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EFFECT OF LOW SOIL TEMPERATURE ON PHOSPHATE NUTRITION OF PLANTS—A REVIEW

By C. D. SUTTON

Introduction

Effects of soil temperature on plant growth were reviewed in 1952 by Richards *et al.*[1] who concluded that the greatest single factor restricting growth at low temperatures was the low absorption of water. Work since 1952 was reviewed by Nielsen & Humphries.[2] They considered that the effect of soil temperature on growth was not clearly understood but that it would be an over-simplification to attribute the deleterious effects of cold soils entirely to reduced water uptake. They concluded that additional nutrients, particularly phosphate, might improve growth at low temperatures. Any adverse effect of low temperature on phosphate nutrition may be due to effects on plant growth and/or to effects on the soil. Nielsen & Humphries dealt with the former but not the latter.

Prominence in this review is therefore given to soil factors which may restrict phosphate supply or availability at low temperatures and to the use of phosphate fertilisers to reduce the adverse effects of cold soils.

Soil factors restricting phosphate uptake

Soil factors may operate by restricting the mineralisation of organic phosphate at low temperature and/or by reducing one or more of the primary soil factors that regulate inorganic phosphate uptake by plants.

Restricted mineralisation of organic phosphorus at low soil temperatures has frequently been suggested as a likely mechanism.[3–5] However, the magnitude of the temperature effect on phosphate uptake in mineral soils and the rapidity with which temperature differentials affect phosphate nutrition, make the suggestion less plausible. In the experiments reported by Case *et al.*[5] for example, phosphate uptake by oats was more than doubled by an increase in temperature from 15 to 25°. Although the importance of organic phosphorus in crop nutrition is not well documented, the general observation that the contribution of organic phosphorus can be ignored in most soil-phosphorus studies, throws considerable doubt on an effect of this magnitude. Again, Case *et al.*[5] showed that beneficial effects from increasing the soil temperature were already evident in the crop after five to eight days, a period too short to allow appreciable contribution from mineralisation.

Mechanisms operating through the soil inorganic phosphate supply seem more likely. Four primary parameters are considered to control this supply[6]: a capacity, measured by the total quantity of inorganic phosphate that is potentially able to enter the soil solution; a rate factor, which describes the rate at which solid-phase phosphate comes into the soil solution when this is depleted by uptake; an intensity of phosphate in the soil solution, for example the concentration; a diffusion factor, a measure of the rate at which phosphate ions move through the soil.

The first parameter is a measure of the total quantity of available phosphate while the other three are concerned with its availability.

The capacity seems least likely to be affected by temperature. The bulk of the potentially available inorganic phosphate is always in the solid phase, but it is in equilibrium with a tiny quantity of phosphate in the soil solution. Where this equilibrium operates by the dissolution (and formation) of phosphate compounds of low solubility, temperature can obviously have no effect on the total quantity of phosphate involved in the whole system, only on the equilibrium value.

Where the solid-phase phosphate is in an adsorbed form, however, it is possible that the bonding strength for some ions may be such that at high temperatures they can exchange with ions in solution, but are held too tightly to do so at lower temperatures. Thus some effect of temperature on the measured capacity can be envisaged.

Gunary[7] reported that at 3° the content of isotopically dilutable phosphate in the one soil studied was only 55% of that in the same soil at 23°. Arambarri & Talibudeen[8] also found an increase in labile phosphate when the temperature was raised from 25 to 35°. This increase occurred primarily in the fraction of the labile phosphate that had the slowest exchange rate with ^{32}P in solution. In a later paper[9] these authors found a similar result only in the soil with the lowest phosphate status of the four used. This result is logical if it is assumed that in such a soil a greater proportion of the labile phosphate will be in the form of adsorbed phosphate than in a soil of high status where precipitated phosphate compounds are more likely to dominate.

Theoretically, temperature must affect the rate of release of phosphate from the solid phase into the soil solution and similarly it must affect the rate of phosphate diffusion. That increasing temperature is also likely to cause an increase in the intensity (the concentration of phosphate in solution) can also be argued on theoretical grounds since a temperature increase will lead to both an increased solubility of sparingly-soluble phosphate compounds and to a decrease in the tightness of bonding of adsorbed phosphate.

There is little experimental evidence for temperature effects on these three parameters. Arambarri & Talibudeen[9] measured rates of isotopic exchange of phosphate in four calcareous soils at different temperatures. Three rates of isotopic exchange were differentiated and the slowest of these

was very temperature dependent. An increase in temperature from 25° to 35° gave an increased exchange rate for all soils. These authors also found that the equilibrium concentrations of phosphate in the soil solution increased by 1 to 2% with each 1° rise in temperature. They stated that these findings were generally consistent with those of Aslyng who had previously examined phosphate concentration in similar soils over the range 0° to 30°.

The amount of phosphate extracted from soil by an anion exchange resin was found to be strongly influenced by temperature.[10] The magnitude of the effect varied between soils but in one soil was as high as a 4% increase per degree over the range 10° to 40°. Resin acts as a zero sink for the phosphate ions in solution, relying on this disturbance of the equilibrium to bring more solid-phase phosphate into solution. Since this is essentially the mode of action of plant roots, it seems highly probable that these too will experience an equally marked temperature effect on phosphate supply.

Thus there is good evidence from laboratory studies that low soil temperatures can reduce the *availability* of soil phosphate to plants and there is evidence that with some soils the *quantity* of available phosphate may also be reduced. It is, therefore, suggested that these effects are likely to contribute significantly to the reduction in phosphate uptake by plants at low temperature.

Ameliorating effects of phosphate fertiliser

That the addition of phosphate fertiliser can, at least in some circumstances, overcome the reduction in plant growth at low temperatures has been shown by many workers.[11–20] Thus with barley, Power *et al.*[21] showed that the soil temperature range in which 80% or more of the maximum yield could be attained was increased by the addition of phosphate as follows:

	P_2O_5 rate (lb/acre)	Temperature range for at least 80% of maximum yield
Soil 1	Nil	(80% not attained)
	35	12·8—16·1°c
	70	10·6—20·6°c
Soil 2	Nil	(80% not attained)
	35	13·3—21·7°c
	70	10·6—23·3°c

The beneficial effect of fertiliser phosphate could result from the fertiliser increasing the quantity of available phosphate present. Additionally, or alternatively, it could result from the fertiliser providing a source of phosphate ions that are at a higher energy level than the bulk of the soil phosphate and hence more able to enter the solution phase at lower temperature. If this latter mechanism predominated, it would result in a more than proportionate uptake of fertiliser phosphate, compared to soil phosphate, at low temperature. This has, indeed, been shown to occur for barley[22], for tomatoes[13] and for oats.[4]

It has also been noted that phosphorus uptake at low temperatures is improved where the fertiliser is placed rather than mixed through the soil and where the phosphate is of high water-solubility.[5,13,17,23] Both placement and high water-solubility would increase the energy level of the phosphate from the fertiliser source by maintaining localised zones of high phosphate concentration.

Thus there is good evidence that the fertiliser phosphate is primarily acting by providing, at least locally, a source of phosphate that is more available and more able to maintain a higher concentration of phosphate in solution than the native soil phosphate.

This is further support for the contention that from the soil point of view it is the reduction in availability rather than the quantity of inorganic phosphate that is responsible for the reduced phosphate uptake at low temperatures.

Effect of temperature on the relationship between phosphorus content and dry matter

At very low soil temperatures the ability of the plant to produce dry-matter, i.e. grow, is restricted to an even greater extent than is phosphate uptake. Power *et al.*,[22] for example, grew barley in pot experiments at soil temperatures of 7, 11, 15, 19, 23 and 27°. From their data they concluded that the lower the soil temperature, the lower was the level of phosphate uptake that was required to satisfy the (reduced) growth requirements of the plants. When fertiliser phosphate was supplied, increased uptake occurred at all temperatures, so that, particularly at 7°, and to a slight extent at 11°, there was an increase in the phosphorus content of the plant tissue. At higher temperatures, however, dry-matter production was limited by the rate of phosphate uptake at all the phosphate levels studied.

In addition, it has been found that at temperatures above the optimum for barley growth (15°), the efficiency of dry-matter production per unit weight of phosphate taken up was decreased.[20,22,24] A higher phosphate uptake was therefore required to maintain yields under these conditions.

Thus a high phosphate content which may accumulate when a low temperature has drastically restricted growth could be of considerable benefit to the plant when the temperature rises and rapid growth is possible. Most of the evidence for the effects of temperature on phosphate uptake, however, comes from glasshouses or growth rooms where temperatures have been kept constant. There have been few attempts to simulate conditions in the field, where the normal pattern is for crops, particularly cereals, to germinate in relatively cold soils, which warm up as the season progresses. Under these conditions an even greater benefit from fertiliser phosphate may be expected.

Practical implications

It is evident that part of the reduction in plant growth at low temperatures is due to restricted phosphate uptake and also that fertiliser phosphate can overcome this aspect of the restriction.

The most obvious practical implication, therefore, is that in cold soils, a starter application of fertiliser phosphate can permit crop growth to start earlier in the season, and, because phosphate uptake can still occur at temperatures too low for growth, it can permit an accumulation of phosphate which will allow growth to proceed more rapidly as soon as there is a seasonal rise in temperature.

Most benefit from added phosphate can be expected where there is an economic advantage in early development or early maturity. For example, phosphate application is extending the northern limit of maize cultivation in Canada by lengthening the growing season. Similarly, an economic benefit is to be expected where early produce is important, as in vegetables, or where a penalty for late growth is incurred, for example where summer droughts occur or where inclement weather can interfere with late harvesting.

An additional practical implication is in the interpretation

of soil analysis. Most laboratory measurements of soil phosphate status are made at ordinary laboratory temperatures (20–25°). It is not surprising that these do not always give good correlations with field experiments if both phosphate availability from the soil and the efficiency of dry-matter production per unit of phosphate taken up are temperature dependent. Climatic conditions should, therefore, be taken into account in interpreting soil-phosphate analyses and their relation to the results of field experiments. It is also possible that some measurements of soil phosphate status might be more meaningful if the extraction stage were made at a temperature nearer to that likely to occur in the field at the time of maximum phosphate demand.

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NATURE OF THE YELLOW COPPER COMPLEX PRODUCED IN CERTAIN ANALYTICAL METHODS FOR THE DETERMINATION OF MALATHION

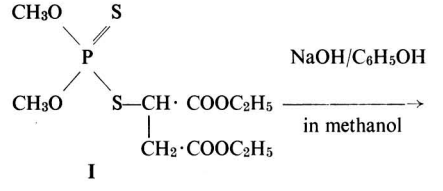
By A. C. HILL

Evidence is presented to show that the yellow copper complex, cupric *O,O*-dimethylphosphorodithioate (C), which is formed in certain colorimetric methods for the determination of malathion, can exist in reversible equilibrium with its two dissociation products, cuprous *O,O*-dimethylphosphorodithioate (A) and bis-(dimethoxyphosphorothiono) disulphide (B). At 20°, in solution in chloroform, equimolar proportions of A and B were found to be 24·86% associated to form C, and the equilibrium constant for the association reaction, A + B → 2C, was found to be 0·4378.

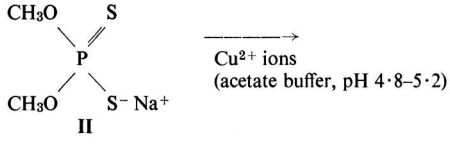
In view of these findings, a correlation is possible between colour intensities of yellow complex solutions obtained by two differing methods. This indicates that the yellow complex obtained by previous authors is initially undissociated. Hence, dissociation is considered to account, at least partly, for the instability of the yellow complex that has been reported by previous authors. The incorporation of B in the extractant, at a level of 450 moles of B per mole of yellow complex, is proposed as a means of limiting dissociation to a maximum of 1%.

Introduction

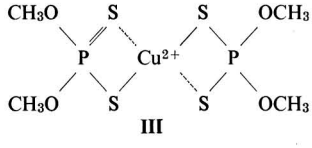
In a recent paper a titrimetric method is reported[1] for the determination of *S*-[1,2-di(ethoxycarbonyl)ethyl] *O,O*-dimethylphosphorodithioate (**I**) in malathion insecticide and in its formulations. This method is based upon quantitative cleavage of **I** by alkali in methanol in the presence of phenol to give sodium *O,O*-dimethylphosphorodithioate (**II**), followed by complete reaction of **II** with cupric ions in the presence of sodium acetate–acetic acid buffer solution to give cupric *O,O*-dimethylphosphorodithioate (**III**).



I



II



III

When formed under these conditions and at the same time extracted into chloroform, **III** forms a yellow or yellowish-brown solution which shows only 2% decrease in colour intensity during one month of storage at room temperature.

This method is based upon the same scheme of reactions as previously reported.[2–6]

All these authors have stated, without evidence, that the yellow copper complex produced in their analytical methods is cupric *O,O*-dimethylphosphorodithioate. However, their conclusions regarding the complex are warranted by the fact that it results from a double decomposition reaction between sodium *O,O*-dimethylphosphorodithioate and copper sulplate.

No report of the preparation and isolation of cupric *O,O*-dimethylphosphorodithioate could be found in previous reports and, in view of this, a preparation of the compound was attempted. This led to the observations which are reported in this paper.

Experimental and Results

Preparation of sodium *O,O*-dimethylphosphorodithioate dihydrate

The sodium salt, which had been prepared by neutralising pure *O,O*-dimethylphosphorodithioic acid with sodium methoxide in dry methanol, was recrystallised from ethyl acetate (Analar grade). At the solution stage, the ethyl acetate was heated below 45°, to avoid possible thermal decomposition of the sodium salt. On cooling to 0°, large colourless crystals of the sodium salt formed. Repeated recrystallisation from ethyl acetate yielded a product which melted at 65–66°. Elemental analysis was C = 11·3, H = 4·8 and P = 14·5; $(CH_3O)_2P(S)\cdot SNa\cdot 2H_2O$ would require C = 11·1, H = 4·7 and P = 14·4.

To confirm this the sodium salt was subjected to iodometric titration by the method of Bode & Arnswald[7] for *O,O*-dialkylphosphorodithioic acids; the results indicated a purity of 99·8% for the dihydrate. Determination by the titrimetric method recently reported for malathion,[1] but omitting the hydrolysis stage, gave a purity of 99·77%. The salt was extremely soluble in water and did not appear to be hygroscopic when exposed to an atmosphere at 57% R.H. and 20° for 24 h.

Formation of the yellow copper complex

Stock solutions of sodium *O,O*-dimethylphosphorodithioate dihydrate in methanol, with the range of concentrations indicated in Fig. 1, were prepared. 10 ml aliquots of these solutions in separating funnels were each treated[1] with 25 ml buffer solution, 50 ml chloroform and 25 ml 0·02 M copper sulphate solution, in that order, and each mixture was shaken for 2 min. The chloroform layer, containing the yellow complex, was drained into a 100 ml measuring flask and the aqueous layer was washed with 3 × 10 ml portions of chloroform. Each extract (80 ml) was made up to 100 ml and allowed to stand for 2 h at 20°, and then its optical density was measured at 420 nm in a 2 mm glass cell against a chloroform blank on a Unicam SP 500 spectrophotometer.

Stock solutions of 99·6% malathion in methanol, with the range of concentrations indicated in Fig. 1, were also prepared. 10 ml aliquots of these were transferred to separating funnels, and treated with methanolic sodium hydroxide in the presence of phenol[1] to convert the malathion to sodium *O,O*-dimethylphosphorodithioate. The hydrolysed malathion was neutralised by addition of 25 ml buffer solution and then converted to the yellow complex, extracted, and made up for colour measurement as described for the sodium salt.

The optical densities of the extracts were plotted against concentration of either the sodium salt or 99·6% malathion to give the curve shown in Fig. 1. The fact that one curve satisfies both sets of plotted points shows that the colour intensities obtained by converting molecularly equivalent quantities of the sodium salt and of malathion are the same; it also indicates that hydrolysis of malathion to the sodium salt under the conditions reported previously[1] is quantitative.

Preparation of cupric *O,O*-dimethylphosphorodithioate

A solution of sodium *O,O*-dimethylphosphorodithioate dihydrate (4·32 g) in water (30 ml) was added to a solution of cupric sulphate pentahydrate (2·60 g) in water (30 ml) in a separating funnel. The dark, yellowish-brown oil which formed was separated from the aqueous phase by several extractions with chloroform (100 ml total). The volume of the extract was reduced to 50 ml by evaporation at room temperature in a draught of air, and the buff-yellow material which separated was collected by filtration and dried (yield 3 g, m.p. 48–50°). Recrystallisation from 100 ml of 50 : 50 n-hexane/chloroform yielded 1·95 g of yellowish-white material, m.p. 150–152° (decomp.). This appeared to be crude cuprous *O,O*-dimethylphosphorodithioate.

The mother liquor from the initial crystallisation yielded 0·70 g of yellow material on evaporation. This material, though mostly microcrystalline, was observed to contain several large colourless crystals. Some of these were separated manually and were found to have a melting point of 47–49°, which corresponded to that of bis(dimethoxyphosphorothiono) disulphide (49–51°).

From these observations it appeared that recrystallisation was dissociating the yellow copper complex, thought to be cupric *O,O*-dimethylphosphorodithioate, into cuprous *O,O*-dimethylphosphorodithioate and bis(dimethoxyphosphorothiono) disulphide.

Subsequently, it was found that the yellow colour could be re-formed by mixing almost colourless solutions in chloroform of the materials separated by crystallisation. Thus, it appeared that recrystallisation was not of itself causing dissociation of the yellow copper complex. Rather it was leading to separation of dissociation products which already existed in equilibrium with the yellow copper complex, which when removed from the system allowed further dissociation to take place.

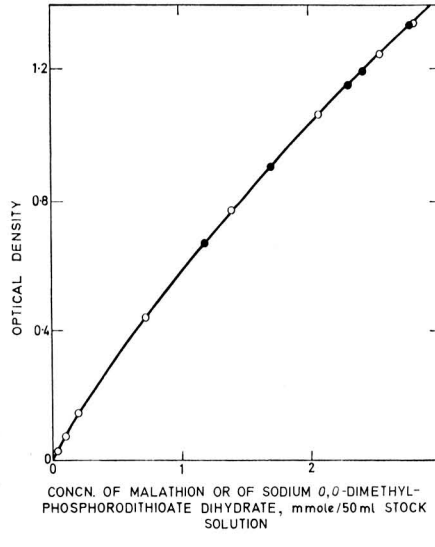


FIG. 1. *Curve showing optical densities of solutions of yellow copper complex in chloroform (100 ml)*

Solutions prepared from 10 ml aliquots of various stock solutions of either malathion 99·6% (●), or sodium *O,O*-dimethylphosphorodithioate, 99·8% (○)

Preparation of bis(dimethoxyphosphorothiono) disulphide

230 ml 0·1 N iodine solution was added slowly with stirring to 5·0 g sodium *O,O*-dimethylphosphorodithioate dihydrate dissolved in 50 ml distilled water. The pale yellow syrup was extracted with chloroform and the chloroform extract evaporated. The residue (3·6 g) was crystallised from n-hexane, yielding colourless crystals (1·93 g), melting point 49–51° (lit. 51–52°).[8] The compound in chloroform solution showed no absorption of light in the visible range.

Preparation of cuprous *O,O*-dimethylphosphorodithioate

Cupric sulphate pentahydrate (3·75 g) was dissolved in 500 ml water and liquid sulphur dioxide (10 ml) dissolved in 50 ml water was added, followed by five drops of 1 N hydrochloric acid. The solution was then heated almost to boiling, and sodium *O,O*-dimethylphosphorodithioate (3·24 g) dissolved in 100 ml of water was added with stirring. The precipitate which formed, at first pale yellow, rapidly became white, and heating was stopped. After cooling, the cuprous complex was collected by filtration and washed with water. The filtrate was colourless and still retained the odour of sulphur dioxide.

The white material was dried for 1 h at 55°, powdered, and dried for a further hour at 55°. This gave the cuprous complex as a chalk-white material. The yield was 3·20 g (96·8%). The material had no definite melting point; when heated it suddenly became brown at 149–151° and did not melt even when heated to 230°.

The cuprous complex was almost insoluble in carbon tetrachloride, chlorobenzene, benzene, n-hexane, methanol,

acetone and water, and sparingly soluble in chloroform. After being washed with chloroform, the material showed no absorption in the visible range of wavelengths.

Elemental analysis on the washed material was C = 10·8, H = 2·6, P = 14·4 and Cu = 29·00; $[(CH_3O)_2P(S)S]_2^{2-}$ Cu^{2+} requires C = 10·9, H = 2·7, P = 14·1 and Cu = 28·8. Initial attempts to determine the copper content by various ashing techniques were unsuccessful owing to interference by phosphorus. The copper was finally determined by digestion with sulphuric and perchloric acids, neutralisation with ammonia until the first permanent blue colour was formed, buffering to pH 10 with an ammonia–ammonium chloride buffer solution and titration of the copper with EDTA using PAN [1-(2-pyridylazo) -2-naphthol] as indicator.

Reaction of the disulphide and the cuprous complex

Various quantities, 1, 2, 5, 10 and 15 ml, of a 0·02 M solution of the disulphide in chloroform were added to 10 ml quantities of a 0·01 M solution of the cuprous complex in a 100 ml measuring flask. The mixtures were made up to 100 ml with chloroform and allowed to stand at 20°. Table I shows the variation of optical density with mole ratio of the disulphide to the cuprous complex that was found after 18 h. The figures show that the disulphide and the cuprous complex are only partially associated in solution to form the yellow complex.

The optical density (1·023) of the solution containing the 1 : 1 mole ratio agrees well with the optical density (1·033) of a '0·002 M' solution of yellow copper complex read from Fig. 1. This shows that the position of equilibrium obtained on association of the disulphide and the cuprous complex (both initially 0·001 M in chloroform) is practically the same as that obtained on dissociation of the cupric complex (initially 0·002 M).

An average value for the degree of association of the cuprous complex and the disulphide when mixed together in equimolecular proportions was calculated from the data in Table I. In making calculations it was assumed that solutions of the yellow complex in chloroform obeyed Beer's Law and that the disulphide (IV) and the cuprous complex (V) associated to form the cupric complex (III) according to the following reaction:

$$\underset{\text{IV}}{(CH_3O)_2P(S)S\text{-}S(S)P(OCH_3)_2} + \underset{\text{V}}{[(CH_3O)_2P(S)S]_2^{2-}\ Cu_2^{2+}} \rightleftharpoons \underset{\text{III}}{2[(CH_3O)_2P(S)S]_2^{2-}\ Cu^{2+}}$$

TABLE I

Variation of optical density (O.D.) with mole ratio of bis(dimethoxyphosphorothiono) disulphide to cuprous *O,O*-dimethylphosphorodithioate in chloroform

Molar conc. of sulphide	O.D. after 18 h, 420 nm, 20°C, 2 mm cell
—	0·000
0·0002	0·407
0·0004	0·625
0·001	1·023
0·002	1·418
0·003	1·688

Then, representing the degree of association at the 1 : 1 mole ratio by 1·023 x, the equilibrium constant, K_e, was $4(1\cdot023x)^2/(0\cdot001-1\cdot023x)^2$; while at the 0·2 : 1 mole ratio K_e was $4(0\cdot407x)^2/(0\cdot0002-0\cdot407x)(0\cdot001-0\cdot407x)$. Equating these two expressions for K_e and solving for x, x was found to be $2\cdot535 \times 10^{-4}$. Similarly, by equating the expression for K_e at the 1 : 1 mole ratio with those for K_e at the other mole ratios, three more values of x, $2\cdot401 \times 10^{-4}$, $2\cdot437 \times 10^{-4}$ and $2\cdot348 \times 10^{-4}$, were calculated. Hence, the average value of x was $2\cdot430 \times 10^{-4}$ and the degree of association at the 1 : 1 mole ratio was $1\cdot023 \times 2\cdot430 \times 10^{-4}$ mole per 0·001 mole of reactant ≡ 24·86%. Finally, substituting $y = 2\cdot486 \times 10^{-4}$ in the expression $4y^2/(0\cdot001-y)^2 = K_e$, K_e was found to be 0·4378.

Since the equilibrium constant contains no volume term, the dissociation products of the yellow copper complex formed from malathion should always be 24·86% associated at 20° regardless of the amount of malathion converted.

The results in Table II show the effect of temperature on the optical density of a solution of yellow copper complex that was 0·002 M expressed as undissociated cupric *O,O*-dimethylphosphorodithioate. This effect was completely reversible on cooling. The increase of optical density with temperature appears to be linear (0·0123/°C) and indicates that association of the dissociation products increases with temperature.

TABLE II

Optical densities (O.D.) at various temperatures of a solution in chloroform (100 ml) of cupric *O,O*-dimethylphosphorodithioate prepared from pure sodium *O,O*-dimethylphosphorodithioate dihydrate (0·4 m mole)

Temp, °C	17	20	25	30	35
O.D., 420 nm, 2 mm cells	0·989	1·022	1·088	1·146	1·210

Unsuccessful attempts were made to calculate the degree of association of the dissociation products at the 1 : 1 mole ratio assuming either that other proportions of the disulphide and the cuprous complex associated to form the yellow copper complex, or that the cuprous complex existed as $Cu^+S(S)P(OCH_3)_2$. Solutions of algebraic equations were obtained which either had no physical significance or did not fit the data.

Discussion

The calculations above prove that one molecule of cuprous *O,O*-dimethylphosphorodithioate and one molecule of bis-(dimethoxyphosphorothiono) disulphide associate to form two molecules of the yellow copper complex. From this it would appear that the yellow copper complex is cupric *O,O*-dimethylphosphorodithioate. No alternative formula seems possible, and, moreover, this formula affords the only reasonable explanation for the formation of the yellow colour.

The dissociation reaction proposed here is analogous in form to one that has been proposed by Busev & Ivanyutin[9] for cupric *O,O*-diethylphosphorodithioate.

There is a possible correlation between the data reported in Table I and in Fig. 1 and the data of Orloski[6] which are the only ones for optical density *versus* malathion concentration reported. From these data, which presumably relate to 1 cm spectrophotometer cells, it can be calculated that a 0·002 M solution of yellow copper complex in cyclohexane would have

an optical density of about 3·98 at 26° in a 2 mm cell, whereas a '0·002 M' solution of yellow complex as prepared by the method described above has an optical density of 1·023 (Table I). However, the '0·002 M' solutions reported here are only 24·86% associated at 20°. Assuming that Beer's Law is obeyed, it can be calculated that a 0·002 M solution of completely undissociated yellow complex would have an optical density of 4·12 at 20° in a 2 mm cell. Allowing for possible differences between actual cell lengths and the nominal lengths used in making the calculations, these figures are in good agreement and indicate that the yellow copper complex as obtained by the method of Orloski is undissociated cupric *O,O*-dimethylphosphorodithioate. Other data reported by Orloski[6] show that the optical density of solutions of the yellow copper complex decreases slightly with increase in temperature. If it were possible to take this effect into account, this would improve agreement between the figures calculated above.

Since the complex as obtained by the method of Orloski appears to be the undissociated complex, it is probable that the instability of this complex is due to dissociation. Orloski reports[6] a 4% loss of colour after only a 15 second delay in measuring its intensity and a 40% loss after a 2 min delay.

From the data reported here, it can be calculated that the addition of ~ 450 moles of the disulphide per mole of the yellow copper complex, produced in any of the colorimetric methods reported for the determination of malathion, would stabilise the yellow copper complex; first by greatly reducing the rate of dissociation and eventually by allowing not more than 1% dissociation. If this procedure were employed in the method of Orloski, it would entail extraction of the yellow copper complex, from a neutral or acetate-buffered aqueous phase, using a 1·6% (by wt.) solution of bis(dimethoxyphosphorothiono) disulphide in cyclohexane instead of cyclohexane alone; the same extractant would have to be used in preparing the standard curves.

Under the conditions reported here and elsewhere[1] for converting malathion to the yellow copper complex, dissociation probably proceeds so rapidly that it cannot be observed visually as fading of colour. The concentrations of yellow complex initially produced are approximately 100 times greater than those produced by the method of Orloski. Since the rate of dissociation is proportional to the square of the concentration of the yellow complex, this means that the rate of dissociation will be 10,000 times faster than that indicated by Orloski's figures for loss of colour with time.

Before the yellow complex in colorimetric methods for malathion is formed, various authors[4–6] have employed a 'ferric-oxidation' step under neutral conditions to remove materials present in hydrolysed malathion, such as mercaptans and thiols, which would interfere by reducing cupric ions to cuprous. The purpose of this was to avoid subsequent reaction of *O,O*-dimethylphosphorodithioate anions with cuprous ions to form cuprous *O,O*-dimethylphosphorodithioate—a colourless complex—instead of with cupric ions to form the required yellow complex. If a colorimetric version of the titrimetric method reported recently[1] is employed, this version may also require the inclusion of a 'ferric-oxidation' step under neutral conditions. However, in such a version, the rôle of the 'ferric-oxidation' step would be different. Its purpose would be to prevent formation of cuprous complex formed by direct reaction of *O,O*-dimethylphosphorodithioate anions with cuprous ions, because curpous complex formed in this way would interfere by altering the position of equilibrium between the yellow complex and its dissociation products.

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SYNTHESIS AND PESTICIDAL EVALUATION OF PHENAZINES

I.—Halophenazines

By B. CROSS, C. L. DUNN, D. H. PAYNE and J. D. TIPTON

The synthesis and biological activity of twenty-six halophenazines is described. The Wohl-Aue reaction and the new method for cyclisation of 2-nitrodiphenylamines by oleum were found to be the most convenient methods of synthesis.

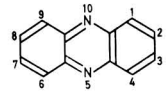
Phytotoxicity of a characteristic type was higher in foliar spray than in pre-emergence tests. Chlorine substitution appeared to confer higher activity than other halogen substituents, and in the chlorophenazines activity decreased with increasing substitution. Both 1- and 2-chlorophenazine were highly effective herbicides.

Acaricidal and fungicidal activity showed similar responses to structural changes, and optimum activity, together with a low level of phytotoxicity, was reached with 1,4-dichlorophenazine.

Introduction

Naturally occurring phenazine compounds have been isolated from bacteria and several of these affect the growth and viability of a wide range of micro-organisms.[1] Indications of potential pesticidal use have until recently been restricted to nematicidal activity[2] and, for phenazine itself, a low level of insecticidal activity[3] coupled with foliar scorch symptoms.[4] The closely related quinoxalines have been developed as pesticides; for example oxythioquinox, (1,3-dithiolo(2,3-b)5-methylquinoxaline-2-one),[5] as an acaricide and powdery mildew fungicide. It was of interest therefore to design and synthesise phenazines as potential pesticides.

The numbering of the phenazine nucleus used in this report is shown below.



Experimental

Chemical synthesis

All melting points are uncorrected (see Table I).

1,2,4-Trichlorophenazine (17) (Method A)

Aniline (20 g), 2,3,5-trichloronitrobenzene (52 g), powdered potassium hydroxide (120 g) and dry toluene (500 ml) were stirred vigorously and heated under reflux for 2 hours. The water eliminated during the reaction was removed by means of a Dean Stark apparatus. The hot mixture was filtered and the filtrate was evaporated to dryness to give a solid, m.p. 182°. Purification by chromatography on neutral alumina using benzene–chloroform as eluant gave, after crystallisation from ethyl acetate, 1,2,4-trichlorophenazine (17), 17% yield, m.p. 185–186° (Analysis, Table I).

2-Chlorophenazine-10-oxide (25) (Method B)

To a stirred concentrated sulphuric acid solution (20 ml) of 4′-chloro-2-nitrodiphenylamine (6·8 g, 0·027 mole) maintained at 20° by external cooling, was added 28% oleum (25 ml). After five minutes, the reaction mixture was slowly poured into a vigorously stirred solution of water (2·5 l) and ethanol (300 ml) containing sodium bicarbonate (150 g). A yellow precipitate separated out; the total mixture was extracted with methylene chloride (2·5 l). After the methylene chloride had been dried over anhydrous sodium sulphate, the solution was evaporated under reduced pressure to give a brown solid (8·4 g). Crystallisation from ethanol gave 2-chlorophenazine-10-oxide (25), 4·05 g (64%) m.p. 176–177°. (Analysis, Table I.)

In a similar manner 2,8-dichlorophenazine-10-oxide m.p. 228–229° was prepared from 4,4′-dichloro-2-nitrodiphenylamine in 77% yield, 2,7-dichlorophenazine-5-oxide m.p. 236–240° from 4′,5-dichloro-2-nitrodiphenylamine in 54% yield, 1,7-dichlorophenazine-5-oxide, m.p. 197–198° in 80% yield from 4′,6-dichloro-2-nitrodiphenylamine and 2,3-dichlorophenazine-5-oxide m.p. 222–223° from 4,5-dichloro-2-nitrodiphenylamine in 5% yield.

Reduction of phenazine-N-oxides

In most examples of Method A and all of Method B, phenazine-*N*-oxides result. These have been reduced to the phenazine by a variety of procedures.

An aniline solution (14 l) of 2-chlorophenazine-5-oxide (1,264 g) was heated under reflux for 8 hours. Excess aniline was distilled off *in vacuo* to a volume of 2·5 l. On cooling, the product crystallised out. This solid was dissolved in benzene and filtered through a short alumina column to remove dark impurities. The solvent was removed. After being washed with light petroleum the product (3), 910 g (76%), m.p. 138–140° was obtained.

2-Chlorophenazine-5-oxide (30·3 kg, 131 moles) was suspended in glacial acetic acid (120 l). The mixture was heated to 40° and iron powder (5·9 kg, 80% iron, 105 g atoms) was slowly added, the temperature not being allowed to exceed 65°. After being stirred for a further hour, the solution was run into water (1,200 l) to precipitate the product. The solid was filtered off, washed successively with water, methanol and light petroleum ether (40–60°) to give 26·3 kg, 122·7 moles (93·5%), identical with 2-chlorophenazine obtained by other procedures.

TABLE I

Preparation and biological activity of halophenazines

	Phenazine	Yield[a], % (Method)	m.p. °C	Analysis, %	Phytotoxicity ratings[k] Post-emergence kg/ha 10	Post-emergence kg/ha 1	Pre-emergence kg/ha 10	Pre-emergence kg/ha 1	Acaricidal[l] activity TI	Fungitoxicity[m] category
1	1-Cl	18 (A)	122[i]	Found C 67·3 H 3·2 N 13·0 Cl 16·7 Req. C 67·5 H 3·3 N 13·1 Cl 16·6	9	7	8	0	300	B
2	2-F	2 (A)	175–176	Found C 67·9 H 3·8 N 12·9 Req. C 67·3 H 3·3 N 13·1	6	1	5	0	40	D
3	2-Cl	62 (B) 30 (A)	139–140[h]	Found C 67·3 H 3·1 N 13·1 Cl 16·8 Req. C 67·5 H 3·3 N 13·1 Cl 16·6	8	6	6	2	300	C
4	2-Br	— (A)	149–150[b]	—	5	3	2	0	400	C
5	2-I	[e]	169–170[e]	—	2	1	0	0	50	—
6	1,2-di-Cl	21 (A)	172–173	Found C 57·7 H 2·4 N 11·0 Cl 28·4 Req. C 57·9 H 2·4 N 11·4 Cl 28·5	6	2	1	0	80	B
7	1,3-di-Cl	1·25 (A)	189–190	Found Cl 27·2 Req. Cl 28·5	2	0	1	0	50	C
8	1,4-di-Cl	40 (A)	191–192	Found C 58·0 H 2·6 Cl 28·2 Req. C 57·9 H 2·4 Cl 28·5	3	1	1	0	450	A
9	1,6-di-Cl	3 (A)	265–266	Found C 57·9 H 2·5 N 11·3 Cl 28·3 Req. C 57·9 H 2·4 N 11·3 Cl 28·5	0	0	0	0	B	D
10	1,7-di-Cl	17 (B)	200–201	Found Cl 27·8 Req. Cl 28·5	0	0	0	0	C	B
11	1,8-di-Cl	4 (A)	219–220	Found C 58·3 H 2·4 N 11·2 Cl 28·4 Req. C 57·9 H 2·4 N 11·3 Cl 28·5	1	0	0	0	C	D
12	1,9-di-Cl	20 (A)	206·5–207·5	Found C 57·9 H 2·6 N 11·2 Cl 28·4 Req. C 57·9 H 2·4 N 11·3 Cl 28·5	2	0	1	0	C	B
13	2,3-di-Cl	0·25 (A) 5 (B)	246–247[j]	—	0	0	0	0	B	C
14	2,7-di-Cl	12 (B)	265·5–268[g]	—	1	0	0	0	B	C
15	2,8-di-Cl	62 (B)	230–231	Found C 57·6 H 2·4 N 11·1 Cl 28·4 Req. C 57·9 H 2·4 N 11·3 Cl 28·5	2	1	0	0	C	C
16	1,2,3-tri-Cl	1 (A)	202	Found C 50·8 H 1·9 Cl 37·7 Req. C 50·8 H 1·8 Cl 37·4	—	—	—	—	< 10	—
17	1,2,4-tri-Cl	17 (A)	185–186	Found C 50·9 H 1·9 N 9·9 Cl 37·8 Req. C 50·8 H 1·8 N 9·9 Cl 37·4	1	0	0	0	< 10	C
18	1,2,9-tri-Cl	1 (A)	204·5–205·5	Found C 50·7 H 1·9 Cl 37·3 Req. C 50·8 H 1·8 Cl 37·5	-	—	—	—	B	B
19	1,4,6-tri-Cl	0·5 (A)	215–216	Found C 50·5 H 1·8 Cl 37·2 Req. C 50·8 H 1·8 Cl 37·5	1	0	1	0	B	B
20	1,4,7-tri-Cl	17 (A)	220–221	Found C 51·1 H 2·2 N 9·6 Cl 37·4 Req. C 50·8 H 1·8 N 9·9 Cl 37·5	0	0	1	0	C	D
21	1,2,3,4-tetra-Cl	90[d]	235[d]	Found C 45·5 H 1·5 N 9·5 Cl 44·4 Req. C 45·4 H 1·3 N 8·8 Cl 44·5	0	0	0	0	B	D
22	1,4,6,8-tetra-Cl	— (A)	210[e]	—	0	0	0	0	C	—
23	1-Cl; 5-O	87	158–158·5	Found C 62·4 H 3·4 N 11·7 Cl 15·4 Req. C 62·5 H 3·0 N 12·1 Cl 15·5	6	4	3	1	100	—
24	2-Cl; 5-O	30 (A)	176–177	Found C 62·5 H 3·0 N 12·0 Cl 15·2 Req. C 62·5 H 3·0 N 12·1 Cl 15·4	6	6	4	0	20	—
25	2-Cl; 10-O	64 (B)	174–176	Found C 62·6 H 3·0 N 12·1 Cl 15·4 Req. C 62·5 H 3·0 N 12·1 Cl 15·5	6	5	5	0	C	—
26	2-Cl; 5,10-di-O	95[f]	182[f]	—	5	2	4	1	B	—

[a] If the synthesis involves an N-oxide intermediate, the yield refers to the overall yield of N-oxide preparation and its reduction to the phenazine.
[b] Ref. 8 m.p. 149–150° [c] Ref. 8 m.p. 169–170°, prepared by the diazotisation of 2-aminophenazine and its reaction with cuprous iodide.
[d] Ref. 9 m.p. 235°, prepared from condensation of *o*-phenylenediamine and tetrachloro-*o*-benzoquinone. [e] Ref 10 m.p. 210°.
[f] Prepared by oxidation of compound 3, 24 or 25 with 30% H_2O_2 in glacial acetic acid at 50°C, lit., m.p. 190–191°.
[g] Ref. 12 m.p. 265–266°. — indicates data not available. [h] Ref. 1 m.p. 140°. [i] Ref. 1 m.p. 122–123°. [j] Ref. 15 m.p. 250–251°
[k] Mean phytotoxicity scores for all seven test species, rated on a 0–9 scale (0 = no effect, 9 = killed).
[l] TI (toxicity index) $= \frac{LC_{50} \text{ of methyl parathion}}{LC_{50} \text{ of phenazine}} \times 100$
B, C = incomplete kill and no appreciable kill in the preliminary screen
[m] Inhibition of cucumber powdery mildew: A = 90–100% inhibition at 50 ppm or equal to Karathane in the same test
B = 90–100% inhibition at 100 ppm or half as active as Karathane in the same test
C = 90–100% inhibition at 300 ppm
D = 90–100% inhibition at 1,000 ppm, or > 50% inhibition at 300 ppm
E = Inactive at the highest concentration tested (300 or 1000 ppm)

Biological evaluation

Herbicide screen

The following species were used for measuring the phytotoxicity of each chemical: maize (*Zea mays*), oat (*Avena sativa*), ryegrass (*Lolium perenne*), pea (*Pisum sativum*), linseed (*Linum usitatissimum*), mustard (*Sinapis alba*), and sugar-beet (*Beta vulgaris*).

For post-emergence application, batches of each of the seven test species were grown to a seedling stage with one or two true leaves. In the pre-emergence test, seeds of the same seven species were sown in bands in John Innes potting medium with a flint grit covering in plastic dishes, and watered shortly before treatment.

Each chemical was dissolved or suspended in 50% acetone in water containing 1·25% Triton X-155 wetting agent. This was sprayed on to the seedling foliage or seed trays by a moving-belt spraying apparatus.

At the end of the test period (7 days for the foliar spray test and eleven days for the pre-emergence test) the results were recorded visually. Phytotoxicity was rated on a 0–9 scale (0 = no effect, 9 = killed). The mean scores for all seven species treated with 1 and 10 kg/ha are shown in Table I.

Acaricide screen

Primary screening of the phenazines was carried out against 7–10 day-old glasshouse red spider mites (*Tetranychus telarius* L.) reared on French-bean plants.

For a preliminary test 0·7% w/v of each chemical was formulated as a solution or fine suspension in 20% acetone in water containing 0·05% Triton X-100 wetting agent. Further dilutions were used to determine dosage-mortality curves. Methyl parathion, formulated as above and applied over the range 0·05–0·005%, was used as the standard.

Discs cut from French-bean leaves and supported on moist filter paper were sprayed by a logarithmic spraying apparatus at a rate equivalent to 40 gal/acre. After being sprayed the discs were left for a ½–1 hour drying period, and then infested with 10 mites. Twenty-four hours after infestation—during which time the discs were kept under normal glasshouse conditions—the mites were examined for mobility. The number of immobile, dead and moribund mites was recorded and, in the preliminary screen, the compounds were rated A for complete or nearly complete kill, B for incomplete kill, and C for no appreciable kill. Compounds rated A in the preliminary screen underwent LC_{50} determinations. The toxicity of each active phenazine was compared with that of the standard and expressed as the toxicity index (TI), (Table I) where:

$$TI = \frac{LC_{50} \text{ of methyl parathion}}{LC_{50} \text{ of phenazine}} \times 100$$

Fungicide screen

As preliminary fungicide screens had shown that the phenazines possess significant activity only against powdery mildew fungi, the disease selected for structure–activity studies was cucumber powdery mildew (*Erysiphe cichoracearum*). Cucumber seedlings (cv. Butcher's Disease Resister) were grown in 3½ in pots of John Innes potting medium until two true leaves were fully expanded.

Each chemical was dissolved or suspended in 5 or 10% acetone in water containing 0·005% Triton X-100 wetting agent. Preliminary tests were carried out with concentrations of 1000 ppm and 300 ppm; active compounds were tested further at 100 and 50 ppm. Karathane (25% dinocap WP) was included in each test as a commercial standard. These aqueous preparations were sprayed, by a DeVilbiss Aerograph hand sprayer, on to the upper surfaces of two leaves per plant, and three plants were sprayed per treatment. The leaves were allowed to dry and then inoculated by dusting-on conidiospores of *Erysiphe cichoracearum* from infected leaves. Readings were made after 7 to 10 days' incubation in normal glasshouse conditions. The powdery mildew infection on each leaf was rated on a visual scale based on percentage of leaf area infected, where 0 = no infection, 1 = 1–10% infected, 2 = 11–20% infected..... 10 = 91–100% infected.

The fungicidal (or fungistatic) activity (Table I) is expressed by the following categories:

A = 90–100% reduction in infected area at 50 ppm, or equal to Karathane in the same test;
B = 90–100% reduction in infected area at 100 ppm, or half as active as Karathane in the same test;
C = 90–100% reduction in infected area at 300 ppm;
D = 90–100% reduction in infected area at 1000 ppm (or > 50% at 300 ppm); and
E = Inactive at the highest concentration tested (300 or 1000 ppm).

Results

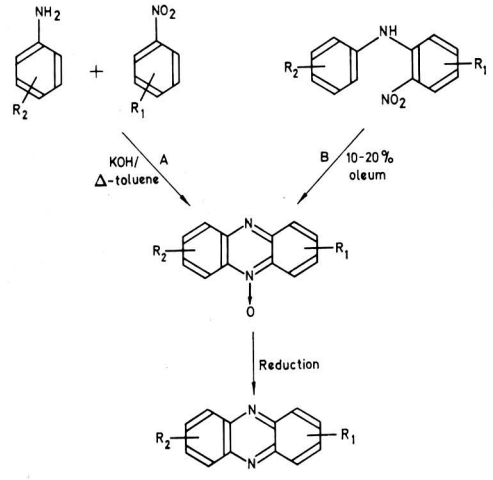
Synthesis of halophenazines

Method A

Halophenazines were most conveniently prepared by the Wohl-Aue synthesis,[6] which is the reaction of an aniline with nitrobenzene in the presence of powdered potassium hydroxide in a solvent such as dry toluene. Yields were generally low but were somewhat better if the halogen group was present in the nitrobenzene rather than the aniline ring. A phenazine-*N*-oxide was isolated except when the expected product had a substituent *ortho* to the *N*-oxide nitrogen, in which case the phenazine was obtained directly e.g. 1,4-dichlorophenazine (8). The halophenazine-*N*-oxides were readily reduced in 80–95% yields to the corresponding halophenazines by either heating under reflux with aniline for several hours or by iron–acetic acid reduction at 40°.

Method B

The new method for cyclisation of halogenated 2-nitrodiphenylamines in the presence of an excess of 10–20% oleum has been developed[7] as a procedure leading to halophenazine-*N*-oxides. It is particularly effective when halogen substitution is in the ring containing no nitro-group. For example 4′,6-dichloro-2-nitrodiphenylamine afforded 1,7-dichlorophenazine-5-oxide in 80% yield, which on reduction by iron–acetic acid gave 1,7-dichlorophenazine.



Phytotoxicity

The symptoms developed by plants treated with phenazines were distinct and characteristic. In particular broad-leaved species (e.g. linseed and mustard) were severely deformed, the leaf margins curling upwards. In the foliar spray test acute necrosis was also caused and, in general, activity was higher in this test than in the pre-emergence soil spray. Not all the phenazines were phytotoxic at the maximum dosage (10 kg/ha) and the level of phytotoxicity varied with the substituent and the substitution pattern. Chlorophenazines appeared to be the most phytotoxic as exemplified by the 2-halophenazine series in which 2-Cl > 2-F $\geqslant$ 2-Br > 2-I. The following observations regarding structure–activity relationships were therefore restricted to chloro substituents. Mono-substitution conferred the highest degree of phytotoxicity, and both 1-chloro and 2-chlorophenazine were highly active. Activity was reduced as the number of substituents was increased, giving the general order mono > di > tri > tetra, although there was considerable variation in activity between positional isomers, e.g. 1,2-, 1,3- and 1,4-dichlorophenazines were all moderately active but 2,3-dichlorophenazine was inactive.

Substitution into both rings conferred less activity than substitution in one ring so that the six examples of dichloro substitution in both rings (9–12, 14, 15) were generally less active than the four examples of dichloro substitution in one ring (6–8, 13). Trichloro substitution, whether in one or both rings, resulted in such low levels of phytotoxicity that no obvious patterns emerged, while tetrachloro substitution rendered the compound inactive (21 and 22).

Chloro-*N*-oxides (23–26) exhibited lower levels of phytotoxicity than the parent phenazines. Activity appeared to be about the same whether substitution was in the 5 or 10 position and was not markedly decreased by di-substitution in both positions.

Acaricidal activity

In the 2-halosubstituted series, the chloro-(3) and bromophenazines (4) showed a high level of acaricidal activity, whereas the fluoro-(2) and iodophenazines (5) were significantly less active. The monochlorophenazines (1 and 3) appeared equally active but the activity of the dichlorophenazines (6–8, 13) with halogen in only one ring varied considerably with positional isomers, in the order 1,4 > 1,2 > 1,3 > 2,3. Trichlorophenazines (16 and 17) were considerably less active, whilst the tetrachloro compound (21) was virtually inactive.

Chlorine substitution in both rings (eg. 9–12) caused almost complete loss of acaricidal activity. Mono-*N*-oxides (23–25) were acaricidal, but at a lower level than their parent phenazines (1,3), the di-*N*-oxide (26) was almost inactive.

Fungitoxicity

In the control of cucumber powdery mildew both 2-chloro- and 2-bromophenazine (3, 4) were more active than 2-fluorophenazine (2), but 1-chlorophenazine (1) was significantly more effective. Dichloro substitution in one ring (6–8, 13) conferred activity which closely paralleled the acaricidal activity. Dichlorophenazines having one substituent in the 1-position were at least as active as 1-chloro-phenazine (1) when the second substituent was in the 2-,4-,7- or 9-position, but were less active than 1-chlorophenazine when the second substituent was in the 3-,6- or 8- position. 1,4-Dichlorophenazine was distinctly more effective than 1-chlorophenazine, with much less phytotoxicity. Dichlorophenazines with both chlorines β to the nitrogens (13–15) were equal in effect to 2-chlorophenazine (3). Further substitution in one or both benzo-rings (17–21) produced no general improvement on the activity of the corresponding dichlorophenazines.

Discussion

The monohalophenazines possess a high degree of general biological activity and could have the same metabolic site of action in the three types of organism. 1-Chlorophenazine is very toxic to plants, mites and fungi, but with other monohalophenazines there appears to be no general pattern relating phytotoxicity, acaricidal activity and fungitoxicity. Possibly the electronic and steric properties of each halogen influence the penetration of the three different biological membranes differently, resulting in some specificity. However, the phytotoxicity is always high enough to make the fungicidal and acaricidal members worth little further consideration for use as plant protectants.

The dichlorophenazines, which are generally less phytotoxic, show some relationship between toxicity to the three organisms and the positions of substitution. This is best shown when one chlorine is in the 1-position, and when the position of the second chlorine substituent is related to arbitrary measures of each of the biological activities.

The fungicidal activity varies with the consecutive substitution positions. This is paralleled up to 6-chloro by the acaricidal activity which then decreases to zero. The variation is also detectable with the phytotoxicity but as part of a trend decreasing to a minimum at 6-chloro and increasing again at 8-chloro and 9-chloro. This trend is shown by the phytotoxicity parallels and possibly reflects the trend in the polarity in the dichlorophenazines reported by Morita.[13]

A similar variation is found in the phytotoxicity and acaricidal activity when one chlorine is in the 2-position, and the position of the second chlorine is again plotted against biological activity. Excluding cases where penetration is presumed to be the limiting factor the response shown by the three types of organism to the positions of substitution indicates a common metabolic mode of action.

Tri- and tetra- substitution reduces the phytotoxicity and

acaricidal activity to a low level. The fungicidal activity of the tri- and tetra-chlorophenazines was generally less than that of related dichlorophenazines. These observations suggest that substitution in excess of two chlorines leads to hindrance of the chemical either at external biological membranes or at the site of action. Oxidation of one or both nitrogens successively reduces the biological activity. It seems probable that the biological action of the phenazines involves single-electron oxidation–reduction reactions at the nitrogen atoms,[14] so that N-oxidation can be considered to block the site of biological activity.

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METHOD FOR DETERMINING 'ALAR' (B–995) RESIDUES IN APPLES

By V. P. LYNCH

A method for determining residues of 'Alar' (B-995) in apples has been developed. It is based on the alkaline hydrolysis of 'Alar' to yield unsymmetrical dimethylhydrazine. The latter is oxidised by selenium dioxide to yield formaldehyde which is determined colorimetrically using 2-hydrazinobenzothiazole.

Introduction

'Alar' is the trade name for succinic acid 2,2-dimethylhydrazide, a plant growth regulator marketed by the United States Rubber Company, Chemical Division. It is effective over a wide range of plant species in modifying growth and resistance to pathogenic and physiological diseases. The application of 'Alar' to edible crops in the United Kingdom has been developed by Mirvale Chemical Co. Ltd. in co-operation with the Murphy Chemical Co. Ltd.

This paper is mainly concerned with the determination of 'Alar' residues in apples. Consideration was given to the method of Lane[1] who determined 'Alar' residues by using trisodium pentacyanoamminoferroate which is a reagent for unsymmetrical dimethylhydrazine (UDMH). UDMH is formed by hydrolysis of 'Alar' in aqueous sodium hydroxide. The method lacks sufficient sensitivity and leads to low recoveries when dealing with levels in the < 1 ppm range. Consequently a method which would give the necessary sensitivity with good reproducible recoveries was sought. Since 'Alar' is soluble in water to the extent of 10% and relatively insoluble in most common water-immiscible solvents a conventional extraction and clean-up procedure could not be employed. Sutton[2] found that UDMH undergoes aerial oxidation to give formaldehyde which was then determined using chromotropic acid. The method involved an oxidation time of 20 hours. An oxidant capable of oxidising UDMH to formaldehyde which would use a short oxidation period and yield reproducible results was therefore sought. Selenium dioxide was found to fulfil these requirements, and in aqueous solution oxidised UDMH to formaldehyde in 30 minutes at 50°. The latter could then be distilled out and determined spectrophotometrically. Sawicki & Hauser[3] employed 2-hydrazinobenzothiazole as a reagent for the colorimetric determination of aliphatic aldehydes. Previous work in these laboratories showed this technique to be superior to that using chromotropic acid and it was therefore used for determining the formaldehyde obtained from the oxidation of UDMH.

Experimental

Apparatus

The apparatus consisted of two distillation units (A and B).

Distillation A (hydrolysis stage)

This consisted of a 700 ml round-bottom flask fitted with a splash head (Quickfit SM7/13) to the other end of which was attached a double surface condenser (effective length 17 cm) and a delivery tube.

Distillation B (distillation of formaldehyde)

This consisted of a 50 ml round-bottom flask fitted with a U adaptor (Quickfit MF22/1) to the other end of which was attached a Liebig condenser (effective length 15 cm) and a delivery tube.

Reagents

2-Hydrazinobenzothiazole (British Drug Houses Ltd.); 0·5% solution in concentrated hydrochloric acid–water (1 : 9, by vol.); potassium ferricyanide, 1% aq. sol; potassium hydroxide, 10% aq. sol; dimethyl formamide, reagent grade; sodium hydroxide, pellets and 50% aq. sol; selenium dioxide, 5% aq. sol; sulphuric acid, 7·5N and 10·0N; formaldehyde solution in water (4 μg/ml); and 'Alar' solution in water (20μg/ml) were used.

Procedure

Preparation of standard curve

Aliquots of the standard formaldehyde solution in the 0–20 μg range were pipetted into 1 × 6 in test tubes. The volume of the solution in the tubes was adjusted to 6 ml with distilled water. 1·25 ml of the 0·5% 2-hydrazinobenzothiazole solution were added to each of the tubes and the solution was allowed to stand for 18 min with intermittent shaking. 1·25 ml of the 1% potassium ferricyanide solution was then added and the solution was allowed to stand for 25 minutes with intermittent shaking. 2·5 ml of dimethyl formamide was added (to dissolve the red precipitate) followed by 2·5 ml of the 10% potassium hydroxide solution. The contents of the tubes were transfered to 25 ml volumetric flasks and made up to volume with water. The absorbence of the solutions was read at 582 nm in 1 cm cells against a reagent blank. The absorbence was plotted against the concentration of formaldehyde.

Application to apples

50 g finely shredded sample was placed in a 700 ml round-bottom flask, 50 ml water was added, the flask was fitted to the distillation apparatus (A) and 30 ml was distilled off. The distillate was discarded. 250 ml of the 50% sodium hydroxide solution plus 40 g of sodium hydroxide pellets were added to the flask and another 30 ml distilled off. This distillate was acidified by adding dropwise 10N sulphuric acid, usually 3–4 drops being sufficient. Depending on the expected residue, all or part of the distillate was transferred to a 50 ml round-bottom flask and concentrated to 4 ml on a rotary film evaporator. 1 ml of the 5% selenium dioxide solution was

added and the solution was allowed to stand in the flask in a water bath for 30 minutes at 50°. The flask was transferred to the distillation apparatus (B), 10 ml of the 7·5N sulphuric acid was added, and 6 ml distilled off into a test tube containing 1·25 ml of the 0·5% 2-hydrazinobenzothiazole solution. The same procedure as for the standard curve was then followed. For conversion of formaldehyde to 'Alar' the readings from the standard curve were multiplied by 160/30.

Results

The results obtained are shown in Tables I, II and III.

Discussion

Under the conditions described, formaldehyde is a stable oxidation product of unsymmetrical dimethylhydrazine. The oxidation is quantitative, one mole of UDMH yielding one mole of formaldehyde.

Acknowledgment

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TABLE I

Recovery of formaldehyde from the selenium dioxide stage

Formaldehyde added μg	% recovered
15·5	103, 98, 104
7·75	96, 103

TABLE II

Recovery of unsymmetrical dimethylhydrazine (UDMH) through entire procedure

UDMH added, μg	% recovered
11·5	110
23·0	104
34·5	96, 99, 103

TABLE III

Recovery of 'Alar' from apples

'Alar' added, ppm	% recovered
0·5	106, 94, 88
1·0	90, 93, 91
2·0	83, 88, 97
5·0	93, 100
20·0	92, 97

ACETALDEHYDE AS A POSSIBLE INDICATOR OF SPOILAGE IN GREEN KONA (HAWAIIAN) COFFEE

By DELIA B. RODRIGUEZ,* H. A. FRANK and H. Y. YAMAMOTO

Kona coffee cherries were demucilaged by either mechanical, enzymic or chemical methods, by the action of bacterial pure cultures, or by natural fermentation. Thirteen volatile components were detected by gas chromatography in all samples of green coffee tested, and these included methanethiol, acetaldehyde, dimethyl sulphide, propionaldehyde, acetone, isobutyraldehyde, butyraldehyde, ethanol, and isovaleraldehyde. Probably, methanol and/or methyl ethyl ketone also were present among the volatile components detected.

The relative concentrations of several volatile components did not vary appreciably among the different lots of coffee demucilaged experimentally. However, acetaldehyde concentration increased as the duration of natural fermentation was prolonged, being markedly higher in grossly over-fermented (spoiled) coffee beans.

All samples of coffee demucilaged experimentally had similar cup-testing quality (Kona grade No. 1), indicating that none of the demucilaging methods enhanced or diminished coffee flavour or aroma. Over-fermented beans, however, were poor in cup-testing quality.

Introduction

In Hawaii, and in countries that produce mild coffee, demucilaging is usually carried out by the 'wet' method of coffee fermentation, being essentially a degradation of the mucilage layer by pectinolytic bacteria in the natural microflora of coffee cherries.[1] Although fermentation is the most common method for mucilage layer removal, other demucilaging methods have been tried, both experimentally and commercially, employing physical, chemical, and enzymic means. Recent studies on demucilaging of coffee cherries by pectinolytic bacteria[2] and yeasts[3] indicated that pure culture inocula could also be substituted for natural fermentation. Other methods including mechanical demucilaging, the action of pectic enzyme preparations and of dilute alkali solutions have been tried in some South American countries but have gained very limited use in commercial coffee processing.

It has been stated that fermentation merely removes the mucilage layer without affecting coffee flavour and aroma.[4,5] Wellman,[6] on the other hand, has written that when demucilaging methods other than fermentation are used, the coffee beans lack 'character' and acidity. One purpose of the present investigation was to determine whether or not fermentation, or other demucilaging methods, affect the quality (i.e., flavour and aroma) of Kona coffee.

Another purpose of this investigation was to determine the volatile components present in green coffee demucilaged by different methods and to detect, if possible, any volatile component(s) that could be used as an index of spoilage. Objective chemical tests for measuring green coffee quality would have substantial value in an industry that relies heavily on sensory evaluation, even though this is conducted by experts. Rhoades[7] has stated that differences in volatile components of green coffee beans could be used for quality grading, and other publications have indicated that volatile components are associated with coffee flavour[8-10] and aroma.[7,11]

Experimental

Samples

Sound, ripe coffee cherries (*Coffea arabica* var. *typica*) were hand-picked in Kona (on the island of Hawaii) and processed the same day. The cherries were divided into several lots, pulped, and demucilaged by one of the methods described below. The completion of demucilaging was determined from the characteristic texture change that accompanies degradation of the coffee cherry mucilage layer.[1,12]

Methods of demucilaging

Mechanical

Lot No. 1 was demucilaged with a Fukunaga mechanical demucilaging machine.[5]

Enzymic

Lot No. 2 was demucilaged with aqueous 0·025% 'Klerzyme 200', a pectinase preparation (Wallerstein Company, Staten Island, New York). The pulped beans were almost entirely covered by the solution and stirred occasionally during the 4 hours needed to complete enzymic demucilaging.

Chemical

A 4% solution of sodium hydroxide was added to lot No. 3 and stirred occasionally during the 8 minutes needed to complete chemical demucilaging.

Bacterial

Inocula were prepared in these laboratories and flown the following day to Kona, where demucilaging was conducted. Three *Erwinia dissolvens* strains, isolated previously and shown to be active demucilagers,[2] were used as inocula for controlled bacterial demucilaging. The bacterial cells were centrifuged from shaken cultures grown for 24 h at 30° in nutrient broth and then refrigerated overnight prior to being taken to Kona. The cells were diluted with tap water, added to the pulped beans, and the mass stirred occasionally during the 9 hours needed to complete demucilaging in all 3 lots. Strain No. 273 was used to demucilage lot No. 4, strain No. 238 for lot No. 5, and strain No. 288 for lot No. 6.

Natural fermentation

A large batch of pulped beans was placed in the fermentation vat, with enough tap water to nearly cover the mass, and allowed to ferment naturally. Portions of the fermenting beans were removed after 12 h (lot No. 7), 17 h (lot No. 8), 41 h (lot No. 9), and 67 h (lot No. 10).

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Processing

Immediately after demucilaging, each lot of beans was washed thoroughly, sun-dried and hulled. A portion of the green beans from each lot was stored at −15° until it could be analysed chemically by gas chromatography. The remainder of each lot was used for coffee quality evaluation by cup-testing.

Coffee quality

Within 1 month of processing, each lot of beans was examined and graded by 3 professional coffee cup-testers in Kona. The grading system[13] is based on physical appearance of the green beans and on sensory evaluation of the flavour and aroma of roasted coffee, employing customary commercial cup-testing procedures.[8] In this system, Kona No. 1 is the top grade.

Gas chromatography

Fifty g of green coffee beans were placed in 190 ml wide-mouth glass jars, a piece of aluminium foil placed over the mouth, and the lid screwed on tightly. After heating the beans for 1 h in a 90° water bath, 2 ml of head space vapour were removed by syringe (through a pin-hole made previously in the lid only) and injected into the gas chromatograph. Duplicate samples were analysed from each lot of coffee.

The volatile components were detected with an Aerograph 200 gas chromatograph (Wilkens Instrument and Research, Inc.) equipped with a flame ionisation detector. The column was a 10 ft × $\frac{1}{8}$ in coil of stainless steel tubing containing a liquid stationary phase of 10% Ethofat (Wilkens) on a solid support of 60/80 mesh acid-washed Chromosorb P (Wilkens). The operating conditions were: column temperature, 50°; detector and injector temperatures, 140° and 105°, respectively; carrier gas (nitrogen) and gas for flame (hydrogen) flow rates, 20 cc/min.

The chromatograms were recorded with a Honeywell Brown Elektronik 1 millivolt recorder with a chart speed of 30 in/h and attenuation, 20. The area (sq. in) for each peak was calculated by multiplying peak height by width at half-maximum height.

Thirteen peaks were detected, and nine of these were identified tentatively from the similarity of their retention times to those of tested samples of authentic compounds. The known reference compounds were selected from those reported to be present in coffee.[7,14–16]

Chromatographic analyses and quality evaluations were conducted on 2 samples of 'spoiled' coffee, i.e., excessive natural fermentation for 5 days (lot No. 12) and 7 days (lot No. 13) respectively. Another lot (No. 11), taken from the same batch of beans, was demucilaged mechanically and served as a control for this experiment.

Results and Discussion

Effect of demucilaging method on coffee quality

Although individual cup-testers had slightly different evaluations for some quality categories, their overall gradings were uniform for the samples experimentally demucilaged. All 3 testers rated the test samples in lots No. 1–10 as Kona grade No. 1, indicating that they found no appreciable differences in quality among the 10 test samples. These results suggest that coffee quality, as evaluated by expert cup-testers, was not affected by the demucilaging method, when conducted under laboratory conditions.

No deterioration in roast, aroma, or flavour quality was observed when natural fermentation was prolonged as much as 41 to 67 h (lots No. 9 and 10). Apparently the controlled conditions employed in this study prevented introduction of spoilage organisms from improperly cleaned equipment and from the microflora that accumulates on fermentation vats during the processing season. However, since commercial operations cannot maintain strict cleanliness, processors should wash fermented beans as soon as demucilaging is completed to avoid the possibility of spoilage. In Kona, where natural fermentation is usually conducted overnight (12 to 16 h), the brevity of the fermentation period probably reduces the incidence of spoilage during demucilaging.

Identification of volatile components

Thirteen volatile components were detected in all samples of green Kona coffee (Fig. 1). Of these, the following 9 compounds were tentatively identified from their gas chromatographic retention times: methanethiol, acetaldehyde, dimethyl sulphide, propionaldehyde, acetone, isobutyraldehyde, ethanol (the major volatile constituent), and isovaleraldehyde. Of the four remaining, one peak (No. 10) was not identified because its retention time was the same as that of methanol and methyl ethyl ketone.

Using gas chromatography, Rhoades[14] found 16 volatile components in 7 varieties of Central and South American

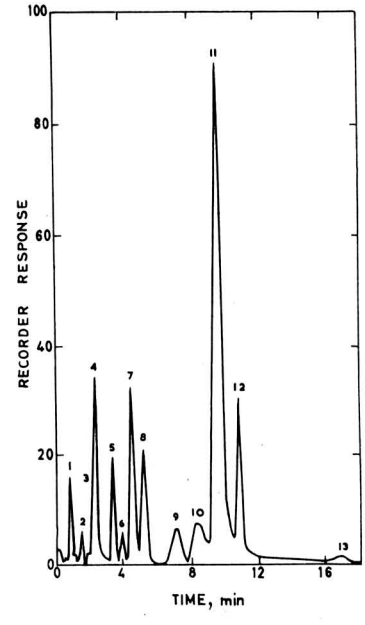

FIG. 1. *Typical gas chromatogram of volatile components in green Kona coffee*

Identification of peaks: 1 unidentified; 2 unidentified; 3 methanethiol; 4 acetaldehyde; 5 dimethyl sulphide; 6 propionaldehyde; 7 acetone; 8 isobutyraldehyde; 9 butyraldehyde; 10 unidentified (methanol and/or methyl ethyl ketone?); 11 ethanol; 12 isovaleraldehyde; 13 unidentified

green coffee. He detected all the compounds reported in this study (plus methanol and methyl ethyl ketone) in addition to hydrogen sulphide, furan, methyl furan, and diacetyl. In contrast with our observations, Rhoades found that methanol was the major volatile component, not ethanol as our data indicate for Kona coffee (Fig. 1). The smaller number of volatile components observed in our study might be attributed to differences in variety, horticultural practices, or climate. However, it is more likely that the fewer volatile components detected in this study resulted from the lower heating temperature (90°) employed to avoid generating compounds not normally present in green coffee but which can form through excessive heating.[14]

Effect of demucilaging method on volatile components

Four volatile components varied noticeably with different demucilaging treatments. These were peaks 4 (acetaldehyde), 5 (dimethyl sulphide), 7 (acetone), and 8 (isobutyraldehyde). In the present study, therefore, the effects on volatile components in terms of changes in the relative concentrations of peaks 4, 5, 7, and 8 alone were compared. The area for each peak was measured as described above and calculated as a fraction (relative percentage) of the total area for all 4 peaks.

Table I shows the effects of different demucilaging methods on the relative concentrations of peaks 4, 5, 7, and 8. The concentrations of these volatile components are quite similar for coffee demucilaged mechanically, enzymically, chemically, or by brief (12 h) natural fermentation. When natural fermentation was lengthened to 17 or 41 h, a slight increase was detected in the relative concentration of peak 5 (dimethyl sulphide). Further prolongation of natural fermentation to 67 h resulted in a noticeable increase in the concentration of peak 4 (acetaldehyde).

From these observations it was not possible to conclude whether the slightly elevated acetaldehyde concentration in lot No. 10 indicated deterioration in quality because this sample also had been rated Kona grade No. 1 by cup-testing procedures. However, Table II shows that coffee subjected to excessively prolonged fermentation for as much as 5 or 7 days had increased acetaldehyde peaks. The cup-testers described both of these samples as 'sour' and rated them as poor in grade.

These results suggest that acetaldehyde could serve as an index of quality deterioration in green Kona coffee and, as seen in the 67 h samples (Table I) may even indicate incipient spoilage before it can be detected by sensory evaluation. Future work will investigate the possibility that the absolute concentration of acetaldehyde might be used to evaluate green coffee quality or to detect early stages of spoilage.

TABLE I

Effect of demucilaging method on several volatile components of Kona coffee

Lot No.	Demucilaging method	Relative concentration, % Peak No.* 4	5	7	8
1	Mechanical	32	10	36	22
2	Enzymic	33	9	37	21
3	Chemical	34	9	35	22
	Bacterial fermentation:				
4	strain 273	41	8	30	20
5	strain 283	37	8	32	22
6	strain 288	36	10	32	21
	Natural fermentation:				
7	12 h	36	8	34	22
8	17 h	33	14	30	22
9	41 h	34	19	27	19
10	67 h	43	20	22	14

* Identification of peaks: 4 acetaldehyde; 5 dimethyl sulphide; 7 acetone; 8 isobutyraldehyde

TABLE II

Effect of over-fermentation on several volatile components of Kona coffee

Lot No.	Demucilaging method	Relative concentration, % Peak No.* 4	5	7	8
11	Mechanical	38	10	24	28
12	5 day fermentation	57	6	19	17
13	7 day fermentation	80	5	7	8

* Identification of peaks: 4 acetaldehyde; 5 dimethyl sulphide; 7 acetone; and 8 isobutyraldehyde

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COLD WATER-EXTRACTABLE PECTIN IN CELL WALLS OF PLANT LEAVES

By P. KOOIMAN

When leaves of *Cyclea barbata* and some other plant species are disintegrated in cold water part of the acidic cell-wall material dissolves. The extracted material is a highly esterified pectin almost free from other polysaccharides; it was identified by its specific optical rotation and by enzymic conversion into D-galacturonic acid. Solubility of such a pure and highly esterified pectin from plant cell walls in cold water seems to occur only in a restricted number of plant species.

During storage of the disintegrated tissue for some hours the pectin is gradually de-esterified by pectin esterase resulting in gelation of the suspension. In Indonesia the gel is known as 'tjintjaoe idjo' and is used as an ingredient for beverages.

Introduction

The leaves of *Cyclea barbata* Miers (Menispermaceae) are used in Indonesia as a delicacy; they are ground with a little water and the juice is pressed through cloth. After a short time a gel is formed which is cut up and added to beverages. The gel is called 'tjintjaoe idjo' and is stated to have a cooling or refreshing effect.[1]

Since *Cyclea barbata* is not common in Indonesia some plant species are used as substitutes for the preparation of 'tjintjaoe idjo'; these include the related species *Stephania hernandifolia* Walp. (Menispermaceae), and the taxonomically remote species *Premna parasitica* Blume (Verbenaceae) and *Gardenia jasminoides* Ellis (Rubiaceae).

The chemical nature of 'tjintjaoe idjo' and the mechanism underlying gel formation are unknown. The present paper contains a study of this problem, which is of interest because of the wider aspect of gel stability in cell walls.

Experimental

Materials

Dried leaves of *Cyclea barbata*, *Stephania hernandifolia* and *Premna parasitica* were obtained from Indonesia. Leaves of other plant species investigated were harvested from plants kept at these laboratories.

The crude enzyme preparation 'Pektolyt' was obtained from De Betuwe, Tiel, The Netherlands. It is a commercial product derived from *Aspergillus niger*. An aqueous extract was treated at 0° with five volumes of cold ethanol, and the precipitate was dried and used as an enzyme preparation.

Paper chromatograms were made on Whatman No. 1 filter paper using the following solvents (v/v): (a) n-butanol–pyridine–water 6 : 4 : 3; (b) n-butanol–malonic acid–water 4 : 1 : 5;[2] (c) ethyl acetate–acetic acid–pyridine–water 5 : 1 : 5 : 3.[3] Sugar and uronic acid spots were made visible by spraying with *p*-anisidine phosphate reagent.[4]

All evaporations were done *in vacuo* below 35°. Melting points were corrected.

Pectin extraction

Extraction of cold water-soluble pectin

Either fresh or dried leaves were kept in boiling 80% ethanol for a few minutes, the liquid was poured off, and the leaves were dried. The product was comminuted and ground in a mortar with water at room temperature. After dilution, the suspension was filtered and the residue was ground twice more with water and filtered. The combined extracts were acidified (pH 2–3) with hydrochloric acid and centrifuged at 10,000 *g* for 20 min, and 4 volumes of 95% ethanol were added to the clear solution. The gelatinous precipitate was washed with 60% ethanol, then several times with 95% ethanol, and finally with ethyl ether. The product was dried *in vacuo*.

Extraction of residual pectin

The residues from the extractions of cold water-soluble pectin were repeatedly extracted with 0·5% ammonium oxalate at 90° for ½ h until the filtrates did not give a precipitate with an excess of 95% ethanol. The combined filtrates were acidified with dilute hydrochloric acid and the pectin was precipitated by the addition of 4 volumes of ethanol. The precipitates were separated by centrifugation and washed and dried as described for cold water-soluble pectin.

Determination of percentage esterification and of pectin content

A weighed sample (200 mg) of the pectin preparation was dissolved in water (25 ml) and 2 N hydrochloric acid (10 ml) was added. The pectin was precipitated by the addition of ethanol (50 ml). After standing for 1–2 hours the precipitate was filtered and washed with 50% ethanol, then with 95% ethanol until the washings were neutral. The pectin was dissolved in water (25 ml) and titrated with 0·1 N sodium hydroxide using phenolphthalein as an indicator (*a* ml consumed). To the neutralised solution 0·1 N sodium hydroxide (10 ml) was added; the stoppered vessel was left overnight and the contents were back-titrated with 0·1 N hydrochloric acid (*b* ml consumed). Then the percentage of esterification (DE) was calculated from:

$$\mathrm{DE} = \frac{10-b}{10+a-b} \times 100\%$$

The content of pectin (*P*)* in the sample (*W* mg) was calculated from:

$$P = \frac{a \times 17{\cdot}6 + (10-b) \times 19{\cdot}0}{W} \times 100\%$$

* Pectin is the material consisting of residues of D-galacturonic acid and its methyl ester

Conversion of pectin into D-galacturonic acid

Pectin (100 mg) was dissolved in water (5 ml), and Pektolyt (10 mg) was added. The mixture was incubated at 37° for 6 days in the presence of a few drops of toluene to preclude microbial contamination. The solution was filtered and passed through a column of Amberlite IR-120 (H^+) to remove the cations. Water was evaporated, and the thin syrup was diluted with ethanol and filtered. D-Galacturonic acid crystallised.

Preparation of α-methyl-D-galacturonide methyl ester

Galacturonic acid (150 mg) was refluxed in 4% methanolic hydrogen chloride (5 ml) for 2 h. The solution was neutralised with silver carbonate, and insoluble salts were removed by filtration. The filtrate was evaporated to dryness and the residue was crystallised and recrystallised from absolute ethanol–ethyl ether. Melting points and specific rotations are recorded in Table I.

Detection of pectin esterase activity in leaves

A medium containing 2% agar and 1% pectin was neutralised towards methyl red and poured into a Petri dish. When the agar gel had set, small holes were punched and filled with brei of *Cyclea* leaves. Blanks were prepared by subjecting a portion of the brei to 100° for a few minutes and filling the holes with the heated material. The Petri dishes were incubated at 30° overnight. If pectin esterase is present in the brei it diffuses into the agar gel and de-esterifies the pectin, resulting in the production of free carboxyl groups; consequently the pH is lowered around the holes, and this is indicated by the methyl red indicator.

Results and Discussion

The middle lamellas and—to a smaller degree—the primary cell walls of leaf parenchyma of *Cyclea*, *Stephania*, *Premna*, and *Gardenia* are stained intensely by ruthenium red and alcian blue, which indicates that they contain considerable quantities of acidic polysaccharides.

When fresh or dried leaves were ground in a mortar with a little water added, part of the acidic cell wall material dissolved. In the course of some hours the viscous suspension, provided it had an appropriate concentration, gelified. Leaves that had been kept in boiling ethanol for some minutes before being ground with water yielded a viscous suspension which did not gelify on standing.

A gelatinous precipitate was obtained when the filtrate of the viscous suspension was treated with excess ethanol. Hydrolysis of the precipitate by heating it in N sulphuric acid at 100° for four hours did not liberate more than 2–3% monosaccharides (chiefly galactose), as roughly estimated visually from spots on paper chromatograms in solvent (a). A commercial pectinolytic enzyme preparation, however, hydrolysed the material, D-galacturonic acid being virtually the sole product. The latter was recognised by its positive reaction with Ehrlich's basic lead acetate reagent,[4] and by its chromatographic behaviour in solvents (a), (b) and (c). It was identified definitely by conversion to α-methyl-D-galacturonide methyl ester (Table I). The extracted material had a specific optical rotation which was very similar to that of pectin. After de-esterification the specific optical rotation was very similar to that of sodium pectate. It was concluded that the extracted material was pectin.

The yields of total pectin (the sum of cold water-soluble and residual pectin) and of cold water-soluble pectin from the four plant species investigated were very different (Table II). The substitutes for *Cyclea* had lower percentages of cold water-soluble pectin, which is probably the reason why they are less suitable for the preparation of 'tjintjaoe idjo' than *Cyclea*. The content of cold water-soluble pectin in adult leaves of *Gardenia* was larger than in growing leaves; the total content of adult leaves was considerably larger than of growing leaves. This was valid when the yields were calculated on the basis of fresh leaves, dried leaves, or dried ethanol-extracted leaves. The content of pectin in the cold water-soluble pectin from *Cyclea* was 94% and from *Gardenia* 92%.

TABLE I

Melting points and specific optical rotations of α-methyl-D-galacturonide methyl ester prepared from pectins obtained from different plant species

Source	Melting point[a]	$[\alpha]_D^b$ ($C\ 2H_2O$)
Cyclea barbata	148°	+137°
Gardenia jasminoides	147–148°	+133°
Premna parasitica	147°	+124°
Apple pectin	146°	+134°

[a] Ref. 5, 148°
[b] Ref. 6, +124° for the monohydrate, which corresponds with +134° for the anhydrous component

TABLE II

Pectins from leaves of some plant species

	Total pectin[a]	Cold water-soluble pectin[a]	cold water-soluble pectin		
			$[\alpha]_D$ (pectin)	$[\alpha]_D$ (sodium pectate)	esterification, %
Cyclea barbata	9·5	7·5	+245°	+258°	68
Gardenia jasminoides (adult leaves)	17·4[b]	6·3[b]	+232°	—	82
Gardenia jasminoides (growing leaves)	10·1[b]	5·2[b]	+237°	—	82
Premna parasitica	5·0	2·7	+234°	+246°	60
Stephania hernandifolia	3·4	2·2	+248°	+260°	40

[a] Percentage on dry leaves
[b] Based on dry ethanol extracted material these figures were for adult leaves 25·2 and 9·1%, for growing leaves 14·0 and 7·2%, respectively

These pectins were therefore essentially pure. Less pure were the residual pectins from *Gardenia*; the contents of pectin were 81% for adult leaves and 73% for growing leaves.

The degrees of esterification varied considerably, being 82% in the cold water-soluble pectin from *Gardenia*, but much lower in the cold water-soluble pectins from the other plant species; the relatively low degrees of esterification in the latter species might well be attributed to the long period of storage (several years) of the dried leaves. The degree of esterification of the residual pectin from adult leaves of *Gardenia* was 53%, and from growing leaves 59%. The cold water-soluble pectin was sharply distinguishable from the residual pectin.

The literature contains only one report of the direct isolation of a pure pectin; it was extracted from sunflower heads with ammonium oxalate–oxalic acid and had a degree of esterification of 37%.[8] In a few instances the isolation of a pure pectin or of a pure pectic acid from a mixture of polysaccharides was reported,[9,10] demonstrating that in the plant materials investigated part of the uronic acid occurred as homoglycans. On the other hand a cold water-extractable pectin was reported to occur in lucerne; however, the pectin was a minor component of the complex material extracted.[11]

Evidently the phenomenon that pure pectins can be extracted from cell walls with cold water is uncommon. Boorsma[1] states that, from about 20 menispermaceous species investigated, only leaves from *Stephania hernandifolia* Walp., *Limacia macrophylla* Miq., and *Cyclea peltata* H.f. et Th. (=*C. barbata* Miers) yielded a gelatinous mass when ground with water. Attempts to extract cold water-soluble pectins from leaves of the following, arbitrarily chosen, plant species gave no results: *Asarum europaeum* L., *Beta vulgaris* L., *Hedera helix* L., *Ligustrum vulgare* L., *Rheum palmatum* L., *Saxifraga crassifolia* L., *Spinacia oleracea* L., *Vinca minor* L.

The gelification of cold water extracts from leaves which had not been treated with boiling ethanol could be explained, if pectin esterase de-esterified the pectin, the resulting pectinic acid forming a gel with the calcium ions present in the leaf extract. Indeed the presence of pectin esterase could be demonstrated with the pectin-agar plate method.

The cold water-soluble pectin is not bound covalently in the cell wall; if it was released by enzymic cleavage of covalent bonds during maceration, different results would have been observed after treatment with hot ethanol. Furthermore either the natural gel structure in the cell wall is not strong enough to survive exposure to water, or factors which stabilise the natural gel structure are removed by grinding the tissue in the presence of water. As Rees *et al.*[12] pointed out, a pectin gel is *in vitro* 'stabilised by the presence of solutes which presumably act in large part at least by diminishing the osmotic pressure which would otherwise be disruptive'. In the cell walls of *Cyclea* and the other species having cold water-soluble pectin osmotic stabilisation might be achieved in a similar way. Evidently this is not a general mode of stabilising pectins in cell walls.

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Dried leaf samples of *Cyclea barbata*, *Stephania hernandifolia* and *Premna parasitica* were obtained by Jr. T. D. Siem and Mr. T. Babock. Their tentative identification by Dr. R. C. Bakhuizen van den Brink and Prof. C. G. G. J. van Steenis, Rijksherbarium, Leiden is gratefully acknowledged.

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BREAKDOWN OF WL 9385, AN AZIDO TRIAZINE HERBICIDE, IN SOILS AND ON WHEAT

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

The breakdown of the herbicide WL 9385 (2-azido-4-ethylamino-6-t-butylamino-1,3,5-triazine) in soils and in spring wheat has been studied in the laboratory by radio-isotope techniques.

In soils the major long-term breakdown product was formed by the reduction of the azido group to an amino group together with smaller amounts of a subsequent de-ethylation reaction product.

On the foliage of spring wheat the reduction reaction also occurred and de-ethylation of the reduction product was also evident, but with some de-ethylation of WL 9385 itself.

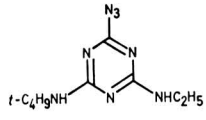
Spring wheat grown in soils treated with the herbicide at 2 kg/ha contained residues of 2·2 ppm (as equivalent ppm of WL 9385) of metabolites in the seed head and 7·4 ppm in the rest of the plant above ground at harvest at 65 days from treatment.

Residues of ^{14}C-compounds could not be detected in the seed head of wheat which had received a foliar application of 10 ppm of ^{14}C-WL 9385 at 100 days before harvest and residues in the rest of the plant above ground were only 0·16 ppm.

The breakdown products are not of higher acute toxicity to rats than the parent compound and the major soil breakdown products are not herbicidal.

Introduction

The chemical, toxicological, and biological properties of the azido triazine herbicide WL 9385,* (I, 2-azido-4-ethylamino-6-t-butylamino-1,3,5-triazine) were first described[1] in 1966.



(I)

The compound has a broad spectrum of pre- and post-emergence herbicidal activity and is particularly effective in controlling seedling annual grasses, post-emergence, at application rates down to 0·5 kg/ha.

The breakdown products formed when WL 9385 is applied to soils or spring wheat or when spring wheat is grown in treated soil have been studied and are described in this paper.

Experimental

^{14}C-WL 9385

A sample of specific activity of 6·4 nCi/μg randomly labelled in the nuclear carbon atoms was prepared and was purified by thin-layer chromatography (t.l.c.) to a radiochemical purity of over 99%.

Soil treatment in the absence of growing plants

Samples (100 g) of unsterilised field soils from East Anglia were treated separately with 1 mg of ^{14}C-WL 9385 in 1 ml acetone and were mixed thoroughly and stored in glass jars in subdued light at 10–20° for 9–12 months.

Soil treatment in the presence of growing plants

The radioactively labelled herbicide was applied to the surface of the soil (John Innes No. 2 Compost) between the growing spring wheat plants (variety Opal) at near 5 days from emergence minimising contact of the herbicide with the foliage of the plants. The ^{14}C-WL 9385 (18 or 30 mg) was applied to the soil in 1 ft square boxes in solution in 200 or 320 ml of 16% (by vol.) acetone in water. The plants were grown to harvest in a glasshouse with sub-irrigation. The plant growth was good.

Foliar treatment of growing plants

^{14}C-WL 9385 in acetone solution (500 μg/ml) was applied by means of a microsyringe to the leaves of young (3–5 leaf stage) spring wheat plants (variety Opal) to give an initial residue of about 10 ppm on the leaves. The plants were grown to maturity in a glasshouse as above.

Extraction

Soils

Soil samples were extracted (1 ml solvent for each 1 g soil) successively by being shaken with methanol for 1 hour, with methanol overnight, with ammonia (0·880 s.g.)–water–methanol (1 : 2 : 2) for 72 h and were finally washed with methanol. The extracts were combined and the soil residuum was dried in a vacuum desiccator.

Plants

The wheat plants were cut off at ground level, and the seed head was cut off and extracted separately. The plants were cut into small pieces and were macerated with acetone (5 ml for each 1 g of plant), and the residua were dried in a vacuum desiccator. The dried residua were re-extracted either with water or with 3% ammonia (0·880 s.g.) in water at 60° for 12 h.

The extracts of the plants and soils were concentrated by rotary evaporation to give a watery residue which was shaken with chloroform. The chloroform and water phases were examined separately.

Radiocounting

The procedures for the radiocounting of extracts and for the determination of the radioactivity in the plant and soil residua were as described previously.[2]

* Shell Registered Trade Number.

Identification of the radioactive components

The nature of the radioactive components in the extracts was examined by t.l.c. on pre-coated Merck F_{254} silica gel plates and the components were located using a Desaga radioscanner.[2] Unlabelled reference compounds were run on the same plates either mixed with the extracts or alongside them and the plates were examined in light of 254 nm wavelength.

The t.l.c. properties of the ^{14}C-components and the reference compounds were compared in at least 6 systems and the systems chosen differed for each component depending on its polarity. Some of the elution systems used and the R_f values of the reference compounds are summarised in Table I.

In some cases the radiochemical component was isolated in sufficient purity, after t.l.c. in several systems, to enable it to be examined by gas–liquid chromatography, mass spectrometry or infra-red spectrophotometry.

Results

Identification of the breakdown products

Soils

Five radiocomponents (A, B, C, D and E) were detected in the soils treated at 10 ppm and a typical radioscan of a thin-layer chromatogram is shown in Fig. 1.

The components A and B were not identified. Their t.l.c. properties did not correspond to any of the compounds in Table I and they were present in too low a concentration for identification by other means.

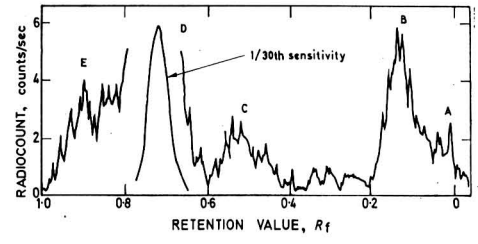


FIG. 1. *Radioscan of thin-layer chromatogram of extract of treated medium loam*

The loam was extracted at 12 months from treatment with ^{14}C-WL 9385 at 10 ppm
Lettered components referred to in text

The t.l.c. R_f values of component C and V (Table I) agreed when they were co-chromatographed in several systems, and they are probably identical.

Component D was identified as **II** (Table I). The R_f values of component D and **II** agreed when they were co-chromatographed on t.l.c. plates with ten different elution systems. Component D was also purified by t.l.c. in several systems and its infra-red spectrum (KBr disc) was identical to that of **II**. In addition component D and **II** had the same retention time on gas–liquid chromatography ($2' \times \frac{3}{16}''$ column of 3% phenyldiethanolamine succinate on 100–120 mesh Celite at 188°) using flame ionisation detection.

Component E and WL 9385 had the same R_f values when they were subjected to chromatography in six t.l.c. systems and they were probably identical.

Crops

Six radioactive components (F, G, H, J, K and L) were detected in the wheat plants that had received a foliar application of ^{14}C-WL 9385 and a typical radioscan of a thin-layer chromatogram of an extract is shown in Fig. 2.

Components F and G were not identified and they did not correspond to any of the reference compounds listed in Table I.

Components H, J and L were identified as compounds **V**, **II** and **I** respectively by the methods used for the soils. Sufficient component H was present to enable it to be purified by t.l.c., and the infra-red and mass spectra of the purified material were the same as those of compound V.

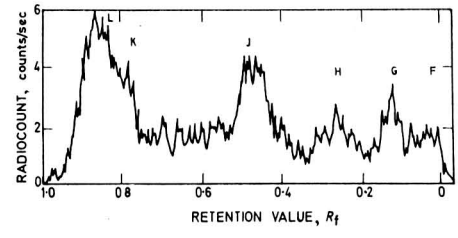


FIG. 2. *Radioscan of thin-layer chromatogram of extract of treated wheat*

The foliage was extracted at 6 days after treatment at 10 ppm.
Lettered components referred to in text

TABLE I

Thin-layer chromatography of WL 9385 and some related compounds

Elution on Merck ready-coated plates with 250 μ layers of silica gel F_{254}

Compound with 2,4,6-substituents on a 1,3,5-triazine ring	Retention value, R_f, in elution system indicated						
	10% (by vol.) ethanol in chloroform	10% (by vol.) ethanol in benzene	2% (by vol.) ammonia (0·880) in acetone	45% (by vol.) acetone and 5% (by vol.) glacial acetic acid in benzene	5% (by vol.) methanol in chloroform	15% (by vol.) acetone in benzene	15% (by vol.) ethanol in chloroform
WL 9385							
I 2-azido-4-ethylamino-6-t-butylamino-	0·87	0·70	0·90	0·92	0·85	0·73	0·90
II 2-amino-4-ethylamino-6-t-butylamino-	0·50	0·30	0·70	0·70	0·50	0·10	0·60
III 2-azido-4-amino-6-t-butylamino-	0·75	0·50	0·87	0·90	0·78	0·33	0·70
IV 2-azido-4-ethylamino-6-amino-	0·65	0·35	0·87	0·87	0·70	—	0·65
V 2,4-diamino-6-t-butylamino-	0·38	0·17	0·65	0·63	0·30	0·18	0·42
VI 2-amino-4-ethylamino-6-amino-	0·20	0·08	0·60	0·50	0·20	0·00	0·20

Component K and compound **III** had the same R_f values when they were co-chromatographed in several systems, and they are probably identical.

Persistence of the radioactivity in the soils and wheat

The concentrations of the ^{14}C-components in the soils and in the wheat plants at different intervals from application are shown in Tables II–V.

Discussion

In the experiments in jars of soil, 71–85% of the applied ^{14}C-activity was still present in the soils after 9–12 months. From 87–99% of the remaining ^{14}C-activity was present as one compound, **II** (Table I), which contained an amino group in place of the azido group in the parent WL 9385. There was little sign that compound **II** was degraded further and it is therefore likely to be persistent under field conditions.

When the foliage of wheat was treated with ^{14}C-WL 9385 several breakdown products were detected in the early stages after application, although most of the applied activity was lost by volatilisation either of the parent compound or of breakdown products. The conversion of WL 9385 to compound **II** occurred on the foliage of the plant and there was also considerable de-alkylation of compound **II** to t-butylmelamine **V** and also de-alkylation of the parent compound to compound **III**. In both cases the de-alkylation reaction resulted in loss of the ethyl group and not the butyl group.

TABLE II

Residues of ^{14}C-WL 9385 and its breakdown products in soils

Laboratory experiments on jars of soil treated with ^{14}C-WL 9385 at 10 ppm and stored at 10–20°c for 12 months for loam, peat and sand and 9 months for clay

Soil type (pH)	Residue of ^{14}C-components,* % applied activity					
	WL 9385	II	V	Components A and B (Fig. 1)	Unextracted radioactivity	Total
Medium loam (pH 8·0)	3·2	77	<0·5	1·3	1·7	83
Peat (pH 6·4)	<0·5	77	<0·5	<0·5	1·2	78
Sandy loam (pH 7·9)	1·1	78	0·7	1·4	4·2	85
Clay loam (pH 8·0)	1·9	62	2·4	2·8	1·9	71

* See Table I for formulae of compounds **I–VI**

TABLE III

Residues of ^{14}C-WL 9385 and its breakdown products in spring wheat at harvest after a post-emergence soil application

The wheat was grown to maturity in a glasshouse in non-sterile John Innes No. 2 compost and received a soil treatment of ^{14}C-WL 9385 at 2 kg/ha about 5 days after emergence and was sampled 65 days after treatment*

Component	Residues of ^{14}C-components, equiv. ppm of WL 9385†	
	Seed head	Rest of plant above ground
WL 9385		
extractable with acetone	<0·03	<0·03
Compound **III**		
extractable with acetone	<0·03	<0·03
Compound **II**		
extractable with acetone	0·33	1·01
unextractable in acetone: isolated by reversed isotope dilution with unlabelled **II**	—**	1·00
extractable with hot aqueous ammonia	0·35	2·53
Compound **VI**		
extractable with acetone	0·49	1·52
unextractable in acetone: obtained in isotope dilution study	—**	0·30
extractable with hot aqueous ammonia	0·30	0·75
Other (polar) components	<0·05	<0·05
Unextracted residue	0·75	0·32
Total residue	2·2	7·4

* The soil at harvest contained 0·65 ppm of ^{14}C-residue in the 0–3 in layer, being mainly **II** with traces of WL 9385 and **V** and 0·01 ppm of residue in the 3–8 in layer

† Untreated (control) plants contained no detectable residue (<0·02 ppm)

** Not done

TABLE IV

Residues of ^{14}C-WL 9385 and its breakdown products from foliar applications to wheat

Young seedlings at 3-leaf stage treated with ^{14}C-WL 9385 (500 μg/ml solution) to give 10 ppm application and maintained by sub-irrigation in a glasshouse till sampled

Interval to sampling, days	Weight of sample relative to time of application	Residues of ^{14} C-components as % of applied activity in whole plant above ground						
		WL 9385	III	II	V	Components G and F (Fig. 2)	Unextractable in acetone	Total residue
1	1·0	19·5	3·9	4·7	2·0	<1	0·9	31
2	1·1	15	<2	5·0	2·0	<2	0·8	24
3	1·0	8·0	1·9	6·0	2·2	1·8	0·8	21
6	1·3	6·0	1·3	3·2	1·8	2·7	2·4	18
10	2·1	4·0	<0·6	4·0	2·0	4·0	14	21
14	2·6	<1·0	<0·8	10	7·5	2·5	6·0	26

TABLE V

Residues of ^{14}C-WL 9385 and its breakdown products in wheat at harvest at 100 days following foliar application

The plants were treated and grown as in Table IV which gives the results at short intervals from treatment

Sample	Residues of ^{14}C-components, equiv. ppm of WL 9385
Seed head	
Extracted	<0·01
Unextracted	<0·01
Total	<0·02
Rest of plant	
Extracted components	0·06
Unextracted components	0·10
Total	0·16

It is thus evident that whilst the reduction reaction (to compound **II**) and the de-alkylation reaction can occur both in soils and on foliage, the reduction reaction predominates in soils whereas the de-alkylation reactions are only important as a metabolic route in plants.

When wheat was grown in treated soil the residues of ^{14}C-components present in the plants at harvest (Table III) were higher than when the plants had received a direct foliar application of WL 9385 (Table V). Under field conditions, when a post-emergence application of this herbicide is made, any residues in the plants at harvest will arise more from uptake from the soil than by persistence of the residues on the foliage.

The breakdown pathways that have been established for WL 9385 in soils and in wheat are summarised in Fig. 3, together with the acute oral toxicities of the compounds.

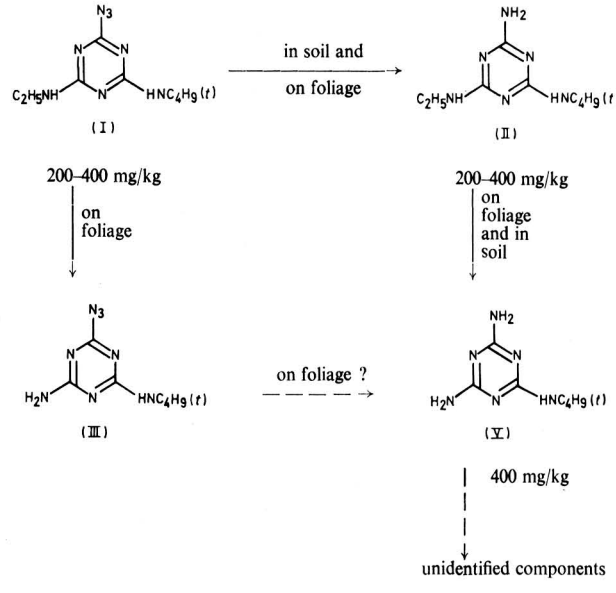


FIG. 3. *Breakdown pathway for WL 9385 herbicide in soils and on wheat*

The known LD_{50} values (acute oral to rats) are quoted for each component

None of the breakdown products has a greater acute oral toxicity to rats than the parent compound, and the main decomposition products in soils, **II** and **V**, are not appreciably herbicidal.

Conclusions

When the azido triazine herbicide WL 9385 was applied to soils, the azido group was converted to an amino group to give the main decomposition product. When the herbicide was applied to the foliage of winter wheat the reduction of the azido group also occurred together with reactions involving the de-alkylation of the ethylamino group in the parent. Whilst the major breakdown products are of no greater acute mammalian toxicity than the parent and have no herbicidal activity, they are likely to occur in wheat grown in the field with post-emergence application of the herbicide. It will thus be necessary to obtain field residue data on the breakdown products if WL 9385 is to be used commercially as a herbicide for cereals.

Acknowledgments

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Woodstock Agricultural Research Centre,
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COMPONENT ANALYSIS – AN APPROACH TO THE INTERPRETATION OF SOIL DATA

By D. A. HOLLAND

The interpretation of soil analyses frequently requires the simultaneous consideration of a number of interrelated nutrient concentrations. Component analysis provides a means of re-expressing such measurements as a set of independent linear functions. When applied to two examples it was found to lead to a meaningful and consistent summary of the data, and it was concluded that a component analysis approach shows considerable promise as an aid to the interpretation of soil analyses.

Introduction

The interpretation of numbers of interrelated measurements is a common biological problem. A typical example is the interpretation of the results of chemical analyses when, between samples, the concentration of one nutrient may be correlated with the concentration of another as a result of antagonistic or synergistic interactions between ions. In these circumstances, separate studies of each nutrient, by the analysis of variance, for example, can be misleading since the outcome of one such study may be related to the outcome of another. In this way they may result in alternative expressions of a single phenomenon rather than a series of separate and independent pieces of information. Further, in repeated experiments, a particular treatment can, when individual nutrients are examined in isolation, appear to give conflicting or inconsistent results.

Sometimes it is possible to overcome these difficulties by studying particular functions (differences, ratios, etc.) of the nutrient concentrations; for example, Cunningham studied the totals of the anions and cations in the nutrient composition of a range of plant species.[1–3] This approach, however, requires *a priori* knowledge of the relevance of such functions.

Unfortunately, this knowledge is frequently lacking and it then becomes necessary to seek out functions of the observed nutrient concentrations which satisfy predetermined criteria.

One method of determining such functions is 'principal component analysis'. It is the purpose of this paper to examine the value of an approach to the interpretation of soil analyses based on this procedure. After first describing the procedure, and giving an example of its application, its extension into a wider, more general approach will be discussed with a further example.

Principal component analysis

The technique of principal component analysis has been described by Kendal[4] and Pearce.[5] Briefly, and in the present context, it is a method of determining from the observed concentrations of different nutrients in a number of soil samples, a set of independent linear functions of the concentrations which could account for the observed variation in the samples, i.e. functions which involve no more than simple addition or subtraction of concentrations, and such that the values of one function are not correlated with the values of another. For example, if X, Y and Z are the concentrations of three nutrients in a sample, then a linear function of these concentrations would take the form:

$$U=(u_1 X+u_2 Y+u_3 Z)$$

where u_1, u_2 and u_3 may be positive or negative. This function must be independent of another, say: $V=(v_1 X+v_2 Y+v_3 Z)$, i.e. the values of U and V for each sample must be uncorrelated. It is usual, when writing these functions, to assume a standard order for the nutrients and then to write only the coefficients of each, thus the above function defining the quantity U would be written as: (u_1, u_2, u_3). This form of notation is referred to as a 'vector' and the convention will be followed throughout this paper.

These functions are derived in such a way that the first accounts for the largest possible proportion of the total variation in all nutrients, the second accounts for the largest possible proportion of the remaining variation and so on until all the variation is accounted for. Thus, the sum of the variances of the functions equals the sum of the variances of the original nutrient concentrations. Functions that satisfy all of these criteria are termed 'principal components'.

In practice it is frequently found that the total variation can —for all practical purposes—be accounted for in terms of fewer components than the number of nutrients initially observed. In this way the analysis can lead to a considerable condensation of the data. Further, because they are independent of each other, the values of these functions are more amenable to study by the usual statistical methods (analysis of variance, regression, etc.) than are the original data.

Example of application of principal component analysis

A 4-acre field was divided, by a 50-foot grid, into 64 squares. From each square, soil samples were taken from the depths 0–6 in, 6–12 in, 12–18 in, 18–24 in, 24–30 in, and 30–36 in. Each sample was analysed for P, K, and Mg (ppm 'available' in 1:2 Morgan's extract, two minutes shaking time).

There were thus 18 observations per grid square and the problem was to map the pattern of variation in these nutrients —this being the sort of information which might be required before the field was used for an experiment.

The simple approach would be to take each nutrient in each stratum and mark its value on a skeleton plan of the field and so attempt to draw a 'contour' map for each of the 18

determinations. While this would afford some simplification in the presentation of the data the number of maps involved would still leave much to be desired.

For each nutrient, however, a principal component analysis can be carried out on the six concentrations resulting from the different strata. When this is done, it is found that in each case two principal components together account for most of the variation, namely 91% of the total variation in P over all depths, 89% of the total variation in K, and 87% of the total variation in Mg. The vectors of these components are shown in Table I. For P and K they correspond to the concentration in the topsoil, and to the difference between the top soil and the rest of the profile. For Mg they represent the total amount throughout the profile (with extra weight attached to the lower strata), and the difference between the top foot and the bottom foot.

If values corresponding to these six functions, i.e., the first two principal components for each of the nutrients P, K and Mg, are calculated and plotted on skeleton maps the problem is reduced from one of 18 maps to one of six, four of which display a considerable degree of 'zoning' or grouping of values (Fig. 1). Thus, high values of 'topsoil P' are discernible to the left, with medium/low values elsewhere; high values of 'topsoil K' are discernible to the right, with medium/low values elsewhere; an area with high values of total Mg throughout the profile is discernible in the centre, tailing off to low values to the left and right, and there is an area to the left where Mg in the lower strata is high relative to that in the upper strata.

The degree of similarity between these maps suggests that the analysis might profitably be taken a stage further by carrying out a principal component analysis on the values of these four derived variates. Table II shows the vectors to result from such an analysis. The first two components are of most importance, the last two, which account for only 10·9% of the total variation, being of little practical significance. Apart from the fact that neither of the first two components appears to be particularly concerned with the concentration of P, they are not easy to interpret since they are, in fact, linear functions of linear functions. It is not difficult, however, to re-express them in terms of the original 18 variates—as has been done in Table III.

TABLE I

Vectors of the first two principal components of the nutrient concentration at successive depths for each nutrient

Component	Vector						% of variation
P1	(0·91	0·40	0·07	0	0·02	0·04)	83
P2	(0·37	−0·73	−0·51	−0·19	0	−0·16)	8
K1	(0·83	0·53	0·11	0·05	0·06	0·03)	70
K2	(0·52	−0·68	−0·47	−0·17	−0·05	−0·05)	19
Mg1	(0·28	0·31	0·30	0·29	0·44	0·66)	49
Mg2	(−0·66	−0·52	−0·18	0·04	0·26	0·41)	38

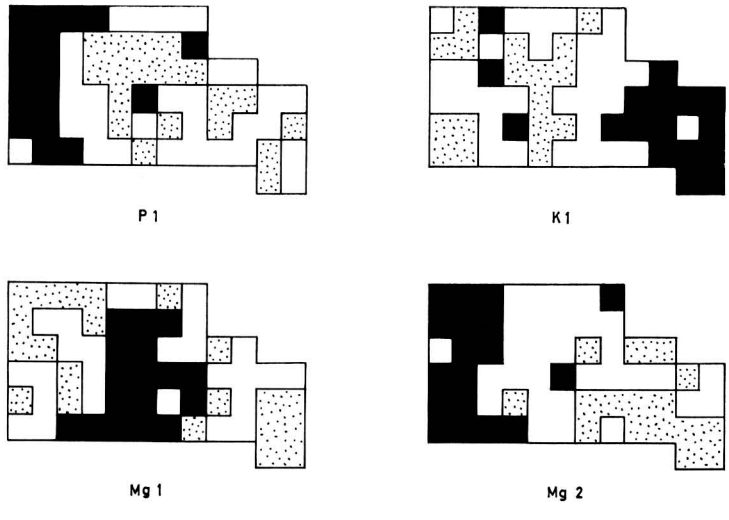


FIG. 1. *Distribution of values of P1, K1, Mg1 and Mg2*

■ High values; □ Medium values; ⁞⁞ Low values

TABLE II

Vectors of the principal components of P1, K1, Mg 1 and Mg 2

Component	Vector				% of variation
X_1	(0	−0·38	0·89	0·26)	50·6
X_2	(0·05	−0·27	−0·38	0·88)	38·5
X_3	(0·03	0·88	0·27	0·38)	10·8
X_4	(−0·99	0·01	−0·01	0·05)	0·1

TABLE III

Vectors of the components X_1 and X_2 in terms of the original determinations

Component	Depth, in	Nutrient			% of original variation
		P	K	Mg	
X_1	0–6	0	0·45	0·10	
	6–12	0	0·29	0·20	
	12–18	0	0·06	0·31	
	18–24	0	0·03	0·38	
	24–30	0	0·03	0·66	
	30–36	0	0·02	1·00	42
X_2	0–6	−0·06	0·32	−1·00	
	6–12	−0·03	0·20	−0·83	
	12–18	0	0·04	−0·40	
	18–24	0	0·02	−0·10	
	24–30	0	0·02	0·10	
	30–36	0	0·01	0·16	32

It can now be seen that the first component, which accounts for 42% of the original variation in all nutrients at all depths, is, broadly speaking, a measure of the amount of K plus the amount of Mg—with emphasis on the top foot for K and the bottom foot for Mg.

The second component, accounting for a further 32% of the original variation, corresponds to a measure of the difference between the amounts of K and Mg in the top foot.

If the values of these two components are now calculated and represented in just two maps, (Fig. 2), wherein 74% of the variation over the field is accounted for, it will be found that the first, K plus Mg, assumes high values in the centre of the field, tailing off to the left and right, while the second, the difference between K and Mg, assumes high values in an area to the left of the field.

In this example, principal component analysis has achieved a considerable condensation of the data by reducing 18 variates into two simple maps with only a slight loss of information. The exercise has, thus far, been purely mathematical with no appeal to nutritional disciplines. However, it may be noted that the distinctive zone demarcated to the left of the field corresponds closely to an area of shallow soil (Fig. 3a) as shown on the soil map of the area.[6] Furthermore, Pearce[7] carried out a similar analysis of various growth measurements made on trees grown in this field. One of the components to emerge from this analysis was a measure of the tree's ability to fulfil its early promise. When quantitative values for this component are calculated and represented graphically as has been done for the soil data, the same distinctive zone (Fig. 3b) results.

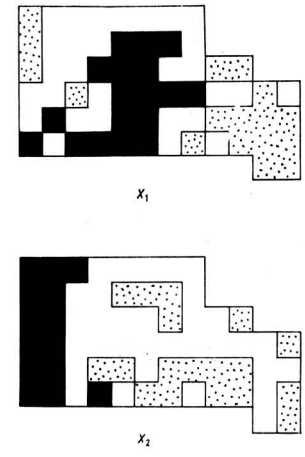
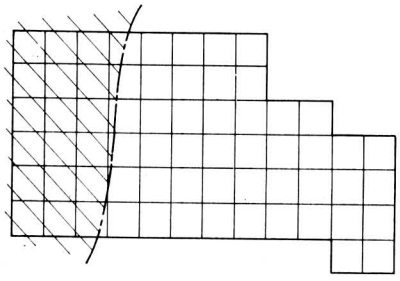
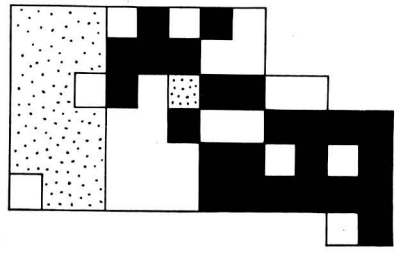


FIG. 2. *Distribution of values of* X_1 *and* X_2
■High values; □ Medium values; ⁞⁞⁞ Low values



(a)



(b)

FIG. 3. a) *Location of area of shallow soil*
▩ Shallow soil
b) Distribution of values of 'ability to fulfil promise'
■ High values; □ Medium values; ⁞⁞ Low values

Extension of the principal component analysis approach

So far principal components have been regarded as simple algebraic expressions of the original observations. There is, however, an alternative, geometric, viewpoint which has the advantage of extending the approach.

This view can best be illustrated through an example. When two variates are observed, the data can be represented by means of a scatter diagram. The shape of the resulting 'cloud' of points would be a feature of the interrelationship between the two variates. The points in the diagram can be re-defined in terms of two new axes, corresponding to the principal axes of the ellipse containing the cloud. This has the advantages that the two new values defining each point will be independent of each other and that while one corresponds to the direction of maximum variation, the other corresponds to the direction of minimum variation. They are in fact the principal components.

Furthermore, the coefficients in the vector of a principal component will be the cosines of the angles the component makes with each of the original axes. This can be constructed in three dimensions while, mathematically, the concept can be extended to any number of dimensions.

Instead of occupying the whole of the available space, the cloud may be represented by fewer dimensions (or at least sufficiently so for practical purposes) and so needs fewer components to define it. For example, three variates may be observed, but all the points might lie in a plane, which only needs two axes to define it.

Having adopted a geometrical, or spatial, concept of principal component analysis it now becomes possible to enquire whether or not some hypothetical component is consistent with a set of observed results. If the hypothetical component is regarded as a direction, the problem is to determine how closely this direction lies within the space defined by the principal components, and thus can be measured as the angle between the hypothetical direction and the direction within the principal component space that is most nearly parallel to it. For example, it may be possible to represent the observations in a plane such as that of this text, and a hypothesis by some direction inclined to the plane. The extent to which the observations are in accordance with the hypothesis can be assessed by the angle between the hypothetical direction and its projection on to the plane. If this angle is small, the hypothesis may be accepted; if it is large, the hypothesis is rejected.

If a hypothetical component is found to be acceptable it may be preferable to use it in any definition of the space occupied by the observations. Thus, while still giving a simple summary of the data it is possible to transform principal components into other components more useful or meaningful to the purpose in hand—even though they will no longer correspond to directions of maximum variation, next greatest variation etc.

Example of extension of principal component analysis

The problem was to identify qualitative effects of ploughing and manuring on the nutritional status of a soil. It arose in the course of an experiment intended to study methods of restoring soil uniformity after grubbing an orchard and before replanting a further trial.[8–10]

Immediately after the mature orchard had been removed, the area was divided into strips which were ploughed to depths of either 9 or 18 in. Each of these strips was further divided into two to receive either a rich or a poor manurial programme during the ensuing rotation of arable crops and grass ley. There were thus four differently treated soils—shallow-ploughed and poorly manured, shallow-ploughed and richly manured, deep-ploughed and poorly manured, and deep-ploughed and richly manured—which can be designated SP, SR, DP and DR, respectively.

Four and a half years after grubbing, soil samples were taken from the top 9 in and analysed for nitrate N, P, K and Mg, and a principal component analysis was carried out on these four nutrients, for each soil separately.

The first two principal components were sufficient to account for most of the variation in each soil (Table IV). Thus, if it were possible to construct a four-dimensional scatter diagram then it would be found that all the points would, very nearly, lie in a plane.

There are certain observable similarities between the components of one soil and those of another. For example, both SP and DP tend to show a component which represents the amount of N alone; the first components of both SP and DR are similar in representing the total of K and Mg. The extent to which these planes are coincident in space, i.e. these pairs of components represent alternative definitions of the same plane, can then be investigated. Data from all soils can be pooled and, after 'between-soils' differences have been eliminated, a further principal component analysis can be carried out to give what might be termed the principal components of the average 'within-soil' space. The first three of these are those given in Table V, the fourth accounting for so little of the variation as to be negligible.

If each of these is taken in turn, the angle it makes with the plane representing each soil can be calculated; the results are set out in Table VI. Both X_1 and X_2 lie close to the plane of the richly manured soils, while only X_3 lies close to the planes of the poorly manured soils.

If only X_3 lies close to the planes of the poorly manured soils, it becomes necessary to determine the other axis, at right angles to X_3 but still in the plane, in order to complete each definition. In both cases this turns out to be—

$$Z=(0\cdot2,\ 0,\ 0\cdot7,\ 0\cdot6)$$

The proportion of the total variation in each soil which is attributable to X_1, X_2, X_3 and Z—which are no longer principal components—can now be calculated (Table VII) and it may be concluded that the poorly manured soils exhibited variation in X_3 (amount of nitrate-N) and Z (total amount of K and Mg) with possibly more variation in N and less in the total of K and Mg when deeply ploughed. while the richly manured soils exhibited variation in X_1 (amount of K) and X_2 (amount of Mg) with less variation in K and more variation in Mg when deeply ploughed.

Analyses of variance on the original data showed only that

TABLE IV

Vectors of the first two principal components of N, P, K and Mg for each soil

Soil	Component	Vector				% of variation
SP	V_1	(0·19	0·04	0·59	0·78)	69
	V_2	(−0·97	−0·01	−0·01	0·23)	24
SR	V_1	(−0·05	−0·05	−0·99	−0·03)	77
	V_2	(0·14	0·02	−0·03	0·99)	17
DP	V_1	(0·43	−0·01	−0·85	−0·30)	49
	V_2	(0·86	0·01	0·29	0·43)	32
DR	V_1	(−0·04	0·02	−0·80	−0·60)	56
	V_2	(0·14	−0·06	−0·60	0·78)	29

TABLE V

Vectors of the first three principal components of N, P, K and Mg for all soils

Component	Vector				% of variation
X_1	(0·06	0·03	0·94	0·34)	59
X_2	(0·16	−0·04	−0·35	0·92)	25
X_3	(0·98	−0·01	0	−0·18)	14

TABLE VI

Angles of coincidence between the components X_1, X_2, and X_3 and the planes representing each soil

Soil	Component		
	X_1	X_2	X_3
SP	34°	57°	3°
SR	3°	4°	89°
DP	29°	64°	24°
DR	4°	12°	86°

TABLE VII

Percentage variation in each soil attributable to X_1, X_2, X_3 and Z

Soil	% variation attributable to:				Total
	X_1	X_2	X_3	Z	
SP	0	0	24	66	90
SR	71	23	0	0	94
DP	0	0	30	42	72
DR	53	46	0	0	99

deep ploughing with rich manuring increased variation in all nutrients while rich manuring increased the mean levels of P and K.

Conclusions

It must be emphasised that principal component analysis is no more than a mathematical manipulation of numerical data with no foundations whatsoever in chemistry or nutritional science. While the mathematics may be valid, the validity of their application in any particular context must be judged by their results.

In the two examples which have been cited, principal component analysis has lead to the definition of independent linear functions of the data with the added property that they represent quantities of maximum variation, next greatest variation etc. By virtue of this last property it has been possible to condense the data. From this summary, and by the application of some geometrical manipulation, it has been possible to derive alternative independent linear functions of the original variates which have the added properties of being either meaningful or consistent.

This approach is not new but it is only in recent years, with high-speed computers to deal with the cumbersome calculations involved, that it has been applied to much extent and found to give useful results in a wide range of problems—social, economic, physical, and biological. From the above examples it would appear that a component analysis approach offers considerable possibilities as an aid to the interpretation of soil analyses.

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QUANTITY/INTENSITY RELATIONS IN SOILS AND THE POTASSIUM NUTRITION OF THE STRAWBERRY PLANT *(FRAGARIA* SP.*)*

By E. G. BRADFIELD

In glasshouse pot experiments over two years, the strawberry plant used potassium primarily from sources which were in instantaneous equilibrium with the soil solution. Leaf potassium concentration at flowering and fruiting was highly correlated with the initial equilibrium potassium activity ratio (AR_K^o) of the soil, and a linear relationship existed between $\frac{1}{\text{Leaf-K}}$ and $\frac{1}{AR_K^o}$. Yield of fruit was less well correlated with AR_K^o than with the quantity of labile potassium in the soil. The gradient of the straight line part of the quantity/intensity (Q/I) graph was increased for 2 of the 5 soils after the period of intensive cropping; this could be related to an increase in the amount of exchangeable calcium + magnesium in the soil.

Introduction

By controlled sand culture experiments it is possible to obtain leaf nutrient concentrations associated with optimum growth and yield of strawberry plants.[1] However, it is still desirable to be able to relate plant performance to measurements of soil nutrient status in order that any major soil deficiency may be corrected, using the minimum of fertiliser, before planting. Leaf analysis may then be used in the following year as a check on the nutritional status of the plant.

The work of Beckett[2,3] has shown that equilibrium soil activity ratios, and the relations between these ratios, (I) and the labile potassium of the soil, (Q), may be useful in studies of the potassium nutrition of plants, and Beckett *et al.*[4] have carried out pot experiments with cauliflower, barley and bean plants over a three-month cropping period to relate potassium uptake by these plants to the amount of labile potassium removed from the soil. For plants grown over a longer period of time (e.g. 1 year), the extent to which the labile soil potassium is replenished from reserves of fixed potassium may also become important.

A glasshouse pot experiment has been carried out over a period of two years to study the relations between the labile potassium in the soil and the uptake of potassium by the strawberry plant, yield of fruit and leaf composition, and whether the forms of the Q/I curves of the soils were unchanged after a period of intensive cropping.

Experimental

Treatments and layout

Five soils were used in the experiment: calcareous high-K, (A); calcareous low-K, (B); acid low-K, (C & D); and organic, (E).

Some of their properties are given in Table I.

The soils were laid out in random plots in three blocks; each plot consisted of three pots. 1500 g of air-dry soil (1200 g for E) was thoroughly mixed with 1500 g (1800 g for E) of a water-washed non-calcareous coarse pit sand and placed on top of a layer of pea gravel, supported by a piece of coarse-mesh Tygan, in a 21·5 cm diameter plastic pot. The pots stood in plastic saucers (to prevent leaching of nutrients), and water was applied to the base until the soil was moist throughout.

TABLE I

Properties of soils used before and after cropping

Soil type		Total K, %	pH (0·01 M-$CaCl_2$)	C.E.C.*	Ex. K	Ex.(Ca + Mg)	C, %
					mequiv./100 g		
A	before	0·67	7·3	15·8	0·615	15·2	2·06
	after		7·7	17·4	0·146	17·3	2·23
B	before	0·35	7·2	11·3	0·185	11·1	1·93
	after		7·6	11·6	0·064	11·5	1·84
C	before	1·38	4·9	9·9	0·128	3·1	1·72
	after		4·6	10·1	0·046	4·9	1·55
D	before	1·38	4·7	10·3	0·108	3·1	1·64
	after		4·6	9·9	0·036	4·1	1·72
E	before	0·20	7·3	26·5	0·346	26·2	4·76
	after		7·2	23·3	0·123	23·2	4·45

* Cation exchange capacity

Values refer to the actual soil : sand mixture used in the experiment

Strawberry runners, cv. Cambridge Vigour, were taken from virus-free mother plants at the end of July 1965, rooted in sand in a mist propagator and planted into the pots at the beginning of September. A previous experiment had shown that the initial potassium content of the runner had no effect on any of the recorded soil/plant parameters, irrespective of the initial potassium status of the soil.

Nutrient solution (without potassium) was supplied to the base of the pots in such quantities that the surface of the soil was kept moist; the composition of the nutrient, based on the standard Long Ashton solution (as described by Hewitt[5]), contained:

NO_3^- 16, SO_4^{2-} 2, PO_4^{3-} 4, Ca 16, Mg 2 and Na 1·33 mequiv./l; Fe (as Fe-EDHPA), 5, Cu 0·064, Mn 0·55, B 0·33, Zn 0·065 and Mo 0·019 ppm. Rainwater was used for all dilution of nutrients.

Pest and disease control

The plants were sprayed with 'Metasystox' in early March against aphids and a cover of dispersable sulphur was maintained on the developing leaves against mildew and *Botrytis*.

Records and methods of analysis

Soil samples were taken at time of potting (September 1965) and final harvest (July 1967) for the determination of potassium activity ratios and *Q/I* relations by the following method, which is essentially that described by Beckett.[2,3] 10 g portions of air-dry soil were shaken for 10 minutes at 25° with 20 ml of solutions containing 0, 0·1, 0·3, 0·5, 0·7, 1·0, 1·4, 1·75 and 2·0 m mole/l potassium and 10 m mole/l calcium.

For each extract, the ratio $\frac{a_K}{\sqrt{a_{Ca+Mg}}}$ i.e. AR_K, *I*, in the equilibrium filtrate was calculated from measurements of the potassium, calcium and magnesium concentrations using activity coefficients from the second approximation to the Debye–Huckel equation and plotted against the amount of potassium (as mequiv./100 g air dry soil) gained ($+\Delta K$) or lost ($-\Delta K$) by the soil, (*Q*).

In 1966, leaf samples were taken at the flowering and fruit-ripening stages of growth, and the concentration of major elements was determined using the methods described by Bould.[1] In 1966 and 1967 the number and yield of fruit were recorded, and the potassium content of a sub-sample was determined. After fruiting, the plant tops were harvested (including dead leaves) and the total weight and K content were measured. The total potassium uptake by the plant over a period of 2 years was calculated from these data. The roots could not be analysed because of soil contamination, but it was considered that their potassium content would be small in comparison with the total present in the leaves and fruit.

Total potassium in the soil was determined by flame photometry after digestion of the sample with perchloric/hydrofluoric acid. The pH of the soils, before and after cropping, was determined in a 1 : 2·5 soil : 0·01 M-$CaCl_2$ suspension. The cation exchange capacities (C.E.C.) of the soils were measured by the method of Bascomb.[6] Exchangeable potassium was determined by leaching with N ammonium acetate. For the calcareous soils (A, B and E) it was assumed that the exchange complex would be saturated with calcium and magnesium; the value taken as exchangeable calcium plus magnesium was therefore the cation exchange capacity minus the exchangeable potassium. For the non-calcareous soils (C and D), soluble salts were removed by leaching with 40% ethanol, followed by removal of exchangeable calcium and magnesium by 0·2 N barium chloride/triethanolamine at pH 8·1. Organic carbon was determined by the rapid Walkley-Black method.[7] All potassium determinations were made by flame photometry and all calcium and magnesium determinations by atomic absorption spectroscopy.

Results

Soil potassium relations

Q/I *relations*

An idealised *Q/I* relation of a soil, before and after cropping, as described by Beckett *et al.*,[4] is shown in Fig. 1. It consists of two parts. The upper linear part describes the exchange behaviour of potassium held at sites showing no marked preferential affinity for K as opposed to (Ca+Mg); extrapolation to $AR_K = 0$ gives K_{ns}, the amount of potassium held at these sites. The gradient of this linear part is called the potential buffering capacity of the soil (PBC_K) and is a measure of the capacity of the soil to maintain the equilibrium soil activity ratio against depletion of labile soil potassium. The lower curved portion represents the exchange behaviour of potassium at sites which have a specific affinity for K as opposed to (Ca+Mg). Extrapolation of the curve to $AR_K = 0$ gives a measure of K_L, the total amount of potassium in equilibrium with the soil solution and ($K_L - K_{ns}$) gives a measure of K_s, the amount of potassium held at sites with a specific affinity for K. K_L and K_s cannot be measured accurately in this way because of inaccuracies in extrapolation. The equilibrium soil activity ration AR_K^o, is the value of $\frac{a_K}{\sqrt{a_{Ca+Mg}}}$ in the equilibrium soil solution at which potassium is neither gained nor lost by the soil. After a period of plant growth, the soil will be depleted of some of its labile potassium

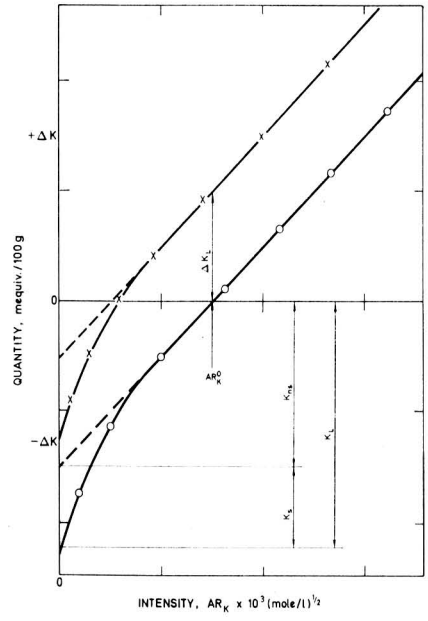

FIG. 1. *Idealised* Q/I *relation of a soil*
○ before cropping; × after cropping

(ΔK_L) and the *Q/I* curve will be transposed vertically by this amount.

Figs 2–6 show the actual *Q/I* relations of the experimental soils before and after cropping. From these graphs, values of K_L, K_{ns} and K_s were measured, and these data together with potassium exchangeable to ammonium acetate (K_{ex}) and the potential buffering capacity (PBC_K) are shown in Table II.

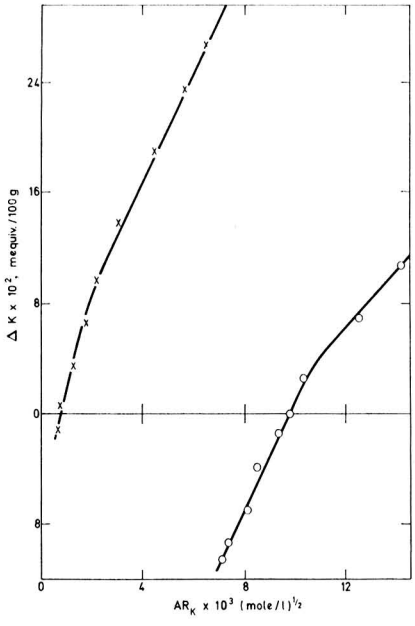

FIG. 2. Q/I *relation of soil A*
○ before cropping; × after cropping

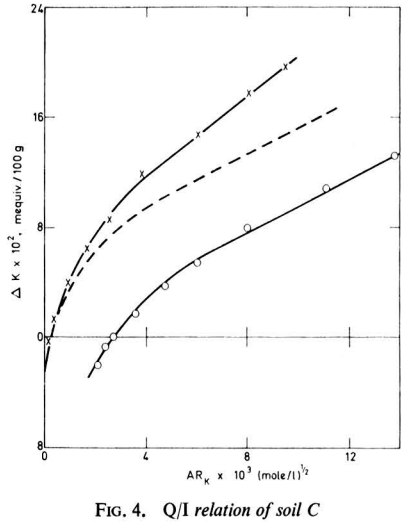

FIG. 4. Q/I *relation of soil C*
○ before cropping; × after cropping
– – – Relation after cropping, corrected to constant complement of exchangeable calcium and magnesium

From Figs 2–6 and Table II the following points may be noted:

The *Q/I* relation of soil E showed no lower curved portion and all the labile soil-K was held at non-specific sites.

For soils A and B it can be seen that the potassium held at the non-specific sites was readily available to the strawberry plant and was reduced to zero after the cropping period; that held at specific sites was less readily available, and K_S had a measurable value, even for the very deficient soils C and D, after the period of plant growth.

The potassium exchangeable to ammonium acetate was greater than the labile-K in equilibrium with the soil solution. Similar findings were reported by Beckett *et al.*[4]

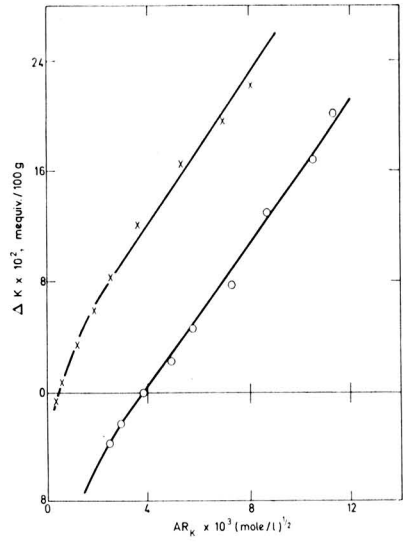

FIG. 3. Q/I *relation of soil B*
○ before cropping; × after cropping

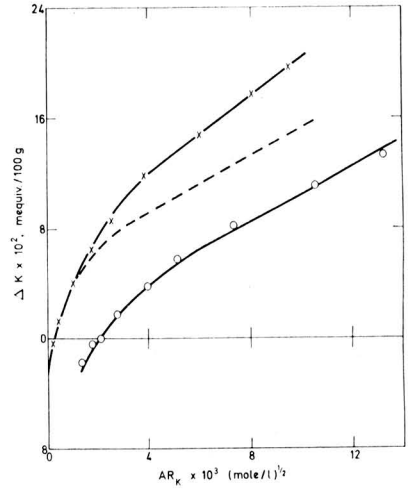

FIG. 5. Q/I *relation of soil D*
○ before cropping; × after cropping
– – – Relation after cropping, corrected to constant complement of exchangeable calcium and magnesium

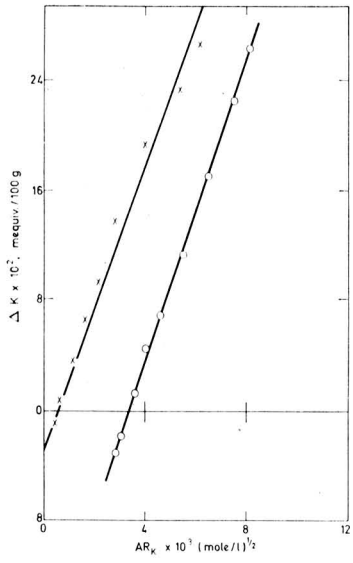

FIG. 6. Q/I *relation of soil E*
○ before cropping; × after cropping

A significant increase was observed in the potential buffering capacity of soils C and D after a period of intensive cropping. Table I shows that the exchangeable (Ca+Mg) of these soils has increased by about 30% over this period. Since the potential buffering capacity of a soil is a function of its complement of exchangeable (Ca+Mg)[3], an increase in PBC_K is to be expected.

Assessment of the total power of the soil to supply potassium

The water percolation technique of Matthews & Smith[8] was used as a measure of the extent to which the labile potassium of the soils was likely to be replenished from non-labile sources. Total potassium released from the soils, before and after cropping, as a function of time of percolation is shown in Fig. 7. For each soil, the potassium removed by continuous water percolation may be divided into two parts, one an easily available portion (K_a) which is readily leached from the soil, and the other, a less available portion which is removed at a constant rate. By extrapolating the constant-rate part of the curve back to its intercept at zero time, a measure of the easily available portion of soil potassium can be obtained. Values for the soils before cropping are shown in Table III together with the labile soil potassium K_L (measured by Beckett's procedure) and the potassium exchangeable to ammonium acetate before and after continuous water percolation.

In these soils the same portion of the soil potassium is measured by K_a and K_L, with the exception of the organic soil E, in which the potassium removed by continuous percolation is greater than the value obtained for the labile-K. Table III also demonstrates that a portion of the potassium exchangeable to ammonium acetate is not in exchange equilibrium with the soil solution. The difference in exchangeable potassium, before and after continuous percolation, is of the same order as the easily available potassium removed from the soil.

TABLE II

Soil-K measurements before and after cropping

Soil type	AR^{o}_{K} $(M/l)^{\frac{1}{2}}$	K_L*	K_{ns}	K_s*	K_{ex}	PBC_K	Sig. diff. between PBC_K measurements ($P = 0{\cdot}05$)
		mequiv./100 g air dry soil					
Before cropping							
A	0·0098	0·560	0·379	0·181	0·615	39	A: 7
B	0·0038	0·148	0·100	0·048	0·185	25	B: 5
C	0·0027	0·100		0·100	0·128	10	
D	0·0021	0·076		0·076	0·108	10	C: 2
E	0·0034	0·182	0·182		0·346	56	D: 2
After cropping							
A	0·0008	0·100		0·100	0·146	36	E: 13
B	0·0004	0·040		0·040	0·064	20	
C	0·0002	0·028		0·028	0·046	13	
D	0·0002	0·026		0·026	0·036	13	
E	0·0005	0·028	0·028		0·123	56	

* Values uncertain because of inaccuracies in extrapolation of the *Q/I* curve

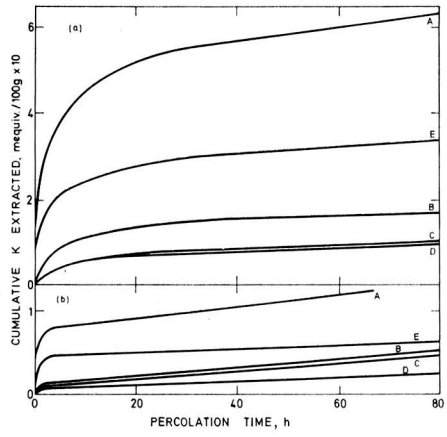

FIG. 7. *Rate of release of potassium from the experimental soils A–E by continuous water percolation (a) before and (b) after cropping*

TABLE III

Measurements of labile potassium in the soils
mequiv./100 g air dry soil

Soil type	K_a	K_L	K_{ex}		Difference
			Before	After	
A	0·513	0·560	0·615	0·064	0·561
B	0·146	0·148	0·185	0·028	0·157
C	0·069	0·100	0·128	0·021	0·107
D	0·064	0·076	0·108	0·028	0·080
E	0·269	0·182	0·346	0·056	0·290

K_a – potassium readily leached from the soil by continuous water percolation
K_L – labile soil potassium from *Q/I* graph
K_{ex} – potassium exchangeable to ammonium acetate before and after continuous water percolation

TABLE IV

Potassium uptake by the strawberry plant and the decrease in amount of labile soil-K as a result of cropping
mequiv. K/100 g air dry soil

Soils	A	B	C	D	E
K removed from soil by crop (K_R)	0·451	0·136	0·087	0·095	0·182
Reduction in labile K (ΔK_L) from Q/I relation	0·396	0·115	0·076	0·068	0·154
Decrease in K_{ex} as a result of cropping	0·469	0·120	0·082	0·072	0·223
Decrease in K_a as a result of cropping	0·436	0·133	0·062	0·056	0·226

Soil-K/plant-K relations

Relations between uptake of potassium by strawberry plants and various soil parameters

The amount of potassium taken up by the strawberry plant over two growing seasons was calculated from weights of dry matter and fruit produced and their respective potassium concentrations. These are shown in Table IV together with ΔK_L values (decrease in labile-K after the period of plant growth), the decrease observed in the potassium exchangeable to ammonium acetate on cropping, and the difference in the amount of easily available potassium released from the soils by continuous percolation, before and after cropping.

Because the gradient of the *Q/I* graph (PBC_K) is greater after the period of cropping, an accurate measurement of ΔK_L is not possible for soils C and D since the vertical separation of the two graphs is not constant. An approximate, but constant, value for ΔK_L in these soils was obtained by redrawing the *Q/I* curves. The graph for the soil after cropping was split into its straight line and curved portion as described by Beckett.[11] The straight-line portion was redrawn after multiplication of the ΔK values by the ratio of $(Ca+Mg)_{ex}$ before and after cropping, and the curved portion was added; this gave a *Q/I* curve based on the value of $(Ca+Mg)_{ex}$ for the soil before cropping. This procedure relies on the assumption that the Gapon constant for the potassium–(calcium+magnesium) exchange reaction in these soils is unchanged by a period of intensive cropping; this is probably justified because application of the above procedure resulted in *Q/I* graphs of unchanged slope, before and after cropping, for soils C and D (Figs 4 and 5).

The evidence thus suggests that most of the potassium available to the strawberry plant in these soils came from that portion which was in rapid exchange equilibrium with the soil solution.

Soil parameters, yield of fruit and leaf composition

Table V shows values for concentration of leaf potassium at flowering (K_{fl}) and fruit ripening (K_{fr}), total yield of fruit (Y) and various soil parameters for the 1966 season only. In 1966 plants growing on soils C and D showed severe symptoms, on B, moderate symptoms, and on E, slight symptoms of potassium deficiency at the fruit-ripening stage of growth. Those growing on soil A showed no such symptoms. Comparison of leaf %K at fruit ripening with the figures suggested by Bould[1] (i.e. that K limits yield below 1·0% and deficiency symptoms appear at K = 0·5%), were generally in agreement with the yields and visual appearance of the plants, although the value of leaf K for plants growing in soil E was less than would be expected in relation to symptoms and yield of fruit. Leaf concentrations of N, P, Ca, and Mg were all above the critical levels suggested by Bould;[1] none of these elements was therefore likely to have limited plant performance.

These data show that the relation between soil K and leaf K or fruit yield is curvilinear and may best be represented graphically by a reciprocal plot, as described by Nelder:[9]

$$\frac{1}{y} = \frac{a}{x} + c$$

where y and x are the related variables and 'a' and 'c' are constants. The limiting value of y as $x \longrightarrow \infty$ is given by $\frac{1}{c}$. In this experiment a linear relationship was found to exist between $\frac{1}{y}$ and $\frac{1}{x}$ and degrees of correlation are shown in Table VI.

Leaf K is well correlated with the initial equilibrium activity ratio of the soil solution, the amount of labile K, the amount of ammonium acetate-exchangeable K and the amount of easily available K removed from the soil on percolation.

TABLE V

Initial potassium in the soil, concentration of potassium in the leaf and yield of fruit in 1966

Soil type	Initial AR^o_K $(M/l)^{\frac{1}{2}}$	Initial K_L	Initial K_{ex} mequiv./100 g	Initial K_a	K_{fl} % dry wt	K_{fr} % dry wt	Yield(Y), g
A	0·0098	0·560	0·615	0·513	2·41	1·47	365
B	0·0038	0·148	0·185	0·146	1·85	0·87	236
C	0·0027	0·100	0·128	0·069	1·54	0·64	162
D	0·0021	0·076	0·108	0·064	1·50	0·57	194
E	0·0034	0·182	0·346	0·269	1·88	0·64	360

Yield of fruit, however, is less well correlated with the activity ratio (a measure of potassium intensity) than with the various measurements of the quantity of labile K in the soil (K_L, K_{ex}, K_a).

The simple reciprocal relation implies that yield of fruit and concentration of leaf potassium approach zero as the soil potassium supply approaches zero, and form an asymptote towards a constant value as the potassium supply increases. This type of equation has been used by Freeman[10] to relate dry weight production and leaf potassium concentration in the nutrient supply for vegetable crops grown in sand culture. Similarly, it is of interest to calculate the following regression equations between the reciprocals of leaf potassium in strawberry leaves and the initial equilibrium potassium activity ratio of the soil solution from the data in Table V.

$$\frac{1}{K_{fl}} = \frac{7{\cdot}09 \times 10^{-4}}{AR^o_K} + 0{\cdot}347$$

$$\frac{1}{K_{fr}} = \frac{29{\cdot}4 \times 10^{-4}}{AR^o_K} + 0{\cdot}458$$

From these equations a limiting value of K_{fl} and K_{fr} may be calculated as the initial intensity of potassium supply in the soil solution (AR^o_K) approaches infinity. These values indicate that the potassium concentration in strawberry leaves, sampled when just fully expanded, is unlikely to exceed 2·9% at flowering and 2·2% at fruit-ripening stages.

Discussion

The *Q/I* relations of the five soils used were of the form described by Beckett[11] with the exception of the organic soil E, the *Q/I* graph of which showed no lower curved portion and in which all the labile potassium was held at non-specific sites. Table I shows that this soil, when mixed with sand in the proportion of 2 : 3, had an organic carbon content of 4·8%. Thus the original soil had a carbon content of 12% (= approximately 20% organic matter). It is generally considered[12] that all the potassium in organic soils is present in a readily exchangeable form, and the results of Salmon[13] show that in a peat soil the *Q/I* relation for potassium is not curved at the lower levels.

After 2 years of intensive cropping, the gradient of the straight-line part of the *Q/I* relation (PBC_K) of the soils was unchanged except for the acid soils C and D for which a significant increase was observed (Table II). The potential buffering capacity of a soil is a function of its complement of exchangeable calcium plus magnesium[3] and if this value increases, either because of an increase in the cation exchange capacity of the soil or by replacement of ions (other than potassium) at the exchange sites by calcium and magnesium, the PBC_K would be expected to increase. Table I shows that soils C and D have an unchanged exchange capacity but an increased value for exchangeable (Ca + Mg), presumably owing to replacement of some exchangeable ions (other than K) by (Ca + Mg) from the nutrient solution applied to the pots. If it is assumed that the Gapon constant for the potassium–(calcium and magnesium) exchange reaction in the soil is unchanged by cropping, then the PBC_K of soils C and D will be directly proportional to their complements of exchangeable (Ca + Mg), before and after cropping. Thus PBC_K after cropping, multiplied by the ratio of exchangeable (Ca + Mg) before and after cropping, should equal PBC_K before cropping. That this is correct, within the limits of experimental error, is shown in Table VII.

The potassium in these soils could be divided into three parts: a part which was in exchange equilibrium with the soil solution and held at sites which were non-specific for potassium; a smaller part, in exchange equilibrium with the soil solution but held at sites more specific for potassium than calcium and magnesium; and a part which was released slowly and at a constant rate on continuous percolation of the soil with water. This potassium was not in rapid equilibrium with the soil solution, as measured by the procedure of Beckett.

TABLE VI

Correlation coefficients between reciprocals of leaf K, yield and various soil parameters

	$1/AR^o_K$	$1/K_L$	$1/K_{ex}$	$1/K_a$	1/Yield
$1/K_{fl}$	0·97**	0·98**	0·95**	0·95**	0·86*
$1/K_{fr}$	0·96**	0·97**	0·94*	0·93*	$0·82^{ns}$
1/Yield	$0·72^{ns}$	$0·82^{ns}$	0·90*	0·93*	—

** P = 0·01; * P = 0·05; ns = not significant

TABLE VII

Values of PBC_K of soils C and D corrected to constant $(Ca+Mg)_{ex}$

	PBC_K before cropping	PBC_K after cropping	PBC_K after cropping corrected to constant $(Ca+Mg)_{ex}$
C	10	13	8
D	10	13	10

The first two portions of potassium are those described by Beckett.[11] That which is held at non-specific sites (K_{ns}) is readily available to the strawberry plant whereas that held at specific sites is not so readily available and cannot supply sufficient potassium for optimum growth. The third portion was of little importance as a source of potassium for strawberry plants. In all the soils studied, 85–90% of the potassium taken up by the strawberry plant came from the part which was in exchange equilibrium with the soil solution (Table IV). The results indicate that some of the potassium measured by ammonium acetate exchange was not in instantaneous equilibrium with the soil solution. All five soils released potassium only very slowly from sources not initially in exchange equilibrium with the soil solution. It was unfortunate that no soils which rapidly released potassium from initially non-labile sources (cf. some of the soils used by Matthews & Smith[8]) were included and that no assessment of the availability of this type of potassium to the strawberry plant could therefore be made.

This preliminary experiment has shown that the strawberry plant primarily takes up potassium from sources which are in rapid equilibrium with the soil solution. The intensity and capacity of this supply can be measured by the procedures introduced by Beckett[3] but, before these functions can be fully utilised as a guide to the potassium fertilisation of soils for strawberry production, more information is needed on the uptake of potassium by the plant at different stages of its physiological development, the minimum potassium intensity required in the soil solution to provide for this uptake, and how the intensity of supply is buffered against depletion in relation to release and fixation of potassium from sources not initially in rapid exchange equilibrium with the soil solution.

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COMPOSITION OF ADIPOSE TISSUE TRIGLYCERIDES OF NEONATAL AND YEAR-OLD LAMBS

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

Triglycerides were isolated from perinephric (internal) and subcutaneous (external) adipose tissue obtained from neonatal lambs and from lambs which had been fed, until they were a year old, on a semi-synthetic ration or on a diet of grass cubes. The triglycerides were analysed for their fatty acid composition (including *trans* unsaturated acids) and for the intramolecular distribution of these acids between the primary and secondary alcoholic groups of the glycerol moiety.

Whereas in the year-old lambs (and in adult sheep previously examined), the triglycerides of internal adipose tissue had a higher content of stearic acid and *trans* unsaturated acid than those of external tissues, the triglycerides from the perinephric and subcutaneous tissues of the neonatal lambs were very similar in fatty acid composition. Palmitic acid and C_{18} mono-unsaturated acid together constituted more than 80% of the total acids. This composition resembles that of the subcutaneous triglycerides of the grown animal and suggests that, at all stages of growth, the triglycerides of external tissues are largely the result of endogenous synthesis.

The fatty acids of the adipose tissues of the neonatal animals did not contain any of the acids of exogenous origin, such as those with *trans* double bonds, which characterise the triglycerides of the growing and mature animals, particularly those of the internal depots. Nevertheless, the intramolecular disposition of the fatty acids in the triglycerides formed *in utero* was similar to that previously observed in triglycerides from both the internal and external depots of the adult sheep. Saturated acids (palmitic and stearic acids) predominated amongst those esterified in the 1- and 3-positions of the glycerol moiety and unsaturated acids (almost entirely oleic acid) were the major components esterified in the 2-position.

While the triglycerides from corresponding body sites in the two groups of year-old lambs were generally quite similar with respect to their content of palmitic acid, stearic acid and C_{18} mono-unsaturated acid, the contribution of *trans* isomer to the total C_{18} mono-unsaturated acid was considerably greater in the tissues (particularly perinephric tissue) of the animals fed on grass cube than in the tissues of those given the semi-synthetic ration. This difference between the two groups of lambs was associated with a corresponding difference in the proportions of C_{18} *trans* unsaturated acid in the lipids of the rumen contents of the animals.

Introduction

Although it has been known for some time that the external (subcutaneous) triglycerides of sheep are more unsaturated than those of internal adipose tissue,[1] it is only recently that analyses of the triglycerides from different body sites of individual sheep have been reported.[2,3] In an earlier paper[3] it was observed that, in common with the triglycerides of most other animal species so far examined, saturated fatty acids predominated in positions 1 and 3 of the glycerol molecules and unsaturated acids (except *trans* isomers) were, for the most part, esterified in the 2-position. As with stearic acid, *trans* isomers (almost entirely C_{18} mono-unsaturated acid) were found in greatest concentration in the internal depot glycerides and were similarly preferentially esterified in the 1- and 3-positions. This suggests that the minimal distortion of an otherwise straight chain of carbon atoms, which is occasioned by the presence of a *trans* bond in a fatty acid, leads to its being treated metabolically as if it were a saturated acid.

These observations have now been extended to cover (i) analyses of the adipose tissue triglycerides of neonatal lambs in order to compare the pattern of fatty acid deposition *in utero* with that which obtains in the adult animal and (ii) a study of the composition of adipose tissue triglycerides of lambs raised to one year of age on diets of known composition, with special reference to the extent of deposition of *trans* unsaturated acids.

Experimental

Animals and diet

Seven new-born lambs were used. Animals subsequently referred to as lambs 1, 2 and 3 were still-born and lambs 4 to 7 were slaughtered shortly after birth.

From weaning at ten weeks of age the year-old lambs had been fed to appetite on one of two diets. Three animals (lambs 8, 9 and 19) were fed on a diet of grass cubes, and the other three (lambs 11, 12 and 13) were given a semi-synthetic, pelleted ration consisting, in parts by wt., of oat straw 60, maize starch 15, sucrose 10, casein 10, salts 3 and molasses 2, together with a supplement of a preparation of vitamins A and D_3. The diets differed in the content and composition of their total fatty acids; the grass cubes contained 1·8 g/100 g diet, of which linolenic acid was the principal component (65%) and the semi-synthetic diet contained 0·5 g fatty acids /100 g, of which linoleic acid comprised 50%.

Sampling of tissues and rumen contents

Perinephric adipose tissue (2–5 g) was taken from each neonatal lamb and subcutaneous adipose tissue (1–2 g) was obtained from the chest and rump region of three of these animals (lambs 5, 6 and 7). Immediately after slaughter of the year-old lambs a representative sample of rumen contents (about 250 g) was obtained from each animal. Samples of adipose tissue, each about 10 g, were subsequently taken from the perinephric region, from over the chest and from near the base of the tail of each dressed carcass.

Extraction and analysis of lipids

Using methods outlined previously by Duncan & Garton,[3] the component fatty acids of the triglycerides of each adipose tissue sample were determined by gas–liquid chromatography (g.l.c.), their content of *trans* unsaturated acid by infra-red spectroscopy and their intramolecular disposition by the pancreatic lipase procedure. Free and esterified fatty acids were prepared from 150 g portions of rumen contents as

described by Feliński *et al.*[4] and their composition determined by g.l.c., as for the fatty acids of the triglycerides.

In the Tables and, as appropriate in the text, fatty acids are designated according to the shorthand nomenclature of Dole *et al.*[5] which indicates the number of carbon atoms/molecule, followed by the number of double bonds.

Results and Discussion

In Table I are presented the values for the fatty acid composition of the triglycerides derived from the perinephric and subcutaneous adipose tissue of the neonatal lambs. As in the adipose tissues of adult sheep[3] and of the year-old lambs (Table II), C_{16} and C_{18} components (16:0, 16:1, 18:0 and 18:1) together comprise a very high proportion of the total fatty acids present in the triglycerides of foetal adipose tissue. This finding is in agreement with previously reported analyses of the total neutral lipids[6] and perinephric triglycerides[7] of neonatal lambs and our results confirm the virtual absence from foetal triglycerides of branched-chain fatty acids and *n*-acids with an odd number of carbon atoms and also the complete absence of unsaturated fatty acids possessing *trans* double bonds. Whilst the presence of such fatty acids of exogenous origin is characteristic of most tissue lipids (including plasma lipids) of the adult ruminant,[8] it would appear that plasma triglycerides do not pass the placental barrier and thus are not available for incorporation into foetal adipose tissue. Furthermore, though maternal phospholipids[6] and free fatty acids[9] can apparently traverse the placenta of the sheep, it seems that fatty acids thus transferred to the foetus are not utilised in the formation of triglycerides which must therefore be dependent on the supply of fatty acids synthesised *de novo*.[10] It is therefore not surprising that, as Table I shows, the triglycerides of the perinephric and subcutaneous adipose tissue of the neonatal lambs are very similar in fatty acid composition. This composition resembles that of the triglycerides derived from subcutaneous depots of the adult sheep[3] and of the year-old lamb (Table II) suggesting that, at all stages of growth, the triglycerides of external tissues are largely the result of endogenous synthesis. By contrast, the triglycerides of the internal adipose tissue of adult sheep[3] and of developing sheep (Table II) are apparently markedly influenced by fatty acids of exogenous origin. The possibility that the extent of uptake by a particular tissue of such fatty acids from circulating plasma lipids may be related to the activity of tissue lipases remains to be investigated,[11] as also does the possible effect of the temperature of the tissue on the

TABLE I

Component fatty acids of the triglycerides of perinephric and subcutaneous adipose tissue of neonatal lambs, mol. %

Lamb	Tissue	Fatty acid					
		14:0	16:0	16:1	18:0	18:1*	Others†
1	Perinephric	0·5	21·3	2·3	11·3	64·2	0·4
2	Perinephric	0·7	23·1	2·8	13·9	59·5	tr
3	Perinephric	0·5	17·3	2·2	13·2	66·8	tr
4	Perinephric	0·5	21·2	2·8	10·9	64·3	0·3
5	Perinephric	0·8	21·8	2·6	10·5	64·2	0·1
	Subcutaneous	0·7	20·1	4·2	10·8	62·8	2·2
6	Perinephric	0·7	23·4	2·6	14·5	58·6	0·2
	Subcutaneous	1·4	25·2	3·8	13·6	54·9	1·1
7	Perinephric	2·7	28·8	5·3	8·6	52·4	0·2
	Subcutaneous	1·6	29·2	3·4	13·7	51·6	0·5

tr = trace
* No *trans* isomer present
† Comprising 12:0, 14:1, 15:0, 17:0, 17:1

TABLE II

Proportions of C_{16} and C_{18} fatty acids in the triglycerides of lambs fed on grass cubes or on a semi-synthetic diet, mol. %, (means of three animals)

Source of triglycerides	Fatty acid					
	16:0	18:0	18:1 (total)	18:1 (*trans*)*	18:2	18:3
Lambs 8 to 10 (Grass cube diet)						
Perinephric	19·6	33·6	31·0	12·0	4·0	2·6
Subcutaneous (chest)	24·4	19·5	38·9	8·8	3·1	3·0
Subcutaneous (tail)	22·3	11·7	45·9	6·9	4·4	3·6
Lambs 11 to 13 (Semi-synthetic diet)						
Perinephric	23·0	33·5	30·3	1·6	1·9	0·5
Subcutaneous (chest)	24·7	20·0	41·3	1·2	1·7	tr
Subcutaneous (tail)	22·6	14·1	49·1	1·1	2·1	0·8

tr = trace
* calculated as elaidic acid, i.e. *trans*-9-octadecenoic acid

nature of fatty acids synthesised *de novo* and on the extent of incorporation into triglycerides of fatty acids of both exogenous and endogenous origin.[12,13]

With regard to the intramolecular distribution of fatty acids in the triglycerides of the neonatal lambs, it can be seen from Table III that saturated acids (16:0 and 18:0) predominate amongst those esterified to the primary alcoholic groups (positions 1 and 3) of the glycerol moiety and that 18:1 (exclusively oleic acid) is present as the major component esterified to the secondary alcoholic group (position 2). This disposition pattern resembles that previously reported[3] for the triglycerides of both internal and external depots of the adult sheep and indicates that, regardless of whether the fatty acids are derived from an exogenous or endogenous source, they are treated metabolically in a similar manner during the assembly of triglyceride molecules.

TABLE III

Positional distribution of palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1) in triglycerides of neonatal lambs, mol. % (to nearest whole number)

	Fatty acid		
	16:0	18:0	18:1
Perinephric (lamb 1)			
Triglycerides	21	11	64
2-Monoglycerides	4	4	89
% in 2-position*	6	12	46
Perinephric (lamb 2)			
Triglycerides	23	14	60
2-Monoglycerides	6	4	87
% in 2-position	9	10	48
Perinephric (lamb 7)			
Triglycerides	29	9	52
2-Monoglycerides	5	3	88
% in 2-position	6	11	56
Subcutaneous (lamb 7)			
Triglycerides	29	14	52
2-Monoglycerides	16	10	66
% in 2-position	18	24	42
Subcutaneous (lamb 5)			
Triglycerides	20	11	63
2-Monoglycerides	12	5	74
% in 2-position	20	15	39

* Derived from the expression $\frac{(\text{mol. \% fatty acid in 2-monoglycerides}) \times 100}{(\text{mol. \% same fatty acid in triglycerides}) \times 3}$

The proportions of the major component fatty acids (16:0, 18:0 and total 18:1) were very similar in the triglycerides from the corresponding adipose tissue sites of both groups of year-old lambs (Table II) though, with respect to the minor components 18:2 and 18:3, these acids were present in relatively greater proportions in the triglycerides of the lambs fed on grass cubes. Though the triglycerides from comparable body sites in both groups of animals contained similar proportions of total 18:1 fatty acid, the contribution of *trans* 18:1 to the total differed markedly between the two groups. The triglycerides of the lambs fed on grass cubes contained much higher proportions of *trans* 18:1 than those given the semi-synthetic diet and, as previously observed[3] in adult sheep, this species of fatty acid is deposited to a relatively greater extent in the triglycerides of perinephric adipose tissue than it is in subcutaneous depots.

Acids with *trans* double bonds arise in the rumen during the process of bacterial hydrogenation of dietary polyunsaturated acids (18:2 and 18:3) and it has been shown by Katz & Keeney[14] that, though small amounts of 18:2 and 18:3 with *trans* bonds are present in rumen contents, *trans* 18:1 predominates and this consists mostly of *trans*-11-octadecenoic acid. Following their intestinal absorption[15] and transport in plasma lipids,[16] the *trans* acids become available for incorporation into tissue lipids. It is therefore of some interest to consider the proportions of *trans* unsaturated acid in the adipose tissue triglycerides of the lambs in relation to the amounts present in the lipids of the rumen contents. The results of the analyses of the lipids of rumen contents obtained, at slaughter, from the lambs are summarised in Table IV. It can be seen by reference to Table II that the proportions of *trans* unsaturated acid present in the adipose tissue triglycerides of each group of lambs reflect those present in the lipids of the corresponding rumen contents. These lipids consist mainly of free fatty acids which result from the rapid hydrolysis of dietary lipids by rumen bacteria;[17,18] in one of these studies[18] it was shown that hydrogenation of unsaturated acids could take place whether or not they had been released from

TABLE IV

Proportions of C_{16} and C_{18} fatty acids in the lipids of rumen contents of lambs fed on grass cubes or on a semi-synethetic diet, mol. %, (means of three animals)

Source of fatty acids	Fatty acid				
	16:0	18:0	18:1 (total)	18:1 (*trans*)*	18:2+18:3
Lambs 8 to 10 (Grass cube diet)					
Free fatty acids in rumen†	12·3	60·7	15·2	9·9	4·6
Esterified fatty acids in rumen	21·2	29·5	12·1	4·5	19·6
Lambs 11 to 13 (Semi-synthetic diet)					
Free fatty acids in rumen‡	20·1	45·9	11·0	1·5	4·7
Esterified fatty acids in rumen	27·6	18·7	14·3	None	10·3

* Calculated as elaidic acid, i.e. *trans*-9-octadecenoic acid
† 80% of total fatty acids (186 mg/100 g rumen contents)
‡ 68% of total fatty acids (103 mg/100 g rumen contents)

ester combination in linseed oil. The presence of *trans* unsaturated acid in esterified form in the rumen lipids of the grass-fed lambs is in harmony with this observation, though the possibility that free *trans* acid can be incorporated into bacterial structural lipids is not excluded.

Despite the efficiency of the rumen microbial systems for converting 18:3 and 18:2 to *trans* 18:1 and stearic acid (18:1), it seems that small amounts of these polyunsaturated acids escape hydrogenation in the rumen and accumulate in the tissues, notably blood plasma lipids,[16] biliary lipids[19,20] and adipose tissue triglycerides; the presence of greater proportions of 18:3 and 18:2 in the adipose tissue triglycerides of the lambs given grass cubes than in those of the lambs reared on the semi-synthetic diet is evidently associated with the correspondingly greater amounts of polyunsaturated acids in the former diet.

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LEAD CONTAMINATION IN MINING AREAS IN WESTERN IRELAND

II.*—Survey of animals, pastures, foods and waters

By P. P. DONOVAN, D. T. FEELEY and P. P. CANAVAN

The determination of detectable amounts of lead contamination in blood, foods and biological materials has been a recent problem in relation to the operation of lead mining in the West of Ireland. Following the mining activities, the incidence of lead contamination was investigated in the following ways: (i) lead values in viscera, blood and faeces of dead animals and control of lead contamination in blood, faeces and milk of live animals in mining areas; (ii) examination of pastures in areas adjoining mining operations and road dust; (iii) analysis of foods, milks and waters in the mine vicinity; and (iv) lead content in workers blood due to exposure in mines.

Investigations under (i), (ii) and (iii) are dealt with in this paper. As a result of the toxicity results found in the laboratory, the mining authorities instituted precautionary steps in the mining operations to eliminate lead 'fallout'.

Introduction

The normal blood levels of animals[1] have been ascertained to be in the range of 0·05–0·25 ppm (5–25 μg/100 g) for goats, sheep, horses, cows and calves, and to be about 35 ppm in faeces. In poisoned animals, the highest values are usually in the liver and kidney, particularly in the renal cortex. If there is other evidence of lead poisoning, values higher than 25 ppm in kidney cortex and 10 ppm in liver are of definite diagnostic significance. Levels of 10 to 25 ppm should be regarded as suspicious.[2] Due to concentration in kidney cortex the equivalent lead content in the whole kidney could be regarded as about one-third of the cortex figure. When analytical data are the only evidence available it is the practice to regard calf kidney cortex values of 25 ppm or more and liver lead concentrations of 20 ppm or more (on a wet tissue basis) as sufficient to warrant a conclusion that lead poisoning has been the cause of death.[2] In the results obtained with animals that died in the vicinity of the mines the figures are set out against the above levels. Corresponding data are shown with regard to examination of blood and faeces of live animals and fluctuations on levels on transfer from contaminated areas.

The examination of grass for lead content provides important data. Normal pastures have been found to contain less than 10 ppm but seasonal variation gives figures up to 40 ppm.[3] The analyses of many samples in the mining area gave very much higher figures and the relation of those values with the lethal effect on animals grazing on those pastures is part of the investigation. The intake of lead by the animals from the contaminated vegetation is related to the 'fallout' of lead dust from the mining operations. With extension and improvement of precautions in the mine to prevent the 'fallout' of the ore dust particles, the lead content of the pastures showed a marked decrease. Roadside soil samples taken along the route of the mine lorries gave high values and this could be attributed to spillage and subsequent drainage.

The analysis of food grown in the mining district must be related to the permissible maximum values of lead allowed for human consumption. The figures relating to vegetables and cereals would be important but as a measure of the actual 'fallout' as distinct from grass results, the coverage and washing effect of the rains must be considered. Pasture investigation lends itself to a closer study since the link with animal intake is more direct than with humans whose food is subject to treatment in preparation for consumption. Samples of milk, butter and cream from the area should not alone provide important data relating to food contamination from dairy cows but their analysis is essential from human health hazards. In view of the discharge of the mine effluent into adjoining river water the determination of the lead content is necessary for the safety of animals grazing nearby and also for control of toxic effect on fish and fish life in the rivers. The latter hazard is very pertinent despite the fact that the effluent is subjected to treatment before discharge into the river.

Experimental

Analysis

For lead determination the samples were normally submitted to the wet oxidation process though the dry ashing technique in the muffle furnace at 500° was found to give satisfactory results with grass and some food samples. The lead was then estimated using di-thizone mixed colour method.[4] The mono-colour method[5] gave confirmatory results.

Some determinations on aliquot portions of the wet oxidation sample were carried out with an atomic absorption spectrophotometer, after complexing with di-thizone and extracting with methyl iso-butyl ketone. The results obtained were in agreement with the colorimetric values but in general it was found that the latter method was more applicable on a routine scale to determination in blood samples and the colorimetric method was used in the present work.

Results

Dead animals

During the period of the survey ten animals which had been grazing in fields adjoining the mine died. Post-mortem examinations were carried out and the lead content of the liver, kidney, ruminal contents and faeces was determined. The results obtained are outlined in Table I.

* Part I: *Chemy Ind.*, 1968, p. 1802

The results showed that the first six animals had absorbed toxic doses of lead in the liver and kidney. The veterinary examination also indicated that the symptoms coincided with lead poisoning. Animals Nos 7 and 8 had absorbed a higher than normal amount of lead in the liver and kidney. Veterinary reports indicated that lead poisoning may have been a contributory factor in the death of these animals. Animals Nos 9 and 10 gave normal lead values in the liver and kidney and it was concluded that lead poisoning was not the cause of the death of these animals.

Live animals

On the discovery of the death of the animals, all livestock was changed to pastures remote from the mining area. A constant monitoring survey was carried out and the blood, milk and faeces of 95 of these animals were examined for lead content over a period of 20 months. The results obtained on four of those animals are outlined in Table II. These four animals in Table II were selected because of the fact that the three indices, namely blood, milk and faeces examination were available in each animal at specific intervals. In the remaining cases, such samples were not available but some results obtained are shown in Table III.

The above results indicate a gradual fall in the lead content of the blood after the first two months. After seven months the lead content of the blood had returned to normal in all cases. The lead content of the milk also shows a gradual decline from December '66 to July '67. The lead content of the faeces, however, is more variable and probably changes with the daily eating of the animals.

TABLE I

Lead content of dead animals, ppm

No.	Animal	Date	Kidney	Liver	Ruminal contents	Faeces
1.	Heifer	22.11.66	10	8	810	1360
2.	Cow	22.11.66	9·9	10·1	675	1250
3.	Horse	2. 2.67	6·0	13·6	144	196·0
4.	Cow	19. 2.68	35·0	55·0	85·0	—
5.	Cow	19. 2.68	50·0	30·0	—	—
6.	Cow	19. 2.68	31·0	17·0	788	—
7.	Horse	8. 5.67	5·6	8·6	26	74
8.	Cow	24. 4.67	5·0	6·5	62	64
9.	Cow	16. 1.67	1·9	1·7	67	25·0
10.	Cow	17. 4.67	0·88	0·43	0·39	21·0

TABLE II

Lead content in 4 live cows, ppm

Animal No.	Date	Blood	Milk	Faeces
52553 Cow	30.12.66	0·46	0·19	133·0
	26. 1.67	0·84	0·18	13·5
	13. 4.67	0·67	0·15	98·0
	10. 7.67	0·09	0·06	33·0
62395 Cow	14.12.66	0·34	0·20	24·0
	13. 1.67	0·28	0·22	43·0
	16. 1.67	0·19	0·15	112·0
	10. 7.67	0·2	0·08	93·0
55111 Cow	14.12.66	0·43	0·11	12·0
	13. 1.67	0·16	0·10	16·0
	16. 3.67	0·10	0·05	10·0
	10. 7.67	0·08	0·03	15·0
1132 Cow	30.12.66	0·45	0·20	58·0
	26. 1.67	0·5	0·18	46·0
	13. 4.67	0·44	0·14	42·0
	10. 7.67	0·24	0·07	18·5

Pastures

During the survey period the lead content of 281 samples of grass from fields in the vicinity of the mine was determined. The results ranged from 27 to 16,300 ppm. The highest values obtained were from those fields adjoining the mine and subject to the 'fallout' of the dust particles in the path of the prevailing wind. The results are outlined in Table IV.

The lead content of samples of grass in areas remote from the mine was also determined. The values obtained from grass taken from six areas are listed in Table V.

The results in Table V may be regarded as representing the normal amount of lead in pastures in areas remote from the mine. A comparison of the results in Table IV with those in Table V indicate that the lead content of the samples of pasture from the fields adjoining the mine is very much in excess of normal values. Animals feeding on these pastures would be in danger of absorbing toxic amounts of lead in a relatively short period of time.

Roadside samples of dust

Samples of dust from along the route taken by ore-carrying lorries gave values ranging from 1350–13,000 ppm when collected within a 10 mile radius of the mine. These results compare with values ranging from 175–501 ppm obtained in

TABLE III

Lead content in live animals, ppm

Animal No.	Date	Blood	Faeces	Milk
6597 Cow	14.12.66	0·9	30	—
	30.12.66	0·75	32·5	0·05
	13. 1.67	0·54	43·0	0·04
1429 Cow	14.12.66	0·8	7·4	—
	13. 1.67	0·15	8·0	0·12
	16. 3.67	0·08	16	0·22
	10. 7.67	0·21	—	0·04
Horse	14.12.66	0·21	—	—
	13. 1.68	0·31	—	—
	16. 3.68	0·078	89·0	—
	10. 7.67	0·11	11·7	—
Horse	14.12.66	0·64	—	—
	13. 1.67	0·4	—	—
	16. 3.67	0·16	—	—
	10. 7.67	0·09	—	—
65354 Cow	14.12.66	1·21	220·0	—
	13. 1.67	0·8	13·0	0·2
	10. 3.67	0·70	108·0	—

TABLE IV

Lead content of 281 grass samples, ppm

% of grass samples	Lead content
7·83	0– 100
5·70	101– 200
11·03	201– 300
10·33	301– 400
13·52	401– 500
9·60	501– 600
8·90	601– 700
8·54	701– 800
4·27	801– 900
2·84	901–1000
9·61	1001–2000
7·83	over 2000

TABLE V

Lead content of grass from 6 areas, ppm

Area	Lead content
1	7·8
2	3·0
3	35·0
4	6·5
5	2·5
6	20·0

samples of roadside dust collected in areas remote from the mine. The significance of this lead content is that the dust particles may be blown or washed into the adjoining fields causing pasture contamination.

Food

The lead content of samples of food produced or grown in the mining area was determined. The foods examined were cabbage, milk, butter, cream and parsley. The results obtained are given in Table VI.

The lead content of the cabbage (outer leaves) is above the permissible limits (5 ppm) in some cases but in practice the outer leaves are discarded in preparation for cooking. Washing would also remove dust particles containing lead. One of the results obtained with parsley is also above the permissible limit. The figures for milk, butter and cream are generally satisfactory with regard to health hazard.

Waters

Samples of the river water receiving the mine effluent were examined for lead content. The results obtained with pH and total hardness figures are given in Table VII.

Sample No. 1 was taken at a point ten yards below the entry of the mine effluent into the river. Samples 2–8 were taken at 100 yard intervals downstream and sample No. 9 was taken one mile below point of entry of discharge from the mine.

International Drinking Water Standards require that the lead content should not exceed 0·1 ppm.

Routine analysis of river waters unconnected with the mine effluent discharge for the purpose of regional supply schemes for drinking water showed that the lead content did not exceed this limit. The river concerned, however, is not utilised for purposes of human consumption and in view of the alkalinity and pH values the water would not be regarded as deleterious to fish or fish life. The problem of cattle grazing on the river bank and drinking the water is one requiring investigation and as a precaution live animals were removed from the field adjoining the river around the point of entry of effluent discharge. Subsequent dilution with river flow should ensure the elimination of the lead hazard in this respect.

Conclusions

The investigations show that in a lead mining area a strict monitoring control must be maintained with regard to the hazard of dust 'fallout' from the mining operations. The scattering of those particles on the pasture of the adjoining fields constitute a direct hazard to animal life. This lead contamination was of such an extent as to cause the death of several cattle. Precautions taken by the mine authorities in the mining area and subsequent processing of the ore are considered to have eliminated this danger and the results in Tables II and III would seem to confirm this view. The establishment of a neutral zone around the mine where animals were not allowed access to the pasture also minimised the hazard to animal life in the vicinity.

Apart from the actual toxicity to the animals concerned the need to test food supplies in the area is observed. As well as the direct food consumption of vegetables there is the danger of contamination in milk and butter from cows grazing on such pastures.

In the particular mining area concerned as a result of the control survey many precautions were taken to control dust 'fallout'. It is essential that a system of laboratory testing control with regard to this type of lead contamination be maintained to ensure the safeguarding of animal life and food in the area.

TABLE VI

Lead content of several food samples, ppm

Cabbage (outer leaves)	Cabbage (inner leaves)	Milk (dairy)	Butter	Cream	Parsley	Wheat
2·3	0·05	0·01	0·10	0·10	2·0	6·0
2·4	0·10	0·05	0·21	0·28	18·0	
2·94	0·29	0·07	0·24	0·45		
3·3	0·40	0·14	0·30			
10·0	0·60		0·34			
14·0			0·45			
16·0			1·0			
40·0						
0·4	0·4					
8·0	0·8					
2·4	0·4					
2·3	0·3					
2·94	0·29					

TABLE VII

Lead content of river water samples, ppm

Sample	Total hardness	pH	Lead
1	280	7·7	4·20
2	276	8·5	0·40
3	316	8·0	0·38
4	320	7·9	0·40
5	314	7·8	0·36
6	321	7·8	0·26
7	300	7·7	0·10
8	290	7·9	0·19
9	295	7·8	0·06

In the particular mining area concerned as a result of the control survey many precautions were taken to control dust 'fallout'. It is essential that a system of laboratory testing control with regard to this type of lead contamination be maintained to ensure the safeguarding of animal life and food in the area.

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IN VIVO 'QUANTUM' SYNTHESIS OF FAT IN RIPENING SEEDS OF TWENTYFOUR PLANT SPECIES

By A. R. S. KARTHA and H. S. NAINAWATI

The proportions of partial (incompletely acylated) glycerides present in oils from ripening seeds at different oil development stages were determined by the gravimetric method for 24 plant species belonging to 16 families. 133 samples were examined and 17 of these represented oil development stages below 10%. No detectable amounts of partial glycerides were present in any case; this suggests the operation of the 'quantum' mechanism of fat synthesis in all the species studied.

Introduction

No detectable proportions of mono- and di-glycerides were present in oils from ripening coconut at a series of low oil development stages and this indicated a 'quantum' synthesis mechanism in the formation of coconut oil.[1] The conditions necessary for this mechanism are such that they help to solve other problems related to fat formation in plants. Hence it was interesting to ascertain whether the above mechanism holds good for large numbers of plants. The proportions of partial glycerides present at different oil development stages in 24 plant species belonging to 16 families have hence been determined.

Experimental

Unripe fruits at different stages of growth were collected as far as possible from the same plant or from a group of plants from the same seeds grown in the same plot. In most cases the fruit-coats were removed directly and the seeds weighed to constant weight at 48–50°, but in some cases the seeds were removed only after partly drying the fruits. Special care has to be taken to see that the kernels do not get crushed or broken during removal from the fruit-coat since the damaged kernels show a tendency to develop some acidity during drying. Fats containing higher free fatty acids tend to a continuous decrease in weight for quite some time when the hydroxyl value is determined by the gravimetric method.[1] This is probably due to a slow volatilisation of the higher fatty acid esters and in cases where any hydrolysis has occurred the extracted fats have to be refined with alkali.

When constant weight was reached the seeds were sorted out visually into different sizes. Each batch was comparatively uniform on the basis of seed weights (±2%) and represented a separate seed development stage. Percentage seed development was calculated on the basis of mature seeds as 100%, and percentage oil development on the basis of oil in mature seeds as 100%. Extraction of oil from seeds was done by the cold percolation procedure using carbon tetrachloride as previously described.[1] Since a large variety of seeds with highly different oil contents, namely from trace to 65%, were used, the details of percolation varied somewhat in different cases and are given below. When seeds were extracted without removal of the testa, as for example with sunflower *Cassia occidentalis*, *Brassica* sp. etc., they were first de-waxed by shaking at room temperature for half an hour with three portions of 25 ml of carbon tetrachloride each. The waxy coating on the testa frequently contains free alcohols and in the case of sunflower seeds the waxes thus removed had hydroxyl values of 60–65. The de-waxed seeds were directly ground up with about 3 times their weight of glass powder (Pyrex glass, acid washed) and 5 times their weight of anhydrous sodium sulphate. The efficiency of the cold percolation procedure in effecting rapid and complete extraction is believed to be due largely to initial spreading of the lipids as a monomolecular layer on the surface of the electrolyte.[2] Hence this grinding to fine powder has to be carefully carried out in all cases, particularly in case of tough low oil content material.

When the seeds contained more than 25% oil, 3–5 g were adequate to obtain the required amounts of oil. In these cases a uniform 70–80 ml of percolate was collected. More than 98% of the carbon tetrachloride-extractable lipids however occurred in the first 5–10 ml when the column was packed tightly.

When the seeds contained less than 25% oil, the volume of percolate collected was 200 ml in view of the large bulk of material to be extracted. The unsaturated mono- and diglycerides are liquid and readily soluble in carbon tetrachloride. The intermediate glycerides of saturated acids are solid and more sparingly soluble. When the latter occur in small proportions only their solubility in carbon tetrachloride will naturally be increased by the presence of liquid intermediate and triglycerides which will normally exert considerable intersolubility effects. The extraction of all intermediate glycerides will hence be complete if no materials are present in the seed meal which are capable of holding up mono- and di-glycerides by adsorption. The absence of such material has been verified by the following standardisation.

1·5–2·0 g of a mixture of tri- and intermediate glycerides (prepared from sesamum oil) with hydroxyl value 18·0 (present technique) was mixed with the carbon tetrachloride insoluble 'marc' left after cold percolation of 4 g of ripe groundnut kernels. The ripe seeds were used because mono- and di-glycerides are generally thought to be absent in these and if such adsorptive substances are actually present in seeds, they will be present in maximum effective concentration in the ripe state. The added mixture was recovered by the standard percolation procedure, 70–80 ml of percolate being collected. The recovered material amounted almost exactly to the original weight and its hydroxyl value was also the same. Carbon tetrachloride insoluble material in seeds thus do not contain substances which can hold back by adsorption any detectable amounts of mono- and di-glycerides.

The hydroxyl values of the carbon tetrachloride extracted oils were determined directly. This was carried out essentially as reported earlier[1] but the following improved procedure was used. After the fat is acetylated and excess acetic anhydride decomposed by boiling with ethyl alcohol the reaction mixture is distilled on a hot-plate till only about 5 ml is left. The residue is transferred to an evaporating dish containing the filter paper ring[1] with hot acetic acid. The dish is never filled to more than half the height of the filter paper ring. The solvent is evaporated off on a briskly boiling water bath. Subsequently the residue is moistened with 2 ml portions of ethyl alcohol for 5 min and the solvent is removed as before. This operation is repeated thrice to complete the decomposition of last traces of acetic anhydride. The dishes are then heated to constant weight at 105°.

Results

The details of plant species studied and oil development stages at which hydroxyl values were determined are given in Table I. The determinations were carried out in general with

TABLE I

Oil development stages (ODS) of ripening seeds at which zero hydroxyl values of oils were observed

Species	ODS	ODS	ODS	ODS	ODS	ODS	ODS
Anacardiaceae							
Anacardium occidentale	100	47					
Mangifera indica	100	46	19	2			
Apocynaceae							
Nerium thevetifolium	100	94	68	25			
Tabernaemontana dichotoma	100	77	41	6			
Caricaceae							
Carica papaya	100	82	70	64	21		
Cruciferae							
Brassica campestris var. *dichotoma*	100	59	32	16	9		
Brassica campestris var. *sarson*	100	52	6				
Combretaceae							
Terminalia catappa	100	16					
Compositae							
Helianthus annus (early var.)	100	80	62	46	40	27	
Helianthus annus (late var.)	100	20	16	10	5	1·5	
Cucurbitaceae							
Lagenaria vulgaris	100	66	26	16	8		
Euphorbiaceae							
Jatropha curcass	100	26	3	0·3	tr		
Jatropha glandulifera	100	79	40	12	1·6	0·4	
Leguminoseae							
Arachis hypogaea	100	82	64	32	16		
Cassia occidentalis	100	53	41	22			
Clitoria ternatea	100	95	91	82	55	11	
Pongamia glabra	100	99	75	62	59	42	37
	25	21	19	11	12	8	3
Linaceae							
Linum usitatissimum	100	66	51	37	34	14	6
Lythraceae							
Punica granatum	100	10					
Malvaceae							
Abelmoschus esculentus	100	75	33	19	7	4	1·6
	0·9	0·4					
Meliaceae							
Azadirachta indica	100	62	52	33	16	10	2
Melia agadirachta	100	77	66	54	31	15	
Moringaceae							
Moringa pterigosperma	100	49					
Rutaceae							
Murraya koenigi	100	69	59	50	42	32	23
Sapotaceae							
Achras zapota	100	75	61	29	20		

0·5–1·0 g of oil. The weights of hydroxylated oil can normally be determined to ±0·4 mg and hence these quantities are adequate for the accuracy desired. No further details of the experiment are given in cases where the weights of oil before and after acetylation agreed within the limits of experimental error.

In cases where there is an initial accumulation of intermediate glycerides during ripening, the hydroxyl value may reach a maximum at low oil development stages due to obvious reasons. Because of this the determinations are more significant at oil development stages below about 10%. However, owing to large and entirely unpredictable differences in the rate of oil synthesis with seed development in different plant species, appreciable amounts of oil at oil development stage below 10% could be procured only in a few cases. It was also not practicable to get the usual 0·5–1·0 g oil in these instances. The details of instances where hydroxyl determinations were made at oil development stages below 10% are given in Table II.

TABLE II

Hydroxyl values of oils from some ripening seeds at oil development stages below 10%

Seed	ODS, %	Fat acetylated, g	Acetylated fat, g
Tabernaemontana dichotoma	6	0·1266	0·1262
Brassica campestris var. *dichotoma*	9	0·5763	0·5770
Brassica campestris var. *sarson*	6	0·1060	0·1060
Lagenaria vulgaris	8	0·5686	0·5686
Jatropha curcass	2·6	0·0192	0·0192
Jatropha curcass	0·02	0·0294	0·0294
Jatropha glandulifera	1·6	0·1846	0·1846
Jatropha glandulifera	0·4	0·0222	0·0226
Pongamia glabra	8	0·4252	0·4254
Pongamia glabra	3	0·3444	0·3442
Linum isitaatissimum	6	0·1642	0·1648
Punica granatum	10	0·1358	0·1358
Abelmoschus esculentus	7	0·2014	0·2016
Abelmoschus esculentus	4	0·1408	0·1406
Abelmoschus esculentus	1·6	0·0980	0·0982
Abelmoschus esculentus	0·9	0·0690	0·0690
Azadirachta indica	2·1	0·1538	0·1538

In the later stages of ripening of linseed and pomegranate (underlined in Table I) significant increases were observed in the weights of acetylated oils. However, the earlier stage did not show any increase in weight. It appeared that the observed increase in weight might have been caused only by autoxidative deterioration of the more unsaturated acids present in larger quantities in the later stages. Hydroxyl values of such samples were carefully redetermined by the procedure of isolating and titrating the combined acetic acid present in the acetylated fat[3] when no significant values were observed in any case.

The results in Tables I and II show that in all the 24 plant species now reported fat synthesis takes place by the 'quantum' mechanism. The 24 species examined to date include a major proportion of oil-bearing plants of economic value. The absence of any exception in this connexion is particularly significant.

Conclusion

The 'quantum' mechanism of fat bio-synthesis appears to be a highly favoured mode of fat formation in ripening seeds of phanerogams.

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EXTRACTION OF PROTEIN FROM EXPELLER- AND SOLVENT-EXTRACTED COCONUT MEAL BY DILUTE ACID, ALKALI, AND SALT SOLUTIONS

By J. CHELLIAH* and N. G. BAPTIST

The extractability of protein (nitrogen) from poonac (the press-cake left after extraction of oil from dried coconut kernel) with dilute aqueous hydrochloric acid, sodium hydroxide, and salt solutions has been investigated. Experiments were carried out on both expeller poonac and solvent-extracted poonac, the protein of the former being more soluble. Approximately 40% and 55% of the poonac protein nitrogen was extractable with 0·15% aqueous acid and alkali, respectively, under optimum conditions. Dilute salt solutions were found to have a comparatively poor solubilising effect under the conditions employed. Increasing the fat content of expeller poonac to 10% increased the nitrogen extracted by acid but not by alkali solutions.

Introduction

Few experimental studies have been published on the extraction of coconut protein from the residues left after the extraction of oil from dried coconut endosperm ('copra'). Recent reports are those of Sreenivasan[1] and Smith[2]; earlier work has been described by Curtin.[3]

Coconut oil is one of the major exports of Ceylon. 'Poonac', the presscake obtained as a by-product after expulsion of the oil is used in Ceylon chiefly as a constituent of animal feeds and agricultural fertilisers and contains 19–22% protein and 5–10% oil. The chief disadvantage of the crude poonac is its high fibre content (10%). Early work on the material was restricted to determining its biological value in experimental animals,[4,5] but no attempt to prepare from it a product which would be fit for human consumption has come to the notice of the present authors.

A study of the extractability of the protein of poonac has accordingly been carried out, bearing in mind that the procedure applied should ultimately be capable of economic and commercial exploitation and of producing a product of unimpaired nutritional value. The experiments and conclusions reported in this paper constitute the first part of this investigation.

Experimental

Materials and Methods

Two 5 lb samples of poonac, one of solvent-extracted and the other of expeller poonac, were obtained from the Oils and Fats Corporation, Seeduwa. A petroleum distillate ('hexane', S.B.P. 62°–82°, Shell Company of Ceylon) is used as solvent in obtaining the solvent-extracted poonac from expeller poonac. The latter is prepared from kiln-dried copra which is subjected to a preliminary process of disintegration, cooking and drying prior to expulsion of the oil in a screw-press. Each sample, was reduced to a fine powder in a laboratory disintegrator ('Atomill', British Jeffrey Diamond Ltd., Wakefield) and finally passed through a 60-mesh sieve. The samples were dried and then stored in an air-tight bottle. Weighed aliquots in duplicate were taken for each experiment, moisture being determined simultaneously.

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Moisture

This was determined by drying at 105° to constant weight.

Fat

The fat content was determined by exhaustive extraction with petroleum ether (b.p. 60–80°) in a Soxhlet apparatus.

Solvents

Aqueous solutions of HCl (0·10–1·00%, wt./vol.); NaOH (0·01–2·00%, wt./vol.); NaCl (0·5–3·9%, wt./vol.); $CaCl_2$ (0·03–0·10%, wt./vol.). All reagents were of analytical reagent quality.

Extraction of nitrogen by shaking with solvent

1 g of the material was shaken with the solvent (25 ml) for an interval of time (1 h or longer), the suspension left to stand for a further period, and shaken again at a given temperature (29°, 45°, 60°) for 2 h in an automatic shaking apparatus prior to filtration or centrifugation. Aliquots of the clear filtrate (No. 1 Whatman filter paper) or supernatant were taken for nitrogen determination. Occasionally it proved necessary to centrifuge the suspension and filter the supernatant to obtain a clear extract.

Extraction of nitrogen by homogenisation with the solvent

0·2 g (approx.) weighed aliquots of the material were homogenised with solvent (25 ml/g poonac) in a glass homogeniser (thick-walled glass tube, 14·5 × 2 cm with a ground surface on the inside, and provided with a motorised ground glass pestle). Homogenisation was carried out until no large aggregates were visible under the microscope (about 30 min). The suspension was then centrifuged, the supernatant filtered if necessary, and aliquots of the clear extract taken for nitrogen determination.

Nitrogen

All determinations were carried out by the micro-Kjeldahl procedure according to Chibnall, Rees & Williams.[6]

Precipitation of protein from the extract with trichloroacetic acid (TCA)

40% TCA solution in water was added to a measured aliquot of the clear extract till a final concentration of 5% was

attained (5 ml extract + 0·75 ml 40% TCA). The precipitate was washed by centrifugation with a small volume of 5% TCA and transferred to a micro-Kjeldahl flask for protein-N estimation.

Precipitation of protein from the extract by heat coagulation

A measured volume of the clear extract was carefully neutralised to the point of maximum turbidity (pH ~ 4·5) and heated to 80–90° when coagulation took place. The solids were separated by centrifugation, washed with water and protein nitrogen determined as before.

Centrifugation

This was usually carried out for 10 min in the M.S.E. Minor centrifuge using the maximum speed position. Room temperature which varied between 28° and 30° is recorded as 29°. Where higher temperatures were used for extraction, a constant temperature water bath was employed. All pH determinations were done with a Cambridge pH meter.

Non-protein N (NPN)

This was determined by shaking or homogenising weighed aliquots of the sample with 5% TCA (25 ml/g poonac) for 3 h at 29°, and taking suitable aliquots of the clear filtrate for estimation of N.

Results and Discussion

The figures obtained for moisture, fat and nitrogen contents of the samples of poonac used are shown in Table I.

The effect of concentration of HCl on the extraction of nitrogen from expeller poonac at room temperature is shown in Table II. The extracted N was found to reach a maximum with acid concentrations in the range 0·4–0·5% regardless of the method of extraction employed; however the increase in extracted N with acid concentrations over 0·2% was relatively less.

In the case of solvent-extracted poonac (Table III), the extracted N was very small, a value of the same order as the water-soluble N of the expeller poonac being obtained. Also, variation in the acid concentration had only a relatively small effect on the extractable N. It is shown later (see Table XIII) that the extractable N from the solvent-extracted poonac by salt solutions is also very small. This suggests that the proteins of the poonac have suffered further denaturation in the solvent-extraction process. This could have nutritional implications. Butterworth & Fox[7] have shown that the nutritive value of coconut deteriorates as processing temperature increases. The superior lysine availability of a solvent-extracted coconut meal prepared for human consumption (UNICEF) over two other solvent-extracted commercial samples (France) which they reported indicates that the protein could suffer damage in the extraction process itself.

In view of the insolubility of solvent-extracted poonac protein, it was decided to use only expeller poonac in studying the effect of other variables like the volume of solvent per unit weight of poonac, the temperature of the medium, the fat content of the poonac and the time of contact of the poonac with the solvent. On the basis of the results shown in Table II and some earlier observations obtained before it was realised that temperature could have a marked effect, 0·15% HCl was chosen as the most suitable and economic concentration of acid, as it gave near-maximum N extractability.

Effect of solvent volume on N extractability

This is shown in Table IV. The extracted N increased to a maximum at a volume of 25–30 ml/g poonac, and thereafter decreased somewhat. A volume less than 15 ml/g poonac produced a semi-solid mass from which a filtrate or supernatant on centrifugation could not be obtained. With increase in volume, a decrease in pH of the mixture occurred as would be expected from the fact that the buffering effect of the poonac protein would be limited. The maximum N extraction was obtained at pH 1·8–2·0.

TABLE I

Composition of expeller poonac and solvent-extracted poonac

	Expeller poonac	Solvent-extracted poonac
Moisture, %	8·7	9·6
Moisture, maximum absorbed on equilibration, %	15·5	15·7
Fat (on anhydrous basis), %	9·4	3·1
Nitrogen (on anhydrous basis), %	3·78	4·20

TABLE II

Effect of varying the concentration of aqueous HCl on the extraction of nitrogen from expeller poonac (Moisture, 11%)

The pH values of the solutions are the values obtained after shaking the sample for 3 h with the acid at 29°C

HCl, % (wt./vol.)	pH of acid	pH of mixture	Extracted N (shaking) (g N/100g)	Extracted N (homogenising) (g N/100g)
0·00	—	5·80	—	0·34
0·10	1·57	2·35	0·55	0·72
0·15	1·42	2·02	0·86	1·26
0·20	1·26	1·75	0·99	1·36
0·30	1·08	1·38	1·03	1·43
0·40	0·97	1·14	1·08	1·70
0·50	0·88	0·99	1·05	1·72
0·60	0·79	0·95	1·05	1·65
0·80	0·67	0·76	0·78	1·35
1·00	0·56	0·66	0·56	—

TABLE III

Effect of varying concentration of aqueous HCl on the extraction of nitrogen from solvent-extracted poonac (Moisture, 11%) at 29°C

HCl, % (wt./vol.)	Extracted N (shaking) (g N/100 g)	Extracted N (homogenising) (g N/100 g)
0·0	—	0·28
0·4	0·36	0·34
0·5	0·41	0·36
0·6	0·37	0·37
0·7	0·37	0·35
0·8	0·34	0·34

TABLE IV

Effect of varying the volumes of 0·15% HCl/g poonac on the extraction of nitrogen from expeller poonac (Moisture, 11%) at 29°C

Volume of acid, ml/g	15	17	20	25	30	35	45	50
pH of extract	2·82	—	2·50	2·02	1·84	1·78	1·75	1·62
N (shaking), g N/100 g	0·33	0·38	0·81	0·88	0·89	0·84	0·75	0·77

Effect of temperature and time of contact on N extraction

This is shown in Table V. The extracted N increased with temperature irrespective of the time of contact. At room temperature (29°), maximum values were obtained after 24 h, while at the higher temperatures, no further increase was obtained after a 3 h period of contact. The N extracted after a 3 h period of shaking at 60° was 177% of the amount extracted at room temperature for a like period. Extraction at higher temperature could therefore obviate the inconvenience of long extraction periods in the cold. The use of temperatures higher than 60° was not investigated due to the likelihood of decreasing the solubility of the poonac protein by increasing denaturation.

Effect of added oil on N extractability

The low solubility of solvent-extracted poonac protein suggested that fat played a critical part in determining the solubility of the protein. Preliminary experiments indicated that addition of oil produced no increase in extracted N in the case of solvent-extracted poonac, but that with expeller poonac, a definite increase was obtained. The extractability of nitrogen from expeller poonac was therefore determined in the presence of added aliquots of coconut oil and varying acid concentrations (Table VI). It was found that with 0·10% HCl, further addition of oil did not increase the extractable N. With 0·15% HCl, maximum extraction was obtained when the fat content of the sample was increased from its original figure of 8·4% to 9·2%; with 0·20% HCl, a figure of 11·1% fat gave maximum extraction of nitrogen. This increase in extracted N in the presence of fat is important in that it adds weight to the argument that the lower solubility of the solvent-extracted poonac protein is due to irreversible denaturation of the coconut lipoprotein complex which starts with the drying of the coconut-endosperm and is completed by the solvents used in the extraction process.

The N precipitable as protein from these extracts is shown in Table VII. It will be seen that 71% of the extractable N (90% of the protein N) can be precipitated by TCA. It was found in further experiments that corresponding amounts of protein could also be recovered by heat coagulation under carefully controlled conditions.

The NPN of poonac amounts to about one-tenth of the total nitrogen. The bulk of this consists of free amino acids.[8] It was found somewhat unexpectedly that elimination of the water-soluble constituents of the poonac improved the extraction of the poonac protein (Table VIII). The nitrogen extracted by 0·15% HCl from washed poonac was found to be almost completely recoverable as protein; and the total extractable protein N was thereby increased from 1·06 to 1·24 g N/100 g poonac, an increase of 17%. The figures also show that the water-soluble protein of the poonac is only a minute fraction of the total protein, amounting to ~ 0·4 g protein/100 g poonac.

By first extracting the water-soluble N and then extracting the residual N with acid, the total extractable nitrogen was also increased, a figure of 1·62 g N (Table VIII) being obtained as against 1·49 g N (Table VII)/100 g poonac. These results suggest that there are certain water-soluble constituents in the poonac which decrease the solubility of the poonac proteins in this acid medium. Tables IX, X, XI and XII give the figures of nitrogen extracted from expeller poonac using dilute aqueous NaOH solutions as solvent under conditions similar to those employed when dilute HCl was used. Though

TABLE V

Effect of temperature and time of contact on the extraction of nitrogen from expeller poonac (Moisture, 11%) by 0·15% HCl

Values given represent g N extracted/100 g poonac

Extraction time, h	29°C	45°C	60°C
3	0·85	1·28	1·51
24	1·06	1·29	1·52
48	1·08	1·25	1·48

TABLE VI

Effect of fat content of the poonac on the extraction of nitrogen from expeller poonac (Moisture, 11%; fat, 8·4%) by varying concentrations of aqueous HCl at 29°C

HCl, % (wt./vol.)	Weight of oil added /g poonac, mg	Fat in sample (calculated), %	Extracted N, g/100 g poonac
0·10	—	8·4	0·55
	15·8	9·8	0·48
	27·2	10·8	0·38
	40·9	12·0	0·40
0·15	—	8·4	0·86
	9·3	9·2	1·14
	19·8	10·2	1·10
	29·0	11·1	1·09
0·20	—	8·4	0·99
	17·6	9·8	1·23
	29·1	11·1	1·30
	37·3	11·7	1·19
0·30	—	8·4	1·03
	17·1	9·9	1·21
	27·7	10·8	1·22
	37·0	11·7	1·25

TABLE VII

Recovery of protein from 0·15% HCl extract of expeller poonac (Moisture, 11%) under conditions for maximum extraction

1 g poonac and 25 ml 0·15% HCl were shaken for 3 h, left to stand for a further 18 h and then heated in a thermostatically controlled water bath at 60°C with shaking for 3 h before filtration and precipitation of protein with TCA

Total extracted N	NPN	Protein N by difference	N in protein ppt	% Total N recovered	% Protein N recovered
	(g N/100 g poonac)				
1·49	0·32	1·17	1·06	71	90

TABLE VIII

Effect of washing expeller poonac (Moisture, 11%) with water on the extraction of its protein with 0·15% aqueous HCl at 60°C

1 g poonac was shaken with 100 ml water for 3 h. After filtering and washing the residue, it was shaken with 25 ml 0·15% HCl for 1 h and then heated at 60°C for 2 h with shaking before filtration and N estimation in the extract. Protein was precipitated with TCA. N in a suitable aliquot of the water-soluble fraction was also determined. Figures represent g N/100 g poonac.

Water-soluble N	Extracted N	Protein N	
0·34	1·28	1·24	(97% of the extracted N)

aqueous NaOH (0·15%, wt./vol.) extracted more nitrogen than the same concentration of HCl, there was the disadvantage that the extract developed a brown colour, the colour increasing in intensity with increase in strength of alkali and temperature. Unlike acid extraction, alkali extraction was not improved by increasing the fat content of the sample. Shaking the sample for periods longer than 3 h produced only a small increase in the extracted N (Table X). The values in Table XII suggest that this increase does not reflect increased solubilisation of protein but rather a breakdown of protein to soluble nitrogenous products. The amount of total N recovered as protein was not increased by prolonging the time of contact of the sample with the alkali solution.

Table XIII shows that dilute salt solutions are comparatively poor extractants of poonac N. Aqueous $CaCl_2$ solutions were not as effective as aqueous NaCl solutions. Here again it is seen that solvent-extracted poonac proteins are more insoluble in the salt solution. When allowance is made for the NPN present, a maximum solubility of protein (corresponding to ~ 0·3 g protein N/100 g poonac) was obtained with 3% aqueous NaCl solution. The nitrogen extractability with aqueous NaCl of expeller poonac, however, showed a marked difference in that maximum solubility (corresponding to ~ 0·6 g protein N/100 g poonac) was achieved with only 0·06% aqueous NaCl solution. The advantage of salt extraction is that no chemical reactions take place during the extraction process, and dialysis of the extract gives a relatively white protein product.

With all solvent media, it was found that the degree of comminution of the poonac was important in relation to the extractable N. If the sample was merely shaken, and not homogenised, the extracted N was less. The effect of homogenisation tended to disappear at higher temperatures of extraction and with longer periods of contact. Improving contact of the poonac proteins with the extracting medium is therefore very important. It is likely that a higher state of subdivision than the 60-mesh product used would have helped considerably in the solubilisation of the poonac protein. These results indicate that it should be possible to devise a fairly simple and economic process for extraction of coconut protein from poonac and similar oilseed residues.

Values for the essential amino acids in three coconut protein isolates prepared by different methods have been reported by Sreenivasan.[1] Table XIV shows the highest values obtained for each amino acid in these preparations, together with values determined by one of the authors (Baptist, unpublished data) in crystalline coconut globulin (N = 17·5%, anhydrous basis) prepared from poonac. The composition of the FAO reference protein[9] is also given for comparison.

The lysine and tryptophan contents/g food N are higher than those of cereals, leafy vegetables, and other oilseed proteins.[9,10] From Table XIV, 'chemical score' values of 82 and 71 are obtainable for the coconut protein isolate and for coconut globulin, the limiting amino acids being methionine and tryptophan, respectively. In comparison, the biological value of poonac protein for the rat has been reported to be 71[5] (solvent-extracted meal) and 77[4] (expeller meal).

Application in human feeding

In converting the tasteless coconut protein to an acceptable human food, it should prove possible to increase the biological

TABLE IX

Effect of varying concentrations of aqueous NaOH at 29°C on the nitrogen extracted from expeller poonac (Moisture, 11%)

NaOH, % (wt./vol.)	Extracted N (shaking) (g N/100 g poonac)	Extracted N (homogenising) (g N/100 g poonac)
0·01	0·45	0·64
0·02	0·51	—
0·03	0·64	0·93
0·04	0·76	—
0·05	0·88	1·47
0·06	1·04	—
0·07*	1·18	—
0·08	1·31	—
0·09	1·52	—
0·10	1·56	1·78
0·15	1·76	—
0·30	2·11	2·05
0·50	2·22	2·08
1·00	2·28	2·27
2·00	—	2·58

* Above this concentration, the extract took on an increasingly brown colour

TABLE X

Effect of temperature and time of contact on the nitrogen extracted from expeller poonac (Moisture, 11%) by 0·15% aqueous NaOH

The experimental procedure was identical with that for Table V. Values given represent g N extracted/100 g poonac

Extraction time, h	29°C	45°C	60°C
3	1·76	1·92	2·13
24	1·83	1·98	2·25
48	1·88	2·03	2·26

TABLE XI

Effect of the fat content of poonac on the nitrogen extracted from expeller poonac (Moisture, 11%) by 0·15% aqueous NaOH

NaOH, % (wt./vol.)	Weight of oil added/g poonac, mg	Fat in sample (calculated), %	Extracted N /100 g poonac, g
0·15	—	8·4	1·78
0·15	24	10·7	1·67

TABLE XII

Protein (5% TCA precipitable) nitrogen in 0·15% aqueous NaOH extract at 60°C of expeller poonac (Moisture, 11%)

1 g poonac was kept in contact with 25 ml 0·15% NaOH for intervals of 1 h and 22 h, and heated with shaking in a thermostatically controlled water bath for a further 2 h before centrifugation. Total N and protein N was determined in the supernatant.

Time of contact, h	Extracted N	NPN	Protein N by difference	Protein N in TCA ppt	Total N recovered %	Protein N recovered %
	(g N/100 g poonac)					
3	2·17	0·32	1·85	1·71	78·6	92·4
24	2·10	0·32	1·78	1·65	78·2	92·7

value of the product still further by admixture with small amounts of animal protein such as milk or fish protein, or of vegetable protein such as leaf protein, manufactured by alternative processes already worked out for such materials.

A further consideration is that the poonac residues remaining after protein extraction still contain over half their original protein content, and could be re-utilised after appropriate treatment for the same purposes for which the original product was used, namely, as a constituent of animal feed or agricultural fertiliser.

A flowsheet that could form the basis of a technological process is shown in the following scheme:

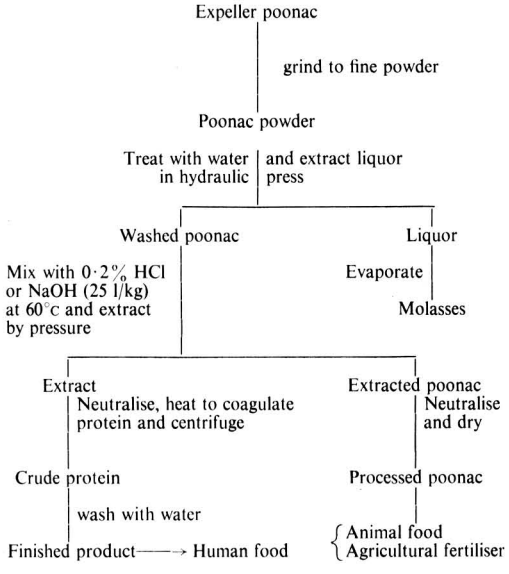

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TABLE XIII

Effect of varying concentrations of salt solutions at 29°c on the nitrogen extracted from expeller poonac (Moisture, 9·6%) and solvent-extracted poonac (Moisture, 11·0%)

Concentration of salt in solution %, (wt./vol.)	N extracted by aqueous NaCl (homogenising) g/100 g expeller poonac	N extracted by aqueous $CaCl_2$ (shaking) g/100 g expeller poonac	N extracted by aqueous NaCl (homogenising) g/100 g solvent-extracted poonac
0·00	0·34	—	—
0·03	—	0·39	—
0·04	0·78	—	—
0·05	0·79	0·39	—
0·06	0·89	—	—
0·08	0·72	—	—
0·10	0·57	0·38	—
0·50	0·45	—	0·27
1·0	0·35	—	0·28
2·0	—	—	0·49
3·0	—	—	0·59
3·5	—	—	0·54
3·9	—	—	0·34

TABLE XIV

Essential amino acids in coconut protein

(Values represent g amino acid/16 g N)

Amino Acid	Coconut globulin (Baptist)	Coconut protein isolate (Sreenivasan)	Reference protein (FAO)
Lysine	4·5	5·1	4·2
Methionine	2·0	1·8	2·2
Tryptophan	1·0	1·6	1·4
Threonine	3·4	2·9	2·8
Isoleucine	4·5	3·5	4·2
Valine	6·6	4·8	4·2
Phenylalanine	(5·1)*	5·1	2·8
Leucine	(7·2)*	7·0	4·8

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ISOLATION AND CHARACTERISATION OF DISULPHIDE PEPTIDES FROM WHEAT FLOUR

By I. K. JONES and P. R. CARNEGIE

A method has been devised for the isolation and fractionation of disulphide peptides from flour. Oxidised glutathione is only a minor member of a family of disulphide peptides, the rest of which are basic and of molecular weight about 2000.

The basic disulphide peptides could not be fractionated into individual components, but they contained some disulphide groups that were very reactive towards glutathione at pH 5·8, which is approximately the pH of dough.

Introduction

Dough is known to be physically altered by the disulphide peptide, oxidised glutathione.[1] There is a possibility that naturally occurring disulphide peptides in flour itself may have similar properties to glutathione and thus have a role in determining the physical properties of the dough made from that flour.

In 1940, Balls & Hale[2] reported the presence of a disulphide compound in the ether extracts of flour. This compound was diffusable through a rubber membrane and soluble in trichloracetic acid, suggesting its molecules were smaller than those of protein. Bell,[3] reporting on the non-protein nitrogenous compounds of flour, did not find cystine, either as the free amino acid, or in hydrolysates of peptides.

Frater & Hird[4] found in flour a disulphide which, when treated with sulphite, liberated a thiol which gave a current–voltage wave with the polarograph. This disulphide was not a substrate for glutathione reductase, but oxidised GSH (glutathione) to GSSG (glutathione-*S*,*S*-glutathione or oxidised glutathione). Proskuryakov & Zueva[5] found a soluble disulphide in flour extracts, and claimed that this was GSSG. In 1954, Kuninori & Matsumoto[6] found both thiol and disulphide compounds which, on gel filtration, had distribution coefficients similar to that of glutathione. More recently, Hird *et al.*[7] have quantitatively investigated the disulphide compounds of several flours, using polarographic and enzymic methods. They established values of 2·2–5·3 μmole/100 g for oxidised glutathione, and 36–50 μmole/100 g of total diffusable disulphide.

Since disulphide peptides might affect the rheological properties of dough, it is important to investigate their levels in flour. This paper reports on the isolation and properties of flour peptides.

Experimental

Materials

Export grade flour, chemically untreated, and milled from Victoria 'Fair Average Quality' wheat in the seasons 1964–65 and 1965–66, was the gift of W. S. Kimpton & Sons, Melbourne.

Glutathione (reduced and oxidised), glutathione reductase III and $HADPH_2$ (reduced nicotinamide adenine dinucleotide phosphate) type II were obtained from Sigma, St. Louis.

SE-Sephadex C-25, course grade and Sephadex G-50, G-25, G-15 and G-10, all bead form, fine, were the products of Pharmacia, Uppsala.

Fluorescein mercuric acetate was prepared according to the method of Karush *et al.*[8]

Acrylamide AM-9 Chemical grout was obtained from Cyanamid, Wayne.

Isolation of disulphide peptides from flour

Extraction

400 g of flour were suspended in 1 litre 1·0M acetic acid at 4° and a final pH of 2·9. This low pH minimises enzymic proteolysis.[9] The slurry was stirred mechanically for 30 min.

Dialysis

The slurry was placed in a dialysis sac (Visking 27/32) and dialysed against 5 l of 1·0 M acetic acid for 72 h, with magnetic stirring of the diffusate.

Ion-exchange

The diffusate at pH 2·9 was passed through a 100 g column (8·6 × 13·0 cm) of SE-Sephadex in the H^+ form in water, followed by 3 l of distilled water. All components with a net positive charge were retained on the column, whereas uncharged carbohydrate was removed during the washing. Aspartic acid was only partly retained on the column.

The retained compounds were removed by displacement development using 6·0 M-NH_3 as the displacer. Visible pigment bands enabled observation of the development. Just before the bands passed off the large column, the effluent stream was connected in series to two smaller columns of SE-Sephadex (1·9 × 6·4 cm and 0·65 × 23·0 cm) in order to sharpen the bands.[10] The order of displacement was: acidic compounds first, followed by neutral compounds, and finally basic compounds (Fig. 1).

Pooling and drying

Following displacement, the fractions were pooled according to the presence or absence of glutathione. This led to two fractions—one acidic (containing glutathione) and the other containing neutral and basic components (Fig. 1). These fractions were dried at less than 40° on a rotary evaporator.

Gel filtration

Following pooling and drying, each fraction was dissolved in 1·0 M acetic acid and applied to a column (4·1 × 3·5 cm)

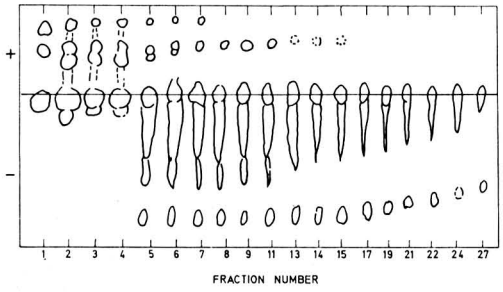

FIG. 1. *Paper electrophoresis at pH 4·0 of the fractions displaced from SE-Sephadex*

Peptides and amino acids were located with ninhydrin. Fractions 1–4 contain only acidic or neutral compounds. The tailing of the basic peptides first appears in fraction 5

of Sephadex G-25 in the same solvent. Fractions were assayed by ninhydrin[11] and by fluorescence quenching of fluorescein mercuric acetate for disulphide groups.[8] Fig. 2 illustrates the elution pattern obtained with the basic fraction. Peptide fractions and amino acid fractions were separately pooled, freeze-dried and stored over desiccant at 4°.

Modification I

The solvent used was 40% ethanol–1% acetic acid adjusted to pH 2·7 with HCl. 4 kg of flour were suspended in 10 l of solvent. The slurry was dialysed against 10 l of solvent which contained 180 g of SE-Sephadex (H^+ form). After dialysis, the Sephadex was transferred to a column.

Modification II

4 kg of flour were suspended in 15 l water and the pH was adjusted to 6·5 with about 3 ml pyridine. The dialysis step was eliminated. The slurry was centrifuged at about 1000 *g* and the supernatant was titrated to pH 2·9 with HCl, before addition of SE-Sephadex (H^+ form, 100 g) to the supernatant. The SE-Sephadex was then transferred to a column.

Fluorometric assay of disulphide

For assay of disulphides,[8] fluorescein mercuric acetate was made to 2×10^{-7} M in 1·0 M-NaOH. Five microlitre samples from each fraction of a column eluate, were mixed with 5 ml of the reagent solution. The relative fluorescence was measured on an Aminco-Bowman spectrofluorometer.

Amino acid analysis

0·20 mg of peptide was hydrolysed for 22 h *in vacuo* at 110° in 3 ml of HCl of constant boiling point. The hydrolysate was dried on a rotary evaporator and analysed on a Technicon amino acid analyser, using norleucine as the internal standard.

Amide nitrogen

This was determined by the method of Smythe *et al.*[12]

Total half-cystine

This was determined as cysteic acid after oxidation with performic acid.[13]

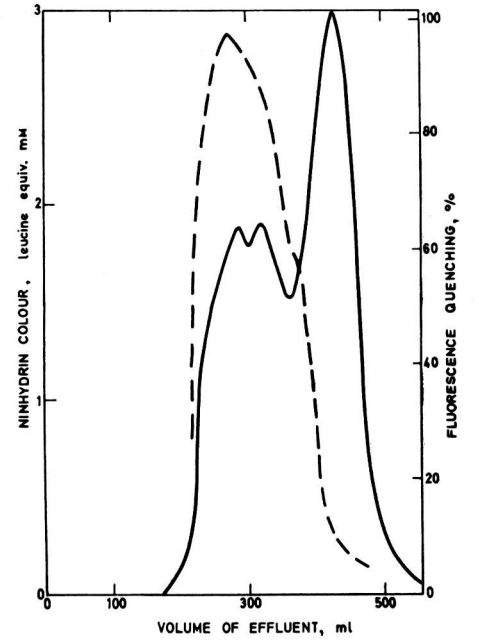

FIG. 2. *Elution of basic peptides and amino acids from Sephadex G-25*

(35 × 4·1 cm, in M acetic acid)
——— ninhydrin colour
— — — Fluorescence quenching of fluorescein mercuric acetate (representing disulphide groups)

Cysteine

Cysteine was determined by the loss of cystine consequent upon alkylation with iodoacetic acid and subsequent amino acid analysis. The reaction solution was 0·1 M potassium phosphate, pH 7·0, 10 mM in EDTA, 8 M in urea, and 40 mM in iodoacetate. This solution was purged with nitrogen and 1·0 ml was added to 1·0 mg peptide and allowed to stand under nitrogen for 3 h at room temperature. The reactants were removed from the alkylated peptide on a column of Sephadex G-10 (1·8 × 39·5 cm). The resulting peptide was hydrolysed and analysed by the normal procedure described above.

Reduction and alkylation

Disulphides were reduced with mercaptoethanol and alkylated according to the procedure of Crestfield *et al.*[14] Reactants were removed from the peptide on the column of Sephadex G-10 prior to acid hydrolysis and analysis.

Tryptophan

This was determined by the spectrophotometric method of Beaven & Holiday.[15]

Polarography

The amperometric back titration procedure of Frater & Hird [16] was employed, using methyl mercuric iodide as the titrant.

Total nitrogen

Total nitrogen was determined by the micro Kjeldahl method.[17]

Total carbohydrate

The method of Immers[18] was used for these determinations.

Peptide map for estimation of molecular size

The method used was that of Carnegie,[19] using gel filtration on a micro-column of Sephadex G-25 in phenol–acetic acid–water (1 : 1 : 1, wt. : v : v), for the first dimension and partition chromatography on paper as the second dimension, the solvent being n-butanol–pyridine–acetic acid–water (80 : 60 : 12 : 48).

Analytical gel filtration

10·0 g Sephadex G-50, 20 g Sephadex G-25, 30 g Sephadex G-15 and 50 g Sephadex G-10 were allowed to swell in solvent (0·01% formic acid) and then poured into four columns of uniform bore (1·8 cm) to give gels of length 33·3, 33·5, 34 and 39·5 cm respectively. Peptide samples (0·5–2·0 mg) were applied to the columns in 0·5 ml solvent. Flow rate was maintained at 90–100 ml/h by a peristaltic pump, and the effluent was scanned at 2050Å in a 0·3 ml quartz flow cell.

Edman degradation

The subtractive Edman procedure described by Konigsberg[20] was employed to determine the proportion of N-terminal residues in the peptides, and thereby obtain an estimate of their average chain lengths.

Reduction of disulphide by glutathione

The procedures used were those described by Hird.[21] GSH reacts with disulphides to form GSSG, which is then reduced by glutathione reductase, using $NADPH_2$. The disappearance of $NADPH_2$ was observed at 3400Å in a recording spectrophotometer. The reaction was performed under nitrogen, and the solutions were freshly prepared, purged with nitrogen and stored in ice. The reaction mixture consisted of 1·4 ml phosphate buffer (0·1 M, pH 6·95 or 5·85), 10 mM in EDTA, 0·1 ml of glutathione reductase (1 unit) in buffer, 0·1 ml $NADPH_2$ (6·7 mM) in buffer, and 0·1 ml GSH (120 mM) in buffer. The exchange reactions were initiated by the addition of 0·1 ml disulphide (3·0 mM) in buffer.

Paper electrophoresis

Paper used was Whatman 3 MM. Buffers used were pyridine–acetic acid–water (15 : 50 : 2250), pH 4·0 and 0·045 N sodium borate, pH 10·0. Voltage applied was 45 V/cm and the paper was cooled to −5° throughout the run. Peptides were located with a ninhydrin stain[22] or with cyanide–nitroprusside.[23]

Gel electrophoresis

Samples containing 70–100 μg peptide were examined on polyacrylamide gels according to the method of Carnegie *et al.*[24]

Results

The unmodified isolation method gave a yield of 79 mg of peptide 100 g flour. By applying a correction to allow for peptide remaining inside the dialysis sac during dialysis (Table I), a value of 125 mg per 100 g for the peptide contact of flour was obtained. Table I also shows that peptides contain only about 1% of the nitrogen of flour, and 3% of the total disulphide content.

The first modification, where 40% ethanol was incorporated in the more dilute acetic acid solvent, did not improve the yield. It had been expected that the ethanol might have dispersed the proteins (particularly prolamines) and diminished hydrogen bonding among proteins and peptides. However, it seems that 1·0 M acetic acid as employed in the original method was already performing this rôle, and ethanol achieved no improvement.

The second modification, where the extracting medium was water, produced yields of only 130 mg per kg of flour. This low yield is presumably due to the absence of any reagents which disperse the gluten proteins or disrupt hydrogen bonding.

TABLE I

Peptide content of flour
100 g of flour

The values presented are based on yields obtained from the unmodified isolation method. Corrections have been applied to allow for equilibrium dialysis

	μ of disulphide	g of dry matter	mg of nitrogen
Whole flour	925	86	1310
Oxidised glutathione	1·6	0·00095	0·134
Basic peptide	26·2	0·125	13·75

Properties of the acidic fraction

The acidic flour peptides and amino acids were examined by paper electrophoresis at pH 4·0, but attention was only directed to sulphur-containing peptides. In most preparations, only one spot was obtained with cyanide–nitroprusside, and this was in the position of GSSG. In some preparations a minor nitroprusside-positive spot was observed in a position corresponding to GSH. Elution and acid hydrolysis of the suspected GSSG and GSH spots liberated the amino acids glycine, half-cystine and glutamic acid. Since glutathione was the only peptide in this fraction to contain half-cystine, the half-cystine content of the acidic fraction will quantitatively represent the amount of glutathione in flour. This value is shown in Table I.

Properties of the basic peptide fraction

General properties

Paper electrophoresis at pH 4·0 showed that this material moved towards the cathode with pronounced tailing to the origin (Fig. 1). Even at pH 10·0 no anionic movement of the peptides was detected, which means that the peptides of this fraction were quite strongly basic. This was confirmed by amino acid analysis (Table II).

Paper techniques were of little value in resolving the basic peptides into their individual components because of the problem of tailing. Tailing is not uncommon in the electrophoresis or chromatography of basic peptides on paper.

The material was heterogeneous, as evidenced by acrylamide gel electrophoresis and by column gel filtration. Gel electrophoresis showed three major and four minor bands after staining and washing. This would suggest a minimum of seven peptides with a possibility that additional peptides (especially those of lower molecular weight) may have been lost in the staining and washing procedure, as is GSSG.

In the case of analytical gel filtration, asymmetric elution patterns typical of complex mixtures were obtained with both Sephadex G-25 and G-50 (Figs 3 (a) and (b)).

TABLE II
Amino acid analysis of basic flour peptides

	μmoles per cent	μmoles per 100 mg
Aspartic acid	7·4	46·0
Glutamic acid	12·2	76·3
Amide nitrogen	10·6	66·2
Lysine	4·1	25·8
Histidine	2·4	15·3
Arginine	5·9	36·6
Serine	6·1	38·3
Threonine	4·2	26·5
Proline	6·2	38·9
Glycine	10·0	62·3
Alanine	9·2	57·2
Valine	7·1	44·5
Methionine	1·1	7·1
Isoleucine	3·4	21·5
Leucine	6·8	42·2
Tryosine	2·8	17·7
Phenylalanine	2·4	15·1
Tryptophan	1·3	7·9
Cysteine*	0·0	0·0
Cystine (half)†	7·1	44·3

* Cysteine was determined after reaction with iodoacetate
† Half-cystine was determined after oxidation to cysteic acid

Molecular size

Evidence regarding molecular size was gained from the dialysis step in the isolation of the peptides, and more usefully, from analytical gel filtration. Under the conditions of the preparative dialysis, cytochrome *c* was unable to pass through the cellophane, whereas the flour peptides were. This suggests that the flour peptides are smaller than cytochrome *c*.

Analytical gel filtration in dilute formic acid showed that the peptides entered both Sephadex G-50 and G-25 whereas 50% and 83% of the material was excluded respectively from Sephadex G-15 and G-10. The spread of the elution patterns (Figs 3 (c) and (d)) is suggestive of a variety of molecular sizes. However, gel filtration in aqueous media does not yield a reliable correlation between molecular size and elution volume if aromatic residues are present in the peptides, owing to retardation by adsorption. For this reason, the peptide mapping technique of Carnegie[7] is preferred, because it employs the dissociating solvent, phenol–acetic acid–water (1 : 1 : 1, wt. : v : v). This procedure revealed that the peptides entered Sephadex G-25 in this solvent, but the distribution coefficient was very small, indicating a molecular weight in the region of 2000. A significant finding was the absence of any peptides with molecular weight less than 1500.

Degradation of the peptides using the subtractive Edman procedure indicated that the average length of peptide chain was of the order of 9 or 10 residues. In the case of disulphide peptides where two chains might be linked, the molecular weight would then be about 2000 to 2500.

Mixed disulphides

The presence of mixed disulphides containing glutathione or cysteine as a half disulphide would be revealed by oxidation with performic acid and estimation of their sulphonic acid derivatives. However, after following this procedure, no glutathione or cysteine derivatives were located by paper electrophoresis, and so the content of these forms of mixed disulphide was assumed to be negligible.

Amino acid analysis

Results of amino acid analysis are shown in Table II. There are some similarities to the gluten proteins such as high glycine and proline. The main differences are in the lower content of amide residues, and the much higher content of half-cystine—four to six times as high as that of gluten. The content of ionisable residues is also higher in the peptide.

The basic residues exceed the acidic residues in quantity, thus leaving the peptide with a net positive charge.

The total nitrogen calculated from amino acid analysis, 12%, was in good agreement with that obtained from determinations by the Kjeldahl procedure. Determination of total carbohydrate showed that the basic peptide material contained only 1·1% carbohydrate.

Amperometric determination of thiol and disulphide

Attempts to titrate thiol groups in the peptide preparation with methyl mercuric iodide were abandoned because, in the presence of the peptide, the polarographic behaviour of the methyl mercuric derivatives iodide was unpredictable and could not be interpreted.

Alkylation of thiol

Attempted alkylation of the peptide with iodoacetic acid, without prior reduction, did not result in a loss of half-cystine residues as determined on the amino acid analyser. This was taken to indicate that there was no free thiol in the peptide.

When reduction with mercaptoethanol preceded alkylation, amino acid analysis revealed a total loss of half-cystine and cysteic acid.

These two observations led to the conclusion that all of the half-cystine was present as the disulphide, none as thiol, and that none had been inadvertently oxidised, during isolation, to cysteic acid or alanine sulphinic acid.

Reduction of peptide disulphide by glutathione

The lability of the disulphide group in the basic peptides to reduction by thiol has important implications in interpretation of rheological data. It was found that at pH 6·95 and 5·8, GSH was able to react with the disulphide according to equations 1 and 2 to form GSSG.

$$\text{Peptide-SS-Peptide} + \text{GSH} \longrightarrow \text{Peptide-SSG} + \text{Peptide-SH} \quad (1)$$

$$\text{Peptide-SSG} + \text{GSH} \longrightarrow \text{Peptide-SH} + \text{GSSG} \quad (2)$$

The rate of reaction is compared in Table III with those of cystamine and lysine vasopressin, a basic decapeptide with an intramolecular disulphide group. At pH 6·95, GSH reduces cystamine at more than twice the rates of reduction of the flour disulphide peptides and lysine vasopressin. However, at pH 5·8 which is the pH of dough, the flour peptides were

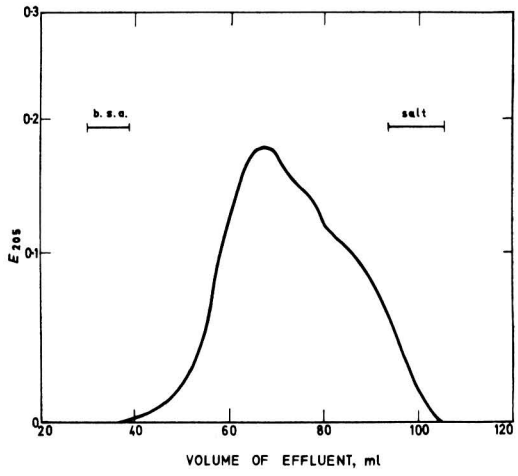

FIG. 3 (a). *Profile of basic peptides eluted from Sephadex G-50*
(33·3 × 1·8 cm, in 0·01% formic acid)
The regions of elution of bovine serum albumin (b.s.a.) and salt are indicated

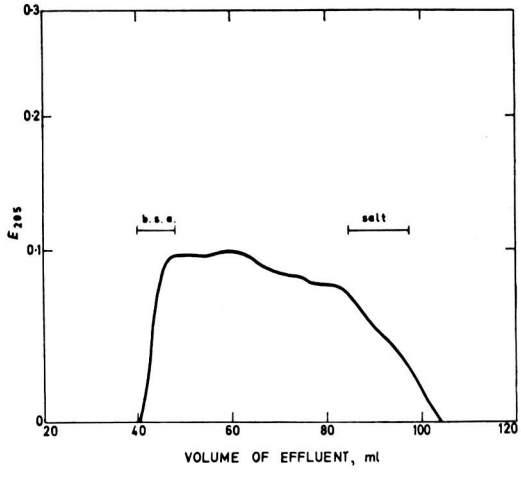

FIG. 3 (b). *Profile of basic peptides eluted from Sephadex G-25*
(33·5 × 1·8 cm in 0·01% formic acid)

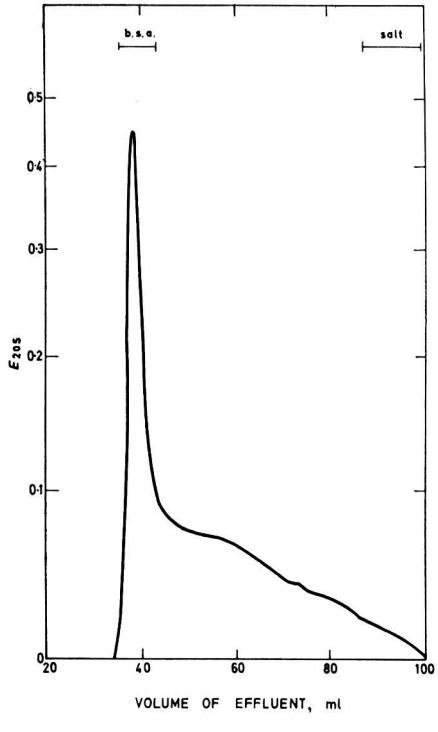

FIG. 3 (c). *Profile of basic peptides eluted from Sephadex G-15*
(34 × 1·8 cm in 0·01% formic acid)

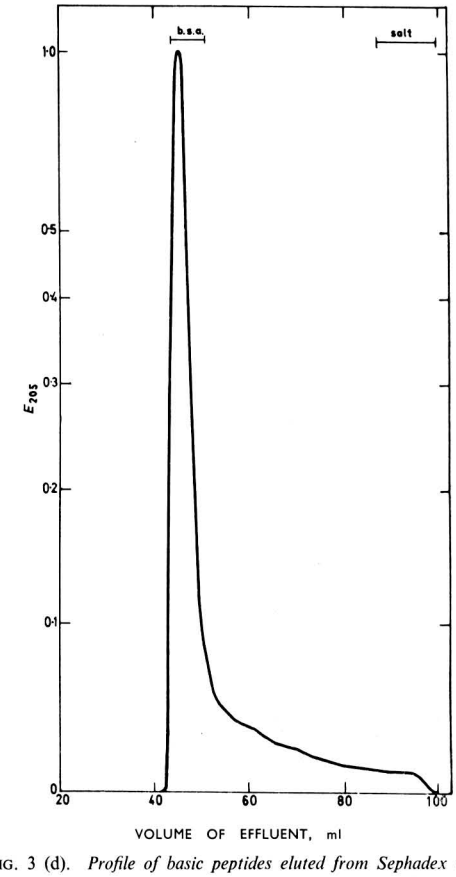

FIG. 3 (d). *Profile of basic peptides eluted from Sephadex G-10*
(39·5 × 1·8 cm in 0·01% formic acid)

the only ones to show any appreciable reactivity. This would imply that under conditions that occur in dough, some disulphide groups of the flour peptides are more susceptible to reduction by thiol than the disulphide groups of cystamine or lysine vasopressin. The reactions at pH 6·95 were studied at 30°, at which the natural decay of $NADPH_2$ was not rapid. At pH 5·8 and 30° the natural decay of $NADPH_2$ was much faster and the temperature had to be lowered to 12° in order to lower the rate of decay to a manageable level.

The reduction of the flour disulphide peptides by GSH at pH 5·8 proceeded in a complex manner. Initially the production of GSSG was rapid, followed by a less rapid phase, followed by a slow phase. This is suggestive of at least three types of disulphide bonds being attacked by GSH.

One of these (disulphide 1) reacted rapidly at 13·7 times the rate of cystamine, but was apparently present in small quantities. Judging by the amount of $NADPH_2$ consumed in its reduction, it represented only about 4% of the flour basic peptide disulphide (Table III). The second disulphide (disulphide 2) reacted less rapidly (8 times the rate of cystamine), and represented about 32% of the total peptide disulphide. The third disulphide (disulphide 3) constituted the remainder of the flour peptide disulphide, and was only slowly reduced by GSH.

After reduction by GSH, the flour peptides, having a thiol concentration of less than 0·34 mM, were able to reduce cystamine at a rate that was 3·7 times as rapid as its rate of reduction by 6·5 mM GSH. This indicates that the reduced peptides contain at least one very reactive thiol group.

TABLE III

Rate of exchange between GSH and disulphides

Disulphide	Rate as percentage of cystamine rate		Concentration of disulphide in reaction mixture
	pH 6·95	pH 5·8	
Cystamine	100	100	0·167 mM
Flour basic peptides	42	—	0·167 mM†
Disulphide 1		1370	0·012 mM*
Disulphide 2	—	800	0·055 mM*
Disulphide 3	—	58	0·100 mM*
Lysine vascopressin	40	68	0·167 mM

† This value represents the total disulphide in the flour peptide sample

* These values represent the concentrations of the individual disulphides, 1, 2 and 3 (see text), and were estimated from the amount of $NADPH_2$ consumed in the reduction of each

Discussion

The results show that oxidised glutathione (GSSG) is only a minor component in the peptide fraction recovered from flour, being only 6% of the total peptide disulphide (Table I). In terms of dry matter, GSSG represented only 0·8% of the total peptide fraction. The recovery of 1·6 μmoles of the GSSG from 100 g of flour is in agreement with the enzymic determinations by Hird *et al.*,[7] but is considerably lower than the estimate of Kuninori & Matsumoto[6] who obtained values of about 13 μmoles per 100 g flour.

Although the isolated material contained no thiol groups, their possible existence in flour peptides cannot be excluded. No thiol-protecting reagents were incorporated in the extraction procedure because, at the higher pH values required for satisfactory reaction of thiols with protective agents, the proteolytic enzymes of flour would be active.[9] Consequently any thiol peptides in flour would probably be oxidised to disulphide peptides during the isolation.

The basic peptides formed a complex group the members of which could not be separated by normal methods. They had similar charge properties, and even when modified by oxidation with performic acid, they could not be separated on an ion-exchange column. It is probable that not all of these peptides contained disulphide groups. In fact the reduction of the disulphides with GSH suggested the presence of three types of disulphide (Table III). Also, if the molecular weight is about 2000, and all the peptides contained one disulphide group, there should be one μmole of disulphide per 2 mg of peptide. Since the disulphide content was only 0·44 μmole per 2 mg, it may be concluded that disulphide peptides constitute slightly less than half the total peptide.

The rapid reaction of some of the flour disulphide peptides with GSH at pH 5·8 suggests that they may also react with protein thiols in dough and alter its rheological properties. However, it must be emphasised that the rapidly reacting disulphides constituted only a third of the total. These peptides had only slight rheological activity in dough. This probably results from high activity of a small proportion of the peptide disulphides rather than from slight activity of the majority.

The slow reaction of cystamine with GSH at the pH of dough suggests that it would have little or no rheological activity in dough. This has been confirmed.

After reduction by GSH at pH 5·8, the flour peptides contained a very reactive thiol that was able to rapidly reduce cystamine. The presence of this reactive thiol could explain the rheological activity of electrolytically reduced peptides. The slight rheological activity may indicate that the proportion of reactive thiol is small.

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RHEOLOGICAL ACTIVITY OF PEPTIDES, SIMPLE DISULPHIDES AND SIMPLE THIOLS IN WHEATEN DOUGH

By I. K. JONES and P. R. CARNEGIE

The disulphide peptides of flour have been separated into acidic (glutathione) and basic peptides, and their effects on the rheological properties of dough have been investigated. The only peptide normally present in flour to have marked activity in dough was glutathione.

The other disulphide peptides of flour were only slightly active in that their sole effect was to diminish the tolerance of the dough to prolonged mixing. Their lack of activity probably results from their disulphide groups being non-reactive at the pH values found in dough. The same peptides in the thiol form were slightly more active in that they decreased development time and maximum resistance.

All types of thiols tested were active in dough, but of the disulphides tested, only the acidic peptides GSSG (oxidised glutathione) and bis-γ-glutamylcystine had significant activity. Possible modes of action of disulphide peptides in dough are discussed.

Introduction

The preceding paper[1] described the existence of appreciable quantities of disulphide peptides in flour. It has long been known that wheaten dough is rheologically very sensitive to chemicals that are capable of modifying thiol or disulphide groups.[2,3] These observations have led to the postulate that thiol–disulphide exchange reactions among the gluten proteins are partly responsible for the peculiar visco-elastic properties of wheaten dough.[4–6] Indirect evidence for such a system has been obtained by a variety of methods.[7–14] Peptide disulphides could be expected to interfere in a thiol–disulphide exchange system by reacting with a protein (PR) thiol to liberate a peptide (R) thiol and form a mixed disulphide (Eqn. 1).

$$PR\text{-}SH + R\text{-}SS\text{-}R \longrightarrow R\text{-}SH + PR\text{-}SS\text{-}R \quad \ldots (1)$$

The effect of small amounts of the disulphide hexapeptide, oxidised glutathione (GSSG), was first reported by Ziegler[15] in 1940. Therefore there seemed to be a strong possibility that the naturally occurring disulphide peptides of flour may, like GSSG, possess a capacity for altering the rheological properties of dough as measured in the farinograph and the extensograph.

The effects of some thiols and disulphides which have not previously been examined for rheological activity have been compared with other simple thiols and disulphides.

Experimental

Materials

Flour was donated by W. S. Kimpton & Sons and was milled from Victorian 'Fair Average Quality' wheat of the 1965/66 season. Moisture content was 14%, nitrogen 1·94% and water absorption 57·4%. It was chemically untreated.

Glutathione (GSH), GSSG, homocystine and oxidised lipoic acid were the products of Sigma, St. Louis. Cystamine and dithiothreitol (Cleland's reagent) were respectively obtained from Light, Colnbrook, and from Calbiochem, Los Angeles.

Oxidised dithiothreitol was prepared by dissolving 30 mg of dithiothreitol in 20 ml of 10% pyridine (final pH 8·6), and bubbling air through the solution for 72 h. Pyridine was removed by repeated drying (at 25° on a rotary evaporator) and bis-γ-L-glutamyl-L-cystine was prepared by incubating GSSG with carboxypeptidase.[16] Carboxypeptidase was removed by precipitation at pH 3·0.

Crude flour peptides were obtained by the same procedure as described in the preceding paper,[1] except that acidic and basic peptides were pooled after displacement from the SE-Sphedax. This meant that the crude flour peptides represented the total extractable peptides of flour.

Basic flour peptides and acidic flour peptides were obtained

from crude peptides by fractionation on a small column (0·8 × 13 cm) of Amberlite CG400 in the acetate form. In 0·01 N acetic acid, basic peptides were not retained. Acidic peptides were retained, and were eluted from the column with 1·0 N acetic acid. Electrophoresis showed that GSSG was absent from the basic fraction.

Preparative electroreduction

The reduction cell consisted of two adjacent Perspex chambers connected by a window in which a piece of cellophane (held between two rubber gaskets) prevented free movement of solution between the two chambers whilst maintaining electrical conductivity. The cathode chamber (4 × 4 × 6 cm) contained mercury to a depth of 1·5 cm (just reaching the 0·7 × 3 cm window). The mercury was cooled by circulating iced water and was stirred magnetically. The anode compartment contained a constantly flowing stream of 0·1 N-H_2SO_4. The anode consisted of a 3 cm piece of coiled platinum wire. 10 ml of the disulphide solution to be reduced was added to the cathode compartment, and electrical conductivity was provided by the addition of 0·05 ml of N-HCl. The current source was a 12 V lead accumulator. The progress of electrical reduction was followed by periodically removing 0·005 ml. samples and assaying for thiols by the fluorescence quenching of fluorescein mercuric acetate at pH 7·5.[17] In the case of flour peptides, the assay solution was 4 M in urea to ensure exposure of all thiols. The reduction of basic flour peptides and GSSG is illustrated in Fig. 1.

Farinograph

Fifty g of flour was mixed in the small bowl of the farinograph for 3 minutes to allow temperature equilibration. After 3 min, 28·7 ml of a solution containing 1·0 g of NaCl and any additives (pH adjusted to 5·9 if necessary) was mixed with the flour. Cystine, homocystine and oxidised lipoic acid, all of which are relatively insoluble, were added as suspensions in the saline solution.

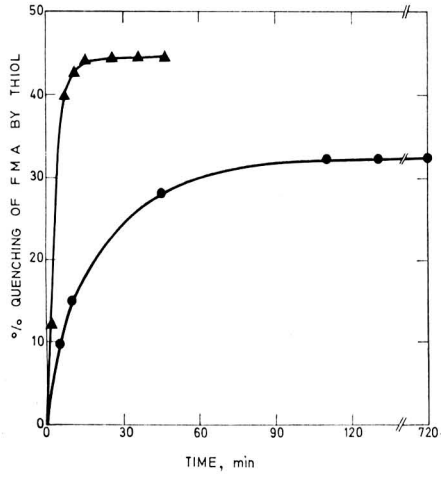

FIG. 1. *Electroreduction of GSSG and basic flour peptides*

Both solutions were initially 30 mM in disulphide. The production of thiol was followed by the quenching of the fluorescence of 4 × 10^{-6} M fluorescein mercuric acetate (FMA) at pH 7·5. The assay of flour peptides was performed in 4M urea. (Absolute quenching values are not comparable for different thiols)

▲——▲ GSSG
●——● Basic flour peptides

Extensograph

Three hundred g of flour were mixed for 3 min in the large bowl of the extensograph, after which 174·5 ml of a solution containing 6 g of NaCl and any additives were mixed with the flour. Mixing was allowed to proceed for a further 6 min. The dough was removed from the bowl and divided into 3 pieces, each of 150 g. Each piece of dough was balled and allowed to rest for 45 min. It was then re-balled and shaped, and stretched after further rest periods of 0, 5, and 10 min.

Results

Rheological effect of thiols

When added to dough in the farinograph, the simple thiol compounds cysteine, homocystine, cystamine, γ-glutamylcysteine, reduced lipoic acid, dithiothreitol and GSH all produced a characteristic decrease of the development time from the normal 6 min to less than 4 min (Fig. 2). In addition, the maximum resistance was increased, and there was a rapid fall in resistance in the 4 to 5 minutes following development. After this stage had been reached, the dough was very tolerant to mixing and further decline in resistance was only slight. Similar rheological activity had previously been reported for GSH,[3,18–20] cysteine,[19–22] dithiothreitol[23] and reduced lipoic acid.[23] The general effect of thiols is typified by γ-glutamylcysteine, depicted in Fig. 2.

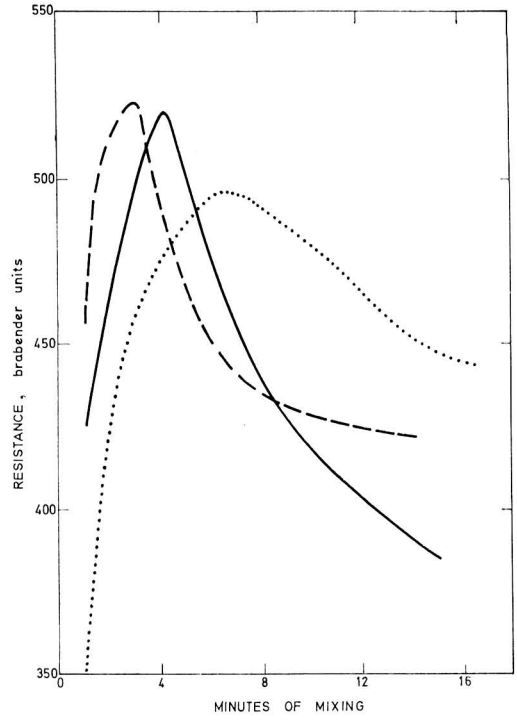

FIG. 2. *Effects of a simple thiol and a simple disulphide on farinograms of dough*

The farinogram tracings are represented by median lines, and the vertical scale has been expanded by a factor of 6·8. The 50 g bowl of the farinograph was used

. . . . . Control dough;
– – – – Dough plus 30 μmole of γ-glutamylcysteine;
——— Dough plus 30 μmole of bis-γ-glutamylcystine

Effect of disulphides

The only simple disulphides found to have appreciable rheological activity in the farinograph were GSSG and bis-γ-glutamylcystine. Cystine, homocystine, cystamine, oxidised dithiothreitol and oxidised lipoic acid were all found to be inactive. Similar results have been reported for cystine,[21] oxidised dithiothreitol[23] and oxidised lipoic acid.[23] However, it has also been reported that cystine is rheologically active.[24]

Bis-γ-glutamylcystine produced a slightly more severe effect than GSSG on a mole-for-mole basis, although the overall effect was very similar. This effect is illustrated in Fig. 2, where it can also be seen that the disulphides differed from thiols in their effect on dough. With disulphides, the decrease in development time was not as great, and, whereas with thiols the resistance reached a relatively steady value after 8 or 9 minutes of mixing, with disulphides the loss of resistance was both rapid and continuous.

Effect of flour peptides

When the crude flour peptides, containing 30 μmole of disulphide, were added to dough, the rheological activity was similar to that of 10 μmole of GSSG. However, when the more purified basic and acidic peptides were separately tested, the rheological activity was found mostly in the acidic fraction (Fig. 3) which had previously been shown to contain GSSG as its sole disulphide.[1] The basic peptides showed only slight activity (Fig. 4). There was no alteration to the development time, but thereafter, the fall in resistance was more rapid than in the controls.

Further, when the relaxation of strains mechanically introduced into dough was studied using the extensograph, it was found that 100 μmole of basic peptide disulphide had no effect on the rate of relaxation of the dough (Fig. 5). In the same circumstances, 50 μmole of GSSG had a pronounced effect in increasing the rate of relaxation. In other experiments as little as 20 μmole GSSG had a definite effect on the rate of relaxation.

In the reduced form, the basic peptides decreased the development time in the farinograph as did the simple thiols, but in contrast to simple thiols, the maximum resistance was significantly lowered (Fig. 4).

Discussion

Flour can be thought of as plant tissue which has been structurally disrupted by the milling process. As such, it will contain a complex mixture of biochemical entities. The peculiar properties of dough will depend to some extent on each one of these components. The present discussion emphasises disulphide bonds and thiol groups because they appear to be important components in defining the properties of dough (see Hird[3]).

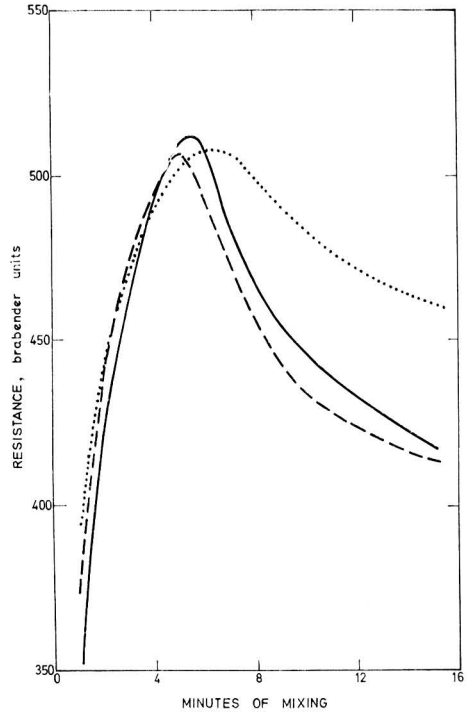

FIG. 3. *Effects of an acidic flour peptide on a farinogram of dough*
Conditions were the same as for Fig. 2
. . . . . Control dough;
— — — — Dough plus 10 μmole of standard GSSG;
———— Dough plus acidic flour peptide containing 8 μmole of disulphide

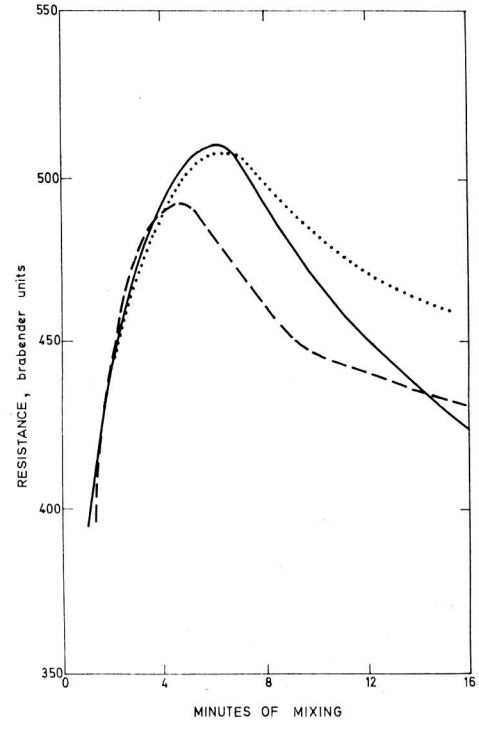

FIG. 4. *Effects of basic flour peptides on farinograms of dough*
Conditions were the same as for Fig. 2
. . . . . Control dough;
———— Dough plus basic flour peptides containing 30 μmole of disulphide;
— — — — Dough plus reduced basic flour peptides containing 60 μmole of thiol

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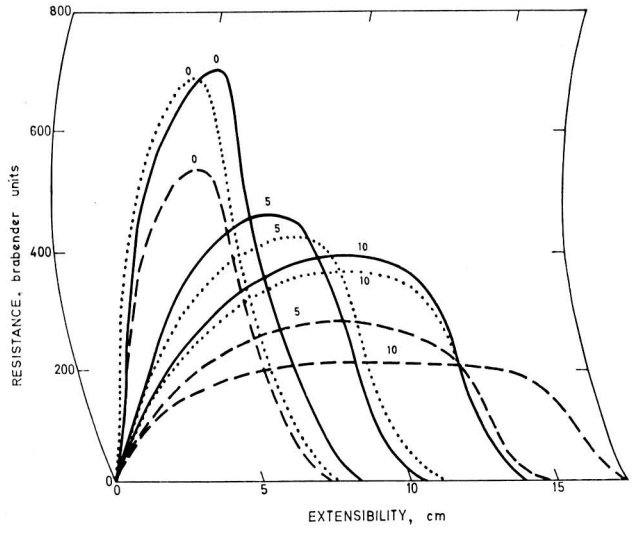

FIG. 5. *Extensograms of doughs tested at 0, 5 and 10 minutes after balling and shaping*

The numbers 0, 5 and 10 refer to the time of testing
. . . . . Control dough;
— — — — Dough plus 50 μmole of standard GSSG per 300 g flour
———— Dough plus 100 μmole of basic flour peptide

Flour disulphide peptides

The endogenous disulphide peptides of flour were tested for rheological activity, and it was found that GSSG was the only peptide that possessed substantial activity. The basic peptides possessed slight activity when tested in the farinograph, and had no effect on the relaxation of dough as measured by the extensograph.

The endogenous level of basic disulphide peptides in flour was found to be 13 μmole of disulphide per 50 g of flour.[1] When an additional 26 μmole of isolated peptide was added to 50 g of flour in the farinograph, the effect was to treble the normal amount of these peptides. This trebling produced only a slight effect on the rheological properties of the dough.

The probable reason for the failure of the basic disulphide peptides to alter the properties of dough is that most of their disulphide groups are relatively non-reactive at the pH of dough. Examination of the reactivity of these disulphide peptides with GSH showed that at the pH of dough, some of the disulphides were rapidly reduced, but the majority were slow to react.[1]

The only disulphide fraction isolated that substantially altered the properties of dough was the glutathione fraction. Although the basic peptides are present in a 5 to 10 fold excess over endogenous glutathione, glutathione appears to be more important in determining rheological behaviour. It follows that the quality of flour, as determined by the rheological behaviour, should be correlated with the small amount of glutathione, rather than with the content of the more numerous basic disulphide peptides (see also Hird *et al.*[25]).

Simple disulphides

Of the simple well characterised disulphides added to dough in the farinograph, GSSG and bis-γ-glutamylcystine were the only ones found to have rheological activity. Cystine, homocystine, oxidised lipoic acid, oxidised dithiothreitol and cystamine were all inactive, although their corresponding thiol forms were highly active.

A possible mode of action of disulphides has been presented in Equation 1. A portion of the thiol produced (RSH) may undergo further reactions as described in Equation 2. However, one important effect of disulphides will be to replace protein thiols with a mixed disulphide.

$$PR\text{-}S\text{-}S\text{-}PR + RSH \longrightarrow PRSH + PR\text{-}S\text{-}S\text{-}R \quad .. \quad (2)$$

It has been suggested[26] that disulphides such as GSSG need to be enzymically reduced in dough before becoming rheologically active. The high activity of bis-γ-glutamylcystine, which is not reduced by wheat extracts,[27] negates this hypothesis. Further, it has been found that the addition of a glutathione reductase system to dough does not in any way alter the response of dough to GSSG.[28] Thus it seems that reductase enzymes do not play an important part in mediating the activity of disulphides. The different rheological activities of disulphides probably result from different chemical activities of their disulphide groups.

Simple thiols

The addition of a thiol such as GSH could be expected to cleave disulphide bonds (Equation 2). The effect of this on the farinogram may depend on whether the bonds so cleaved were intra-molecular or inter-molecular.[10,29,30] Cleavage of inter-molecular bonds would presumably increase the fluidity

of the dough. The effects of cleavage of intra-molecular bonds of insoluble proteins are not known, but one could envisage some increase in viscosity resulting from increased dispersion of the proteins.

Electrolytically reduced flour peptides

When added to dough in the reduced form, the basic peptides differed in their effect from simple thiols. First, their effect was comparatively slight, and second, whereas simple thiols increased the resistance early in mixing, the flour peptides decreased it. These peptides were considerably larger than any of the simple thiols tested, and one possible explanation of their different effects is that being larger, the basic peptides would have less access to protein bonds—particularly intra-molecular bonds.

Conclusion

The basic disulphide peptides of flour are less important than endogenous glutathione in determining the rheological behaviour of dough.

Of several disulphides examined, only GSSG and bis-γ-glutamylcystine were capable of substantially altering the physical properties of dough.

Acknowledgments

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ERRATA

In the paper by Ewart in *J. Sci. Fd Agri.*, 1968, **19**

Page 618 left-hand column, line 22 *for* $\frac{(2n)!}{n!\,2n}$ *read* $\frac{(2n)!}{n!\,2^n}$

Page 621 caption to Fig. 6(a) should read

(a) Three glutenin molecules under tension

SS denotes an interchain S.S bond
▬ denotes a polypeptide chain of mol. wt. ~20,000
—— denotes remainder of molecule, of similar structure to the part shown in more detail

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JANUARY, 1969

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Genesis, mineralogy, and related properties of West Indian soils. I. Montserrat series, derived from glauconitic sandstone, Central Trinidad. II. Maracas series, formed from micaceous schist and phyllite, Northern Range, Trinidad. N. Ahmad, R. L. Jones and A. H. Beavers (*J. Soil Sci.*, 1968, **19**, 1–8, 8–19).—Characteristics of the two series are presented. A. H. CORNFIELD.

Mica genesis in Hawaiian soils. T. C. Juang and G. Uehara (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 31–35).—A soil mica with the formula $Na_{0\cdot11}(NH_4)_{0\cdot06}K_{1\cdot24}$ (Al, Si) (Al, Fe, Mg) O_{20} $(OH)_4$ was identified in wet upland Hawaiian soils. It contains 7·2% K_2O and is predominantly a mixed-layer mica with vermiculite–montmorillonite and chlorite. The mica is concentrated in the surface horizons and its concn. in the soils increases with rainfall and elevation. Biological recycling of K is proposed as a mechanism for its formation. A. H. CORNFIELD.

Influence of pseudogleying on various chemical characteristics of loess soils. H. Wiechmann (*Z. PflErnähr. Bodenk.*, 1968, **120**, 20–31).—In the course of soil development in the B_V/C_V horizons of uncultivated soils, Fe- and Al-oxides are set free and a close positive correlation is found between clay and these oxides. A positive correlation also exists between clay and dithionite-sol. Fe (Fe_d). No relations exist between oxalate-sol.-Fe (Fe_{ox}) and -Al (Al_{ox}) and the clay content. Fe_d is negatively correlated with pH only where the B_V/C_V horizon is undisturbed by cultivation; liming has little effect and poor drainage has no effect on Fe_d. The content of Fe_{ox} depends primarily on the intensity of weathering and increases with decreasing pH in all horizons. This correlation is closer where poor drainage exists. The degree of activity of Fe oxides and pH are more closely related and the activity decreases with increasing depth. The same applies to Al_{ox} except that drainage has a much smaller effect. M. LONG.

Soil trends and variability across selected landscapes in Iowa. P. H. Walker, G. F. Hall and R. Protz (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 97–101).—Soil profile features measured in sample grids on various Iowa landscape segments were represented in polynomial trend equations. A systematic variation in max. and min. of soil properties was observed from upland to lower pediment slopes using this model. A statistical fit of soil landscape data was provided by the polynomials. A. H. CORNFIELD.

Limitations of quantitative soil clay mineralogy. B. L. McNeal (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 119–120).—Analysis of the effects of varying the parameters assigned to soil mineral species for the purpose of quant. mineralogical analysis suggests that absolute determination of mineral contents may be precluded by the variations in soil mineral properties. Rejection of those variations that produce unreasonable results for specific groups of soils indicate that mineral content for these soils is often valid to within ±5%. A. H. CORNFIELD.

Micromorphological study of soil crusts. D. D. Evans and S. W. Buol (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 19–22).—Photomicrographs of thin sections of soil crusts indicated differences due to soil type and management. The value of the technique in relation to water intake and air permeability is discussed. A. H. CORNFIELD.

Factors affecting the production and measurement of colours in montmorillonite and kaolinite clays and natural soils. M. A. K. Majlis (*Diss. Abstr.* B, 1967, **28**, 431–432).—Colour changes in the minerals caused by additions of Fe, Mn or org. matter under controlled pH conditions are examined. The pH of suspensions of the minerals and the colour sources, singly or in combination were adjusted to various levels with NaOH or $CaCO_3$. After washing, filtering, air-drying and sieving (0·25 mm) the products, the reflectancies were measured at a range of wave-lengths, data being converted into I.C.I. and Munsell colour units. Effects of additions of the colorants are recorded. Small differences in colour of soils and minerals were measurable spectrophotometrically. With diminution in particle size, reflectance and visual efficiency in dark soils increased and colour purity in red soils was more readily defined. Colour change in clays following additions of the colorants may be a two-stage phenomenon in which an initial surface coating may be bonded to the clay, followed by a build-up of the deposit with material not necessarily bonded. A. G. POLLARD.

Calcium release and weatherability of some primary minerals assessed with jack pine (*Pinus banksiana*, Lamb) and weathering solutions. D. Burger (*Diss. Abstr.* B, 1967, **28**, 394).—Primary minerals were separated from relatively young soils in recently glaciated areas, ground, and equal vol. of the size-fraction (0·104–0·147 mm) were taken for examination. The minerals were added to the nutrient medium in greenhouse experiments, as sources of Ca for the pines. In laboratory tests, portions of the ground minerals were mixed with 0·0012 N-H_2SO_4 and subjected to a gentle rolling motion in polythene bottles. At weekly intervals the acid was decanted and replaced by fresh acid. The conductivity, pH and Ca content of the acid extracts were determined. Two stages of weathering of the minerals are recognised, viz., (*a*) an early-weathered stage during which Ca is released gradually with decreasing conductivity and pH and (*b*) a semi-weathered stage during which these values become relatively stabilised. The rates of release of Ca from the minerals by the dil. H_2SO_4 were in the descending order: calcite (I), dolomite (II), apatite (III), bytownite (IV), diopside (V), grossularite (VI), augite (VII), and labradorite (VIII), hornblende (IX), oligoclase (X), and albite. Rates of release of Ca by the acid, by a nutrient solution alone and by presence of seedlings were significantly correlated. The weatherability index based on Ca released from semi-weathered minerals by acid was I > II > IV > III > VIII > X > V > VII > IX > VI. A. G. POLLARD.

Effect of grass cover on overnight heat losses from soil, and factors influencing thermal conductivities of soils. M. W. Gradwell (*N.Z. Jl Sci.*, 1968, **11**, 284–300).—Losses of sensible heat down to depths of 40 cm during radiation (frost) nights were calculated from recorded soil temp., moisture contents and *d* (dry-wt.) for silt loam with and without grass cover. A short-grass cover decreased losses more than did reduction (0·4 g/cm³) in dry wt. *d* by soil loosening. Keeping the soil clean-cultivated and compact in an orchard gives 30% savings in artificial-heating costs. Calculated relations between soil thermal conductivity (λ), moisture and *d* (1·2–1·5 g/cm³) show that an increase of 25 units of moisture-content (by dry-wt.) has the same effect as an increase of 0·3 g/cm³ dry-wt. *d*, viz., the λ is doubled. The λ of most N. Zealand soils can be calculated approx. from *d* and moisture-content using these relations. W. J. BAKER.

Comparison of two methods of determining the thermal diffusivity of a moist soil. A. Hadas (*Proc. Soil. Sci Soc. Am.*, 1968, **32**, 28–30).—Comparison of a sine wave type of heating and a square wave type showed that the latter method yielded higher values for the thermal diffusivity of a soil at various moisture contents and with different heating periods. A. H. CORNFIELD.

Methods of determining the moisture characteristics of soils. P. J. Salter (*Expl. Agric.*, 1967, **3**, 163–173).—Field and laboratory methods of measuring the upper and lower limits of availability, from which available-water capacity is determined, are described. The errors associated with the use of the different techniques on a wide range of soils were calculated and suggestions are made regarding the choice of method for particular circumstances. A. H. CORNFIELD.

Instrument for *in situ* measurement of soil moisture flow and suction. J. W. Cary (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 3–5).—The construction of a soil moisture flux transducer and results obtained in the laboratory under steady-state conditions in a silt loam are described. The main advantage of the method is that the hydraulic conductivity of the soil need not be known in order to measure unsaturated flow in the field. A. H. CORNFIELD.

Applicability of Darcy's law. D. Swartzendruber (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 11–18).—The magnitude of the various reported deviations from Darcy's equation are considered and their importance in flow processes in soils is discussed. A. H. CORNFIELD.

Measurement of soil suction by a rapid method. M. J. Dumbleton and G. West (*J. Soil Sci.*, 1968, **19**, 40–46).—Apparatus is described for rapidly measuring soil suction up to pF 3·35 without changing the moisture content of the sample. The apparatus can be constructed in a form suitable for field use. A. H. CORNFIELD.

Device for calculating available water capacity (*AWC*) of soils. J. B. Williams (*Expl. Agric.*, 1967, **3**, 159–162).—The device is basically a 'slide rule', constructed in either straight or circular form, which facilitates the procedure of calculating *AWC* based on measuring the thickness of each soil horizon and assessing its texture, and summing the products of the thickness of each horizon and the mean values of *AWC* for the relevant textural classes. A. H. CORNFIELD.

Soil reflectance, temperature, and fallow water storage on exposed subsoils of a Brown soil. A. L. Black and B. W. Greb (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 105–109).—Cross slope levelling of a loessial silt loam exposed different horizons of subsoil which varied in reflectance and other physical properties. Soil water storage during fallow was negatively correlated with mean soil temp. to a depth of 7·6 cm, but subsequent crop yields were not related to the amount of soil water stored at the end of the fallow period. Soil temp. was influenced by soil reflectivity. A. H. CORNFIELD.

Soil water diffusivity from horizontal infiltration. F. D. Whisler, A. Klute and D. B. Peters (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 6–11).—A method for measuring soil water diffusivity, based on γ-ray absorption measurements of the water content as a function of time at a fixed position in a horizontal infiltration system, is described. A. H. CORNFIELD.

Air permeability of different fractions of the pore volume [in soils]. K. H. Hartge (*Z. PflErnähr. Bodenk.*, 1968, **120**, 31–45).—Core samples from three para-Brown Earth profiles (loess, glacial till) were equilibrated with soil tensions of 20–300 cm water columns. Air permeability at each tension was measured. The vol. of pore fractions calculated from the soil water characteristic were compared with those calculated from air percolation by the Hagen-Poiseuille equation. From the data obtained the no. and length of pores involved in percolation were estimated. Air permeability increased with rise in soil moisture tension, with a min. value at 100–200 cm tension. Interrelationships between the various factors are discussed. A. G. POLLARD.

Density, conductance and infra-red studies of clay–water systems. P. Jorgensen (*Diss. Abstr.* B, 1967, **28**, 430).—The density of water adsorbed on Na- and Li-bentonites was determined by use of density-gradient tubes and pycnometers. Over the range of concn. (up to 34%) used there was no change in partial sp. vol. Water with abnormal d causes an apparent change of 0·06 g/cm³ in the d of the mineral; this accords with the view that each clay particle is surrounded by two layers of water with d differing from that of pure water by 2%. Data for the conductance of sols and gels having different concn. of clay showed that activation energy for conductance increased with clay concn. whereas the wt. conductance showed min. at 0·2 and at 3·5–4·0 g of clay per 100 cm³ of suspension. Particle mobility is apparently lost between these two points probably because of particle bonding. With clay concn. > 4·0 g/100 cm³ the particles were immobile and conductance was due entirely to ions. Use of i.r. data for the Na bentonite showed the clay-ion system to influence the bending vibration for the water mol. as well as the amount of H-bonds formed. When the water adsorbed on the clay surfaces corresponded with a monolayer between the silicate layers, optimum restriction on the bending vibration was reached and the amount of H-bonding attained its max. at this point. H-bonding was min. with ~ 2 water mol. layers. A. G. POLLARD.

Cationic diffusion in clay minerals. I. Homogeneous and heterogeneous systems. T. M. Lai and M. M. Mortland (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 56–61).—Diffusion values for Na^+ or Cs^+ in vermiculite saturated with Na^+, K^+, or protonated *p*-phenylenediamine, and in kaolinite and bentonite saturated with Na^+ or K^+, were measured at various times. Heterogeneous and homogeneous diffusion systems were suggested on the basis of change or lack of change in apparent diffusion coeff. with diffusion time. This was in accord with the structure of the clay minerals used and conformed with a mathematical model derived originally for solving the grain boundary problem. The diffusion coeff. of external and interlayer surfaces of Na^+ in Na-vermiculite were evaluated. A. H. CORNFIELD.

Thermodynamics of cation exchange in montmorillonite clay. A. R. Swoboda (*Diss. Abstr.* B, 1967, **28**, 434).—Heats of exchange of Na^+, Li^+ and K^+ in montmorillonite were measured calorimetrically; ΔH° values were determined from equilibrium const. at different temp. in apparatus designed to record heat exchange measurements. Heat changes of 0·01 cal. in 265 ml of dil. clay suspension could thus be measured. For the exchange of Na by K, of Li by K and of Li by Na ΔH° values were −956, −771 and −101 respectively. Enthalpy changes calculated from equilibrium data did not agree with values obtained by direct calorimetry; the discrepancy is attributed to difficulty in measuring accurate thermodynamic equilibrium const. at different temp. Changes in free energy values approximated to ΔH° for the Na-K exchange; ΔG° for the Li-K exchange was approx. double the ΔH°. For the Na-Li exchange ΔH° and ΔG° were approx. the same but the energy transfers were in opposing directions. This and other data show the affinity of montmorillonite for the three cations to be in the order K > Li = Na. A. G. POLLARD.

Correlation between pH and exchangeable sodium percentage in saline and alkali soils. R. K. Shah, J. C. Vora and A. J. Gandhi (*Indian J. appl. Chem.*, 1967, **30**, 96–99).—A number of arid, semi-arid and humid coastal salines from eleven different localities were used. The correlation between pH and *ESP* (exchangeable Na %) was extremely poor. It is assumed that many other factors other than *ESP* influence the soil, such as the presence of gypsum, the $CaCO_3$ content, extent of salinity and maturity of the soil complex, and these factors do not necessarily influence pH and *ESP* to the same extent. I. DICKINSON.

Effect of humic acid on iodide adsorption by soils. R. C. Jee and S. K. De (*Indian J. appl. Chem.*, 1967, **30**, 80–83).—Humic acid (**I**) helped considerably in shifting the negative adsorption of I^- either towards positive or to a lesser negative adsorption. This effect depended on the type of soil surface and also on the concn. of **I**. The optimum concn. of application of **I** to the soil type used, to obtain max. I^- adsorption, was 4 g/10 g of soil. I. DICKINSON.

Metabolism of humus substances in soils by continuous cultivation. I. Matsura, F. Kunts, D. I. Nikitin and R. A. Lokhmacheva (*Microbiology* [*USSR*], 1967, **36**, 584–590).—The possibility of carrying out continuous observations is proven. The apparatus and working conditions are described; the experimental results are discussed and their interpretation is attempted. The methods employed include simultaneous estimation of enzyme activity, the number of micro-organisms present on different media and the content of humus substances in the fractions. (19 references.) C.V.

Preparation of soils for microbiological analysis by ultrasonic treatment. D. G. Zvyagintsev and G. M. Galkina (*Microbiology* [*USSR*], 1967, **36**, 910–916).—Compared with grinding, shaking, etc., it was found that subjecting a soil suspension to low frequency, low power ultrasonics, gave more satisfactory results relating to the number of bacteria and actinomycetes present, although the effectiveness depended on the peculiarities of the type of soil. Humus-gley soil showed an increase of over 1000 times, chernozem 20, red soil 5–8 and turf-podzol had factors of 2–5 times. Wet soils did not show such marked increases but this treatment was equally applicable. The number of fungi did not increase as markedly and after prolonged treatment the number was found to drop, a phenomenon not found with bacteria and actinomycetes. (14 references.) C.V.

Clay minerals and microbial ecology. G. Stotzky (*Trans. N.Y. Acad. Sci.*, 1967, **30**, 11–21).—The marked influence of clay minerals (*CM*) on the activity, ecology and population dynamics of micro-organisms in natural habitats, specially soil, is discussed. The influence differs with bacteria, fungi and actinomycetes and appears to be related to the physico-chemical properties of the clays. It is suggested that when all the problems are solved it may be possible by manipulation of the *CM* composition to control the

undesirable micro-organisms in their natural habitat. (27 references.) C.V.

Distribution of Caulobacter in certain soils. N. A. Krasil'nikov and S. S. Belyaev (*Microbiology [USSR]*, 1967, **36**, 906–909).—Members of this group were found in humus horizons of podzolic turf soils at pH 5·5–7·0; the techniques used did not show their presence in more acid soils. The numbers of cells found ranged from 100–10,000 per g soil. Using pedoscopes, it was shown that these cells frequently surrounded the hyphae of the fungi. (11 references.) C.V.

Self-diffusion of phosphorus in clays and soils. I. Effect of phosphorus rate. R. E. Phillips, G. A. Place and D. A. Brown (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 41–44).—For each clay and soil there was a linear correlation between rate of P application (10–320 ppm PO_4^{3-}-P) and self-diffusion of PO_4^{3-} at near-neutral pH. Diffusion coeff. in kaolinite and montmorillonite clays were approx. 40 times above those in illite and in silt loam and clay soils. For individual clays and soils diffusion coeff. were highly correlated with water-sol. PO_4^{3-} and Al-bound PO_4^{3-}, but for the materials as a whole self-diffusion coeff. were not correlated with any PO_4^{3-} fraction. A. H. CORNFIELD.

Interaction of limestone particle size and phosphorus on the control of soil acidity. H. L. Barrows, A. W. Taylor and E. C. Simpson (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 64–68).—Previous application of PO_4^{3-} (150 ppm P soil basis) did not reduce the efficiency of limestone and calcite in neutralising soil acidity when these materials were applied in < 80-mesh form. When the materials were applied in coarser form efficiency of neutralisation decreased with increasing coarseness. 12–14 mesh material reduced efficiency by 8·5%. The extent of reduction of efficiency of neutralisation increased with level of PO_4^{3-} previously applied. The reduced efficiency was attributed to surface coatings of phosphatic materials. A. H. CORNFIELD.

Inositol penta- plus hexa-phosphate content of Canadian soils. R. B. McKercher and G. Anderson (*J. Soil Sci.*, 1968, **19**, 47–55).—The inositol penta- + hexa-phosphate contents of 18 surface soils ranged from 20 to 71 ppm P and of 12 subsoils from 18 to 43 ppm P, and were higher in forest than in grassland soils. The amounts were correlated with total org. P and total soil P at $P < 0·001$, with PO_4^{3-}-retention capacity at $P < 0·01$ and with total N at $P < 0·05$. The amounts accounted on an average for 6% of the total P and for 17% of the org. P. A. H. CORNFIELD.

Mineralisation of organic phosphorus in soils as affected by addition of inorganic phosphorus. D. R. Wier and C. A. Black (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 51–55).—The increased mineralisation of soil org. P resulting from incubation of soils with added PO_4^{3-}, as reported by other workers could not be confirmed in incubation tests with mineral and org. soils. A. H. CORNFIELD.

Dissolution and availability to plants of rock phosphates of igneous and sedimentary origins. R. H. Howeler and C. M. Woodruff (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 79–82).—Missouri apatite (igneous origin) had a strong cryst. structure, showed a slow rate of dissolution in dil. HCl and released its P very slowly to crops in greenhouse studies. Sedimentary rock phosphates have a smaller degree of crystallinity and decompose more easily. Florida rock phosphate was the most effective in supplying P to plants, whilst the availability of P from Arkansas rock phosphate was low because of its high $CaCO_3$ content. A. H. CORNFIELD.

Forms of inorganic phosphorus extracted from soils by N-sulphuric acid. R. Shah, J. K. Syers, J. D. H. Williams and T. W. Walker (*N.Z. Jl agric. Res.*, 1968, **11**, 184–192).—The value (P_a) for the amount of inorg. P extracted by N-H_2SO_4 from an unignited soil sample exceeded acid-extractable Ca-P for all samples, the P_a fraction being composed of acid-extractable Ca-P plus a part of secondary inorg. P. For weakly-weathered soils P_a exceeded the sum of acid-extractable Ca-P and non-occluded P, whereas in strongly-weathered soils in which acid extractable Ca-P was absent, non-occluded P exceeded P_a. The decline in P_a with increasing degree of soil development is attributed to loss by leaching and conversion into org. P and occluded forms of inorg. P. (20 references.) E. G. BRICKELL.

Acid-free vanadate-molybdate reagent for the determination of total phosphorus in soils. H. L. S. Tandon, M. P. Cescas and E. H. Tyner (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 48–51).—The determination of total P in soils using an acid-free vanadate-molybdate colorimetric method following digestion with $HClO_4$ is described. The optimum acidity for colour development is derived from the residual acidity of the $HClO_4$. A. H. CORNFIELD.

Potassium reserves in British soils. I. The Rothamsted classical experiments. O. Talibudeen and S. K. Dey (*J. agric. Sci., Camb.*, 1968, **71**, 95–104).—Soils (34) were exhaustively cropped with ryegrass under glass. The concn. and yield of K in the ryegrass tops and the K intensity (*KI*) in the soil were measured every 4 weeks. The *KI* is defined as $(K)/(Ca)^{0·5}$, where (K) and (Ca) are the activities of the K and Ca ions in solution in equilibrium with the unextracted soil. The rate of change in *KI* was related to periods of intense and limited exhaustion and partial recovery of the soil during cropping. The cumulative K yield of ryegrass was highly significantly related to *KI* of uncropped soil, the 16-week yield being better related than the 60-week yield. For Park Grass soils allowance for pH changes improved the relation. *KI* for all soils decreased to nearly 10^{-3} $(M)^{0·5}$ after 16 weeks, although large yield differences persisted after this. The K buffer capacity (*BC*) of the soil per unit clay content was inversely related to *KI* of the uncropped soil. For K-rich soils but not for poor soils values of *KI* and *BC* were related. Where N was applied the value of *KI* was smaller than that in the same soil with no N applied. Field liming also decreased *KI* and increased *BC*. A rapid method is described for the determination of *KI*. M. LONG.

Potassium exchange equilibria and yield responses of oats, barley and maize in Quebec soils. H. G. Zandstra and A. F. MacKenzie (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 76–79).—The K/(Ca + Mg) activity ratios of eight soils were compared with growth responses of three species to added K in field tests. The activity ratios did not correlate as well with crop response to K as did 'K potential' (product of total exchangeable K and potential buffering capacity). A. H. CORNFIELD.

Rapid method for determining movement of applied manganese in soil. L. R. Hossner (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 125–126).—A rapid qual. method for estimating the rate and distance of movement of Mn^{2+} from fertiliser granules in soil is presented. Filter paper saturated with 5 N-HNO_3 is pressed on the soil surface and sol. Mn is absorbed by the paper, which is then removed and dusted with $NaBiO_3$ to develop the MnO_4^- colour. A. H. CORNFIELD.

Association of manganese and cobalt in soils. R. M. Taylor (*J. Soil Sci.*, 1968, **19**, 77–80).—Soil Mn was mineralised either as birnessite or lithiophorite in five of seven soils sampled in Bermuda, Europe, and the Middle East. The majority of the soil Co was associated with these minerals, agreeing with earlier work on Australian soils and suggesting that these two are the most common forms of secondary Mn minerals. A. H. CORNFIELD.

Effect of phosphorus source on the movement and uptake of band-applied manganese. L. R. Hossner and G. E. Richards (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 83–85).—When mixed with $MnSO_4$ and band-applied in a silt loam (pH 7·0), $NH_4H_2PO_4$ (MAP) and NH_4 polyphosphate (APP) were much more effective than were $Ca(H_2PO_4)_2$ (MCP) and $(NH_4)_2HPO_4$ (DAP) in increasing Mn uptake by soyabeans. The uptakes of Mn and P were highly correlated. The amount of Mn absorbed per increment of P decreased in the order APP = MAP, MCP, DAP. Exchangeable-plus easily reducible-Mn as influenced by P source decreased in the order of APP, MAP, MCP = DAP. A. H. CORNFIELD.

Availability of manganese and zinc in fusions with sulphur. A. E. Ludwick (*Diss. Abstr.* B, 1967, **28**, 430).—The value of the products of fusion of Mn- or Zn-compounds with S as slow-release sources of the two trace elements for plants is examined. In greenhouse trials on a sandy loam (pH 7·6), max. yields of ryegrass and Mn uptake were obtained by additions of $MnSO_4$, $MnCO_3$ or the porous Mn–S fusion products; MnO_2 was ineffective. The uptake of S was directly related to the surface area of the granules; rates of oxidation of S were not adversely affected by the presence of MnO_2 or $MnCO_3$. The Mn uptake of the crop was related directly to the solubility of the Mn source and inversely to the size of granules; the total yield of the crop was not affected by the Mn treatments. In field trials with soyabeans and oats 40–80 mesh and 80 mesh particles of the fusion products gave best results. Of the Zn products, fusions of ZnO or $ZnCO_3$ with S gave the highest yields of dry matter and provided a favourable and constant Zn supply. The influence of particle size on solubility of Zn and oxidation of S was similar to that on corresponding Mn products. Oxidation of S was increased by raising the pH of soil from 5·2 to 7·6; at pH < 4 the oxidation was temporarily suppressed. A. G. POLLARD.

Sulphate distribution in a solonetzic soils with a water table. S. U. Khan and G. R. Webster (*J. Soil Sci.*, 1968, **19**, 20–24).—In a solonetzic loam column subjected to a moving water table, reten-

tion of SO_4^{2-} was greater in the surface soil than at lower depths. SO_4^{2-} retention was lowest in the soil layer (pH 8·41) having the highest acid-extractable PO_4^{3-}. SO_4^{2-} tended to move more slowly than water in soil. A. H. CORNFIELD.

Solubility and redox criteria for the possible forms of selenium in soils. H. R. Geering, E. E. Cary, L. H. P. Jones and W. H. Allaway (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 35–40).—The solubility of seven soils indicates that Se concn. in solution is governed primarily by a ferric oxide–selenite-adsorption complex (Se oxidation state +4). Under certain conditions Se may also exist in the oxidation states +6, 0, and −2. The proportions of Se in the four oxidation states are treated theoretically as they are affected by redox potential, soil pH, and ions with which Se combines. A. H. CORNFIELD.

Relationship of total and hot water-soluble boron, and fixation of added boron, to properties of podzol soils. U. C. Gupta (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 45–48).—Total and hot water-sol. B contents were higher in fine- than in coarse-textured soils and in soils derived from Permocarboniferous rocks. The % of the total B in hot water-sol. form was also higher in the fine-textured soils. 12–20% of the added BO_3^{3-}-B was fixed, against extraction with hot water, during incubation of soils for 12 weeks, and extent of fixation was little affected by varying moisture content from 50 to 100% of the field capacity. Hot water-sol. B was positively correlated with soil total B and org. matter content. A. H. CORNFIELD.

Method for extracting organic matter from soil. G. J. Gashko and F. J. Stevenson (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 117–119).—The method involves pre-treatment of the soil with 0·3 N-HF followed by extraction of org. matter with 0·02 M-$Na_4P_2O_7$ and then with 0·03 N-NaOH. After each treatment sol. inorg. constituents are removed by dialysis. Most of the solubilised org. matter is recovered as an acid-insol. material low in ash. A. H. CORNFIELD.

Oxidation-reduction potential of a saturated forest soil. F. T. Bonner and C. W. Ralston (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 111–112).—When a forest soil was waterlogged and incubated for 25 days redox potentials fell to a much greater extent with incubation at 21–27° than at 5°. Addition to the soil before incubation of 5% sucrose, ground pine needles or yellow-pine leaves greatly increased the fall in redox potentials. The addition of a complete nutrient had no effect on the soil incubated with sucrose, but reduced the drop in potential in soil incubated at the higher temp. with needles and leaves. A. H. CORNFIELD.

Effect of nitrate and nitrous oxide on hydrogen and methane accumulation in anaerobically-incubated soils. D. Laskowski and J. T. Moraghan (*Pl. Soil*, 1967, **27**, 357–368).—During anaerobic incubation addition of KNO_3 to the soil or of N_2O to the atm. above the soil reduced the rate of accumulation of CH_4. No H_2 accumulated either in the absence or presence of KNO_3 or N_2O. A. H. CORNFIELD.

Effect of the size of soil aggregates on nutrient supply. I. S. Cornforth (*J. agric. Sci., Camb.*, 1968, **70**, 83–85).—Pot trials using soil aggregates of different sizes showed that both P and NO_3^--N uptakes were more rapid from fine than from large particles. M. LONG.

Effect of crop rotation and fertilisers on nitrification in sod-podzolic soil. A. I. Chunderova and T. P. Zubets (*Microbiology [USSR]*, 1967, **63**, 917–920).—Long-term experimentation showed that a significant change occurred in the biological activity of slightly cultivated podzolic soil with considerable activation of nitrification, this being used as a criterion of increase in soil fertility. (10 references.) C.V.

Limiting values of plant mineral contents and their use for the estimation of fertiliser requirements. A. Finck (*Z. PflErnähr. Bodenk.*, 1968, **119**, 197–208).—Values of deficiency, normal range and toxicity levels of major and of some trace elements are tabulated for a few crops. Their use is discussed. M. LONG.

Use of nitric acid in the manufacture of fertilisers. D. A. Mitchell (*Proc. Symp. Sulphur Sulphuric Acid India, Indian Chem. Mfrs Ass.*, 1967, 45–53).—A review and discussion are presented, with emphasis on the NP/ASU [nitric acid, phosphate/$(NH_4)_2SO_4$] process. E. G. BRICKELL.

NH_4NO_3 neutraliser pressures. J. C. Maunders and A. J. Grant (*Chem. Process Engng*, 1968, **49**, No. 4, 70–71).—Production of fertiliser-grade NH_4NO_3 as high-*d* prills requires the use of $\lesssim$ 99·5% NH_4NO_3 solutions, which produces corrosion and explosion hazards. Use of Ti for corrosion prevention is expensive. Both hazards can be prevented by carrying out the neutralisation process at lower temp. in a vac. evaporator. Smooth neutralisation is achieved by injecting into the bottom leg of the neutraliser a stream of recycle NH_4NO_3 solution with HNO_3 injected just above and mixed with the NH_4NO_3 before contacting the NH_3 injectors. The heat of neutralisation is thus controlled and dispersed, and as a result of the vac. in the neutraliser, concn. to >97% is achieved at reduced temp., leaving a greatly reduced heat load for the falling-film evaporator at the prilling tower. The saturation temp. of the steam leaving the neutraliser and passing through the NH_3 superheater and vaporiser and HNO_3 preheater, $\sim$ 80°, is low enough to avoid corrosion of the preheater. Stainless steel can be safely used in this process. T. M. BARZYKOWSKI.

Ammonium nitrate. A. J. Payne and P. G. Glikin (*Chem. Process Engng*, 1968, **49**, No. 4, 65–69).—A survey is given of uses of NH_4NO_3 in fertilisers, physical and chemical properties, manufacture (with flowsheets) of prilled and granulated NH_4NO_3 (Stamicarbon and ICI methods), instrumentation (Stengel process), safety precautions, maintenance and process costs. T. M. BARZYKOWSKI.

Fertilisers from domestic refuse. Anon. (*Engineer, Lond.*, 1968, **225**, 60).—A plant to manufacture a clean, cheap fertiliser from unsorted domestic refuse has been developed. A typical plant handling 100 tons/day would yield 300 tons fertiliser a week, of three main grades, one containing no chemicals and being suitable for conditioning, and two being chemically supplemented for horticultural and commercial applications. All types of refuse (bicycle frames, furniture, carpets, shop, office and domestic) are handled, this being first pulverised and shredded to a specific size before digesting, where the natural process of fermentation raises it to a temp. sufficiently high to destroy pathogenic micro-organisms. C.V.

Nitrification studies on neem cake, mixture of neem cake and superphosphate, processed neem cake and their effect on soil aggregation. N. P. Sinha and K. C. Gulati (*J. Instn Chem. India*, 1967, **39**, 199–202).—Max. nitrification was observed in processed neem cake, followed by neem cake with single superphosphate and untreated neem cake. The effect of cakes on the formation of water-stable aggregates appears to be temporary. E. G. BRICKELL.

Estimation of free acid in superphosphates by high frequency titrimetry. A. D. Pandey and A. K. Roy (*Technology, Q. Bull. Fertil. Corp. India*, 1967, **4**, 26–27).—The free acid in the superphosphate (single or triple) is extracted with Me_2CO/Et_2O (1 : 1), the organic solvents are evaporated and the residue, dissolved in water, is titrated against standard alkali in a high frequency (8 Mc/sec) titrimeter. The first break in the titration curve is sharp and corresponds to the first neutralisation of free H_3PO_4 and complete neutralisation of H_2SO_4 (if present). E. C. APLING.

Availability of phosphate in sheep dung. D. Gunary (*J. agric. Sci., Camb.*, 1968, **70**, 33–38).—Pot trials indicated that the inorg. phosphate content of sheep dung is initially easily available but becomes less so after contact with soil. The org. P has only a low availability. Dung phosphate availability is dependent on the extent of its contact with plant roots. M. LONG.

Evaluation of phosphate fertilisers. I. Immediate value of dicalcium phosphate, nitrophosphates, Gafsa rock phosphate, basic slag and potassium metaphosphate for barley and ryegrass. G. E. G. Mattingly and A. Penny **II. Residual values of [the last four compounds] for potatoes, barley and swedes in rotation, with reference to changes in soil phosphorus status.** G. E. G. Mattingly (*J. agric. Sci., Camb.*, 1968, **70**, 131–138, 139–156).—I. Over the whole season total ryegrass yields in 1960 were the same with each of three granular nitrophosphates (**I**), potassium metaphosphate (**II**), Gafsa rock phosphate (**III**), basic slag (**IV**), granular superphosphate (**V**) and powdered dicalcium phosphate (**VI**), although growth was slower with **I** (containing 5% water-sol. P), **III** and **IV** for the first 12 weeks. More P was taken up by grass from **VI** and **II** than from **V**. For barley **VI** was only 60% as efficient as **V**, **III** was almost inert and **II** and **IV** were 22 and 23% as efficient. The efficiency of **I** was about the same as the water-sol. P content, i.e., 1, 25 and 53% for the 5, 26 and 50% water-sol. grades respectively.

II. Average residual values of these fertilisers calculated from yield response, P uptake and soil analysis were found to be 100 for **V**, 100–102 for **I**, 95 for **II**, 94 for **IV**, and 92 for **III**. Mean 'fresh' superphosphate equiv. of residues from all fertilisers for potatoes and swedes respectively, were 17 and 26% after the first rotation and 11 and 15% after the second. Mean yields of crops increased by 0·24 ± 0·037 ton/acre for potatoes, 0·22 ± 0·08

cwt/acre for barley and 1·16 ± 0·148 ton/acre for swedes for each ppm $NaHCO_3$-sol. P in the soil at harvest. M. LONG.

Results of an experiment at Rothamsted testing farmyard manure and N, P, and K fertilisers on five arable crops and permanent grass. III. Yields 1961–1965. F. V. Widdowson and A. Penny (*J. agric. Sci., Camb.*, 1968, **70**, 53–58; cf. *ibid.*, 1963, **60**, 353).—The results of trials, started in 1956, and carried out during the years 1961–1965 are reported. N decreased rotational ley (**I**) yields, slightly increased potato (**II**) yields after **I** and greatly increased wheat (**III**), barley (**IV**), kale (**V**) and permanent grass (**VI**). P increased all crop yields except **VI**. **IV, V** and **VI** were little affected by K which was beneficial to **I** and **III** and especially so to **II**. Farmyard manure (*FM*) increased all crop yields, especially of **II**. Fertilisers decreased the effect of *FM*. M. LONG.

Superphosphate on wheat. Cumulative effect of repeated applications on yield response. V. F. McClelland (*Aust. J. agric. Res.* 1968, **19**, 1–8).—In experiments over 26 years (10 cycles of a rotation system) with annual applications of 30–120 lb/acre of superphosphate, the cumulative effects gradually gained in significance in comparison with the current effects, especially at the higher levels of application. (10 references.) P. S. ARUP.

Crop response to applied zinc in ammoniated phosphate fertilisers. J. J. Mortvedt (*J. agric. Fd Chem.*, 1968, **16**, 241–245).—Various granular ammoniated phosphate fertilisers were compared as carriers containing 2% Zn as ZnO, $ZnSO_4$ or Zn–EDTA for maize grown in greenhouse plots. Zn uptake by maize was low when inorg. Zn was incorporated with NPK ammoniated fertilisers, but satisfactory when Zn–EDTA was used. The actual effectiveness of $ZnSO_4$ increased with decreases in its concn. in the granules, especially when the granules had been powdered, and decreased with the degree of ammoniation of the fertiliser. (12 references.) P. S. ARUP.

Relative effectiveness of various magnesium fertilisers on a magnesium-deficient pasture. D. E. Hogg and J. Karlovsky (*N.Z. Jl agric. Res.*, 1968, **11**, 171–183).—Fine (**I**) and coarse dolomite, fine and coarse dolomite-reverted superphosphate, Mg phosphate, kieserite (**II**) and serpentine superphosphate (**III**) were compared against normal superphosphate in a field trial on a Mg-deficient pasture. Highest yields of herbage dry matter were obtained with **I, II** and **III** treatments. (13 references.) E. G. BRICKELL.

Controlling urea hydrolysis in soils. Imperial Chemical Industries Ltd. (Inventor: T. E. Tomlinson) (B.P. 1,094,802, 1.10.63).—The soil is treated (either before, simultaneously or after addition of a source of urea) with a hydrolysis-inhibiting compound or compounds. These are (*i*) dithiocarbamates or their deriv. of formula $-[CH_2NRC(S)SZ]_2$ (where each R is independently H or alkyl and Z is monovalent metal, NH_4 or alkylammonium) or the corresponding cyclic dithiocarbamates of divalent metals, or of formula $NHYC(S)S_nX$ (where $n=1$ or 2, X and Y are ⩾12C alkyl or aryl, optionally halo-substituted, or X can also be NH_4, and Y can also be acetamido or H) or (*ii*) *N*-R^2-dichloromaleimides (where R^2 is optionally halo-substituted ⩾12C alkyl, cycloalkyl or aryl). The compound(s), e.g., Zn ethylene-bis-dithiocarbamate or *N*-methyldichloromaleimide can be used together with a hydrolysis-inhibiting haloalkane such as hexabromoethane. S. D. HUGGINS.

Improved assimilability of phosphorus by plants. Societa Edison (B.P. 1,088,800, 1.6.66. It., 7.6.65 and 4.4.66).—Land is treated with fertilisers containing P, and one or more of the following compounds: tartronic acid, Me-, Et-, Pr^n-, Bu^n, Bu^i- and Bu^t-tartronic acids and their deriv. substituted in the alkyl group by ⩽1 COOH, OH, NH_2 or NH_2 substituted with Me and/or Et. The compound/s are present in the fertiliser in amounts of 400 kg to 4 metric tons/metric ton of P in the fertiliser, calculated as P_2O_5. S. D. HUGGINS.

Expanded soil-treating agent. Grunzweig und Hartmann A.-G. (B.P. 1,090,033, 11.2.65. Ger., 12.2.64).—The agent, with prolonged action, is obtained from a substantially closed cell expanded thermoplastic which is only slowly degradable in the soil, particularly a homo- or co-polymer of styrene, with a fertiliser and/or other active substance distributed in the thermoplastic. The mixture of polymer with fertiliser, trace elements, pesticide, plant growth regulant, peat etc., together with a low-boiling blowing agent is continuously expanded by extrusion at ⩾120°. S. D. HUGGINS.

Fertilisers. W. R. Grace and Co. (B.P. 1,094,781, 16.2.65. U.S., 15.4.64).—The trace metals Zn, Mn or Fe are supplied to plants as a composition containing at least one metal salt (sulphate, phosphate, borate etc.) (1–11 pt. by wt.) and at least one metal aminoacetate (a nitrilotriacetate, an ethanol nitrilodiacetate or a diethanol nitriloacetate) (1 pt.). Thus, a suitable composition (claimed) consists of $ZnSO_4$ (5), Zn nitrilotriacetate (1) and a NPK fertiliser (100 pt.). S. D. HUGGINS.

Plant Physiology, Nutrition and Biochemistry

Determining the stoicheiometry of photosynthetic phosphorylation. A. A. Horton and D. O. Hall (*Nature, Lond.*, 1968, **218**, 386–388).—Determinations are described of the P : 2e ratio (μmoles ATP per 2 electrons transferred), normally regarded as unity, when different new buffers are used. Based on $[Fe(CN)_6]^{3-}$ and TPN^+ as electron acceptors, the overall P : 2e ratio with both oxidants was between 1·5 and 1·9 and the 'stimulated' ratio was between 2·71 and 2·75. An interpretation involving possible cyclic photophosphorylation is offered; the presence of mitochondria in chloroplast prep. or exchange reactions between P and ADP and/or ATP are ruled out. Existence of > 1 site for phosphorylation is neither proved nor disproved. The effects of Mg^{2+} and ADP on the overall and stimulated ratios are reported. It is concluded tentatively that the accepted stoicheiometry of non-cyclic photophosphorylation using $[Fe(CN)_6]^{3-}$ may need revision, and that the stimulated ratio is of doubtful significance. (36 references.) W. J. BAKER.

Physiology of sugar-cane. IX. Factors affecting photosynthesis and sugar storage. J. C. Waldron, K. T. Glasziou and T. A. Bull (*Aust. J. biol. Sci.*, 1967, **20**, 1043–1052).—Studies were made of the effects of light intensity, CO_2, temp., leaf age and sugar concn. in stalks and leaves on photosynthesis in sugar-cane. Photosynthetic efficiency appears to be linked to availability of sinks but may also be altered by metabolic imbalance induced by environmental shock. End product repression of photosynthesis by sugars was not the operative control in detached leaves exposed to long light treatments. (10 references.) E. G. BRICKELL.

Productivity of tropical pasture plants. I. Growth analysis, photosynthesis and respiration of Hamil grass and Siratro in a controlled environment. M. M. Ludlow and G. L. Wilson (*Aust. J. agric. Res.*, 1968, **19**, 35–45).—Under optimum conditions, the growth rate of the grass (*Panicum maximum*) was almost twice that of the legume (*Phaseolus atropurpurpeus*), although the leaf-area ratio of the grass was the lower. A comparative trial by the Watson and Hayashi method showed that the high net assimilation of the grass was due to a higher photosynthetic rate, despite a higher respiration rate than in the legume. (38 references.) P. S. ARUP.

Use of the Cionco model to obtain further information on the nature of leaf boundary layers. L. A. Hunt (*Can. J. Bot.*, 1968, **46**, 177–178).—The Cionco mathematical model for air flow within a vegetative canopy was used to estimate the diffusion resistance of the leaf boundary layer in a maize crop. Comparison with values calculated for transfer through a laminar boundary layer suggested that the layer was either turbulent or in a state of transition from laminar to turbulent flow. (11 references.) J. L. WALPOLE.

Cuticular transpiration and wax structure and composition of leaves and fruit of *Vitis vinifera*. J. V. Possingham, T. C. Chambers, F. Radler and M. Grncarevic (*Aust. J. biol. Sci.*, 1967, **20**, 1149–1153).—The fine structure of the surface wax of sultana vine leaves was examined using the C replica technique. Leaf wax is morphologically similar to that on the surface of grapes, and consists of overlapping hydrophobic platelets. Cuticular transpiration was markedly increased by brief exposure to light petroleum vapour, thus disorganising the structure of the platelets, which may thus be important in controlling the transpiration in both the fruit and leaves. E. G. BRICKELL.

Radioisotopes in [determination of salts and labelled complexes in] plant physiology. A. van den Hende (*Atompraxis*, 1968, **14**, 294–296).—The absorption of salts and labelled complexes after foliar application is studied, the method being used to control absorption of ions (e.g. PO_4^{3-}) from the soil. ^{22}Na was used for foliar absorption, Na and K being applied to soils of varying texture. Mn salts and complexes were also studied. (22 references.) C.V.

Uptake and translocation of $^{45}CaCl_2$ after spot application on apple skins at different states of fruit development. J. Wienecke (*Atompraxis*, 1968, **14**, 305).—'Cox Orange Renette' was used, ^{45}Ca translocation being most effective in the early stages of development and decreasing with further development of the fruit. ^{45}Ca-

pesticide mixtures were studied but transport through the skin into the fruit was not substantially influenced. (18 references.) C.V.

Cation–anion relationships in crop nutrition. VI. Effects of part, age and species of plant and some soil characteristics. R. K. Cunningham (*J. agric. Sci., Camb.*, 1968, **70**, 237–244).—There is a positive relationship between the sum of cations (*C*) in plants and their % total N when N is supplied exclusively as NO_3-N. The proportion of ions entering the roots from the soil solution is not constant. The cation/anion relationships (*CA*) are independent of the part of the plant analysed, age or species of plant and of the soil type. The relationships have the same form regardless of species but dicotyledons have values of *CA* twice as great as do monocotyledons at the same level of total N and org. N respectively. Values of *C* and *CA* are greater in grass grown on alkaline than on acid soils. These relationships vary little with yield. M. LONG.

Uptake of rubidium ion in different plants exposed to sudden fall in temperature. F. Zsoldos (*Z. PflErnähr. Bodenk.*, 1968, **119**, 169–173).—The uptake of Rb by sugar melon, rice, cucumber and sorghum exhibits a min. below certain temp. The fall in uptake is attributed to changes in the intensity of respiration, the so-called cold shock effect. Below certain temp. the membranes become more permeable so that Rb uptake increases again. M. LONG.

Influence of molybdenum on iron nutrition of tomato. J. A. Berry and H. M. Reisenauer (*Pl. Soil*, 1967, **27**, 303–313).—Max. accumulation of Fe in the tomato plant occurred at marginally-adequate Mo concn. (0·001 μM) in the nutrient. Higher and lower Mo nutrient levels depressed Fe uptake. Enhanced Fe absorption due to increasing Mo supply was related to increased reductive capacity of the plant roots. Low Mo supply also limited the translocation of Fe from veinal to interveinal tissue. A. H. CORNFIELD.

Differences in molybdenum uptake by micro-organisms from the rhizosphere of radish *Raphanus sativus*, grown in two soils of similar origin. M. W. Loutit, J. S. Loutit and R. R. Brooks (*Pl. Soil*, 1967, **27**, 335–346).—The accumulation of Mo in the tissue of micro-organisms from the rhizosphere region of radish growing on two soils of similar origin, but which showed different uptake of Mo, was studied. Micro-organisms from the soil which produced high-Mo radish accumulated less Mo in culture tests than did those from the soil which produced low-Mo radish. There were no significant differences in the frequency of morphological types in the isolates of micro-organisms from the two soils. Results are discussed in relation to the difference in incidence of dental caries between the two areas. A. H. CORNFIELD.

Interactions between aluminium and phosphorus on root surfaces and cell wall material. D. T. Clarkson (*Pl. Soil*, 1967, **27**, 347–356).—When barley seedlings were grown in nutrient containing Al^{3+}, most of the Al absorbed was recovered in the cell wall fraction of the roots. Al^{3+} absorbed by prep. of cell wall material was not appreciably exchangeable with Ca^{2+} or Na^+, and the Al-treated cell wall material adsorbed appreciable amounts of PO_4^{3-}. In experiments with root segments the P associated with Al was almost completely exchangeable. A. H. CORNFIELD.

Phosphorus–zinc imbalance in plants. L. C. Boawn and J. C. Brown (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 94–97).—Beans and potatoes were grown using a split-medium technique which allowed Zn to be supplied to the plant from soil and P to be supplied from solution. Intervenal chlorosis and stunting occurred only with high P supply, but was not accompanied by a reduction in plant Zn %. Increasing soil Zn supply produced normal plants. Normal metabolism in plants depends on a physiological balance between P and Zn. A. H. CORNFIELD.

Phosphorus–zinc interaction in two soyabean varieties differing in sensitivity to phosphorus nutrition. G. M. Paulsen and O. A. Rotimi (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 73–76).—Nutrient solution studies of P-tolerant and P-sensitive soyabean varieties given varying levels of P and Zn showed that high P decreased growth of the P-sensitive variety more than that of the P-tolerant variety, but decreased Zn % in both varieties to the same extent. Extra Zn^{2+} overcame the effect of P on the tolerant, but not on the sensitive, variety. Zn deficiency affected growth of all plant parts similarly but had little effect on P content of the plants except when Zn was supplied to deficient plants, when leaf Zn % decreased. A. H. CORNFIELD.

Supply of calcium, strontium, manganese, and zinc to plant roots growing in soil. E. H. Halstead, S. A. Barber, D. D. Warncke and J. B. Bole (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 69–72).—In a growth chamber experiment the calculated supply of Ca^{2+}, Sr^{2+}, Mn^{2+}, and Zn^{2+} to four plant species by root interception plus mass flow was highly correlated with their uptake. Plant species influenced Ca^{2+} and Sr^{2+} but not Mn^{2+} and Zn^{2+}, uptake. Of the methods used to calculate root interception the one giving the max. value was most highly correlated with uptake. A. H. CORNFIELD.

Cation effects on phosphate absorption from solution by *Trifolium subterraneum*. D. G. Edwards (*Aust. J. biol. Sci.*, 1968, **21**, 1–11).—Phosphate absorption was markedly increased by increasing the Ca level in the nutrient solution to 25 or 250 μM, and by raising the Mg concn. when Ca was absent. Both rates of absorption were independent of the K level. The effects of Ca and Mg on the rate of phosphate absorption occurred before the effects of Ca and K on growth rates became apparent. Mg was unable to replace Ca as an essential element for plant growth. (25 references.) E. G. BRICKELL.

Effects of salinity on salt and water uptake by maize roots. E. L. Klepper (*Diss. Abstr.* B, 1967, **28**, 510).—Effects of the osmotic pressure (*OP*) of the growth medium on the exudation behaviour of single, unbranched root tips of maize are examined. Seven-day-old roots were excised and left to exude for 18 h in aerated Hoagland solution, with added NaCl, the *OP* of which was thus raised to 0·61–3·16 bars. Comparable roots were allowed to exude into unconditioned Hoagland solution. The two sets of roots yielded practically the same vol. of exuded sap per cm^2 of root surface, *OP* being generally greater in roots of plants in the salinised nutrient. The *OP* of the root tissue was ~5 bars higher than that of the exudate from that root and the *OP* of roots increased linearly with increase in *OP* of the medium. An explanation of these observations is presented. A. G. POLLARD.

Early products of radioactive phosphate esterification by barley roots. I. H. M. Elbagouri (*Diss. Abstr.* B, 1967, **28**, 395).—The absorption of ^{32}P-labelled M-KH_2PO_4 (pH 5·0) by exised barley roots was studied during 30 min.; enzymic activity in the roots was halted at intervals (1–30 min.). The accumulated ^{32}P in the roots was fractionated into (*a*) acid-sol.-P, (*b*) phospholipin-P, (*c*) nucleic acid-P and (*d*) protein-bound-P. Results suggest that within a few minutes of contact with the phosphate solution a considerable proportion of the absorbed P was incorporated into (*d*). The P content of this fraction represented < 3% of the total P content of the roots. In presence of inhibitors (chloramphenicol, 2,4-dinitrophenol) (*d*) was the only fraction showing changes in ^{32}P-labelling pattern. The incorporation of ^{32}P into (*d*) was closely associated with the process of accumulation of P in roots and the occurrence of ^{32}P in other fractions appeared to be the outcome of the utilisation of the accumulated protein-P. A. G. POLLARD.

Protein content and amino-acid composition of varieties of grain sorghum. T. K. Virupaksha and L. V. S. Sastry (*J. agric. Fd Chem.*, 1968, **16**, 199–203).—A negative correlation was found between the protein content (8·6–18·2%) and the lysine content (0·117–0·41%) of grains from 48 genetic varieties of sorghum. One variety, however, had high concn. of both protein and lysine in the seed. Prolamine and glutelin were the main protein fractions; high protein levels were mainly due to increases in prolamine. Amino-acid compositions of nine varieties and of the protein fractions of two varieties are given. (21 references.) P. S. ARUP.

Effect of varying nitrogen and potassium nutrition on the content of soluble amino-compounds in the aerial parts of oats. K. Mengel and M. Helal (*Z. PflErnähr. Bodenk.*, 1968, **120**, 12–20).—Increasing N application increased the sol. amino-content, especially in the case of glutamic acid, glutamine, aspartic acid, asparagine, alanine, threonine, serine and proline. A higher supply of K gave a lower concn. of glutamine, asparagine, aspartic acid, leucine and valine. In view of the marked effect on the glutamic acid concn. by N, this amino-acid was considered to be the best indicator of the N status of the plants. K had the most marked effect on the amides, especially on glutamine. M. LONG.

Root exudates of plants. III. Effect of temperature and 'cold shock' on the exudation of sugars and amino-acids from seeds and seedlings of maize and cucumbers. V. Vancura (*Pl. Soil*, 1967, **27**, 319–328).—The extent of exudation of 24 NH_2-acids and 17 sugars from germinating maize seeds and of nine sugars from germinating cucumber seed increased with temp. (8–28°). There were some differences due to temp. in the pattern of exudation. When seedlings grown initially at a favourable temp. were exposed to cold shock for 3 days exudates increased several-fold from maize roots and showed a marked increase from cucumber roots. Maize roots subjected to cold shock exuded three new oligosaccharides, fructose and saccharose in addition to previously detected substances. A. H. CORNFIELD.

Fluoro-organic compounds in plants. I. Metabolism of 2-^{14}C-fluoroacetate. P. W. Preuss, A. G. Lemmens and L. H. Weinstein (*Contr. Boyce Thompson Inst. Pl. Res.*, 1968, **24**, 25–31).—Application of Na 2-^{14}-C-fluoroacetate to sterile seedlings of *Acacia georginæ*, peanut, castor bean, and 'Pinto' bean caused $^{14}CO_2$ evolution and incorporation of ^{14}C into water-soluble fractions and lipids. An enzyme capable of cleaving the C–F bond appears to be present. (17 references.) E. G. BRICKELL.

Oxidation-reduction enzymes in wheat. G. R. Honold (*Diss. Abstr.* B, 1967, **28**, 474–475).—Enzymes extracted from wheat seed and from five milling fractions of two hard red winter wheats (Triumph, Bison) and two hard red spring wheats (Lee, Selkirk) were examined. Isozyme bands, detected on gels after polyacrylamide disc electrophoresis, varied from one band for lactate, α-ketoglutarate and isocitrate dehydrogenases to eleven bands for malate dehydrogenase. Two catalase bands and eight peroxidase bands (two of which migrated towards the cathode at pH 8–9) occurred in all samples. Enzyme activities, measured spectrophotometrically, manometrically or by densimeter showed oxalate dehydrogenase to be 100–1000 × more active than the other dehydrogenases examined. Peroxidase and catalase were highly active; polyphenol, ascorbate and indolylacetic acid oxidases together with lactate, succinate and glutamate dehydrogenases had low activities. Relationships between these and other enzymes during germination and subsequent growth of the plants, and the resulting chemical changes within the plants, especially concerning proteins, are detailed. A. G. POLLARD.

Oxalyl-coenzyme A synthetase and the neurotoxin β-*N*-oxalyl-*l*-α,β-diaminopropionate. G. A. R. Johnston and H. J. Lloyd (*Aust. J. biol., Sci.*, 1967, **20**, 1241–1244).—Seeds of *Lathyrus sativus* yielded extracts containing oxalyl-CoA synthetase, the properties of this enzyme being remarkably similar to those observed in extracts of pea seeds. Labelled β-*N*-oxalyl-*l*-β-diaminopropionate was produced when ^{14}C oxalate was incubated with *l*-αβ-diaminopropionate and *L. sativus* 55% extracts under conditions appropriate to high activities of oxalyl- CoA synthetase. (15 references.) E. G. BRICKELL.

Accumulation of citric acid in fruit. M. Tishel (*Diss. Abstr.* B, 1967, **28**, 1836).—The acetone powders of citric acid accumulators (e.g. orange, lemon, grapefruit, strawberry, tomato, apple, lime, and in particular the Bartlett pear) were assayed for the activity of enzymes associated with citrate metabolism. Aconitase, isocitric dehydrogenase and malic dehydrogenase showed activities which did not differ considerably from one fruit to another. Citric synthase varied markedly from fruit to fruit, but no correlation between citric acid content and this activity was apparent. The org. acid profile in the fruit tissues examined does not correlate with the enzyme activity profile. Labelled precursor experiments corroborate the enzyme survey finding. The intracellular distribution of newly-synthesised org. acids indicates the possibility of compartmentation as a factor in acid accumulation in fruits. F. C. SUTTON.

Anomalous growth response to cyanide of bean plants in iron-deficient media. G. F. Israelstam (*Nature, Lond.*, 1968, **218**, 390–391).—Experiments similar to those reported by Tissières (*Biochem. J.*, 1951, **50**, 279) showed that plant growth is less severely inhibited by CN^- in an Fe-deficient than in an Fe-rich medium, contary to the effect which would be expected from interference of CN^- with cytochrome oxidase and other enzymes. 10^{-5} M-CN^- retarded the growth of *Phaseolus vulgaris* seedlings much more severely in presence of Fe than in its absence, whether EDTA was present or not. Various explanations of the very complex action of CN^- in whole plants are offered; response is altered by both temp. and CN^- concn. W. J. BAKER.

Effect of copper on development of nitrogen-fixing mycobacteria utilising molecular and bound nitrogen. I. K. Il'ina (*Microbiology [USSR]*, 1967, **36**, 809–813).—Cu is not necessary for N-fixation and cannot replace Mo; it is not a component of the N-activating enzyme system of mycobacteria. It does however possess a stimulatory effect which is enhanced by trace elements Zn, Mn and B, this increase becoming more marked when Mo is present. These findings relate to *M. flavum* 301 but are not found with *M. roseo-album* 368 showing that a marked difference in behaviour can occur. C.V.

Physical environment and symbiotic nitrogen fixation. IV. Factors affecting the early stages of nodulation. V. Effect of time of exposure to unfavourable root temperatures. A. H. Gibson (*Aust. J. biol. Sci.*, 1967, **20**, 1087–1104, 1105–1117).—IV. The most rapid initial nodulation was observed at 30°, and plants growing at this temp. had the highest rate of nodule appearance. Transfer experiments suggested that the infection of root hairs was stimulated by 30° root temp. and that infection continued at a rapid rate up to 33° but was then severely retarded. Shoot temp. in the range 18–25° had little effect on nodule formation. With 4- and 8-h daily light periods, initial nodulation was retarded; for plants receiving 12, 16, or 20 h light/day, the 'time to first visible nodule' values were similar. (17 references.)

V. Symbiotic N fixation by *Trifolium subterraneum* L., inoculated with three strains of *Rhizobium trifoli* Dang., was studied for root temp. 8–28°. The total amount of N fixed during any period was determined by the root temp. during the period, the amount of N previously fixed, % N in the plants at the start of the period, and the bacterial strain that formed the nodules. (11 references.) E. G. BRICKELL.

Rapid germinability test with exudates from seed. K. Takayanagi and K. Murakami (*Nature, Lond.*, 1968, **218**, 493–494).—When seeds are soaked aseptically in distilled water at 30° for 24 h and the exudate is analysed qual. (for sugars) by paper chromatography and quant. (for total sugars) by the anthrone spectrophotometric method, it is found that seeds of poor or no germinability exude much more sugar per g (70–200 μg) than do good seeds (0·3–5 μg). A rapid test makes use of urine-sugar analysis paper (Tes-Tape) which, when immersed in the exudate from 5 h-soaking of seeds, turns green with infertile seed but remains yellow (even after a 20 h-soak) with good seeds. W. J. BAKER.

Mechanism of action of ethylene. I. Effects of ethylene on mitochondria prepared from bean cotyledons. II. Effects of ethylene on mitochondria from rat liver and yeast, and on mitochondrial adenosine triphosphatase. A. O. Olson and M. Spencer (*Can. J. Biochem.*, 1968, **46**, 277–282, 283–288).—I. Spectrophotometric determinations at 520 nm on suspensions of the intact mitochondria were used for the measurement of their vol. changes. The changes induced by additions of adenosine di- or tri-phosphate were accelerated by the presence of dissolved C_2H_4, but spontaneous changes or those caused by PO_4^{3-} or Ca^{2+} ions were not affected. The enzyme affected by C_2H_4 is probably mitochondrial adenosine triphosphatase; ouabain, a known inhibitor of this enzyme, prevented the response to C_2H_4. (21 references.)

II. The effects of C_2H_4 described in Part I were also observed for mitochondria from rat liver and yeast. The effects were limited to the enzyme *in vivo*, and could not be reproduced on a partly purified sample of adenosine triphosphatase. (13 references.) P. S. ARUP.

Effect of benzimidazole and kinetin on nicotinamide nucleotide content of senescing wheat leaves. D. Mishra and E. R. Waygood (*Can. J. Biochem.*, 1968, **46**, 167–178).—The nicotinamide nucleotide content of wheat leaves floated on dil. solutions of benzimidazole and/or kinetin increased during 5 days, whereas little or no change occurred in leaves floated on water. In both treatments NADPH increased at the expense of NADH during the photoperiod, and *vice versa* during darkness; similar changes in the ratio NADH to NADPH (a decrease followed by an increase) also occurred in both treatments. Chloroplasts from leaves floated on water lost all their NADP, but those from leaves floated on the dil. solutions increased or maintained their level of NADP. (32 references.) P. S. ARUP.

Inhibitor of receptacle growth in non-pollinated strawberry fruit. P. B. Goodwin (*Nature, Lond.*, 1968, **218**, 389–390).—Experimental evidence confirms that lack of growth near non-pollinated carpels arises from production, by the carpel, of an inhibitor. It is suggested that the calyx produces a substance essential for growth of fertile carpels in tissue culture of *Fragaria vesca semperflorens* and that non-growing carpels (fertile or non-fertile) inhibit receptacle growth. The calyx is not essential for fruit growth on the intact plant, but the presence of even healthy fertilised achenes tended to inhibit growth of the receptacle under all conditions. Attempts to extract the growth inhibitor, using isolated receptacles in culture for assaying activity, are summarised. The inhibitor was probably not any of the many listed chemicals tested in the medium. (11 references.) W. J. BAKER.

Purine derivatives in lucerne as growth stimulants for *Bacillus subtilis*. E. M. Bickoff, R. R. Spencer, S. C. Witt, B. E. Knuckles and J. B. Stark (*J. agric. Fd Chem.*, 1968, **16**, 246–251).—A procedure is described for the isolation of the active growth factors from conc. aq. extracts of lucerne, involving acidification and removal of the pptd. matter, fractionation over three cation-exchangers, countercurrent partition with the use of an H_2O–AcOH–Pr^iOH–Bu^nOH system, and two-dimensional paper chro-

matography, with the activity during the fractionation being followed by microbiological assay using *B. subtilis*. The main factors were identified as adenine, adenosine, and guanosine, which accounted for almost all the activity; hypoxanthine, xanthine, inosine, isocytosine and cytidine were present in trace amounts. (35 references.) P. S. ARUP.

Presence of a growth inhibitor in the tubers of nutgrass (*Cyperus rotundas* L.). S. P. Singh (*Proc. Indian Acad. Sci.*, B, 1968, **67**, 18–23).—Tuber extract of nutgrass, when applied to the growing medium, inhibited the germination and growth of 10 crop species under laboratory conditions, thus confirming the presence of some phytoxic substance(s). Inhibition varied with different crop species. (13 references.) E. G. BRICKELL.

Synthesis of (2-chloroethyl)-trimethyl-$^{14}C_3$-ammonium chloride (Clormequat). J. Falecki (*Bull. Acad. pol. Sci. Sér. Sci. chim.*, 1968, **16**, 29–33).—The synthesis of labelled Chlormequat is described, by reaction of labelled Me_3N with ethylene dichloride. Chlormequat is an exogenic systemic factor inhibiting internode elongation in plants like *Chrysanthemum* sp., *Poinsettia* sp., tomato and wheat. (13 references.) (In English.)
T. M. BARZYKOWSKI.

Carotenogenesis in ripening mangoes. V. V. Modi and V. V. R. Reddy (*Indian J. expl. Biol.*, 1967, **5**, 233–235).—Alfanzo mangoes were studied in order to show the reduced NADP regenerating system in ripening mangoes. Utilisation of geraniol (**I**) and farnesol (**II**), (**I** > **II**) in the cell-free extracts of mangoes for carotene formation suggests that they are precursors of carotenoids. The presence of **I** in mango extracts further suggests that it is an intermediate in carotenogenesis. (22 references.)
E. G. BRICKELL.

Influence of *X*-irradiation on sprouts and metabolism of potato tubers during storage. W. Scheid and F. Heilinger (*Atompraxis*, 1968, **14**, 299–302).—Storage of the Maritta variety was at 12–4°; they were irradiated with low dosage (400–3200 R) twice, in February while unsprouted and in July when weakly or pronouncedly sprouted. They were then stored at 20–1°. Sprouting can be inhibited for 10 days by irradiation at 3200 R, the inhibition being more marked at temp. < 20° or with higher radiation dosages. (12 references.) C.V.

Effect of ionising radiation on the respiratory metabolism of sprouting seeds. A. Amburger and A. Süss (*Atompraxis*, 1968, **14**, 296–299).—A single radiation of grains of barley (1–100 R) resulted in a slight acceleration of sprouting and in initial development of the sprouts. In the glutathione ascorbic acid—ascorbic acid oxidase system, the SH groups and the enzyme ascorbic acid oxidase proved to be specially sensitive to radiation. The amount of dehydroascorbic acid decreased more under the influence of radiation than did that of ascorbic acid. During the 10 day observation period regeneration occurred in varying degree. (17 references.) C.V.

Chemical radiation protection against damage to cytoplasm and nuclei in plant cells by α- and β-radiation. R. Biebl and W. Url (*Atompraxis*, 1968, **14**, 302–305).—This damage is discussed; it may arise by direct action upon an essential life mol. or act upon the aqueous phase of the protoplasm, forming radiochemical disintegration mol. The former results in cell death in 24 h, the latter in a slow death measured in days. Pretreatment with radio-protective substances such as thiourea, thioacetamide, methylallylisothiourea etc., delays 'cytoplasmic death' probably by inactivation of the disintegration products and if the radiation has been sufficiently low, 'nuclear death' may be delayed or averted. (26 references.) C.V.

Plant growth regulant mixture. Badische Anilin- und Soda-Fabrik A.-G. (Inventors: H. Oettel, H. Frohberg and K.-H. Koenig) (B.P. 1,092,138, 12.3.65. Ger., 13.3.64).—The claimed composition contains a chlorocholine salt (chloride) and a choline salt (chloride) in the mol. ratios of 1 : 0·05–1 : 20 respectively; the length of plants is reduced, and stem wall thickness is increased so that there is a lessening in the lodging of certain types of grain, e.g. wheat. The compositions have low mammalian toxicity.
S. D. HUGGINS.

Plant growth stimulants. Midwest Research Institute (Inventors: C. C. Chappelow and J. T. Byerley) (B.P. 1,096,885, 14.12.64).—The claimed compounds (**I**) $Ar_2CH{\cdot}CH(Ar){\cdot}(CHX)_3{\cdot}CH_2X$, where Ar is Ph or alkylphenyl, and X may be -OR, -OCOR, -OCOOR^I or -OCO·NR$_2$ (R is H or alkyl or aryl of 1–10 C, R^I is alkyl or aryl of 1–10 C) are contained in a composition with surface-active agent, auxiliary agricultural chemical colouring agent, corrosion inhibitor, adhesive, powder diluent or oil. An example of **I** is a 1,1,2-tris(3,4-dimethylphenyl)-1,2-didesoxyhexitol; the compounds stimulate germination and growth of e.g. cereals, grasses, beets, tomatoes. S. D. HUGGINS.

Crops and Cropping

Comparison of light and heat transmission of horticultural glass and translucent PVC. C. C. Tunnicliffe (*N.Z. Jl agric. Sci.*, 1968, **11**, 219–222).—Transmittance of visible light was 0·92 for glass, and 0·70 for new and 0·18 for weathered translucent PVC. PVC sheet transmitted heat faster than did glass. E. G. BRICKELL.

Effects of potassium and magnesium fertilisers on yield and composition of successive crops of ryegrass, clover, sugar-beet, potatoes, kale and barley on a sandy soil at Woburn. J. Bolton and A. Penny (*J. agric. Sci., Camb.*, 1968, **70**, 303–311).—Results from an 8-year field trial indicate that K_2SO_4 and to a smaller extent $MgSO_4$, whether applied alone or together, increase the yield of all crops. The K/Mg ratio has no effect on yield but does affect the ratio of these elements in the crop. The order of response to K is potato > clover = barley > sugar-beet > kale > ryegrass, ranging from 17–28% crop increase, whilst response to Mg ranges from 3–10%. Changes in exchangeable Mg in the soil reflect differences in applied Mg and crop uptake. Changes in exchangeable K were less than expected, due to release of K in low-K plots and K-fixation in high-K plots. Increase in Mg deficiency incidence is attributed to low Mg content of local liming materials and to reduced use of farmyard manure. M. LONG.

Improvement of cereal yields (of wheat). D. Bertrand, M. Raverdy and A. de Wolf (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 211–215).—In field experiments with wheat on plots with N-fertilisation at different levels, additions of Mo increased the soil-N but did not increase yields to any statistically significant extent; yields from plots receiving 1·6 kg/ha of Mo were, however, on an average, 6·3% higher than those from control plots. Yields were greatly affected by the amount of N added. (11 references.)
P. S. ARUP.

Mineralisable nitrogen in the soil under various leys and its effect on the yields of following wheat. J. K. R. Gasser (*J. agric. Sci., Camb.*, 1968, **70**, 323–329).—The increase (*I*) in mineral N following incubation of rewetted air-dry soil gave the best correlations with grain yields of spring wheat ± fertiliser N and N uptake following ploughing up of ryegrass, clover and ryegrass/clover leys, compared with mineral N and the increase of mineral N following incubation of fresh soil. Correlations of *I* with yield and N uptake were also best when N fertilisers were applied. Values of *I* depended on grass species and were increased by application of N to the ley. Differences disappeared after 18 months. With N fertiliser application yields of winter wheat, following mixed leys, tended to the same value, regardless of the mineralisable N in the soil. In the case of grass leys the max. yield of following spring wheat given N increased with increasing mineralisable N in the soil.
M. LONG.

Effects of different levels of nitrogen, phosphorus and potassium on the yields, nitrogen content and kernel weights of malting barley (var. Proctor). T. F. Gateley (*J. agric. Sci. Camb.*, 1968, **70**, 361–367).—The average yield increase at more than 13 sites was 5·1 and 8·2 cwt. from applications of 35 and 70 lb/acre of fertiliser N respectively. A mean yield increase of 44 cwt followed an application of 27 lb P/acre at four sites where soil P extracted by a modified Morgan's extractant fell below 2 ppm. K applied at rates of up to 168 lb K/acre produced a yield increase at only one site and led to a reduction at another. The mean grain N content without N application was 1·47%; applications of 35 and 70 lb N/acre led to increases of 0·06 and 0·19% respectively. P and K had little effect on the N content. N applications of 35 and 70 lb/acre increased the 1000 kernel wt. by 0·7 and 1·0 g respectively, except when lodging occurred. P and K had no effect. M. LONG.

Reaction of barley varieties to nitrogen fertiliser. S. Dubetz and S. A. Wells (*J. agric. Sci. Camb.*, 1968, **70**, 253–256).—Varietal responses were independent of whether the plants were grown with supplementary light in late Sept. or without it in March. Differences in yield and protein content between varieties were small at low levels of N but pronounced and consistent at high N levels. Results of yields obtained in the pot trials were in line with field behaviour except that 'Betzes' yielded more than 'Hannchen'. These two varieties yielded most but contained the least protein.

'Palliser' was intermediate and 'Compana' gave the lowest yields but contained the most protein. M. LONG.

Response of some barley varieties to irrigation and nitrogen fertiliser. E. J. M. Kirby (*J. agric. Sci., Camb.*, 1968, **71**, 47–52).—Four varieties of barley were compared with variety Proctor under two levels of irrigation and two levels of N fertiliser. The varieties from a Mediterranean climate and Japan yielded less grain and total dry matter than the adapted varieties, but differed in their response to irrigation, due largely to their ability to form extra ears when irrigated. N had no effect on yield, although the high level produced more ears. High N increased the yield of N in both grain and whole plant and one exotic variety, Algérie 48, was found capable of high N yields in both grain and straw. Varietal differences in response to irrigation are discussed and related to development and possible drought resistance. M. LONG.

Effect of water supply on growth and yield of barley. A. A. Abd el Rahman, K. H. Batanouny and N. H. Ezzat (*Pl. Soil*, 1967, **27**, 369–382).—In a desert calcareous sandy loam region max. vegetative growth and grain yields of barley were obtained when the greater part of the irrigation water was applied in the last stage of vegetative growth and ear formation. A. H. CORNFIELD.

Physiochemical properties of brown rice from *Oryza* species and hybrids. C. C. Ignacio and B. O. Juliano (*J. agric. Fd Chem.*, 1968, **16**, 125–127).—The protein and amylose contents and the gelatinisation temp. are tabulated for 29 samples, including 11 wild species and two interspecific hybrids of the genus *Oryza*. Amino-acid analyses are given for 17 samples. The results indicated that the wild species and some of their hybrids with *Oryza sativa* would offer no advantage in a breeding programme. P. S. ARUP.

Comparative studies of upland and swamp rice varieties (*Oryza sativa* L.).I. Effect of soil moisture on growth and nutrient uptake. II. Effect of varying supply of manganese on growth and yield. B. A. C. Enyi (*J. agric. Sci., Camb.*, 1968, **71**, 1–13, 15–17).—I. Rice varieties Agbede (**I**) and BG 79 (**II**) were subjected to soil moisture regimes of 60, 80 and 100% saturation and flooding. Except under flooding conditions **II** attained greater total dry weight than **I**. Both produced most dry wt. under conditions of 100% saturation; on flooded plots **II** outyielded those at 80% saturation while the reverse was true of **I**. Total leaf numbers were higher with **II** and leaf number increased with increasing soil moisture up to 100% saturation; leaf area was highest with **I** during tillering and mid-vegetative stages; for both species leaf area during these stages was highest with 100% saturation. **II** produced more shoots than **I**; during tillering saturated and flooded conditions favoured shoot production in both **I** and **II**. Shoot mortality % in **I** was unaffected by soil moisture, whereas in **II** it increased with driest and with saturated conditions. Mean relative leaf growth rate and net assimilation rate were greater with **II**, the converse applying to leaf wt. ratio. Mn concn. fell with **I** and **II** under flooded conditions.

II. Spraying **I** and **II** with Mn (20 ppm) decreased the total dry wt. and dry wt. of leaf laminae, stems, leaf sheaths and root when the plants were grown on soil at 80 or 100% moisture saturation; under these conditions, 10 ppm Mn increased the ear wt. of **I**. On saturated soil, 20 ppm Mn also increased ear wt. In flooded soil, both 10 and 20 ppm treatments increased dry wt. of **I**, but only 20 ppm treatment increased ear dry wt. of **II**. Ear wt. of **I** receiving 0 or 10 ppm Mn decreased with increasing soil moisture; that of **II** increased. It is probable that Mn supply limits yield of **I** grain on flooded soil. M. LONG.

Influences of associated salts on maize root development and the availability of banded phosphates. W. R. Kussow (*Diss. Abstr.* B, 1967, **28**, 397–398).—The possible effect of N and K fertilisers, drilled in the seed row, on phosphate availability to maize roots is examined. The P uptake of roots increased during the first 4–6 weeks after emergence, the effect being probably due to the non-phosphatic nutrients. The K requirement of the crop was met by 34 lb of K/acre placed in the row or by broadcast application of 150 lb of KCl/acre. Inclusion of N fertiliser with the mixture applied in the row markedly increased the P intake and the final crop yield regardless of the broadcast treatment. Grain yields were increased by in-the-row fertilisers only when K was not broadcast. The action of non-P fertilisers included with P for in-the-row treatment probably improves root distribution in and around the fertiliser band. In greenhouse trials P intake by roots was directly related to the extent of root development in the fertiliser zone. No osmotic effects were apparent. Provided soil pH remained < 7 the NH_4^+-N accounted for all major variations in root development, the max. effect occurring with N at 500 ppm. With high levels of urea as N source the soil pH became > 7 and root growth was restricted regardless of NH_4^+ present. In greenhouse soils of pH 7·1 and 7·2, NO_2^- was formed when urea comprised 15% of the N in the in-the-row fertiliser; replacement of urea by NH_4NO_3 prevented the formation of NO_2^-.
A. G. POLLARD.

Utilisation of phosphorus sources of different availability by mycorrhizal and non-mycorrhizal maize. C. L. Murdoch, J. A. Jackobs and J. W. Gerdemann (*Pl. Soil*, 1967, **27**, 329–334).—The inoculation of maize plants with an endotrophic mycorrhizal fungus (unnamed *Endogone* species) increased yields and tissue P % on a soil of low P availability. Availability of P from a readily available source [$Ca(H_2PO_4)_2$] was unaffected by inoculation, but that from difficultly available sources [rock phosphate and $Ca_3(PO_4)_2$] was increased. A. H. CORNFIELD.

Effect of potassium on the carbohydrate and nitrogenous fraction of maize. W. C. Liebhardt (*Diss. Abstr.* B, 1967, **28**, 431).—The mechanism of the premature breakdown of parenchyma in the lower part of K-deficient maize plants is investigated. The breakdown appears to result from inhibition of protein synthesis and consequent disorganisation of the cellular structure and ultimate death of the cells. In commercial hybrids grown with or without sufficient K, max. yields of carbohydrates and dry matter occur at the late dent stage. In actual K deficiency these changes occur somewhat earlier. Varietal differences in yields of starch and sugar with uptake of K were considerable, but among varieties the % K in the tissues at the late dent stage and the final yield of grain were closely related. A. G. POLLARD.

Effect of plant spacing and sowing time on grain production of hybrid and open-pollinated maize. K. I. Hussain, M. Ahmad and M. Hussain (*W. Pakistan J. agric. Res.*, 1967, **5**, 17–23).—The optimum sowing time was found to be the first half of Aug. Spacing at 1 ft × 1 ft gave better yields than did spacing at 3 ft × 9 in. The best yielding varieties were G.H.134 > U.S.13 > Khanpur local > Swabi White. P. S. ARUP.

Effect of temperature on tillering of grain sorghum seedlings. R. W. Downes (*Aust. J. agric. Res.*, 1968, **19**, 59–64).—No tillering occurred when seedlings of the Combine Kaffir variety of *Sorghum vulgare* Pers. were grown at temp. > 18°. Tillering was induced by growing the plants at lower temp. Genetic, physiological and agronomic implications are discussed. P. S. ARUP.

Selection for freedom from after-cooking darkening in a potato breeding programme. C. D. Dalianis (*Diss. Abstr.* B, 1967, **28**, 412).—Tests for after-cooking discoloration were made with 437 clones and a first selection of 11 clones was examined in detail, using standard methods of prep. and cooking. Discoloration was dependent on genetic differences and on environmental factors. Tests with single tubers can afford a reliable prediction of discoloration. In the early generations of selections of varieties based on samples from non-replicated plots, at least four tubers should be used as test samples. The precision of the estimate was not greatly improved by use of eight tubers. In the later stages of selection of varieties the most representative results of discoloration tests were obtained by increasing the no. of years and of replications within years from which the sample is taken. Four tubers from each of two replications from each location or year are suggested, together with a system of colour classification using four tubers per clone. A. G. POLLARD.

Determination and economic analysis of relationships between plant population and yield of maincrop potatoes. P. R. Sharpe and J. B. Dent (*J. agric. Sci. Camb.* 1968, **70**, 123–129).—Field experiments were carried out with Desirée potatoes on a medium loam soil overlying clay. Optimum yields were obtained with a spacing of 17·1 in. in the row and 28 in. between rows. Mathematical relationships between crop yields, spacing data and costs are established. A. G. POLLARD.

Interaction of nutrient supply and plant density in relation to maximal yield of the swede crop. R. W. Lang and J. C. Holmes (*J. agric. Sci., Camb.*, 1968, **70**, 369–373).—With low plant densities a high nutrient supply was required to reach max. yield of roots, whilst at high plant densities the same nutrient supply depressed yield. Over the normal range of plant density this is of little importance for the range of nutrient supply used. On high density crops, such as swedes for human consumption, the normal rate of fertiliser usage should not be exceeded owing to increased cost and lowered yield. More fertiliser and higher plant density were needed to give max. yields of roots and tops than of roots alone.
M. LONG.

Commercial growing of horseradish. Anon. (*Leafl. U.S. Dep. Agric.*, 1968, No. 547, 6 pp.).—Commercial growing, fertilisers, diseases and insect pests, harvesting, storing, and marketing is described. E. G. BRICKELL.

Effect of 'hardening' radish seeds. A. T. Abdel Hafeez and J. P. Hudson (*Nature, Lond.*, 1967, **216**, 688).—Results for plants grown from 'hardened' and 'unhardened' seeds in wet and dry soil at three levels of soil fertility show that 'hardening' seeds (by initial wet–dry cycles) is sometimes more beneficial under favourable than under adverse growing conditions. Improved growth and more dry-wt. were obtained with hardened seeds in moist soil. W. J. BAKER.

Use of polyethylene sheeting below ground to separate the roots of herbage species. B. F. Bland (*J. Br. Grassld Soc.*, 1967, **22**, 252–256).—Black polyethylene used to separate the roots of different herbage species remained a satisfactory barrier below ground for at least 4 years. The sheeting can be punctured by rhizomes of couch grass. A. H. CORNFIELD.

Results of nitrogen-level tests on grassland. W. D. Jagtenberg (*Landbouwvoorlichting*, 1968, **25**, 190–193).—Tests were carried out over 3 years on 24 different soil types, with adequate PK supplies. With 100 kg of N per annum, the yields of dry matter were increased by 11–20 kg/kg of N. Responses were somewhat lower with 200 kg of N p.a., and still smaller and sometimes irregular with 300 kg. The max. response to the first 100 kg of N p.a. occurred on wet peaty soils. Responses in general were influenced more by soil moisture than by soil-type. P. S. ARUP.

Comparison of anhydrous ammonia and ammonium nitrate as nitrogenous fertilisers for grassland. R. S. L. Jeater (*J. Br. Grassld Soc.*, 1967, **22**, 225–229).—Trials at three sites where N was applied at rates ranging from 45 to 295 lb per acre showed that anhyd. NH_3 was not as effective as NH_4NO_3 (in split application) in increasing dry matter yields of herbage. Splitting the NH_3 dressing was more effective than a single application, but was still not as effective as NH_4NO_3. Response from a single application of NH_3 was more effective when given in the spring than in the autumn. Split NH_4NO_3 application gave more uniform yields of herbage through the season than a single application of NH_3. A. H. CORNFIELD.

Competition between wheat and undersown pasture in the year of sowing and the effect of undersowing on the yield of pasture in the following year. K. Santhirasegaram and J. N. Black (*J. Br. Grassld Soc.*, 1967, **22**, 239–244).—Sowing pasture species with wheat reduced the yield of the pasture species, more so when wheat was drilled in 7 in. than in 14 in. row spacing, and was least when the two crops were in alternate 7 in. rows. The pasture also reduced the yield of wheat, but the effects were very much less marked than those of wheat on pasture. Pasture yield early in the following season was related to the seed yields in the year of establishment, but later in the season differences due to treatments disappeared. A. H. CORNFIELD.

Effects of grazing on the nutrition of pastures. N. J. Barrow (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 254–262).—Grazing increases pasture requirements for mobile nutrients such as K and S which are not strongly retained by the soil and are leached out by rainfall. but may have little effect for an immobile nutrient such as P, except where the level is already sub-optimal. Imbalance also occurs where grazing tends to be restricted to certain parts of a pasture. (54 references.) J. L. WALPOLE.

Comparison of the yield of three grass species at various levels of nitrogenous fertiliser sown alone or in a mixture. D. W. Cowling and D. R. Lockyer (*J. agric. Sci., Camb.*, 1968, **71**, 127–136).—There was no evidence that mixed swards of S.24 perennial ryegrass, S.37 cocksfoot and S.48 timothy produced higher yields of dry matter than did these species when grown alone; there was no evidence of a beneficial or antagonistic effect of one species on another. The botanical composition changed during the course of the experiment, cocksfoot becoming especially dominant at the highest level of N. Single grass swards are suggested as the most suitable approach to grassland husbandry. M. LONG.

Influence of light intensity, temperature and nitrogen fertilisation on dry matter production, protein content and nitrate-nitrogen accumulation in forage crops. J. R. George (*Diss. Abstr.* B, 1967, **28**, 438).—In two-year trials with timothy, smooth bromegrass and orchard-grass the effects of various applications of N (0–300 lb N per harvest) are examined. Dry matter yields increased with the N treatment, except that the highest application reduced yields, notably with timothy. Protein contents increased in all species and in both years, with the N dressing; the 300 lb application produced a 38–44% increase in protein as compared with the crop without added N. Crops receiving > 37·5 lb N contained NO_3-N in amounts increasing linearly with the fertiliser application. In growth chamber experiments NO_3-N in the crops increased with the N application regardless of temp. or light intensity; reduction of light intensity with rise in temp. increased the NO_3 contents progressively at 15°, 22·5° and 30°. NO_3 contents continued to increase over a 7-day period at low light intensity. With constant light and temp. conditions the NO_3 in the crop increased with the N dressing but the accumulation of NO_3-N in the crop at a given level of N treatment declined with advancing crop maturity. Application of 75 or 150 lb of N to a tall fescue pasture increased the NO_3 accumulation in the crop but there was no reduction in the gain in wt. of grazing animals attributable to the high NO_3 levels. A. G. POLLARD.

Salinity effects on roots and tops of Bermuda grass. V. B. Youngner and O. R. Lunt (*J. Br. Grassld Soc.*, 1967, **22**, 257–259).—Bermuda grass top-growth decreased with increasing salinity (80–320 mequiv. NaCl + $CaCl_2$ in equivalent amounts) of the nutrient solution. Root wt. increased with salinity up to intermediate levels and then decreased with further increasing salinity. There were differences in tolerance to salinity among nine varieties. A. H. CORNFIELD.

Effects of single compared with split applications of fertiliser nitrogen on the yield and seasonal production of a pure grass sward. M. E. Castle and D. Reid (*J. agric. Sci., Camb.*, 1968, **70**, 383–389). Nitram (prilled NH_4NO_3) was applied to S.24 perennial ryegrass at the rates of 100, 200 and 300 lb/acre either as a single dressing or as two, three, four or five split dressings at successively later dates in the growing season over a two-year period. Herbage was cut at about 5-week intervals. Dry matter yields were increased by increased N application rate and by applying three or four equal dressings. Mean crude protein was increased by increasing N application rate, but reduced by split applications. NO_3-N content was higher with a single heavy dressing than after split dressings, amounting to the same amount of N. The optimal number of applications for 100 lb/acre was three; and for 200 and 300 lb applications, four. M. LONG.

Uptake of fertiliser and soil nitrogen by ryegrass as affected by carbonaceous residues. G. L. Terman and M. A. Brown (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 86–90).—Studies with ^{15}N-labelled $(NH_4)_2SO_4$ applied in a pot test with ryegrass showed that the % of applied N recovered by the grass increased with amount applied (40–300 ppm). Addition of 0·5% ground maize forage resulted in reduced recovery of applied N, particularly at the lower levels. After six cuttings the recovery of applied N by tops and roots was 75% and 72% from a fine sandy loam and a silty clay loam respectively, where the highest rate of N was applied. The maize residues immobilised 14% and 16% of the applied N in the sandy loam and clay loam respectively, with the highest rate of N. The % of applied N immobilised decreased, but actual amounts increased, with increasing amount applied. A. H. CORNFIELD.

Non-sward densities for the assessment of yield in Italian ryegrass. II. Convenient plot and block size and shape. F. England (*J. agric. Sci. Camb.*, 1968, **70**, 105–108).—In two uniformity trials described, S.22 and Italian ryegrass were grown with 6-in. spacing and harvested in basic units of 1 yd.^{2}. When no allowance was made for guard-rows the smallest plots were the most efficient. For comparative purposes the size of plot used was that required to show a difference of 7% of the mean. If allowance was made for one guard row round each plot, 2 yd.^{2} plots were as efficient as those of 1 yd.^{2} and required fewer replications. In general, long narrow plots in short wide blocks were the most efficient. Choice of shape of plots and blocks was important; for a given size of plot, a poor shape may be less than half as efficient as one of good shape. A. G. POLLARD.

Practice of haymaking. P. J. J. Philipsen and P. S. Hak (*Landbouwvoorlichting*, 1968, **25**, 161–177).—Practical advice is given on the operations involved in the grass-crushing and hay-ventilation system, and a suitable ventilating system is described. The efficiency of ventilation-drying is greatly increased by the crushing process, and the use of the system as a whole shortens the period of field-treatment. P. S. ARUP.

Factors affecting change in botanical composition in a ryegrass–white clover pasture under continuous grazing. W. Harris and R. W. Brougham (*N.Z. Jl agric. Res.*, 1968, **11**, 15–38).—Swards developed under lax and moderate grazing systems were virtually pure stands of the two sown species, whereas under continuous close grazing

marked ingress of *Agrostis tenuis*, *Poa* spp., *Oxalis corniculata*, *Hydrocotyle moschata*, *Sagina procumbens*, and *Taraxacum officinale* occurred. *A. tenuis* showed marked negative association with *Lolium perenne* and with *Poa* spp., whereas ryegrass and *Poa* spp. were positively associated with *Trifolium repens*. *A. tenuis* had a well-defined association with low soil N and high soil moisture sites, and the death of Manowa ryegrass in the sown mixture hastened its ingress into continuously-grazed sward. (31 references.) E. G. BRICKELL.

Influence of herbage height at treading and treading intensity on the yields and botanical composition of a perennial ryegrass–white clover pasture. K. R. Brown (*N.Z. Jl agric. Res.*, 1968, **11**, 131–137).—Four rates of sheep treading were investigated, and higher yields of total herbage and sown species in winter and spring, but not autumn and summer, were recorded for pastures trodden before herbage removal. At the highest treading intensity, marked reduction in herbage yields were recorded in all seasons, but at intensities of > 24 sheep/acre, reductions were much less marked in some seasons. E. G. BRICKELL.

Varieties of alfalfa [lucerne]. Anon. (*Fmrs' Bull. U.S. Dep. Agric.*, 1968, No. 2231, 12 pp.).—Thirteen principal and twenty new varieties are described. E. G. BRICKELL.

Vetch culture and uses. P. R. Henson and H. A. Schotch (*Fmrs' Bull. U.S. Dep. Agric.*, 1968, No. 1740, 22 pp.).—Climatic, soil and moisture requirements, seeding time, rate and method, fertilisers, inoculation, winter killing, uses of vetch, seed production, insect pests and diseases are described, together with details of the various cultivated vetches in the U.S. E. G. BRICKELL.

Rotation responses of cotton in the Sudan Gezira. II. Effect of fertiliser nitrogen on the response to rotation. J. E. Jackson and H. O. Burhan (*J. agric. Sci., Camb.*, 1968, **70**, 257–263).—Fertiliser applications were superimposed on a rotation trial already 30 years old. Various combinations of cotton and fallow and alternating crops of dura and lubia (*Dolichos lablab*. L.) were investigated. The results support the idea that low yields in the absence of N are due to N deficiency. Response to N was in general greater on rotations leading to low cotton yields, rotational effects being greatly reduced when N was applied. Generally cotton yields were higher when cotton followed dura and lubia than when cotton–fallow only rotations were used. Cropping in the Gezira could be considerably intensified without loss of cotton production. M. LONG.

Influence of low night temperature on the growth and development of cotton. J. R. Gipson (*Diss. Abstr.* B, 1967, **28**, 438–439).—Two cotton varieties were grown under four different temp. regimes. Mean night temp. > 60°F retarded vegetative growth and altered fruiting patterns, low temp. favouring more and smaller bolls. Boll periods (period from open bloom to open boll) were inversely related to night temp. Rates of fibre elongation and the length of mature fibres and their fineness were all positively correlated with night temp. (43–70°F). The % cellulose and crystallinity of mature fibre diminished in low temp., as also did the degree of polymerisation in one, though not in a second variety. The oil and N contents, the germinability of the seed and in some cases, the lint index and seed index were reduced by low temp.; both oil and N contents of the seed were significantly correlated with % germination. Temp. did not affect the plant nutrient status. The carbohydrate (sugar and starch) status of stems and roots was increased by low temp.; total carbohydrates (roots, stems and leaves) were increased in one but not in a second low-temp. year. A. G. POLLARD.

Pollination of apple trees (*Malus sylvestris* Mill.). III. Varieties Granny Smith, Kidd's Orange Red and Golden Delicious. T. Palmer-Smith and P. G. Clinch (*N.Z. Jl agric. Res.*, 1968, **11**, 149–154).—Studies of insect visitation and pollination of these trees showed that in all cases the only pollinating insects were honey bees, on which fruit set in the apple trees depends almost entirely. E. G. BRICKELL.

Variations in potassium content in leaves of grafted walnut trees. *Juglans regia*/*Juglans nigra* cultivated under natural conditions. J. Gagnaire and C. Vallier (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 81–86).—Trees grafted on the root stock *Juglans regia* are liable to rot-disease, whilst trees grafted on *Juglans nigra* are resistant. At the age of > 30 years, however, the latter grafts show symptoms of graft incompatibility accompanied by decreases in leaf-production, foliar-K and in the ratio of foliar K/Ca. P. S. ARUP.

Regulation of fruit set in the grape vine. M. G. Mullins (*Aust. J. biol. Sci.*, 1967, **20**, 1141–1147).—Small immature fruits developed on defoliated and decapitated vine cuttings, on cuttings in which leaves, apices, and roots were removed as they appeared, and on inflorescences which were cultured in the light *in vitro* on a medium devoid of exogenous growth substances. These results indicate that fruit set is regulated by org. nutrients rather than by specific hormonal stimuli originating from organs external to the developing bunch. (18 references.) E. G. BRICKELL.

Improvement of the yield of the hop plant (*Humulas lupulus*) by means of shoot selection. R. H. J. Roborgh (*N.Z. Jl agric. Sci.*, 1968, **11**, 215–218).—Hop shoots of high potential yield were found to be characterised by a stagnation of increase in the lengths of their first four fully elongated internodes. E. G. BRICKELL.

The Avocado crop. A. A. Gogolashvili (*Pishch. Tekhnol.*, 1968, No. 2, [63], 25–28).— C.V.

Commercial growing of asparagus. Anon. (*Frms' Bull. U.S. Dep. Agric.*, 1968, No. 2232, 22 pp.).—Climatic and soil requirements, fertilising and maintaining fertility, varieties, growing and handling crowns, planting, cultivation, harvesting and marketing are described, with notes on insect pests and diseases. E. G. BRICKELL.

Protein content of spring and winter varieties of field beans (*Vicia faba* L.) sown and harvested on the same dates. D. A. Bond and G. Toynbee-Clarke (*J. agric. Sci., Camb.*, 1968, **70**, 403–404).—Spring and winter varieties of field beans were sown and harvested on the same dates. The crude protein content of seeds of the spring variety was higher on average by $3{\cdot}1 \pm 0{\cdot}9$, $2{\cdot}8 \pm 0{\cdot}7$ and $2{\cdot}9 \pm 0{\cdot}3$% in respective trials. M. LONG.

Survey of the analytical composition of field beans (*Vicia faba* L.) A. Eden (*J. agric. Sci., Camb.*, 1968, **70**, 299–301).—Modern strains of beans were examined with respect to proximate and mineral composition. Most of the winter strains were of the Throws M.S. variety and of the spring strains, Minors. Spring beans averaged 31·4% crude protein (dry matter basis); the value for winter beans was 26·5%, the differences being highly significant. True protein values followed a similar trend. The figures for crude fibre were, spring beans 8% and winter beans, 9%, also a highly significant difference. Apart from P (which was much higher in modern strains) both strains contained the normally accepted levels of oil and main mineral components. M. LONG.

Growing pumpkins and squashes. Anon. (*Fmrs' Bull. U.S. Dep. Agric.*, 1968, No. 2086, 21 pp.).—Varieties, culture and weed control, curing and storing, and diseases and insect pests and their control are described. E. G. BRICKELL.

Influence of nitrogen and potassium fertilisation on the growth and flowering of azaleas (*Rhododendron simsii*, Planch.). K. Rathsack and A. Jungk (*Z. PflErnähr. Bodenk.*, 1968, **120**, 1–11).—Azaleas, var. Schäme, were grown outdoors in peat and subjected to various levels of N and K applied in solution at the rate of 5 l/m²/week. Sources of N were NH_4NO_3, $(NH_4)_2SO_4$ and NH_4Cl. N had more effect on growth and flower production than did K, supra-optimal concn. having a greater depressant effect than K on yield. Optimal concn. for N ranged from 375 to 500 ppm, the spread probably arising from variations in yearly pptn. The corresponding range for K was 250–500 ppm, although little difference in plant response could be detected in the range 125–1000 ppm. With increasing N the optimal range for K contracted. $[NH_4^+]$ influenced the plants more than did the NH_4/K ratio. M. LONG.

Irrigation of sugar-cane. R. A. Yates (*Aust. J. agric. Res.*, 1967, **18**, 903–920).—Irrigation trials on sugar-cane during four seasons included measurements of the potential evapotranspiration of cane (E_{tc}) or of grass (E_{tg}), pan evaporation (E_p), mean air temp. (T_a) and the calculation of Penman's potential evaporation (E_o). E_{tc} consistently exceeded E_p ,and E_{tg} slightly exceeded E_p in summer; the correlation of E_t with the other parameters was also studied. Yield responses to irrigation were about 10 times as great on a red volcanic loam as on a heavy alluvial clay and reasons for this are proposed. Growth, and growth response to irrigation are very severely curtailed when the mean temp. falls below 70°F, soil temp. appearing to be the critical factor. The growth rate is too susceptible to temp. and other factors to be reliable as a guide to the need for irrigation. (35 references.) J. L. WALPOLE.

Effects of early drought and transplanting on subsequent development of the tobacco plant. J. M. Hopkinson (*Aust. J. agric. Res.*, 1968, **19**, 47–57).—Plants subjected to early water stresses, as in commercial hardening and transplanting, eventually thrived better than untreated controls. The increased assimilation and growth rates that occurred after recovery were accompanied by changes in

leaf-distribution and a delay in flower initiation occurring at a higher node, which increased the no. of leaves, and prolonged vegetative growth. (12 references.) P. S. ARUP.

Pruning to prevent wind damage. Anon. (*Plrs' Bull. Rubb. Res. Inst. Malaya*, 1967, No. 91, 147–157).—Certain high-yielding clones of rubber trees are particularly susceptible to trunk snap and other forms of wind damage, and this can be greatly reduced by pruning to improve the shape and stability of the crown. Pruning techniques are discussed, relating to the age and branching habit of the trees as well as to the layout of the plantation. The effect of fertiliser schedules on wind-susceptible clones is discussed. J. L. WALPOLE.

Covers and fertilisers for immature rubber. Anon. (*Plrs' Bull. Rubb. Res. Inst. Malaya*, 1967, No. 89, 66–72).—Creeping legume cover-crops promote quicker growth and earlier maturity of new rubber plantings, but where this is not practicable the same result can be achieved by applying larger amounts of nitrogenous fertiliser. In either case, adequate amounts of K must be included in the fertiliser schedule. The results of field trials are tabulated. J. L. WALPOLE.

Fuel composition. Mobil Oil Corp. (B.P. 1,090,704, 1.10.65. U.S., 5.10.64).—A smokeless fuel composition, capable of burning without a flame, for use in orchards, vineyards, etc. during frosty weather, comprises a briquetted mixture of petroleum coke 50–90 (60–80), an oxidising agent capable of supporting combustion (a nitrate, perchlorate, peroxide, or permanganate) 2–15 (5–10), a binder (starch) 1–10 (3–5), wood-sawdust 3–20 (5–15) and/or charcoal 4–40 (7–20), and, as a water-proofing agent, a paraffin wax emulsion 0·4–2 wt.-%. A mixture of the petroleum coke, wood-sawdust and charcoal is blended with an aq. solution of the binder and the oxidising agent, mixed with the wax emulsion, and the product is briquetted. One face of the briquette is coated with readily ignitable material consisting of the above composition from which the petroleum coke has been excluded. J. M. JACOBS.

Soil coating composition. Esso Research & Engng Co. (B.P. 1,095,965, 7.9.66. U.S., 16.9.65).—Agricultural crops are established and sustained in semi-arid areas by coating the soil with a composition containing 30–70% of a petroleum product (which forms a continuous film that is penetrable by seedlings but impervious to water and is devoid of herbicidal properties), water and 0·1–5·0% by wt. of a polyisobutylene, mol. wt. 600–50,000, the composition being acidic (HCl present) and containing an emulsifying agent and, optionally a solvent. Thus a suitable composition contains asphalt (50%), Duomeen T (0·3%), HCl (0·47%), naphtha (3%), PIB (1%) and water (45·23%). S. D. HUGGINS.

Pest Control

Organic insecticides. Synthesis of 1,2,3,4,7,7-hexachlorobicyclo-[2,2,1]hept-2-ene-5,6-dimethyl *N*-alkyl- and *N,N*-dialkyl-carbamates. A. F. Anishchenko, S. D. Volodkovich, N. N. Mel'nikov and S. I. Shestakova (*Zh. obshch. Khim.*, 1967, **37**, 2132–2134).—1,2,3,4,7,7-Hexachloro[2,2,1]-hept-2-ene-5,6-dimethyl *N*-alkyl- and *N,N*-dialkyl-carbamates were prepared in good yields by conducting the reaction in a medium of inert, org. solvent at low temp., using a 2-fold excess of amine. With increase in number of C atoms in the *N*-alkyl, fungicidal activity of the carbamates was reduced. 1,2,3,4,7,7-Hexachlorobicyclo[2,2,1]hept-2-ene-5,6-dimethyl carbamate had the highest activity. A.L.B.

Chemistry of the new insecticidal phosphoric acid ester GS 13005. K. Rüfenacht (*Helv. chim. Acta*, 1968, **51**, 518–526).—The syntheses of *O,O*-dimethyl-*S*-[(2-methoxy-1,3,4-thiadiazole-5(4*H*)-one-4-yl)-methyl] dithiophosphate and of intermediates, byproducts and degradation products are described. The heterocyclic ring is formed by reaction of K methylxanthogenate with hydrazine hydrate and subsequent treatment of the product with $COCl_2$ to furnish 2-methoxy-1,3-4,thiadiazole-5(4*H*)-one (**I**). Reaction of **I** with aq. HCHO gives 2-methoxy-4-hydroxymethyl-1,3,4-thiadiazole-5(4*H*)-one, which with $SOCl_2$ gives 2-methoxy-4-chloromethyl-1,3,4-thiadiazole-5(4*H*)-one (**II**). Esterification of **II** with K *O,O*-dimethyldithiophosphate follows. Shorter processes comprise a direct condensation of the hydroxymethyl intermediate with dithiophosphoric acid and of the 2-methoxy-1,3,4-thiadiazolone with the salt of *O,O*-dimethyldithiophosphoric acid and HCHO. Selectively labelled compounds containing ^{14}C on three positions are made in the same way. The preparation of the degradation products is also described. M. SULZBACHER.

Esters of phosphoramidodithioic acid. Ya. A. Mandel'baum, G. L. Abramova, N. N. Mel'nikov and L. M. Golovleva (*Zh. obshch. khim.*, 1967, **37**, 2540–2544).—A series of amides and hydrazides of *O*-alkyl *S*-aryl (alkyl) esters of phosphorodithioic acid, possessing fungicidal and insecticidal properties was synthesised by three methods: (*a*) by use of *O*-alkyl *S*-aryl esters of phosphorochloridodithioic acid as starting material, (*b*) by a one-stage process from the N-substituted phosphoramidothioic dichloride, Na benzenethiolate (**I**) and the corresponding alcohol and (*c*) by reaction of an *O*-alkyl ester of N-substituted phosphoramidochloridothioic acid with an alkane- or arene-thiol. Method (*c*) was satisfactory. Caustic soda or metallic Na was used in prep. of **I**. An excess or a stoicheiometric amount of NEt_3 or pyridine was used as HCl acceptor. A.L.B.

Fungicidal activity of aromatic derivatives of dichloroacetamide. I. G. Khaskin, E. A. Shomova and A. L. Stolper (*Microbiology [USSR]*, 1967, **36**, 853–856).—The action of dichloroacetamide (**I**) and 24 of its aromatic deriv. was studied in relation to the growth of five phytopathogenic fungi, *Botrytis cinerea*, *Fusarium oxysporum*, *Alternaria radicina*, *Aspergillus niger* and *Rhizoctonia violacea*. Unsubstituted **I** possesses negligible fungicidal activity but dichloroacetanilide inhibits the growth of fungi to a much greater degree. The most active compound was 2,2-dichloro-*p*-chloroacetanilide. Theoretical interpretation of the findings is attempted. C.V.

Recovery of *Pratylenchus loosi* from soil samples. P. Sivapalan (*Tea Q.*, 1967, **38**, 29–35).—The Baermann funnel technique gives wide variation among replicates and recovers only 4–5% of root eelworms present. Modification of the technique is described resulting in four times the number being extracted and the time required is considerably reduced. C.V.

Comparison of *Pratylenchus* spp. (Nematoda) population densities in grass and apple roots. O. A. Egunjobi (*N.Z. Jl agric. Res.*, 1968, **11**, 142–148).—*Pratylenchus* spp. populations from the roots of apple trees were compared with those extracted from the roots of grasses growing under the tree canopies. All results indicated a significant predominance of the grass root populations over those from apple roots, and the economic significance of this is discussed. (11 references.) E. G. BRICKELL.

Collar and branch canker (*Phomopsis theae* Petch) of young tea. I. Incidence. II. Influence of soil moisture. N. Shanmuganathan and W. R. F. Rodrico (*Tea Q.*, 1966, **37**, 221–228; 1967, **38**, 320–330).—I. A general review, it being concluded that although green shoots could become infected, and even die, in general the infection rarely progressed further and no serious damage to the bush occurred. However pycnidia could be produced and this provided inoculum for stem infection.

II. Although infection could occur at any time of the year, max. canker development only occurred when plants were infected during the drier months. Low soil moisture favoured this state. (13 references). C.V.

Laboratory assay for determining pathogenicity of *Phytophthora* spp. to tomato. G. I. Robertson (*N.Z. Jl agric. Res.*, 1968, **11**, 211–214).—The radicals of tomato seedlings, grown on moist seed testing paper, were inoculated *in situ* with strips of agar inoculum using 13 isolates of the water mould *Phytophthora* spp. Elongation subsequent to inoculation and lesion length were then measured to assess disease severity, and the results are tabulated and discussed. The method described is also suitable for other root pathogens and hosts, and may be useful in distinguishing resistant varieties and for race classification. E. G. BRICKELL.

Lomasomes in wheat leaves infected by *Puccinia graminis* and *P. recondita*. M. A. Ehrlich, J. F. Schafer and H. G. Ehrlich (*Can. J. Bot.*, 1968, **46**, 17–20).—The nature and occurrence of lomasomes (boundary formations) in mesophyll cells of wheat plants infected with *Puccinia graminis* and *P. recondita* were studied. Lomasomes were found in host cells from both resistant- and susceptible-type infection centres and were particularly massive and frequent in plants grown at low temp. (65–70°F.). The observed lomasomes may be associated with infection-induced alterations in the mesophyll cell wall. J. L. WALPOLE.

Physiology of host–parasite relations. XIX. Further observations on nucleoprotein changes in wheat leaf nuclei during rust infection. P. K. Bhattacharya, M. Shaw and J. M. Naylor (*Can. J. Bot.*, 1968, **46**, 11–16).—Cytophotometric measurements were made on infected and uninfected leaves of Little Club wheat at intervals after inoculation with stem rust fungus (*Puccinia graminis tritici* Erikss. and Henn.). No change in host DNA was observed within

6 days after inoculation, but there were marked decreases in protein-bound lysine and arginine within 2 days. The DNA/lysine and DNA/arginine ratios were higher in rust-affected host nuclei, but infection did not alter the ratio of protein-bound lysine to arginine. Profound changes in nuclear metabolism of the host are induced shortly after rust infection. (50 references.)
J. L. WALPOLE.

Effect of rust infection on DNA, RNA and protein in nuclei of Khapli wheat leaves. P. K. Bhattacharya and M. Shaw (*Can. J. Bot.*, 1968, **46**, 96–99).—The effects were determined of stem rust fungus infection on the levels of nuclear DNA, RNA, histone and total protein, and on the size of the nuclei in the mesophyll cells of the primary leaf of Khapli wheat. Nuclear size, RNA, and protein content increased and the histone content had decreased by three days after inoculation, while the DNA content decreased from four days after inoculation. These results for the resistant variety Khapli are similar to those reported earlier for the susceptible variety Little Club, but the changes occur more rapidly after inoculation. (23 references.) J. L. WALPOLE.

Transmission and biology of sunflower downy mildew. P. G. Goossen and W. E. Sackston (*Can. J. Bot.*, 1968, **46**, 5–10).—Sunflower seedlings grown in soil naturally infested with *Plasmopara halstedii* may not show symptoms of downy mildew even though the pathogen can be demonstrated in the plant tissues. Sporulation can be induced by placing infected seedlings in a saturated atm. for 12 h, while inoculating seeds with a suspension of zoosporangia gave a high % infection within 14 days. Damping-off of sunflower seedlings induced by *P. halstedii* was observed in some cases. Spore concn. was not critical for infection in the ranges tested (100–150,000 zoosporangia/ml). Spores stored at −20° on infected leaves germinated about 75% after 3 weeks but none did so after 24 weeks storage. When added to untreated soil, zoosporangia remained infective for only 7 days, but some remained infective for 14 days in pasteurised and sterilised soils.
J. L. WALPOLE.

Respiratory activity of fungal associations in zones of heart rot and stain in sugar maple. H. M. Good, J. T. Basham and S. D. Kadzielawa (*Can. J. Bot.*, 1968, **46**, 26–36).—Heart rot disease in maple trees was studied by comparing the total activity of the decay region (as measured by CO_2 output), water content, pH and predominant micro-organisms in zones selected across the affected area. Trees from which *Fomes igniarius* was isolated gave consistent results with the central, severely decayed parts of the tree being slightly acid and containing the fungus in abundance. Corresponding results were obtained with rot caused by *Polyporus glomeratus*. Trees showing no *F. igniarius* evinced a different pattern: the pH of the decayed area was consistently alkaline and the moisture content and CO_2 production were very high. (12 references.) J. L. WALPOLE.

Effect of ioxynil on the free amino-acid and protein content of the leaves of tartary buckwheat and spring wheat. D. Paton and S. Zalik (*Can. J. Bot.*, 1968, **46**, 89–92).—Spring wheat seedlings sprayed with ioxynil showed little change in the overall concn. of either the soluble or protein amino-acids after two days, whereas tartary buckwheat showed marked changes in the balance between soluble and protein amino-acids. J. L. WALPOLE.

Change in the photic reaction due to DDT in the grain weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). C. A. Barlow (*Can. J. Zool.*, 1968, **46**, 35–40).—Lethal doses of DDT produce a change in photic orientation from the normal photonegative toward photopositive, the extent and time of occurrence after exposure depending somewhat on dosage. The survival value of the change in photic reaction is discussed. (13 references.)
E. G. BRICKELL.

Effect on honey bees of dichlorvos and bromophos applied as sprays to white clover (*Trifolium repens* L.). T. Palmer-Jones and P. G. Clinch (*N.Z. Jl agric. Res.*, 1968, **11**, 138–141).—Both dichlorvos and bromophos proved safe to honey bees when applied to white clover at the rates of 0·5 pint/15 gall water/acre and 2 pints/13 gall water/acre, respectively. E. G. BRICKELL.

Effects of using organochlorine sprays in an orchard. N. Collett and D. L. Harrison (*N.Z. Jl Sci.*, 1968, **11**, 371–379).—Evidence of exposure, symptoms, and tissue analyses on bird carcasses, soil, worms, apple leaves and harvested apples proved that the increased mortality among blackbirds and thrushes towards the end of a 12-year period of applying DDT (3 sprays yearly), DDD and BHC (one spray of each) to the orchardgrass was attributable to organo-Cl insecticide poisoning. Total accumulated residues of organo-Cl compounds in the soil (and mostly near the surface) were ~ 15–20 lb/6-in. acre, but residues in harvested apples were negligible. (17 references.) W. J. BAKER.

Organophosphorus poisoning. [A] Properties of avian esterases. [B] Diagnosis of poisoning in pheasants owing to a number of common pesticides. P. J. Bunyan, D. M. Jennings and A. Taylor (*J. agric. Fd Chem.*, 1968, **16**, 326–331, 332–339).—[A] Normal contents of brain, kidney and liver esterases, determined by electrophoresis, were sufficiently consistent for the detection of abnormal levels in pheasants and pigeons. Thimet poisoning caused complete inhibition of brain cholinesterase and certain changes in isoesterase pattern. The effects were still observed 12 days *post mortem*. (12 references.)

[B] Lethal and sublethal doses of six organophosphorus pesticides were fed to 36 pheasants, and esterase levels were measured in liver, kidney and brain extracts. Pesticide dosages and residue levels, changes in tissue electrophorograms due to pesticides, and tissue-enzyme levels in dosed birds are tabulated. The use of the procedures described for the diagnosis of poisoning by organo-P pesticides is discussed. (16 references.) P. S. ARUP.

Toxic effects of insecticide Telodrin in poultry. M. P. Verma, H. S. Bagha and B. K. Soni (*Indian J. expl Biol.*, 1967, **5**, 245–248).—The effects of Telodrin on white Leghorn cockerels were studied. Symptoms of acute Telodrin poisoning appeared in 70–120 min. after oral administration, the affected birds showing increasing cyanosis of comb and wattle and accelerated respiration followed by extreme nervousness and hyperexcitability with convulsions. The LD_{50} value was determined as 3·85 mg/kg body wt. The liver and kidneys showed varying degrees of degenerative and haemorrhagic changes, and the gall bladder was distended.
E. G. BRICKELL.

Precautions in the use of weedkillers. Anon. (*Plrs' Bull. Rubb. Res. Inst. Malaya*, 1967, No. 89, 77–80).—The acute oral LD_{50} values and the toxicity hazards for 12 of the most important herbicides are tabulated. The risks attendant on mixing and spraying operations are described and general precautions and safety rules given. J. L. WALPOLE.

Occurrence and control of root-lesion eelworm (*Pratylenchus loosi*) in nurseries. A. Kerr and M. K. Vythilingam (*Tea Q.*, 1967, **38**, 22–28).—Nurseries on 52 estates mostly in up-country plantations were sampled, infestation being heavy in 2, light in 13, very light in 16 and absent in 24. MeBr was better than DD fumigation for control. There is no direct evidence to show that nurseries can become reinfested by eelworms carried by water. C.V.

Control of grape diseases and insects in the Eastern U.S. J. R. McGrew and G. W. Still (*Fmrs' Bull. U.S. Dep. Agric.*, 1968, No. 1893, 28 pp.).—Twelve diseases and fourteen pests are described together with their control by fungicides, insecticides, spreaders and stickers. E. G. BRICKELL.

Possible use of *Bacillus thuringienis thuringienis*, Berliner, in the control of crop pests. II. Susceptibility of some lepidopterous pests to *B. thuringiensis*. T. V. Venkatraman and R. Chander (*Proc. Indian Acad. Sci.* B, 1967, **66**, 231–236).—All larval stages of *Papilio demoleus* L. were highly susceptible to two commercial prep. of *B. thuringiensis*, while *Prodenia litura* (F.) showed moderate susceptibility and *Plusia orichalcea* (F.) and the pyralids *Leucinodesorbonalis* Guen. and *Chilo partellus* (Swinh.) showed less susceptibility. *P. demoleus*, a serious pest of young citrus, may be efficiently controlled by *B. thuringiensis* sprays. E. G. BRICKELL.

Poria control by soil fumigation with methyl bromide. N. Shanmuganathan and S. R. A. Fernando (*Tea Q.*, 1967, **38**, 311–319).—*Poria hypolateritia* (**I**), causative agent of root disease, can be effectively controlled by MeBr applied at a concn. 0·5 lb/100 sq. ft and little or no penetration of the fumigant occurs outside the treated area. Control is best when divided in 200 sq. ft units, each being separately dealt with. A covering period of 48 h is adequate and planting can take place thereafter. There is a significant increase in growth, an increase in total available N and NH_4-N in the soil, stimulation of fungi antagonistic to **I**. such as *Trichoderma viride* and *Penicillium* spp. and suppression of weed growth for ~2 months and no adverse effects have been noted. Black root disease in tea (*Rosellinia arcuata*) is also controlled. (11 references.) C.V.

Weed control by 2,4-D amine/sodium chlorate mixtures. Anon. (*Plrs' Bull. Rubb. Res. Inst. Malaya*, 1967, No. 91, 139–146).—A promising degree of weed control in rubber plantations was given by spraying with 2,4-D amine/$NaClO_3$ mixtures, which were effective replacements for sodium arsenite. Susceptible and

resistant weed species are tabulated, and for better results against the dominant Buffalo grass (*Paspalum conjugatum*) some of the $NaClO_3$ should be replaced by MSMA. Spraying should be directed away from the tree trunks to avoid the risk of bark damage.
J. L. WALPOLE.

Possible use of translocations to fix desirable genes in insect pest populations. C. F. Curtis (*Nature, Lond.*, 1968, **218**, 368–369).—Explains, in terms of genetics, how the prolonged decrease in fertility after the release of translocation homozygotes could eradicate a pest. W. J. BAKER.

Retention and volatilisation of lindane and DDT in the presence of organic colloids isolated from soils and leonardite. K. Porter and W. E. Beard (*J. agric. Fd Chem.*, 1968, **16**, 344–347).—When suspended in solutions of lindane or DDT in hexane, colloids isolated from soils and leonardite decreased losses of the insecticides on evaporation of the hexane, compared with the losses observed on evaporation without the colloids. Lindane and DDT were completely volatilised from the residues without colloids at 60 and 80°, respectively, but in the presence of the colloids appreciable volatilisation occurred only on heating to 186°. P. S. ARUP.

Use of carbon to reduce uptake of insecticidal soil residues by crop plants. Effects of carbon on insecticide adsorption and toxicity in soils. E. P. Lichtenstein, T. W. Fuhremann and K. R. Schulz (*J. agric. Fd Chem.*, 1968, **16**, 348–355).—In laboratory pot experiments, active C (Norit) reduced the uptake of aldrin into pea roots and leaves by 96%, at 1000 ppm in quartz sand and at 4000 ppm in loam. Similar results for dieldrin, heptachlor, and heptachlor epoxide were achieved with C at 2000 ppm. The presence of C hindered the extraction of the insecticides from loam and reduced their toxicity to *Drosophila melanogaster*; the residue binding effects increased with time (0–15 days). Results in field plots were less marked, but C would still be useful for reducing insecticidal residues in crops. (18 references.) P. S. ARUP.

Colorimetric methods for determination of simazine and related chloro-*s*-triazines. M. T. H. Ragab and J. P. McCollum (*J. agric. Fd Chem.*, 1968, **16**, 284–289).—Four sensitive, rapid and reliable methods were developed for determination of chloro-*s*-triazine herbicides, based on their reactivity in the Zincke reaction (heating with aq. pyridine and treatment with aq. NaOH) and condensation of the unstable, yellow reaction product with Et cyanoacetate, barbituric acid, or 2-thiobarbituric acid. Extinction measurements were made at 436·5, 550, 582 and 625 nm, and sensitivities were 0·033, 0·020, 0·025 and 0·017 ppm, respectively. Reaction mechanisms are outlined. (20 references.) P. S. ARUP.

Gas chromatographic determination of malathion and its oxygen analogue, malaoxon. C. Corley and M. Beroza (*J. agric. Fd Chem.*, 1968, **16**, 361–363).—In this rapid method, malathion and malaoxon (diethylmercaptosuccinate *S*-ester with *O,O*-dimethylphosphorothioate), and also diazinon and diazoxon residues, extracted from plant or animal tissues, or milk, are purified by a simple solvent partition method and analysed by g.l.c. with flame-photometric detection. Recoveries were 90–100% at the 0·05–2 ppm level. P. S. ARUP.

Polarographic determination of dithiocarbamates and their heavy-metal complexes. D. J. Halls, A. Townshend and P. Zuman (*Analyst, Lond.*, 1968, **93**, 219–223).—Procedures are described for the determination of mono- and di-alkyldithiocarbamates (alone or mixed) and of the Na, Zn and Mn ethylenebisdithiocarbamates used as pesticides, *viz.*, nabam, zineb and maneb. The polarographic method is based mainly on adjustment of pH, all anodic waves registering between −1·0 and −0·2 V. Reproducibility is ±2–4% for pure solutions and ±5–15% in presence of biological material. (25 references.) W. J. BAKER.

2-Substituted benzoxazoles. Monsanto Chemicals (Australia) Ltd. (B.P. 1,087,101, 16.12.65. Australia, 18.12.64).—Used as biocidal agents (herbicides, fungicides) the 2-trichloromethylbenzoxazoles are substituted in the benzene nucleus by one or more substituents, chosen from halogen, NO_2, OH, alkyl, alkoxy, aryl, aryloxy, carboxy, carbalkoxy, halo- or alkoxy-alkyl, or halo-, nitro-, alkyl- or alkoxy-aryl, preferably in the 5- and/or 6-positions. They are prepared from e.g., an *o*-aminophenol and an alkyl trichloroacetimidate. Thus, $1,2,4,6\text{-}OH\cdot NH_2\cdot C_6H_2\cdot Cl_2$ is refluxed with Me trichloroacetimidate in EtOH for 2 h to give 2-trichloromethyl-5,7-dichlorobenzoxazole, m.p. 84° (MeOH).
S. D. HUGGINS.

2-Substituted benzoxazoles. Monsanto Chemicals (Australia) Ltd. (B.P. 1,087,779, 16.12.65. Australia, 18.12.64).—Used as herbicides and fungicides, the 2-trichloromethylbenzoxazoles, optionally substituted in the benzene ring by halogen, NO_2, OH, alkyl, alkoxy, aryl, aryloxy, carboxy, carbalkoxy, or substituted alkyl (halogen or alkoxy) or substituted aryl (halogen, NO_2, alkyl or alkoxy) are obtained from *o*-aminophenol, optionally substituted in the benzene ring, and an alkyl trichloroacetimidate of formula $RO\cdot C{:}NH\cdot CCl_3$, where R is alkyl, at 60–150° under anhyd. conditions. The substituents are preferably in the 5, 6 or 5,7-positions. The compounds can be prepared in various ways (see B.P. 1,087,101) but the claimed method is by reaction of an *o*-aminophenol with an alkyl trichloracetamidate. S. D. HUGGINS.

Isonitriles. CIBA Ltd. (B.P. 1,087,532, 13.10.65. Switz., 14.10.64).—Biocidal (insecticidal, fungicidal, herbicidal etc.) prep. contain dioxan deriv. of formula $R^1R^2(O_2C_4H_4)R^3(N{:}C)$, wherein R^1 and R^2 are each H, optionally substituted alkyl, alkenyl, cycloaliphatic, aromatic or heterocyclic or R^1 and R^2 together represent an alkylene group of 1–6 C which may be substituted and R^3 is an alkyl of 1–4 C. E.g. 2-(*p*-chlorophenyl)-5-isocyano-5-methyl-1,3-dioxan, m.p. 115–116° (MeOH) is obtained by reacting 2-(*p*-chlorophenyl)-5-formylamino-5-methyl-1,3-dioxan (prep. also described, starting from the 5-nitro deriv.) with $POCl_3$ in dry pyridine, with cooling; the product is then heated at 60° for 15 min and poured on to ice. S. D. HUGGINS.

Dihydric fluoroalcohols and salts. Allied Chemical Corp. (B.P. 1,087,805, 10.8.66. U.S., 11.8.65).—Used as pesticides (insecticides, herbicides, etc.) the title compounds (**I**), e.g. 1,3-bis (1,3-dichlorotetrafluoro-2-hydroxy-2-propyl)-2-phenyl-1-propene, have the formula $(CF_2X)_2OH\cdot C\cdot CH{:}RC\cdot CH_2\cdot C\cdot OH(CF_2X)$, wherein R is Ph or alkyl of 1–3 C and X is F or Cl. The mono- and di-alkali metal and ammonium-salts are prepared from **I** by reaction with e.g. NaOH or NH_3. **I** are prepared from perhaloacetones and α-olefins by the method described in B.P. 964,755.
S. D. HUGGINS.

Substituted tetrahydrothiophene 1,1-dioxides. Uniroyal Inc., (B.P. 1,090,308, 6.10.66. U.S., 19.10.65 and 19.8.66).—Possessing pesticidal and/or bactericidal activity, the tetrahydrothiophene 1,1-dioxides are substituted in the 3-position by X and R^1 (X is Cl or Br, R^1 is H, Cl or Me) and in the 4-position by R^2 (H, Cl or Me) and $Z\cdot C_6H_{5-n}\cdot Y_n$ (Z is S or sulphonyl, Y is Cl, Br or C_{1-8} alkyl, *n* is 0, 1 or 2), or in the 3-position by R^1 only, in the 4-position by R^2 and X and in the 5-position by $Z\cdot C_6H_{5-n}\cdot Y_n$. A 2,5- or 2,3-dihydrothiophene 1,1-dioxide is reacted with an aryl sulphenyl halide at 20–60° in a solvent, e.g., 3-chloro-4-phenylthiotetrahydrothiophene 1,1-dioxide, (**I**), m.p. 80–82° (Et_2O) is obtained by reacting 2,5-dihydrothiophene 1,1-dioxide in $CHCl_3$ with benzenesulphenyl chloride. The sulphonyl deriv,. m.p. 174–175°, is obtained by oxidising **I** with H_2O_2/AcOH. S. D. HUGGINS.

Phenazine derivatives. Shell Internationale Research Mij N.V. (Inventors: B. Cross and R. E. Woodall) (B.P. 1,090,899, 26.8.66).—Used for protecting crops, the phenazines (**I**), substituted on the benzene rings by up to 8 R groups, where R is halogen, aryl, alkyl, aralkyl, alkaryl, alkoxy, thioalkyl, alkyl- or aryl-sulphonyl, OH or optionally substituted NH_2, can be obtained by reducing the corresponding 2,2′-dinitrodiphenylamine with N_2H_4 in alcoholic alkali- or alkaline-earth metal hydroxide at > 20° in the presence of activated C or metal of Groups Ib, IIb or VIII. E.g. 2-chlorophenazine, m.p. 131–133° is obtained in 74% yield from 4-chloro-2,2′-dinitrodiphenylamine by reduction with N_2H_4 in ethanolic KOH, in presence of Raney Ni. S. D. HUGGINS.

Chloro-pivalolactones. Eastman Kodak Co., Assee of W. J. Jackson, jun., and J. R. Caldwell (B.P. 1,095,213, 6.1.65. U.S., 7.1.64).—The title compounds (**I**), e.g. 2-chloromethyl-2-methyl-propiolactone contain 1–3 Cl atoms and at least one of Me and $CHCl_2$ groups, and are prepared by chlorinating with Cl_2 in presence of promotor (u.v., visible light, or free radicals) at 0–80°; concn. of HCl is reduced by presence of $CaCO_3$ or of sufficient water to avoid hydrolysis of the product. **I** containing 2–3 Cl atoms, and low mol. polymers thereof are useful as herbicides, fungicides and insecticides. The high mol. products form fibres, films, etc. S. D. HUGGINS.

Pesticidal compositions [azide solutions]. Pittsburgh Plate Glass Co., Assee of W. O. McConnell and H. W. Rahn (B.P. 1,095,796, 6.4.65. U.S., 7.4.64).—KN_3 is claimed as a pesticide (insecticide, fungicide, herbicide etc.) and may be applied before planting, before or after emergence of crop. NaN_3 is a possible substitute. Soil life of biodegradable herbicides is prolonged by addition of sufficient KN_3 to the soil to kill micro-organisms.
S. D. HUGGINS.

Oxime ethers and pesticidal preparations containing them. CIBA Ltd. (B.P. 1,096,037, 20.1.66. Switz., 22.1. and 9.7.65) (28 pp.).—Compositions for combating pests, especially harmful insects, acarides, nematodes, and micro-organisms (e.g., phytopathogenic fungi and bacteria) and for controlling weeds, contain 2,4,1-$R^{III}(R^{IV})C_6H(O\cdot N{:}CR^IR^{II})R^VR^{VI}$ wherein R^I–R^{II} are aliphatic, cycloaliphatic, araliphatic, aromatic, or heterocyclic residues or R^I is H or R^I and R^{II} together represent part of a saturated or unsaturated carbocyclic or heterocyclic ring; R^{III} and R^{IV} are negative groups; and R^V-R^{VI} are H, halogen, alkyl, OH, acyloxy, alkoxy, aryloxy, SH, acylthio, alkylthio, arylthio, NH_2, alkyl- or dialkylamino, or are as R^{III} and R^{IV}. A typical agent is *p*-anisoldehyde(**I**)*O*-(2,4-dinitrophenyl)oxime, m.p. 187–187·5° ($HCONMe_2$-EtOH), prepared by adding a solution of Na in EtOH to a solution of **I**-oxime in EtOH, then adding a solution of 1,3,4-$(NO_2)_2C_6H_3Cl$ in EtOH dropwise at 40–65°. F. R. BASFORD.

Propargyloxybenzene derivatives and their use as pesticides. Fisons Pest Control Ltd. (Inventors: P. L. Carter, A. J. Lambie and G. T. Newbold) (B.P. 1,097,255, 15.8.64).—**4-Propargyloxy**-azoxybenzene (**I**), -diphenyl sulphide, and -1-benzyloxybenzene are pesticides, especially acaricides. In an example, 4-propargyloxy-azobenzene (**II**) m.p. 83° (aq. EtOH) is prepared in 83% yield by boiling a mixture of *p*-$OH\cdot C_6H_4N_2Ph$, propargyl bromide, K_2CO_3, and acetone during 4 h. **I** (m.p. 85° from aq. EtOH) is obtained from **II** by oxidation at 90° for 30 min. with AcOH–H_2O_2.
F. R. BASFORD.

Phenylamine ammonium salts. Farbenfabriken Bayer A.-G. (Inventors: W. Daum and H. Scheinpflug) (B.P. 1,094,882, 16.9.66. Ger., 27.9.65).—Compounds, $C_6X_5NR^I\cdot A\cdot NR_3^{II}\cdot Y$, are active against phytopathogenic bacteria (A is C⩽$_2$-aliphatic chain which may be interrupted by arylene or hetero atoms; X is Cl or Br; Y is anion; R^I is H or alkyl of 1–4 C; and R^{II} is arylmethyl optionally substituted by halogen, NO_2, alkoxy, and/or alkyl, or is aliphatic hydrocarbon radical optionally containing halogen—but only 1 R^{II} is arylmethyl). A mixture of $C_6Cl_5\cdot NMe\cdot[CH_2]_6\cdot NMe\cdot CH_2Ph$ and *cis*, *trans*-1,3-dichloropropene is kept at 80° during 18 h, then treated with light petroleum (**I**). After 2 h solid is filtered off and washed with **I** to give [6-(pentachlorophenyl-methylamino)hexyl] methyl benzyl (3-chloroallyl) ammonium chloride; the activity against *Xanthomonas malvacearum* is described.
F. R. BASFORD.

Fluorinated benzimidazoles and compositions containing them. Fisons Pest Control Ltd. (Inventors: D. E. Burton, A. J. Lambie and G. T. Newbold) (B.P. 1,087,561, 16.2.63).—Compounds claimed, are: 2-trifluoromethyl- or 2-pentafluoroethyl-benzimidazoles (and salts thereof) optionally substituted in the 4-, 5-, 6-, and/or 7-position by OH, alkyl, alkoxy, NO_2, halogen, pseudohalogen (e.g., CN), substituted alkyl, COR (where R is OH, alkoxy, or optionally substituted NH_2), amino, SH, alkylthio, or SO_2R. In an example, a mixture of $1,2\text{-}(NH_2)_2C_6Cl_4$ and $CF_3\cdot CO_2H$ is heated at 100° during 16 h, then excess of acid and water are removed/<1 atm. The powdered residue is boiled with benzene, then the filtered solution is cooled, with separation of 4,5,6,7-tetrachloro-2-(trifluoromethyl)benzimidazole, m.p. 285°. The products are pesticides, especially molluscicides. F. R. BASFORD.

Molluscicides. Shell Internationale Research Mij. N.V. (Inventors: R. G. Barker) (B.P. 1,092,389, 31.3.66).—The compositions contain a solid carrier and, optionally, a surface-active agent, together with a compound of formula Ph_3CNHR, wherein R is phenyl substituted by ⩽1 alkyl group of 1–7 C, ⩽1 NO_2 or NO_2 and alkyl of 1–7 C, e.g. *N*-trityl-*m*-toluidine. The compounds are prepared from RNH_2 and tritylcarbinol on heating in a polar, aprotic solvent ($HCONMe_2$). A table of toxicity towards molluscs (*Australorbis glabratus*) and towards fish (*Lebistes reticulatus*) is given. S. D. HUGGINS.

Coumaranyl-carbamic acid esters. Farbenfabriken Bayer A.-G. (Inventors: R. Heiss, E. Bocker, W. Behrenz and I. Hammann) (B.P. 1,087,006, 18.4.66. Ger., 25.5.65).—Possessing insecticidal and acaricidal properties, the claimed coumaranyl-*N*-acyl-*N*-methyl carbamic acid esters are obtained by acylating with an acid anhydride the corresponding coumaranyl-*N*-methyl carbamic acid esters at 70–170° in the presence of H_2SO_4 catalyst. The compounds, e.g. 2-methyl-7-coumaranyl-*N*-acetyl-*N*-methyl carbamate, b.p. 135–136°/0·45 mm Hg, can have 1-4 alkyl (C_1–C_3) groups in the 2- and 3-positions of the five-membered ring. S. D. HUGGINS.

Methylenedioxyphenyl compounds and pesticidal compositions containing them. Shell Internationale Research Mij. N.V. (Inventors: G. J. Popjak, E. Bonthrone, J. C. Felton and P. N. Manis) (B.P. 1,087,017, 8.7.65).—Insecticidal compounds claimed have the formula $1,2,4,5\text{-}CX\cdot NRR^I\cdot C_6H_2Y\cdot(O)_2CH_2$ wherein X is O or S; R and R^I are H, or may be alkyl when X is O; Y is H or halogen. A typical product is 2-chloro-4,5-methylenedioxy-thiobenzamide, m.p. 138–140° (benzene). It is obtained by passing H_2S during 3 h at ⩾55° through a solution of $1,2,4,5\text{-}CN\cdot C_6H_2Cl\cdot(O)_2CH_2$ in $HCONMe_2$ and $NHMe_2$. The compounds have synergistic action on insecticides against e.g. *Musca domestica*. F. R. BASFORD.

Azido substituted organophosphorus compounds, their preparation, and pesticidal compositions containing them. Commonwealth Scientific & Industrial Research Organisation and Imperial Chemical Industries of Australia and N. Zealand Ltd. (B.P. 1,087,066, 9.12.64. Australia, 20.12.63) (36 pp.).—Products claimed are effective against, e.g., *Boophilus microplus*, *Calandra granaria*, *Plutella maculipennis*, *Lucilia cuprina*, *Aphis rosae*, *Musca domestica*, and *Tetranychus telarius*. They have the formula $N_3\cdot A\cdot PO(X\cdot E)\text{-}Z\cdot L$ wherein A is CRR^I or $-C_6H_4\cdot CRH\text{-}o$(or *p*); R and R^I are H, alkyl, aryl, aralkyl, alkaryl, or cycloalkyl; X and Z are O, S, or NR^{II}; and R^{II}, E, and L are as R or may be H (R^{II} only) alkenyl, aralkenyl, alkynyl, or cycloalkenyl, or E and L together may form a heterocyclic residue, and R–R^{II}, E, and L may be substituted by halogen, CN, OH, NH_2, alkyl- or dialkylamino, alkoxy, aryloxy, alkylthio, arylthio, carbalkoxy, carbaryloxy, NO_2 or N_3, provided that no C carries >1 N_3 group and the total of such groups is ⩾2. In an example (112 claims), a mixture of $(OEt)_2PO\cdot CH_2Cl$, NaN_3 and Me_2SO is heated at 90–95° during 30 h, then the cooled product is dissolved in 5 N-HCl. The solution is extracted with ether during 24 h, and oil recovered from the extract is distilled, to give, *inter alia*, Et$_2$(azidomethyl)phosphonate, b.p. 62°/0·5 mm, n_D^{20} 1·441. F. R. BASFORD.

Tricyclic ketones, their preparation, and pesticidal compositions containing them. Hooker Chemical Corp. (Inventor: P. E. Hoch) (B.P. 1,087,817, 1.12.64. U.S., 2.12.63) (31 claims).—Compounds claimed are pesticides and comprise $1\text{-}X^I\text{-}2\text{-}R\text{-}3\text{-}R\text{-}4\text{-}X^{II}\text{-}6\text{-}X^{III}\text{-}7\text{-}X^{IV}$ $7\text{-}X^V\text{-}5\text{-}$oxo-bicyclo[2,2,1]heptanes wherein X^I, X^{II}, X^{IV} and X^V are H, halogen, or optionally halogenated alkyl or alkenyl (at least 2 of them being halogen), X^{III} is H or halogen and the 2 R together represent a $CHR^I\cdot O\cdot CHR^{II}$ chain optionally substituted by up to 4 halogen (R^I and R^{II} are H, alkyl, or halogenoalkyl). They are prepared by reacting a $1\text{-}X^I\text{-}2\text{-}CHR^I\cdot OH\text{-}3\text{-}CHR^{II}\cdot OH\text{-}4\text{-}X^{II}\text{-}$ $5\text{-}X\text{-}6\text{-}X^{III}\text{-}7\text{-}X^{IV}\text{-}7\text{-}X^V$-bicyclo[2,2,1]hept-5-ene with MOR (X is H if X^{III} is halogen or *vice versa*; R is H, alicyclyl, or optionally substituted alkyl or aryl; M is metal), then treating the resulting cyclic ether with a strong acid (at 40–120°). E.g., 1,2,3,4,7,7-hexachloro-5,6-di-(hydroxymethyl)-bicyclo[2,2,1]hept-2-ene is added during 2 h to a solution of NaOH in boiling MeOH, then after a further 2 h solvent is distilled off. The residue is poured into water, and ppt. is collected and recrystallised from CCl_4, to give the ether (m.p. 157–158°), which is added to 97% H_2SO_4 at 93–95°, then after 5 h the mixture is worked up, to give 1,4,6,7,7-pentachloro-2,3-(2-oxapropylene)bicyclo[2,2,1]-heptan-5-one active against *Musca domestica* at a concn. of 1000 ppm (100% knock-down in 2 h). F. R. BASFORD.

Sulphur-containing oxime carbamates. Shell Internationale Research Mij. (Inventors: C. Donninger, J. H. Davies and R. H. Davis) (B.P. 1,090,986, 10.11.65).—Insecticidal agents especially active against nematodes have the formula $NR^{III}R^{IV}\cdot CO_2N{:}CR^{II}\cdot SR^I$ wherein R^I–R^{II} are optionally substituted hydrocarbon groups or R^{II} is CN; R^{III}–R^{IV} are H or optionally substituted alkyl or aryl. In an example, a solution of ClCMe:NOH in MeOH is added dropwise to a solution of MeSNa in MeOH, then after 30 min. at the boil the cooled, filtered solution is evaporated and the residue is chromatographed on SiO_2 gel. Product recovered is recrystallised from benzene–light petroleum, to give 1-(methylthio)acetaldoxime, m.p. 92°. This is dissolved in CH_2Cl_2, then 2 drops of NEt_3 are added, followed by MeNCO. After 30 min. at the boil the product is worked up chromatographically to give 1-(methylthio)acetald-oxime-methylcarbamate (*O*-methylcarbamyl 2-oximino-2-methyl-thioethane), m.p. 77–77·5° (benzene–light petroleum).
F. R. BASFORD.

Phthalimide derivatives and compositions containing them. Sumitomo Chemical Co. Ltd. (B.P. 1,091,352, 21.1.66. Japan, 25.1., 30.4., 8.6., and 1.9.65).—Phthalimides substituted on the N by $X\cdot C{\equiv}CH$ (wherein X is $[CH_2]_2$, CHMe, or $CHR\cdot CO\cdot X^1$; R is H or Me; X^1 is CH_2, $[CH_2]_2$, or CHMe) are claimed and are synergists for insecticides. In an example, a mixture of 3-chlorobutyne, K phthalimide, and $HCONMe_2$ is heated at 120° during 30 min. and at 150–160° during 30 min., then the mixture is poured on to ice and extracted with $CHCl_3$. The extract is washed with N-KOH, water, and 0·5 N-HCl, then solvent is removed, leaving

N-(1-methylprop-2-ynyl)-phthalimide, m.p. 110–112°, in 87% yield. The synergists are relatively cheap, are less toxic to mammals than conventional products and are effective not only with pyrethrin-type but also with carbamate-type insecticides such as carbaryl.
F. R. BASFORD.

Unsaturated cyclic ketones. Roussel-Uclaf (B.P. 1,091,394, 24.1.66. Fr., 22.1.65).—2-R-3-methyl-4-hydroxycyclopent-2-enyl-ones (R is H or aliphatic radical) (intermediates in the synthesis of insecticides of the pyrethrin type) are readily prepared by interaction of $CO \cdot CH_2CO \cdot CHR \cdot CMe \cdot CH(OR^1)_2$ (of B.P. 1,091,395) (R^1 is alkyl) with acid in aq. medium. E.g., 5-methyl-5-(diethoxymethyl)-3-oxo-δ-valerolactone is boiled in 2% aq. HCl during 3 h, then water is distilled off azeotropically in presence of benzene. A solution of the residue in CH_2Cl_2 is passed through Al_2O_3 which is then eluted with solvent. The eluate is distilled, to give 3-methyl-cyclopent-2-en-4-ol-1-one, b.p. 110°/0·5 mm. F. R. BASFORD.

***O,O*-Dialkyl *S*-(2-halogeno-1-phthalimidoethyl)phosphorodithioates and insecticide compositions thereof.** Hercules Inc. (B.P. 1,091,738, 9.12.65. U.S., 10.12.64).—In the title compounds alkyl is Me or Et and halogen is Br or Cl. In an example, $(OMe)_2PS_2NH_4$ is added during 18 h at 30° to a solution of *N*-(1,2-dibromoethyl)-phthalimide in MeCN. After a further 2 h at 50–60° ppt is filtered off, the filtrate is evaporated, and the residue is dissolved in benzene. The solution is washed with 5% aq. $NaHCO_3$ and water, and worked up to give *O,O*-Me_2*S*-(2-bromo-1-phthalimidoethyl) (thiothionophosphate, m.p. 102–103° (toluene–light petroleum). Its activity against larvae of *Prodenia eridania* and plum curculio (*Conotrachelus nenuphar*) is described. F. R. BASFORD.

Pesticidal compositions. CIBA Ltd. (B.P. 1,091,964, 31.3.65. Switz., 10.4.64).—The active substances in the composition comprise (*a*) a compound of formula $(R^1O)R^2O(S)P \cdot O \cdot C_6H_3 \cdot NO_2(R^3)$, wherein R^1, R^2 and R^3 are lower alkyl radicals of 1–2 C, preferably Me and (*b*) a compound of formula $[R(O)n\text{-}1]R^I(O)_{m-1}:P(Z)O \cdot CR^{II}:C(X)A$, (wherein R and R^I are alkyl of 1–5 C which may be interrupted by O or phenyl, R^{II} is H or Me, Z is O or S, A is Cl or Br, a lower alkyl or chloralkyl of 1 or 2 C, X is Cl or Br and *n* and *m* each = 1 or 2) or a halogenation product of dimethyl dichlorovinyl phosphate, the components (*a*) and (*b*) being in a ratio of 3 : 1 to 10 : 1. The compositions are active against *Prodenia litura*, *Platyedra gossypiella*, *Musca domestica*, etc.
S. D. HUGGINS.

Phosphoric acid esters. Farbenfabriken Bayer A.-G. (Inventors: G. Schrader, W. Behrenz and I. Hammann) (B.P. 1,097,373, 16.9.66. Ger., 29.10.65).—Compounds of outstanding insecticidal and acaricidal properties are obtained by reacting $OR^I(OR^{II})\text{-}PO_2 \cdot CHBr \cdot CCl_2Br$ (I) with $SM \cdot CO_2R$, wherein R is alkyl of 1–6 C; R^I–R^{II} are alkyl of 1–6 C which may contain Cl, M is alkali metal or NH_4. E.g., I (R^I=R^{II}=Me) is added at 30–40° to a solution of $SK \cdot CO_2Et$ in MeCN. After a further 1 h at 40° the mixture is diluted with benzene, washed with water, and distilled, to give a 62% yield of Me_2 2,2-dichloro-1,2-di-carbethoxythio-ethyl phosphate, b.p. 70°/0·01 mm. Its effect on a variety of pests is presented. F. R. BASFORD.

Derivatives of phenyl *N*-methylcarbamates. Toa Noyaku K.K. (Inventors: I. Seto, H. Tanaka, Y. Ishii, Y. Nagae and S. Kitakata) (B.P. 1,099,084, 8.2.66).—Compounds of the formula $NHMe \cdot CO_2 \cdot C_6HXRR^IR^{II}$-1,2,3,4,5 are active against injurious insects (houseflies, plant-hoppers, leaf-hoppers, etc.) (R^I is H or Me; when R^I is H then X is halogen or Me, R is H, halogen or Me, and R^{II} is H or halogen—but X, R, and R^{II} are different; and when R^I is Me then X is halogen, R is Me, and R^{II} is H). A representative product is 2-chloro-3-methylphenyl methylcarbamate, m.p. 110–111° (toluene–light petroleum), obtained by adding MeNCO to a cold mixture of $1,2,3\text{-}OH \cdot C_6H_3Cl \cdot Me$, toluene, and NEt_3 in a pressure vessel, then after a while heating at 100° during 8 h.
F. R. BASFORD.

Heterocyclic carbamates and thiocarbamates and their use in insecticides. FMC Corp., Assee. of W. G. Scharpf (B.P. 1,099,691, 22.1.65. U.S., 23.1. and 12.10.64).—Compounds $NR^IR^{II} \cdot CX \cdot OR$ are active against arthropods and nematodes wherein R^I and R^{II} are H or aliphatic hydrocarbon radicals of 1–3 C; X is O or S; R is 2,3-dihydrobenzofuran-4(or 7)-yl optionally substituted in the 2- and 3-position by aliphatic radicals of 1–3 C and in the benzene nucleus further by 1–3 alkyl or alkenyl of 1–3 C, halogen, halogenoalkyl, NO_2, NH_2, substituted NH_2, CN, carbalkoxy, acyl, alkoxy, alkylthio, or other carbamate group (there being Me and another group in the 2-position when the 3-position is unsubstituted). In an example, a mixture of 7-hydroxy-2,2-dimethyl-2,3-dihydrobenzofuran (prep. described), ether, MeNCO, and NEt_3 is stirred at room temp., with formation of 2,2-dimethyl-2,3-dihydrobenzofuran-7-yl methylcarbamate, m.p. 151–152° (methylcyclohexane).
F. R. BASFORD.

8-Quinolyl-carbamic acid esters. Farbenfabriken Bayer A.-G. (Inventors: K. Goliasch, H. Scheinpflug and H. Jung) (B.P. 1,087,122, 2.3.66. Ger., 16.3.65).—Fungicidal agents of the formula $CO_2R \cdot NH \cdot A \cdot R^1$ are claimed in which R is quinol-8-yl; A is alkylene; and R^1 is Cl, alkoxy, or alkylthio. They are effective against *Archimycetes*, *Phycomycetes*, *Ascomycetes*, *Basidiomycetes*, and *Fungi imperfecti* (especially *Piricularia oryzae* in rice, also *Cochliobolus miyabeanus* and *Corticium sasakii*), also *Mycosphaerella*, *Cercospora corticium*, *Alternaria*, *Botrytis cinerea*, *Phialophora cinerescens*, *Verticillium alboatrum*, *Fusarium dianthi*, and *F. cubense*. A typical agent is quinol-8-yl (3-ethoxypropyl)carbamate, m.p. 104–105°, prepared in 97·4% yield by heating a mixture of 8-hydroxyquinoline, pyridine, acetone, CH_2Cl_2 and $NCO[CH_2]_3\text{-}OEt$ during 2 h. F. R. BASFORD.

Aryl esters of β-isothiuroniumethane sulphonic acid. Vsesoyuznyi Nauchno-Issledovatel'skii Institut Fitopatologii (Inventors: N. K. Bliznyuk, A. F. Kolomiets, R. N. Golubeva, E. F. Granin, Yu. N. Fadeev, L. S. Vrublevskaya, S. L. Varshavskii, L. P. Kofman and K. N. Vikhanskii) (B.P. 1,087,280, 27.10.65).—The title compounds have fungicidal activity and are prepared by interaction of $CH_2:CH \cdot SO_3R$ (I) (R is aryl), with thiourea (II) and an acid or with a II salt. E.g., a solution saturated with I (R=Ph) in BuOH is mixed with a saturated solution of II-hydrochloride in BuOH at 60–80°, then after 1 h at 80–90° the mixture is cooled. Ppt. (85–90%) comprises Ph isothiuroniumethanesulphonate hydrochloride, m.p. 165–166°. The activity of some of the many compounds described against *Alternaria solani* and *Botrytis cinerea* is tabulated. F. R. BASFORD.

***N*-Cyanoalkyl haloacetamides.** Nippon Soda K.K. (B.P. 1,090,608, 18.7.66. Jap., 21.7.65).—The claimed fungicides have the formula $X \cdot CH_2CO \cdot NH \cdot YCN$, where X is Cl or Br and Y is C_1–C_5 alkylene (branched or unbranched), particularly $\text{-}CHR \cdot CH_2\text{-}$ where R is H or C_1–C_3 alkyl. E.g. $ClCH_2CO \cdot NHCH_2CH_2CN$, m.p. 95–96·5° [dichloropropane (I)] is obtained by reacting $NH_2CH_2CH_2CN$ with $ClCH_2COCl$ in I at 15–25°, boiling under reflux for 1·5 h and working up the mixture. The products are active against phytopathogenic fungi such as *Pseudoperonospora cubens*. S. D. HUGGINS.

Thiocarbamylmorpholine derivatives. Shell Internationale Research Mij. N.V. (Inventor: F. D'Angeli) (B.P. 1,091,578, 1.11.65). —Compounds of the formula $R \cdot CS \cdot NH[CH_2]_nX$ have fungicidal properties (R is morpholino; X is NCS or NHCSR; *n* is 1–6). In an example, a solution of $[CH_2]_4(NCS)_2$ in acetone is added dropwise to a solution of RH in acetone, then ppt. is filtered off. The filtrate is diluted with ether, more ppt. is removed, and the liquor is evaporated. The residue is dissolved in ether, and the washed extract is freed from solvent, leaving oily 4-[(4-isothiocyanatobutyl)-thiocarbamyl]morpholine (40% yield), active against *Phytophthora infestans*. F. R. BASFORD.

***N*-(1-Halo-1-nitroalkylthio)-dicarboximides.** Chevron Research Co. (Inventors: G. K. Kohn and J. G. E. Fenyes) (B.P. 1,094,250, 7.9.66. U.S., 7.9.65).—Used as fungicides, the title compounds contain the dicarboximide group: $(CO)_2NSR$, attached [in the 1,2-position for case (*i*)] to one of the groups: (*i*) C_6 ring containing 0–3 double bonds, (*ii*) $R^ICNH\text{-}$, (*iii*) $R^{II}C:CHNSR$ or (*iv*) $C(O)NSR$

(where R^I and R^{II} are H or 1–6 C alkyl, R is a 1-halo-1-nitroalkyl group, the halogen being Cl or Br and the alkyl portion containing 2–6 C atoms). The compounds are obtained by reacting the corresponding imide salt with a 1-halo-1-nitroalkylsulphenyl halide. E.g. K phthalimide in ether is reacted with 1-chloro-1-nitroethyl-sulphenyl chloride for 15 min.; after washing and drying, the product *N*-(1-chloro-1-nitroethylthio)-phthalimide has a m.p. of 110–112°. S. D. HUGGINS.

***N*-(Substituted allyl)rhodanines.** Shell Internationale Research Mij N.V. (B.P. 1,096,580, 17.8.65.U.S., 19.8.64).—The claimed compounds (I) have a 2-substituted allyl moiety bonded by the N, e.g., 3-(2-chloroallyl)rhodanine. They are prepared by adding $CH_2:CRCH_2Cl$, wherein R is CN, halogen, halogen-substituted C_1–C_4 alkyl, NO_2 or dialkylamino of 2–8 C, to rhodanine (II). Alternatively, the corresponding isothiocyanate $H_2C:CR \cdot CH_2 \cdot NCS$ is reacted with a mercaptoacetic acid or ester. I, e.g., 3-(2-cyano-allyl)rhodanine (m.p. 60–62°), prepared by reacting II in MeCN with 2-(chloromethyl)acrylonitrile at 5–20° in presence of Et_3N,

are useful as bacteriostats and as fungicides against plant pathogens (*Rhizoctonia solani*, etc.). S. D. HUGGINS.

***N*-Substituted amino derivatives of cycloheptatriene and their conversion to dihydrotropone.** Shell Internationale Research Mij. N.V. (B.P. 1,088,016, 9.3.66. Neth., 11.3.65) (60 claims).—A 7-NRR^1-cyclohepta-1,3,5-triene is claimed (R and R^1 are org. radicals or R^1 is H, or NRR^1 is heterocyclyl- but NRR^1 is not NHMe) and prepared by adding an amine to a solution of $\leqslant$ 1 tropylium salt (of B.P. 887,693 or 961,910) or a slurry of such a solution and a further undissolved portion of said salt (excluding the case when $NHMe_2$ is added to aq. tropylium bromide). The product is isomerised by heating at $\leqslant$ 80° to a 1-NRR^1-analogue which is converted into a 2-7-dihydrotropone by hydrolysis. Some of the products have herbicidal activity. E.g., a solution of $NHMe_2$ in water is mixed quickly with water containing tropylium tetrafluoroborate at room temp. The mixture is extracted with ether and the extract is distilled to give an 84% yield of 7-dimethylaminocycloheptatriene, b.p. 64–64·5°/8 mm, n_D^{20} 1·5233. This is heated at 140° in N_2 during 85 min., then distillation affords 1-dimethylaminocycloheptatriene, b.p. 88–90°/10 mm, n_D^{30} 1·593, in 76% yield. The latter is converted into 2,7-dihydrotropone, b.p. 53–55°/8·5 mm in 80% yield by heating it in dil. HCl at 60° during 5 min. F. R. BASFORD.

Substituted acylanilides and their use as herbicidal compounds. Monsanto Co. (B.P. 1,088,397, 8.12.64. U.S., 9.12.63. Addn. to B.P. 1,008,851, 10.11.61).—Compounds of the formula 2,6,1-$C_6H_3R^IR^{II}\cdot N(COR^{III})CHR^{IV}A\cdot R^V$ are claimed in which the benzene nucleus may contain a further 1–3 substituents selected from halogen, alkyl, or alkoxy and R^I is t-alkyl; R^{II} is alkyl or alkoxy, A is O or S; R^{III}–R^V are optionally halogenated hydrocarbon or R^{IV} and R^V are H or R^V is furfuryl, hydrocarbon radical containing OH, or oxa-alkyl, when A is O, or is furfuryl, hydrocarbon radical optionally containing halogen or is oxa-alkyl when A is S. In an example, a solution of $C_{16}H_{33}NMe_3Br$ in ice-water is mixed with 2,6,1-$C_6H_3EtBu^t\cdot N(CH_2Cl)CO\cdot CH_2Cl$ and stirred during 19·5 h, temp. rising to 18° within 2·5 h, then the sticky product is dissolved in benzene. The org. layer is washed with water, evaporated, and a solution of the residue in hexane is chilled, to give a ppt. of *N*-hydroxymethyl chloroacet-2-ethyl-6-t-butylanilide, m.p. 67–70°. Its herbicidal effect on 19 different plants is described. F. R. BASFORD.

Dichlorothiobenzoylhydrazines and their use as herbicides. Shell Internationale Research Mij. N.V. (Inventors: J. Yates and E. Haddock) (B.P. 1,090,146, 17.6.65).—The herbicides have the formula 2,6,1-$C_6H_3Cl_2\cdot C(SH){:}N\cdot NRR^1$ wherein R and R^1 are optionally substituted aliphatic, cycloaliphatic or aromatic groups or can form a heterocyclic ring. In an example, a mixture of 2,6,1-$C_6H_3Cl_2\cdot COCl$, NMe_2NH_2, and ether is stirred during 7 h, then pptd, 1-(2,6-dichlorobenzoyl)-2,2-dimethylhydrazine, m.p. 190–192° is collected washed with hot water and ether, and boiled with P_2S_5 in toluene overnight. The decanted liquor is concentrated, and solid is boiled with light petroleum, to give 1-(2,6-dichlorothiobenzoyl)-2,2-dimethylhydrazine, m.p. 166–168°. F. R. BASFORD.

Carbamidoximes. Farbenfabriken Bayer A.-G. (Inventors: K. Dickore, K. Sasse, L. Eue and H. Hack) (B.P. 1,090,357, 12.10.66. Ger., 28.10.65).—Herbicidal compounds of the formula 4,3,5,1-$OR^1\cdot C_6H_2X_2\cdot CH{:}N\cdot O\cdot CONHR^{II}$ are claimed wherein X is Cl, Br, or I; R^1 is H, COR, or CONHR; and R and R^{II} are C_{1-4}-alkyl or aryl which may contain halogen, NO_2, and/or C_{1-4}-alkyl or -alkoxy. An example is *O*-(methylcarbamyl)-3,5-dichloro-4-hydroxybenzaldoxime, m.p. 120–122° (EtOAc), made by adding MeNCO at 20° to a suspension of 4,3,5,1-$OH\cdot C_6H_2Cl_2\cdot CH{:}NOH$ in CH_2Cl_2, then working up after 24 h. F. R. BASFORD.

Herbicidal compositions. Rhône-Poulenc S.A. (Inventors: J. Desmoras and P. Jacquet) (B.P. 1,090,950, 15.4.65. Fr., 20.4.64).—The composition contains $\leqslant$ 1 pyridazine deriv., containing O attached to the 1-position; the 3-position is substituted by alkoxy of 1–4 C, the 4-position is substituted by NO_2 and the 6-position by alkyl of 1–4 C. The diluent used is a mineral, animal or vegetable oil or a liquid containing a wetting, dispersing or emulsifying agent or is a solid; the active ingredient constitutes 0·005–50% by wt. of the composition, used to limit weed growth. The deriv., e.g. 3-methoxy-4-nitro-6-methylpyridazine-1-oxide, are prepared by nitration of the corresponding substituted pyridazine oxides. S. D. HUGGINS.

3,5-Dialkylhydantoins. U.S. Borax & Chemical Corp. (B.P. 1,092,962, 24.11.65. U.S., 16.12.64).—Used in herbicidal compositions, the title hydantoins (I) are substituted in the 3-position by alkyl of 3–6 C and in the 5-position by alkyl of 1–4 C, and are applied to the foliage of weeds at a rate of 4–25 lb/acre. The I, e.g., 3-isopentyl-5-methyl hydantoin, are obtained by converting an amino-acid ester to the isocyanate deriv., which is then reacted with a C_3–C_6 alkylamine to give the hydantoate; the latter is cyclised with dil. acid to give I. S. D. HUGGINS.

Herbicides. Badische Anilin- und Soda-Fabrik A.-G. (Inventors: A. Fischer, M. Seefelder and H. Armbrust) (B.P. 1,094,605, 1.4.65. Ger., 2.4.64).—The active ingredients have formula X^1(X^2)-$C_6H_3[NRCOCH{:}NOH]_m$, wherein X^1 and X^2 are H, Cl, NO_2, carboxy, COMe, Ac or Me, R is H or Me and m=1 or 2, and are used in conjunction with a solid or liquid carrying agent. A typical compound is isonitrosoaceto-*p*-toluidide. S. D. HUGGINS.

Indan compounds. L. Givaudan & Cie S.A. (B.P. 1,095,718, 7.9.65. U.S., 28.9.64) (24 pp.).—Acylindans useful as preselective herbicides (and in some cases musk odorants) are claimed, optionally substituted in the 1-, 2-, and/or 3-position by $\geqslant$ 5 alkyl of 1–3 C and further substituted in at least one of the 4-, 5-, 6-, or 7-positions by acyl group (of a fatty or cycloalkanoic acid) and in $\leqslant$ 2 of the remaining positions by acyl or alkyl of 1–6 C (but if positions 1 and 3 have quaternary C atoms then there is no acyl in position 4 or 7). They are prepared by a process of acylation. E.g., a solution of 1,1,4,6,7-pentamethylindan (I) in CCl_4 is added dropwise at 0° to a solution of $AlCl_3$ and AcCl in CCl_4 during 2·5 h while keeping at 1–3°. After a further 2·5 h the mixture is poured into ice/water, then the org. layer is washed and worked up, to give 5-acetyl-1,1,4,6,7-pentamethylindan, m.p. 64–65° (EtOH). The prep. of I, m.p. 75–76°, from pseudocumene and isoprene is described. F. R. BASFORD.

Herbicidal mixtures. Badische Anilin- und Soda-Fabrik A.-G. (Inventors: G. Scheuerer, A. Zeidler and A. Fischer) (B.P. 1,099,553, 15.4.65. Ger., 20.4.64 and 31.3.65).—The claimed 2,4-dioxotetrahydroquinazolines, e.g. 1-acetyl- and 1-methyl-**3-isopropyl-2,4-dioxotetrahydroquinazoline** are substituted in the 1-position by R^1[R^1 is H or -(CO)$_n$·A, where n=0 or 1 and A is a linear or branched alkyl, alkenyl or cycloalkyl which may be substituted by Cl, Me or PhO, or Ph optionally substituted by Cl, Br or NO_2] and in the 3-position by R (R is a linear or branched alkyl or alkenyl of 1–4 C, which may be substituted with Cl, OH or alkoxy). Numerous methods of prep. are described. They are contained, for application to wheat, barley, etc., in a conventional solid or liquid extender, solvent, emulsifying agent or dispersing agent. S. D. HUGGINS.

Animal Husbandry

Mixed cropping of berseem with oats and barley for fodder production. M. M. Hasan (*W. Pakistan J. agric. Res.*, 1967, **5**, 95–101).—Sowings of berseem at 16 lb/acre with oats at 40 lb/acre gave fodder yields 12·5% over those obtained from berseem alone at 16 lb/acre. Similar mixed sowings with barley gave increases of 4·5%. The mixed croppings ensured supplies of green fodder during Jan. and Feb. (period of fodder scarcity), not attainable with individual crops alone. P. S. ARUP.

Quality and storage losses of silages made in bunkers, stacks, and by vacuum compression. R. J. Lancaster (*N.Z. Jl agric. Res.*, 1968, **11**, 63–70).—High-moisture grass was ensiled in three 30-ton capacity silos: (1) bunkers covered with polyethylene film (I), (2) by vac. compression within I and (3) stacks covered with I. Mean dry matter recoveries of edible silage were: vac. 86, bunkers 80 and stacks 77%. With dry matter containing about 20% of sugar, fermentation characteristics of the silages were little affected by storage methods, but as levels decreased to 10%, these effects increased substantially, indicating greater stability of the vac. silage. (18 references.) E. G. BRICKELL.

Microbiology of grass silage. R. Whittenbury (*Process Biochem.*, 1968, **3**, No. 2; 27–31).—Incidence of lactic acid bacteria (*LAB*) on growing silage crops is compared with those found in the silage pit; sol. carbohydrates present in *Lolium multiflorum* are shown with special reference to the fructose: glucose ratio and the main products of carbohydrate fermentation by *LAB* are tabulated. Examples are given of clostridial fermentation. C.V.

Comparison of rumen microbial inhibition resulting from various essential oils isolated from relatively unpalatable plant species. Hi Kon Oh, M. B. Jones and W. M. Longhurst (*Appl. Microbiol.*, 1968, **16**, 39–44).—Essential oils were isolated from eight plant species which were relatively unpalatable to sheep and deer. The

inhibitory potency was compared in terms of total gas (*TG*) and volatile fatty acid (*VFA*) production. The inhibitory effect can be divided into four groups: (1) Essential oils from vinegar weed and California bay inhibited rumen microbial activity most; (2) lesser inhibition was produced by rosemary and Californian mugwort oils, (3) blue-gum eucalyptus and sagebrush oils were next in order and (4) Douglas fir and Jerusalem oak produced least inhibition, 0·3 ml of each oil being used. A highly significant correlation coeff. was found between *TG* and *VFA* indicating the validity of either method to measure the activity of rumen micro-organisms. The selectivity and voluntary consumption of ruminants is discussed in relation to the characteristic odour and anti-bacterial action of these oils. C.V.

[A] **Use of guar meal in poultry ration.** [B] **Nature of chick-growth inhibiting factor in guar meal.** M. Y. Malik and A. A. Sheik (*W. Pakistan J. agric. Res.*, 1967, **5**, 107–115, 116–124).—[A] The meal from *Cyamopsis psoraloides* ('guana') was shown to contain a chick-growth inhibiting factor that was only partly inactivated by toasting or by autoclaving with water. The use of the meal in place of sesame cake also caused pancreas hypertrophy. (10 references.)

[B] Treatment of the meal with N-HCl followed by autoclaving partly destroyed the growth-inhibiting factor. Additions of 2–3% of the gum obtained from guar meal, or 4% of guar-splits to ordinary rations produced effects similar to those obtained with additions of 15% of the raw meal. (14 references.) P. S. ARUP.

Metabolisable energy of field beans (*Vicia faba* L.) for poultry. K. J. Carpenter and C. L. Johnson (*J. agric. Sci. Camb.*, 1968, **70**, 391–392).—Metabolisable energy, determined in feeding trials with chicks is 2·69 kcal/g at 87% moisture content. This value is similar to that predicted from analytical data. M. LONG.

Nutritional evaluation of meat meals for poultry. VI. Association of growth promotion, protein digestibility and hot-water soluble protein content, with effect of amino-acid supplementation. B. S. Sathe and G. L. McClymont (*Aust. J. agric. Res.*, 1968, **19**, 171–179).—In rations based on wheat, skim milk, and meat meals, variations in protein digestibility and hot-water sol. protein were not correlated with chick-growth promotion. Meals of both high and low protein quality gave improved growth rates with additions of amino-acids, especially lysine and methionine, but a second limiting factor is suspected which may be one or more of vitamins, minerals and antibiotic. (24 references.) P. S. ARUP.

Intensive beef production from molasses and urea. T. R. Preston, A. Elias, M. B. Willis and T. M. Sutherland (*Nature, Lond.*, 1967, **216**, 721–722).—The apparent incompatibility of feeding molasses and forage together was resolved by decreasing the forage to the min. level consistent with normal rumen function. Results of such a diet (plus minerals and vitamins) fed to 48 bulls having free access to molasses containing 0·67% urea show that, after 24 weeks, wt. gains, feed conversion, killing-out %, and NH_3 concn. in rumen closely resemble those for cattle feeding on grain, whilst carcass composition, rumen pH and flora/fauna simulate those of animals fed much forage. Of the N intake, only ~11% came from true protein, with ~59% from urea and $(NH_4)_2SO_4$, 20% from molasses and 11% from forage. Despite high energy-intake, the concn. of volatile fatty acids in the rumen was very low. The new system is being tried out on 300 bulls. W. J. BAKER.

Finishing beef cattle. Anon. (*Fmrs' Bull., U.S. Dep. Agric.*, 1968, No. 2196; 30 pp.).—Feeding systems, kinds of cattle to feed, roughages and pasture, finishing feeds, supplements (protein, minerals, vitamins) additives and hormonal implants and balancing rations are described. E. G. BRICKELL.

Plane of nutrition effects in late pregnancy and during lactation on beef cows and their calves to weaning. G. K. Hight (*N.Z. Jl agric. Res.*, 1968, **11**, 71–84).—High plane pre-calving cows gained 58 lb in live wt. (*LW*), and low plane pre-calving groups lost 80 lb between their initial *LW* and last precalving *LW*. After calving a difference of 131 lb in *LW* between high and low pre-calving treatments was produced. Low plane calves were 13 lb or 22% lighter than high plane calves at birth. The effects of the plane of nutrition on cow and calf deaths, cow *LW* and subsequent fertility and calving date and *LW* are discussed. (41 references.)
E. G. BRICKELL.

Effects of drenching cows with oxidised oils on fat composition and meat quality. L. Hartman, F. B. Shorland, Z. Czochanska, P. Woodhams and A. H. Kirton (*N.Z. Jl Sci.*, 1968, **11**, 122–130).—Drenching cows daily for 21–28 days before slaughter with partly oxidised tallow, peanut oil or paraffin oil under conditions similar to those for bloat control affected neither the animals' health nor the flavour nor quality of the beef before or after refrigeration. Peroxide values of kidney and meat fats of treated and control animals increased during storage at −15° for 6–8 months. (17 references.) W. J. BAKER.

Absorption of volatile fatty acids from the reticulo-rumen and abomasum of sheep. V. J. Williams, T. R. Hutchings and K. A. Archer (*Aust. J. biol. Sci.*, 1968, **21**, 89–96).—Under similar initial conditions of tonicity, pH, and volatile acid concn., total fatty acid was absorbed at approx. equal rates from both the reticulo-rumen and the abomasum, and in the latter the absorption of individual fatty acids varied directly with the no. of C atoms in the molecule. NH_3 in acid solution was not absorbed from either organ. (15 references.) E. G. BRICKELL.

Volatile fatty acids in the caecum of sheep. G. J. Faichney (*Aust. J. biol. Sci.*, 1968, **21**, 177–180).—The concn. of HOAc was higher in the caecum than in the rumen, and propionic and butyric acid concn. were lower. The proportions of higher fatty acids did not differ greatly for the two organs. Levels of NH_3-, residual- and protein-N were higher in the caecum. Changes in acid and N levels after time of feeding are plotted, and the curves are discussed. (14 references.) E. G. BRICKELL.

Protozoan, bacterial and volatile fatty acid changes associated with feeding tylosin. N. Satapathy and D. B. Purser (*Appl. Microbiol.*, 1967, **15**, 1417–1421).—Tylosin (**I**) was fed to two of six wethers for 79 days; to a second two for 28 days. The addition of **I** to the daily feed resulted in a two-fold rapid increase in protozoal concn. and change in composition, or characteristics, or both, of the bacterial population. Total acid concn. were initially depressed but appeared to be greater than those of the controls at the termination of the experiment. Deletion of **I** from the ration resulted in a rapid fall in the protozoal concn. but changes in the bacterial population did not occur for a further 30 days. (15 references.)
C.V.

Activity of enzymes in the endometrium, caruncles, and uterine rinsings of progestogen-treated and naturally cycling ewes. R. N. Murdoch and I. G. White (*Aust. J. biol. Sci.*, 1968, **21**, 123–131).—Of progestogen-treated ewes 86% came into oestrus 2 or 3 days after removal of the hormone and the uterus contained higher levels of amylase (**I**) and alkaline phosphatase (**II**) than did those of naturally cycling ewes. **I**, succinate dehydrogenase (**III**), glutamate–oxaloacetate transaminase (**IV**) and acid and alkaline phosphatase activities were maximal during the luteal phase, and glycerylphosphorylcholine diesterase (**V**) activity also increased but did not reach a max. until just prior to oestrus. Lactate dehydrogenase (**VI**) and glucose-6-phosphate dehydrogenase activity remained unchanged throughout the cycle. Levels of **I, III, IV** and protein were greater in the caruncles than in the endometrium, whereas the reverse was true for **II** and **VI**. Enzyme activity of uterine rinsings was very variable, though the activity of **II** and **V** changed significantly during the cycle. (41 references.)
E. G. BRICKELL.

Effect of feeding dried beet pulp on growth rate of Awassi sheep. I. I. Al-Azzawi, K. E. Ghoneim, H. El-Haidary and N. T. Kazzal (*J. agric. Sci. Camb.*, 1968., **70**, 109–110).—Lambs aged 7–13 months were fed a control ration containing ground barley or one of three others based on varied proportions of dried sugar-beet pulp but without barley. Male lambs were initially heavier and gained in wt. more rapidly than did females. Lambs having rations containing sugar-beet pulp grew better than did those given the control ration. The most economical rates of gain in wt. resulted from the inclusion of 40% beet pulp in the concentrate part of the ration. A. G. POLLARD.

[A] **Determination of selenium in biological materials.** [B] **Retention of selenium by growing lambs.** R. C. Ewan, C. A. Baumann and A. L. Pope (*J. agric. Fd Chem.*, 1968, **16**, 212–215, 216–219).—[A] Sub-μg quantities of Se were determined using a fluorimetric method which combined the wet digestion of org. samples, co-pptn. of Se with As and measurement of fluorescence with 2,3-diaminonaphthalene. The recovery of org.^{75}Se was 95%, and, when selenite was added to plants, 103%. Concn. of Se down to 0·01 ppm could be rapidly determined, with results in good agreement with those obtained by other methods. (13 references.)

[B] Most of the Se administered orally to lambs deficient in vitamin E and/or Se as ^{75}Se-labelled selenate was found in the urine during the first week, but appreciable amounts also appeared in the faeces at first, and smaller amounts persisted during the experimental period. Supplementation of the deficient diet with α-tocopherol appeared to increase the excretion of Se during the first few days. More Se was retained in the liver than other tissues.

The results suggest that the appearance of nutritional muscular dystrophy may depend on the levels of both tocopherol and of Se. (20 references.) P. S. ARUP.

Purpose-grown wools. I. Modification of the felting properties of wool through the sheep's feed. A. Johnson (*J. Text. Inst.*, 1968, **59**, 243–252).—The shrinking property of wool can be modified during growth by the administration of chemical additives to the sheep's diet, so as to alter the character of the fibre half-way through the growth of the staple. Addition of Na_2MoO_4 to the lucerne–chaff feed during the first half of the growth of the staple, followed by addition of Na_2SO_4 for the second half gave strong root–weak tip fibres which enhanced shrinkage, and reversal of the order of administration of the additives gave weak root–strong tip fibres which imparted shrink resistance. Shrinkage control of the wool has hitherto been confined to chemical treatments of the shorn fibre. The method described presents an opportunity for the mass production of a large variety of other special-purpose wools. W. H. KEMP.

Ubiquinone, vitamin A and sterol levels in liver of neonatal pigs. W. E. J. Phillips and R. L. Brien (*Can. J. Biochem.*, 1968, **46**, 291–293).—The concn. of ubiquinone in the liver fat at birth was approx. double that found in mature pigs, and after a small increase during two weeks, it gradually declined to adult levels. Liver vitamin A increased rapidly during the first two weeks and then more slowly. The concn. of sterols in the liver fat at birth was similar to that found in mature pigs. (17 references.) P. S. ARUP.

High level cereal diets for the growing/finishing pig. III. Comparison with a control diet, of diets containing high levels of maize, flaked maize, wheat and barley. T. L. J. Lawrence (*J. agric. Sci., Camb.*, 1968, **70**, 287–297).—Six diets were fed to Large White type weaner pigs individually and restrictively. Five of the diets contained 85% of either maize, flaked maize, sorghum, wheat or barley in the starter diets and 90% of these grains in the finisher. The sixth diet was similar to the barley diet except that 25% weatings replaced that amount of barley. Maize contained the highest digestible energy in the starter diets, whilst wheat and sorghum had the highest in the finisher. Flaked maize was superior to barley and control, but lower than the other three. Some of the pigs on the sorghum diet developed leg weakness, although pantothenic acid could not be shown to be deficient. Growth rates with all six starter diets were similar, but maize and barley were superior to wheat and sorghum with regard to food conversion. Maize and wheat were superior to all others in the finisher diets. Overall, maize was superior to flaked maize, sorghum and control rations. Carcass lengths did not differ, but the control and barley rations produced most lean. The control ration produced the highest % of leg and shoulder in the carcass and the highest % of lean in the middle. M. LONG.

The nutrition of the female mink (*Mustela vison*). I. The metabolic rate of the mink. II. The energy requirement for maintenance. III. The water requirement for maintenance. D. J. Farrell and A. J. Wood (*Can. J. Zool.*, 1968, **46**, 41–45, 47–52, 53–56).—I. The resting heat production of two female mink was minimal at 25 ± 2°. Basal heat production (76·5 kcal/kg$W^{0.73}$, where W = body wt.) of sleeping females was within the range predicted from other work. (14 references)

II. Energy intake rate is described by the equation, intake = 0·226 wt. + 14, under conditions of body wt. stasis. This is higher than predicted from work with other species, and cage size influenced the energy requirement. (35 references.)

III. Under non-stress conditions, the females consumed 2·8 g total water/g of dry feed or 0·63 g/kcal of apparent digestible energy intake, which is in reasonable agreement with values reported for other species. (16 references.) E. G. BRICKELL.

Fertility in rabbits inseminated with diluted or washed spermatozoa. R. G. Wales and T. O'Shea (*Aust. J. biol. Sci.*, 1968, **21**, 181–183).—Sperm no. rather than dilution of seminal plasma and the associated leaching of intracellular constituents is the important factor in regulating fertility. E. G. BRICKELL.

Effect of egg separation on hatching time, and the source of clicking sounds in the embryo of the domestic chicken. J. A. McCoshen and R. P. Thompson (*Can. J. Zool.*, 1968, **46**, 243–248).—Synchronised hatching was studied using White Leghorn and Rhode Island Red embryos and chicks. There was no difference in the mean hatch times of separated vs contact eggs, though the former were more variable in their hatch times. The source of clicking was associated primarily with the right abdominal air sac and possibly with the right posterior thoracic air sac of the respiratory system. (13 references.) E. G. BRICKELL.

Progress in Canadian oyster hatchery development. R. E. Drinnan and J. P. Parkinson (*Stud. Fish. Res. Bd Can.*, 1967, Pt 1, 309–322).—A review of developments at the Experimental Oyster Hatchery at Ellerslie, Prince Edward Island. E. G. BRICKELL.

Transferable drug resistance. E. S. Anderson (*Science J.*, 1968, **4**, No. 4, 71–76).—Apart from the normal aspects of drug resistance, this special form is discussed. Resistance may be to a single drug, or to several. The mechanism of this resistance transfer factor is discussed; some 26 different patterns were found with phage type 29 of *Salmonella typhimurium* showing drug resistance to ampicillin (*A*), chloramphenicol (*C*), kanamycin (*K*) neomycin (*N*), streptomycin (*S*), sulphonamides (*Su*), tetracyclines (*T*), and furazolidone (*F*), the commonest resistance spectra being *SSuTF*, *ASSuTF*, *KNSSuTF*. The steep rise of bovine, mainly calf, infections of the type discussed, and the cause, is discussed and is examined and the wisdom of prophylactic and preventive therapy is questioned and compared. C.V.

A nephrotoxin from *Aspergillus fumigatus* and its possible relationship with New Zealand mucosal disease-like syndrome in cattle. R. H. Thornton, G. Shirley and R. M. Salisbury (*N.Z. Jl agric. Res.*, 1968, **11**, 1–14).—Extracts of mycelium of *Aspergillus fumigatus* injected intravenously into mice and sheep caused severe cortical tubulo-necrosis of the kidneys, a lesion similar to that in cattle described as a mucosal disease-like syndrome. A nephrotoxin of fungal origin appears to be the likely cause. (11 references.) E. G. BRICKELL.

Use of 'Pluronics' administered in the drinking water as a means of bloat control in cattle. D. S. M. Phillips (*N.Z. Jl agric. Res.*, 1968, **11**, 85–100).—'Pluronic L64' (an antifoaming, nonionic surfactant based on polyoxypropylene–polyoxyethylene block polymers) administered in the drinking water of 25 cows grazing on red clover over 9 days, was highly effective as a preventative for bloat, which was closely related to the 'Pluronic' intake during the preceding 12 h. A level of 0·2 oz per cow in 12 h was necessary, on average, to eliminate serious bloat. (13 references.) E. G. BRICKELL.

Liver flukes in cattle. Anon. (*Leafl. U.S. Dep. Agric.*, 1968, No. 493, 8 pp.).—Life cycle, symptoms, diagnosis, treatment, and control are described. E. G. BRICKELL.

Retention of insecticides in beet-top silage. G. Wildbrett, A. Djarrahbachi-Razawi and F. Kiermeier (*Milchwissenschaft*, 1968, **23**, 213–216).—Retention of DDT (**I**) and parathion (**II**) was studied, the leaves being sprayed and then ensiled one day later, in a special 9·5 l container. In the first 25 days the intense microbial activity fell and the concn. of insecticide was sharply reduced; *p*-nitrophenol was formed from **II** and from the findings it was assumed that enzymic action is primarily responsible for this breakdown. With **I** this is entirely attributable to enzymes. After 150 days, the silage still contained 0·6–1·6 mg **II** per kg, **I** being reduced by 55% during the same period. The concn. of **I** and **II** applied, considerably higher than that expected as residues in practice, had no effect on acid development or quality of this silage. (17 references.) C.V.

Micro-organism cultivation. Esso Research & Engng Co. (B.P. 1,090,093, 23.3.65. U.S., 6.5.64).—Yields of 70–80% protein food supplement for animals (and/or humans) is obtained by inoculating, with *Micrococcus cerificans*, water and O_2, a feed mixture of C_1–C_{35} n-hydrocarbons (containing < 0·1% by wt. aromatics), water, a N compound, a P compound and water-sol. inorg. salts of Na, K, Mg, Ca, Fe and Mn. The upper froth zone of the biosynthesis bath has a wt. ratio of froth micro-organism cells to froth hydrocarbons of > 1·0 to 1 and the lower slurry zone is at pH 6–8 and 20–55°. A table of amino-acid composition of the product is given. S. D. HUGGINS.

Animal feedstuff. Pfeifer and Langen (Inventors: C. Heller and P. Reiners) (B.P. 1,091, 264, 16.9.66).—A protein-forming feed for ruminants is obtained from compressed diffusion cossettes of sugar-beet, having a dry substance content of 13–25% (17%) at 60–70°, by spraying them with an aq. solution of urea, a phosphate and molasses, at 70–80°, and drying at ~100°. Preferably the solution consists of (*i*) urea, (*ii*) a phosphate solution produced by heating together $Na_2HPO_4 \cdot 12H_2O$ (2) and $NaH_2PO_4 \cdot 2H_2O$ (1 pt.) and (*iii*) molasses having a dry substance content of 75–85%, the contents of (*i*), (*ii*) and (*iii*) being e.g. 21·5%, 17·5–16% and 61–55% respectively, and the pH being ~7. Yeast and vitamins can also be added. S. D. HUGGINS.

Silage. Albright and Wilson (Ireland) Ltd. (B.P. 1,091,681, 4.1.66. Ire., 8.3. and 14.5.65).—The grass or green crop is treated with peat containing (*i*) an acid e.g. H_3PO_4, sufficient to maintain a pH value of 3·0–6·5 in the crop during anaerobic fermentation, and optionally (*ii*) molasses. By adding the acid to the pulverised peat (which should not undergo appreciable carbonisation or hydrolysis during treatment) and not directly to the crop, even distribution is readily achieved. S. D. HUGGINS.

Proteinous animal food. A/S Christiania Portland Cementfabrik (Inventor: L. Jantzen) (B.P. 1,092,628, 26.11.64).—Lignoprotein (**I**) for use in admixture with conventional animal food diluents (88%), is pptd. from proteinous waste water by treatment with nearly pure high mol. wt. lignosulphonic acids or torula yeast-fermented sulphite waste liquor; the lignosulphonic acids used are prepared by treating sulphite waste liquor with tanned leather and recovering the acids by adding NH_4OH. The **I** obtained by this method is easy to filter and process, and contains ~ 60% of digestible protein. S. D. HUGGINS.

Ruminant nutrient compositions. Pfizer Ltd. (Inventors: L. A. Davey and R. J. Boscott) (B.P. 1,093,907, 25.11.65).—The composition consists of a propionyl-substituted urea, particularly mono-propionyl urea, hydrolysable by rumen flora to propionic acid, and a non-toxic solid carrier containing cereal, mineral elements and vitamins. The product is suitable for treating or preventing metabolic disorders due to low blood glucose levels, e.g. pregnancy toxaemia in sheep. S. D. HUGGINS.

Nitrogenous foods. Institut National de la Recherche Agronomique and Produits Chimiques et Celluloses Rey (B.P. 1,099,583, 3.2.66. Fr., 3.2. and 8.2.65).—Of vegetable, animal or synthetic origin, the animal feeds are preserved against microbial deamination by reacting the nitrogenous feed in a moist medium at $\geq 70°$ with from 3–40% tanning agent(s) by wt., until no sol. N can be detected. The product is then dried $\geq 80°$. E.g. chestnut tannin powder is used in aq. solution at pH 8–9 and the reaction mixture is allowed to stand for 2–16 h at < 22°. S. D. HUGGINS.

2-(Chloro-2′-furyl)benzimidazoles. Merck & Co., Inc. (B.P. 1,087,345, 1.12.64. U.S., 31.12.63).—The title compounds, useful as anthelmintic agents (in domestic animals and humans) are prepared by a variety of exemplified methods and comprise 2-(mono- and dichlorofur-2-yl)benzimidazoles substituted in the 5- or 6-position by OPh, SPh, halogen, Ph, halogenophenyl, or C_{1-5}-alkyl, -alkoxy, or -alkylthio, and optionally in the 1-position by alkyl of 1–5 C, aralkyl, or acyl. E.g., a solution of 3,4-dichlorofuroyl chloride in benzene is added to a solution of *o*-$NH_2C_6H_4$-NO_2 in benzene–pyridine, then chloroform is added until clear. The solution is washed with water and dil. HCl, then solvent is removed, to leave *N*-(3,4-chlorofuroyl)-*o*-nitroaniline, m.p. 188–189°. A suspension of this in AcOH, and dil. HCl is treated with Zn dust, and after 1 h at 95–100° the mixture is worked up, to give 2-(4-chloro-2-furyl)benzimidazole, m.p. 253–255°.
F. R. BASFORD.

Benzimidazole, benzoxazole and benzthiazole derivatives. Merck & Co. Inc. (B.P. 1,088,096, 4.11.64. U.S., 6.11. and 23.12.63).—There are claimed 2-R-5-R^I-6-R^{II}-benzox(thi- and imid-)azoles, the last being optionally substituted in the 1-position by alkyl of 1–5 C, aralkyl, alkanoyl, aroyl or aralkanoyl, also methods of prep., and the use thereof as anthelmintic agents (especially active against swine ascarids), wherein R is pyrrolyl, furyl, thienyl, thiazolyl, isothiazolyl, or thiadiazolyl; R^I is H and R^{II} is $C_{10}H_7$ or Ph which may contain halogen, R^{III} (alkyl of 1–5 C), OR^{III}, SR^{III}, NHR^{III}, or NR^{III}_2, or *vice versa*. In an example, a mixture of thiazole-2-carbonyl chloride, 1,4,2-NH_2·C_6H_3Ph·NO_2, and pyridine is stirred at room temp. during 30 min. and at 75° during 1 h, then poured on to ice. Ppt. is collected, washed, and recrystallised from EtOAc, to give thiazole-2-carb-*o*-nitro-*p*-phenylanilide. This is reduced at 50° with H_2 in presence of EtOH and 5% Pd-on-C, then the filtered solution is treated with aq. HCl, with pptn of 2-thiazol-2-yl)-5(6)-phenylbenzimidazole.
F. R. BASFORD.

Substituted benzthiazolium compounds and complexes thereof for use in animal husbandry. Dow Chemical Co. (Inventor: R. L. Klingbail) (B.P. 1,097,053, 9.3.65. U.S., 16.3.64).—Parasites which dwell for at least part of their life cycle in host animals (especially domestic animals and poultry), e.g., *Nematodirus*, *Ascaris*, *Haemonchus*, *Chabertia*, *Trichostrongylus*, *Strongyloides*, are controlled by applying to the host or to the parasites in the phase of their life cycle outside the host, a complex of a 2-R-3-R^1-benzthiazolium halide and urea, thiourea, guanidine or its hydrochloride, biuret, or $C_6H_{3m-n-a}X_n(NO_2)_a(\cdot OH)_m$, wherein X is halogen, 1–6 C alkyl or alkoxy of 1–4 C; *m* is 1–6; *n* is 0–5; *a* is 0–2 (but $m + n + a$ is 1–6); R is benzene residue substituted in the *p*-position by NR^{II}_2 and optionally elsewhere by Me; R^I and R^{II} are alkyl of 1–4 C; and the benzo portion of the benzthiazolium salt may contain 1–2 alkyl of 1–4 C. In an example, a hot solution of urea in MeOH is added to a boiling solution of 2-(*p*-dimethylaminophenyl)-3,6-dimethylbenzthiazolium chloride (**I**) monohydrate in MeOH. On cooling, a 1 : 1-urea-**I** complex separates out as a monohydrate, m.p. 213·5–215·5°. It is 100% lethal to *Ascaris lumbricoides*, in a feed containing 0·012%. F. R. BASFORD.

Acid-soluble veterinary medicaments. Farbenfabriken Bayer A.-G. (Inventors: W. von Bonin, M. Federmann and R. Strufe) (B.P. 1,097,054, 24.2.66. Ger., 24.2.65).—A medicament for oral administration to ruminants comprises an active agent, e.g., 2,2′-dihydroxy-3,3′-dinitro-5,5′-dichlorodiphenyl, coated with or embedded in an acid-sol., alkali-resistant material composed of a copolymer of CH_2:CR·CO·NH$[CH_2]_n$$NR^IR^{II}$ with, e.g., styrene, methyl-styrene, $MeCO_2CH$:CH_2, $EtCO_2CH$:CH_2, CH_2:CH·CN, CH_2:CMe·CO_2Me, or an acrylate (R–R^{II} are alkyl of 1–4 C or R is H; *n* is 2–4). F. R. BASFORD.

Halogen-containing 2,2′-methylene-diphenols. N.V. Nederlandsche Combinatie voor Chemische Industrie (B.P. 1,097,552, 6.7.65. Neth., 11.7.64).—Compounds, 2,3,5,6,1-OH·$C_6HCl_5CH_2$·C_6H_2-XX^I·OH-1,3,5,2, and monophosphates thereof are claimed wherein X and X^I are Cl, Br or I (X and X^I are not both Cl) (cf. B.P. 760,342). They are useful in combating *Fasciola hepatica* (liver fluke) especially in sheep. In an example, conc. H_2SO_4 is slowly added at 70° to a mixture of 3,5,6-trichlorosaligenin, 1,2,4-OH-$C_6H_3Br_2$ and water, then after 30 min. at 90–100° the cooled mixture is filtered. Solid is washed with water and purified, to give a 56% yield of 4′,6′-dibromo-3,4,6-trichloro-2,2′-methylenediphenol (3,5,6-trichloro-3′,5′-dibromo-2,2′-hydroxydiphenylmethane), m.p., 175–176·5° (product of same formula but with m.p. 155–157° is excluded). F. R. BASFORD.

Improvement in meat quality. Yamada Denki Kogyo K.K. (B.P. 1,098,757, 20.4.65. Jap., 21.4.64).—A male esculent animal or bird is injected with 5–90 μmol/kg body wt. of a compound RCO·OC_6H_4·A·C_6H_4OCO·R (**I**) dissolved in vegetable oil. R is an alkyl of 4–15 C and A is a diethylethylene or diethylvinylene group. Normal rearing is then continued for 2 weeks—5 months, before killing. Examples of **I** are hexoestrol-divalerate, -dicaproate and -dienanthylate. S. D. HUGGINS.

4-Chloro-3-t-butylphenyl-*N*-methylcarbamate and insecticidal compositions containing it. Fisons Pest Control Ltd. (Inventors: P. J. Brooker and G. T. Newbold) (B.P. 1,099,368, 28.11.63).—The title compound m.p. 150–151° (light petroleum) is prepared by adding MeNCO and NEt_3 to a solution of 1,4,3-OH·C_6H_3Cl·Bu^t in light petroleum and keeping the mixture at room temp. during several h. It has insecticidal properties (effective against *Pieris brassicae*, *Rhipicephalus appendiculatus*) and is especially useful as a cattle dip or spray. F. R. BASFORD.

Anticoccidial compositions [and feed supplements]. Merck and Co. Inc. (B.P. 1,087,958, 22.10.65. U.S., 30.10.64).—Poultry feeds contain agents of formula (COOY)·$C_6H_3(X)$·NR^1R^2, (**I**), wherein R^1 and R^2 are H, C_{1-5} alkyl, C_{1-6} alkanoyl or Bz, Y is H, C_{1-6} alkyl, alkali metal or an equiv. of alkaline earth-metal and X is Cl, C_{1-5} alkyl or NO_2. For control of coccidiosis amounts of **I** of 0·006–0·03 wt. % are sufficient; the 2-substituted-4-aminobenzoic acid compounds can be used (up to 30%) as feed supplements.
S. D. HUGGINS.

2,4-Diaminobenzoic acid derivatives. Merck & Co. Inc. (B.P. 1,089,477, 1.11.65. U.S., 3.11.64).—Used as animal feed supplements for the treatment and prevention of coccidiosis, the deriv. have the formula 1,2,4-COOY·C_6H_3·NHR·NH_2, wherein Y is H, a C_{1-4} alkyl radical, alkali metal or equiv. of an alkaline earth metal and R is C_{1-4} alkyl. They are prepared by reacting 2-(C_{1-4} alkyl) amino-4-nitrobenzoic acid with H_2 in the presence of a Pt catalyst (or other noble metal) and constitute 1–30% by wt. of the poultry feeds. The compound 2-ethylamino-4-aminobenzoic acid is particularly claimed. S. D. HUGGINS.

Naphthoquinone derivatives. Merck & Co. Inc. (B.P. 1,091,810, 5.9.66. U.S., 14.9.65).—Compounds claimed are coccidiocidal agents and comprise 1,4-naphthoquinones substituted in the 2-position by OH, in the 3-position by $[CH_2]_3$·C_6H_4(O·C_6H_4X-*p*)-*p* and in the 8-position optionally by OH (X is H or halogen—but is not H if the 8-position is unsubstituted). In an example, a mixture of 2,8-hydroxy-1,4-naphthoquinone, (O·CO·$[CH_2]_3$·C_6H_4·OPh-*p*)$_2$ and AcOH is heated at 95–100° during 2 h, then evaporated/ < 1

atm. A solution of the residue in ether is extracted with aq. saturated $NaHCO_3$, then with dil. aq. HCl, and water, and worked up to give 2,8-dihydroxy-3-[3-(*p*-phenoxyphenyl)propyl]-1,4-naphthoquinone, m.p. 105–109° (benzene–light petroleum).
F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Biochemical and breadmaking characteristics of wheat grain. N. A. Il'vitsky and L. K. Videneyva (*Pishch. Tekhnol.*, 1968, No. 1, [62], 22–25).— C.V.

Oxygen and fermentative oxidation of lipids in grain. G. E. Lapshina and A. P. Nechayev (*Pishch. Tekhnol.*, 1968, No. 1, [62], 26–38).—(78 references.) C.V.

Wheat lipids. V. G. Baikov, U. F. Kl'Yushkina, A. P. Nechayev and L. I. Puchkova (*Pishch. Tekhnol.*, 1968, No. 3, [64], 25–27). C.V.

Investigations on cereal lipids. R. Drapron and N. Fisher (*Getreide Mehl*, 1968, **18**, 7–12).—A review, covering the definition and classification of lipids, modern extraction and separation methods, lipid composition of cereals, hydrolysis and oxidation of lipids, and the rôle of lipids in breadmaking. (95 references.)
E. C. APLING.

Investigations on fat–protein complexes in cereals. II. Composition of protein and lipid components. M. Rohrlich and T. Niederauer (*Fette Seifen AnstrMittel*, 1968, **70**, 58–62).—A proteolipid, which was isolated from either rye-, wheat- or oat-flour, resembled a single mol. species when submitted to t.l.c. or electrophoresis. During lipophilisation the molecule undergoes an irreversable decomposition, and the liberated proteins and fats can be isolated. The amino-acid components resemble the protein constituents found in wheat-, rye- or oat-flour, and are similar to the 'Zwickel' proteins. Some eight to nine compounds are found in the amino-acid fraction, and three to four fractions in the proteolipid fraction. G. R. WHALLEY.

Drying of boiled groats. G. Y. Masloboyev and P. V. Ser'yogin (*Pishch. Tekhnol.*, 1968, No. 1, [62], 77–79).— C.V.

Determination of ash content in flour and groats. (*Getreide Mehl*, 1968, **18**, 13–19).—Results of collaborative experiments conducted by the ICC in 1962, 1964 and 1966 are reported, statistically analysed and discussed. No significant differences were found between results for ash determined at 600, 800 and 900°, in Au–Pt, pure Pt or porcelain dishes, but SiO_2 dishes were found to be unsatisfactory, particularly at 900°. The variability of ash results was significantly greater for groats than for flour, but no significant effect of particle size on the determination of ash content was shown. The results suggest that extended time of heating at 900° results in a lower ash figure, and further investigation is recommended. E. C. APLING.

Endosperm reduction in hard red spring wheat. N. L. Kent and A. D. Evers (*NWest. Miller*, Dec. 1966 [reprint, 5 pp.)].—Studies were made of the effects of moisture variation in the wheat (when roller milled) and in the flour (when pin milled) on particle size reduction of inner and sub-aleurone endosperm. % Reduction (to below 35 μm dia.) during pin-mill regrinding was 29–81 for inner and 1–50 for sub-aleurone endosperm, according to moisture content of wheat and flour. In both cases reducibility was greater at higher wheat grinding moisture and at lower flour regrinding moisture. After partial separation by sedimentation–flotation in CCl_4/C_6H_6 mixtures (*d* from 1·39 to 1·45) the protein contents of the endosperm fractions were estimated to be 9–18% (inner) and 31–35% (sub-aleurone). E. C. APLING.

Importance of moisture and pressure in the milling of flour for air classification. N. L. Kent (*NWest. Miller*, April 1966 [reprint, 6 pp.]).—Experimental milling of five wheat types showed that when the first break stage of roller milling was carried out with the wheat at 22% moisture content (*MC*), followed by subsequent stages at normal *MC* (14·5–17%), the flour yields approached or exceeded those obtained from milling at normal *MC* throughout, but the degree of reduction (% of particles < 35 μm in dia.) and total protein spread in the air-classified fractions were greater than normal and approached those obtained from wheat milled at 22% *MC* throughout. Total protein spread was also increased by preliminary damping (to 35–40% *MC* for Manitoba wheat or to 25% for soft wheats) and drying (by gentle circulation of air below 40°) before milling at normal *MC*. Pressure deformation of grains at 20–22% *MC* before milling gave a slight increase in yield of roller mill flour and in % breakdown of coarse particles during regrinding on the pin mill. It is concluded that endosperm fragmentation is increased by the application of pressure, with or without shear, to wheat in a sufficiently moist and plastic condition.
E. C. APLING.

Sulphydryl and disulphide contents of wheat flours, doughs and proteins. D. Mecham (*Baker's Dig.*, 1968, **42**, No. 1, 26–28, 30, 59).—A review of methods which are sensitive enough to be useful for flour and a discussion on problems which are still unanswered. (40 references.) I. DICKINSON.

Flour requirements for continuous breadmaking. G. W. Schiller (*Baker's Dig.*, 1967, **41**, No. 2, 44–46, 87).—The continuous method is compared with the conventional method of bread baking. Requirements for flour intended for use in continuous breadmaking are reviewed, e.g., the flour should flow easily and quickly through the pneumatic handling systems, and the flour colour should be measured by the new instruments which have outmoded the ash test. The flour's protein quality and quantity are decisive in determining the amount of mixing required for complete dough development. It is advisable to determine the appropriate level of oxidation and the level of diastatic supplement at the mill. In addition to short mixing time, flour should have the capacity for rapid hydration. I. DICKINSON.

Evaluation of methods for the determination of germination damage in rye on the basis of high amylose activity. E. Drews (*Getreide Mehl*, 1968, **18**, 1–7).—Results by various routine methods for the estimation of diastatic activity are reported for 24 samples of rye (eight varieties, three locations, 1966 harvest). Correlations between the results of different methods are shown graphically and the limitations of individual tests for the evaluation of the baking value of rye flour are discussed. (24 references.)
E. C. APLING.

White and wholemeal bread and flour in the diet of caries-susceptible rats. T. H. Grenby (*Br. dent. J.*, 1966, **115**, 26–29).—Neither white nor wholemeal bread was cariogenic under the conditions of an experiment with 8-week old rats fed variations of a diet containing 66% flour, dried bread or sucrose, 32% skim milk powder and 2% liver powder. Caries incidence was related to the dietary sucrose content and not to the flour or bread content. (11 references.) E. G. BRICKELL.

Storage and delivery of flour. D. Reimelt (*Brot. Gebäck*, 1968, **22**, 5–8).—General principles of modern systems for flour storage, transport, weighing and mixing are outlined and illustrations of typical installations are given. E. C. APLING.

Effect of pressure treatment on the granular structure of different starches and their susceptibility to enzymic attack. C. Mercier, R. Charbonnière and A. Guilbot (*Stärke*, 1968, **20**, 6–11).—Commercially prepared potato and cereal starches with moisture contents of 1–36% were subjected to hydraulic pressures of up to 6000 kg/cm² for periods of 1 min. The damage was assessed by optical microscopy, a differential coloration method and crystal structure examination by *X*-ray diffraction. The changes in solubility and degradation by α- and β-amylase were also determined. The extent of pressure damage depends on moisture content with a min. at 19% for potato and 14% for cereal starches. The appearance of the damaged grains is different at lower and higher hydration levels. (16 references.) (In French.)
J. B. WOOF.

Changes in maize starch resulting from irradiation and temperature. V. A. Yakovenko, N. V. Romensky and L. V. Masenko (*Pishch., Tekhnol.*, 1968, No. 3, [64], 42–44).—(11 references.)
C.V.

Drying of potato starch with infra-red rays. H. Pfennig (*Stärke*, 1968, **20**, 16–26).—An apparatus is described for studying the drying of starch of differing moisture contents under i.r. irradiation. Using this equipment, temp. changes, drying rates and the effects of air velocity and radiation intensity were studied. Data on the equilibrium moisture contents has permitted one section of the desorbtion isotherm to be elucidated. The discontinuity found in the drying process was studied by *X*-ray structure analysis and i.r. spectroscopy of the starch at different moisture contents in hexachlorobutadiene solution. (58 references.) J. B. WOOF.

Biostimulants of breadmaking yeast [obtained] from waste materials from flour and groats milling, and confectionery. N. K. Palagina (*Pishch. Tekhnol.*, 1968, No. 1, [62], 67–71).—10 references.
C.V.

Effects of combined magnesium and phosphate supply on the energy-producing degradation reactions of bakers' and fodder yeast cells. W. Nordheim (*Mschr. Brau.*, 1968, **21**, 196–203).—Analyses carried out on *Candida utilis* grown normally and with PO_4^{3-} deficiency showed that when PO_4^{3-} and Mg^{2+} are subsequently made available to the cell the uptake of the two is directly related; effects of P and Mg under both aerobic and anaerobic conditions are discussed both for this yeast and for bakers' yeast (*Saccharomyces cerevisiae*). (37 references.) J. B. WOOF.

Aspects of research on industrial enzymes. G. L. Solomons (*Chem. Engr, Lond.*, 1968, CE9–CE12).—The use of α-amylase, protease, glucoamylase and pullulanase enzymes in breadmaking is considered. Problems associated with producing these fungal and bacterial enzymes are discussed, particularly the provision of an adequate O_2 supply to viscous mould broths by new forms of agitation, the selective inactivation of enzymes by pH and temp. control, and induction of enzymes by substrates such as maltose. (10 references.) J. LAMBORN.

Biochemical aspects of breadmaking. H. R. Stafford (*Process Biochem.*, 1967, **2**, No. 4; 18–20).—A brief summary. C.V.

Minor ingredients as a factor in bread properties. R. H. Kilborn (*Baker's Dig.*, 1967, **41**, No. 2, 24–27, 29, 32).—The combination of $KBrO_3$ and an *l*-cysteine–bromate–whey blend can lead to gas holes in bread and a sharp reduction in fermentation tolerance. Variations in flour show different tolerance levels. The effect of a fungal enzyme supplement was different in two flours containing 20 and 30 ppm $KBrO_3$ respectively, although they showed the same farinograph development times and thus had the same mixing requirements. *l*-Cysteine–bromate–whey and fungal enzyme supplements are best suited for plant bakeries where a constant source of flour is used. I. DICKINSON.

Factors affecting bread flavour. W. Pence (*Baker's Dig.*, 1967, **41**, No. 2, 34–36, 85).—A general review; factors discussed include ingredients, mixing, fermentation, make-up and baking. I. DICKINSON.

Protein value of crust and interior of leavened bread. Habibullah and S. M. Ali (*Pakist. J. scient. ind. Res.*, 1967, **10**, 141–142).—Although protein contents of crust and interior of loaf are the same, the net protein utilisation operative of the crust is ~50% lower and thus so is the net dietary protein calories % (NDP Cal %). The NDP Cal % inside the loaf is approx. equiv. to that of wheat flour, whilst the available lysine in the crust is ~15% less than in the corresponding flour. Results indicate that excessive baking should be avoided. W. J. BAKER.

Storage and cooling of bread. A. Jaus (*Brot. Gebäck*, 1968, **22**, 1–5).—Requirements for large scale forced cooling of bread for slicing and/or packing are briefly discussed and typical installations are illustrated. Cooling curves for the crumb and crust of bread cooled in still air and with forced convection at different R.H. levels are presented, and the effect of cooling conditions on taste and keeping quality (including mould contamination) are discussed. E. C. APLING.

Quality control for the pie baker. I. Ingredient specifications. K. N. Tsourides (*Baker's Dig.*, 1968, **42**, No. 1, 32–33, 36, 38).—Specifications and methods of analysis for ingredients for pies are given. The type of flour, shortening, starches, and dried milk and egg products are discussed. The amylose and amylopectin contents of some commonly used starches are given, and a comparison of a typical fat analysis and specifications is made. I. DICKINSON.

Biscuit manufacture. H. R. Stafford (*Process. Biochem.*, 1968, **3**, No. 3, 58–62).—A general review. C.V.

Foodstuffs derived from rice. G. Cantoni (B.P. 1,092,245, 19.7.65. It., 20.7.64).—A foodstuff containing a high % of water-sol. components (e.g. 85% water-sol. carbohydrate and ~6% water-sol. protein) is obtained by heating unfinished hulled rice under pressure in an autoclave and in presence of dil. acid (e.g. H_3PO_4 or glycerophosphoric acid). The cooled product is neutralised with e.g. $NaHCO_3$, dried and ground to give a powder rich in P, suitable as a food or food supplement for addition to milk, beer, soup etc. P.P.R.

Sugars and confectionery

Acid hydrolytic preparation of starch syrup. G. Tegge (*Stärke*, 1968, **20**, 45–49).—Pilot-plant studies were carried out on the continuous conversion of corn starch to syrup by acid hydrolysis. Optimum hydrolysis with a min. of coloured impurity formation is achieved if reaction time is reduced to a min. At constant reaction time, the temp. can be reduced by using higher acid contents though this is ultimately limited by the practical considerations of refining. The purer the intermediate products obtained by these means, the more efficient is the final decolorisation stage with activated charcoal or resin. During refining, separation of the protein sludge reduces by 20% the protein content of the liquor after charcoal treatment, though this had no effect on colour or hydroxymethylfurfural content. J. B. WOOF.

Purification of sugar-beet syrup by dialysis. I. F. Zelikman and D. M. Leibovich (*Pishch. Tekhnol.*, 1968, No. 1, [62], 80–82).— C.V.

Adsorption of some non-sugars from refined sugar syrups. I. L. Zdanovich and I. F. Zelikman (*Pishch. Tekhnol.*, 1968, No. 2, [63], 66–70).— C.V.

Interaction of soluble carbohydrates with silicomolybdic acid. V. N. Khrustal'yova, M. F. Kachalova and V. V. Kozlov (*Pishch. Tekhnol.*, 1968, No. 3, [64], 38–41).— C.V.

Sucrose solubility in the presence of non-sugars. M. I. Daishev, I. F. Zelikman and L. M. Daisheva (*Pishch. Tekhnol.*, 1968, No. 3, [64], 32–37).—23 references. C.V.

Estimation of tutin and hyenanchin in honey. I. Comparison of thin-layer chromatography and intracerebral injection methods. II. Toxicity of honey from test hives during 1962 to 1967. P. G. Clinch and J. C. Turner (*N.Z. Jl Sci.*, 1968, **11**, 342–345, 346–351).—I. Analyses of 150 honey samples by t.l.c. and intracerebral injection of mice showed that results for the two methods were in good agreement if it was assumed that samples were positive by t.l.c. only when they produced spots having R_F values for both tutin (**I**) and hyenanchin (**II**), or the spot of **II** only. Some samples contained unknown substance(s) having R_F approx. equal to that of **I**. Toxicities of samples containing **II** only were calculated as mg **I** per 100 g honey.

II. Honey from North Island, N. Zealand, contained **I** and **II** as revealed by oral dosage of guinea-pigs, intracerebral injection of mice, and t.l.c. A mouse-test procedure (fivefold more sensitive than the guinea-pig method) is described for determining the concn. of toxins in honey, expressed as **I**-equiv. (mg per 100 g). The toxic samples had **I**-equiv. from 0·1 to 6·7. W. J. BAKER.

Creamed honey-fruit spreads. R. Berthold, jun. and A. W. Benton (*Fd Technol. Champaign*, 1968, **22**, 83–85).—Products with commercial possibilities were made by blending sun- or freeze-dried apricots, pineapples or strawberries with finely-cryst. honey. (10 references.) P. S. ARUP.

Critical evaluation of the A.O.A.C. method for determining milk protein in milk chocolate when applied to crumb chocolate. R. J. Motz (*Analyst, Lond.*, 1968, **93**, 116–117).—The results obtained for crumb chocolate tend to be unreliable because of comparatively small differences in temp. used during crumb drying. Loss of total N is small over the range 104–140°, but loss of protein-N can amount to ~90% at drying temperatures around 140°, although only ~10% is lost on heating for 6 h at 95°. Since losses of lactose are >5% during manufacture, the lactose content, in association with other results, should provide a more reliable guide to milk content. W. J. BAKER.

Free-flowing icing sugar. Henkel and Cie. G.m.b.H. (B.P. 1,092,770, 28.10.66. Ger., 30.10.65). Addn. to B.P. 1,084,949).—The sugar is obtained by spraying molten finely-divided fat, of m.p. 30–35° (e.g. hydrogenated groundnut oil), into freshly sifted pulverulent sugar, which is either falling freely or is in a retarded state of fall (obtained by passing air upwards through the sugar). The final product contains 5–40 (15)% of fat; flavourings, powdered milk etc., can be added to the sugar before treatment. S. D. HUGGINS.

Fermentation and Alcoholic Beverages

Dissolved oxygen measurement in continuous aseptic fermentation. A. N. Roberts and P. G. Shepherd (*Process Biochem.*, 1968, **3**, No. 2; 23–24).—The process is used for monitoring. Comparison is made with results obtained with a galvanic probe which is adequate for short periods. The probe used by Phillips and Johnson (*Biochem. Microbiol. Tech. Engng*, 1961, **3**, 261) is used. This consists of 40 ft × ⅛ in. dia. tube of PTFE coiled inside the

fermenter. A slow stream of N_2 is passed through this to pick up the O_2 which has diffused from the broth through the tube wall, the final O_2 concn. being estimated by a Beckman O_2-analyser. This system has been in use since mid-1966. C.V.

Continuous saccharification and fermentation in alcohol production. I. Y. Veselov, I. M. Grachova, L. E. Mikhailova, S. A. Babayeva and B. A. Ustinnikov (*Pishch. Tekhnol.*, 1968, No. 1, [62], 108–110). C.V.

Fermentability as a criterion for the selection of hybrid barleys suitable for brewing. A. Fritz, E. Ulonska and W. Lenz (*Brauwissenschaft*, 1968, **21**, 217–222).—Hybrid strains of some barley varieties were studied to determine whether fermentability of the derived wort could be used as a criterion for selection of promising varieties. The existing Congress method for mashing was modified, which resulted in a more consistent sugar composition in the worts, and this in turn produced more favourable fermentation performances. Consistent differences in the behaviour of brewing barleys were revealed by the fermentation test. The results show that fermentability can be applied to the selection of barleys with a high extract potential and good fermentation properties. (12 references.) I. DICKINSON.

Rôle of phospholipase B in malted barley during brewing. L. Acker and J. Geyer (*Brauwissenschaft*, 1968, **21**, 222–226).—Phospholipase B (**I**) liberates fatty acids from lecithin (**II**) and lysolecithin (**III**), which occur in similar concn. in malted barley. Although **I** is formed during the malting process, only 37 mg of the choline ester of glycerophosphoric acid (**IV**) and 35 mg of free choline (**V**) were found in 100 g of malt. The variation of **V**-containing fractions was observed throughout the brewing process. The most extensive variations occurred during mashing. The finished beers contained only traces of **II** and **III** (50 μg/l), but 141 mg/l of free **V** and 78 mg/l of **IV** were found. Two thirds of the **V** introduced by the malt passed into the beer due to the action of **I** and subsequent enzymic actions. 43% of the **V** compounds were extracted from the hops. (10 references.) I. DICKINSON.

Enzyme synthesis in micro-organisms. M. D. Lilly (*Process Biochem.*, 1967, **2**, No. 2, 17–20, 28).—The induction and repression of enzymes is reviewed, these processes being governed by genetic instructions and feed-back mechanism. Beer wort fermentation is given as an example; after the monosaccharides have been used, galactosidase and permease are induced to hydrolyse to disaccharides. These different mechanisms are specially important when non-carbohydrate nutrients are used or when a batch process is to be converted to a continuous one. (24 references.) C.V.

Dextrinising activity of α-amylase in barley-malt extracts. I and II. W. Błaszków (*Roczn. Technol. Chem. Żywn.*, 1967, **14**, 27–37, 39–49).—I. To show that the dextrinising capacity of α-amylase increases 2–3 times during the drying of green malt, the extract, total-, protein- and amine-N, dextrinising ability, saccharifying capacity and pH were determined in enzymic extracts from green and dry malt. Individual fractions of proteins, segregated according to their mol. wt. by mol. filtration through Sephadex G-100 were examined spectrophotometrically for the presence of α-amylase. The results show that during the drying period of green malt, the dextrinising capacity of α-amylase increases, and the colour reaction with I_2 at various pH indicates that changes in the structure and mol. wt. of α-amylase take place. (23 references.)

II. Further studies using mol. filtration, colorimetric, turbidimetric and chromatographic methods, and examination of protein u.v. spectra were carried out. The difference between dextrinising activity of α-amylase in green and dry malt extracts disappears at pH < 5 and changes in the macromol. structure of α-amylase are associated with increase in its dextrinising capacity. Addition of cysteine to enzymic prep. also affects the dextrinising property of α-amylase. The increased dextrinising ability of α-amylase is accompanied by a slight increase in its saccharifying capability and also by the reduction of SH groups. (16 references.) T. M. BARZYKOWSKI.

Inactivation of barley malt β-amylase by photo-oxidation. N. A. Zherebtsov and V. V. Z'yuz'ina (*Pishch. Tekhnol.*, 1968, No. 2, [63], 48–52).—14 references. C.V.

Change in composition of light malt protein on thermal treatment. E. I. Velikaya and M. Y. Ushakova (*Pishch. Tekhnol.*, 1968, No. 1, [62], 47–50). C.V.

Hydrolysis of unmalted barley protein by proteolytic ferments of malt. L. M. Boiko, P. M. Mal'tsev and V. P. Mat'yukhina (*Pishch. Tekhnol.*, 1968, No. 2, [63], 45–47).— C.V.

Kaffircorn malting and brewing studies. XX. Isolation of starch by laboratory wet milling of kaffircorn and malted kaffircorn after a neutral chemical steep. E. M. Montgomery and E. van Assen (*Stärke*, 1968, **20**, 60–63).—Two lots of Short Red kaffircorn and 3- and 6-day malts were washed in water containing 0·1% Triton X-100 before steeping for 48 h at 38° in the presence of Na_2S or diethyldithiocarbamate. The extracted sol. pigment solution was drained off and the grain conditioned with 5% NaCl solution. The grain was milled in a Fryma mill and the starch isolated by a series of sieving, settling and centrifuging procedures. The starch was obtained in 89–92% yield and the protein content was 0·2–0·23%. Ash was 0·11–0·2% of the starch. The pH of the starch suspension was 5·9–6·0 and the swelling and other properties were not unlike those of ordinary maize starches. (In English.) J. B. WOOF.

Continuous fermentation of brewer's wort. A. D. Portno (*J. Inst. Brew.*, 1968, **74**, 55–63).—Owing to the loss of activity of yeast in high concn. (especially apt to occur in homogeneous systems) it is important both to allow for partial escape of the yeast and to maintain a certain degree of concn. gradient. Conditions ensuring the continuous exposure of the yeast to a high nutrient concn. can be ensured by the use of an elongated fermentation vessel or of a series of homogeneous units with a regulated feed-back of yeast cells. (22 references.) P. S. ARUP.

Wort composition. A review. I. C. MacWilliam (*J. Inst. Brew.*, 1968, **74**, 38–54).—Values for the concn. of individual compounds present in wort are collated and grouped into sections. The effects of variation of grist composition and of washing and boiling conditions on wort composition, and the fate of individual constituents during fermentation are discussed. (122 references.) P. S. ARUP.

Application of hop extracts in brewing. VI. The use of pre-isomerised hop extracts in brewing trials. H. Weyh (*Brauwissenschaft*, 1968, **21**, 240–244).—Bitter substances in the beer did not decrease appreciably with increasing boiling time of the pre-isomerised hop extract (**I**). An ethanolic solution of **I** added to pitching wort or green beer resulted in sharp aromas, and the beer was rejected by a tasting panel, but unpleasant flavour components disappeared to a great extent when the **I** was boiled for approx. 60 min. The protein stability of beer prepared with **I** was similar to that obtained with whole hops of this year's crop. The foam stability of beers prepared with **I** was better the later **I** was added during the brewing process. I. DICKINSON.

Preparation of hop extracts for fermentation. S. Kh. Abdurazakov, M. E. Lekar'yova and B. V. Rakhimova (*Pishch. Tekhnol.*, 1968, No. 2, [63], 86–87).— C.V.

Aroma of hops. I. Origin and identification of hop aroma substances. M. De Mets and M. Verzele (*J. Inst. Brew.*, 1968, **74**, 74–81).—G.l.c. analyses were carried out on headspace atm. of fresh hops, on Et_2O and tetralin extracts, and on dry vac. distillates of pressed and stored hops. The aroma from fresh hops contained mainly myrcene which disappeared as the hops aged, and also small amounts of Me_2CO and dimethylvinylcarbinol. About 25 compounds were formed during storage, by degradation of the α- and β-acids. The compounds found in the extracts and dry distillates were not present in hop aroma; in addition to hydrocarbons, carbinols, aldehydes, ketones and esters, they included S compounds, chiefly Me_2S, Me_2S_2 and methyl dithioacetate. The quality of the hops deteriorated when the volatiles were allowed to escape. P. S. ARUP.

Adsorption of hop substances on yeast cell wall. I. J. Dixon and A. A. Leach (*J. Inst. Brew.*, 1968, **74**, 63–67).—The absorption (measured by the method of Dixon) on to two top and one bottom yeasts followed the pattern of the Freundlich equation, the log of the proportion of the hop substances adsorbed per unit mass of yeast bearing a linear relation to the log of the final concn. of the hop substances in the beer. The three yeasts adsorbed 1·1–7·8% of the substances originally present in the beer from similar worts, but the amounts differed from one series of fermentations to another. Overall losses were ~23%. P. S. ARUP.

4-Acetylhumulinic acids. M. Van Boven and M. Verzele (*J. Inst. Brew.*, 1968, **74**, 81–83).—A solution of humulone in MeOH was made 0·067 N with NaOH, refluxed for 30 min. and poured into ice-cold 1 N HCl. The product, known as Weichharz B, was a mixture of the A and B diastereoisomers of 4-acetylhumulinic acid in 25% yield. The mixture was separated by countercurrent distribution with a phase system of iso-octane and buffer of pH 3·7. A reaction mechanism and the structures of the products are presented. P. S. ARUP.

Non-biological haze in beer. J. Pasfield (*Process Biochem.*, 1968, **3**, No. 3; 49–52).—The constitution of polypeptide-tannin complexes is discussed and the reactions involved in this type of haze formation are considered. The use of synthetic polymers such as nylon or the more effective polyvinyl pyrrolidone to absorb tannins is reviewed and compared with the use of very low concn. H_2O_2 in the mash liquor which results in oxidation taking place during normal conversion, the pptd. tannins being retained in the mash tun. (19 references.) C.V.

Beer-foam stabilising substances. G. Kamm (*Brauwissenschaft*, 1968, **21**, 209–217).—Three groups of beer-foam stabilising substances are described: (1) proteins, (2) metallic salts and (3) high mol. wt. substances which mainly increase the viscosity. In group (1), albumins improved foam stability at concn. of 5–10 g/100 l and gelatine was effective at 50 mg/l. Hydrolytic degradation of 'spent grains' also yielded foam-stabilising materials. Of metallic salts only Fe can be used, added in concn. of 1 mg/l, since Co and Ni are hazardous to health. In group (3) agar-agar showed good activity at a concn. of 50 mg/l and alginic acid and its deriv. applied at rates of 40–80 mg/l. The effects of foam-destroying substances were largely reversed by the use of polypropylene glycol alginate. Gum arabic (45–300 g/100 kg) added to barley during malting, can have foam improving activity on the derived beer. The addition of foam stabilisers is forbidden in Germany and it is recommended that beer shall be prepared without the addition of such stabilisers. (32 references.) I. DICKINSON.

Phenolic constituents of beer and brewing materials. III. Simple anthocyanogens from beer. J. W. Gramshaw (*J. Inst. Brew.*, 1968, **74**, 20–38).—A description is given of the paper chromatographic separation and structural investigation of (+)-catechin (**I**) and six simple anthocyanogens obtained by extraction from polyamide absorbates from beer. One of the anthocyanogens was probably a stereoisomeric form of 5,7,3′,4′,-tetrahydroxyflavan-3,4-diol with the epicatechin group at C_2 and C_3 (**II**), another (**III**) a biflavan derived by condensation between **I** and **II**, and a third a triflavan derived by condensation between **II** and **III**. The remaining anthocyanogens were mixtures containing, in addition to deriv. of **I**, compounds that yield (+)-epicatechin on acid hydrolysis. The bearing of anthocyanogen structure on beer stability is considered. (55 references.) P. S. ARUP.

Continuous fermentation for wine production. I. Cornejo, C. Llaguno and J. M. Garrido (*Revta Ciencia apl.*, 1968, **22**, 21–26).—Using the yeast *Saccharomyces ellipsoideus* 87 in a continuous fermentation process the must needs to remain in the fermentation vessel < 4 days and the yeast maintains its fermenting power for at least 20 days. Aeration activates the yeast without spoiling the wine. The wine produced is satisfactory analytically, but organoleptic properties are inferior to normal. L. A. O'NEILL.

Alteration in nitrogenous substances in continuous 'champagnisation'. S. P. Avak'yants and V. S. Gul'yayeva (*Pishch. Tekhnol.*, 1968, No. 3, [64], 59–63).— C.V.

Characterisation of wines by their volatile constituents. II. F. Prillinger and A. Madner (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18**, 1–9).—Further to Part I (*ibid.*, 1967, **17**, 271–279). the g.l.c. examination was extended to red, marc, sediment, raisin, and artificial wines. Red wines differed from white wines in their content of small or trace amounts of Et caproate, hexyl acetate and similar compounds. Raisin, sediment, and artificial wines contained no hexanol (**I**) but comparatively large amounts of lactate (**II**). The ratio of **II** to **I** was ~0·5 in red or white wines; artificial wines contained up to 25% of **II**. P. S. ARUP.

Possibility of avoiding use of sulphurous acid in production of sweet white wines. M. Flanzy (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 100–104).—An example is described of the production of a sweet white wine of good quality and totally unaffected by oxidative spoilage, without the use of SO_2. The wine was produced by the (practically) anaerobic fermentation of non-clarified must from Grenache grapes infected with *Botrytis cinerea*. The destruction of oxidative enzymes (plentiful in Grenache grapes) is considered as having been the operative factor. P. S. ARUP.

Table wine manufacture. R. W. Joswell (*Process Biochem.*, 1967, **2**, No. 1, 5–11).—15 references. C.V.

Contaminating yeasts and yeast-like micro-organisms in wine cellars. E. Minarik (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18**, 10–16).—Various micro-organisms other than true wine yeasts found as contaminants in three Czechoslovakian wine cellars did not affect the stability of the wines. P. S. ARUP.

Acetic acid bacteria: properties and applications. T. Leisinger and J. Muller (*Process Biochem.*, 1967, **2**, No. 4; 10–15).—The classification of these organisms is discussed and they are divided into two genera capable of producing dihydroxyacetone, 5-ketogluconic acid, 2-ketogluconic acid and L-sorbose from a variety of substrates. (53 references.) C.V.

Manufacture of Scotch malt whisky. A. C. Simpson (*Process Biochem.*, 1968, **3**, No. 1, 9–12).—A descriptive review. The ppm concn. of alcohols, esters, acids, carbonyl and phenolic compounds are tabulated and a comparative note on other whiskies is included. (44 references.) C.V.

Composition of the aroma of some brands of whisky and rum analysed by customary methods and by gas chromatography. L. Nykänen, E. Puputti and H. Suomalaien (*Kem. Teollisuus*, 1968, **25**, 399–404).—The determination of the EtOH, aldehydes, fusel alcohols and ester content and the composition of these fractions of spirits are briefly described. The differences found between some Scotch, Bourbon and imitation whiskies, and some rums are detailed and their significance is discussed. (In English.) (15 references.) K. GRAUPNER.

Substances [controlling] ultra-violet absorption of cognac alcohols and cognacs. M. Skurikhin (*Pishch. Tekhnol.*, 1968, No. 2, [63], 88–93).—11 references. P.P.R.

Formation of crotonaldehyde in rectification and heat treatment. Y. D. Silva, P. S. Tsyganov and V. F. Sukhodol (*Pishch. Tekhnol.*, 1968, No. 1, [62], 111–113).—12 references. C.V.

Determination of alcohol content in beverages by phase titration. D. B. Walters, I. D. Chawla and D. W. Rogers (*J. agric. Fd Chem.*, 1968, **16**, 259–261).—Phase titration with BzOH to the point of turbidity gives results for EtOH in wines and spirits that are comparable with those obtained by distillation and pycnometry. Differences in results by the two methods were 0·11–0·88%. Vol.-% were determined by reference to a graph of titration results against the EtOH content of known mixtures of EtOH and water. Further accuracy might be achieved by the use of graphs based on results obtained with mixtures of EtOH and water with suitable additives to compensate for the small errors due to the presence of flavouring and colouring substances. (15 references.) P. S. ARUP.

Chill stability and foam adherence of beer. F. and M. Schaefer Brewing Co. (B.P. 1,088,324, 12.3.65. U.S., 20.3.64).—Beer is improved against microbial growth by addition of a C_7H_{15}-*p*-hydroxybenzoate, or an alkali- or alkaline earth-metal salt thereof, admixed with (*i*) a propylene glycol alginate (20–120 ppm of total composition) and (*ii*) 0·1–10 ppm of a Co salt or of the composition FS-26W, which consists of 70% $ZnSO_4 \cdot 7H_2O$, 2·5% $CaNa_2$ EDTA salt, 2·5% glycine and 25% gum arabic. The Co salt or FS-26W overcome the adverse effects on chill stability and foam adherence caused by adding the heptyl hydroxybenzoate. S. D. HUGGINS.

Brewing malt beverages. Phillips Petroleum Co. (B.P. 1,094,358, 9.12.64. U.S., 13.12.63).—The process consists of preparing a malt mash, heating, filtering to give a wort and boiling the wort in a brew kettle using 1·3–5·0 barrels of water/100 lb brew materials so that a diluted wort having an extract of < 11° Balling is obtained. This is then freeze-concentrated to remove 30–80% by wt. water, leaving a conc. wort which can be readily fermented. Hop extract may be added at any stage after boiling. S. D. HUGGINS.

Fruits, Vegetables, etc.

Lactonic compounds of apricot. C. S. Tang and W. G. Jennings (*J. agric. Fd Chem.*, 1968, **16**, 252–254).—Volatiles adsorbed from Blenheim apricots on active C, separated by g.c. and characterised by i.r. spectroscopy, contained benzyl alcohol, caproic acid, epoxydihydrolinalool, γ-caprolactone, δ-octalactone, δ-decalactone, and γ-dodecalactone, here reported for the first time. Possible biogenetic routes to the γ- and δ-lactones via hydroxy fatty acids are postulated. (17 references.) P. S. ARUP.

Dielectric properties of potatoes and potato chips. W. E. Pace, W. B. Westphal, S. A. Goldblith and D. Van Dyke (*J. Fd Sci.*, 1968, **33**, 37–42).—Dielectric properties were determined at different frequencies and moisture contents to select the optimum frequency and range of moisture content in which microwave energy can be used most efficiently. Measurements were made at 300, 1000 and

3000 MHz on raw potatoes at 25° and at three moisture levels on chips at 25, 52 and 82°, and total lipids and N contents were also determined. The high moisture content and presence of various dissolved salts in raw potatoes gave very high dielectric values. Chips showed a rapidly decreasing dielectric loss as the moisture content was reduced. For finish drying of chips, a frequency of 3000 MHz causes 3 to 4·5 times greater power production than 1000 MHz in the chips. This difference is due entirely to the difference in frequency, since the difference in dielectric loss values at the two frequencies is small. (19 references.) I. DICKINSON.

Effect of variety and length of storage on carbohydrate contents and table quality of sweet-potatoes. M. K. Ali and L. G. Jones (*Pakist. J. scient. ind. Res.*, 1967, **10**, 121–126).—Raw and baked roots were sampled at harvest, after curing, and after 4–21 weeks of storage at 60°F and 72% R.H. Softness (penetrometer) of baked roots increased with storage time, whilst during baking dry matter (I), reducing sugar (II), total sugar (III) and dextrin (IV) increased in all four varieties although starch content (V) decreased sharply and non-reducing sugar (VI) remained approx. constant. During storage all varieties gained in II, III, IV and VI, but decreased in I. Softness of baked roots correlated positively with VI or III, i.e. the higher the sugar the softer the variety, but negatively with V. i.e. the softer the variety the lower the starch. (23 references.)
W. J. BAKER.

Volatile carbonyl compounds during fermentation of cabbage. (*Brassica napus* var. *chinensis*). J. Hrdlička, D. Čurda and J. Pavelka (*Sb. vys. Sk. chem.-technol. Praze, Potraviny*, 1967, **E15**, 51–54).—Volatile carbonyl compounds were isolated from raw cabbage and cabbage fermented for 7, 14, 21, 64 and 88 days, and separated by paper chromatography; 19 carbonyl compounds were found. In the first seven days of fermentation there was an increase in certain volatiles, particularly in diacetyl and acetaldehyde, but other compounds progressively reduced in amount, and at 21 days (organoleptic optimum) only six carbonyls remained detectable. (In English.) E. C. APLING.

Change in amino-acid composition of cucumbers in heat treatment. E. I. Petropavlovsky, S. S. Shikhaliyev and E. M. Sobolev (*Pishch. Tekhnol.*, 1968, No. 1, [62], 106–107).— C.V.

Non-alcoholic beverages

Scientific-technical Commission [on fruit juices], Berlin. (*Rep. Int. Fed. Fruit Juice Producers*, 1966, **7**, 331 pp.).—This includes the following papers: **Use of diethyl pyrocarbonate for preservation of fruit juices; its influence as regards maintenance of natural purity.** (41 references.) K. Wucherpfennig. **Tolerance limits for aluminium and cadmium in fruit juices.** H. Rentschler. **Tin content in fruit juices.** (22 references.) S. Weiss. **Biochemical, physiological and nutritional significance of flavonoids of fruit origin.** (15 references.) V. L. S. Charley. **Occurrence of an alkaloid in a tropical fruit (*Passiflora edulis*).** K. Wucherpfennig. **Occurrence of histamine in beverages.** K. Millies. **Methods for determination of formic acid in fruit juices.** (27 references.). K Gierschner. **Determination of heavy metals in fruit juices.** (29 references.) K. Gierschner and D. List. **Grading of citrus juices on basis of special analytical data with special reference to formol values.** (22 references.) K. Gierschner and G. Baumann. **Changes in quality of fruit juices during storage in plastic bottles.** G. Bretthauer. **Dependence of pasteurisation conditions on counts and fruit tissue content of juices.** I. Franke. **Preparation of beverages from dates.** P. Dupaigne and J. P. Richard. **Further investigations on preservation of apple juices with diethyl pyrocarbonate.** (22 references.) K. Gierschner and H. Treptow. **Resistance of moulds in fruit juices to irradiation and heating.** (11 references.) M. Jensen.

Scientific-technical Commission [on fruit juices], Madrid. *Ibid.*, 1967, **8**, 285 pp.).—This includes the following papers: **Correlation of subjective and objective analyses on research on processed fruit and vegetable flavour.** (14 references.) W. L. Stanley and D. G. Guadagni. **Correlation of microbiological and chemical methods for estimation of orange juice quality.** (18 references.) J. M. Sharf. **Measurement and characterisation of aroma in varieties of black currants and in their juices (aroma value, gas chromatographic aroma analysis, and organoleptic evaluation.** (27 references.) Taina Kuusi and Terffu Kuusi. **Detection of adulterations in citrus juices. IX. Mineral composition of whey of single strength orange juices manufactured in Spain and the United States.** E. Primo Yúfera and J. Royo Iranzo. **Rapid determination of total sulphur dioxide content of wines and grape juice.** L. R. Mattick and J. C. Moyer. **Objective method for evaluation of content of pulp in suspension in fruit beverages.** (13 references.) P. Dupaigne. **Irradiation effect on aroma of apple juice. I. Exploratory studies.** L. Gascó, R. Barrera and F. de la Cruz. P. S. ARUP.

Storage of fruit juices. R. H. Robbins (*Process Biochem.*, 1967, **2**, No. 6, 47–49).—Storage for 12 months or more was studied with special reference to microbial spoilage, colour change and off-flavours due to enzymic action. Preservation by CO_2 saturation, sterile filtration, pasteurisation, evaporation and spray drying are summarised and the technique of fractional distillation of volatile components with their subsequent return to the bulk of the juice is also considered; this latter is employed in the production of instant-coffee and -tea. (18 references.) C.V.

Influence of pasteurisation on the colloidal system of grape juice. A. N. Yatsyna, V. I. Makukhin and V. I. Reva (*Pishch. Tekhnol.*, 1968, No. 3, [64], 52–54).—21 references. C.V.

Concentration of raw strawberry juice by a freezing method. Z. Niedzielski (*Roczn. Technol. Chem. Żywn.*, 1967, **14**, 59–67).—Highly conc., high-quality strawberry juice, containing almost the same amount of vitamin C and aroma as a raw juice, was obtained by freezing out the water from fresh juice at −25° with subsequent separation of ice crystals by centrifuging. The concentrate thus produced contained 69·6 dry matter and 92·4 vitamin C, compared with 100 as the basis in a raw juice. The concentrate obtained by freezing-concn. surpasses the concentrate obtained by evaporation in flavour, colour and aroma. (15 references.)
T. M. BARZYKOWSKI.

Organic acids of pomegranate juice. S. Kh. Aburazakova and L. B. Gabbasova (*Pishch. Tekhnol.*, 1968, No. 1, [62], 51–55).— C.V.

Quantitative comparison of isoamyl alcohol, pentanol, and hex-3-en-1-ol in tomato juice. Varietal and harvest differences and processing effects. J. H. Johnson, W. A. Gould, A. F. Badenhop and R. M. Johnson, jun. (*J. agric. Fd Chem.*, 1968, **16**, 255–258).—Concentrates of the volatiles of tomato juice were obtained by vac-steam distillation, extraction of the distillate, containing dissolved $(NH_4)_2SO_4$, with ether and evaporation of the extract to small bulk. The concentrates were analysed by g.l.c. for iso- and optically active amyl alcohols (considered together), n-pentanol, and *cis*-hex-3-en-1-ol. Varietal and seasonal variations are reported for 10 varieties of tomatoes. The total amounts of the three constituents were 7·4–40·2 ppm. Processing losses varied with the variety and the compound. (13 references.)
P. S. ARUP.

Tea, coffee, cocoa

Quality of coffee in Kenya/Tanzania. J. A. N. Wallis (*Café*, 1967, **8**, Nos. 1/2, 2–25).—A review. (208 references.) C.V.

Utilisation of spent coffee grounds from soluble coffee industry. C. P. Natarajan and N. G. Rao (*Indian Coff.*, 1968, **32**, 25–29).—Essentially, the volume of waste available at a site is the answer to worthwhileness of recovery. The following % average is the chemical composition: oils and fatty acids 13·9, proteins, amino-acids and complexes 14·2, reducing sugars 5·80; the composition of the fatty acid fraction is 2·0, 2·3 myristic acid; 28·8, 23·3 palmitic acid; 4·5, 6·5 stearic acid; 1·6, 2·5 arachidic acid; 0·2, 0·0 behenic acid; 18·9, 19·4 oleic acid, and 44·0, 48·0% linoleic acid for *C. Arabica* and *C. Robusta* respectively. Heating value of the dry grounds is compared with that for coal, oil or wood; this value is higher than that of wood, approx. ⅔ that of coal and ½ that of oil. C.V.

Production of tea. Marshall's Tea Machinery Co. Ltd. (Inventor: F. A. Hopper) (B.P. 1,097,661, 2.8.63).—After the tea is fired, essential oil extracted from tea leaf is introduced into the tea. The oil can be extracted from the tea leaf while the latter is being subjected to a conventional tea-producing process.
S. D. HUGGINS.

Instant coffee product. Procter and Gamble Co. (B.P. 1,099,810, 3.1.67. U.S., 3.1.66).—The water-sol. extract which produces little or no coffee foam or scum when reacted with water contains 50–500 ppm by wt. of a substituted di- or tri-carboxylic acid having 3–6 C and containing ≮1 hydrophobic substituent of formula $RCH_2\cdot$ or $RCOO\cdot$, where R is a hydrocarbon group of 9–21 C e.g. the reaction product of malic acid and stearoyl chloride or of Na malonic ester and lauroyl chloride. S. D. HUGGINS.

Milk, Dairy Products, Eggs

Measurement of the quantitative effects of inherent and environmental factors in the composition of the milk of individual cows and of herds, with particular reference to lactose content. J. P. Walsh, J. A. F. Rook and F. H. Dodd (*J. Dairy Res.*, 1968, **35**, 91–105, 107–125).—I. The quant. estimation of the effects of various factors on milk lactose from analyses of the milk of individual cows within herds is described. Potential lactose content in the milk of individual cows is predicted from the observed K/lactose ratio in the milk. The difference between the predicted potential and the actual lactose content is partitioned into fractions that are attributed to effects due to age, inter-quarter difference and changes with stage of lactation. The results of the application of the method to two commercial herds are presented. (27 references.)

II. The above method is applied to the measurement of the effects of genetic or environmental factors on the lactose content of milk of eight commercial herds. (10 references.) M. O'LEARY.

Soil and climatic factors controlling caesium-137 content in New Zealand milk. J. R. Bray and R. H. Jackman (*N.Z. Jl Sci.*, 1968, **11**, 352–362).—Lack of positive correlation between rainfall and ^{137}Cs in the milk suggested that soil factors were also involved. Results from nine different areas revealed that, relative to rainfall, the ^{137}Cs in milk collected from areas with volcanic, allophanic soils is greater than that from non-volcanic areas. Two factors [%C and rate of release of fixed K (K_c)], largely dependent on soil mineralogy, were correlated ($P < 0.02$) with milk-Cs over a two-year period, and when each factor was combined with rainfall an even closer correlation ($P < 0.001$) was obtained. Varying fixation of Cs by soil colloids rather than varying concn. of competing K is the critical factor governing milk-Cs content; this is confirmed by the positive correlation ($P < 0.001$) between K_c and relative decrease in milk-Cs arising from the decline of ^{137}Cs after the 1965 fall-out max. The indices (rainfall × %C) and (rainfall/K_c) should permit prediction of milk-Cs values in unmonitored areas. (15 references.) W. J. BAKER.

Methionine as a precursor of methyl sulphide in cows' milk. J. R. Dunham, G. Wood, R. Bassette and M. C. Reddy (*J. Dairy Sci.*, 1968, **51**, 199–201).—The Me_2S content of milk from cows fed either alfalfa (lucerne) hay alone, lucerne hay plus 26 g/day 90% *d,l*-methionine, or lucerne hay plus 5 g/day S was determined by chromatographic analysis. Methionine yielded more ($P < 0.005$) Me_2S in milk than S or hay alone. There was no significant difference in the Me_2S contents of the milks of cows fed hay or S. *In vitro* fermentation of methionine, methionine sulphoxide, and methionine sulphone each with ground lucerne and rumen fluid inoculum resulted in the production of more Me_2S ($P < 0.01$) than did ground lucerne with rumen fluid. In the first 5 h of fermentation more ($P < 0.01$) Me_2S was produced from methionine and methionine sulphoxide than from methionine sulphone. After 6 h the level of Me_2S produced from methionine sulphone approached that produced from the other amino-acids. (14 references.) M. O'LEARY.

Influence of calcium salts on milk acidity. Ngo-Loi and P. F. D'yachenko (*Pishch. Tekhnol.*, 1968, No. 3, [64], 80–81). C.V.

Proteolytic activity of *S. lactis*, *S. cremoris*, *S. diacetilactis*, *L. helveticus* and *L. casei*. J. Doležálek (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E15**, 59–67).—The causes of proteolysis of albumin in sterile skimmed milk maintained at pH 5·4 by addition of $CaCO_3$, inoculated with pure cultures of the named organisms and maintained at 30° over 30 days, were investigated. Liberated amino-acids were determined by column chromatography. With all cultures, the amino-acids liberated in greatest proportion were glutamic acid, proline and leucine. Differences between cultures were mainly quant. in nature, the proteolytic activity of the Streptococcus spp. being less than that of the Lactobacilli. Notable qual. differences were that *S. cremoris* did not liberate aspartic acid, *L. helveticus* did not liberate aspartic acid or phenylalanine, with *S. lactis* free histidine and lysine were not present until after 30 days of proteolysis, and arginine was liberated only by *L. casei*. (In English.) E. C. APLING.

Linkage between the peptide and sugar moieties in cow's κ-casein. A.-M. Fiat, C. Alais and P. Jollès (*Chimia*, 1968, **22**, 137–139).—Preliminary studies of the peptide–sugar linkages in whole κ-casein and in the κ-caseinoglycopeptide part of casein suggest the presence of an *O*-glycosidic linkage between threonine and galactosamine. (18 references.) (In English.) M. SULZBACHER.

Coagulation of milk proteins. N. K. Rostrosa and P. F. D'yachenko (*Pishch. Tekhnol.*, 1968, No. 3, [64], 78–79).— C.V.

Rôle of lysine residues in the coagulation of casein. R. D. Hill and B. A. Craker (*J. Dairy Res.*, 1968, **35**, 13–18).—Treatment of casein with dimethylaminonaphthalene sulphonyl chloride inhibited its coagulation by rennin. The effect was due to substitution on lysine side-chains of the κ-casein fraction. Inhibition was complete when 2–3 lysine residues/κ-casein mol. were blocked. Other properties of the κ-casein, such as the release from it of non-protein nitrogen by rennin and its ability to stabilise α_s- and β-caseins in the presence of Ca^{2+}, were not affected. The evidence indicates that lysine side-chains on κ-casein take part in the coagulation of rennin-treated casein. (11 references.) M. O'LEARY.

Seasonal variation in the viscosity index and adhesive strength of casein from the milk of individual cows. C. R. Southward and R. M. Dolby (*J. Dairy Res.*, 1968, **35**, 25–30).—The viscosity of casein samples from six cows showed a general decline from August to May while the adhesive strength showed a general increase. The casein from one of the cows had a lower than average viscosity, which is attributed to the presence of a rare genetic variant of α-casein. The variations in viscosity and adhesive strength are due to lactational rather than seasonal changes. M. O'LEARY.

Factors affecting the viscosity of caseinates in dispersions of high concentration. J. F. Hayes, P. M. Southby and L. L. Muller (*J. Dairy Res.*, 1968, **35**, 31–47).—Physical effects of various cations on caseinate dispersions of high concentration over a range of temp. and pH were studied. In the presence of Ca and Sr, casein viscosity decreased rapidly at 30 to 40° and a gel which re-liquefied on cooling formed at 50–60°. This phenomenon was not observed in the presence of Ba, Al or Mg. Conditions for reversible gel formation to occur are high concentration of Ca caseinate, Ca content of ~ 1% of the protein and pH range 5·2–6·0. (27 references.) M. O'LEARY.

Identification and quantitative determination of 2-furfurol in sterilised concentrated milk. R. G. Arnold and R. C. Lindsay (*J. Dairy Sci.*, 1968, **51**, 224–226).—Quant. g.c. showed that the amount of 2-furfurol in sterilised concentrated milk increased rapidly through 26 weeks storage at 27°. After 26 weeks the amount of 2-furfurol in the milk exceeded its flavour threshold (10–12·5 ppm). M. O'LEARY.

Spectrophotometric method for determination of heat-activated sulphydryl groups of skim-milk. M. Koka, E. M. Mikolajcik and I. A. Gould (*J. Dairy Sci.*, 1968, **51**, 217–219).—A description is given of an adaptation of the 5,5′-dithiobis(2-nitrobenzoic acid) reagent method for the above spectrophotometric determination. The activation of SH groups in heated skim-milk was found to follow first order reaction kinetics, with rate constants of 0·078, 0·142, 0·384 and 0·805 for 75, 80, 85 and 90°, respectively. (10 references.) M. O'LEARY.

Determination of molybdenum in milk with zinc dithiol. R. E. Stanton and A. J. Hardwick (*Analyst, Lond.*, 1968, **93**, 193).—The sample is submitted to wet oxidation with HNO_3–H_2SO_4, the mixture is heated to fuming and, when cool, is diluted with water to < 10 M in H_2SO_4. Ppt. of $CaSO_4$ is allowed to settle for 12 h and the Mo is then determined in an aliquot of the clear solution by the dithiol procedure used for soils, etc. (*Idem, ibid.*, 1967, **92**, 387), except that standards are prepared with 4 M-H_2SO_4 and without addition of Fe. Standard deviation for ~ 25 μg of Mo was ± 3·4 μg (25 results). W. J. BAKER.

Optimum dilution of milk and liquid milk products in the analysis of [degree of] fat dispersion. A. F. Andreyev and V. D. Surkov (*Pishch. Tekhnol.*, 1968, No. 3, [64], 71–73).— C.V.

Modification of the acid degree value test for lipolytic rancidity in milk. A. C. Hunter, J. M. Wilson and G. W. Barclay (*J. Dairy Res.*, 1968, **35**, 19–24).—The modification consists of using an increased quantity of BDI reagent to overcome the difficulty of extracting the fat of milk from newly-calved cows and of milk having a high fat content. M. O'LEARY.

Method for determining volatile acids in cultured dairy products. W. L. Hempenius and B. J. Liska (*J. Dairy Sci.*, 1968, **51**, 221–222).—Samples of the products are steam distilled and the first 100 ml fraction of the distillate is collected. HOAc content is then determined by conventional titration against NaOH or by g.c. M. O'LEARY.

Nutrition and cancer; experiments with milk. J. Jacquet, C. H. Huynh and S. Saint (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 112–122).—The prophylactic effects of milk against the develop-

ment of cancerous tumours are demonstrated in feeding experiments with rats. (32 references.) P. S. ARUP.

Nutritive values of milk and milk products. Fat-soluble vitamins in milk and milk products. S. Y. Thompson (*J. Dairy Res.*, 1968, **35**, 149–169).—A review is given of papers published from January 1962 to January 1967. (~ 130 references.) M. O'LEARY.

Effect of γ-irradiation on milk vitamins. I. Milostić (*Bull. scient. Cons. Acads RSF Yougosl.*, 1967, **12**, 334).—The vitamin contents of milk, γ-irradiated milk and thermally processed milk were compared. The results show that milk pasteurised by ionising radiation (^{60}Co) is preferable to thermally processed milk. The changes in organoleptic properties are discussed. (In English.) W. E. ALLSEBROOK.

Folic acid activity of some milk foods for babies. J. E. Ford and K. J. Scott (*J. Dairy Res.*, 1968, **35**, 85–90).—Folic acid activity of samples of human milk and of raw and pasteurised cows' milk was similar, equivalent to about 54 μg folic acid/l. Values for goats' milk were about 6 μg/l. Values for various reconstituted commercial baby milks ranged from 9 to 65 μg/l. M. O'LEARY.

Quality of commercial buttermilks. T. W. Keenan, F. W. Bodyfelt and R. C. Lindsay (*J. Dairy Sci.*, 1968, **51**, 226–227).—Data on flavour, microbial quality, acetaldehyde and diacetyl contents, and volatile acidity are presented for buttermilks from 10 regional dairies. M. O'LEARY.

Change in the properties of the fat globule membrane during the concentrating of milk. G. C. Cheeseman and L. A. Mabbitt (*J. Dairy Res.*, 1968, **35**, 135–147).—Studies indicated that the formation of the casein micelle–fat globule complex during boiling of milk in a climbing-film evaporator is due to a combination of the κ-casein components of the micelles and the fat globule membrane. The reaction is not significantly affected by increases in concn. of individual constituents or by changes in temp. or pH. The high whey retention of curds made from re-diluted conc. milks indicates that curd properties, other than fat retention, are also altered during milk concn. M. O'LEARY.

Effect of dietary fat on the production of fatty acids in goat milk. I. Effect of amount of dietary fat on butterfat content and fatty acid composition of the milk. II. Effect of the supplementation of low-fat diets by C_{18} fatty acids on the composition of blood plasma and milk. J. Delage and P.-M. Fehr (*Annls Biol. anim. Biochim. Biophys.*, 1967, **7**, 437–444, 445–457).—I. In 16 dairy goats, transfer to a low-fat diet during the second of four periods of 28 days (of which 7 days were regarded as the adaptation period) led to a drop in milk yield. At the same time the fat content of the milk and the proportion of C_{18} fatty acids in the fat decreased and the proportion of shorter-chain acids (C_{10}–C_{16}) increased. The low-fat diet had no apparent effect on wt. gain, but although the fatty acid composition of the milk returned to normal when oil was added to the diet, production of milk and butterfat remained low during subsequent periods. (26 references.)

II. Results of a latin square-design experiment on four dairy goats with one low-fat diet and three fat-free diets with or without the addition of stearic or linolenic acid are reported. Milk fat content and yield and lipid and cholesterol ester contents of blood plasma varied directly with intake of dietary fat. Modification of the composition of the dietary fat led to similar changes in the fatty acid composition of milk and plasma triglycerides. Addition of stearic acid to the diet resulted in an increased % of stearic and oleic acids in the butterfat; linolenic acid induced an increase in the proportions of all the C_{18} acids. (36 references.) E. C. APLING.

Milk lipids. I. Fatty acids of cows' milk fat and fat globule membrane lipids. J. Hladík and L. Forman (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E15**, 69–74).—Comparative analyses (g.c. of the Me esters) of the fatty acid composition of fat globule membrane lipids and of raw milk fat are tabulated for three milk samples. The membrane lipids contained more palmitic, and less lauric, capric, caprylic and oleic acids than the raw milk. Total saturated and mono-enoic acids in globule membrane lipids were 62·9–64·5 and 28·4–29·5% and in raw milk were 59·7–60·5 and 31·9–32·9%, respectively. (In English.) (14 references.) E. C. APLING.

Influence of seasons on the chemical composition of milk fat. J. Doležálek, L. Forman and I. Hodaňová (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E16**, 19–26).—Analyses of the fatty acid composition of milk fat (by g.l.c. of the Me esters) are reported for 24 samples of bulk milk drawn at fortnightly intervals. 25 different fatty acids were determined, and the most significant variations with season appeared to be in the contents of palmitic acid (25–41%; min. in Jan., max. in Aug.) and stearic acid (4·1–9·8%; min. in Dec. and April and max. in Feb. and Aug.). Saturated acids comprised ~ 70% of the total acids from Nov. to Mar. and ~ 60% from April to Oct. Variations in composition were especially marked during the winter season. The importance of these variations in relation to the consistency and keeping quality of butter is emphasised. (In English.) (11 references.) E. C. APLING.

Determination of degree of fat hardening, in high fat cream and butter by specific heat capacity [measurements]. A. K. Avvakumov and G. V. Tverdokhleb (*Pishch. Tekhnol.*, 1968, No. 1, [62], 168–170).— C.V.

Continuous low temperature preparation of cream in butter manufacture by churning. G. V. Tverdokhleb and E. M. Mal'yarova (*Pishch. Tekhnol.*, 1968, No. 3, [64], 82–86).— C.V.

Fatty acids of 'drawn' butter stabilised by antioxidants after prolonged storage. A. N. Valeyeva and M. I. Gor'yaev (*Pishch. Tekhnol.*, 1968, No. 1, [62], 41–44).— C.V.

Coefficient of the dilution of butter serum. IV. E. Pijanowski, K. Klonowska and S. Zmarlicki (*Roczn. Technol. Chem. Żywn.*, 1967, **14**, 135–147).—The use of the coeff. of the dilution of butter serum in studying the relationship between the acidity of butter serum and the acidity levels of the respective buttermilk or sour cream was investigated. T. M. BARZYKOWSKI.

Fatty acid composition of Turkish and German butters. M. Metin (*Milchwissenschaft*, 1968, **23**, 276–278).— C.V.

Effects of variations in pH, of the removal of calcium and of the addition of sulphur-bond inhibitors on the rate of setting of renneted milk. W. Tuszyński, J. Burnett and G. W. Scott Blair (*J. Dairy Res.*, 1968, **35**, 71–83).—Reduction of pH from 6·66 to 6·18 did not affect the coagulation time or max. firmness of renneted skim-milk, but the rate of firming up was reduced and subsequent softening of the curd was accentuated. Similar effects were observed as a result of a reduction in Ca content. The addition of *N*-ethyl maleimide, a reagent specific for sulphydryl groups, had no effect. (30 references.) M. O'LEARY.

Relationship between calcium equilibrium and coagulation of renneted milk. W. Tuszyński and K. Zielinska (*Milchwissenschaft*, 1968, **23**, 208–211).—Increases in sol. Ca and P in whey, relationship to pH values of renneted milk, speed of coagulation, finer curd, etc., were studied by chemical, rheological and photometric methods. With decreasing pH values max. values for speed of coagulation were attained as well as greater firmness of curd. (17 references.) C.V.

Influence of vibration on cheese formation. B. G. Mirgorodsky (*Pishch. Tekhnol.*, 1968, No. 2, [63], 82–85).— C.V.

Cheddar cheese manufacture. R. Scott (*Process Biochem.*, 1967, **2**, No. 2; 5–10; 23–28).—A quite detailed review. (53 references.) C.V.

Quality improvement in Emmental and Gruyère cheese by inoculation with *Streptococcus faecalis* in low-count raw-milk. J. A. Kurmann (*Milchwissenschaft*, 1968, **23**, 193–198).—Improvement in body, openness and aroma is claimed. (35 references.) C.V.

Carbon dioxide production by *Leuconostoc citrovorum*. B. Holmes, W. E. Sandine and P. R. Elliker (*Appl. Microbiology*, 1968, **16**, 56–61).—Using a Gilson differential respirometer it was found that this organism produced 500–900 μl CO_2 in 6·5 h from milk whereas using non-fat milk containing 0·33% yeast, 800–1500 μl were produced. Cell extracts of *Streptococcus cremoris*, *S. lactis*, *Lactobacillus lactis*, *L. casei* and *L. helveticus* also enhanced gas production from 20 to 70%. Autolysates of these bacteria present in the ripening of certain cheeses may stimulate *Leuconostoc citrovorum* to produce gas resulting in the slit-open defect in Cheddar cheese. Yeast extract (I) caused increased acid and gas production per cell but did not increase growth. One metabolic source of CO_2 was the decarboxylation of pyruvate produced during catabolism of citric acid; this was stimulated by I by 16%. (18 references.) C.V.

Biochemical changes in 'Queso blanco' cheese during storage at high temperatures; its potential for developing countries. L. G. Siapantos (*Diss. Abstr.* B, 1967, **28**, 408–409).—Only small no. of lactose-fermenting clostridia were present in 5-day old cheese but these increased to very large no. after storage for a month at 80–100°F. Under these conditions the lactose content of the cheese decreased from 2·5% to 0·13% in 2 months; the volatile matter included butyric, acetic, propionic and formic acids, butyl-,

isopropyl- and ethyl-alcohols, acetone and acetaldehyde in decreasing order of concn. Heat-sealing the cheese in polythene–cellophane packets in a vac. caused gas to accumulate in 2–3 weeks, increased sol. fatty acids by 200–300%, and resulted in slight proteolysis of β-lactoglobulins but not of casein. No food-poisoning organisms were found. The most acceptable flavour was obtained by storage for 1 month at 80–100°F. During the manufacture of the cheese, lactic, tartaric, citric and phosphoric acids in specific proportions were used as coagulating agents at 180°F. The relationship between titratable acidity of the milk and the acid required was linear and indirect. Possible modifications and improvements in manufacturing detail are discussed.
A. G. POLLARD.

Kinetics of diffusion of salt in cheese. T. Jakabowski (*Milchwissenschaft*, 1968, **23**, 582–588).—A formula is developed which enables the NaCl content in cheese dry matter to be calculated from the salt content of the brine. Various shapes and volumes of cheese 1 cm^3–5000 cm^3 are considered. (36 references.) C.V.

Sensitive method for quantitative determination of nitrite in cheese. A. Lembke, O. Moebus and F. Wasserfall (*Milchwissenschaft*, 1968, **23**, 211–213).—Guajazulene is used instead of α-naphthylamine. Comparative tests show that the new method gives better reproducibility. C.V.

Cheesemaking. Alfa-Laval A.B. (B.P. 1,094,440, 14.7.66. Swed., 14.7. and 9.9.65 and 2.2.66).—The milk is partially dehydrated to a dry solids content of 13–60% by wt., and cooled to a temp. suitable for cold renneting; rennet is added, the mixture warmed to effect curdling and the curd divided into pieces. Any whey present is removed. The heating of the milk after renneting is effected by steam injection or high frequency radiation. By this process, lactose can be removed as crystals at the cooling stage, also the whey-removal step can often be omitted.
S. D. HUGGINS.

Edible Oils and Fats

Characteristics of the fatty acid composition and biochemistry of some fresh-water fish oils and lipids in comparison with marine oils and lipids. R. C. Ackman (*Stud. Fish. Res. Bd Can.*, [1967], 1968, Pt 2, 465–480; *Comp. Biochem. Physiol.*, 1967, **22**, 907–922).—The fatty acid composition of oils from fresh-water sheepshead, tullibee, maria, and alewife were compared with those from the marine species Atlantic herring and cod. Total C_{16} and C_{18} fatty acids were higher in the fresh-water fish. Palmitic acid comprised ~ 60% of total saturates in both types; total di- and tetra-enoic acids were twice, and trienoic acid 3–4 times, as high in the fresh-water oils as in the marine oils. The ratio of total linolenic to total linoleic acids was lower in the fresh-water oils. (61 references.)
E. G. BRICKELL.

Quantitation problem in the open tubular gas chromatography of fatty acid esters from cod liver lipids. R. C. Ackman, J. C. Sipos and P. M. Jangaard (*Stud. Fish. Res. Bd Can.*, [1967], 1968, Pt 2, 379–385; *Lipids*, 1967, **2**, 251–257).—Although esters of saturated and monounsaturated fatty acids of marine origin can be quantitatively analysed on open tubular g.c. columns with a flame ionisation detector, there are serious losses of the long-chain (C_{18}–C_{22}) highly unsaturated fatty acids of marine oils on the column. Correction factors, derived from comparisons of chain-length composition and I_2 values, are suggested which permit reasonably accurate analyses. (53 references.) E. G. BRICKELL.

Spectrophotometric determination of linoleic and linolenic acids in walnut oil. V. I. Dorodneva and A. L. Shinkarenko (*Pishch. Tekhnol.*, 1968, No. 1, [62], 1771–1772).— C.V.

Determination of the fatty acid composition of 'oleic fat'. A. N. Valeyeva and M. I. Gor'yaev (*Pishch. Tekhnol.*, 1968, No. 2, [63], 33–39).— C.V.

Distribution of the fat content of various oil plants. II. Linseed, mustard and soyabean. W. Schuster (*Fette Seifen AnstrMittel*, 1968, **70**, 155–159).—The fat contents of a number of commercial plants were determined, and the tabulated results are related to the plant variety, location and the year of growth. The following min. and max. values were obtained: rape 33·7–49·9%, sunflower (seed) 49·3–63·7%, sunflower (fruit) 29·0–42·0%, linseed 29·8–46·7%, mustard 24·1–38·4% and soyabean 9·8 to 23·9%.
G. R. WHALLEY.

Determination of caesium-137 in oil-bearing seeds and fruits. A. Seher and G. Bürjes (*Fette Seifen AnstrMittel*, 1968, **70**, 53–58).—A method is described for the determination of the ^{137}Cs content of oils derived from plants and seeds, and includes procedures for phosphate removal and the co-pptn. of the Cs. The method is preferable to the usual ashing technique, and has less interference from other materials. Ion-exchange is used with $K_2[CoFe(CN)_6]$ for the enrichment of the ^{137}Cs, which is subsequently estimated by an impulse-measuring technique. The max. error of the method was −3·4 to +0·2% when model determinations were carried out on linseed and palm kernel oils. (15 references.)
G. R. WHALLEY.

Determination of tocopherols in oils and fats. Influence of the tocopherol content of groundnut and soyabean oils on the course of oxidation of the oils on heating. I. H. E. Schmidt (*Fette Seifen AnstrMittel*, 1967, **69**, 913–916).—A sensitive method is described for the determination of the α-, γ- and δ-tocopherol content of groundnut oil, in which the components are isolated from the unsaponifiable matter by t.l.c. and then estimated photometrically after spraying with a solution containing 0·1% $FeCl_3$ and 0·25% 4,7-diphenyl-1,10-phenanthroline. When groundnut oil is heated there is a reduction in the total tocopherol content which affects subsequent oxidation, and this is followed by use of 4-hexylresorcinol. The blue dye thus obtained is identified as a trimethine deriv.
G. R. WHALLEY.

Determination of tocopherols in oils and fats. Influence of the tocopherol content in groundnut and soyabean oils on the course of oxidation of the oils on heating. II. H. E. Schmidt (*Fette Seifen AnstrMittel*, 1968, **70**, 63–67).—The influence of the total tocopherol content at 70° was investigated by following the changes in the peroxide and thiobarbituric acid values of the oils, and by the hexylresorcinol test. Accurate estimations show that refined groundnut oils containing 100 and 70 ppm of γ- and α-tocopherol when heated to 70° for 175 h contain no free tocopherol, and these oils assume a yellow coloration with absorption max. at 460, 260 and 269 nm. These are the absorption max. exhibited by the γ- and α-tocopherol quinones. (11 references.) G. R. WHALLEY.

Determination of tocopherols in oils and fats. Influence of the tocopherol content of groundnut and soya oils on the course of oxidation of the oils on heating. III. H. E. Schmidt (*Fette Seifen AnstrMittel*, 1968, **70**, 159–161).—The experimental techniques used are described in detail, and include methods for determining the tocopherol content, method of heating the oils, preparation of the unsaponifiable matter, t.l.c. of tocopherols, photometry of the tocopherols, the hexylresorcinol test and its modification, and the preparation of dyestuffs from glutaconic dialdehyde and hexylresorcinol.
G. R. WHALLEY.

Oxidation of lipids in full-fat soyabean meal on heating. J. Pokorný, H. Zwain and G. Janíček (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E18**, 55–60).—Oxidative changes in the fat of soyabean meal during heating at 105–180° for 15–300 min. were investigated. Fat changes were not appreciable < 130°, but deterioration was rapid at higher temp. Peroxide values were irregular, but at higher temp., acid value and benzidine and thiobarbituric acid values rose with increasing time and temp. of heating. (In English.) (13 references.) E. C. APLING.

Pressing sunflower husks in a closed vessel. N. N. Dovgal' and V. P. Borod'yansky (*Pishch. Tekhnol.*, 1968, No. 3, [64], 91–94).— C.V.

Effect of copper and iron ions on the autoxidation of fats. J. Pokorný, S. S. Kondratenko, H. Zwain and G. Janíček (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E17**, 93–114).—The autoxidation of lard, groundnut and sunflowerseed oils at 60° in the presence of added Cu or Fe (as chlorides or myristates) was studied. Autoxidation was followed by determinations of peroxide value (taken as indicator of primary oxidation products) and of benzidine and 2-thiobarbituric acid values (taken as indicators of secondary oxidation products). The pro-oxidant effect of Cu was much greater than that of Fe, but the effect of myristates, though similar, was not identical to that of chlorides. Max. reaction rate was little affected, but the ratio of secondary products to peroxide content increased with concn. of Cu (and, less definitely, Fe). Probable reaction schemes are discussed. (In English.) (76 references.)
E. C. APLING.

Hydrocarbons in pig lard. A. Rutkowski and W. Korzeniowski (*Roczn. Technol. Chem. Żywn.*, 1967, **14**, 51–57).—T.l.c. and g.l.c. analysis of the 0·11% of unsaponifiable matter present in the lard showed that it consists of about 45% sterols, 42% hydrocarbons and 13% di- and tri-terpenic alcohols. Examination of the hydro-

carbon fraction confirmed the presence of hydrocarbons with chain length from C_{19} to C_{35}. Most abundant are C_{28} hydrocarbons (16% of the total quantity), followed by C_{26} (12), C_{24} (11), C_{25} (10), C_{27} (9), C_{23} (8) and C_{29} (7%). T. M. BARZYKOWSKI.

Effect of the degree of unsaturation on the course of fat autoxidation. J. Pokorný, H. Zwain and G. Janíček (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E19**, 53–67).—Studies of the course of autoxidation are reported for nine fats of varying degrees of unsaturation (iodine values 39–182). Peroxide values were determined at intervals over up to 80 days, and peroxide decomposition products were estimated on the basis of benzidine and thiobarbituric acid values and by column chromatography. The initial content of double bonds affects not only the initial and max. rates of autoxidation, but also the ratio of peroxides to peroxide decomposition products. Probable reaction mechanisms are discussed. (In English.) (15 references.) E. C. APLING.

Composition and properties of margarine blends. III. Content of some trace elements. IV. Stability against autoxidation. M. Karvánek, E. Mareš, I. Zeman and J. Pokorný (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E15**, 75–80, 81–87).—III. Mean values of Cu, Fe, Ni and Mn found in 30 samples (five in each successive month) were 14·1, 76·7, 11·6 and 3·6 μg/100 g of fat, respectively. Variations from month to month (i.e., blend to blend) were small and significant differences due to blend were found only for Cu and Mn. (In English.)

IV. Results of stability tests are reported for 30 margarine blends. Stability was determined by the Schaal test at 60° (iodometric determination of peroxide value at suitable intervals, with induction period taken as the time necessary to reach a value of 100 mequiv./kg). Induction periods varied from < 10 days (25% of samples) to 20–150 days (25% of samples). No simple relationship between iodine value and stability was observed, but average values of linoleic acid content of the least, average and most stable samples were 5·4, 3·9 and 2·5%, respectively. Individual variations (from 1·7–7·3% for the middle group) were, however, too large for prediction of stability from linoleic acid content. (In English.) (13 references.) E. C. APLING.

Meat and Poultry

Composition of food. III. Nutritive value of beef from intensively reared animals. J. M. Harries, A. W. Hubbard, F. E. Alder, M. Kay and D. R. Williams (*Br. J. Nutr.*, 1968, **22**, 21–31).—A general survey. The *longissimus dorsi* had more non-protein N and more nicotinic acid, but less iron and riboflavine than the superficial digital flexor muscles. Also there was less vitamin A and carotene in the liver samples in the intensively reared animals as compared with the controls. The limitations of the experiment must be kept in mind but otherwise little significant difference was noted between the two groups. (27 references.) C.V.

Browning and associated properties of porcine muscle. J. A. R. Bowers (*Diss. Abstr.* B, 1967, **28**, 1783).—A split-plot design was used to study browning and associated properties of LD muscles from 12 Duroc and 12 China barrows. Pigs were (1) untreated, (2) sugar-fed 1 week before slaughter and (3) fasted 48 h, then exercised to exhaustion before slaughter. One half of each carcass was cooled at 30°F and the other at 42°F. Loins and hams were evaluated for 'quality'. Muscles chilled at 42°F had lower firmness, colour, and marbling scores and higher reducing sugar values than those at the lower temp. Multiple regression analyses indicated that ether extract and reducing sugar were the important factors affecting browning. F. C. SUTTON.

Discoloration in cooked ham. G. A. Gardner (*Process Biochem.*, 1967, **2**, No. 3, 49–52).—Discoloration can generally be attributed to the effect of an uneven distribution of curing salts on bacterial activity such as that of *Lactobacillus viridescens* which, in the absence of catalase, produces H_2O_2; this oxidises porphyrins. C.V.

Quality of broth from cooled and frozen chicken-meat muscle. K. N. Bogdanova (*Pishch. Tekhnol.*, 1968, No. 1, [62], 97–99).— C.V.

Meat treatment. Ajinomoto Co., Inc. (B.P. 1,089,084, 12.4.66. Jap., 12.4., 16.7. and 13.8.65).—The organoleptic properties of meat (and fish) are improved and an enhanced quality and yield of the meat when cooked are obtained by treatment with one or more basic amino-acids or their salts. Lysine, arginine, histidine or ornithine (≮ 0·02% by wt.) is added to the meat, either raw or partially cooked. Treatment of pork, beef, whale, cuttlefish meat, chicken, etc. is described. S. D. HUGGINS.

Sausage meats of high active vitamin content. Fabrica de Productos Quimicos y Farmaceuticos Abello, S.A. (B.P. 1,095,513, 20.5.65. Spain, 20.5.64).—Suitable animal and/or vegetable enzyme extracts are mixed with minced or sliced liver at 40–50° and pH = 7, so that partial proteolysis of the liver occurs. This gives a higher active vitamin content without unfavourably affecting the colour, taste or aroma. The treated liver is then mixed with further meat and additives to produce the claimed sausage meat. S. D. HUGGINS.

Curing emulsified meat products. Griffith Laboratories Ltd. (B.P. 1,095,517, 21.7.65. U.S., 7.8.64 and 15.3.65).—Accelerated curing, adaptable to automation, is achieved by emulsifying a meat mass, at pH < 7 (due to lactic acid) containing a curing salt including alkali metal nitrite and heating the emulsion, in presence of a non-shorting quantity of acid sufficient further to reduce the pH, in a casing at 93–149°, so that an internal temp. of ≮ 65° is attained. The cured meat is removed and cooled. A non-shorting quantity of lactone, e.g. glucono-δ-lactone, which slowly hydrolyses to edible acid, may be included in the mass to be emulsified, thus achieving the desired pH reduction at the curing stage. Colour formation is accelerated by adding an ene-diol (Na erythorbate). S. D. HUGGINS.

[A] and [B]. Method and machine for producing boneless comminuted meat. Stephen Arthur Paoli (B.P. 1,125,161–2, 27.8.65, [A] U.S., 2.9.64 and 27.8.65. [B] div. out of [A]).—The process is particularly applicable to gutted, unfilleted fish, and to poultry wings, drumsticks, etc., hitherto difficult to process economically. P.P.R.

Fish

Chemical and physical alterations of frozen whitefish and bovine skeletal muscles. A. A. Awad (*Diss. Abstr.* B, 1967, **28**, 1566).—Storage temperatures of −10° for whitefish muscle (**I**) and −4° for bovine muscle (**II**) were selected so that dramatic alterations would occur within short periods of frozen storage, i.e. 16 weeks and 8 weeks respectively. Results indicate that progressive insolublisation of myofibrillar proteins took place during frozen storage in both **I** and **II**. The solubility of sarcoplasmic protein in **II** gradually decreased over an 8-week storage period; yet with frozen **I**, the extractability of sarcoplasmic protein did not change significantly over a 16-week period. F. C. SUTTON.

Cholesterol content of fish fillets for the characterisation of the fish quality. J. Wurziger and G. Hensel (*Fette Seifen AnstrMittel*, 1967, **69**, 937–942).—In order to assess the quality of fish meat, a series of cholesterol and fat content determinations were carried out on a wide variety of fish and shell fish meats. The results show that the cholesterol content is independent of the fat content, and such determinations are of use in assessing fish meat quality in only a few cases. Similar results were obtained with commercial fish products. (10 references.) G. R. WHALLEY.

Influence of lipids on fish quality. R. C. Ackman (*Stud. Fish. Res. Bd Can.*, [1967], 1968, Pt 2, 353–365; *J. Fd Technol.*, 1967, **2**, 169–181).—Lipids in fish muscle influence product quality through interaction with other components. In frozen storage of lean fish, hydrolysis of phospholipids gives free fatty acids capable of interacting with protein to produce texture deterioration; rancidity is more commonly associated with lipids in fatty fish. The mechanism of these effects is discussed. (68 references.) E. G. BRICKELL.

Heavy metal ions and the development of rancidity in blended fish muscle. C. H. Castell and D. M. Spears (*J. Fish. Res. Bd Can.*, 1968, **25**, 639–656).—1–50 ppm of 10 different ions were added to blended muscle from fresh cod, haddock, flounders, redfish, herring, mackerel, scallops and lobster, and the resulting rancidities were determined by thiobarbituric acid values and odours. With some exceptions, Fe^{2+}, V^{2+} and Cu^{2+} were the most active catalysts in promoting lipid oxidation. Fe^{2+} was always more effective than Fe^{3+}, Cd^{2+} and Co^{2+}, and Zn^{2+} produced rancidity with the fatty species only. Ni^{2+}, Co^{3+}, Ce^{2+} and Mn^{2+} did not accelerate rancidity in any of the muscles. Relative rancidities produced in muscle of different species by a particular metal varied considerably. Frozen storage rendered the muscle less susceptible to subsequent metal-induced rancidity. E. G. BRICKELL

Oxidative metabolism of nonprotein nitrogen components by fish spoilage bacteria and their physiology of psychrotrophic growth during storage of fish (English sole). D. Bannerjee (*Diss. Abstr.* B,

1967, **28**, 2046).—Dominance of *Pseudomonas* spp. in English sole was shown to be due to their rapid growth at ice temp. and their efficient use of the N-containing substances in the water-sol. fraction of fish tissue. Nonprotein nitrogen material such as amino-acids and creatine was oxidised by these organisms yielding NH_3 and other substances characteristic of spoiling fish. Alanine oxidation was studied as this amino-acid was used preferentially by the spoilage bacteria. The spoilage *Pseudomonas* were found to be psychrotrophic growing over a range from 0 to 35°. Cells grown at 8° produced an alanine oxidase system which was active below 12°, indicating a temp.-induced psychrophilic oxidase which was produced in addition to the normal mesophilic oxidase system. It was assumed that a psychrophilic isozyme was involved. The general distribution of such inducible systems in psychrotrophic bacteria would explain their ability to shift to high activity at low temp. after a suitable lag phase period. F. C. SUTTON.

Nucleotide degradation and organoleptic quality in fresh and thawed mackerel muscle held at or above ice temperature. D. I. Fraser, D. P. Pitts and W. J. Dyer (*J. Fish. Res. Bd Can.*, 1968, **25**, 239–253).—Excellent correlation of taste with inosine monophosphate (**I**) and hypoxanthine (**II**) content, with various simple measures of I dephosphorylation, was obtained under various handling conditions including delayed icing, holding at elevated temp., and after thawing. U.v. absorption at 248 mμ of a Dowex-treated perchloric acid extract, and ratio of u.v. absorption of extracts at 251 mμ after Dowex treatment to that before treatment, proved equally as good indices of progressive quality loss to the unacceptability level as the more complex estimation of **I** or **II**. (27 references.) E. G. BRICKELL.

Incidence and growth of some pathogens in freshwater crayfish (*Procambarus clarkii* Girard). J. A. Barkate (*Diss. Abstr.* B, 1967, **28**, 1980).—In live, unhandled, S. Louisiana crayfish it was indicated that *E. coli* and fecal streptococci are normally found in the product taken from natural sources, and may be carried over during the processing of the tailmeat. At refrigeration temp. (5°) type E toxin was produced in 33 days in raw or cooked crayfish tailmeat or in crayfish étouffé. There was a definite relation between the pH of the product and inactivation of type E toxin. At pH 8·0–8·5 the toxin was inactivated. F. C. SUTTON.

Nutritional value of freshwater crayfish waste meal. R. T. Lovell, J. R. Lafleur and F. H. Hoskins (*J. agric. Fd Chem.*, 1968, **16**, 204–207).—Dried, waste material from crayfish processing plants (shell, muscle, viscera) was subjected to inorg. and org. analyses using atomic absorption spectrometry and conventional foodstuff analysis. The most accurate method for the determination of chitin in the meal was based on that of Black and Schwartz (cf. *Analyst, Lond.*, 1950, **75**, 185) with the use of cold instead of hot 5% HCl for decalcification. The meal contained 14·1% of chitin; of the total N, 19·8% was in the form of chitin. In feeding trials with rats, the digestibility coeff. for the chitin-free protein (~87%) was the same as that for methionine-supplemented soyabean meal. Full analyses are tabulated for ten samples of the meal. (11 references.) P. S. ARUP.

Food product. White Fish Authority and Herring Industry Board (Inventor: E. P. Sidaway) (B.P. 1,094,684, 12.5.65).—The consistency of pork sausages or pork luncheon meat is claimed for chopped mixtures of fillets of white fish, pork fat, rusk and water (46·9–75%, 10–30%, 1·5–15·0% and 1–10%, or 55–75%, 10–25%, 5–15% and 1–3·5% or 46·9–70%, 12–30%, 5–11·6% and 1·5–10%, respectively). Up to 70% of rusk is replaceable by soya flour. The products, together with flavourings, etc., are stuffed into casings to form sausages, smoked if desired, and cooked. S. D. HUGGINS.

Spices, Flavours, etc.

Determination of traces of volatile solvents in spice oleoresins by gas chromatographic analysis of the headspace. B. Labruyère, C. Olsthoorn-de Leeuw and F. Smeenge (*Perfum. essent. Oil Rec.*, 1968, **59**, 206–211).—A headspace g.l.c. technique was used for the determination of small quantities of Me_2CO, MeEtCO, n-hexane, n-nonane and C_6H_6 in viscous spice extracts, which are immiscible with water and contain essential oils. A repeatability of 7·2% was obtained where the resin contained 47 ppm of Me_2CO, although differences of 28·8% (in duplicates) were obtained, due to heating and solvent loss from the small samples. Reversing the carrier gas after the appearance of the last solvent peak prevents column contamination. Me_2CO has been found to be a natural constituent of pepper. (14 references.) G. R. WHALLEY.

Quantitative colorimetric determination of vanillin with mixed heteropoly acids in food products. M. F. Kachalova, V. N. Khrustal'yova and V. V. Kozlov (*Pishch. Tehknol.*, 1968, No. 2, [63], 188–190).—10 references. C.V.

5′-Guanylic acid. Kyowa Hakko Kogyo Co. Ltd. (Inventors: H. Samejima, H. Teranishi and M. Ito) (B.P. 1,091,365, 19.5.65. Jap., 19.5.64).—The title acid is obtained by heating an aq. solution of guanosine di- and tri-phosphates, of pH 11–14, preferably 12–13, at 100–120°. It is used for making chemical seasonings. S. D. HUGGINS.

Recovery of 5′-guanylic acid. Merck & Co. Inc. (B.P. 1,093,463, 1.6.65. U.S., 11.6.64).—Used as a flavouring agent, the product is obtained by mixing 1,4-dioxane with an aq. acidic solution, pH 0·5–5 (1·0–3), containing 5′-guanylic acid (**I**) to produce a crystalline dioxanate (**II**). An aq. solution of **II** reacts with NaOH to give guanosine-5′-monophosphate (Na salt). The process can be used to recover **I** from enzymic ribonucleic acid hydrolysates. S. D. HUGGINS.

***l*-Perillalcohol.** Nippon Terpene Chemical Co. Ltd. (B.P. 1,094,875, 9.12.64. Jap., 9.12.63 and 19.6.64).—Used as an intermediate for l-perillaldehyde and perillartine, the food flavouring agents, the claimed alcohol is obtained by oxidising β-pinene (**I**) at 30–100° in presence of a Pb salt of formula $Pb(OOC{\cdot}C_nH_{2n+1})_4$ and of a saturated fatty acid of formula $C_nH_{2n+1}COOH$ (**II**) where $n = 0$–18. The resulting products are then heated at 150–300° after, optionally, the removal of part of the saturated fatty acid and/or water. The Pb salt can be formed *in situ* by reaction of red lead with **II**; a myrtenyl acylate, a *l*-nopinene glycol diacylate or a *p*-menthene-1,7-diol acylate can be used in place of **I** as a starting material. S. D. HUGGINS.

Xanthine-type compounds. Kyowa Hakko Kogyo Co. Ltd. (B.P. 1,099,564, 7.2.66. Jap., 8.2.65).—Used as flavouring material, 5′-xanthylic acid, xanthosine or xanthine is produced by culturing a micro-organism capable of producing the compounds, e.g. *Pseudomonas fluorescens*, in an aq. nutrient medium, containing 30 μg–2 mg decoyinine/l, under aerobic conditions at 20–40° and pH 5·5–9·0. S. D. HUGGINS.

Nucleoside-5′-monophosphates. Asahi Kasei Kogyo K.K. (B.P. 1,099,858, 1.7.65. Jap., 2.7.64).—Used as seasoning materials, the title products are obtained by reacting nucleosides (e.g. uridine, cytidine, xanthosine) with Me_2CO, POX_3 ($POCl_3$) and water, adding pyridine or t-amine 1–4 h after the start of the reaction (carried out at −20 to +50°), adding water to produce a 2′,3′-*O*-isopropylidene 5′-nucleotide and hydrolysing the product with acid. S. D. HUGGINS.

Preservatives

Sulphurous and ascorbic acids in urine; Mechanism of antioxidant action. Y. D. Tagunkov (*Pishch. Tehknol.*, 1968, No. 1, [62], 56–59).—11 references. C.V.

Influence of phosphate compounds on certain fungi and their preservative effect on certain cherry fruit (*Prunus cerasus* L.). F. J. Post, W. S. Coblentz, T. W. Clou and D. K. Salunkhe (*Appl. Microbiology*, 1968, **16**, 138–142).—Na-hexametaphosphate (**I**) Na-tripolyphosphate, (**III**), Na-tetraphosphate (**III**), and tetra-Na-pyrophosphate (**IV**) were examined to determine their effect on fresh cherries and to ascertain their antimycotic effects against the most common fungal spoilers *Penicillium expansum*, *Rhizopus nigricans* and *Botrytis* sp. **III** appeared to be the most effective and had the greatest *in vitro* effect, 10%, (applied as a dip) inhibiting fungal growth for 30 days at 34°F and 94% R.H. Untreated controls showed growth within 14 days. The order of effectiveness was **III** > **I** > **II** > **IV**. (11 references.) C.V.

[A] **Cooking poultry with phosphate.** [B] **Poultry treatment with solid phosphate.** Albright & Wilson (Mfg) Ltd. (B.P. 1,087,289–90, 23.9.66. U.S., 27.9.65).—[A] Cooked poultry is prepared by cooking in the solutions claimed in [B] at ≮ 167°F.

[B] Uncooked poultry is preserved by coating with a finely-divided solid non-cyclic phosphate of Na or K, or a mixture of these, having a ratio of metal oxide to P_2O_5 of 0·9 : 1–2 : 1 and allowing contact until ≮ 0·1% of the phosphate is absorbed into the raw tissue. Na tripolyphosphate is claimed as suitable for the treatment. S. D. HUGGINS.

Pesticides in Food

Effect of direct steam heating and vacuum treatment on levels of

pesticide residues in milk. R. A. Ledford, J. H. Chen and W. F. Shipe (*J. Dairy Sci.*, 1968, **51**, 219–220).—The passage of milk containing pesticide residues through commercial steam distillation–vacuum processing equipment, originally developed to expel volatile off-flavours, resulted in the removal of the following % of the pesticides: dieldrin 3·0; heptachlor 0; lindane 23·8; and DDT (*p,p'*-) 8·4. M. O'LEARY.

Effect of various physical treatments on certain organochlorine hydrocarbon insecticides found in milk fat. M. Kroger (*J. Dairy Sci.*, 1968, **51**, 196–198).—Freeze-drying and mild deodorisation treatments were shown to be ineffective in eliminating heptachlor epoxide and dieldrin from butteroil. Steam-deodorisation at 180–195° and 0·01–0·5 mm Hg for 5 h completely removed the insecticides from the butteroil. (14 references.) M. O'LEARY.

Gas chromatographic analysis of Ciba C-9491 [*O*-(2,5-dichloro-4-iodophenyl)-*O*,*O*-dimethylphosphorothioate], its oxygen analogue, and its phenolic hydrolysis product in sweet maize and milk. M. C. Bowman and M. Beroza (*J. agric. Fd Chem.*, 1968, **16**, 280–283).—Ciba C-9491 (**I**), the dimethylphosphate analogue (**II**), and the phenol (2,5-dichloro-4-iodophenol) (**III**) were extracted from maize into C_6H_6 and from milk into $COMe_2$ and then CH_2Cl_2, the latter extract being defatted by solvent partition between hexane and MeCN, and then dissolved in C_6H_6. **I** was separated from **II** and **III** by elution of the extracts with benzene through a column of SiO_2-gel and one of Al_2O_3 successively; **III** was then eluted from the SiO_2-gel column with $COMe_2$ and **II** from the Al_2O_3 column with MeOH. G.l.c. of **I** and **II** was carried out with flame photometric, and of **III** with electron-capture detection. Recoveries of **I** were 93–100%, of **II** 82–93%, and of **III** 53–71%. The method was sensitive to < 0·01 ppm of **I**, **II**, and **III**. P. S. ARUP.

Effect of soil systemic insecticides on flavour and residue in coffee. J. G. Rodriguez, J. E. Fahey and C. E. Fernandez (*J. agric. Fd Chem.*, 1968, **16**, 276–278).—No foreign odour or flavour was detected in tests on coffee made from beans from trees that had been treated with phorate, disulfoton or Bidrin. No insecticide residues were found in the green beans, harvested 40 days after the treatment. P. S. ARUP.

Behaviour of DDT in potatoes during commercial and home preparation. F. C. Lamb, R. P. Farrow, E. R. Elkins, R. W. Cook and J. R. Kimball (*J. agric. Fd Chem.*, 1968, **16**, 272–275).—Commercial washing operations removed ~ 20%, lye peeling + washing removed ~ 94%, and in home prep. procedures washing + peeling removed > 91%, of the small residues of DDT isomers present in potatoes that had been grown in soil treated for 5 years with DDT. No decrease occurred when potatoes with skins were boiled or pressure cooked, or during storage at 7° for 5 weeks. (23 references.) P. S. ARUP.

Thin-layer chromatographic–enzyme inhibition procedure for organophosphorus pesticides in plant extracts without elaborate clean-up. C. E. Mendoza, P. J. Wales, H. A. McLeod and W. P. McKinley (*Analyst*, *Lond.*, 1968, **93**, 173–177).—The procedure is applicable to 50-g sub-samples of apple, beet, carrot, lettuce, pea and potato. Azinphos-methyl, carbophenothion, diazinon, ethion, malathion, mevinphos and parathion are extracted with MeCN and then partitioned into hexane. A 10-ml aliquot (≡ 150 mg of sample) of hexane extract is concentrated to 0·5 ml and submitted to enzymic treatment with steer-liver homogenate and 5-bromoindoxyl acetate spray at pH 8·3 on a Kieselgel G-HR-coated t.l.c. plate, using acetone–hexane to develop the chromatogram. Pesticides are revealed as white spots on deep blue background. Advantages of this semi-quant .method are indicated; it permits screening for any of the seven pesticides in routine samples without elaborate clean-up. W. J. BAKER.

Fumigant residues in wheat and flour: solvent extraction and gas chromatographic determination of free methyl bromide and ethylene oxide. S. G. Heuser and K. A. Scudamore (*Analyst*, *Lond.*, 1968, **93**, 252–258).—Procedures are described for extraction of traces of the two compounds, from partly aerated wheat or flour, with Me_2CO–H_2O (5 : 1) at ~ 20°, and for their determination by g.l.c. Methods are also given for determining (1) the efficiency of extraction (by a combination of g.c. and chemical absorption) and (2) the loss of fumigant caused by reaction with cereal constituents before recovery. The recoveries of free fumigant average ~ 95% for flour and > 98% for wheat grains; the sensitivity is 0·3 ppm. (13 references.) W. J. BAKER.

Food Processing, Refrigeration

Progress of food irradiation work. (*Fd Irrad.*, 1968, **8**, No. 3, 2–51).—Research work carried out in the various countries is summarised, future programmes also being indicated. Publications are listed. C.V.

Significance of temperature and duration of sterilisation. M. S. Aminov (*Pishch. Tekhnol.*, 1968, No. 1, [62], 104–105).— C.V.

[Multistage] drying of food forage maize grain. A. S. Korchak and V. I. Zhidko (*Pishch. Tekhnol.*, 1968, No. 1, [62], 75–76).— C.V.

Seed drying: Influence of preliminary heating. V. I. Zhidko and V. I. Aleinikov (*Pishch. Tekhnol.*, 1968, No. 2, [63], 58–61).— C.V.

Intensified drying of persimmon. M. A. Grishin and R. R. Dzhindzholiya (*Pishch. Tekhnol.*, 1968, No. 3, [64], 152–154).— C.V.

Treatment of high fat cream by high frequency electric current. E. P. Shalapugina, V. V. Vologdin, M. S. Kovalenko and V. V. Sychov (*Pishch. Tekhnol.*, 1968, No. 3, [64], 87–90).— C.V.

Cream processing. J. Rothwell (*Process Biochem.*, 1968, **3**, No. 1, 19–24).—A review. (13 references.) C.V.

Peculiarities found in fat hydrogenation, using a rotary apparatus. P. I. Chechevitsyn and V. A. Maslikov (*Pishch. Tekhnol.*, 1968, No. 1, [62], 94–96).— C.V.

Hydrogenated fat hardening. I. V. Nikonov (*Pishch. Tekhnol.*, 1968, No. 2, [63], 40–44).— C.V.

Processing of raw onion to improve microbial quality of the dehydrated product. J. S. Gurvitz (*Diss. Abstr.* B, 1967, **28**, 1566–1567).—A jet of compressed air was used to remove the paperskin and K_2SO_3 was used in all dilution media. Five different heating methods and exposures to two org. oxides were studied as a means for reducing initial contaminations of onions. The most effective method in yielding a final product low in standard plate count and having satisfactory pungency and colour, was to heat whole onions with humid air (130°F) for 60 min., in a through-air-flow chamber which gave 132-fold reduction in the number of micro-organisms with about 8% reduction in alliin and a bleaching effect on the colour. F. C. SUTTON.

Modern refrigeration. II. Preservation of food by cold. W. B. Gosney (*Jl R. Soc. Arts*, 1968, **116**, 474–491).—A review of modern refrigeration practice is given, covering causes of food spoilage (attack by micro-organisms, biochemical reactions, loss of water), chilling and freezing, storage temp., refrigerated spaces, insulation, refrigerated transport, commercial and domestic refrigeration and some special requirements. (14 references.) E. G. BRICKELL.

Blast freezing and cold storage of bakery products. (*Mod. Refrig. Air Control*, 1968, **71**, No. 845; 55–56, 58, 61).—Production economies, resulting from the introduction of this approach, are described together with the technical details of the plant and equipment. C.V.

Freezing-concentration of sucrose solutions. Z. Niedzielski, S. Zagrodzki and A. Kułagowska (*Roczn. Technol. Chem. Żywn.*, 1967, **14**, 69–81).—Freezing concn. of a 15% model solution of sucrose was studied, and out of several methods, two were selected as the most profitable. In one, freezing out of water was carried out spontaneously without stirring the solution, while in the other a rotating crystalliser (2 r.p.m.) was used, with initial inoculation of the solution with very fine ice crystals. The latter method proved the more efficient, the sucrose solution being concentrated to 60% compared with 55% in the former method. Optimum freezing was attained at −25° carried out for 90 min. (10 references.) T. M. BARZYKOWSKI.

Fruit storage project. Anon. (*Mod. Refrig. Air Control*, 1968, **71**, No. 844; 45).—The provision of additional space to provide for 1425 tons of refrigerated and controlled atm. fruit storage using an existing building is described in some detail. When complete the whole unit will hold 14,000 tons fruit. C.V.

Changes during thermal and hydrothermal processes. VII. Volatile carbonyl compounds in apple juice concentrated by freezing and in apple cider. VIII. Volatile carbonyl compounds in celery processing. IX. Volatile carbonyl compounds in carrot processing. X. Effect of various types of ovens in the origination of volatile carbonyl compounds in bread during the baking process. XI. Changes in the nutritive value of soya meal with the addition of certain saccharides. T. Hrdlička (VII–XI), V. Vík (VII), G. Janíček (VII, XI), D. Čurda (VIII, IX), J. Pavelka (VIII, IX), E. Honischová (X) and P. Čuda (XI) (*Sb. vys. Šk. chem.-technol. Praze*, *Potraviny*, 1967, **E14**, 39–43, 45–48; **E15**, 55–58; **E16**,

63–70; **E18,** 29–54).—VII. A simple paper chromatographic comparison is presented of the volatile carbonyl components of 'apple cider' (an apple juice concentrated by evaporation at 55°, and sweetened with sugar) and a freeze-conc. apple juice. The comparison indicates a general loss of volatile carbonyls, but an increase in furfural content, as a result of the conventional evaporation process; it is concluded that freeze-concn. is very suitable for production of concentrates of good aroma. (In English.) (24 references.)

VIII. Semi-quant. (paper chromatographic) determinations of individual volatile carbonyl components of fresh, dried and canned celery are reported. Content of volatile carbonyls was increased as a result of the canning process, but gradually reduced during storage for 14–42 days. (In English.)

IX. Volatile carbonyls were isolated from fresh carrots and from canned carrots immediately after processing and following storage for 7 and 21 days. Paper chromatographic comparisons showed an increase in carbonyls on sterilisation with a decrease on subsequent storage. (In German.) (10 references.)

X. Comparative analyses of aroma volatiles are reported for breads baked in grate, steam-tube drawplate and travelling tray ovens. Main variations were in the amounts of 2- and 3-methylbutanal, furfural, HCHO, MeCHO, Me_2CO + propanal, and hexanal. The amount of aroma volatiles was highest in bread baked in the grate oven and least in that from the travelling ovens; the former was also rated highest by organoleptic evaluation. (In English.) (20 references.)

XI. The effects of heat treatment (30–120 min. at 130–160°) on soya meal in the presence of additions of 1–50% of glucose, sucrose, maltose and starch were studied. Colour of the ethanolic extract increased and total and water-sol. N and digestibility by pepsin and trypsin all decreased with increasing time and temp. of heating. The extent of these changes was increased slightly by additions of starch and sucrose, moderately by maltose and greatly by glucose; additions of maltose and glucose also caused a fall in pH on heating. The major changes are a result of Maillard reaction which is promoted by glucose and maltose but not by sucrose and starch. Increase in extract colour and decrease in protein digestibility may be used as indicators of undesirable changes in nutritive value resulting from heat treatment. (In English.) (24 references.)

E. C. APLING.

Cold storage of summer tomatoes. S. A. Khan (*W. Pakistan J. agric. Res.*, 1967, **5,** 43–46).—Semi-ripe tomatoes could be kept in suitable condition for canning during 15–20 days at 0° and R.H. 85–90%. The storage life of ripe tomatoes was ~ 5 days shorter.

P. S. ARUP.

Effect of freezing temperature on the hydration capacity of defrosted meat. J. Brendl and S. Klein (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E16,** 39–48).—Determinations of hydration capacity are reported for meat frozen at −190°, −70° and −15° after defrosting either at 2–4° over 24 h or rapidly in a water bath at 30°. All the freezing treatments reduced hydration capacity compared to that of unfrozen meat, and the greatest reduction resulted from freezing at −15°. Hydration of meat frozen at −70° was unaffected by defrosting procedure, but meat frozen at −190° was better after rapid thawing, and meat frozen at −15° was best when thawed slowly. (In German.) (18 references.)

E. C. APLING.

Effect of some salts on the hydration capacity of freeze-dried meat. J. Brendl, S. Klein and M. Kociánová (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E14,** 49–63).—'Bound' water in reconstituted ground freeze-dried meat was determined by two methods: (1) separation of 'free' water by centrifugation at 1000 *g* for 5 min., and (2) a pressure method (*cf.* Gran, et al., *Biochem. Z.*, 1953, **325,** 1). The effect of NaCl and/or sodium hexametaphosphate (**I**) on rehydration of freeze-dried meat was studied by determinations of 'bound water' in ground samples rehydrated in various solutions for 30 min. In undenatured freeze-dried meat the amount of bound water was increased by NaCl (up to 5%, fresh meat basis), by **I** (up to 1%) or by a combination of NaCl and **I**. Heat treatment for 3 h at 40, 60 or 80° before freeze drying progressively reduced water-binding capacity and response to additions of **I** (no response after 3 h at 80°). Measurements of water bound after reconstitution with, e.g., 2% salt and 2% salt + 0·5% of **I**, might serve as useful criteria in the evaluation of thermal denaturation in freeze-dried meats. (In German.) (19 references.)

E. C. APLING.

Ultrasound backscatter in fresh and thawed animal tissue. M. Freese and D. Makow (*J. Fish. Res. Bd Can.*, 1968, **25,** 605–606).—Backscatter of high-frequency ultrasound from beef and fish tissue, measured using a pulsed transducer ringing at 3·16 MHz for 1·5 μsec, differed markedly for fresh tissue and for tissue which had been frozen and then thawed. Noise amplitude appears to correlate with 'freezing history' and general physical condition, and is highly dependent on the rate of freezing and the number of times a sample has been frozen.

E. G. BRICKELL.

Air blast freezing of fish. G. P. Hill, J. H. Merritt and J. K. des Bordes (*Process Biochem.*, 1967, **2,** No. 6, 42–43).—Fish fillets placed in closed Al-trays, stacked in horizontal supports and frozen by air blast at 12 ft/sec at −32° were studied to investigate the influence of contact between fish and lid upon rate of cooling. Variations in lid pressure, in contact area and in air space were studied.

C.V.

Effect of stage of rigor and of freezing–thawing processes on storage quality of refrozen cod. J. A. Peters, W. A. MacCallum, W. J. Dyer, D. R. Idler, J. W. Slavin, J. P. Lane, D. I. Fraser and E. J. Laishley (*J. Fish. Res. Bd Can.*, 1968, **25,** 299–320).—Freezing before rigor mortis is preferable to freezing post-rigor, and thawing by means of microwaves is preferable to thawing by means of water. Free fatty acids contents increased sharply as a result of thawing and refreezing, and the rapid increase continued during the first 2 months of frozen storage. Taste panel scores correlated significantly with free fatty acids (1% level) and with extractable protein (0·5% level). (38 references.)

E. G. BRICKELL.

Preserved fruits manufacture. J. Ballerini (B.P. 1,089,729, 7.10.66).—Fruits, particularly bananas, are preserved by steeping them (preferably after pricking with a needle), for a long period in an aq. solution of $CaCO_3$ and $(NH_4)_2CO_3$ until they are saturated, citric acid and alum being optionally added. The treated fruit is then washed with cold water and plunged into hot, conc. sugar syrup.

S. D. HUGGINS.

Sterilised, dehydrated and powdered foodstuffs. T. Nakamura (B.P. 1,093,508, 12.5.66. Addn. to B.P. 1,032,204).—The fish, vegetables, fruit, etc., are treated with an aq. medium at or above the b.p. of the latter (e.g. by steaming in an autoclave); the softened product is then immersed in EtOH (or aq. EtOH) at −30° or lower to replace at least part of its water content by EtOH, dried in air and powdered. The process eliminates undesirable smells from the foodstuff and gives a product which can be packaged and stored without addition of preservative.

S. D. HUGGINS.

Packaging

New processes and aids in packaging techniques. W. K. Sterling (*Fette Seifen AnstrMittel*, 1968, **70,** 122–126).—Modern developments in packaging technology are discussed, and include equipment for heat sealing, the formation by air of different plastic shaped containers, carton sealing, fully pneumatic drum filling of liquids, semi-continuous labelling and case-banding.

G. R. WHALLEY.

Filling packages with loose products. V. F. Pet'ko and G. D. Gal'perin (*Pishch. Tekhnol.*, 1968, No. 3, [64], 108–113).—(10 references.)

C.V.

Insect-resistant shipping bags. R. L. Brett (*Soap chem. Spec.*, 1967, **43,** No. 12; 100, 102, 104, 274).—Multi-wall paper bags treated with piperonyl butoxide (**I**) and pyrethrins (**II**) are recommended as suitable containers for foodstuffs, with adequate protection against insect attack. Such bags, which also have tape-over-stitch, or pasted-open-mouth closures, prevent the actual entry of insects. The F.D.A. permit the use of such bags of 50 lb or more capacity, with a coating of 50 ± 10 mg of **I** and 5 ± 1 mg of **II** per ft^2 of paper. The tolerances of these materials on the contents of the bag are 10 ppm of **I** and 1 ppm of **II**.

G. R. WHALLEY.

[Modern applications of plastics in] bottling and transport of beverages. O. Pasquarelli (*Materie plast.*, 1967, **33,** 1309–1314).—Recent Italian developments in the use of plastics in handling of beverages and alcoholic liquids in large tanks, and applications of bottle crates are reviewed, with many illustrations. Individual crate manufacturers' products are described briefly.

C. A. FINCH.

Plastics in the dairy industry. V. Cleaning and disinfection of plastic surfaces. I. Special characteristics. G. Wildbrett (*Fette Seifen AnstrMittel*, 1968, **70,** 185–196).—Detailed descriptions of the cleaning of plastic surfaces are given. Angle of contact measurements showed that the adhesion of fatty soil on to PVC and polyethylene surfaces is greater than the corresponding

adhesion of the cleaning solution usually used in the cleaning of metal or glass surfaces. Soft PVC surfaces retain water which is in contact with the cleaning solution and the amount of liquid retention depends mainly on the nature and quantity of the plasticiser and the wash temp. The special requirements for cleaning plastic surfaces are considered and disadvantages are discussed. (112 references.) G. R. WHALLEY.

Packing of whole and cut loaves. E. Schultze (*Brot Gebäck*, 1968, **22**, 21–24).—The properties of packaging foils are briefly reviewed and examples of their use in the German baking industry are illustrated. E. C. APLING.

Corrosion and protection of aluminium [containers] in the storage of processed vegetables. I. I. Ar'yamova, I. A. Makolkin and M. S. Sytilin (*Pishch. Tekhnol.*, 1968, No. 3, [64]) 66–68).— C.V.

Determination of the permeability of packing materials and of the diffusion coefficient for oxygen by the coulometric method. J. Davídek, D. Čurda and J. Severová (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E19**, 25–34).—The method is based on coulometric determination of the O_2 by electroreduction on a powdered Ag cathode against a Pt anode in 5 N-KOH as electrolyte. Ar is used as the inert gas; the effluent from the brass diffusion cell is passed at const. velocity through the cathode and the consumption of electricity (applied voltage 1·5 V) recorded. Application to the determination of the O_2 permeability of foil materials and to the determination of the permeability and diffusion coeff. of O_2 in hardened fats is described. Relative standard deviation (for foils) was ~ 10%. (In English.) E. C. APLING.

Inhibition of iron corrosion in citric and malic acid solutions using agar. L. E. Chernenko, E. P. Gilinskaya, I. N. Putilova and I. N. Smirnova (*Pishch. Tekhnol.*, 1968, No. 3, [64], 49–51).— C.V.

Extractive photometric determination of small amounts of tin in tinned material using quercetin. M. Karvánek, D. Čurda and A. Kelarová (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E19** 45–52).—After wet oxidation of the sample with H_2SO_4/HNO_3, the solution is buffered to pH 4–6 with NaOAc solution, 0·4% ethanolic solution of quercetin (5 ml) is added and the Sn–quercetin complex is extracted into 2 × 20 ml of ethyl acetoacetate/CCl_4 (4 : 1). The combined extracts are filtered, diluted to 50 ml with EtOH, and the absorption is measured at 435 nm. Within the range 0–70 μg of Sn per 50 ml, the calibration curve is linear and the max. error is ± 5%. (In English.) (21 references.) E. C. APLING.

Packaging meats. American Can Co. (B.P. 1,097,637, 3.10.66. U.S., 21.10.65).—The freshness and red colour of meat is preserved for relatively long periods in a meat package consisting of raw meat, a first sealed container of O_2-permeable material containing the meat and a second sealed container of O_2-impermeable material surrounding the first container. The meat and the interiors of both containers are freed of O_2, flushed with an inert gas (N_2) and the containers are then sealed. S. D. HUGGINS.

Insecticidal compositions. Cooper, McDougall and Robertson Ltd. (Inventors: J. P. Brooke and P. H. Lomax) (B.P. 1,098,838, 12.11.65).—Used to coat packaging materials, such as paper or cardboard, the compositions contain at least one insecticide, e.g. pyrethrum, neopynamin, allethrin, dissolved and/or dispersed in a film-forming synthetic resin consisting completely or predominantly of a copolymer of styrene with a dibasic unsaturated aliphatic acid half-ester and, optionally, a synergist based on the methylenedioxy phenyl structure e.g. piperonyl butoxide. Tests were carried out on coated and uncoated cartons; the insecticidal coatings greatly reduced attack by *Plodia interpunctella*, *Angasta kuhniella* and *Tribolium casteneum*. S. D. HUGGINS.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Nutrition and civilisation. T. Mayer (*Trans. N.Y. Acad. Sci.*, 1967, **29**, 1014–1032).—A review. (23 references.) C.V.

Symposium on enrichment and fortification of foods with nutrients. [A] **Enrichment and fortification of dairy products and margarine.** S. T. Coulter and E. L. Thomas. [B] **Enrichment and fortification of cereals and cereal products with vitamins and minerals.** C. L. Brooke. [C] **Fortification of cereals and cereal products with proteins and amino-acids.** G. K. Parman. [D] **Enrichment of sugar and sugar products.** J. M. Navia. [E] **Enrichment of fruit products and fruit juices.** R. H. Bunnell. [F] **Enrichment of special dietary food products.** L. J. Filer, jun. (*J. agric. Fd Chem.*, 1968, **16**, 158–162, 163–167, 168–171, 172–176, 177–183, 184–189).—

[A] An account is given of the enrichment of liquid low-fat and separated milk and margarine with vitamins A and D in the U.S.A., and milk fortification with thiamine, riboflavin, niacin, Fe and I_2. Action should be taken to permit the fortification of dried separated milk for domestic use. New standards and possible future developments are discussed. (26 references.)

[B] Enrichment of cereal products and flours with B vitamins and thiamine, niacin, riboflavin, Fe, vitamin A, etc. is described. (21 references.)

[C] Fortification of staple cereals to alleviate malnutrition in developing countries is described. The possibilities of using protein concentrates from groundnuts, cottonseed, coconut and soyabeans, and fish, milk and yeast proteins for the supplementation of various foods are discussed. Problems connected with supplementation and the possibility of the use of pure amino-acids are considered. (10 references.)

[D] The advantages of the enrichment of foods rich in sugar with minerals and vitamins, especially thiamine and niacin, in order to make them metabolically self-sufficient, are described. Supplementation with cariostatic phosphates is also recommended. (46 references.)

[E] A review is given of the nutritional and technological aspects of ascorbic acid enrichment of fruit products. (28 references.)

[F] A review, dealing mainly with additions of ascorbic acid, vitamin B_6, vitamin D, Ca, P, Mg, Fe and Zn to infants' foods. (28 references.) P. S. ARUP.

Engineering problems associated with the fermentation of hydrocarbons. I. A. E. Humphrey (*Chemy Can.*, 1968, **20**, 28–33).—Some potentially commercial hydrocarbon fermentations are described, e.g., microbial dewaxing of crude oil fractions, n-alkane oxidation by micro-organisms and production of single-cell protein, and yeast, the principal advantage of which is rapid mass doubling of various organisms, besides its economical application. Disadvantages of the system are costs and acceptability in food development. Engineering problems associated with single-cell protein production include oxygen transfer, heat transfer, oil dispersion, residual oil content of the cells, cell recovery, cell wall destruction and protein isolation. The applications of the single-cell proteins in animal and human foods are discussed. M. DUDLEY.

Methane as a carbon substrate for the production of microbial cells. G. Hamer, C.-G. Hedén and C.-O. Carenberg (*Biotechnol. Bioengng*, 1967, **9**, 499–514).—Soil *Methanomonas* was grown aerobically in submerged culture in order to study the possibility of producing protein from CH_4. A medium consisting entirely of mineral salts was used in a baffled, impeller agitated, sparged, batch fermentor with a sealed gas recirculation system. At O_2 concn. < 12·1 vol.-% (explosion limit), growth was not nutrient limited although cell densities were relatively low. To maintain higher densities with the same gaseous phase, more efficient contacting devices are necessary. The organism used required no additional growth factors. (17 references.) J. B. WOOF.

Interrelationships between fatty acid biosynthesis and acyl-lipid synthesis in *Chlorella vulgaris*. B. W. Nichols, A. T. James and J. Breuer (*Biochem. J.*, 1967, **104**, 486–496).—Fatty acid synthesis from [2-^{14}C] acetate by *Chlorella vulgaris* grown and incubated in the dark is limited almost exclusively to formation of saturated and monoenoic acids, whilst saturated and polyunsaturated fatty acids are rapidly synthesised in cells incubated in light. Two groups of lipids are present in both dark- and light-incubated cells. The first group, which contains phosphatidyl glycerol, monogalactosyl diglyceride, lecithin, and neutral glyceride, has a very high turnover rate for certain fatty acids. The second group comprises digalactosyl diglyceride, sulpholipid, phosphatidylethanolamine, and phosphatidylinositol, and has a slow turnover of fatty acids. The lipids with a rapid turnover of fatty acids may be involved in the sequences of saturated and unsaturated fatty acid synthesis. A classification of lipids based on their suggested functions is proposed. (21 references.) J. N. ASHLEY.

Food value of wool. F. B. Shorland and J. R. Matthews (*N.Z. Jl Sci.*, 1968, **11**, 131–136).—Preliminary results are reported for feeding trials with casein, finely-ground wool and wool solubilised in aq. Na_2S, pptd. with HOAc and dialysed (i.e. reduced wool), respectively, as source of protein for rats. Casein ensured normal growth, but ground wool was largely rejected from the beginning, and deaths after 11 days ended the trial. Animals fed on reduced wool did not gain wt. but survived during the 31-day trial, the wool being generally eaten eagerly. The wool was mixed with a protein-free diet containing essential nutrients. (19 references.) W. J. BAKER.

Improvement of protein value of cottonseed protein isolate with fish flour and skim-milk powder. I. A. Shaikh, M. Arshad, M. Y. Ikram-ul Huq and S. M. Ali (*Pakist. J. scient. ind. Res.*, 1967, **10**, 86–88).—The net protein-utilisation value of the isolate was increased from 41 to 80–90% by controlled addition of fish flour (**I**) or skim-milk powder (**II**), and the concentrate obtained is a cheap additive for infant or invalid foods. Suitable mixtures are 65·8 cottonseed protein isolate + 71·43 **I** + 362·77 g maize starch and 65·8 + 139·2 + 295 g when **II** replaces **I**. The former mixture has a protein value comparable with that of **II** + maize starch.
W. J. BAKER.

Effect of organic solvents on proteins extracted from groundnuts. N. J. Neucere and R. L. Ory (*J. agric. Fd Chem.*, 1968, **16**, 364–365).—Proteins extracted by buffer-dialysis from untreated groundnuts and from groundnuts that had been defatted with CCl_4, C_7H_{16}, or $COMe_2$, were compared by column chromatography on DEAC-cellulose with gradient elution with a phosphate buffer containing 0–0·6 M-NaCl. The solvent treatments reduced the solubility of the albumin and α-conarachin fractions; treatment with $COMe_2$ also reduced the solubility of the α-arachin fraction. Possible constitutional changes in the protein fractions are considered. (10 references.) P. S. ARUP.

Determination of the relative nutritive value of proteins. Factors influencing precision and validity. D. M. Hegsted, R. Neff and J. Worcester (*J. agric. Fd Chem.*, 1968, **16**, 190–195).—Relative nutritive values were estimated by the slope-ratio assay, using a computer for evaluation of the validity and precision of the results. Results obtained with rats using body wt. gain, body water and body N as measures of response were similar, and body water is the easiest to determine. In assaying the values of six proteins, reasonably satisfactory results can be obtained with the use of as few as nine rats per test protein, fed at three levels of each protein. (18 references.) P. S. ARUP.

Effect of heat on digestibility of leaf proteins. I. Toxicity of lipids and their oxidation products. F. H. Shah, Riaz-ud-Din and A. Salam (*Pakist. J. scient. ind. Res.*, 1967, **10**, 39–41).—Treatment of leaf-protein concentrates with human proteolytic enzymes at ~100° decreased their digestibility, probably due to oxidation of highly unsaturated lipids. These oxidation products and their polymers with proteins were toxic to trypsin, pepsin and enzymes in pancreatic extract. Digestibility of heated or fresh protein, however, increased when the protein was defatted with $CHCl_3$–MeOH.
W. J. BAKER.

Protein quality and riboflavin. R. K. Lakhanpal (*Diss. Abstr.* B, 1967, **28**, 1786). The effects of protein quality and riboflavin (**I**) on the relation between concn. of free amino-acids in plasma, riboflavin coenzymes and lipids in liver were studied by feeding weanling rats diets containing casein, gluten or gluten + lysine as protein sources, supplemented with two levels of **I**. The diets were adequate in all other known nutrients. The results indicate that although plasma amino-acids may have value in defining protein nutrition, other factors may be involved. Values for **I** coenzymes and lipids in tissues showed that supplementation of gluten with lysine did not decrease lipids or increase the coenzymes to values comparable to those observed in animals fed casein.
F. C. SUTTON.

Course of casein hydrolysis. I and II. T. Skrabka-Błotnicka (*Roczn. Technol. Chem. Żywn.*, 1967, **14**, 5–14, 15–26).—I. The kinetics of casein hydrolysis were investigated by determining the contents of amino-N and of protein in partial hydrolysates. By determination of the N-terminal and C-terminal amino-acids present in the original casein sample and in casein dissolved in 85% HCO_2H, it was established that under the reaction conditions HCO_2H alone does not induce the rupture of peptide bonds. (17 references.)

II. Free, N-terminal and C-terminal amino-acids were determined in partial hydrolysates and in their individual fractions, obtained by filtering hydrolysates through columns filled with G-50 or G-25 Sephadex. The amounts and compositions of free and bound amino-acids, the no. of peptide particles and N-terminal and C-terminal amino-acids in the individual fractions were calculated. The bonds most resistant to hydrolysis are those of lysine, phenylalanine, valine, leucine, isoleucine, methionine and proline; dicarboxyl- and hydroxy-amino-acids are the most easily liberated. (23 references.) T. M. BARZYKOWSKI.

Assay for amino-acids in foods. Assay of tyrosine. I. Chibata, H. Ito, S. Ishikawa and K. Kawashima (*A. Rep. Tanabe Seiyaku Co. Ltd, Tech. Pt*, 1967, **12**, T 182–T 186; *J. Jap. Soc. Fd Nutr.*, 1966, **18**, 37–41).—The optimum conditions for tyrosine analysis in the presence of carbohydrates were investigated. The highest assay value was given by hydrolysis at 120° for 15 h with 4 N-NaOH or -HCl, followed by chemical assay with 1-nitroso-2-naphthol. Alkaline hydrolysis is preferable when the carbohydrate content is high. In alkaline hydrolysis there is some degradation to a deriv. which nevertheless reacts as tyrosine with the colour reagent. (From English summary.) (10 references.) E. J. H. BIRCH.

Unclassified

Pepsin D, a minor component of commercial pepsin preparations. D. Lee and A. P. Ryle (*Biochem. J.*, 1967, **104**, 742–748).—Isolation and purification of pepsin D (**I**) are described. **I** accounts for ~ 10% of the enzymic activity in commercial prep. of pepsin. **I** resembles pepsin, has mol. wt. ~ 35,000, and has the same C-terminal amino-acid sequence and N-terminal isoleucine residue, but it has no phosphate residue. Like pepsin, it digests haemoglobin and gelatin, but it is twice as active in the clotting of milk. It has the same specificity as pepsin in its action on the β-chain of oxidised insulin. **I** is probably formed in commercial prep. of pepsin by activation of gastric pepsinogen D. (22 references.)
J. N. ASHLEY.

Asparaginic acid as a medium for dissolution of rennet. I. A. Mikhailus (*Pishch. Tekhnol.*, 1968, No. 1, [62], 53–55).— C.V.

Determination of proteolytic activity in ferment preparations. N. M. Pavlenko (*Pishch. Tekhnol.*, 1968, No. 2, [63], 191–192).— C.V.

Influence of low doses of γ-rays on the growth and some enzymic functions of *Streptococcus lactis*. H. Oberman and A. Makiedońska (*Roczn. Technol. Chem. Żywn.*, 1967, **14**, 83–107).—The sensitivity of *Streptococcus lactis* (isolated from cows' milk and used in various souring processes in food industry) to low doses of γ-ray radiation was studied. It was established that specimens surviving γ-irradiation retained their parental characteristics. In examined substrates no correlation was established between radiation inactivation of cells and their respiratory functions. It was corroborated that a lethal dose of γ-radiation for *Streptococcus lactis* is 350,000 rad and the L_{99} dose is 38,000 rad. (30 references.)
T. M. BARZYKOWSKI.

Food sanitation. R. L. Shelton, jun. (*Baker's Dig.*, 1968, **42**, No. 1, 40–42).—The U.S. Food and Drug Aministration (FDA) is maintaining an inspection programme based on the illegality of gross filth and is placing increasing emphasis on bacteriological indications of plant sanitation conditions. Bacteriological findings are used to determine whether a food has been prepared, packed or handled under sanitary conditions. I. DICKINSON.

Possible contamination of frozen foods by airborne particles. G. Setter (*Dt. LebensmittRdsch.*, 1968, **64**, 242–243).—The dangers of adsorption of airborne pollution on cooled foods or ice is pointed out. It is necessary to protect the food under these circumstances or at least to circulate the air. J. B. WOOF.

Effects of flue gases on foods and fodder. S. W. Souci (*Dt. LebensmittRdsch.*, 1968, **64**, 235–241).—The effects of exposure of food to smoke during curing or unintentionally during drying, roasting and baking operations are discussed. The extent of absorption of harmful constituents of the smoke depends on the type of food, subsequent processing, the type of fuel used and the operating conditions. Lead deriv. and polycyclic aromatic hydrocarbons are the most dangerous and a table of structures and properties of some of the latter compounds is given. (43 references.) J. B. WOOF.

Salmonellae associated with 'further processed' turkey products. F. L. Bryan, J. C. Ayres and A. A. Kraft (*Appl. Microbiol.*, 1968, **16**, 1–9).—These organisms isolated from swab samples were found in 12% of chilled, eviscerated carcass, in 27% of finished products and 24% of processing equipment. The same serotypes were found throughout the plant on any one visit and were recovered from 31% of the rinse samples taken from hands and gloves of the processing personnel; over 48 visits, they were found in 37. A greater no. of recoveries was obtained on days when freshly killed turkeys were being processed, 87%, as compared with 59% when frozen-defrosted carcasses were used. Among the 32 serotypes recovered, *S. sandiego* and *S. anatum* were the most frequent. Most of the serotypes have been associated with human salmonellosis and one implication is that these products have been inadequately refrigerated. (25 references.) C.V.

Spoilage bacteria in canned foods. I. Canned asparagus and thermal death time. Chau-Ching Lin, Bih-Keng Wu and Dar-Kuan Lin (*Appl. Microbiol.*, 1968, **16**, 45–47).—*Bacillus stearo-*

thermophilus was the causative organism in the Taiwan experimentation. The F_{250} and Z values of the isolates were 14·2 min. and −7·9°, respectively. (13 references.) C.V.

Bacteriological survey of the frozen prepared foods industry. III. Potato products. IV. Frozen breaded fish. B. F. Surkiewicz, R. J. Groomes [and A. P. Padron (III), L. R. Shelton, jun. (IV)] (*Appl. Microbiol.*, 1967, **15**, 1324–1331; **16**, 147–150).—III. During a series of inspections, 29 potato processing firms were visited; they produced 2544 finished products and 1654 samples were collected for examination. Results were somewhat obscured by the lethal effect of the dehydration temp. but line samples collected at each step reflected the conditions and provided bacteriological support for inspectional evidence of plant insanitation.

IV. Samples (604) from 573 finished products produced by 23 firms were bacteriologically examined. No more than 20% within each group produced under good conditions of sanitation were positive for *Escherichia coli* or coagulase-positive staphylococci. However it is noted that ~ 30% of the groups processed under poor sanitary conditions did not exceed these viable bacterial counts because of the effect of the terminal fry to which the product is subjected. C.V.

Detection of coliform organisms in dietetic foods. G. Obiger and E. Scheubner (*Milchwissenschaft*, 1968, **23**, 269–276).—A general discussion of suitable methods. (22 references.) C.V.

A 2,4-dinitrophenylhydrazine spray for the identification of aflatoxin B_1 on thin-layer chromatoplates. E. V. Crisan (*Contr. Boyce Thompson Inst. Pl. Res.*, 1968, **24**, 37–38).—A freshly prepared, filtered solution of 2,4-dinitrophenylhydrazine (2 g in 10 ml conc. HCl and 90 ml abs. EtOH) is a useful reagent for detecting concn. of ≮ 0·5 μg aflatoxin B_1, giving a yellow/orange colour. If the chromatoplate is first sprayed with conc. HCl/EtOH, aflatoxin B_1 in concn. ≮ 0·025 μg exhibits a distinctive yellow-green fluorescence in u.v. light. E. G. BRICKELL.

Detection of emulsifiers in foodstuffs. E. Kröller (*Fette Seifen AnstrMittel*, 1968, **70**, 119–121).—The detection of emulsifiers in foodstuffs is achieved by a t.l.c. method, using kieselgel G as the adsorbent and eluting with an 80/20 benzene–methanol mixture. Three emulsifiers based on sorbitol mono- and tri-esters of fatty acids are extracted from 100 g of sample with 150 ml of benzene at room temp. The R_F values for these emulsifiers are given for a wide variety of eluting mixtures, as well as a range of colour reactions. Some paper chromatographic methods are also discussed. G. R. WHALLEY.

Oxidation processes in unstable foodstuffs. V. Kyzlink (*Sb. vys. Šk. chem.-technol. Praze, Potraviny*, 1967, **E17**, 5–25).—A review of the chemistry of the most frequently occurring redox processes involved in undesirable changes in foodstuffs. (In English.) (10 references.) E. C. APLING.

Dessert gel. Marine Colloids Inc. (B.P. 1,096,328, 24.11.65. U.S., 24.11.64 and 26.1.65).—Carrageenan is extracted from a sea plant using an alkaline aq. medium at increased temp. Thus, *Eucheuma spinosum* or *Agardhiella tenera* is extracted with aq. $Ca(OH)_2$ solution [3·7–15% by wt. $Ca(OH)_2$ based on dry sea plant wt.] at pH 10·2–11·2 and 60–100° for 1–8 h, the pH being reduced to < 9 after 2 h by addition of acid. The product forms an elastic gel when dissolved in water, and together with flavourings etc. makes a satisfactory dessert. S. D. HUGGINS.

Acid-active stable amylase. Taisho Pharmaceutical Co. Ltd. (Inventors: J. Sawada, T. Misaki, M. Okabe, H. Yasui, K. Hanada and T. Okazaki) (B.P. 1,099,446, 14.3.66).—Useful as a clarifier in beer, a tenderiser in meat, as an enzyme for ripening materials etc., the claimed enzyme, superior to those obtained from *Aspergillus niger*, *A. aureus*, *A. oryzae* or *Paecilomyces varioti*, has amylolytic, dextrinogenic and saccharifying activities at pH 2·5–6·0 and heat stability at 50°. *Paecilomyces subglobosum* is pre-incubated on a sterilised liquid or solid medium for seed koji at 30° for 70 h under aerobic conditions. The koji is then inoculated and cultivated on a main medium at 30°, under aerobic conditions, until the amylase accumulates in the culture. It is then recovered by pptn. with EtOH at −20°, followed by drying. S. D. HUGGINS.

Artificial plant product. General Foods Corp. (Inventor: A. S. Szczesniak) (B.P. 1,099,820, 3.12.65).—Artificial fruits and vegetables, resembling the natural products, are obtained by contacting an aq. solution of a water-sol. alginic acid salt (≯5% by wt.) with a solution of edible alkaline earth metal ions to form an insol. alginate film at the interface of the solutions. The interface is maintained by a dialysis membrane, so that metal ions diffuse through the film at a constant rate of 3·5–11·5 mg of ion per g of alginic acid salt. The product is slowly and uniformly frozen to < 0° giving a fruit-like, cellular structure. If required, the alginate solution can contain sugars, starches, fats, gums or proteins. S. D. HUGGINS.

3.—SANITATION, WATER, etc.

Sonics: Synergistic effects in sonochemical sterilisations. R. M. G. Boucher, M. A. Pisano, G. Tortova and E. Sawicki (*Appl. Microbiol.*, 1967, **15**, 1257–1261).—Using *Bacillus subtilis* var. *niger* the combined action of ethylene- or propylene-oxide (**I**) with high intensity airborne sound waves (34·8 kc/sec) was examined. Reductions of ~ ½ of the time required for sterilisation by **I** alone were observed. The basic mechanism appeared to be more a physical (accelerated gas diffusion) than a chemical one. **Use of ultrasonic energy in assessing microbial contamination of surfaces.** J. R. Puleo, M. S. Favero and N. J. Petersen (*ibid.*, 1345–1351). Using the same organism, it was shown that ultrasonic energy was more reliable in recovering surface contaminants than mechanical agitation. (15 references.) C.V.

Dichlorodifluoromethane–ethylene oxide mixture as sterilant at elevated temperatures. Tien Szu Liu, G. L. Howard and C. R. Stumbo (*Fd Technol. Champaign*, 1968, **22**, 86–89).—The lethal effects of an atm. of ethylene oxide (12%) and dichlorodifluoromethane (88%) at R.H. 33% on the spores of *Bacillus subtilis* (exposed on glass or paper discs) were examined. The no. of min. taken to kill 90% of the spores (D values) ranged from 15 min. at 40° to 0·7 min. at 80°. A 10 D reduction in spore population would probably require 150 min. at 40° or 7 min. at 80°. By extrapolation the times required at 100° or 110° would be 92 sec or 42 sec, respectively. (14 references.) P. S. ARUP.

Death kinetics of spores of *Clostridium botulinum* 62 A on exposure to a dichlorodifluoromethane–ethylene oxide mixture at elevated temperatures. L. M. Kuzminski (*Diss. Abstr.* B, 1967, **28**, 1567).—A non-explosive, non-flammable mixture of ethylene oxide and dichlorodifluoromethane (12/88 wt.-%) was an effective sterilant especially at elevated temp. A preconditioning and exposure R.H. of 3% was most effective for destruction of *C. botulinum* spores with Oxyfume Sterilant 12. F. C. SUTTON.

Problems in the analysis of disinfectants. Testing the efficiency of disinfectants containing ampholytic surfactants using membrane filters. H. Bellinger (*Tenside*, 1968, **5**, 202–207).—Model experiments are described using solutions of disinfectants which contain ampholytic surfactants, and suspensions of *S. aureus* and *E. coli*. When disinfectants of this type are passed through membrane filters, some of the active matter is adsorbed, and the subsequent passage of the test organisms causes retarded growth when they are incubated on nutrient broth or agar. Rinsing the filter discs with sterile water causes little improvement. Only by the incorporation of a neutralisation mixture (3% Tween 80, 0·3% lecithin *ex ovo* and 0·1% Texapon N 25) into the sterile water and nutrient agar, can the disinfectant activity be accurately assessed. The effect of the additives on suspension tests is examined. G. R. WHALLEY.

Effects of various photoperiods on the life cycle and susceptibility of the housefly, *Musca domestica*, L., to insecticide residues. A. T. Fernandez (*Diss. Abstr.* B, 1967, **28**, 726).—Cultures of the housefly were established under photoperiods of light: dark ratios (*PR*) varying from 10/14 to 16/8 h, and some others in continuous light, the temp. and R.H. being constant throughout. With *PR* = 14/10 flies were consistently susceptible to DDT, endrin and dieldrin. With *PR* at 16/8 or 13/11, flies showed no consistent difference in susceptibility from those grown at *PR* 14/10. At *PR* 13/4 or 14/10 flies were the most susceptible to endrin residues. Susceptibility to DDT and dieldrin was lowest in flies grown with *PR* 15/9. Among flies reared with *PR* 14/10 relative susceptibilities, as based on LD_{50} values, diminished (approx. 4·5-fold) between the subjective dawn and 9 h later. All flies were more susceptible at the dawn than at 9 h afterwards, regardless of the *PR*. The onset of light or darkness probably is not a factor influencing susceptibility although an apparent rhythm in sensitivity to trichlorfon was indicated. Flies grown under shorter light periods developed more slowly from egg to adult stage, the reverse being the case with those reared under long-light periods. Females outlived males under all *PR* and those developing at *PR* 15/9 outlived all others. A. G. POLLARD.

Cannery effluent problems. D. Anderson (*Process Biochem.*, 1967, **2**, No. 4; 7–9, 15).—A general review; the question of water re-use, and the factors involved are also examined. C.V.

Gas chromatographic determination of Abate residues in water. F. C. Wright, B. N. Gilbert and J. C. Riner (*J. agric. Fd Chem.*, 1967, **15**, 1038–1039).—Abate is extracted from the acidulated sample with $CHCl_3$, and after washing with aq. NaOH the $CHCl_3$ extract is conc. for g.l.c. with flame ionisation detection. The emulsifier Triton X-100, used for preparing comparative standard dilutions, caused no interference. Average recoveries were ~ 70%. The min. detectable amount was 0·05 ppm. P. S. ARUP.

Determination of temporary and common [total] hardness in water in one test. A. I. Zinov'yev (*Pishch. Tekhnol.*, 1968, No. 1, [62], 175).— C.V.

Stabilised halogenosalicylanilide germicides. Stecker International S.p.A. (B.P. 1,085,617, 27.1.65. U.S., 29.1.64).—A germicidal mixture containing a 3,5,4′-trihalogenosalicylanilide, optionally together with a 5,4′-dihalogenosalicylanilide (halogen is Cl, Br or I) is stabilised against decomposition by addition of 5–45 wt.-% of e.g., 3,5-dibromo-3′-(trifluoromethyl)salicylanilide, which exerts a synergistic effect upon the activity of the germicide(s). F. R. BASFORD.

Crysanthemic acid esters. Sumitomo Chemical Co., Ltd. (B.P. 1,087,016, 6.7.65. Jap., 8., 11. and 15.7.64).—Used in insecticidal compositions, the title compounds have the formula R^5ACH_2-OCOBCH:CMe_2, where A is the imide ring (C_3HGO_2N), G being R^1R^2C or R^3R^4C:C and R^1–R^4 are H or Me, or R^3–R^4 can also be Ph; R^5 is H, Me or Et; B is the dimethylcyclopropane ring. They are prepared e.g. by reacting the appropriate imide (with -CH_2OH attached to the N-atom) with crysanthemic-acid, -anhydride or -chloride (**I**). Thus *N*-(hydroxymethyl)-succinimide or -itaconimide is reacted with **I** in PhMe in presence of pyridine to give the corresponding ester. The products are active against housefly, mosquito and cockroach, and are suitable for sanitary and domestic use, due to their low toxicity to warm-blooded animals. S. D. HUGGINS.

4.—APPARATUS AND UNCLASSIFIED

Influence of taper of homogenising valve on efficiency of milk processing. V. D. Surkov and G. A. Koml'yakov (*Pishch. Tekhnol.*, 1968, No. 2, [63], 73–75).— C.V.

Determination of amino-sugars in mixtures containing glucosamine, galactosamine and muramic acid. D. E. S. Stewart-Tull (*Biochem. J.*, 1968, **109**, 13–18).—A colorimetric method is described for determination of the three sugars in cell-wall hydrolysates without pretreatment of the hydrolysate. The method involves use of a modified Cessi-Piliego method (*Biochem. J.*, 1960, **77**, 508) combined with the Cessi and Serafini-Cessi method (*Biochem. J.*, 1963, **88**, 132). With the former method, glucosamine (**I**) and galactosamine (**II**) form a volatile chromogen after reaction with acetylacetone, whereas muramic acid (**III**) does not. This characteristic allows direct determination of **III** present in the mixture after removal of the volatile chromogens formed from **I** and **II**. In the latter method, **II** is determined in presence of **I** by condensation with acetylacetone to give a pyrrole which is converted into a volatile chromogen. Neither **I** nor **III** forms a volatile chromogen. (21 references.) J. N. ASHLEY.

Formula for determination of time in drying of tobacco. Y. R. Nikitenko and V. F. Trubnikov (*Pishch. Tekhnol.*, 1968, No. 1, [62], 123–124).— C.V.

Carbonyl compounds [present] in tobacco smoke. I. G. Mokhnachov and S. V. Kamenshchikova (*Pishch. Tekhnol.*, 1968, No. 2, [63], 101–106).— C.V.

Micro-organisms of cured tobacco. B. M. Green (*Process Biochem.*, 1967, **2**, No. 1, 12–14).—Cured tobacco is fairly resistant to microbial spoilage provided dampness is excluded. If present, bacteria, yeasts and fungi grow, the types depending on the prevailing conditions. Pipe tobacco has a higher moisture content than that used for cigarettes and there is a greater possibility of biological contamination. Handling and packaging are reviewed. C.V.

Long-term storage of micro-organisms. V. I. Kuznetsova and N. V. Novotel'nov (*Microbiology [USSR]*, 1967, **36**, 921–924).—*Bacillus subtilis*, *Aspergillus oryzae* and *A. terricola* 3374 were studied; these organisms actively produce proteolytic enzyme. Best results were obtained by storage under vaseline oil. Tables show the comparative results obtained over a 20-month period with (1) periodical subculturing, (2) storage under mineral oil and (3) storage of cultures dried by lyophilisation. (13 references.) C.V.

Formamide breakdown reaction for the differentiation of enterobacteriaciae. B. Serény (*Acta microbiol. hung.*, 1967, **14**, 1–5).—A simple alkalisation test which divides the *Enterobacteriaceae* (*E*) into rapid formamide (**I**)-decomposing organisms (*Morganella* and some *Klebsiella* and *Rettgerella* strains), those that decompose **I** slowly (*Serratia*) and the negative-**I** bacteria other than *E.* None of the *E* examined attacked dimethyl-**I**, *p*-aminosalicylic acid, *p*-aminobenzoic acid, β-naphthylamine, *o*-phenylenediamine, aniline, diethylamine, urethane or hexamethylenetetramine. C.V.

Polyisoprenoid hydrocarbons. International Flavors and Fragrances Inc. (B.P. 1,087,774, 25.11.65. U.S., 16.2.65).—Used to enhance tobacco flavours or for making tobacco flavouring agents, the title compounds are obtained by polymerising isoprene at 15–70°, if desired in the presence of a lower alkanoic acid, and of an acid catalyst. The polyisoprenoid-hydrocarbons and -esters are then removed from the lower boiling components (if alkanoic acid is added) and the ester can be saponified to the corresponding alcohol if required. The hydrocarbons have the general formula $H[CH_2C(CH_y)\cdots CH_xCH_2]_nH$, where n is 4–15, x is 1 or 2 and y is 2 or 3, the bond between C and CH_y and C and CH_x being a single or double bond, and the values of x and y are determined by the position of the double bond. S. D. HUGGINS.

Agents for repelling birds, rodents, and leporine animals [rabbits, etc.]. Farbenfabriken Bayer A.-G. (Inventors: B. Anders, R. Hiltmann, E. Kühle, K. Sasse, H. Wollweber and G. Hermann) (B.P. 1,098,687, 31.10.66. Ger., 9.11.65).—The agents have the formula $NR^{II}R^{III}\cdot C(:NR^{I})NR^{IV}R^{V}$ wherein R^{I}–R^{V} are H, halogenophenyl (optionally substituted by halogenoalkyl of 1–4 C), cycloalkyl, or aliphatic hydrocarbon radical which may contain aryl, alkoxy, aryloxy, alkylthio, arylthio, COR (R is OH, alkoxy, or NH_2), OH, SH, or CN), only 1 of them being optionally substituted monohalogenophenyl, or any 2 of them together with N form a heterocyclic radical, or R^{II} is $X\cdot NR^{III}\cdot C(:NR^{I})NR^{IV}R^{V}$ (X is alkylene optionally interrupted by O, S, or N) or R^{II} and R^{III} together represent $(X^{I})_2N\cdot C(:NR^{I})NR^{IV}R^{V}$ (X^{I} is alkylene). An example (of many named, some of which are new) is *N*-(*p*-chlorophenyl)-*N′N′*-diethylguanidine. F. R. BASFORD.

Carbohydrate-derived polymers. Milk Marketing Board (Inventors: T. P. Bird and E. T. Dewar) (B.P. 1,099,372, 3.3.65).—New high mol. wt. water-sol. linear polymers (**I**) are obtained by (*a*) reacting anhyd. galactose with a ketone (acetone) to form the 1,2:3,4-diketal(di-isopropylidenegalactose) (**II**), (*b*) esterifying **II** with (the anhydride of) an αβ-unsaturated aliphatic mono- or di-carboxylic acid (acrylic or methacrylic anhydride to form the 6-acrylate or -methacrylate), (*c*) polymerising the resulting ester in presence of free radicals (to obtain **polydi-isopropylidenegalactose-6-acrylate or -methacrylate**), (*d*) subjecting the resulting polymer to hydrolysis to remove diketal residues to form **I [polygalactose-6-acrylate (III) or -methacrylate (IV)]** by dissolving the polymer in formic acid and gradually diluting the solution with water. **III** and **IV** each form further useful polymer deriv. Possible uses of all polymers and deriv. are discussed; they resemble the industrially useful alginates, pectates, carrageenan, etc. H. L. WHITEHEAD

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JANUARY, 1969

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

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