. G. BRICKELL.

Apparent free space of plant roots. L. Bernstein and R. H. Nieman (*Plant Physiol.*, 1960, **35,** 589—598).—A technique is described using Indian ink to tag the external medium and mannitol and chloride as the test solutes. The apparent free space (AFS) of pea and bean roots changed during leaching of the roots, especially if salt solutions were subsequently used in estimating AFS. The effect is attributed to a loss of exchangeable Ca which by cross-linking carboxyl groups maintained a certain structure in the cell walls. A tendency towards increased AFS under conditions of high transpiration was noted. E. G. BRICKELL.

Plant diseases in long-term manuring experiments. A. Weber (*Tidsskr. Planteavl*, 1960, **64,** 305—330).—A report on the effects of deficiencies in N, P, K, N + P, N + K and P + K in ~30 different plants, grown in open ground and in large cement cylinders. The chief observations are concerned with the effects of K- or N-deficiency and with K-induced Mg-deficiency. P. S. ARUP.

Quantitative considerations in iron nutrition of higher plants. J. J. Oertli and L. Jacobson (*Plant Physiol.*, 1960, **35,** 683—688).—Buckwheat, maize, cotton, lupin, pea, sunflower, tobacco and tomato were grown in nutrient culture with different levels of Fe. Min. Fe concn. depended on plant species, the form of Fe supplied, and the ratio to other nutrients, as did also metabolic or internal requirements as judged by Fe-chlorophyll relationships. Fe-deficient plants take up relatively smaller amounts of anions (NO_3^-) than of cations. A function of Fe in N metabolism is indicated. E. G. BRICKELL.

Chlorosis in soya-bean as related to iron, phosphorus, bicarbonate and cytochrome oxidase activity. G. W. Miller, J. C. Brown and R. S. Holmes (*Plant Physiol.*, 1960, **35,** 619—625).—PI-54619-5-1 and Hawkeye soya-beans did not develop chlorosis when grown using split-medium or split-root techniques with roots in $NaHCO_3$

(10 mequiv./l.) at comparatively low P concn. but in comparison PI soya-beans developed chlorosis in a complete solution containing $NaHCO_3$ (2 mequiv./l.). Cytochrome oxidase activity decreased with increasing [HCO_3^-] in a complete solution but was dependent on P in the split-root experiments. In solution cultures HCO_3^- increased the sol. P concn. but did not directly cause Fe chlorosis or inactivate cytochrome oxidase. E. G. BRICKELL.

Effects of fertilisers and farmyard manure on the copper manganese, molybdenum and zinc removed by arable crops at Rothamsted. R. J. B. Williams, A. Stojkovska, G. W. Cooke and F. V. Widdowson (*J. Sci. Fd Agric.*, 1960, **11**, 570—575).—Farmyard manure (F.Y.M.) and a complete NPK fertiliser mixture had similar effects on the proportions of Cu, Mn, Mo and Zn in wheat, barley, clover, potatoes and kale; in general Mn contents were increased; Cu, Mo and Zn were decreased. Clover removed more of each element than was removed by wheat, barley or potatoes; clover and kale removed more Mo than did the other crops. If total quantities of micronutrients in the surface layer of the soil under test, become " available," these reserves will last for many rotations, otherwise micronutrient deficiencies could occur in crops grown with ordinary fertilisers and without F.Y.M. A dressing of F.Y.M. (15 tons/acre) will replace micronutrients removed, whereas ordinary fertilisers supply only insignificant amounts. (10 references.) E. M. J.

Effect of supra-optimal boron levels on respiration and carbohydrate metabolism of *Helianthus annuus*. E. G. Scott (*Plant Physiol.*, 1960, **35**, 653—661).—Respiration rate is higher in B-treated leaves but the respiratory quotient of both treated and control leaves is unity; concn. of reducing sugars, sucrose, and total sugars plus starch is higher and starch content lower, in treated leaves. *In vitro* studies using starch phosphorylase show that B completely inhibits the formation of starch, probably through complexing with the active site of starch phosphorylase. E. G. BRICKELL.

Absorption of caesium by excised barley roots. G. G. J. Bange and R. Overstreet (*Plant Physiol.*, 1960, **35**, 605—608).—Steady-state absorption was studied under conditions of low temp. and anoxybiosis. The results are explained on the basis of a two-fold absorption mechanism. K, Rb, and to a less extent NH_4^+, inhibited Cs uptake in a way suggesting competition for the same binding site; Li and Mg had little effect, Na and Ca affected only the overall absorption capacity for Cs, Na in a negative and Ca in a positive sense. E. G. BRICKELL.

Analysis of the structural carbohydrates of herbage. I. H. Bath (*J. Sci. Fd Agric.*, 1960, **11**, 560—566).—The proposed method involves (*a*) extraction with ethanol–benzene, 95% ethanol, to remove lipids, pigments, sol. sugars; (*b*) extraction of fructosan and pectin with hot water; (*c*) treatment with acid chlorite to remove lignin and yield holocellulose; (*d*) extraction of holocellulose with KOH to give α-cellulose and hemicellulose. The polysaccharides of the hot water extract, α-cellulose and hemicellulose are hydrolysed, the constituent sugars are separated chromatographically and identified spectrophotometrically. (38 references.) E. M. J.

Quantitative determination of nucleic acids. H. Kern (*Planta*, 1960, **55**, 259—273).—Current methods are critically examined, and an improved analytical procedure is presented. (32 references.) P. S. ARUP.

Elimination of interference by phosphorus and other elements in the flame photometric determination of calcium and magnesium in plant tissue. C. G. Wells and R. B. Corey (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 189—191).—Interference of P, Fe and Al in the flame photometric determination of Ca and Mg in plant material was removed by addition of $FeCl_3$ to the acid plant ash extract followed by pptn. of P, Fe and Al, by addition of aq. NH_3 to pH 5·6. Clogging of the burner capillary by NH_4Cl was prevented by addition of NH_4OAc. Interference of K was eliminated through compensation by addition of 500 p.p.m. K to all solutions. A. H. CORNFIELD.

Salt injury to crops. K. Kreeb (*Z. PflKrankh.*, 1960, **67**, 385—399).—Published and other experimental data are discussed. The % and rate of germination of seeds diminish with rise in salt concn. of the medium. The effect was influenced by plant species but was mainly dependent on the osmotic pressure rather than on the intrinsic toxicity of the dissolved salts. Salt effects due to heavy use of fertilisers are scarcely possible in temperate climates. (75 references.) A. G. POLLARD.

Naturally occurring quinones in wheat and barley and their toxicity to loose smut fungi. M. E. Mace (*Dissert. Abstr.*, 1960, **20**, 4237—4238).—The fungicidal compounds, 2-methoxy-*p*-benzoquinone, 2,6-dimethoxy-*p*-benzoquinone and 2-methoxyquinol, were isolated from wheat germ. -SH compounds known to act as inhibitors of the toxic action of these quinones did not interfere with control of loose smut on wheat or barley grains, but entry of the -SH compounds into the grain could not be demonstrated. All three quinones were highly toxic *in vitro* to the mycelia and spores of the fungi of loose smut (*Ustilago* spp.), perhaps because of ability to combine with or oxidise -SH groups. Wheat and barley germ held anaerobically or in water lost 22 to 28% of the 2-methoxyquinol glucoside originally present; the appearance of free methoxyquinol was indicated by colour tests, but not confirmed by paper chromatography. M. D. ANDERSON.

Effect of auxin on loss of calcium from cell walls. R. Cleland (*Plant Physiol.*, 1960, **35**, 581—584).—Loss of Ca from *Avena* coleoptile and maize mesocotyl cell walls is not enhanced by auxin nor does it cause a redistribution of Ca between pectin and protopectin. It would appear that the removal of Ca cross-linkages is not mediated by auxin and that the Ca-bridge hypothesis is incorrect. S. G. BRICKELL.

Ethionine and auxin-action in *Avena* coleoptile. R. Cleland (*Plant Physiol.*, 1960, **35**, 585—588).—The rate of transfer of methyl groups from methionine to the hot-water-sol. fraction of *Avena* coleoptile cell walls is strongly inhibited, and auxin-increase in methylation entirely prevented, by ethionine. Inhibition occurs 1 h. after treatment. An immediate inhibition of the reversible elongation of the tissues and that of irreversible elongation after 3—6 h. also occurs. EDTA will reverse the inhibition of the reversible but not the irreversible elongation. E. G. BRICKELL.

Photolysis of 2-indolylacetic acid [IAA] by ultra-violet light: dependence on pH and oxygen concentration. W. Klingmüller (*Planta*, 1960, **55**, 283—291).—The max. rate of destruction (in citric acid–phosphate buffered 1% ethanolic solution) occurs (under air or N_2) at pH 4·5. The (faster) rate under air depends solely on the concn. of IAA. The destruction under N_2 probably occurs as a second-order reaction. The theoretical significance of the observations is considered. (18 references.) P. S. ARUP.

Effects of coumarin on growth and development processes, and its mobility in plant tissue. E. Gantzer (*Planta*, 1960, **55**, 235—253).—The effects of coumarin (**I**) alone and in combination with IAA on the growth of the oat-coleoptile are studied by means of three tests. In high concn. ($1 : 10^4$—$1 : 10^2$), **I** probably acts as a poison, and in lower concn. as a stimulant; its action does not depend on a *trans–cis* change. In the *Raphanus* hypocotyl, **I** is transported in both morphological directions. Several theories are considered in connection with these and other observations. (19 references.) P. S. ARUP.

Differential effects of 2,3,6-trichlorobenzoic acid on growth and geotropic curvature of *Avena* coleoptiles. A. R. Schrank (*Plant physiol.*, 1960, **35**, 735—741).—2,3,6-Trichlorobenzoic aicd (TCBA) increased the growth of floating sections in the absence of exogenous indolylacetic acid, and increased the elongation of 15-mm. apical segments. Geotropic curvature of segments stimulated in the horizontal position, was extensively inhibited by 1×10^{-3} and 1×10^{-4}M-TCBA but lower concn. had no effect on negative geotropism. TCBA had no effect on elongation of 15-mm. apical segments for a period of 24 h. at 4° but geotropic curvature was inhibited at this temp. Positive phototropic curvature was not affected by concn. of TCBA from 1×10^{-6}—1×10^{-3}M. E. G. BRICKELL.

Metabolism of plant growth regulators. I. 2,4-Dichlorophenoxyacetic acid in leaves of red- and of blackcurrant. II. Decarboxylation of 2,4-dichlorophenoxyacetic acid in leaves of apple and strawberry. L. C. Luckwill and C. P. Lloyd-Jones (*Ann. appl. Biol.*, 1960, **48**, 613—625, 626—636).—I. The metabolism of ^{14}C-labelled 2,4-D in detached leaves of blackcurrant, which is very susceptible to damage by the herbicide, and of redcurrant, which is highly resistant to the herbicide, was studied. Over 1 week 50% of the 2,4-D in the redcurrant leaf was decarboxylated as compared with only 2% in the blackcurrant leaf. The redcurrant leaf also metabolised the side chains of other hormone-type materials. Other chemical changes occurring in the leaf are reported. The redcurrant is killed by low concn. of 2,4,5-T even though this compound was broken down in the leaf as rapidly as 2,4-D.

II. Detached leaves of Cox, which is resistant to 2,4-D, showed a high rate of decarboxylation of 2,4-D in comparison with those of Bramley's Seedling, which is susceptible to 2,4-D. High rates of decarboxylation were also found in varieties having Cox in their parentage, whilst varieties unrelated to Cox showed low rates. High decarboxylation rate was found in 2,4-D-resistant varieties of strawberry. Tests with 16 other species showed that all but one, whether they were susceptible or resistant to 2,4-D, had low decarboxylation rates. *Syringa vulgaris*, a 2,4-D-resistant species, had a high decarboxylation rate. A. H. CORNFIELD.

Plant-growth activity of α-alkylphenylacetic acids and 1,2-benzcycloalkene-3-carboxylic acids. K. Kawazu, T. Fujita, T. Mitsui, J. Kato and M. Katsumi (*Nature, Lond.*, 1960, **187,** 694—695).—Effect on pea straight-growth of five α-alkylphenylacetic acids and five corresponding homologues in the 1,2-benzcycloalkene-3-carboxylic acids is reported and discussed in respect of the spatial relation of the CO_2H group to the ring system and the hydrophil–lipophil balance. Degree of activity is dependent on sterochemistry of the mol. as in the hydrogenated 1-naphthoic acids (*ibid.*, 1959, **184,** 1415). The abrupt fall in activity from C_{11}- to C_{12}-acid in each series is as yet unexplained. W. J. BAKER.

Effects of temperature and humidity on foliar absorption and translocation of 2,4-D and benzoic acid. J. E. Pallas, jun. (*Plant Physiol.*, 1960, **35,** 575—580).—With increasing temp. (20—30°) increased absorption and translocation of both compounds were found and the effect was further increased by high R.H., which correlated with the degree of stomatal opening. Movement of 2,4-D in the leaf was confined, in general, to the vascular bundles and followed the route of the assimilate stream out of the leaf and into the stem, bud and roots. Benzoate-treated plants showed in addition a diffuse movement away from the treated areas to all parts of the treated leaf. E. G. BRICKELL.

Action of gibberellic acid on lettuce seed germination. H. Ikuma and K. V. Thimann (*Plant Physiol.*, 1960, **35,** 557—566).—Gibberellic acid (**I**) at 60 p.p.m. induces max. germination; below this level the effect is linearly proportional to concn. if the solution is injected directly into the seed 1 p.p.m. is fully effective. The time of greatest sensitivity occurs after about 1½ h. of presoaking in water, this being identical with the most sensitive period of the seeds to red light. Far-red light does not inhibit seeds which have been treated to produce 90% germination but partial inhibition occurs if treatment is given to yield 50% germination. Heavy doses of far-red desensitise the seeds to gibberellin. **I** acts by initiating one of the chemical reactions which result from the light reaction so that its end product is the same as that produced by light. E. G. BRICKELL.

Effects of gibberellic acid on *Mentha piperita*. C. D. Ogzewalla (*Dissert. Abstr.*, 1960, **20,** 4085).—Spraying peppermint plants weekly with gibberellic acid reduced the fresh wt. of the plants, but did not affect the yield of peppermint oil. When a second crop was allowed to grow after harvesting, without further treatment, yields of herb and of oil were reduced. Gibberellic acid caused lengthening of cells in the stem, with elongation of stems and internodes; cells were fewer in no. Leaves of treated plants were thinner. Oils from treated plants had a higher sp. gr. and *n*, were more laevorotatory, and contained more esters (as menthyl acetate) and alcohol (as menthol) than oils from untreated plants. Only one oil from treated plants was within U.S.P. specifications. M. D. ANDERSON.

Antimitotic action of cyanamide on bulbs of *Allium cepa*, L. O. T. Rotini (*Ric. sci.*, 1960, **30,** 869—872).—Solutions of cyanamide of ⩽0·01525% (prepared by treatment of aq. $CaCN_2$ with CO_2) exert an appreciable antimitotic action on the bulb roots of *Allium cepa*, L. Below this concn. the N of the $NH_2{\cdot}CN$ is metabolised by the plant without drawback. A mechanism of action based on cytological tests is proposed. L. A. O'NEILL.

Plant growth in presence of hippuric acid, benzoic acid and glycine. N. Guerrucci and O. T. Rotini (*Ric. sci.*, 1960, **30,** 842—848).—The antimitotic limit of hippuric acid on bulbs of *Allium cepa* L. lies at 0·025%, and of benzoic acid at 0·0085%. Glycine does not show any antimitotic action up to 0·084%. The inhibitory action of hippuric acid appears thus to be related to its enzymic scission. (21 references.) L. A. O'NEILL.

Trypsin inhibitor of lucerne. J. S. Ramirez and H. D. Mitchell (*J. agric. Fd Chem.*, 1960, **8,** 393—394).—Material strongly inhibiting the digestion of casein by trypsin *in vitro* was isolated from dried lucerne by extraction with water, filtering, concentrating the filtrate under reduced pressure, dialysing the extract against water, concentrating the residual extract further, pptg. by excess of ethanol, and separating the ppt. by centrifuging. The active material was readily sol. in water; electrophoresis indicated two components. Activity was gradually destroyed at 98°. The material yielded amino-acids on hydrolysis; it appears to be a polypeptide or non-coagulable protein. Inhibition of trypsin was non-competitive. M. D. ANDERSON.

Crops and Cropping

Utilisation of nitrogen by wheat when water or light are available in insufficient amount. Relations with water utilisation. Y. Coic (*C. R. Acad. Agric. Fr.*, 1960, **46,** 486—490).—Results of pot experiments in the period after flowering are reported. Reduction in water supply reduced yield and utilisation of N; limitation of light reduced yield but had little effect on the need for N_2. The physiological significance of the findings is discussed. E. C. APLING.

Relation of nitrogen to disease development in rice seedlings infected with *Helminthosporium oryzae*. S. B. Chattopadhyay and J. G. Dickson (*Phytopathology*, 1960, **50,** 434—438).—Water-cultured rice was inoculated with *H. oryzae*, and grown in media containing varying levels of N. The plants showed symptoms of N deficiency in media containing NO_3^--N, 105 p.p.m. Max. infection with seedling blight also occurred at this N-level or with NO_3^--N, 217 + NH_4^+-N, 122 p.p.m. Both deficiency and excess of NH_4^+-N affected the development of the disease. NO_3^--N was not utilised by rice from non-aerated media. A. G. POLLARD.

Effect of initial treatments of beet seed on biochemical composition of the tubers. S. Gassian, M. Marchand and J. Séchet (*C. R. Acad. Sci. Paris*, 1960, **251,** 433—435).—Exposure of germinating beet seed at 2° for 30—40 days, or washing them in running water for ~48 h., increased the wt. of tubers and leaves at harvest. In the dry matter of treated tubers the total N and protein contents diminished and that of sol. sugars increased. Exposure of the seed at 23° produces the opposite effects. W. J. BAKER.

Wounding of potato tubers. F. Hansen and J. B. Henriksen (*Tidsskr. Planteavl*, 1960, **64,** 244—293).—Spoilage by wounding varies from negligible after the tubers have been thrown from a height of 85 cm. on to a smooth surface and then stored at 1—5°, to severe after falling on to a rough surface followed by storage at 10—15°. (64 references.) P. S. ARUP.

Influence of manuring on mineral content of pasture crops. A. Hendriksen (*Tidsskr. Planteavl*, 1960, **64,** 1—50).—Undesirable increases in the K/(Ca + Mg) ratio (liable to cause grass tetany) are chiefly due to the presence in the sward of timothy or (especially) cocksfoot grass, and particularly when N or N + K are applied: the effects are most pronounced on soils rich in K and/or poor in Mg. K-manuring should be postponed until June or July, viz. until after the seasonal max. (in May) for the K/(Mg + Ca) ratio. (22 references.) P. S. ARUP.

Free amino-acids in red clover as related to flowering and winter survival. M. D. Wilding, M. A. Stahmann and D. Smith (*Plant Physiol.*, 1960, **35,** 733—735).—A 40—50% increase in amino-acids was shown in the hardier plants which were prevented from flowering but several specific amino-acids failed to show this change. The increase was not associated with a change in total N content of the roots. E. G. BRICKELL.

Nitrogenous content of leguminous creeping covers. Anon. (*R.R.I. Plant. Bull.*, 1960, pp. 35—39).—After one year of growth the "natural" covers (a mixture of *Trema, Ficus, Macaranga, Hornstedtia* spp. and others) produced the greatest wt. of green material and litter, while *Mikania scandens* produced the least total bulk. A cover of leguminous creepers (normal mixture of *Pueraria phaseoloides, Calopogonium mucunoides* and *Centrosema pubescens*) contained rather more N than did the "natural" cover, while both contained very much more than either grass (*Axonopus compressus*) or *Mikania*. E. G. BRICKELL.

Increase in yield and protein content of native bluebunch wheatgrass from nitrogen fertilisation. J. L. Mason and J. E. Miltimore (*Canad. J. Plant Sci.*, 1959, **39,** 501—504).—Very marked increases in forage yields from *Agropyrum spicatum* (by 65—154%) followed annual applications of NH_4NO_3 (especially at 60 lb. of N per acre); corresponding increases in protein per acre were 59—312%. P. S. ARUP.

Effects of frequency and intensity of grazing on the genotypic structure of a ryegrass population. R. W. Brougham, A. C. Glenday and S. O. Fejer (*N.Z. J. agric. Res.*, 1960, **3,** 442—453).—Changes in the plant population as a result of the application of four different grazing systems to New Zealand short rotation ryegrass (*Lolium perenne* × *L. multiflorum*) over a period of 2 years are reported. A selection towards the perennial type plants was observed in the frequent and intensive grazing systems, and towards the Italian type plants in the less intensive and long-spelled grazing systems. The results show that management systems applied to pastures have a rapid influence on natural selection. (21 references). E. C. APLING.

Fruit-thinning of apples by spraying with plant growth substances: 1955—58. H. Øhlers (*Tidsskr. Planteavl*, 1960, **64,** 213—243).—Sprayings with naphthyl-acetic acid or -acetamide under standard conditions have given average fruit-thinnings of 34 and 16%, and increases in fruit-wt. of 18 and 10%, respectively. Somewhat better results are obtained by spraying at petal fall than 8—10 days later. Suitable concn. (10—20 p.p.m. of naphthylacetic acid) are given for different varieties of apples. Any damage caused to the foliage is purely temporary. P. S. ARUP.

Diseases of *Mangifera indica*. XI. Effect of boron on mango necrosis. S. N. Das Gupta and C. Sen (*Phytopathology*, 1960, **50**, 431—433).—Spraying with aq. borax (6—8 lb./100 gal.) twice during the flowering season markedly lowered the incidence of tip necrosis of the fruit. A. G. POLLARD.

Maintaining high soil-nutrient levels for greenhouse tomatoes without excess salt accumulation. R. E. Lucas, S. H. Wittwer, and F. G. Teubner (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 214—218).—The use of heavy dressings of KH_2PO_4, $(NH_4)_2HPO_4$, KNO_3 and NH_4NO_3 spread through the growing season resulted in high yields (up to 109 tons per acre) of tomatoes in greenhouses without increasing the sol. salt content of the soils to toxic values. Seasonal changes in soil nutrient status of a no. of soils are presented. A. H. CORNFIELD.

Failure of peas in " goradu " soils of Gujarat. S. R. Vyas and N. Prasad (*Proc. Indian Acad. Sci.*, 1960, **51B**, 242—248).—The high sand content of this soil gives no protection to the pea rhizobia against high temp. in the summer months and the reduction in no. restricts nodulation. E. G. BRICKELL.

Effect of saline water on growth and chemical composition of beans. I. Influence of soil dilution. J. Lunin and M. H. Gallatin (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 231—234).—Application of saline irrigation water decreased bean yields to a greater extent on a clay soil which had been diluted with an equal wt. of sand than on the original clay soil. There was little difference in the K, Ca, Mg and Na contents of plants between the whole and diluted soils. The Ca, Mg and Na contents of the plant increased with increasing salinity of the irrigation water, but the K content was little affected. A. H. CORNFIELD.

Selection of cotton plants without gossypol. J.-B. ROUX (*C. R. Acad. Agric. Fr.*, 1960, **46**, 613—622).—Breeding experiments indicate the possibility of producing a variety yielding gossypol-free seeds, without the introduction of disadvantageous factors. P. S. ARUP.

Manuring and liming of spinning flax. A. Larsen (*Tidsskr. Planteavl*, 1960, **64**, 102—148).—Yields decrease with soil-pH decreasing <7. In long-term experiments, somewhat better and more constant average yields are obtained with 100 kg. per hectare each of Nitro-chalk and 18% superphosphate, and 150 kg. of 40% K plus the residual effect of an equiv. amount of farmyard manure, than by the application of double the amount of the chemical fertilisers. The latter treatment has, however, proved advantageous after seasons of heavy rainfall. Heavy applications of N are prejudicial to the general quality of the flax, and increase losses by lodging. Deficiency of any one of the nutrient elements causes losses of 10—17% in the yields. (19 references.) P. S. ARUP.

Varietal and environmental effects on rapeseed. II. Fatty acid composition of the oil. B. M. Craig and L. R. Wetter (*Canad. J. Plant Sci.*, 1959, **39**, 437—442).—Results obtained by gas–liquid phase chromatography agree with those obtained by fractional distillation of the Me esters (cf. *Canad. J. Technol.*, 1956, **34**, 335, and *Canad. J. Chem.*, 1951, **29**, 871). The erucic acid contents (~22—49% for seven varieties) are highest for Golden, Argentine, Regina II and Swedish, intermediate for Gute and Arlo, and lowest for Polish. The linoleic acid content (~10—20%) is higher in the Argentine than in the Polish types. Variations in the other acids are comparatively small. P. S. ARUP.

Sulphur deficiency in coffee. W. L. Lott, A. C. McClung and J. C. Medcalf (*IBEC Res. Inst.*, 1960, Tech. Note 22).—The effect of low-S soils on the growth of coffee plants was studied from leaf analyses. Extreme deficiency was marked by severe chlorosis, growth depression (16% normal) and 60 p.p.m. average leaf sulphate-S. Normal plant leaves contained 221 p.p.m. using soils treated with 200 lb. of S per acre as gypsum. Efficiency of present fertiliser practice is discussed, and a min. of 200 p.p.m. leaf sulphate-S proposed as a critical value. (15 references.) P. M. KINGSTON.

Effect of fertiliser rate, method of fertiliser application and plant spacing on yield, quality and burn of Maryland tobacco. C. G. McKee (*Dissert. Abstr.*, 1960, **20**, 3004—3005).—Yield, and price per lb., of tobacco increased with the amount of fertiliser applied in the range 750—2000 lb. of 4–8–12 formulation per acre. The method of applying fertiliser usually had no effect, but in one year fertiliser gave better results when applied in bands than when broadcast. A population of 7128 plants per acre had a higher average cash value than populations of 4900 or 8712 per acre. Higher populations should be used only with larger amounts of fertiliser. High populations, small amounts of fertiliser, and application of fertiliser in bands, all favoured a longer duration of leaf-burn ; long leaf-burn (good fire-holding capacity) was correlated with low temp. of ignition. M. D. ANDERSON.

Effects of soil calcium, potassium and phosphorus on pigmentation in Better Times roses. J. W. Abernathie (*Dissert. Abstr.*, 1960, **20**, 2999—3000).—The red component of the colour of Better Times roses was increased, and the blue component decreased, by low P and low K in the soil. Colour was not affected by soil Ca. In cold storage, red decreased and blue increased ; the change was retarded by low P and K, and high Ca conditions during growth. Roses cut in hot weather were redder ; cooling the greenhouses to lower peak temp. by 10° to 15° increased blue colour. The pigments responsible included anthocyanins, anthoxanthins and unidentified substances. M. D. ANDERSON.

Effect of allyl alcohol on micropopulation of prairie soils and growth of tree seedlings. M. Yatazawa, D. J. Persidsky and S. A. Wilde (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 313—316).—Application of allyl alcohol (100—200 gal. per acre) to prairie soil, which normally supports only poor growth of trees, resulted in considerable increase in growth rate of Monterey pine seedlings and increased uptake of all major nutrients, especially P and K. The treatment resulted in a rapid replacement of native fungal population by *Trichoderma viride*. Inoculation of the virgin soil with mycorrhizal fungi also stimulated growth of the seedlings, but not to such a great extent as did the allyl alcohol treatment. A. H. CORNFIELD.

Conifer establishment on coal spoils as influenced by site factors and organic additions at planting time. G. L. Lowry (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 316—318).—Tests with eight conifer species on spoils of varying acidity showed that on acid spoils (pH 2·1—5·8) pitch pine gave the best results over 2 years for survival and height growth, although white, jack and ponderosa pines showed promise. Shortleaf pine was consistently poor. On mildly acid spoils (pH 5·8—7·3) northern white cedar was the best, but eastern red cedar and ponderosa and jack pines were also satisfactory. Mulching the root zone at planting time with sawdust increased survival in acid sandy spoils, but decreased it in spoils high in silt and clay. An equation relating pine survival with soil moisture equiv., sand content and pH is presented. A. H. CORNFIELD.

Pest Control

Fluoroacetamide and some derivatives as pesticides. I. M. A. Phillips (*Agric. vet. Chemicals*, 1960, **1**, 93—94, 108).—A review. A. G. POLLARD.

Pesticidal phosphorus compounds. I. Fungicides, insecticides and acaricides derived from 3-amino-1,2,4-triazole. B. G. van den Bos, M. J. Koopmans and H. O. Huisman (*Rec. Trav. chim. Pays-Bas*, 1960, **79**, 807—822).—The prep. of compounds prepared by substitution of a phosphoryl group for a H atom in 3-amino-1,2,4-triazole (**I**) or its 5-substituted deriv. are described. Highest biological activity was found when the P-containing substituent was a bis-(dimethylamido)phosphoryl group, and the 5-substituent was H, alkyl (≯11-C), or phenyl. (17 references.) E. C. APLING.

Insecticidal and toxicological properties of *OO*-dimethyl 2,2-dichlorovinyl phosphate (DDVP). R. L. Tracy (*Soap, N.Y.*, 1960, **36**, No. 7, 74—76, 105).—The v.p. of DDVP is ~0·1 mm. at 30° and several h. after application ~2—7% of the total dose is found in the air ; the fumes possess poor penetrating power, but are readily adsorbed on to surfaces and released more slowly. With concn. of 0·5 mg./cu. ft. *Musca domestica*, L., the stable fly and other *Dipterae* were killed in 2—5 h. ; with 1·0 mg./cu. ft., 50% of German cockroaches survived 24 h. With 2·0 mg. all, including cigarette beetles, were killed within 18 h. Normal insecticidal doses are harmless to human beings and cattle and hazard is related to acute intake rather than to accumulation. (11 references.) C. V.

Preparation and fungitoxicity of trialkyl phosphorotetrathioates. C. B. Scott, J. W. Yale, jun., and S. Hashimoto (*J. agric. Fd Chem.*, 1960, **8**, 303—306).—Homologous series of symmetrical and unsymmetrical trialkyl phosphorotetrathioates were prepared, and tested as fungicides for eight spp. of phytopathogenic fungi. Variations between fungi were small, and structure–toxicity relations consistent. In general, fungicidal activity decreased as alkyl groups were lengthened, except for a marked increase when n-decyl replaced n-octyl in the side chain. Some of the compounds, e.g., Me_3 phosphorotetrathioate, were more toxic to fungi than were standard commercial fungicides. M. D. ANDERSON.

Uptake of radioactive ethyl *NN*-di-n-propylthiocarbamate (EPTC-^{35}S) and translocation of sulphur-35 in various crops. S. C. Fang and P. Theisen (*J. agric. Fd Chem.*, 1960, **8**, 295—298).—EPTC containing ^{35}S, applied to soil the day after seeds were planted, was absorbed by all crops examined. Uptake of ^{35}S increased with increased dose, but not proportionately. In bean, pea and maize plants, above-ground parts of the plant contained slightly more ^{35}S than the roots ; in carrots, radishes, cabbages, cucumbers, etc., the above-ground parts contained 70—94% of the ^{35}S. M. D. ANDERSON.

The dithiocarbamates. D. G. Hessayon (*Agric. vet. Chemicals*, 1960, **1**, 31—33).—The fungicidal action of certain groups of dithiocarbamates is discussed in relation to mol. structure.
A. G. POLLARD.

Toxaphene residues on Pangola grass. C. H. Van Middelem, W. G. Genung, E. G. Kelsheimer, L. C. Kuitert and R. E. Waites (*J. agric. Fd Chem.*, 1960, **8**, 289—292).—The U.S. Food and Drug Administration has established a tolerance of 7·0 p.p.m. of toxaphene in the fat of beef cattle; cattle may be fed up to 25 p.p.m. of toxaphene in the diet for 10 weeks without causing the amount in their fat to exceed the tolerated level. After application of three different forms of toxaphene to Pangola grass, at two different rates, the highest residues found 1 week later were below 20·0 p.p.m., and 2 weeks later 12·0 p.p.m. An interval of 7 days is recommended between application of toxaphene to the grass, and allowing cattle to graze on it.
M. D. ANDERSON.

Reversal of fungitoxicity of 8-quinolinol by amino-acids and other chelators. G. A. Zentmyer, S. Rich and J. G. Horsfall (*Phytopathology*, 1960, **50**, 421—424).—The toxicity of 8-quinolinol (**I**) to spores of *Aspergillus niger* was reversed by cysteine (**II**), histidine (**III**), tryptophan (**IV**), casamino acids, dithizone and Versene but not by glutamic acid (**V**), asparagine (**VI**) or glutathione (**VII**). The toxicity of **I** to mycelium of *A. niger* was reversed by **II**, was unaffected by **III, IV, VI** and **VII** and increased by **V**. The action of **I** on *Botryosphaeria ribis* was reversed by **II** but not by **III, IV, V, VI, VII,** glycine or methionine. Dithizone and quinaldic acid reversed the toxicity of **I** to *Stemphylium sarcinaeforme* and *Monolinia fructicola*. The mechanism of the toxic action of Cu quinolinolate is discussed.
A. G. POLLARD.

Biochemical studies of *Cochliobolus miyabeanus*. IV. Fungicidal action of polyphenols: rôle of the polyphenoloxidase. H. Oku (*Phytopath. Z.*, 1960, **38**, 342—354).—The toxic action of catechol on the mycelium of *C. miyabeanus* (the causal organism of leaf blight of rice) was lowered by Na diethylthiocarbamate and that on spore germination was lessened by reducing agents (ascorbic acid, glutathione). The brown product of oxidation of catechol by the polyphenol oxidase of the fungus was itself toxic to the fungus, thus explaining the fungicidal action of catechol. The quinone form of chlorogenic acid is unstable in water and is rapidly polymerised, the process being catalysed by the enzyme prep. of *C. miyabeanus*.
A. G. POLLARD.

Triphenyltin compounds in crop protection and the determination of residues. E. Kröller (*Dtsch. LebensmittRdsch.*, 1960, **56**, 190—193).—Celery plants were sprayed with 1·8 kg./ha. of Brestan, and residues of triphenyltin compounds and their water-sol. decomposition products were determined at intervals in the leaves and tubers. Triphenyltin residues on leaves fell quickly and were undetectable after 45 days. Sol. Sn compounds appeared in the leaves after 11 days, increasing until 21—25 days and then decreasing to zero after 40 days. Analytical methods used are described in detail.
E. C. APLING.

Residue analysis of a chlorinated insecticide (Thiodan) by combination of gas chromatography and infra-red spectrophotometry. G. Zweig, T. E. Archer and D. Rubenstein (*J. agric. Fd Chem.*, 1960, **8**, 403—405).—Thiodan extracted from plant materials by hexane was separated by gas chromatography on a column of Chromosorb coated with silicone grease, and was determined by i.r. spectrophotometry at 8·37μ, and comparison with a standard curve. The method is sensitive to amounts of 20 μg. per ml., 0·2 ml. being required to fill the micro-cell. Results agreed well with determinations of S; determinations of Cl gave consistently higher results. (12 references.)
M. D. ANDERSON.

Microcoulometric gas chromatography of pesticides. D. M. Coulson, L. A. Cavanagh, J. E. De Vries and B. Walther (*J. agric. Fd Chem.*, 1960, **8**, 399—402).—Chlorinated pesticides, such as lindane, aldrin, dieldrin, endrin, DDT, toxaphene, chlordane, heptachlor, Ronnel, DD, etc., are separated by gas chromatography on a column of specially prepared Chromosorb, coated with silicone grease. A coulometric system, based on combustion of the eluted substances, and continuous titration of the Cl^- in the resulting gases with Ag^+ generated in the titration cell, is used to follow the elution of the pesticides from the column. If the combustion is followed by catalytic hydrogenation, S is converted into H_2S and may also be determined. The time required for each pesticide to pass through the separation column is needed to identify the pesticides present. The method may be applied to crude extracts of plants without preliminary clean-up, and a quant. analysis takes only 1 h.
M. D. ANDERSON.

Invert emulsions. J. H. Kirch and J. R. Sterry. (*Agric. vet. Chemicals*, 1960, **1**, 35—37).—The use of invert emulsions as a means of minimising "drift" of herbicides particularly when applied from the air, is discussed.
A. G. POLLARD.

Insecticide treatments for aphid control in relation to spread of barley dwarf virus. E. A. Dickason, W. B. Raymer and W. H. Foote (*Plant Dis. Reptr*, 1960, **44**, 501—504).—Soil application of Thimet (Phorate, 15—22 oz.) or Di-syston (20—32 oz.) prior to sowing, or spray application of demeton (4 oz.) or parathion (4 oz. per acre) to barley initially suppressed aphid populations and in some cases delayed virus infection, but all plants eventually became infected. The treatments had no effect on yields or quality of barley.
A. H. CORNFIELD.

Insecticide studies on the maize stalk borer, *Busseola fusca* (Fuller) in E. Africa. P. T. Walker (*J. econ. Ent.*, 1960, **51**, 321—351).—Control of the insect was obtained by two applications of endrin (2 oz./acre, as 12% dust or as 0·03—0·04% emulsion) or of DDT (3·5 oz./acre as 0·05% emulsion or 5% dust). Malathion and γ-BHC lacked persistence.
A. G. POLLARD.

Use of soil insecticides for controlling potato aphids and virus diseases. P. E. Burt, L. Broadbent and G. D. Heathcote (*Ann. appl. Biol.*, 1960, **48**, 580—590).—Application of Thimet [*OO*-diethyl *S*-(ethylthionomethyl) phosphorothiolothionate, 9·5 lb. per acre] in the furrow mixed with the fertiliser or Rogor [*OO*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate, 10·5 lb. per acre] mixed with activated C and applied in individual doses beneath the tubers controlled aphids from a week after emergence to early Aug. The treatments prevented or greatly reduced the spread of leaf-roll virus, but depressed only slightly the spread of virus Y. Although tubers harvested from treated plots contained very small amounts of insecticide, shoots from these tubers grew slower and showed a lower aphid infestation than did shoots from tubers from control plots.
A. H. CORNFIELD.

Broad spectrum fungicides for control of melting-out of Kentucky bluegrass and *Sclerotinia* dollar spot of Seaside bentgrass. H. B. Couch and L. D. Moore (*Plant Dis Reptr*, 1960, **44**, 506—509).—All the nine materials tested significantly reduced the incidence of the leaf lesion phase of melting-out of Kentucky bluegrass caused by *Helminthosporium vagans*. Dyrene (4—8 oz), Upjohn GAB-5 (2—4 oz.) and California Spray ML-373 (4—8 oz. per 1000 sq. ft.) were the most effective in this respect. Fair control of *Sclerotinia* dollar spot of Seaside bentgrass was obtained by application of Upjohn GAB-5 (4 oz.), actidione RZ (1 oz.), California Sprays ML-372 and ML-373 (4 oz.), Dyrene (6 oz.) and Tersan OM (5 oz. per 1000 sq. ft.).
A. H. CORNFIELD.

Control of *Cercospora* leaf-spot of bananas with applications of oil sprays based on the disease cycle. H. H. Klein (*Phytopathology*, 1960, **50**, 488—490).—Promising results in preventing the development of the disease were obtained by spraying with a petroleum oil (η, 99·3 SUS at 100°F, unsaponifiable residue 85%, d_{20} 0·8785) (0·8—1·5 gal./acre, by aeroplane) the timing of the spray being decided by counting the no. of initial yellow streaks on the leaves.
A. G. POLLARD.

Occurrence of chlorogenic acid in strawberry plants affected with crinkle and yellows viruses. R. D. Durbin, B. S. Castillo and T. H. King (*Plant Dis. Reptr*, 1960, **44**, 536—537).—Water extracts of leaves and/or crowns of nine commercial strawberry varieties known to be infected with crinkle and yellows viruses contained chlorogenic acid (**I**). No **I** could be detected in water extracts of two varieties and seedling selections known to be free of virus infection. Extracts of *Fragaria vesca* (commonly used as an indicator of viruses in strawberry grafts) infected with latent A or Frazier's mild mottle viruses did not give positive tests for **I**.
A. H. CORNFIELD.

Evaluation of soil fungicides against *Fusarium solani* isolated from feeder roots of citrus trees. R. C. Cetas and R. Whidden (*Plant Dis. Reptr*, 1960, **44**, 465—469).—Fungicides were tested against *Fusarium solani* isolated from feeder roots of citrus affected with "spreading decline" disease. In tests with infected soil columns DAC-649 (3,3,4,4-tetrachlorotetrahydrothiophen-1,1-dioxide) (50 p.p.m.) was effective in the percolating water and Vapam and nabam at 200 p.p.m. Captan was relatively ineffective, except in the upper soil layer.
A. H. CORNFIELD.

[A] ***Rhizoctonia* disease of bean as affected by decomposing green plant materials and associated microfloras.** G. C. Papavizas and C. B. Davey, [B] **Effect of dry mature plant materials and nitrogen on *Rhizoctonia solani* in soil.** C. B. Davey and G. C. Papavizas (*Phytopathology*, 1960, **50**, 516—522, 522—525).—[A] Incorporation of green plant material in soil infested with *Rh. solani* considerably increased the no. of soil fungi, streptomycetes and bacteria antagonistic to *Rh. solani*. Young maize and oat plants were highly effective in protecting susceptible bean varieties from infection.

[B] Dry, mature plant material together with sources of N (NH_4NO_3 or slowly available Uramite) were added to a soil infested with *Rh. solani*. After 4 weeks beans were sown. Dried material from soya-bean, maize or oats effectively lowered the incidence of

the disease; oak sawdust was less active. The two forms of N were equally effective except when soya-beans formed the org. supplement and even when applied alone diminished the disease. The comparative efficiency of the org. materials + N was related to the intensity of microbial activity in the soil and, in part, to the C/N ratio of the plant matter but not to the soil pH. A. G. POLLARD.

Interaction of potassium gibberellate and a stunting bean virus on *Phaseolus vulgaris*. W. D. Yerkes, jun. (*Phytopathology*, 1960, **50**, 525—527).—Treatment of bean plants with K gibberellate (**I**) (50 p.p.m.) rapidly increased the growth rate of the plants by lengthening the internodes. The virus restricted growth which practically ceased 14 days after inoculation and largely counteracted the effect of **I**. A. G. POLLARD.

γ-BHC liquid seed dressing for control of turnip flea beetle. H. R. Jameson (*J. Sci. Fd Agric.*, 1960, **11**, 528—534).—Germination was not impaired when viscous aq. γ-BHC (> 16%, 50 ml./lb. of seed,) was applied as seed dressing against flea beetle attack on turnips and kale. Seedlings recently emerged from brassica seed treated with γ-BHC (4% in aq. suspension) were highly toxic to flea beetle in the first week and were protected against material damage for another 2 or 3 weeks. E. M. J.

The hydrocyanic acid content of flax in relation to flax wilt resistance. E. J. Trione (*Phytopathology*, 1960, **50**, 482—486).—The linamarin (**I**) content of flax tissue was high in young plants and decreased (per unit wt. of fresh tissue) with advancing age: it was greater in daylight than in darkness. Experimental data did not support the theory that liberation of HCN from **I** is associated with the resistance of flax varieties to wilt (*Fusarium oxysporum* f. *lini*). A. G. POLLARD.

Action of a high-percentage seed dressing in controlling the rape flea-beetle and the cabbage gall weevil. C. Buhl (*Z. PflKrankh.*, 1960, **67**, 321—326).—The dressing (75—8% lindane) applied to winter rape seed (50 g./kg. with 10 ml. of petroleum) effectively protected the plants against the larvae of the flea-beetle for a period of several months. Attack by the gall weevil but not that by the cauliflower leaf-stalk miner was also prevented. A. G. POLLARD.

The white coffee borer, *Anthores leuconotus*, Pasc., and its control. R. G. Tapley (*J. econ. Ent.*, 1960, **51**, 279—301).—Coffee trees were protected from the borer by spraying the lower part of the stems with dieldrin (0·5%, 1 gal./acre), a marker (methylene blue) being used to indicate treated trees. Protection persisted up to 27 months. A. G. POLLARD.

Influence of environmental conditions on the development of yellow spot disease in cress (*Nasturtium officinale*, R. BR). D. Spire (*C.R. Acad., Agric. Fr.*, 1960, **46**, 505—507).—Low temp. or other adverse conditions for the plant, e.g., attack by *Spongospora*, enhance development and transmission of the disease. Covering with frames covered with polyvinyl chloride film raises the temp. of the air and water in the trenches sufficiently to reduce symptoms and produce healthy growth from initially infected plants. E. C. APLING.

Control of cutworm in forest nurseries. J. R. Aldhous (*Forestry*, 1959, **32**, 155—165).—Aldrin 3 lb. (as a 30% miscible oil) or dieldrin 1·5 lb. (as a 15% miscible oil) in 60—100 gal. of water per acre applied from mid-June to mid-July gave good control of cutworms without damage to seedling conifers. Late application (Aug.—Sept.) caused injury. A. G. POLLARD.

Insecticidal control of the pine looper in Gt. Britain. I. Aerial spraying. M. Crooke (*Forestry*, 1959, **32**, 166—191).—DDT (1 lb. in 3 gal. of liquid per acre) applied by aeroplane, in summer, timed to coincide with adult emergence from pupae, controlled the looper. A. G. POLLARD.

Needle blight of red cedar, *Juniperus virginiana*, L., and its control. A. Kelman, C. S. Hodges and H. R. Garriss (*Plant Dis. Reptr*, 1960, **44**, 527—531).—Characteristics of the disease are described. The fungus *Exosporium glomerulosum*, (Sacc.) Hohn. was associated with the disease. Application of Ortho Phaltan 50W (*N*-trichloromethylthiophthalimide, 2 lb. per 100 gal.) at 10—14-day intervals and after heavy rains over the period May 23 to Sept. 16 gave excellent control of the disease. A. H. CORNFIELD.

Control of blue stain at Thetford Chase, 1957. D. Small and J. N. R. Jeffers (*Empire Forest. Rev.*, 1960, **39**, 211—219).—Scots and Corsican Pine (*Pinus sylvestris*, *P. nigra* var. *calabrica*) were tested with Santobrite (Na pentachlorophenate) (**I**) and Na *o*-phenylphenate. The amount of stain was significantly reduced but not to the degree required by British Grading Rules. Those treated with **I** had the least stain at the first assessment, but there was no significant difference between the two chemicals in their effect on the amount of staining at the later assessment. There were no significant effects from species, or any interactions of the sites, or treatments, but there was a highly significant interaction between the effects of peeling and the dates of felling. E. G. BRICKELL.

Effects of fungicide–insecticide seed treatments on emergence, growth and yield of irrigated cotton in the Sudan Gezira. S. A. J. Tarr (*Ann. appl. Biol.*, 1960, **48**, 591—600).—Treatment of cotton seed with 0·043% methoxyethylmercuriacetate (MEMA) + 0·12—0·24% dieldrin reduced post-emergence wilting and death of seedlings due to termite attack, and also reduced pre-emergence mortality caused primarily by soil insects, but had little effect on the incidence of termite damage to tap-roots of mature plants at the end of the season. The beneficial effects of the treatment were greater in short than in long rotations. Although the treatment was slightly phytotoxic, the beneficial effects derived from it more than outweighed this drawback. A. H. CORNFIELD.

Reaction of *Rhizoctonia* isolates to chemicals. J. B. Sinclair (*Plant Dis. Reptr*, 1960, **44**, 474—477).—Five different isolates of *R. solani* differed in their toxicity to cotton seedlings as well as in their sensitivity to pentachloronitrobenzene, captan and dichlone. This may explain the erratic performance of these chemicals when used for control of cotton seedling damping-off. A. H. CORNFIELD.

Control of cotton root-knot nematodes by seed treatment with 1,2-dibromo-3-chloropropane. J. H. O'Bannon and H. W. Reynolds (*Plant Dis. Reptr*, 1960, **44**, 484—486).—Good control of root-knot nematodes was obtained by treating cotton seed with 1,2-dibromo-3-chloropropane (1·7—4·3 lb. per 25 lb. seed) prior to planting. Germination was adversely affected only with air temp. > 31°. A. H. CORNFIELD.

Nitrogen supplement as a possible control for *Verticillium* wilt of cotton. D. C. Neal and J. B. Sinclair (*Plant Dis. Reptr*, 1960, **44**, 478).—Application of $NaNO_3$ (32 lb. N per acre) in late July to eight varieties of cotton growing in a *Verticillium* wilt-infested area increased cotton yields and reduced the no. of plants with above-ground symptoms of *Verticillium* infection in six of the eight varieties. A. H. CORNFIELD.

Use of the onion test as quantitative method for determining the distribution of emulsifiable 1,2-dibromo-3-chloropropane in soil. A. Rinkov, S. D. van Gundy, R. L. Rackham and M. J. Garber (*Plant Dis. Reptr*, 1960, **44**, 510—515).—The % reduction in growth of onion seedlings was highly correlated with the concn. of 1,2-dibromo-3-chloropropane (**I**) in sand or soil over the range 0—10 p.p.m. The distribution of emulsifiable **I** in soils as measured by the onion test was related to the extent of control of the citrus nematode. The onion test was faster and more reliable than taking nematode counts. The rate of downward movement of **I** into soil when applied in the irrigation water decreased with increasing clay content of the soil. A. H. CORNFIELD.

Effects of soil fumigants on occurrence of nematodes in field bins. E. B. Minton, E. J. Cairns and A. L. Smith (*Plant Dis. Reptr*, 1960, **44**, 479—483).—Trials with a variety of nematocides are reported. A. H. CORNFIELD.

Control of European wireworm *Agriotes obscurus*, L. in Nova Scotia with insecticides applied to the soil. C. J. S. Fox and G. G. Smeltzer (*Canad. J. Plant Sci.*, 1959, **39**, 498—500).—Single applications of aldrin or heptachlor at 4 lb., or of lindane at 1·25 lb. per acre markedly reduced wireworm populations during 4 years, and increased yields of hay for 3 years after application. Similar results were obtained during 2—3 years with single applications of aldrin or heptachlor on oats or leguminous forage crops, but not on potato. P. S. ARUP.

Seed treatment with non-volatile liquid dressings. C. P. Hampson (*Agric. vet. Chemicals*, 1960, **1**, 39—41).—The "mist" method of applying seed dressings reduces the risk of "over-dressing" and wet treatments avoid injury to germination and early seedling growth. A. G. POLLARD.

Chemical and biological control of the glasshouse red spider *Tetranychus urticae*, Koch. L. Bravenboer (*Versl. landbouwk. Onderz.*, 1959, 65.5, 85 pp. + 5 appendix sheets).—A study is made of the effects of temp., the nature of the host plant, and of chemical treatments on the life history of the mite and of its predators, *Stethorus punctillum* Weise and *Typhlodromus longipilus* Nesbitt. Chemical treatments appear to be gradually losing their efficacy owing to increased resistance of the mite, but satisfactory control of the mite can be achieved by one treatment with a selective acaricide (e.g., Tedion) in the presence of the two predators in sufficient relative numbers. (129 references.) P. S. ARUP.

Molecular size versus herbicidal activity of anilides. C. W. Huffman and S. E. Allen (*J. agric. Fd Chem.*, 1960, **8**, 298—302).—Many active herbicides (carbamates, phenoxyacetic acids, ureas, phenols) have an extended dimension of 13 Å, and a collapsed dimension of 10 or 11 Å, although some active compounds have smaller

mol. A no. of substituted anilides were prepared; those with mol. sizes above or below 13 Å extended and 10—11 Å collapsed were inactive. Those of the desired dimensions varied from very active to inactive. Max. effect was associated with 3,4-chlorination of the phenyl ring; Cl at the 2-position greatly decreased activity. Thioanilides were as active as anilides. (20 references.)
M. D. ANDERSON.

Effects on honey bees of some chemical weedkillers. T. Palmer-Jones (*N.Z. J. agric. Res.*, 1960, **3**, 485—490).—The hormone weedkillers, the butyl and butoxyethanol esters of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) do not constitute a hazard to bees. The phenolic weedkillers 50% dinitro-*o*-butylphenol (DNBP) plus 10% dinitro-*o*-s-amylphenol (DNAP) and 40% pentachlorophenol (PCP) are toxic but also highly repellent to bees; there was no transfer of the poison or lasting hive disorganisation from bees killed by the sprays. E. C. APLING.

Influence of the [butyl] ester of 2,4-D applied at various growth stages for control of Russian thistle (*Salsola pestifer*, Nels.) in flax. J. J. Sexsmith (*Canad. J. Plant Sci.*, 1960, **39**, 458—465).—Over five years max. control was obtained by spraying during the periods 19—30 and 45—60 days after sowing, with 6 oz. of the ester per acre. The effects on flax height or time of maturing were slight, provided the flax was not sprayed whilst in bloom. (21 references.)
P. S. ARUP.

Use of monuron and diuron for controlling weeds in sugar cane. E. W. Zomosa (*Agric. vet. Chemicals*, 1960, **1**, 97—100).—The successful use of these herbicides is reported. Factors arising in various systems of cultivating sugar cane and which affect weed growth are noted in relation to the most effective use of the two materials. A. G. POLLARD.

Herbicides for control of weeds in rice in Trinidad. W. A. B. Marshall, C. E. J. Morley and P. H. Rosher (*Empire J. exp. Agric.*, 1960, **28**, 244—254).—Post-emergence control of *Fimbristylis miliacae*, *Sphenoclea zeylanica* and *Jussiaea* spp. without damage to the crop was effected by 2,4-D, MCPA or a mixture of these applied at the tillering stage. A. G. POLLARD.

Field experiments with chemicals for total control of vegetation. H. I. Petersen and E. J. Petersen (*Tidsskr. Planteavl*, 1960, **64**, 331—348).—Weed eradication by means of 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) or (especially) by 2-chloro-4,6-bis-(ethylamino)-5-s-triazine (Simazin) (both at 50 kg. per hectare) is much more efficient and permanent than eradication by chlorates, trichloroacetate or NH_4 sulphamate (all at 200 kg. per hectare). Borax and petroleum prep. containing dinitro-*o*-cresol give the least satisfactory results. (10 references.) P. S. ARUP.

Herbicidal action of 1,1′-ethylene-2,2′-dipyridylium dibromide. G. C. Mees (*Ann. appl. Biol.*, 1960, **48**, 601—612).—Although light was not essential for the herbicidal action of 1,1′-ethylene-2,2′-dipyridylium dibromide (Diquat) on broad beans, the rate of kill increased with light intensity up to about 10 k-lux. The light effect was inhibited by *N*-(4-chlorophenyl)-*NN′*-dimethylurea or the absence of O_2, but not by KCN or in a CO_2-free atm. The herbicide usually depressed respiration, but sometimes caused an initial stimulation of respiration followed by a depression.
A. H. CORNFIELD.

Use of herbicides to break the life cycle of the bentgrass nematode, *Anguina agrostis*, (Steinbuck 1799), Filipjev 1936. W. J. Apt, H. M. Austenson and W. D. Courtney (*Plant Dis. Reptr*, 1960, **44**, 524—526).—Application of dalapon (5 lb.), Amitrol (5—10 lb.) or maleic hydrazide (8—16 lb. per acre) to Colonial bentgrass, *Agrostis tenuis*, depressed heading of the grass in the year of application, thus breaking the life cycle of the bentgrass nematode. In the year following treatment the % florets with galls was greatly reduced and seed yields were increased in comparison with untreated controls. A. H. CORNFIELD.

Use of desiccants permits direct combining of red clover seed. S. C. Wiggans and W. F. Buchele (*Down to Earth*, 1960, **15**, No. 4, 17—18).—Application of 4,6-dinitro-*o*-s-butylphenol (2 lb. in 6 gal. of diesel oil per acre) rapidly defoliated the clover with resultant increased seed yield after combine harvesting; germination rate was unaffected. A. G. POLLARD.

Δ^3-Thiazolines. Veb Leuna-Wierke Walter Ulbricht (Inventors: F. A. Singer and M. Thiel) (B.P. 824,113, 25.4.56).—The prep. is described of Δ^3-thiazoline deriv., e.g., 4,5-dimethyl-2,2-pentamethylene-Δ^3-thiazoline, useful as pest control agents, weed killers, etc. H. S. R.

Triazolylurea compounds. American Cyanamid Co. (B.P. 824,541, 10.4.58. U.S., 26.4.57).—Compounds R·NH·CO·NR′R″ (R is 1,3,4-triazol-2-yl; R′ and R″ are H, alkyl, alkenyl, aryl or substituted aryl) are claimed; they may be obtained by treating R·NH_2 with an aliphatic or aromatic isocyanate or with a carbamic acid halide. The products exert a strong parasiticidal action against fungi, bacteria, mites and insects, and may be compounded with inert diluents to provide compositions for combating household and agricultural insect pests (aphids, beetles, roaches) and for the eradication of bacterial and fungus infections. In an example, interaction of PhNCO with 2-amino-1,3,4-triazole in dioxan yields *N-phenyl-N′-(1,3,4-triazol-2-yl)urea*, m.p. 160—161°. This, in aq. dispersion at 0·001% concn., effects a 95% kill of spores of *Sclerotinia fructigena* and *Macrosporium sarcinaeforme*. F. R. BASFORD.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 825,477, 17.6.58. Ger., 24.6.57).—Compounds CH_2·[S·PS(OR)·OR′]$_2$ are claimed (R and R′ are alkyl of 1—4 C); they are useful as insecticides (especially active against spider mites) and are obtained by interaction of a methylene dihalide with a salt of OR(OR′)·PS_2H in aq. solution at high temp. *Methylene-bis-OO-dimethyl phosphorothiolothionate*, b.p. 116°/0·01 mm. (13% yield), is prepared.
F. R. BASFORD.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 826,037, 7.9.56. Ger., 7.9.55).—Compounds $(OR)_2PS·O·A·S·CH_2R'$ are claimed [R is alkyl or Ph; A is alkylene of 2—4 C; R′ is esterified CO_2H, the radical of an org. or inorg. ester of a hydroxyalkanethiol, alkylthio, arylthio, alkoxy or aryloxy, or may be hydroxyalkylthio or t-aminoalkyl (which may be heterocyclic) when R is Ph]. The products which possess insecticidal properties (systemic action against aphids, flies and mites), are obtained by interaction of R′·CH_2·S·A·OH with $(OR)_2PS·X$ (X is halogen) in presence of acid-binding agent in an inert solvent. Thus, $(OEt)_2PSCl$ and *Et(2-hydroxyethylthio) acetate* (prep. described) afford *Et$_3$ 2-(ethoxycarbonylmethylthio)ethyl phosphorothionate*, b.p. 170°/2 mm. The ester is lethal to flies at 0·01% concn. F. R. BASFORD.

Organic phosphorus derivatives. Société des Usines Chimiques Rhône-Poulenc (B.P. 826,814, 11.2.58. Fr., 4.3.57).—Compounds useful as pesticides comprise 2-methyl-4-oxo-2,4-pyran-5-yl dialkyl (>5 C) phosphates (or thionophosphates). *Pr$^i_2$ 2-methyl-4-oxo-1,4-pyran-5-yl phosphorothionate* is prepared. F. R. BASFORD.

Neutral spiro-heterocyclic esters of phosphoric acid. Union Carbide Corp., Assee of W. M. Lanham (B.P. 824,587, 17.10.57. U.S., 24.10.56).—Compounds useful as insecticides effective against bean aphid, red spider mite and Mexican bean beetle larvae comprise spiro-phosphorinane-cyclohexane deriv., e.g., *spiro-2-ethoxy-2-thione-1,3,2-dioxaphosphorinane-5,1′-cyclohexane*, m.p. 73—77°, the prep. of which is described. H. S. R.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 823,732, 22.11.56. Ger., 22., 24., and 26.11.55).—Compounds of high insecticidal activity (useful against flies, mosquitoes, aphids, mites, etc.) and low toxicity (to humans) comprise thiophosphoric acid esters, viz., $(OR)_2PX·S·A(R')·Y·R''$ (X is O or S; Y is S, SO, or SO_2; R and R″ are alkyl of 1—4 C; A is alkylene of <2 C; R′ is alkyl of 1—4 C, optionally substituted by Cl). *OO-Dimethyl S-1-(ethylthio)prop-2-yl phosphorothiolate*, b.p. 83°/0·01 mm.
F. R. BASFORD.

Phosphate esters suitable as pest control agents. C. H. Boehringer Sohn (Inventors: R. Sehring and K. Zeile) (B.P. 823,505, 3.2.58).—Compounds OR(OR′)·PO·O·CH:CCl_2, useful as pesticides (especially against flies and mites), are obtained by treating $(OR)_2P(OR')$ (R is Me or Et; R′ is $CHR''·CO_2R'''$; R″ is H, Me or Et; R‴ is H or straight or branched, saturated or unsaturated aliphatic hydrocarbon radical of 1—8 C) with chloral at 0—25° in an inert solvent (ether, dioxan, benzene, toluene or light petroleum), then saponifying the resulting ester (if desired when R‴ is not H).. Where R‴ is H, salts of the above compounds are also claimed. In an example, chloral (32) is interacted with Me_2 butoxycarbonylmethyl phosphite at 15° in ether to yield *Me carbobutoxymethyl 2,2-dichlorovinyl phosphate*, b.p. 132—136°/0·2 mm. (with decomp.).
F. R. BASFORD.

Phosphoric and thiophosphoric acid esters. C. H. Boehringer Sohn (B.P. 823,186, 22.11.57. Ger., 22.11.56).—Compounds OR·PO(O·CR^I:$CR^{II}R^{III}$)·X·$R^{IV}SO_n$·R^V, useful as insecticides (contact and internally active poisons) of low toxicity to warm-blooded animals, are obtained by oxidation of OR·PO(O·CR^I:$CR^{II}R^{III}$)·X·R^{IV}·SR^V with, e.g., Cl_2 or H_2O_2 at −20° to +20° in presence of water and optionally an inert org. solvent (chlorinated hydrocarbon or water-sol. alcohol) (R is alkyl of 1—4 C; R^I is H or alkyl of 1—3 C; R^{II} is H, alkyl of 1—3 C or carbalkoxy; R^{III} is halogen or as R^{II}; X is O or S; R^{IV} is alkylene; R^V is alkyl of 1—6 C, aryl, aralkyl or heterocyclic; *n* is 1 or 2). *Me 2,2-dichlorovinyl 2′-ethylsulphoxyethyl phosphate* is prepared.
F. R. BASFORD.

Organo-mercapto-methylene amides of *OO*-dialkylthio (or dithio)-phosphorylacetic acids. Montecatini Società Generale per l'In-

dustria Mineraria & Chimica (B.P. 825,397, 2.6.58. It., 3.6.57).—The compounds, useful as pesticides, are obtained by treating $(OR)_2PX{\cdot}S{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}OH$ with R'SH at 0—150° in presence of an acidic condensing agent (H_2SO_4, HCl, $ZnCl_2$ or $CaCl_2$) in a solvent or a dispersing agent; or by interaction of $(OR)_2PX{\cdot}SM$ with $SR'{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}CH_2Y$ under similar conditions (R is alkyl of 1—4 C; R' is alkyl, alkenyl, cycloalkyl or aryl optionally containing a substituent; X is O or S; Y is halogen; M is Na, K, NH_4 or alkaline-earth metal). OO-*Diethyl* S-(*methylthioacetamido*)*methyl phosphorothiolothionate*, m.p. 49—49·5°, is prepared.

F. R. BASFORD.

Phosphorus- and halogen-containing condensation products. CIBA Ltd. (B.P. 823,415, 11.9.56. Switz., 12.9.55).—Compounds, useful as pesticides, comprise P- and halogen-containing condensation products, probably $OR(OR'){\cdot}PO{\cdot}CCl_2{\cdot}CO_2R''$, obtained by interaction of $(OR)_2P(OR')$ with $CCl_3{\cdot}CO_2R''$ (R and R' are aliphatic radicals of 1—4 C, optionally substituted by Cl, CN or CNS; R'' is aryl optionally substituted by alkyl, alkoxy, cycloalkyl, Cl or NO_2). In an example, *Me*$_2$ [*dichloro*-(p-*chlorocarbophenoxy*)*methyl*]-*phosphonate*, b.p. 130—135°/0·05 mm., is prepared as a water-insol., colourless oil. This is compounded with octylphenolpolyglycol ether, oleic acid and mineral oil to give a water-dispersible concentrate which at 1% concn. is (as a dormant spray) effective in controlling overwintering stages of fruit pests (winter eggs of aphids, eggs of winter moths or psyllae, and overwintering cochineals).

F. R. BASFORD.

Halogenated adipates. Rohm & Haas Co. (B.P. 825,107, 21.6.57. U.S., 2.7.56).—Halogenated adipates, viz., $X{\cdot}CH_2{\cdot}CX(CO_2R){\cdot}CH_2{\cdot}CH_2{\cdot}CHMe{\cdot}CO_2R$ (X is Cl or Br; R is alkyl of 1—14 C, alkoxyalkyl of 2—14 C aralkyl of 7—14 C, or 3—14 C -cycloalkyl or -alkylcycloalkyl) are made in high yield by halogenation of the corresponding methyleneadipic acid at −70° to +100° (−20° to +50°) in presence of a catalyst (viz. a disubstituted amide or a *N*-substituted lactam). The compounds are useful as insecticides, fungicides (especially active against *Stemphylium sarcinaeforme*), and plasticisers (especially for PVC). The prep. is described of *Me*$_2$ *α-chloro-α'-methyl-α-chloromethyladipate* (*Me*$_2$ 1,2-*dichlorohexane*-2,5-*dicarboxylate*) b.p. 125—129°/1 mm., n_D^{20} 1·47.

F. R. BASFORD.

Benzoylpimelates. Rohm & Haas Co. (B.P. 825,381, 5.4.57. U.S., 30.4.56).—*Dimethyl benzoylpimelate* (3-*benzoylpentane*-1,5-*dicarboxylate*) and higher alkyl (up to 18 C) or aromatic or araliphatic esters and also analogues with alkyl substituents in the pentane chain, are claimed as insecticides especially useful against aphids and mites, and are non-phytotoxic.

H. S. R.

Fungicide. Allied Chemical Corp. (B.P. 823,329, 22.10.57. U.S., 13.11.56.).—A highly active fungicidal composition comprises mucochloric anhydride (bis-3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl ether) and a solid carrier (talc, clay, Fuller's earth) or liquid carrier (water or aq. dispersion in org. solvent). Thus, 1% acetone solution of mucochloric anhydride is dispersed in water to give an aq. spray which (at a concn. of 1 p.p.m. of fungicide) is 100% lethal to spores of *Sclerotinia fructicola*.

F. R. BASFORD.

Dimethyldithiocarbamic acid complex. UCLAF (B.P. 825,901, 31.3.58. Fr., 18.9.57).—Alkali metal- or alkaline-earth metal dimethyldithiocarbamate is treated with Cu_2Cl_2 in aq. solution in presence of alkali metal chloride and bisulphite ions, to give Cu^I complex of dimethyldithiocarbamic acid (Cu 47—50%) also containing Cl (17—19%). The complex is useful as a fungicide.

F. R. BASFORD.

Pyridazine derivatives and fungicidal compositions comprising them. J. R. Geigy A.-G. (B.P. 823,078, 15.6.56. Switz., 17.6.55).—Compounds characterised by fungicidal properties comprise pyridazines or phthalazines substituted by Cl and $X{\cdot}S{\cdot}CCl_3$ (X is O or S) in the positions adjacent to each N. As an example, the prep. of 6-*chloro*-3-(*trichloromethylsulphenoxy*)*pyridazine*, m.p. 156—157°, from the corresponding 3-hydroxy compound is outlined.

F. R. BASFORD.

Quaternary ammonium xanthates. B. F. Goodrich Co. (B.P. 823,395, 28.3.56. U.S., 1.4.55).—Compounds characterised by biological properties which make them useful as bacteriostatic agents, bactericides, fungicides, herbicides, algicides, insecticides and preservatives for cellulosic materials comprise quaternary NH_4 xanthates $NRR'R''R'''{\cdot}S{\cdot}CS{\cdot}OY$ (R and R' are Me; R'' is Me, Ph or CH_2Ph; or R—R'' together with N comprise a pyridine nucleus; R''' is aliphatic hydrocarbon group of 8—20 C, aralkyl, ester of a carboxyethyl group, or *p*-di-isobutylphenoxyethoxy when R and R' are Me and R'' is benzyl, or may be Ph when R—R'' are not Me and Y is not Et or Pr; Y is alkyl or alkenyl of 1—4 C). As an example of prep., an aq. solution of Na isopropylxanthate is added to 33% aq. solution of lauryltrimethylammonium chloride at 50—60°. The oily layer is a product active (at a concn. of 100 p.p.m.) against *Alternaria oleracea* and *Sclerotinia fructicola*.

F. R. BASFORD.

Anthelmintic compositions. Boots Pure Drug Co. Ltd. (Inventors: J. W. G. Leiper and G. W. Cranch) (B.P. 822,978, 15.4.57).—A composition suitable for use as a veterinary anthelmintic comprises the double salt of piperazine dihydrochloride and NH_4Cl compounded with a carrier (edible solid, e.g., animal foodstuff, or fluid, e.g., animal digestive juices, in a capsule).

F. R. BASFORD.

Gastropod combating compositions. Farbenfabriken Bayer A.-G. (B.P. 824,345, 19.9.56. Ger., 26.9.55).—A composition for combating molluscs (snails and slugs) contains a 2-hydroxybenzanilide, viz., $R{\cdot}CO{\cdot}NH{\cdot}R''$ (R is o-$C_6H_4{\cdot}OR$ optionally substituted by 1—2 R''; R' is H or acyl of 1—5 C; R'' is halogen, alkyl of 1—5 C or NO_2; R'' is Ph optionally substituted by 1—2 R, at least 1 R being NO_2; and there are also present NO_2, F, Cl or Br radicals, at least one of which is halogen). Thus, an aq. solution containing 5,2',5'-trichloro-4'-nitrosalicylanilide or 2-acetoxy-3-methylbenzanilide in $10^{-6\cdot5}$ concentration is 100% lethal to snails.

F. R. BASFORD.

Compositions containing phenylsulphonamides for use as molluscs repelling agents. J. R. Geigy A.G. (B.P. 825,466, 29.1.58. Switz., 31.1.57).—A composition for the control of molluscs (especially snails) contains at least one compound of the type $R{\cdot}SO_2{\cdot}NR'R''$ (R is Ph substituted by X_m and Y_n; X is Cl, Br, NO_2 or alkyl of 1—2 C; Y is similar to X except for NO_2; m is 1—2; n is 0—2; R' is H alkyl of 1—2 C, or inorg. cation, e.g., alkali metal or alkaline-earth metal; R'' is alkyl of 1—2 C or Ph optionally substituted by NO_2, alkyl of 1—2 C, or Cl). A typical compound (prep. described) is the Na salt of 2,4-*dichlorobenzenesulphonamide*, m.p. 176—178°.

F. R. BASFORD.

Substituted phenoxypropionic acid esters and compositions containing them. Boots Pure Drug Co. Ltd. (Inventors: H. A. Stevenson, R. F. Brookes, G. B. Lush and J. Fraser) (B.P. 825,875, 27.6.56).—Compounds $4,2,1\text{-}Cl{\cdot}C_6H_3Me{\cdot}O{\cdot}CHMe{\cdot}CO_2R$, useful as herbicides are obtained by interaction of $1,2,4\text{-}OH{\cdot}C_6H_3MeCl$ with $MeCHX{\cdot}CO_2R$ or by esterification of the carboxylic acid with ROH (R is alkyl of ⩽3 C, alkoxyalkyl, alkoxyalkoxyalkyl or Ph substituted by alkyl; X is halogen). The prep. of *Bu* α-(4-*chloro*-2-*methylphenoxy*)*propionate*, b.p. 138—140°/2 mm., is described.

F. R. BASFORD.

α-(Halogenophenoxy)propionic acids and compositions thereof. Boots Pure Drug Co. Ltd. (Inventors: H. A. Stevenson, R. F. Brookes and G. B. Lush) (B.P. 826,995, 9.11.56, 20.6.57).—Compounds useful as herbicides (active against *Chenopodium album*, *Stellaria media*, *Sinapsis arvenis*, *Avena fatua* and *A. ludoviciana*) comprise α-(4-bromo-3,5-dimethylphenoxy)-, (m.p. 151·5—152·5°), α-(3,4-dichloro-5-methylphenoxy)-, and α-(3,4,5-trichlorophenoxy)-**propionic acid** and their salts and esters. The method of prep. is detailed.

F. R. BASFORD.

Weed killing composition. Montecatini Società Generale per l'Industria Mineraria & Chimica (Inventors: G. Pellegrini, P. Scrivani and A. Bugiani) (B.P. 824,577, 29.5.58).—A herbicidal composition, especially useful in the control of weeds in rice fields destined for sowing or transplanting, comprises granules formed from a mixture of 2,4-dichlorophenoxyethanol and a fertiliser diluent, e.g., urea or $(NH_4)_2SO_4$. The composition may also contain a superphosphate or phosphorite, or bentonite-type mineral.

F. R. BASFORD.

Killing weeds and influencing plant growth. Farbenfabriken Bayer A.-G. (B.P. 824,534, 21.1.58. Ger., 21.1.57).—A herbicidal and plant-growth inhibiting composition contains a carbamide oxime, viz., $CRR':N{\cdot}O{\cdot}CO{\cdot}NHR''$ (0·5—95 wt.-%), an inert solid (talc, clay, pyrophyllite, diatomaceous earth, flour or fat) or liquid (water, cyclohexanol, acetone, etc.), and optionally a surface-active dispersing agent (⩾30, and preferably ⩾5 wt.-%) (R and R' are H, alkyl of ⩾8 C; or R and R' are alkenyl, alkyl substituted with or interrupted by hetero atoms or substituted by NO_2, alkyl, OH, alkoxy or heterocyclyl, or together with C form a cyclic radical; or R'' is aryl, or alkyl and aryl radical linked to 2 NH_2 groups at the same time). In an example, a premix of (*N*-phenylcarbamoyl-acetoxime (0·1 g.), acetone (3 c.c.), and benzyl hydroxy diphenyl polyglycol ether of ~15 glycol residues (2 drops) is diluted with water (to 100 c.c.), and the resulting solution is applied to mustard plants (with two leaves) and oats of height ~15 cm. After 14 days, the observed damage to the plants is 100% and 0% respectively.

F. R. BASFORD.

Herbicidal compounds. Boots Pure Drug Co. Ltd. (Inventors: H. A. Stevenson, R. F. Brookes and G. B. Lush) (B.P. 823,208, 14.6. and 23.9.55).—Compounds useful as herbicides (especially useful against cleavers and chickweed) comprise α-(3-*chloro*-2-*methylphenoxy*) (**I**)-, α-(2-*chloro*-3-*methyl*-, α-(2-*chloro*-5-*methyl*-, α-(2,4-*dichloro*-3-*methyl*-, α-(4,5-*dichloro*-2-*methyl*-, α-(2,4-dichloro-5-methyl- and α-(3,5-*dichloro*-2-*methyl*-**phenoxy)propionic acid** and salts, esters and amides thereof. The method of prep. is exemplified by that for

a mixture of 3-chloro-*o*-cresol, ethyl α-bromopropionate, K_2CO_3 and acetone. F. R. BASFORD.

Compositions for treating nematodes. Spencer Chemical Co. (B.P. 826,532, 3.12.57. U.S., 11.12.56).—A composition for combating nematodes comprises a liquid or solid carrier and *p*-phenylazoaniline or an acid-addition salt thereof. A conc. composition may contain >50 wt.-% of active ingredient. F. R. BASFORD.

Animal Husbandry

Sampling techniques for better feed quality control. A. B. Poundstone (*Cereal Sci.*, 1960, **5**, 174—175, 178).—The types of samplers or triers used on feeds in the U.S. are reviewed briefly. A detailed description of a 42-in., single-slot trier is given. The methods of taking samples, and of handling them are discussed. S. G. AYERST.

Metabolic aspects of the pasture/animal association. F. Hickey (*N.Z. J. agric. Res.*, 1960, **3**, 468—484).—Determinations of the resting–lactating heat production of dairy cows on different grazings are recorded. Grazing of high-protein ryegrass–clover pastures raised the excess heat production 22 to 24% above that on poorer swards. The excess heat production presumably results from the disposal of excess N compounds in the pasture diet. Possible methods of rectifying the energy–protein imbalance and the effects of other factors which might contribute to reduced energy efficiency are discussed. (23 references.) E. C. APLING.

Immature forage mixtures with citrus pulp versus more mature forage without additive for silage. W. J. Miller, H. L. Dalton and J. K. Miller (*J. Dairy Sci.*, 1960, **43**, 993—998).—The early-cut ensiled mixtures of grasses or grasses and rye give higher milk yields than do the later crops; they are, however, of doubtful economic value, owing to the greater amounts of concentrates required, and the lower yields per acre. P. S. ARUP.

Determination of dry matter and volatiles in silage. P. McDonald and W. A. Dewar (*J. Sci. Fd Agric.*, 1960, **11**, 566—570).—Of volatile constituents of silage (28 samples) dried at 100°, volatility of AcOH was 87·9, butyric acid 89·4 and lactic acid 1·4—16·4%; in silages of high pH, losses of N occurred. (14 references.) E. M. J.

Nutritional quality of forage crops adapted to South-western Saskatchewan as determined by digestibility and dry matter intake when fed to sheep. J. E. Troelsen and J. B. Campbell (*Canad. J. Plant Sci.*, 1959, **39**, 417—430).—As judged by chemical analysis, nutritive value, voluntary consumption and gains in wt., *Elymus junceus* Fisch. or *Medicago media* Pers. provide satisfactory maintenance crops. *Agropyron cristatum*, L. Gaertn. or *A. riparium*, Scribn. and Smith, *A. intermedium*, (Host) Beauv., *A. elongatum*, (Host) B.P. and *Phalaris arundinacea*, L. are (in descending order) less satisfactory. Top dressing with NH_4NO_3 does not increase digestibility or palatability. (30 references.) P. S. ARUP.

Effect of cutting interval and stage of maturity on digestibility and yield of lucerne. W. C. Weir, L. G. Jones and J. H. Meyer (*J. Anim. Sci.*, 1960, **19**, 5—19).—The yield of digestible protein from lucerne was greatest with a 4-week cutting interval; the total digestible nutrient reached optimum yield with a 5-week interval. In a 3-season comparison of cuttings made at different stages of maturity, dry matter yields were higher at the bloom than at the bud stage; the yields of protein and of total digestible nutrient were greatest at the 1/10 bloom stage and that of total digestible protein at the bud stage. A. G. POLLARD.

Vitamin E and selenium in animal and poultry nutrition. F. J. Tagwerka (*Agric. vet. Chemicals*, 1960, **1**, 23—25, 78—84).—Diseases associated with deficiency of vitamin E and an org. "Fraction 3" are discussed in relation to the protective action of Se, notably in cases of muscular dystrophy. Se is a possible constituent of Fraction 3. A likely mechanism of the Se–vitamin E relationship is postulated. (About 120 references.) A. G. POLLARD.

Excretion studies in swine fed arsanilic acid. L. R. Overly and D. V. Frost (*J. Anim. Sci.*, 1960, **19**, 140—144).—Pigs receiving arsanilic acid (**I**) (30—90 g. per ton of feed) excreted more As in faeces than in urine. Intake and excretion reached balance after 10 days. After cessation of **I** feeding the output remained steady for 2 days and then decreased rapidly. Unchanged **I** appeared in faeces (about 5% of the total consumed) but was not detectable in urine. A. G. POLLARD.

Arsanilic acid in growing-fattening rations of identical twin calves. D. E. Hodge, M. P. Plumlee and W. M. Beeson (*J. Anim. Sci.*, 1960, **19**, 38—43).—Addition of arsanilic acid to the ration at the rate of 50 or 150 mg. per head daily, did not affect growth rates. Higher dosages (250—350 mg.) retarded growth and slightly diminished appetites. Feed efficiency and carcass grade were lowered by the 350 mg. dosage. Accumulation of As occurred largely in liver, kidneys and in the rumen wall but not in the skin, heart or fat. A. G. POLLARD.

Chloropromazine residues in beef tissues. R. L. Hendrickson, G. V. Odell, W. J. Costello and H. W. Reuber (*J. Anim. Sci.*, 1960, **19**, 26—33).—Cattle receiving injections of chloropromazine hydrochloride (**I**) (0·19—0·4 mg./lb. live wt.) eliminated 11—12% of the dose in 24-h. urine. In carcasses of animals slaughtered 8 hr. after a 0·4 mg. dosage small amounts of **I** occurred in the fat, brain, heart, lungs and kidneys. No residues appeared in animals killed 72 h. after the injection. Muscle tissue held **I** in a bound form which did not disappear during the normal cooking period nor was it destroyed by heating. A. G. POLLARD.

Toxicity to rabbits and other animals of the fluorofatty acid present in seeds of *Dichapalum toxicarium*. R. A. Peters and R. J. Hall (*J. Sci. Fd Agric.*, 1960, **11**, 608—612).—Compared with fluoroacetic acid, the fluoro-oleic acid present in *D. toxicarium* is approx. 3 times more toxic to rabbits and 2—3 times less toxic to sheep. Data on the toxicity to other animals are given. Death, which may be delayed (e.g., in sheep up to 5 days or in rabbits given 1 mg./kg. in feed, up to 6—8 h.), occurs suddenly. E. M. J.

Diethylstilboestrol and length of preliminary period in utilisation of crude biuret and urea by lambs. II. Various aspects of nitrogen metabolism. G. A. McLaren, G. C. Anderson, J. A. Welch, C. D. Campbell and G. S. Smith (*J. Anim. Sci.*, 1960, **19**, 44—53).—In lambs receiving rations in which much of the N was provided in non-protein form (urea, biuret), diethylstilboestrol (**I**) did not affect the faecal excretion of metabolic N, endogenous urinary N, creatine or allantoin, or the protein-bound I of the plasma. The effect of **I** in accelerating the absorption of N results from its direct action in the tissues of promoting the utilisation of non-protein-N rather than from indirect effects on the thyroid gland. A. G. POLLARD.

Relative value of carotene and vitamin A fed at medium levels in a milk replacer. A. P. Grifo, jun., J. E. Rousseau, jun., H. D. Eaton and D. G. Goslee (*J. Dairy Sci.*, 1960, **43**, 1003—1006).—Judged by the efficiency of blood-vitamin A concn. maintenance in calves, the effectiveness of vitamin-A oil is 42% of that of carotene beadlets. (14 references.) P. S. ARUP.

Comparison of a protein supplement and shelled maize for dairy cows on good pasture. H. T. Bryant, R. E. Blaser, R. C. Hammes, jun., and W. A. Hardison (*J. Dairy Sci.*, 1960, **43**, 988—992).—The two supplements, viz. a grain mixture containing 20% of protein, and ground shelled maize support milk production equally well. (12 references.) P. S. ARUP.

Physiological effects of shading dairy cattle. A. R. Quartermain (*N.Z. J. agric. Res.*, 1960, **3**, 454—460).—Results of a small-scale study of the effects of shade on the respiration rate and rectal and skin temp. of Jersey cows in New Zealand summer conditions (71—85°F) are reported. A slight effect on rectal temp. and a highly significant effect on skin temp. were observed, but it is doubtful if the cooling effect of shade is sufficient to justify its economic use. (16 references.) E. C. APLING.

Effect of level of roughage during rearing period on utilisation of foods by adult cattle. C. C. Balch, R. C. Campling, V. W. Johnson and J. Roy (*Brit. J. Nutr.*, 1960, **14**, 379—390).—Two levels of hay and concentrates were used to study appetite and digestive efficiency. Under the conditions of the experiment, no differences could be determined. C. V.

Bulk feeds for milk production. I. Influence of level of concentrate feeding in addition to silage and hay on milk yield and composition. H. W. Holmes, G. W. Arnold and A. L. Provan (*J. Dairy Res.*, 1960, **27**, 191—204).—Increases in the level of concentrate feeding (1·25—5 lb./gal.) gave small but significant increases in milk yields and the protein and solids-not-fat contents of the milk. In group feeding at the low concentrate level, the cows maintained their wt. and yielded 2·5 gal. of milk daily during 11 weeks, and then completed their lactations similarly to those fed at the highest level. (13 references.) P. S. ARUP.

Variations in rate of milk secretion in milking intervals of 2—24 hours. G. M. Elliott, F. H. Dodd and P. J. Brumby (*J. Dairy Res.*, 1960, **27**, 293—308).—Rates of secretion of milk and solids-not-fat were constant for periods of $\gtrless$16 h.; in three of six experiments they declined during longer intervals. The rates remained depressed during $\lessgtr$16 h. after the long milking intervals. Rates of secretion of butterfat were not affected in any of the experiments. Probable reasons for these results are considered. (25 references.) P. S. ARUP.

Fission products and dairy cows. II. Aspects of metabolism of alkaline-earth elements calcium, strontium and barium. R. J.

Garner, H. G. Jones and B. F. Sansom (*Biochem. J.*, 1960, **76**, 572—579).—After simultaneous oral administration of any two of ^{45}Ca (**I**), ^{89}Sr (**II**) and ^{140}Ba (**III**) (as chlorides) to lactating cows, the mean recoveries of **I**, **II** and **III** after 8 days were: from faeces, 71, 89 and 98%; from urine, 0·4, 1·3 and 1·1%; and from milk, 16, 1·9 and 0·6% of the dose. The corresponding values in the 8 days after intravenous injection are: from faeces, 16, 18 and 36%; from urine, 1·2, 21 and 34%; and from milk, 32, 16 and 10%. Absorptive discrimination is most important in the overall distinction between **I**, **II** and **III** during their passage from diet to milk. Mammary secretion plays a considerable part with **II** whilst with **III** the effects of renal excretion, endogenous secretion into the gut, and mammary secretion are about equal. The rates of removal of each isotope in the whole body, and the rates of incorporation into the non-exchangeable fraction of the whole skeleton, are determined. The metabolism of these isotopes in the cow is compared with that in the rat. (16 references.) J. N. ASHLEY.

Effect of a high-protein, high-potassium ration on the mineral metabolism of lambs. J. P. Fontenot, R. W. Miller, C. K. Whitehair and R. MacVicar (*J. Anim. Sci.*, 1960, **19**, 127—133).—Rations containing high levels of protein and K, similar to those of grazings causing "wheat pasture poisoning", increased the faecal excretion of Mg and lowered Mg retention and plasma-Mg levels. A. G. POLLARD.

Influence of fatty acids on digestibility of ration components by lambs on cellulose digestion *in vitro*. K. L. Davidson and W. Woods (*J. Anim. Sci.*, 1960, **19**, 54—59).—In digestion trials with lambs addition of maize oil 5, mixed fatty acids 5, lauric acid 1, stearic acid 5 or oleic acid 5% to a ration containing 46% of maize cobs, diminished the digestibility of the dry matter, org. matter and cellulose and increased that of the ether extract and, except with lauric acid, of the ash. In *in vitro* tests with rumen organisms, butyric and valeric acids and glycerol increased, acetic and caproic acids did not affect and saturated C_{10}—C_{18} acids and also oleic and linoleic acids depressed cellulose digestion. A. G. POLLARD.

Variation in feed intake as a cause of variation in wool production of grazing sheep. P. G. Schinckel (*Aust. J. agric. Res.*, 1960, **2**, 585—594).—Sheep of varying levels of wool production per unit of body wt. when grazing in the field, and when fed in pens were compared. Differences between sheep and between groups were less than when they were grazing in the field, or fed *ad lib.* in pens. The group with the highest wool production showed a higher efficiency of conversion of feed to wool, and higher intake of food when fed *ad lib.* Production of wool was 22% above that of the group of lowest production when food intake was equal, and 37% greater when they were fed *ad lib.* M. D. ANDERSON.

Efficiency of conversion of food to wool in five Merino strains. A. A. Dunlop, C. H. S. Dolling and J. F. Kennedy (*Aust. J. agric. Res.*, 1960, **2**, 576—584).—Groups of four wethers, representing five strains of Merino (four replicates of each group), were maintained at constant body wt. for 4 months on a diet of lucerne chaff and wheat, and food consumption was compared with wt. of fleece produced during the period. The difference in efficiency of conversion of energy and protein into wool was significant between strains but other differences were not. (11 references.) M. D. ANDERSON.

Fertility responses in ewes treated with thyroxine. D. S. Hart (*N.Z. J. agric. Res.*, 1960, **3**, 565—578).—Ewes were given implantations of L-thyroxine and over three breeding seasons the lambs produced were increased by 9·5%. The effects of different dose levels and the differences in responses found with mature and two-tooth ewes are discussed. (14 references.) E. C. APLING.

Influence of nutritional state of pig on limiting basal metabolism. G. Charlet-Lévy (*C. R. Acad. Sci., Paris*, 1960, **251**, 455—456).—The O_2 consumption at thermal neutrality, after a 15-h. resting fast, show that the amount of N ingested per meal rather than that of dry matter determines the limiting basal metabolism. W. J. BAKER.

Effects of varying protein and energy intakes on the growth and carcass quality of swine. P. R. Noland and K. W. Scott (*J. Anim. Sci.*, 1960, **19**, 67—74).—Rations containing various protein levels (12—20%) and energy contents (950—1200 kcal./lb.) were compared. Differences in growth rate due to the ration were mainly limited to the period from weaning to 75 lb. live wt., during which the protein effect was highly significant and a significant protein × energy interaction was established. From 40 to 75 lb. live wt. the optimum kcal./protein ratio was 49—75; a ratio of 100 produced fastest growth at a later stage. Rations providing 1200 kcal./lb. produced fatter carcasses than did those of lower kcal. content. Carcasses were longer with 16—20% protein than with 12% protein in the ration. A. G. POLLARD.

Effect of condition at farrowing upon the subsequent milk yield and the efficiency of production. D. M. Smith (*N.Z. J. agric. Res.*, 1960, **3**, 598—616).—The yield and composition of the milk and the efficiency of production are reported for two groups of sows, in low and in high condition at farrowing. Both groups were fed on a low ration during lactation. The sows in low condition lost less wt. and produced slightly less milk and milk energy than those in high condition. The methods used for estimating energy efficiency, and the effects of various feeding regimes at different stages of the reproductive cycle, are discussed. E. C. APLING.

Utilisation of maize oil, lard and tallow, by chickens of various ages. R. Renner and F. W. Hill (*Poultry Sci.*, 1960, **39**, 849—854).—The metabolisable energy as % of gross energy for maize oil was 91—96% for chicks from 2 weeks to 8 weeks of age and 90% for 38-week-old adult hens. For lard values were 89—92% for chicks and 82% for adult hens. For tallow values increased from 70% at 2 weeks to 78% at 8 weeks of age and was 77% for the adult hen. When utilisation was measured by absorbability of the materials values for maize oil by chicks and all materials by hens were slightly higher than where metabolisable energy was used. A. H. CORNFIELD.

Utilisation of phosphorus from defluorinated and colloidal phosphate by chicks and laying hens. J. N. Baruah, R. E. Davies, B. L. Reid and J. R. Couch (*Poultry Sci.*, 1960, **39**, 843—849).—When added to a basal diet containing 0·43% of P, colloidal phosphate (CP) at 0·2—0·3% P did not support optimum growth of chicks to 8 weeks of age. The best growth responses were obtained when 25% of the supplemental P was supplied as CP and 75% as $CaHPO_4$ or defluorinated rock phosphate (DRP). No better growth response was obtained with 0·3% of added P than with 0·2%. Addition of 0·1% P as CP plus 0·2% P as DRP to the basal diet (containing 0·3% P) of laying hens did not reduce egg production, feed efficiency with respect to egg production, egg quality, or P level in yolk or blood in comparison with supplemental P supplied completely as DRP. Feed efficiency with respect to egg production was reduced when 66% of the supplemental P was supplied as CP. A. H. CORNFIELD.

Phosphorus availability from the ash of unidentified growth factor sources. J. N. Baruah, R. E. Davies, B. L. Reid and J. R. Couch (*Poultry Sci.*, 1960, **39**, 840—842).—Addition of the combined ash of unidentified growth factor sources (dried whey, distiller's dried solubles, fish solubles, lucerne meal and dried brewer's yeast) (containing 0·2% of P) to a P-deficient diet increased the growth rate of chicks to 4 weeks of age to the same extent as did defluorinated rock phosphate and colloidal phosphate, but was not as effective as was Na_2HPO_4. When supplied together with colloidal phosphate the growth factors ash did not improve the utilisation of P as measured by bone ash determinations. A. H. CORNFIELD.

Amino-acid inter-relationships in the chick with special reference to the rôle of glycine and arginine in alleviating amino-acid toxicities. D. C. Snetsinger (*Dissert. Abstr.*, 1960, **20**, 2996—2997).—Addition to chick diets of excess of lysine (**I**), phenylalanine (**II**), or histidine (**III**) caused inhibition of growth, D-lysine having about 60% the effect of L-lysine. Protein supplements partly overcame the inhibition caused by **I**, as also did glycine (**IV**) and arginine (**V**), singly or jointly, their effects being additive. **IV** also partially overcame inhibition caused by **III** or **II**. **IV** could not be replaced by Na acetate, nor **V** by creatine. The **IV** requirement on a soya-bean–cerelose diet was 1%; on the same diet with 4% of L-lysine, it was over 3%. **IV**-supplemented diets gave high gain-to-feed ratios. All essential amino-acids (**VI**) injected intraperitoneally were toxic to the chicks, producing high blood-NH_3 and high mortality. **IV** or **V** injected at the same time as or earlier than **VI** did not decrease mortality, but **IV** 1 h. before **I** did decrease blood NH_3. M. D. ANDERSON.

Galactose toxicity in male and female chicks. J. H. Nordin, D. R. Wilken, R. K. Bretthauer, R. G. Hansen and H. M. Scott (*Poultry Sci.*, 1960, **39**, 802—812).—Female chicks showed a higher mortality rate than did male chicks when supplied with a diet containing 15% of galactose. Diethylstilboestrol treatment reduced, whilst testosterone enhanced, the sensitivity of female chicks to galactose, but neither treatment affected the sensitivity of male chicks. Blood absorption rate of ingested glucose was similar with both sexes, but males utilised galactose more readily. Uridine diphosphate acetylhexosamine nucleotides of chick liver decreased with galactose toxicity. A. H. CORNFIELD.

Stimulation of chick growth with lucerne concentrates. J. A. Liuzzo, J. G. Lee, A. B. Watts, E. A. Fieger and A. F. Novak (*Poultry Sci.*, 1960, **39**, 823—827).—Concentrates prepared from dehydrated lucerne leaf meal by the method of Novak *et al.* (*J. Amer. pharm. Ass.*, 1958, **47**, 413) and which had been shown to be active for *Neurospora sitophila* were tested in a practical chick ration.

When added at a level ≡ 5% lucerne meal, concentrates A, B and C stimulated chick growth to 8 weeks of age.
A. H. CORNFIELD.

Feed materials for young mammals. Iowa State College Research Foundation (B.P. 826,033, 30.5.56. U.S., 20.10.55).—A growth-promotant feed especially adapted for feeding pigs at ages younger that 8 weeks, comprises a nutritionally balanced composition containing a proteinaceous material (e.g., soya-bean protein) (10—65% by wt.) as principal ingredient in admixture with an active proteolytic enzyme (e.g., pepsin). The composition is substantially in the dry condition. E. ENOS JONES.

Sulphur-containing piperazine compounds. C. Pfizer & Co. Inc. (B.P. 825,995, 20.11.57. U.S., 9.5.57).—S-containing piperazine deriv. are prepared by contacting piperazine (2—4 mol.) with SCl_2 (1 mol.) at −15° to 100°, to produce deriv. containing 26—45% of chemically bound S. The products are useful anthelminthics. E. ENOS JONES.

[A] Substituted carbanilides. [B] Therapeutic compositions for treating poultry. Merck & Co. Inc. (B.P. 825,921—2, 6.9.56. U.S., 26.9.55, [B] divided unit of [A]).—[B] Compositions for treatment of coccidiosis in poultry contain an inert carrier and an *N*-substituted carbanilide, [A] especially *N*-methyl- and -ethyl- (**I**), *NN'*-dimethyl- and -diethyl-4,4'-dinitro(dicyano or dicarboxy)carbanilide. These are obtained by interaction of *p*-nitrophenylisocyanate or a *p*-nitrophenylcarbamoyl chloride with an amine. **I** has m.p. 203—205°.
F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Handling properties of cereal products. R. R. Irani and C. F. Callis (*Cereal Sci.*, 1960, **5,** 198—201 and 214).—The prevention of poor flowability or caking of cereal products by the use of conditioning agents such as tricalcium phosphate, basic $MgCO_3$ and Santocel C (93% SiO_2) was studied. An effective conditioner must adhere to the surface of the material and not merely mix with it. For each material–conditioner combination an optimum conditioner level exists, beyond which the flow may be retarded. Conditioned materials can be blended easier than unconditioned. Curves were plotted showing the improvement in the flow of barley malt flour, bread or cake flour, cocoa and powdered sugar on addition of various conditioning agents. It is stressed that all additives must satisfy the requirements of the Food and Drug Administration. (10 references.) I. DICKINSON.

By-products of rice-milling. G. Brückner (*Getreide u. Mehl*, 1960, **10,** 78—81).—The chemical analyses of various fractions obtained during milling of rice, in particular, vitamin and protein contents, are reported. The limited use of the fractions as feeding stuffs, and their technical applications are discussed. (34 references.) J. V. RUSSO.

Histology and histochemistry of raw and cooked rice kernels. R. R. Little and E. H. Dawson (*Food Res.*, 1960, **25,** 611—622).—Cross sections from 23 varieties were examined. The pericarp and aleurone layers were thickest along the dorsal " ridge " and were separated by a moisture-resistant corky layer. In the starchy endosperm, cell sizes and shapes differed according to position and variety, starch granules being smaller and less crowded in peripheral cells. Protein material lined endosperm cell walls and encased all starch granules, and was more plentiful in cells where starch was less crowded. The fragile cell walls showed presence of cellulose and pectic substances. In brown rice the bran with thick dorsal ridge and water-resistant layer delayed water penetration and limited expansion of gelatinising starch. (13 references.) E. M. J.

Differential reaction of milled white rice varieties to a Millon reagent containing trichloroacetic acid and mercuric acetate. R. R. Little and G. B. Hilder (*Cereal Chem.*, 1960, **37,** 475—482).—Rice flour from 25 varieties was treated with the reagent and differences in sedimentation behaviour and in the appearance of the starch granules were observed. Relationships between these effects and grain length, palatability and other quality factors are discussed. (11 references.) S. G. AYERST.

Suitability of different varieties of mangoes for the preparation of mango cereal flakes. Girdhari Lal, G. V. Krishnamurthy, N. L. Jain and B. S. Bhatia (*Food Sci., Mysore*, 1960, **9,** 121—123).—Pulp and flakes of five South Indian varieties, *Badami, Raspuri, Totapuri, Neelam, Padri* and three U.P. varieties, *Safeda* (Malihabad), *Safeda* (Lucknow) and *Dusehri* were analysed. Sol. solids at 20°, moisture, pH, β-carotene, glucose, fructose, sucrose and colour were determined in the pulp; moisture, β-carotene, colour and flavour in the flakes. Pulps of *Badami* and *Dusehri* varieties were suitable for the manufacture of flakes, but those prepared from other varieties lacked flavour or colour or both. When pulp of poor flavour and dull colour was blended with the more suitable pulp, the resulting flakes were superior to those prepared from the former pulps. E.g., flakes from blended pulps of *Badami* and *Raspuri*, *Badami* and *Totapuri*, and *Safeda* and *Dusehri*, in the ratio of 2 : 1 were satisfactory and constituted a rich source of carbohydrates, minerals, ascorbic acid and β-carotene. I. DICKINSON.

Carbohydrates of Graminae. X. Quantitative study of carbohydrates of wheat germ. M. Dubois, W. F. Geddes and F. Smith (*Cereal Chem.*, 1960, **37,** 557—568).—The carbohydrates of commercial wheat germ, and of germ obtained by hand dissection, were determined and compared. The methods of hand dissection, and micro-extraction of carbohydrates, are described. The effect of moisture on the sugars in the germ is discussed. (25 references.)
S. G. AYERST.

Mineral matter in oat grain and its distribution in rolled oat manufacture. K. G. Bergner and K. Wagner (*Getreide u. Mehl*, 1960, **10,** 81—84).—Methods of determining Ca, SiO_2, Fe and Co in various fractions produced during the manufacture of rolled oats are described and analytical data are presented. (18 references.)
J. V. RUSSO.

Grain storage studies. XXXI. Changes occurring in low-molecular-weight compounds in deteriorating wheat. R. L. Glass and W. F. Geddes (*Cereal Chem.*, 1960, **37,** 568—572).—The reducing and non-reducing sugars were determined chromatographically in wheat samples stored in N_2 at 18% moisture content and 30°, and also in control samples. Among other substances, D-galactose, myoinositol and glycerol were obtained from the deteriorating wheat. (11 references.) S. G. AYERST.

Changes in the soluble carbohydrates during browning of wheat embryos. P. Linko, Y. Y. Cheng and M. Milner (*Cereal Chem.*, 1960, **37,** 548—556).—Changes in the sol. carbohydrates in embryos of wheat stored at moisture contents ranging from 8·9 to 25% and temp. ranging from 29 to 50° were investigated chromatographically. The correlation of browning with these changes is discussed. At some moisture levels several unknown sugar-like compounds appeared. (21 references.) S. G. AYERST.

Rapid determination of DDT and Gammexane in flour and grain. G. Paulig (*Dtsch. LebensmittRdsch.*, 1960, **56,** 223—224).—The method involves extraction with n-hexane, purification of the extract by chromatography and washing with conc. H_2SO_4, and measurement of the i.r. spectrum of a solution in CS_2. DDT is determined from its typical double band at 12·8 to 13μ and Gammexane at 14·6μ. E. C. APLING.

Relation of particle size to certain flour characteristics. B. Sullivan, W. E. Engebretson and M. L. Anderson (*Cereal Chem.*, 1960, **37,** 436—455).—A hard-wheat, 90% patent flour was air-classified in six particle-size ranges and the separated fractions were analysed for particle-size distribution, ash, protein, maltose value, gassing power and η. These findings and the relationship of starch damage to maltose value and gassing power are discussed. (43 references.)
S. G. AYERST.

Lipids of flour. I. Effect of chlorine dioxide treatment on essential fatty acids. N. W. R. Daniels, P. W. R. Eggitt and J. B. M. Coppock (*J. Sci. Fd Agric.*, 1960, **11,** 658—664).—Essential fatty acid (E.F.A.) contents of white flour (70% extraction) treated with twice and with 20 times the present usage rate of ClO_2 were examined by gas–liquid chromatography and by u.v. spectrophotometry of the isomerised oil. Little change was observed after 5 days, but with air storage the E.F.A. content fell by 70·6% between the 5th and 12th day after treatment; and with N_2 storage by 16·1%. The lower the level of treatment of the flour, the less is the destruction of protective tocols, and at current treatment (1·8 g. of ClO_2 per sack) there is little danger of loss of E.F.A. caused by air oxidation during storage. (18 references.) E. M. J.

Changes in the lipids of flour induced by treatment with chlorine dioxide or chlorine, and on storage. D. G. H. Daniels (*J. Sci. Fd Agric.*, 1960, **11,** 664—670).—The C_{16} and C_{18} fatty acids in flour lipids were measured by a gas chromatographic method and changes in effectiveness of the natural antioxidants were assessed by measuring the induction periods of intact flours subjected to an accelerated oxidation test. Flour treated with ClO_2 (120 p.p.m.) stored for 15 weeks showed no changes in the fatty acid composition; after storage for 27 weeks untreated and treated (33 p.p.m. ClO_2) showed changes in acetone extracts. These findings were correlated with measurements of induction periods of flours heated at 100° in O_2.

Treatment and storage diminished induction periods. In Cl_2-treated flour linoleic acid content decreased. Cl_2 treatment caused long induction periods. Moderate doses of ClO_2 or Cl_2 have little effect on the contents of " essential " and other fatty acids, but have marked effects on the natural antioxidant substances in the flour. (14 references.) E. M. J.

Cereal catalase. M. Rohrlich and R. Lenschau (*Getreide u. Mehl*, 1960, **10**, 73—75).—An electrochemical method is used to determine the effects of HCN and of temp. on wheat catalase (**I**) activity, of DDT on rye **I** activity, of inorg. and org. salts, and of amino-acids on wheat and rye **I** activities. J. V. Russo.

Relation of viability and storage deterioration to glutamic acid decarboxylase in wheat. P. Linko and L. Sogn (*Cereal Chem.*, **37**, 489—499).—Germ damage, germination, fluorescence, fat acidity and dehydrogenase activity were determined in 1-year-old and new crop wheat samples and correlated with glutamic acid decarboxylase activity. The value of this information in estimating storage deterioration is discussed. (42 references.) S. G. Ayerst.

Polarimetric study of flour diastatic value. A. H. Woodhead and C. A. Wyatt (*Cereal Chem.*, 1960, **37**, 543—547).—The diastatic values of flour samples measured polarimetrically differ in a regular and simple manner from those obtained by chemical reduction methods of analysis. The reducing substances present in bran and germ were not recorded by the polarimeter. S. G. Ayerst.

Control of diastatic states in rye. C. E. Albertsson (*Getreide u. Mehl*, 1960, **10**, 85—92).—Methods for the determination of diastatic activity are reviewed in order to find one which is suitable for modification to an automatic method. Autolysis of the starch by heating at 70° or gelatinisation by the addition of KCNS, followed by η measurements are both suitable methods. (13 references.) J. V. Russo.

Enzymic reduction of protein in high-amylose maize starch. C. Vojnovich, R. A. Anderson and E. L. Griffin, jun. (*Cereal Chem.*, 1960, **37**, 576—578).—The effect of variation in pH, amount of enzyme present, temp. and time, on the reduction of protein by trypsin and papain were investigated. S. G. Ayerst.

Differential response of rice starch granules to heating in water at 62°. R. R. Little and G. B. Hilder (*Cereal Chem.*, 1960, **37**, 456—463).—In 24 varieties of rice the alteration of starch granules after heating in water was observed microscopically. The correlation with taste panel scores for cohesiveness indicates that rice cooking characteristics may be indicated by this test. (11 references.) S. G. Ayerst.

Effects of fats and non-ionic surface-active agents on starch pastes. E. M. Osman and M. R. Dix (*Cereal Chem.*, 1960, **37**, 464—475).—Eleven different fats, each added to a 6% maize starch paste, caused η to increase at progressively lower temp. There was no difference in their effect on gelatinisation or on the cooling curves of the paste. Addition of surface-active agents to the starch–water–fat mixture increased the temp. at which η increased. The relationship of the effects to the character of the molecule of the surface-active agent is discussed. (12 references.) S. G. Ayerst.

Microflora of wheat starches. G. Spicher (*Brot u. Gebäck*, 1960, **14**, 130—133).—The contents of micro-organisms (aerobic, anaerobic and spore-forming bacteria and moulds) in powdered and lump starch from various sources are tabulated. J. V. Russo.

Specific rotation of cereal and legume starches. R. D. Patel, R. P. Patel and R. S. Patel (*Cereal Chem.*, 1960, **37**, 500—502).—Specific rotation values of starch from 16 spp. of cereals and legumes ranged only between 202° and 204°. Genetic and growth factors thus appear to have little influence on this property of starch. S. G. Ayerst.

Effect of various sugars on formation and character of gluten. D. P. Meiske, M. F. Jones and E. M. Jones (*Cereal Chem.*, 1960, **37**, 483—488).—The effects of various sugars added at 4% of flour wt. were studied by baking gluten balls prepared in three ways, and then measuring their vol. and tenderness. The drip loss during mixing was also measured. The effects of varying the amounts of different sugars added were studied in the same way. S. G. Ayerst.

Vital wheat gluten by drum drying. II. Pilot-plant studies and cost estimates. C. Vojnovich, V. F. Pfeifer, R. A. Anderson and E. L. Griffin, jun. (*Cereal Chem.*, 1960, **37**, 422—435).—In pilot-plant investigations, wheat gluten dispersions in dil. AcOH were prepared by several methods. The gluten was then drum dried and ground. Various types of standard equipment were used and the methods can be easily adapted in commercial plants. A cost estimate for drying 6 million lb. of gluten annually is given. S. G. Ayerst.

Phytin as a buffering substance in leavened dough. M. Rohrlich (*Brot u. Gebäck*, 1960, **14**, 127—130).—The relationship between ash content and buffering capacity of rye flour is determined by potentiometric titration using lactic acid as the titrating agent. The effect of adding phytin, to various types of flour, on the rate of formation of acids during fermentation is determined experimentally. J. V. Russo.

Determination of yeast growth in doughs. J. A. Thorn and J. W. Ross (*Cereal Chem.*, 1960, **37**, 415—421).—A method for isolating yeast cells from dough by enzymic degradation of the insol. starch and protein is described. The cells were readily counted in a Petroff–Hausser chamber. The amount of growth of compressed and active dry yeast in straight doughs, sponges, sweet dough sponges and brew doughs, was determined. In sponges both yeasts grew ~50—60%, in straight doughs ~35%, in sweet dough sponges or in flour brews no growth occurred, the fermentations lasting about 4 h. S. G. Ayerst.

Methods for measuring reactivity of chemical leavening systems. I. Dough rate of reaction. J. R. Parks, A. R. Handleman, J. C. Barnett and F. H. Wright (*Cereal Chem.*, 1960, **37**, 503—518).—Standardised methods for measuring the dough rate of reaction of a chemical leavening system are described in detail. Operations of the apparatus are electronically programmed. Test results were influenced by flour differences and age, presence or absence of milk and shortening, and differences in the performance of the apparatus. (35 references.) S. G. Ayerst.

Bread making without bulk fermentation: effect of mixing and stage of addition of potassium iodate. T. A. Mitchell and C. A. Crawford (*N.Z. J. Sci.*, 1960, **3**, 290—292).—The effects of mixing time and of stage and level of addition of KIO_3 on a continually mixed dough, prepared from a 78% extraction commercial flour, on loaf vol. and maturity, are tabulated. J. V. Russo.

Vitamin content of flour and bread—technological principles. P. F. Pelshenke and A. Rotsch (*Brot u. Gebäck*, 1960, **14**, 121—126).—The B-vitamin contents of German bread cereals, wheat and rye, of commercial flours and of different types of German bread, are reported. Loss of natural B-vitamins during processing (i.e. storage, chemical treatment, aeration and baking) is discussed. Possibilities and limitations of vitamin enrichment of lightflours, by addition of embryo material of cereals, are discussed. (16 references.) J. V. Russo.

The rôle of wheat flours in mixed bread manufacture in germinating years. L. Weith (*Brot u. Gebäck*, 1960, **14**, 137—140).—Bread baking tests using mixtures of rye and wheat flours (in varying proportions) show the impairment of quality that is produced by using even a low % of " spoiled wheat flour " (flour from sprouting grain in wet harvest conditions) with good quality rye flour. J. V. Russo.

Quality of the protein in selected baked wheat products. B. M. Kennedy and A. R. Sabiston (*Cereal Chem.*, 1960, **37**, 535—543).—Baked biscuits, muffins, griddle cakes and cookies, and their unbaked ingredients, were fed to rats. The N and protein efficiency ratios (P.E.R.), food intakes and wt. gains were determined. Baking reduced the P.E.R. The addition of egg and milk protein to wheat products increased the P.E.R. S. G. Ayerst.

Disappearance of bromate during baking of bread. W. Bushuk and I. Hlynka, with addendum by C. C. Lee and R. Tkachuk (*Cereal Chem.*, 1960, **37**, 573—576).—Bread was baked from flour containing 5—80 p.p.m. of $KBrO_3$. The disappearance of bromate during baking was investigated by the amperometric titration technique. Previous findings by Lee and Tkachuk are amended to fall into line with these results. S. G. Ayerst.

Predominantly occurring bread defects. A. Schultz (*Brot u. Gebäck*, 1960, **14**, 141—147).—Faults in vol., crust, crumb and flavour in 12 types of bread both whole and sliced are tabulated. Of these, uneven texture was the most commonly occurring, followed by close crumb, slight unfinished flavour, etc.; and uneven crust formation and impurities the least. J. V. Russo.

Sugars and confectionery

Mycological production of citric and oxalic acids from cane molasses. II. Effects of some enzyme inhibitors. III. Effect of mixed substrates. I. R. Shimi and M. S. Nour el Dein (*J. Sci. Fd Agric.*, 1960, **11**, 613—619, 619—622; cf. J.S.F.A. Abstr., 1961, **11**, i, 31).—II. Mats of *Aspergillus niger* (strains NI and B_8), initially developed on molasses medium were floated on solutions containing molasses (**I**), sucrose (**II**) or citric acid and various concn. of Na arsenite or Na iodoacetate, or (on solutions containing **I** or **II**) 2,4-dinitrophenol. Quant. determinations of pyruvic and α-keto-glutaric acids in the metabolism solutions containing arsenite were made by chromatography of the 2,4-phenylhydrazones. Data are discussed in relation to findings by other workers. (18 references.)

III. In presence of acetate, pyruvate and ethanol in the substrate

the yields of citric acid are higher in the molasses solutions than in those containing sucrose, but yields of oxalic acid are higher in sucrose solutions. In general the presence of acetate, pyruvate and ethanol and optimum concn. of ferrocyanide/phosphate stimulated the accumulation of citric acid and uptake of sugar, acetate being the most suitable. In cultures of NI strain the concn. of methanol used were all toxic to the organism. (10 references.) E. M. J.

Chromatography for examination of variations of carbohydrate complexes in sugar beet after long storage. O. V. Braun (*Zh. prikl. Khim.*, 1959, **32,** 2084—2090).—Paper chromatography was used. The changes reported consisted chiefly of decomposition of sucrose, formation or accumulation (or both simultaneously) of raffinose, formation and accumulation of ketoses, and accumulation of invert sugar. A. L. B.

Maple syrup. XIV. Ultra-violet irradiation effects on growth of bacteria and yeasts. I. S. Schneider, H. A. Frank and C. O. Willits (*Food Res.*, 1960, **25,** 654—662).—Of two bacterial strains (**I**) and one yeast strain (**II**), suspended in maple sap exposed to u.v. radiations of different intensities and lengths of time, **I** were more sensitive than **II**. As time of exposure increased, the no. of living cells correspondingly decreased of all organisms regardless of method of irradiation. U.v. irradiation may be used to disinfect sap when put into storage and to suppress growth during storage. (26 references.) E. M. J.

Application of the Karl Fischer method to the determination of water in sugar confectionery materials. D. Sandell (*J. Sci. Fd Agric.*, 1960, **11,** 671—678).—Details are given of the Karl Fischer reagent, the development of a suitable titration apparatus and of a suitable technique for extracting water from material under test so that it may be completely titrated. The method is rapid (7 or 8 min. for many materials and $\gtrsim$40 min. for any material tested), with a precision of $\pm$1% of the water content. Results are in good agreement with those of oven-drying methods but discrepancies occur in some cases. E. M. J.

Non-enzymic browning in foods. The mechanism of sugar–organic acid system. J. R. Iyengar and N. S. Kapur (*Food Sci., Mysore*, 1960, **9,** 124—126).—Sucrose and citric acid (present in most fruit products) were refluxed in 12 varying proportions. In samples taken at intervals of 2 h., pH, titrable acidity, optical density at 420, 490 and 530 mμ and optical density at peak positions in the region 250 to 300 mμ were determined. The pH of the solution containing decimolar concn. of sugar and citric acid decreased from 2·68 to 2·50 during 10 h. refluxing. When the proportion of acid to sugar was increased, the drop in pH also increased, when the sugar : acid was increased the drop in pH decreased. Browning reaches its max. after 8 h. of refluxing, in solutions containing equimolar proportions of sugar and acid. Decimolar solutions did not show appreciable browning. I. DICKINSON.

Setting time and setting temperature of pectin jellies. J. J. Doesburg and G. Grevers (*Food Res.*, 1960, **25,** 634—645).—The influence of jelly grade was studied ; pectins with various degrees of polymerisation were prepared by enzymic and thermal decomposition and by the combined action of ascorbic acid and H_2O_2. The partial breakdown by the non-enzymic treatments caused an increase in setting time (**I**). The **I** of jellies containing pectins with low ash content increases with decrease of degree of esterification of pectins to ~50% ; jellies from pectins with lower degrees of esterification show shorter **I**. Ca added to jellies causes an increase in setting temp., the effect being stronger when the pectins have lower degrees of esterification. Pectins with a degree of esterification of 45–55% can be used as rapid set pectins. (19 references.) E. M. J.

Molasses treatment. International Minerals & Chemical Corp. (B.P. 826,164, 11.10.56. U.S., 15.11.55).—A process for the removal of sugar from sugar beet molasses such that the nitrogenous values of the molasses are preserved (and are subsequently recovered in the form of glutamic acid) comprises treating the molasses (at 40—70% solids content) for 0·5—5 h. at 50—85° with BaO or $Ba(OH)_2$ ($\lesssim$60 wt.-% on sugar content of molasses), with pptn. of Ba saccharate which is separated. Mother liquor contains glutamic acid (resulting from the hydrolysis of its precursors originally present in the molasses) and is subsequently recovered. F. R. BASFORD.

Dry or substantially dry product from molasses. G. Scott & Son (London) Ltd., W. T. Fosh and H. A. S. Sanders (B.P. 826,949, 19.7.56).—Apparatus is figured and claimed for prep. of a dry powdered or granulated product by heating in high vac. a highly conc. molasses. F. R. BASFORD.

Fermentation and Alcoholic Beverages

Control of contamination in alcoholic fermentation of sugar cane [blackstrap] molasses by penicillin and tetracycline. E. Aquarone (*Appl. Microbiol.*, 1960, **8,** 263—268).—Penicillin (**I**) (7500 units/l.), is effective even in fermentation using the seeding bacteriological process. Highly contaminated mashes can be recuperated by seeding back with a **I**-containing mash. The action of yeast is not directly influenced by **I** even when present in concn. >500,000 units/l. Tetracycline (**II**) does not influence the fermentation time and is an inhibitor of contamination in 1—30 mg./l. Riboflavin does not interfere with **II** and ~60% of **II**-hydrochloride which has been added is found in the fermented mashes. (22 references.) C. V.

Continuous pressing of grapes. N. Ordódy (*Mitt. Wein- u. Obstbau, Wien*, 1960, **10A,** 70—72).—A reply to a criticism of the process of Marek and Epp (cf. J.S.F.A. Abstr., 1959, i, 273). Satisfactory results can be obtained with proper prep. of the vintage and avoidance of over-compression in the continuous press. P. S. ARUP.

Treatment of [grape] mash and must with sulphurous acid. F. Paul (*Mitt. Wein- u. Obstbau, Wien*, 1960, **10A,** 38—53).—The maintenance of an excess of SO_3^{2-} in the mash and (up to the time of fermentation) in the must is shown to prevent discoloration and to conserve the reductones which contribute largely to the fruity taste and aroma of the wine. The consumption of SO_3^{2-} in the mash (to which the equiv. of 70—100 mg. of SO_2 per l. must be added) is considerably greater than that in the must. The best results are obtained by delaying the pressing until the mashing vessel has been filled and the K pyrosulphite has been evenly distributed. Routine control should be kept of the content of SO_3^{2-} and reductones. (22 references.) P. S. ARUP.

Detection of sugar-assimilation by yeasts by paper-chromatography. E. Minarik, L. Laho and A. Navara (*Mitt. Wein- u. Obstbau, Wien*, 1960, **10A,** 23—27).—The incubated (liquid) test media obtained by the Wickerham and Burton method are tested for the sugars under consideration by a previously described chromatographic technique. Assimilation is definitely indicated by the failure of the spot for the sugar in question to appear in its expected place. The results thus obtained are sometimes at variance with those obtained by the current method, but they can be regarded as more reliable. P. S. ARUP.

Addition of ascorbic acid to wine and its effects. H. Konlechner and H. Haushofer (*Mitt. Wein- u. Obstbau, Wien*, 1960, **10A,** 73—82). —The addition of ascorbic acid (**I**) (30—70 mg. per l.) immediately before bottling quickly eliminates all free O_2 and preserves the original fruity character of the wine. Although **I** cannot replace SO_2, its presence reduces the amount thereof necessary to check microbial and enzymic oxidation and to bind the aldehydes. The chemical mechanism of the synergistic effect of **I** and SO_2 is explained. (10 references.) P. S. ARUP.

Use of glucose oxidase in dry white wines. C. S. Ough (*Mitt. Wein- u. Obstbau, Wien*, 1960, **10A,** 14—23).—Satisfactory removal of O_2 from wine can be achieved at 25° and pH 3—4 by the addition of 0·013—0·026 g. of the enzyme (Dee O powder) in presence of $\lesssim$1 g. of glucose per l. The process is very little affected by the concn. of EtOH ; it is retarded by SO_2 and accelerated by ascorbic acid. Discoloration can be avoided by storing the treated wine out of contact with O_2 during 1—3 weeks or by short-time pasteurisation at 60°. (14 references.) P. S. ARUP.

Determination of ammoniacal nitrogen and of macromolecular nitrogenous substances by a cation-exchange method. V. Dimotaki-Kourakou (*Ann. Falsif., Paris*, 1960, **53,** 337—348).—Classical methods for determination of NH_3 in wines are critically reviewed. A method utilising the ion-exchange resin Amberlite IRC-50, which avoids interference from sugars and anions, is described and the results compare favourably with older methods. Macromolecular N substances are determined using the exchange resin Dowex-50. (21 references.) J. V. RUSSO.

Pasteurisation of beer. R. Scriban (*Brasserie*, 1960, **15,** 246—256).—Two methods, pasteurisation in bottle and flash pasteurisation, are discussed in relation to conditions prevailing during the brewing and drawing-off processes to give good biological stability and a favourable development of organoleptic qualities ; and to the type of beer destined for long storage or that to be consumed within a short time. (11 references.) E. M. J.

Prevention of haze formation in beverages. A.-B. Pripp & Lyckholm (Inventor : N. S. Berntsson) (B.P. 826,862, 8.6.56).—Haze formation in beverages (malt beverages, fruit juices, and wines) is prevented by addition of polyvinylpyrrolidone, mol. wt. 15,000—2,500,000 (preferably 5—50 g. per hectolitre and in any case in an excess of $\lesssim$50% over the amount necessary for max. haze pptn.). F. R. BASFORD.

Treatment of beer. American Tansul Co. (B.P. 826,706, 4.6.57. U.S., 6.8.56).—Beer is stabilised against formation of haze (on

repeated chilling or after agitation during transport) and its appearance and foaming properties are improved by treatment (after fermentation and prior to finishing) with SO_2 (5—25 p.p.m.). If desired, a stabiliser clay (montmorillonite) may also be added (before the SO_2). F. R. BASFORD.

Fruits, Vegetables, etc.

Effect of borates and other inhibitors on enzymic browning in apple tissue. II. Mechanism. K. Bedrosian, M. P. Steinberg and A. I. Nelson (*Food Technol.*, 1960, **14,** 480—483).—Catechol (**I**) or purified polyphenol (**II**) extract of apple used as substrate and commercial phenol oxidase (max. activity pH 7) enzyme prep. as catalyst was incubated for 30 min. at 25° and optical density measured at 430 mμ. Borate (or H_3BO_3) inhibition was increased by increasing pH and increasing borate concn. Use of either **I** or **II** extract of apple gave similar results. Increasing amounts of **I** added to aq. H_3BO_3 decreased pH. Borate inhibited oxidation of **I** in absence of enzyme. Addition of borate decreased absorption by **I** of u.v. light. Evidence was that borate inhibits the browning reaction by forming a complex with the substrate, thus preventing oxidation. E. M. J.

Relationship of apple maturity to applesauce quality. R. L. LaBelle, R. S. Shallenberger, R. D. Way, L. R. Mattick and J. C. Moyer (*Food Technol.*, 1960, **14,** 463—468).—Pilot plant studies were made of apples of 1957 and 1958 harvests. Sol. solids–acid ratio changed rapidly prior to and at harvest and had a direct bearing on quality of the product. The ratio was closely correlated with heat unit accumulation in the above two seasons. Sauce colour, flavour and grain improved as harvest was delayed to allow the fruit to ripen on the tree, especially if the apples were processed immediately after harvest; sauce from late-harvest apples was better. Pressure test and calendar date of harvest were inferior indices of maturity; heat unit accumulation and days after full bloom were about equally effective. (15 references.) E. M. J.

Sand in canned strawberries. D. Dickinson and T. W. Raven (*Analyst*, 1960, **85,** 521—523).—In a survey of different packs of canned strawberries sand is determined as the residue removed by a standard washing procedure, wet ashed, dried and weighed. The average sand content of all cans examined including some deliberately packed with dirty fruit was 105 p.p.m. When the packs of dirty fruit and those containing >200 p.p.m. were excluded, the average was 55 p.p.m. The average from all unwashed packs was 220 p.p.m. It is concluded that if a pack contains <100 p.p.m. on average and 100 to 200 p.p.m. in ≯10% of the cans sampled, the canner has taken reasonable precautions. A. O. JONES.

On the formation of metal chelates in canned fruit. K. Heintze (*Dtsch. LebensmittRdsch.*, 1960, **56,** 194—198).—Studies of the formation of chelates of Fe and Sn by various polyphenols present in fruits are reported and their bearing on the distribution of Fe and Sn in the flesh, skin and syrup of canned fruits is discussed. Tin^{2+} forms sol. chelates with anthocyanins, flavones and catechins which are stable in acid fruit juices, but Fe in these conditions forms chelates only sparingly or not at all. E. C. APLING.

Paper chromatographic identification and estimation of free amino-acids in 32 fruits. R. L. Silber, M. Becker, M. Cooper, P. Evans, P. Fehder, R. Cray, P. Gresham, J. Rechsteiner and M. A. Searles (*Food Res.*, 1960, **25,** 675—680).—Collected data presented in this survey furnish approx. quant. amounts and the possibility of the presence of amino-acids not yet identified. Grapes and tropical fruits (with the exception of citrus) contained the highest no. of amino-acids; and apple, gooseberry, etc., the least amounts. (23 references.) E. M. J.

Carotenoids of Japanese persimmons. A. L. Curl (*Food Res.*, 1960, **25,** 670—674).—Persimmons (*Diospyros kaki*) are an excellent source of cryptoxanthin and to a lesser extent of zeaxanthin and antheraxanthin. (11 references.) E. M. J.

Optical density of tomato serum from concentrates as a measure of heat induced changes in product corrosivity. H. H. Hernandez and J. F. Feaster (*Food Technol.*, 1960, **14,** 468—471).—The colour of tomato serum is the best current test for commercially evaluating burning of tomato paste: (*a*) the colour of unburned tomato serum after dilution to a given concn. is relatively constant, (*b*) the amount of change in optical density is proportional to the amount of burning, and (*c*) the colour of tomato serum can be used to predict accurately the expected service life. A method is suggested for reporting results in terms of a colour index that is independent of the equipment used to make the measurements. E. M. J.

Non-alcoholic beverages

Germicidal effect of orange peel oil and D-limonene in water and orange juice. I. Fungicidal properties against yeast. D. I. Murdock and W. E. Allen (*Food Technol.*, 1960, **14,** 441—445).—Orange peel oil(**I**) and D-limonene (**II**) (0·02% in water or 0·1% in orange juice at 25° and pH 7) were lethal to *Zygosaccharomyces major*, **II** being more effective. **I** and **II** were more effective at pH 6·0 and 7·0 than at pH 3·0 and 4·0. Orange juice at pH 7·0 (NaOH) containing 0·1% of **II** was sterile 3 h. after inoculation with yeast cells (10^6/ml.). In juice at pH 3·0 (citric acid), 10^5 cells/ml. were viable after 3 h. The preservative properties of Na benzoate were enhanced by addition of **I** and **II** in concn. of 0·02%. E. M. J.

Determination of the percentage of pulp in fruit and vegetable juices. P. Dupaigne (*Fruits d'outre mer*, 1960, **15,** 325—327).—The most satisfactory method of determining the amount of pulp present is by centrifuging the juice in graduated tubes and measuring the depth of pulp. The effects of variation in the speed and time of centrifuging, and in the size and shape of pulp particles, on the deposition of the pulp, is discussed. S. G. AYERST.

Effect of citric acid concentration on the formation of diacetyl by certain lactic acid bacteria. N. B. Rushing and V. J. Senn (*Appl. Microbiol.*, 1960, **8,** 286—290).—The production of buttermilk off-flavour (diacetyl) (**I**) spoilage in frozen orange concentrates is discussed; **I** is produced by *Lactobacillus brevis* and growth is in direct relation to the amount of citric acid (**II**) in the medium, the nutrient remaining constant. The enzyme producing **I** is adaptive and **I** accumulated only in a citrate-containing medium. Only **II** was a precursor of **I** for this species. No medium was found on which *Lb. plantarum* var. *mobilis* would grow without producing substantial amounts of **I.** *Leuconostoc mesenteroides* and *L. dextranicum* produce **I** in natural orange juice media but do not grow on a synthetic medium at the same pH, 3·8—4·0. (15 references.) C. V.

Construction, procedure and viewpoints on the choice of plant for evaporation and aroma recovery. H. Siegrist (*Fruchtsaft-Industr.*, 1960, **5,** 215—224).—Physical and chemical properties of fruit juices are reviewed. The design of evaporating plant used in the production of fruit concentrates is described in detail. Methods and plant for recovering steam volatile compounds which are lost during evaporation are discussed. (16 references.) J. V. RUSSO.

Management and heat economics of evaporating and aroma recovery plants. W. Pilnik and F. Emch (*Fruchtsaft-Industr.*, 1960, **5,** 245—267).—The relative costs of labour, plant, heating and maintenance in the juice concentration process are discussed with a view to designing a process of optimum efficiency. (16 references.) J. V. RUSSO.

Tea, coffee, cocoa

Soluble extract and coefficient of extraction of coffee. P. Navellier, R. Brunin, F. Chassevent and A. Isaac (*Ann. Falsif., Paris*, 1960, **53,** 326—336).—Methods of determining sol. coffee extract by cold maceration, digestion at 50°, 80° and on a water bath, decoction and 3 h. extraction are discussed. Digestion at 80° is considered to give the most reproducible results. (11 references.). J. V. RUSSO.

Rheology of cocoa butter. II. Effect of storage temperature on apparent viscosity. C. Sterling, F. Shimazu and J. J. Wuhrmann (*Food Res.*, 1960, **25,** 630—633).—The relative apparent η increased throughout the period of storage; the least change occurred at 0° and the greatest at 60°. A constant increase in η, yellowness and rancidity was a direct function of temp. and time of storage. Results were related to oxidative changes which brought about polymerisation of the component fats. (12 references.) E. M. J.

Chocolate. S.p.A. Macchine Industria Dolciaria Carle & Montanari (B.P. 826,502, 5.11.56. It., 30.11.55).—Hot fluid chocolate is divided into at least two portions, each under thermal control, which are simultaneously cooled to at least two different predetermined temperatures. The separate streams are then re-combined, intimately mixed, and discharged as a single fluid stream prior to use in moulding and covering operations. This method of processing ensures that the chocolate mass is brought to the most suitable physical state for moulding. Apparatus is figured. F. R. BASFORD.

Milk, Dairy Products, Eggs

Reviews of progress of dairy science. Section D. Nutritive value of milk and milk products. I. W. A. McGillivray. **II.** J. W. G. Porter (*J. Dairy Res.*, 1960, **27,** 309—321, 321—334).—I. A review covering general aspects and fat-sol. vitamins. (135 references.)

II. A review covering milk proteins, and amino-acids, water-sol. vitamins, mineral salts, and milk and milk products for the pre-ruminant calf. (154 references.) P. S. ARUP.

Relation between composition and consumer acceptance of milk beverages. J. W. Stull and J. S. Hillman (*J. Dairy Sci.*, 1960, **43**, 945—957).—Acceptance is very significantly improved by the addition of 1% of solids-not-fat to whole, low-fat or separated milk. Slight but significant preferences are shown for low-fat milk with 1% of added solids-not-fat, in comparison with natural whole milk, and for fortification with solids-not-fat rather than with fat. P. S. ARUP.

Errors in estimation of lactational yields of milk, fat and solids-not-fat from individual cows. N. R. Thompson, W. K. Stone, C. G. Graf, C. Y. Kramer and R. J. Freund (*J. Dairy Sci.*, 1960, **43**, 951—957).—Significant errors in fat, but not in solids-not-fat %, can arise through failure to take aliquot vol. from the individual milkings. Standard errors for the Babcock and the Watson lactometer tests are $\pm 0{\cdot}05$ and $\pm 0{\cdot}04\%$ for fat and solids-not-fat, respectively. The lactometer test shows an average bias of $-0{\cdot}02\%$ of total solids, which varies with the state of lactation. Sampling errors (which constitute the chief factor in the overall error) for lactational yields of milk, fat and solids-not-fat show approx. equiv. coeff. of variability. (15 references.) P. S. ARUP.

Nomenclature of proteins of bovine milk. First revision. Report of Committee on milk Protein Nomenclature, Classification, and Methodology of Manufacturing Section of A.D.S.A., 1958—59. J. R. Brunner, C. A. Ernstrom, R. A. Hollis, B. L. Larson, R. McL. Whitney and C. A. Zittle (*J. Dairy Sci.*, 1960, **43**, 901—911).—The nomenclature of the constituents of the α-casein fraction is discussed, and recommendations are made concerning that of the β-lactoglobulins. (62 references.) P. S. ARUP.

Studies on casein. III. Preparation of a carbohydrate-rich fraction and a calcium-sensitive fraction from α-casein. M. E. Q. Pilson, G. O. Henneberry and B. E. Baker (*J. Sci. Fd Agric.*, 1960, **11**, 640—644).—An α-casein solution was kept at pH 12 for 45 min. then HCl was added until pH was 7·0. $CaCl_2$ was added and the α-casein was fractionated into fraction A (13·4%) and α-casein — fraction A (56·7% of original α-casein). Sugar and hexosamine contents of fraction A were 3·94 and 4·58 mg./g., respectively, and of α-casein — fraction A 0·68 and 0·68 mg./g., respectively. α-Casein — fraction A, but not fraction A, was precipitated at pH 7·0 by addition of Ca^{2+}. No ppt. was formed on addition of 0·1M-$CaCl_2$ to a solution of α-casein — fraction A (3) + fraction A (1 pt.), but a ppt. was formed if fraction A was pretreated with rennin. (13 references.) E. M. J.

Purification of rennet by column chromatography and paper electrophoresis. R. Schober, N. Heimburger and I. Prinz (*Milchwissenschaft*, 1960, **15**, 506—511).—Rennet (**I**) of varying degrees of purity was fractionated by gradual elution on a carboxymethylcellulose column and the zones obtained were electrophoretically analysed. Crystalline rennin (**II**) decomposes when left in solution and unpurified **I** is separated by chromatography into four zones; the inter-relationship of these is discussed; the main zone is only about half as active as pure **II**. (11 references.) C. V.

Approach to rapid test for antibiotics in milk. H. E. Kennedy and W. J. Harper (*J. Dairy Sci.*, 1960, **43**, 999—1000).—The test, carried out on 0·5-in. filter-paper antibiotic assay discs, depends on the inhibition by Terramycin of the ability of *Streptococcus cremoris* to reduce 2,3,5-triphenyltetrazolium chloride. Reduction is indicated by a pink coloration developed after incubation at 30° during ≮25 min. Reduction is completely inhibited by samples of milk (0·15 ml.) containing 10 μg. of Terramycin per ml. Lower concn. can be estimated by the intensity and area of the colour developed. P. S. ARUP.

Spectrophotometric determination of fluoral and uranine in milk. E. A. Corbin (*J. Dairy Sci.*, 1960, **43**, 920—924).—It is proposed to use the dyes as markers for antibiotic prep, infused into the udder. A quant. aq. extract of uranine for the determination can be prepared by the addition of N-NaOH to the milk at 45—50°, and filtration. For quant. extraction of fluoral, the sample, previously treated with Na tungstate + H_2SO_4 is shaken (mechanically) with several successive amounts of ether. For uranine and fluoral, duplicate results agree within 5 and 8%, and detectable amounts are 1 and 2 μg., respectively. Recovery values for both are 94—103%. P. S. ARUP.

Behaviour of *Lactobacillus acidophilus* in mixed culture with lactic streptococci. B. Mehnert (*Milchwissenschaft*, 1960, **15**, 491—496).—*Lactobacillus acidophilus* (**I**) can be maintained as a pure culture for years without losing the characteristic properties. If grown in a mixed culture with *Streptococcus lactis*, Taette (**II**) and a milk yeast *Geotrichum candidum*, several of the chief characteristics disappear after a few transfers and a re-isolated culture of **I** is unable to split sugars; aberrant forms are also noted and it is difficult to distinguish **I** from *L. helveticus* or *L. bulgaricus*. The original properties of **I** could only be regained by cultivating the original type on solid medium and it is suggested that the variants are related to *Thermobacteria Orla-Jensen* of the genus *L. Beijerinck*. (32 references.) C. V.

Variability in *Lactobacillus acidophilus*. T. Baumgärtel (*Milchwissenschaft*, 1960, **15**, 496—499).—A discussion. (20 references.) C. V.

[A] Non-biological properties of sterilised milk in relation to treatment and temperature of heating. [B] Redox potential of sterilised milk. [C] Alcohol test applied to sterilised milk. C. Higginbottom and M. M. Taylor (*J. Dairy Res.*, 1960, **27**, 235—244, 245—257, 259—265).—[A] Decreases in pH and increases in titratable acidity, browning, yellowing of the filtrate in the Aschaffenburg test, and in the acid ferricyanide-reducing substances (all observed) with increases in sterilising temp. of homogenised milk, are more marked in milk sterilised in bottles with evacuated headspace than in milk sterilised in open bottles. These differences are attributed (in part at least) to the observed slower cooling of the milk under evacuated headspace; in crown-capped bottles, they are dependent on the efficiency of the closure. (13 references.)

[B] Milk sterilised in bottles under reduced pressure shows, for samples taken and measured under N_2, values of -280 to -300 mV; values for milk similarly sterilised in open bottles are $+10$ to -30 mV.

[C] Negative results for the 80% EtOH test are given by sterilised milk sparsely inoculated with *Bacillus subtilis*, *B. licheniformis* or *B. cereus* (and then incubated at 37°) until a few days after the max. population (10^5 per ml.) has been reached. For these bacteria no appreciable differences are observed as between "inhibitory" milk (heated under reduced pressure) and "non-inhibitory" milk (heated in open bottles). With *B. brevis* and *B. circulans*, however, lower max. populations are found in the inhibitory than in the non-inhibitory milk; in such cases, positive tests are obtained for populations $<10^{-5}$ per ml. The EtOH test after 24 h. at 37° is unreliable as an indicator of the keeping quality of sterilised milk at 22°. (10 references.) P. S. ARUP.

γ[-Ray] activity in milk. A. S. Chhabra and R. K. Hukkoo (*J. sci. industr. Res.*, 1960, **19B**, 302—305).—Examination of milk powder samples from different parts of the world showed that activity found was due to the natural ^{40}K content and to ^{137}Cs from fallout. The fallout rate plotted against latitude shows a strong peak in the middle latitudes of the northern hemisphere and lowest values in the polar and equatorial regions. Method of measurement is given. E. M. J.

Strontium in milk. III. Distribution in cream, separated milk, Cheddar cheese and whey. B. J. Demott and R. G. Cragle (*J. Dairy Sci.*, 1960, **43**, 925—930).—Cream (with 40% of fat) from the milk of cows dosed with ^{89}Sr and ^{45}Ca contains ~50% of the isotopes present in the milk. The $^{89}Sr/^{45}Ca$ ratio found in the milk 6 h. after dosing is higher than the constant ratio obtaining for subsequent milkings. Cream can be freed from ^{89}Sr by washing followed by re-separation. The above ratio is slightly higher for the cheese, and slightly lower for the whey than for the original milk. P. S. ARUP.

Fishy flavour in dairy products. I. General studies on fishy butterfat. E. G. Pont, D. A. Forss, E. A. Dunstone and L. F. Gunnis. **II. Volatile compounds associated with fishy flavour in butterfat.** D. A. Forss, E. A. Dunstone and W. Stark (*J. Dairy Res.*, 1960, **27**, 205—209, 211—219).—I. A flavour of fish-oil is developed in dry butterfat containing nordihydroguaiaretic acid (0·005%) and citric acid (0·01%, or the equiv. of lactic acid). Both ingredients (added in propylene glycol or diacetin solution) are essential to the reaction, which is accelerated, but not initiated, by Cu. (11 references.)

II. Gas-chromatographic analysis of the steam-volatile (under reduced pressure) constituents of the fishy-flavoured butterfat (see previous abstract) reveals two fractions as responsible for the flavour, viz., one of oily flavour containing n-hexanal, n-heptanal, hex-2-enal and heptan-2-one, and the other, of metallic flavour containing (in relatively small amount) an unidentified single-carbonyl compound. In addition to these, 17 other compounds have been identified by other chromatographic methods applied to their dinitrophenylhydrazones. The bearings of these findings on the flavour problem are discussed. (20 references.) P. S. ARUP.

Application of vapour phase chromatography to the control of the purity of butter. J. P. Wolff (*Ann. Falsif., Paris*, 1960, **53**, 318—323).—A vapour phase chromatographic method using a column of brick impregnated with polyglycol adipate, and He as the entraining gas, to separate the C_8—C_{20} acid components of palm kernel, groundnut and coconut oils and of butter, is described. Prior to analysis, the methyl esters of the fatty bodies are formed by acid or alkaline interesterification and these esters are injected on to the column. It is suggested that the method be used to replace the Reichert–Meissl method to assess the purity of butter or to determine the

relative proportion of coconut and palm kernel oils in a mixture. (15 references.) J. V. Russo.

Biochemistry of cheese. G. M. Moir (*J. N.Z. Inst. Chem.*, 1960, **24**, 93—105).—A discourse dealing with casein, rennet, manufacturing process, vat operations, starter bacteria, bacterial development, counting bacteria, chemical changes in the vat, gas production, flavour and defects is presented. A special study of tallowy discoloration, also called white streaks, is outlined. The Cu content was lower and the Fe content higher in the defective than in the normal portions. The distribution of metals, especially Fe, can be altered during salting operations. Evidence was that tallowy discoloration in cheese is similar to oxidative changes occurring in food and other natural products. Such changes are accelerated by haematin systems. Cheese made from milk with added small amount of cow's blood developed cracks and the typically tallowy flavour. During salting the blood cells are partially haemolysed and during pressing, the brine solution of haematin gathers in lenticular pockets in the cheese. Rough handling causes these to link with cracks in the rind through which O_2 enters. The conc. haematin with the higher Fe content stimulates the oxidation of the carotene and the butter fat and so causes the tallowy discoloration. I. Dickinson.

Creaming of cottage cheese. D. E. Emmons and Walter V. Price (*J. Dairy Sci.*, 1960, **43**, 931—944).—A standardised test is described in which the unabsorbed dressing (milk and cream containing 12—18% of fat) in creamed cottage cheese in cartons is measured by drainage from funnels fitted with wire mesh discs. Increases in retention of dressing and (accompanying) decreases in curd firmness are promoted by increasing the holding time (up to 24 h.), the pH (above 5·2), the fat % in the finished cheese, and the NaCl (up to 2%), and by the addition of stabilisers. The probable mechanisms involved in the process are discussed. P. S. Arup.

Analysis of volatile food flavours by gas–liquid chromatography. I. The volatile components from dry Blue cheese and dry Romano cheese. J. R. Coffman, D. E. Smith and J. S. Andrews (*Food Res.*, 1960, **25**, 663—669).—Of 29 neutral substances isolated from Blue cheese, the major components were: heptanone-2, nonanone-2, undecanone-2, an unidentified mixed ketone, heptanol-2 and nonanol-2. Of the acids, chromatographed as Me esters, (26 peaks) 10 were predominant in quantity, the major acids having 4, 5, 6, 7, 8 and 10 C. The major components in volatiles from Romano cheese were straight-chain acids of 4, 6, 8 and 10 C. (16 references.) E. M. J.

Irradiation of eggs and egg products. J. Brooks, R. S. Hannan and B. C. Hobbs (*Int. J. appl. Radiation and Isotopes*, 1959, **6**, 149—154).—Small-scale treatment of salmonella-infected frozen egg in 10-kg. tins with ^{60}Co γ-rays at dose-levels of 0·3, 0·4 and 0·5 Mrad is reported. No salmonellae were detected after irradiation and the baking qualities of the material were unimpaired. The avoidance of handling or thawing of the product would make the process attractive if its effectiveness is confirmed. Other aspects of the irradiation of eggs and egg-products are also shortly discussed. (23 references.) E. C. Apling.

Edible Oils and Fats

Effect of heat treatment on chemical properties of animal and vegetable fats; study of their physiological properties. J. Wurziger and H. Ostertag (*Fette Seif. Anstrichm.* 1960, **62**, 895—903).—Changes in the acid, peroxide, epoxide and I_2 values, % aldehyde and fatty acid contents (arachidonic, linolenic and linoleic acids) of soya-bean oil, lard, and hydrogenated lard and vegetable oils, have been determined after heating the oils at 180° for 12 h. in presence or absence of O_2. The physiological changes in body fats obtained from rats following oral and subcutaneous administration have also been studied. G. R. Whalley.

Activity of phospholipases in dried food and model mixtures. L. Acker (*Fette Seif. Anstrichm.*, 1960, **62**, 906—910).—The activity of phospholipases in foodstuffs is briefly reviewed. The decomposition of lecithin by phospholipase B and D in pearl malt barley at 30° is dependent upon the water content of the latter; the way such water is bound also influences the enzymic activity. The activities of the B and D phospholipases have been examined in model mixtures. (17 references.) G. R. Whalley.

Chemical changes produced in lipids by irradiation. B. Coleby (*Int. J. appl. Radiation and Isotopes*, 1959, **6**, 71—75).—A review of recent studies of the effects of irradiation on lipids, including the effects of removal of O_2 of antioxidants and of temp. during irradiation and subsequent storage. (18 references.) E. C. Apling.

Margarine. T. Hedley & Co. Ltd. (Inventors: G. H. Scott and C. M. Colquhoun) (B.P. 826,554, 5.12.56).—A ripened milk of low bacteria count, suitable for use in the manufacture of margarine, is produced by inoculating milk with a desired culture, keeping the inoculated milk under suitable temp. conditions to effect development of desired flavour, then (after optional addition of 10—12·5 wt.-% of NaCl) heating the ripened milk, e.g., at 120—125°F for 4—6 min. (at pH 4·2—4·6) to destroy substantially all of the culture bacteria present (final count $\not>$20,000 bacteria per g.). F. R. Basford.

[A] **Margarine.** [B] **Foodstuffs containing oil and/or fat.** Rootry Exploitatie Maats. N.V. (B.P. 826,226, 826,940, 16.11.55. Neth., 16.11.54).—Addition to [A] the oil and/or fat used in the prep. of margarine, [B] oils or fats used in baking, shortening, salad dressing, etc. (other than margarine), of a polysaccharide ester ($\not>$10%) of an $\not<$8 C acid (e.g., amylose palmitate) stabilises the viscosity so that the product is less sensitive to changes of temp. F. R. Basford.

Meat and Poultry

Committee on slaughtering methods. (*Acta Polyt. scand.*, 1959, No. 7, 35 pp.).—The effects of electric stunning on the quality of pork and whether this method of slaughter could cause haemorrhages in ham and, in general, in the more perishable meat were studied. Deleterious punctate haemorrhages, e.g., in ham can be avoided if the time interval between the interruption of current flow and sticking is reduced to ~5 sec. No differences were found organoleptically and chemically in the meat from stunned and unstunned hogs and the pH in macerate of *gracilis* muscle from hams was the same. E. M. J.

Chilling, freezing and prepackaging of beef. A. Howard (*Food Pres. Quart.*, 1960, **20**, 2—8).—Problems associated with the production of unprocessed meat, control of changes in the carcass, packaging, shipping, e.g., from Australia to Britain are reviewed. Attention is drawn to the economic advantage of the prepacked frozen cut exported in cartons to U.S.A. E. M. J.

Changes in hydration and charges of muscle proteins, during freeze-dehydration of meat. R. Hamm and F. E. Deatherage (*Food Res.*, 1960, **25**, 573—585).—Freeze-drying of beef results in a drop of water-holding capacity only in the isoelectric pH range of muscle, causing a tighter network of protein structure. This may be stabilised by the formation of new salt and/or H bonds. Freeze-drying causes an increase of acidic groups of proteins in the basic range of the isoelectric point. This reaction is not identical with the changes occurring during heat denaturation of muscle. (20 references.) E. M. J.

Changes in hydration, solubility and charges of muscle proteins during heating of meat. R. Hamm and F. E. Deatherage (*Food Res.*, 1960, **25**, 587—610).—Heating of beef from 20 to 30° results in very little change in muscle proteins; from 30 to 40° mild denaturation occurs, with unfolding of protein chains and formation of new salt and/or H bonds; from 40 to 50° strong denaturation occurs with formation of new stable cross-linkages; the amount of negatively charged groups of the muscle proteins decreases with increasing temp.; from 50 to 55° denaturation continues and >55° the negatively charged groups decrease again. At 65° it is nearly complete. The stepwise change of water-holding capacity of meat and of its pH by heating at its normal pH is determined by stepwise decrease of the acidic groups in muscle proteins. Heat denaturation does not cause a significant decrease in the amount of basic groups in muscle proteins. (69 references.) E. M. J.

Influence of freezing and thawing on hydration and charges of the muscle proteins. F. E. Deatherage and R. Hamm (*Food Res.*, 1960, **25**, 623—629).—Quick freezing (−55°) of ground and cut meat results in small but significant increase of water-holding capacity, but no denaturation of the muscle proteins. A mechanical loosening of tissue structure is probably caused by formation of tiny ice crystals within the cells. Slow freezing (−15°) of meat decreases the water-holding capacity probably caused by destruction of protein structure by formation of large ice crystals between the cells. (24 references.) E. M. J.

Storage trial on New Zealand beef and mutton. A. R. Prater, N. W. Vere-Jones and E. A. Roberts (*Commonw. sci. industr. Res. Org., Aust., Div. Fd Pres. Transp.*, 1960, tech. Paper No. 19, 12 pp). —Frozen beef pieces approx. 3 × 3 × 0·5 in. and mutton mince were vac. dried in fat. The effects of cut, temp., length of storage and gas packing were studied. Dried prime cuts of beef were slightly inferior in quality to those of poorer cuts; the prepared material had an off-flavour, was slightly dry, slightly tough, woolly in texture, dark in colour and not easily reconstituted. Shelf life was poor. The dried mutton was acceptable initially in quality, but had a limited shelf life. E. M. J.

Comparative heating efficiencies of a microwave and a conventional electric oven. G. A. Pollak and L. C. Foin (*Food Technol*, 1960, **14**, 454—457).—In heating water, the overall electrical efficiency was 34% for microwave (**I**) and conventional (**II**) ovens; that of a conventional hot plate was 63%. In roasting beef (8 lb.) the values were 36·7% for **II** and 33·4% for **I**. The product prepared in **II** yielded 13% more of edible meat using 24% less electrical energy; 74 B.Th.U. per oz. of yield for **II** as compared with 119 B.Th.U. per oz. of yield for **I**. The difference in energy was attributed to the energy required to evaporate a greater amount of water in microwave cooking. E. M. J.

Fat content in liver sausage. R. Grau (*Fleischwirtschaft*, 1960, **12**, 802, 805).—506 samples were examined; a 40% fat concentration was considered reasonable but 45—50% provided medium to top grade quality. Those giving >55% were considered unacceptable and this value was generally due to lack of homogeneity. C. V.

Modified Teichert method for the estimation of fat in meat. W. V. Falkenhahn (*N.Z. J. Sci.*, 1960, **3**, 333—337).—A rapid butyrometric method for the determination of fat in meat products is described. A finely minced sample is digested with H_2SO_4 in a hot water bath and the vol. of separated fat is measured. The results agree well with those obtained by gravimetric methods (Schmid–Bondzynski–Ratzlaff and Soxhlet). (11 references.) J. V. Russo.

Comparison of rate of cooking and doneness of fresh-unfrozen and frozen defrosted turkey hens. G. E. Goertz, A. S. Hooper and D. L. Harrison (*Food Technol.*, 1960, **14**, 458—462).—The rate of cooking and doneness of fresh chilled (**I**) and frozen defrosted birds (**II**) with similar initial internal temp. and oven temp. were studied. Total cooking time was longer ($P < 0.05$) for birds roasted to 95° in the thigh than for those roasted to 90° in the breast. Total cooking time was shorter and volatile losses lower for **I** than **II**. Doneness scores for light and dark meat were higher for turkeys stored for 1 month than for those of other turkeys. E. M. J.

Effects of irradiation on quality of meat and poultry. B. Coleby (*Int. J. appl. Radiation and Isotopes*, 1959, **6**, 115—121).—A review of the results of storage and tasting trials. Radiation sterilisation of meats usually produces unacceptable changes in colour, odour and flavour. Radiation pasteurisation may be of value, but advantages over traditional storage methods are likely to be marginal. (12 references.) E. C. Apling.

Changes in spoilage pattern of chicken meat as a result of irradiation. M. Ingram and M. J. Thornley (*Int. J. appl. Radiation and Isotopes*, 1959, **6**, 122—128).—Studies of the storage micro-flora of anaerobically-canned chicken meat pasteurised with 0·25 Mrad of ^{60}Co γ-rays and stored at 5°, and of whole eviscerated chickens dosed with 0·8 Mrad and stored at from 1 to 3° in loose film bags, are reported. In both cases spoilage was delayed for several weeks. The spoilage flora on the whole birds consisted entirely of normal non-pathogenic meat spoilage organisms, but the canned meat also showed prevalent faecal streptococci. E. C. Apling.

Treatment of meats with ionising radiations. IV. Comparison of the deterioration in quality during storage of eviscerated chicken carcasses treated with chlortetracycline or radiation. B. Coleby, M. Ingram, H. J. Shepherd and M. J. Thornley (*J. Sci. Fd Agric.*, 1960, **11**, 678—684; cf. J.S.F.A. Abstr., 1960, i, 258).—The progressive decline in quality of carcasses treated with chlortetracycline (CTC) or irradiated with 0·3 and 0·6 Mrad when stored up to 26 days at 0° was observed. Microbial counts were made at intervals. With 0·6 Mrad the carcasses deteriorated most rapidly being inferior after storage for 9 days; with 0·3 Mrad or CTC quality remained high, but by the 20th and 26th day of storage these were significantly inferior to frozen control carcasses. The deterioration of the irradiated carcasses was not due to microbiological spoilage. (15 references.) E. M. J.

Strained meat product. General Products Co. (B.P. 826,250, 2.7.56).—A cooked, strained meat product (for use as infants food) in which clumping is minimised, is produced by mixing water with comminuted meat; straining the resulting slurry; partly coagulating the protein content of the slurry (prior to canning) by means of heat; then cooking the resulting product in containers. F. R. Basford.

Fish

Phosphorus-containing fractions of sterile lingcod muscle during storage at 0°. N. Tomlinson, V. M. Creelman and K. G. Reid (*J. Fish. Res. Bd Can.*, 1960, **17**, 371—376).—In samples of sterile lingcod muscle stored at 0° for periods up to 3 weeks, no significant change occurred in the phospholipid, ribonucleic acid and deoxyribonucleic acid fractions. In the acid sol. fraction, inorg. P increased from 75% to approx. 96% of the total P. The P-containing fractions from the fish held in ice for the same period did not differ appreciably from those of the sterile muscle. An aq. extract of lingcod muscle formed orthophosphate from a variety of phosphorylated compounds. (18 references.) S. G. Ayerst.

Phospholipid hydrolysis in cod flesh stored at various temperatures. J. Olley and J. A. Lovern (*J. Sci. Fd Agric.*, 1960, **11**, 644—652).—Phospholipid hydrolysis in cod flesh stored at temp. ranging from +20 to −29° is due to tissue enzymes, non-enzymic reactions and bacteria present on iced cod playing negligible rôles. Freezing activates the system, eliminating the initial lag at 0° and causing nearly as rapid hydrolysis at −14 as at 0°. At all temp. studied the products accumulating from phospholipid degradation are free fatty acids and water-sol. P deriv. Phospholipid hydrolysis and protein denaturation follow a similar course in frozen fish. (28 references.) E. M. J.

Chemical composition of raw, precooked and canned tuna. I. Core sampling methods. C. J. Carlson, C. E. Thurston and M. E. Stansby (*Food Technol.*, 1960, **14**, 477—479).—Small size fish were chosen, 7—12 lb. in wt., with an average of ~9 lb. The use of four ¾-in. cores (taken according to described plan) per fish gave homogeneous results with respect to protein and moisture contents, but this method of sampling is not recommended for determining rancidity by the thiobarbituric acid test. E. M. J.

Inosine in the muscle of Pacific salmon stored in ice. V. M. Creelman and N. Tomlinson (*J. Fish. Res. Bd Can.* 1960, **17**, 449—451).—Investigation of the inosine, hypoxanthine and ribose content of the muscle of several spp. of Pacific salmon showed that after the fish had been stored in ice for 6—10 days, the major portion of the ribonucleotides were only degraded to inosine. S. G. Ayerst.

Processing and quality studies of shrimp held in refrigerated sea water and ice. II. Comparison of objective methods for quality evaluation of raw shrimp. J. Collins, H. Seagran and J. Iverson (*Comm. Fish. Rev.*, 1960, **22**, No. 4, 1—5).—The following tests were suitable: (i) trimethylamine and volatile acids as indices of bacterial spoilage; (ii) amino-N, non-protein N and total N as indices of enzymic action; and (iii) total solids and total chloride for general characterisation. E. M. J.

Physico-chemical properties of the enzymes involved in shrimp melanogenesis. M. E. Bailey, E. A. Fieger and A. F. Novak (*Food Res.*, 1960, **25**, 557—564).—Data presented describe properties of the enzyme(s); they are generally similar to those for certain phenolases. Shrimp pyrocatecholase (**I**) is a zymogen which can be activated by heating under controlled conditions. The inactivation rate at 35° is first order and the half life of this enzyme is ~1h. under these conditions. Michaelis constants were determined for **I** by manometric and colorimetric methods and for 3,4-dihydroxyphenyl alanine (dopa) oxidase activity by colorimetric methods. Cu^{2+} ions are essential for the activity of **I**, a characteristic of the phenolase group. (22 references.) E. M. J.

Phenol oxidase in shrimp and crab. M. E. Bailey, E. A. Fieger and A. F. Novak (*Food Res.*, 1960, **25**, 565—572).—Phenolase(s) were partially purified from shrimp head press juice and blood. Shrimp phenolase (**I**) catalyses the oxidation of mono- and *o*-dihydric phenols, is present in the exoskeleton and adhering epicuticle, in antennae and blood. **I** from blood is the most active. A protein localised in blood leucocytes is stereospecific for the L-isomer of 3,4-dihydroxyphenylalanine (dopa). Cryst. haemocyanin from the crab catalyses the oxidation of pyrocatechol. Free L-tyrosine occurred in all shrimp samples tested as a substrate for melanin formation in shrimp, identified as dopa melanin. Substances which combine with Cu, e.g., carboxy acids, reducing and phenol-sequestering agents inhibit dopa and pyrocatechol oxidation. Usefulness of ascorbic acid, and sulphites in retarding shrimp melanogenesis was substantiated. (14 references.) E. M. J.

Control of iron sulphide discoloration in canned shrimp (*Xiphopeneus sp.*). I. M. H. Thompson and M. E. Waters (*Comm. Fish. Rev.*, 1960, **22**, No. 8, 1—7).—When the pH of the contents of the can was decreased to <6·6 by addition of a lemon juice concentrate or citric acid solution, only slight discoloration of the cans occurred after storage of approx. 1 year. Best pack contained 3·6 g. of salt, ≯0·52 g. per can of citric acid. A 1 : 40 concn. of lemon juice concentrate No. 309 did not appreciably affect the flavour of the shrimp. The lemon juice was more effective in preventing blackening than was the citric acid. E. M. J.

Shrimp-waste meal: effect of storage variables on pigment content. J. E. Rousseau, jun. (*Comm. Fish. Rev.*, 1960, **22**, No. 4, 6—10).—Shrimp-waste meal containing astaxanthin, a carotonoid pigment, is used as a supplement to the diet of hatchery-raised trout to give desirable colour. A method of analysis of the pigment was

studied and evidence was that it is mainly esterified astaxanthin. Santoquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline), but not BHT (2,6-di-t-butyl-4-hydroxytoluene), was effective in decreasing rate of pigment destruction and development of oxidative rancidity. E. M. J.

Detection and identification of tritolyl phosphate, smoke and spice compounds in oil-containing foodstuffs. J. Wurziger, F. Günther and U. Chandra (*Dtsch. Lebensmitt Rdsch.*, 1960, **56**, 224—227).—The detection of tritolyl phosphate by the indophenol blue reaction is described. Under the same conditions certain substances from smoke and spices also react and a paper chromatographic procedure is described for their distinction. E. C. APLING.

Construction and operation of an inexpensive fish smokehouse. M. E. Waters and D. J. Bond (*Comm. Fish. Rev.*, 1960, **22**, No. 8, 8—12).—An inexpensive fish smokehouse capable of producing 150 lb. (~240 mullet) per operation is described comprising a 4 × 4 × 4 ft. smoker + steel fire box and permitting a fairly accurate control of smoke temp. E. M. J.

Spices, Flavours, etc.

Recent progress in consideration of flavouring ingredients under the Food Additives Amendment. R. L. Hall (*Food Technol.*, 1960, **14**, 488—495).—A review. Substances (some 1300) are listed as (i) no further action (NFA), (ii) food, (iii) generally recognised as safe (GRAS), (iv) food additive. E. M. J.

Degradation of vanillin, ethyl vanillin and *p*-hydroxybenzaldehyde by auto-oxidation. S. Stoll and Y. Prat (*Ann. Falsif., Paris*, 1960, **53**, 316—317).—A paper chromatographic method for separating the acidic degradation products from vanilla beans, natural extracts and products with added vanillin is described and it is suggested that it may be utilised for indicating spoilage. J. V. RUSSO.

Colouring matters

Food colours: their status under the [U.S.A.] law. A. T. Schramm (*Food Technol.*, 1960, **14**, 503—505). E. M. J.

Preservatives

Biological screening techniques for food additives. M. Ives and E. M. Nagler (*Food Technol.*, 1960, **14**, 499—502).—Screening techniques to determine whether the direct food additives, or migrants of unknown biological activity may be toxic to animals are described. E. M. J.

Antibiotic preservation of beef with subsequent feeding to experimental animals. R. B. Sleeth, J. C. Armstrong, H. S. Goldberg and H. D. Naumann (*Food Technol.*, 1960, **14**, 505—507).—Raw and cooked meat containing low levels of oxytetracycline when fed over a period of 16 weeks to rats (2·4—2·8; or 0·8—1·0 μg./g./day) caused no detectable residue in blood serum, heart, kidney or muscle. No significant changes occurred in the gut flora. On this basis, potential health hazard appears to be small. E. M. J.

Food Processing, Refrigeration

Characteristics required of vegetables for processing. J. Shipton (*Food Pres. Quart.*, 1960, **20**, 13—16).—The development of vegetable marketing is reviewed. General requirements in the harvesting and processing of raw materials and specific requirements, e.g., dehydration are discussed in connexion with tomatoes, green peas, beans, onions, cauliflower and potatoes. E. M. J.

Use of calcium hydroxide for firming canned green and red sweet bell pepper. M. W. Hoover (*Food Technol.*, 1960, **14**, 437—440).—Of Ca compounds tested $Ca(OH)_2$ was the most effective firming agent. Best results were obtained with cubed pepper when lime (0·2—0·4% by wt.) was applied for ~30 min. by soaking or dipping in lime water or direct application of the dry material. The lime residue was removed by rinsing prior to blanching and heat processing. Green pepper responded better than red pepper to firming treatments. E. M. J.

Drying of seaweeds and other plants. III. Through-circulation drying of *Zostera marina*. J. H. Merritt (*J. Sci. Fd Agric.*, 1960, **11**, 629—632).—Pilot-plant tests on through-circulation were conducted with a batch dryer. A max. feasible load for *Z. marina* (grasswrack) was ~3·8 lb./sq. ft./layer in four layers. The effects of variations in rate of air flow, air-flow reversal and temp. (160—200°F) were studied in relation to output and efficiency. Best results were obtained by recirculating air towards the end of a run and for even drying a reversal of air flow about half-way through the run. E. M. J.

Modified technique for production of dehydrated meat. R. Grau and A. Schultheiss (*Fleischwirtschaft*, 1960, **12**, 827—828, 831—832).—Fresh or processed meat dried in vac. at >36° over dehydrating material, e.g., silica gel, is suitable for most purposes. Rehydration is more rapid and effective than with pre-cooked dehydrated meat, and storage in vac., N_2 or in double sealed films is satisfactory. C. V.

Effects of partial pumping on the shrinkage, rancidity development, and palatability of aged hams. G. C. Skelley, jun., J. D. Kemp and W. Y. Varney (*Food Technol.*, 1960, **14**, 446—448).—Hams were (*a*) dry-cured or (*b*) partially pumped with an 85° pickle made from the dry-cure mixture 4, 6, 8, 10% respectively, followed by rubbing with dry-cure mixture, equal total curing ingredients being used. All the hams were cured for 21 days at 36—40°F, smoked at 100°F, then aged for 6 months at 65°F and R.H. of 55%. Shrinkage increased especially during the first month of ageing. Pumping reduced shrinkage. Peroxide no. and free fatty acid content increased. Salt content was higher in pumped hams, with a general increase as the pumping rate increased. Dry-cured hams were sounder, had a more desirable colour, and were scored significantly higher for saltiness, flavour and over-all satisfaction. Hams pumped 4% were similar in quality. E. M. J.

Preparation of fish for canning. W. A. Empey (*Food Pres. Quart.*, 1960, **20**, 8—12).—Selection, handling and storage and pretreatment to improve colour, flavour, texture of the canned product are reviewed. E. M. J.

Application of infra-red in food processing. E. A. Asselbergs, W. P. Mohr and J. G. Kemp (*Food Technol.*, 1960, **14**, 449—453).—Data are presented to show how the depth of heat penetration was influenced by wavelength characteristics, voltage input and energy output of the radiator (three types). General quality of i.r.-blanched samples (celery, apples, peas and prep. of french-fried potatoes) was better than that of samples blanched by steam or boiling water methods. Colour, texture and flavour of i.r.-braised, canned meat was considered superior to that, e.g., of beef which had been parboiled in preliminary treatment in the prep of beef stew. (17 references.) E. M. J.

Combination processes. M. Ingram (*Int. J. appl. Radiation and Isotopes*, 1959, **6**, 105—109).—Possible applications of irradiation combined with refrigeration, vac.-packing, antibiotic additions, curing or heating in the storage of foods are reviewed. A combination of heating and radiation is particularly interesting from the point of view of improvements in food processing, but dangers arising from an altered pattern of microbiological spoilage need careful study. (21 references.) E. C. APLING.

Preservation of animal and vegetable tissues. Rhein-Chemie G.m.b.H. (Inventor: R. Kern) (B.P. 826,477, 13.4.56).—In the preservation of animal or vegetable tissues by drying, the material is first cooled to −35° to −70°, then rapidly heated to +25°, after which removal of water (at −15° to + 5°/0·1—2 mm.) is immediately commenced. The biological activity of material (e.g., foetal liver, testis of young animal) treated thus is increased. F. R. BASFORD.

Packaging

Preservation of fruit in a controlled atmosphere within enclosures bounded by plastic film. C. Leblond (*Fruits d'outre mer*, 1960, **15**, 307—316).—Large containers, with walls of plastic sheeting, hold several standard boxes of fruit, and are easily and cheaply made. The atm. within the containers is controlled by the selective diffusion of gases and other substances through the plastic. The type of plastic used can be varied, according to the respiratory quotient of the fruit to be stored. William pears and Golden Delicious apples were stored in these containers at 7°, 12° and 20° for varying lengths of time, and the resulting effects on colouring, loss of wt., firmness of flesh, taste and smell, and diseases, are discussed. S. G. AYERST.

Problems relating to the extractability of food container components. L. E. Schniepp, H. O. Etian and J. M. Jackson (*Food Technol.*, 1960, **14**, 496—498).—Org. chemical ingredients now in use in manufacture of food containers either do not contribute additives or the use is in compliance with established regulations. Problems associated with food products containing appreciable quantities of fats and oils, with regard to migration of components of containers material are discussed. E. M. J.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Residues of aldrin and dieldrin on carrots and their influence on the biological value. W. Schuphan (*Z. PflKrankh.*, 1960, **67**, 340—

351).—Data covering three years showed that residues of aldrin and dieldrin on carrots varied between 0·05 and >2·0 p.p.m., in some cases causing liver trouble in babies. No correlation was apparent between the total or protein-N, mono- or di-saccharide, total acid, ascorbic acid or carotene contents of the roots and the nature of the treatment with chlorinated insecticides. A. G. POLLARD.

Utilisation of synthetic chelates in a study of calcium metabolism. M. Rubin, R. Alexander and G. Lindenblad (*Ann. N.Y. Acad. Sci.*, 1960, **88,** 474—478).—A brief review. (21 references.) C. V.

Treatment of proteins to render them resistant to putrefaction. Borden Co. (B.P. 826,326, 8.1.58. U.S., 2.7.57).—Normally putrefiable protein (e.g., casein, soya-bean or groundnut protein) containing metal (viz., Fe and/or Cu impurities) is rendered resistant to deterioration (due mainly to the metal impurity) by keeping in presence of a source of halogen, especially Cl, e.g., HCl, H_2O_2, a chelating agent for the metal and water (enough to dissolve the halogen source and moisten the protein, e.g., 2—70 pt.) at >0° until reaction between the various components is complete. The preferred chelating agent is an alkali metal polyphosphate, but citric acid and polyaminetetra-acetates, e.g., the Na_2 salt of EDTA, are also effective. F. R. BASFORD.

Mixtures of amino-acids. K. A. J. Wretlind (B.P. 825,193, 4.9.57. Sw. 10.9.56).—An amino-acid mixture, suitable for use as nutrient material (for therapeutic purposes) is economically obtained by subjecting protein (e.g., casein) to enzymic hydrolysis, then (optionally after concentration) heating the product at >30(75—80)° to transform at least part of the liberated glutamic acid and its derivatives into pyrrolidonecarboxylic acid (**I**), and purifying the mixture by dialysis. Preferably the final product contains ≮1·5—2(4—8)% of **I**. F. R. BASFORD.

Unclassified

Polarographic studies on the concentration of oxygen in broth and oxygen uptake rate of mycelium in submerged fermentation of *Penicillium chrysogenum*. R. S. Gondhalekar and R. S. Phadke (*J. sci. industr. Res.*, 1960, **19C,** 183—186).—Different strains derived from *P. chrysogenum* Wis 51—20 and from the Russian strain were used. O_2 levels in fermentations of strains producing pellety mycelium were lower than in those giving filamentous mycelium. The polarographic residual currents of the broth filtrates were high in fermentations with low yields. Storage of the broth at 5° lowered the mycelial wt. and O_2-uptake rates. E. M. J.

Value of pulses as supplements to media in the production of streptomycin. S. Narayanan and V. Iyer (*J. sci. industr. Res.*, 1960, **19C,** 187—190).—Increased streptomycin yields were obtained when powdered indigenous pulses (0·5%) were added to cultures of *Streptomyces griseus*. Ungerminated pulse powders and hydrolysates give better results than prep. from germinated pulses. (13 references.) E. M. J.

Thermal resistance of micro-organisms to dry heat: design of apparatus, operational problems and preliminary results. I. J. Pflug (*Food Technol.*, 1960, **14,** 483—487).—An apparatus is described. The resistance of 5230 (similar to *Bacillus subtilis*) under controlled conditions of temp. (300—380°F ± 1°F) and time (±0·1 min.) was studied. The lag correction factor of the sample cups was evaluated as a function of gas flow for air and superheated steam. The dry-heat resistance of organism 5230 spores to superheated steam was $LD50_{350}$ of 2·4 min. and a z of 42°F. (11 references.) E. M. J.

Canned chocolate nut roll experimentally inoculated with spores of *Clostridium botulinum* types A and B. R. O. Wagenaar and G. M. Dack (*Food Res.*, 1960, **25,** 646—653).—Chocolate nut roll (24 lots) was prepared (212°F for 3 h.), stored at 90°F, cycled weekly for 16 h. at 40°F for intervals up to 12 months. In the first series (lots 1—12) toxin was found in approx. ⅓ of the inoculated samples. Most of the positive samples were from groups having moisture levels >42%. All cans of chocolate nut roll of the composition usable for rations (27% of sucrose; 29·41—29·95% of moisture) were negative for botulinum toxin at all analyses up to and including 12 months of storage at 90°F. In the second series, after storage for 2 weeks, 1, 3, 6 and 12 months at initial pH 5·8—6·0, moisture levels ≯39·75% were limiting to growth and toxin production of *C. botulinum* at all sucrose levels tested. E. M. J.

3.—SANITATION

Control of corrosion produced by cleaning and sterilising solutions. G. Wildbrett and F. Kiermeier (*Milchwissenschaft*, 1960, **15,** 511—519).—The occurrence of corrosion due to these causes is discussed. (14 references.) C. V.

Eradication of *Glossina morsitans submorsitans* and *G. tachinoides* in part of a river flood plain in Northern Nigeria by chemical means. II. W. W. Kirkby and P. Blasdale (*J. econ. Ent.*, 1960, **51,** 253—264).—The flies were eliminated by a single application of DDT (3·75% aq. suspension) to limbs of larger trees in shade up to 5 ft. from the ground during the dry season. A. G. POLLARD.

Effect of perfumery chemicals on the insecticidal efficiency of pyrethrum. J. Pickthall, F. G. S. Whitfield and A. H. Baker (*Pyrethrum Post*, 1960, **5,** No. 4, 16—21).—Of 13 perfumery compounds (**I**) used in insect control formulations containing pyrethrins, only phenylacetaldehyde had a slightly detrimental effect on pyrethrin potency. Each of **I** was used at a rate of 0·5% whereas in a formulation each is unlikely to occur in amount >0·05%. A fall in potency would be too slight to be detected in normal use of the insecticidal mixture in pest control practice. E. M. J.

Incidence of *Ascaris* ova in Pretoria sludge and their reduction by storage (maturation) in large heaps. H. M. Murray (*J. Inst. Sew. Purif.*, 1960, 337—341).—Storage or maturation of dried digested sludge with moisture contents of 45—60% in large heaps for ≮2 months can reduce high counts of viable *Ascaris* ova by as much as 100%. Inactivation is not always complete because of non-uniformity of heat distribution; covering the heap with, e.g., matured sludge is valuable in this respect. N loss during maturation is estimated at ~⅓ from limited tests; contents of P, K and Na are unaffected. O. M. WHITTON.

Survival of eggs of *Taenia saginata* (human beef tapeworm) after mesophilic anaerobic digestion. P. H. Silverman and K. Guiver (*J. Inst. Sew. Purif.*, 1960, 345—347).—Experimental investigations suggest that storage for 20 days at 35° or brief retention (1—5 days) under mesophilic anaerobic conditions is sufficient to inactivate tapeworm eggs. O. M. WHITTON.

Cleaning compositions. W. Pearson Ltd. (Inventors: M. A. Phillips, C. A. Pearson and M. L. Bird) (B.P. 825,960, 17.1. and 2.5.57).—The composition, useful for removal of grease from cooking ovens, is in the form of a stick solid at room temp., and contains Na stearate 5—15, tetrahydrofurfuryl alcohol 5—15, propylene glycol 5—15, and NaOH 5—12 wt.-%, with remainder water. J. M. JACOBS.

Fly repellant. L. Ratner (B.P. 823,255, 16.7.56. U.S., 12.3.56).—A synergistic fly repellant composition comprises at least one Pr_2 or Bu_2 ester of a dicarboxylic acid of ≯5 C (e.g., maleic, fumaric or succinic acid) (≮2%), a synergist, viz., piperonyl butoxide, octyl sulphoxide of isosafrole, 2-ethylhexylimide of *endo*methylenetetrahydrophthalic acid, butoxypolypropylene glycol, or an unsaturated fatty acid or ester, and a solvent carrier (e.g., oil of mol. wt. 254—349). F. R. BASFORD.

4.—APPARATUS AND UNCLASSIFIED

Estimation of sulphate in plant materials and blood. K. G. Hogan and J. N. Breen (*N.Z. J. agric. Res.*, 1960, **3,** 498—502).—A modified reducing mixture for use in the methylene blue colorimetric method of Johnson and Nishita (*Analyt. Chem.*, 1952, **24,** 736) and a suitable apparatus for use in the routine application of the method are described. The method is applicable to a wide range of biological materials and is sufficiently sensitive to determine from 1 to 300 μg. of sulphate S in 10 to 20 mg. of dried pasture or in 1 ml. of blood. (12 references.) E. C. APLING.

Direct-reading spectrometric determination of zinc, copper and lead in plant material. A. Strasheim and D. J. Eve (*Appl. Spectrosc.*, 1960, **14,** 97—100).—Samples are wet-ashed and the Zn, Cu and Pb are extracted in a $CHCl_3$ solution of dithizone. The solution is sprayed on to a rotating-disk electrode and excited by an interrupted d.c. arc, and the relevant spectral lines are recorded. Repeat analyses on a single sample gave coeff. of variation of 5·1 for Zn, 7·3 for Cu and 6·1 for Pb. Limits of detection were <1 Zn, <0·2 Cu and <0·1 p.p.m. Pb. P. T. BEALE.

Colorimetric determination of phosphoric acid in plant substances by vanadium-molybdenum method. M. Koter and H. Panak (*Chem. anal., Warsaw*, 1960, **5,** 317—324).—A colorimetric method using NH_4 metavanadate and molybdate is given. Addition of 2 c.c. of 60% perchloric acid had no influence on the results. With addition of more acid the error of analysis was greater, especially if absorption was measured after prolonged standing of prepared solution. Results were consistent with those of the gravimetric Lorenz method on 40 plant samples. Mineralisation of samples with a mixture of HNO_3 and $HClO_4$ is also discussed and a method of P_2O_5 determination in prepared samples is also given. L. SMAKOWSKI.

Journal of Applied Chemistry

The following papers are appearing in the February, 1961, issue

Formation of lead telluride films by vapour plating
By E. H. Cornish

Liquid–liquid equilibrium in inorganic nitrate–water–organic solvent systems
By W. J. McManamey

The dissolution of metals in dioxan–water solutions of hydrochloric acid
By M. Allbutt and G. Tolley

Fungitoxicity of rhodanine derivatives
By F. J. Allan, G. G. Allan and G. Crank

Improved preparation of phthalazine and quinazoline
By W. L. F. Armarego

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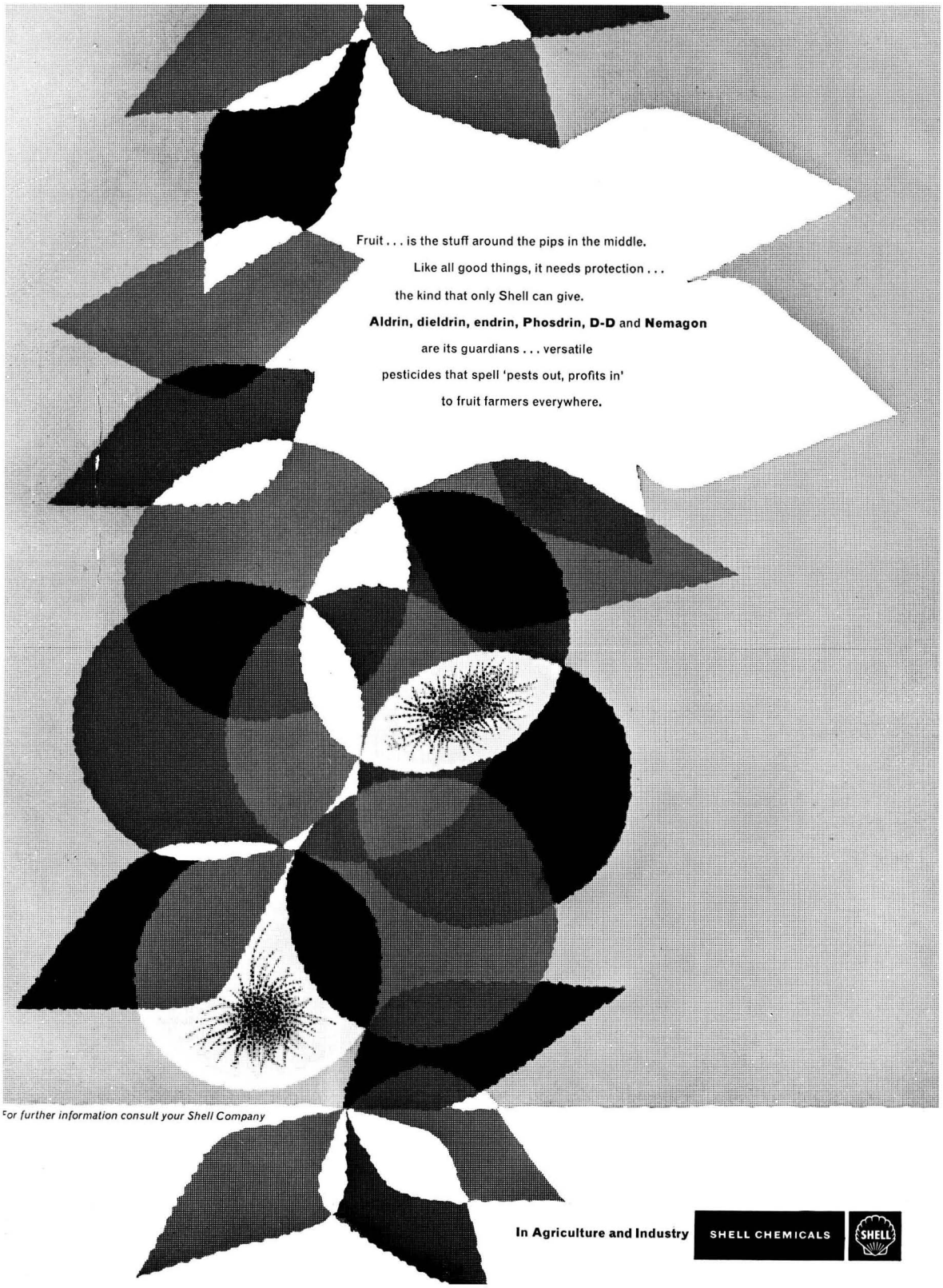

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