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CHANGES IN AVAILABLE NITROGEN CONTENT OF SOILS DURING STORAGE

By B. R. SINGH* and Y. KANEHIRO

Nitrification of mineralised NH_4-N was enhanced by long-term laboratory storage in polyethylene bags. Such storage caused a slight decrease in soil pH, possibly due to release of acids from decomposing organic matter. Long-term oven-drying of soils released substantial amounts of NH_4-N; the NO_3-N remained practically unchanged. The magnitude of released NH_4-N was noted to be dependent upon the organic matter content of soils. It was speculated that the released NH_4-N came from the splitting of ammonium from N-bearing organic compounds in these soils.

Introduction

Before analysis is carried out, soil samples are often stored for varying lengths of time in various containers and bags, and are either exposed to or excluded from the atmosphere. Sometimes they are even air- or oven-dried to facilitate grinding prior to analysis. Such practices may cause marked changes in physical and chemical properties of soils; they may also affect the rate, direction and magnitude of various chemical and biological transformations in a soil. A major change that may occur is in the matter of organic N transformation to available ($NH_4 + NO_3$) forms. Munro & MacKay[1] noted that the air-drying of soils increased nitrate production by 15–20 ppm N over moist samples. Effect of incubation following short-term heating or partial sterilisation, and wetting–drying cycles on soil-N transformation has been studied intensively, and in most of the cases there has been a considerable increase in soil available N.[2–8]

The present investigation was concerned with the effect of long-term storage at room temperature and in an oven on available N content of two Hawaiian soils differing widely in their organic matter content.

Experimental

Two Hawaiian soils, Akaka silty clay (Typic Hydrandept) from the Hilo Coast, Hawaii and Wahiawa silty clay (Tropeptic Eutrorthox) from North Wahiawa, Oahu, were used for this study. The organic carbon contents of the two soils were 14·6 and 1·4%, respectively. Thus these two soils differed widely in their organic matter content. The Akaka soil, developed from volcanic ash under conditions of over 200 in. annual rainfall, is dominantly amorphous and contains large amounts of allophane and hydrated oxides of iron and aluminium. The Wahiawa soil, derived from basalt under 50 in. annual rainfall, contains predominant amounts of kaolin and some iron oxides. The Wahiawa soil was passed through a 60-mesh sieve but the Akaka soil, because of its high moisture content, was passed through a 10-mesh sieve.

About 10 pounds of the two soils were stored in polyethylene bags at room temperature (air-conditioning regulated at 27° ± 3°c). The same two soils were also placed in an electric oven at 90° ± 5°c. Storage in room and oven continued for ten months. Changes in NH_4- and NO_3-N were examined at two-month intervals. Twenty-five grams (oven-dry basis) of a homogeneous subsample were extracted with 150 ml of a 2 N-KCl solution, and NH_4- and NO_3-N were determined by the method of Bremner.[9] Soil pH was also measured at each interval using an Expandomatic Beckman pH meter in a 1:2·5 soil–water mixture that was allowed to stand for 12 h with occasional stirring. The available N and pH determinations were made on duplicate samples.

Results and Discussion

Changes in available N in soils stored at room temperature

There was a four-month lag period during which the NH_4- and NO_3-N in the Akaka soil stored at room temperature remained practically unchanged. While NH_4-N remained the same even after this period, the NO_3-N increased rapidly afterwards (Fig. 1). The four-month lag period in this soil

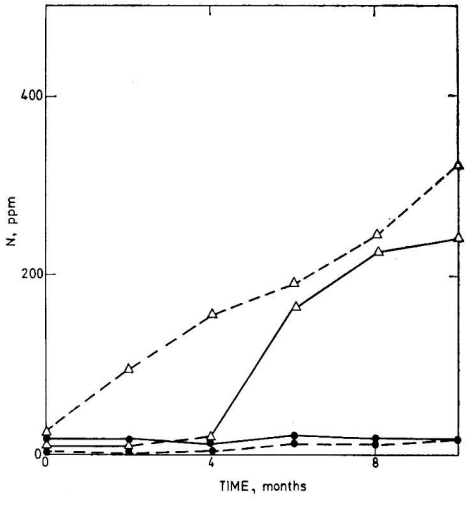

FIG. 1. *Changes in NH_4-N (●) and NO_3-N (△) in the Akaka silty clay and Wahiawa silty clay stored at room temperature* (27°c)
——Akaka silty clay; — — — Wahiawa silty clay

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may have been due to a disturbance in microbial activity after the soil was removed from the field and brought to the laboratory. Apparently, the micro-organisms took a long time to adjust to the new environment before resuming their activity. However, there was no such lag in the Wahiawa soil. While NH_4-N remained virtually constant in this soil, the NO_3-N increased consistently with duration of storage (Fig. 1). The moisture contents of the Akaka and Wahiawa soils during storage were 168 and 30%, respectively. Lower nitrification in the former soil is probably due to its excessive moisture content which impeded nitrification. Thus, despite the presence of high organic matter in the Akaka soil, organic N transformation to inorganic forms was restricted by the excessive moisture during storage. From the data in Table I it is evident that the available N at any given time is greater in the Wahiawa than in the Akaka soil when both are stored at room temperature.

Little or no increase in NH_4-N in these two soils stored at room temperature does not mean that there was no ammonification; in fact the mineralised NH_4-N was nitrified. This is shown by the rapid increase of NO_3-N in the Akaka soil after four months, and in the Wahiawa soil from the very beginning (Fig. 1). Air-drying of soils has been reported to increase nitrification.[1,10] Results presented in Fig. 1 suggest that nitrification is also enhanced by laboratory storage in polyethylene bags where soils suffer little or no moisture loss. Enhanced nitrification could be attributed to increased microbial activity during storage at room temperature.

Changes in available N in soils kept in oven

Long-term storage in an oven at 90°C increased NH_4-N substantially in the Akaka soil. The pattern of NH_4-N release in this soil can be described by observing three regions in the curve (Fig. 2). The first and second regions, corresponding to 0–4 and 4–8 months storage, respectively, show a linear increase in NH_4-N release with the former region showing a faster rate than the latter. The portion of the curve where NH_4-N release almost levels off, comprises the third region. The pattern of NH_4-N release in the Wahiawa soil is similar to that in the Akaka soil, except that the distribution of the three regions over the entire period of storage is different (Fig. 2). The third region for this soil starts after four months and shows a slight upward tendency instead of levelling off as in the Akaka soil. Evidently, the magnitude of released NH_4-N is several fold higher in the Akaka than in the Wahiawa soil, probably because of the high organic matter content of the former.

TABLE I

Changes in available N (ppm) in soils during storage

Months	Available N			
	Akaka silty clay		Wahiawa silty clay	
	Room temp.	Oven 90°C	Room temp.	Oven 90°C
0	19	19	20	20
2	18	727	101	402
4	28	1043	156	458
6	182	1159	199	468
8	240	1250	258	488
10	258	1256	338	517

Unlike results obtained during storage at room temperature of these two soils, there was almost no change in NO_3-N during storage in the oven (Fig. 2). Gibbs[11] reported that *Nitrosomonas* and *Nitrobacter* are killed at 53–55° and 56–58°C, respectively. The fact that there was virtually no increase in NO_3-N, therefore, could be attributed to the death of nitrifiers during long-term heating in the oven.

Lawrence[6] reported that while nitrifiers are killed by partial sterilisation, ammonifiers survive in fair number. Unlike the thin-walled nitrifying bacterial cells, ammonifying bacteria produce thick-walled cells and spores, which can survive even at 100°C. But under long-term heating in an oven, such as in the present experiment, the survival of ammonifiers is questionable, and even if they do survive, they will not be functional. Therefore, the substantial increase in NH_4-N on continuous heating of the experimental soils could not be attributed to surviving ammonifiers.

Heat treatment possibly brings about chemical transformation of soil organic matter and causes N release. Birch[12] noted that the solubility of organic matter in aqueous solution increased with heating. He thought this was due to some chemical alteration of organic matter. Jenkinson[5] attributed this increase to the killing of micro-organisms because the death and subsequent lysis of organisms would be accompanied by an increase in water-soluble organic matter.

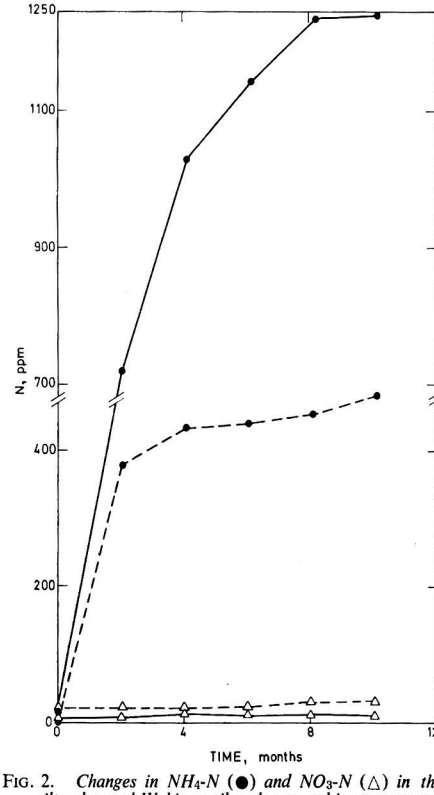

FIG. 2. *Changes in NH_4-N (●) and NO_3-N (△) in the Akaka silty clay and Wahiawa silty clay stored in an oven at 90°C*

—— Akaka silty clay; — — — Wahiawa silty clay

TABLE II
Effect of storage on soil pH

Months	Soil pH			
	Akaka silty clay		Wahiawa silty clay	
	Room temp.	Oven 90°C	Room temp.	Oven 90°C
0	4·3	4·3	5·8	5·8
2	4·3	3·7	5·8	6·2
4	4·3	3·8	5·7	6·2
6	3·8	4·0	5·7	6·2
8	3·9	4·2	5·7	6·3
10	3·9	4·3	5·7	6·2

Cairns[13] reported that the effect of heat on NH_4-N release could be due to either the chemical oxidation of humus or the release of not readily exchangeable NH_4^+ fraction. The latter reaction is improbable with the experimental soils because they contain very little 2:1 clays which are responsible for NH_4^+ fixation. It is speculated that the NH_4-N release in the experimental soils is at least in part due to the splitting of NH_4^+ from N-bearing organic compounds, especially protein and amino acids. The greater NH_4-N release in the Akaka soil could be due to its high organic matter content.

Effect of storage on soil pH

The pH was measured at each storage interval, and the data are given in Table II. Storage after four months at room temperature caused a slight decrease in the pH of the Akaka soil. This could be attributed to the release of certain acids from decomposing organic matter during storage. The other explanation could be the release of H^+ ions during nitrification that started after four months (Fig. 1). However, the very small decrease in the pH of the Wahiawa soil, where nitrification was greater under similar conditions, fails to substantiate the latter explanation.

Upon oven-drying, the pH of the Akaka soil decreased at the 2-month period and gradually increased with increasing periods of oven-drying. This pattern apparently was not related to change in available N status in this soil. The pH of the Wahiawa soil stored in the oven increased at the 2-month period but did not change with the length of oven-drying.

Practical implications

The change in the available N content of soils upon storage at room temperature suggests that soil samples sent to soil testing laboratories for determination of available N and subsequent fertiliser recommendation should be analysed immediately.

Results of long-term oven-drying show that prolonged high temperatures in tropical and subtropical parts of the world would have a great effect on the availability of N to agronomic crops grown in the following season. Intentional practices, such as the burning of crop residues, and unintentional occurrences, such as forest fires, would be expected to increase exchangeable NH_4^+ at the soil surface.

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STUDIES ON POTASSIUM NUTRITION OF PLANTS

IV.*—Effect of potassium nutrition on the composition of leaves of cabbage seedlings *(Brassica oleracea capitata)*

By G. G. FREEMAN and N. MOSSADEGHI

The effect of potassium concentration in the nutrient medium on the composition of leaves of cabbage seedlings has been investigated. The plants were grown in sand culture with seven treatments ranging from extreme deficiency to relatively high potassium concentration. The total amounts of the mineral components were not influenced by the level of potassium nutrition. Similarly, proteins, amino acids and other organic nitrogen components, taken as a whole, did not vary appreciably as shown by the constancy of the Kjeldahl nitrogen values.

Sugar (reducing sugars + sucrose) content rose with increasing potassium concentration in the medium to a maximum (11·0%) at 2·5 mequiv./l and fell slightly at the highest K^+ concentration. Starch content varied in a similar manner but values were, in general, less than half those of the corresponding sugar contents. Total organic acids content was at a maximum (18·6%) when K^+ concentration in the medium was 0·1 mequiv./l and fell to a minimum (7·7%) at a K^+ concentration of 2·5 mequiv./l. 'Other carbohydrates' (the difference between content of 70% [by vol.] alcohol-insoluble residue and Kjeldahl nitrogen × 6·25) also contributed to the balance of percentage composition; the content rose to a maximum (35·2%) at a K^+ concentration of 2·5 mequiv./l and fell off both at higher and lower K^+ concentrations. Within the limits of experimental error, the totals of the leaf components determined were constant over the range of potassium concentrations of 0·1–5·0 mequiv./l in the medium and, on average, 90·7% of the dried leaf was accounted for.

Similar differences in mineral composition and 'protein', sugars and starch contents were found in plant fractions of different leaf ages in potassium deficiency. The differences were related to differences in potassium content which decreased with increasing leaf age.

Introduction

Several authors[1–7] have described how potassium deficiency influenced the total quantities of organic acids in leaves and the relative proportions of certain acids. In the cases of broad bean (*Vicia faba*),[2] *Bryophyllum diagremontianum* Berger,[3] tomato,[4] orchard grass,[5] and cabbage, red beet and potato leaves,[7] potassium deficiency resulted in large increases of total organic acids. It follows that there must be a corresponding decrease in the content of some other component or components to maintain the balance of leaf composition. The object of the present work was to determine how the balance of percentage dry weight composition was maintained over a wide range of potassium nutrition in the leaves of cabbage seedlings (*Brassica oleracea* var. *capitata* L. cv. Primo). The bulk of the published work on the effect of potassium nutrition on plant metabolism has been concerned with a restricted range of components and the importance of maintenance of a balance of total percentage composition has, to a great extent, been neglected. Earlier work indicated that the mineral components of cabbage leaves do not vary appreciably in the aggregate with changes in organic acid content because the sum of the cation equivalents (and therefore approximately the total percentage cation composition) was constant over a wide range of potassium levels (Freeman, G.G., unpublished data, cf.[8]). Similarly, proteins, amino acids and other organic nitrogen compounds, taken as a whole, are probably not involved because the Kjeldahl nitrogen values were practically independent of potassium nutrition over a wide range in sand culture.[8] The question, however, of changes in the nature of the nitrogenous components in potassium deficiency is discussed below. Preliminary experiments on cabbage leaves, grown in sand culture, which received two widely different levels of potassium in the nutrient medium (0·1 and 5·0 mequiv./l) showed that the starch and sugar (reducing sugars + sucrose) components increased with increasing leaf potassium content.[9] This observation was consistent with Ward's[10] data on potato leaf starch. Other reports on the influence of potassium nutrition on leaf carbohydrates include those of Jones,[11] Cooil & Slattery,[12] Scheck[13] and Ward.[14]

Experimental

Sand culture of seedlings

Cabbage seedlings were grown in sand culture as described previously[7,8] and the plants received the modified nutrient media described by Freeman.[15] The nutrient solutions and the corresponding plant samples were distinguished by the symbols K_0, $K_{0\cdot05}$ ..... $K_{5\cdot0}$, where the subscript numeral denoted the potassium concentration of the nutrient medium in mequiv./l. The numbers of plants in each of these groups were 28, 29, 19, 20, 15, 10 and 10, respectively (cf. Table I).

Chemical analysis

Total organic acids were determined in the fresh leaves by the method of Wager & Isherwood[16] as described previously[7] and results were calculated to dry weight basis. It is known that the concentrations of organic acids and certain other leaf components exhibit diurnal variation. This potential variable was eliminated by harvesting the plants at a constant time (09.00 h) and rapidly arresting enzyme action by immersion of the leaves in boiling absolute ethanol. The

* Part III: *J. Sci. Fd Agric.*, 1970, **21**, 121

TABLE I

Analytical data on oven-dried leaves of cabbage seedlings at a series of levels of potassium in the nutrient medium

	Percentage of component at potassium concentrations in the nutrient media, mequiv./l						
	0·0	0·05	0·1	0·15	0·25	2·5	5·0
K	0·20	0·22	0·25	0·32	0·42	3·09	4·29
P	1·10	1·10	0·89	0·68	0·62	0·47	0·45
Ca	3·88	3·91	3·78	3·75	3·27	3·28	3·21
Mg	0·93	1·00	1·06	1·06	1·03	0·90	0·89
Na	5·65	5·58	4·93	4·11	3·01	1·08	0·76
'Protein'	23·5	29·4	29·3	29·8	30·2	25·2	27·0
Sugars	n.d.	4·00	6·18	7·75	10·4	11·0	9·50
Starch	n.d.	2·00	2·50	3·50	4·40	4·60	4·70
'Other carbohydrates'	n.d.	22·9	21·5	22·4	24·2	35·2	28·0
Total organic acids	n.d.	13·1	18·6	17·0	14·7	7·68	10·2
Total	n.d.	83·2	89·1	90·5	92·3	92·5	89·0
Dry matter, % of fresh weight	14·7	9·94	8·99	7·93	7·47	9·37	9·60
Sum of K + P + Ca + Mg + Na	11·8	11·8	10·9	9·92	8·35	8·82	9·60
Organic acids + sugars + starch	n.d.	19·1	27·3	28·3	29·5	23·3	24·4
Insoluble residue	n.d.	54·3	53·3	55·7	58·8	65·0	59·7

n.d. = not determined

leaf residue, after extraction with 70% (by vol.) aqueous ethanol, was washed twice with absolute ethanol, twice with ether and dried *in vacuo* over P_2O_5 to constant weight. This fraction is referred to as 'insoluble residue'. Samples of the leaves were dried to constant weight in an air-circulating oven at 80°c and the residues, after grinding, were submitted to chemical analysis as described previously.[8] Standard errors of the mean values of the mineral components determined under these conditions have been reported.[8]

Determination of sugars

Sugars were determined in the 70% ethanolic extract by the anthrone method as follows. The combined ethanolic extracts were concentrated *in vacuo* to about 10 ml volume and the concentrate was shaken with light petroleum ether (b.p. 80–100°c) to remove lipids, etc.[16] The aqueous phase was evaporated to dryness on a water bath and the residue was dissolved in water (10 ml). An aliquot portion (1 ml) was diluted to 100 ml and a suitable quantity (e.g., 1 ml) was used for sugar determination. The use of anthrone as a qualitative[17] and quantitative reagent for the determination of carbohydrates is well established. The improvement described by Loewus[18] has been used as slightly modified by R. B. Austin (1967, personal communication). The carbohydrate solution (1 ml) was acidified with 81% sulphuric acid (6 ml) and the mixture was cooled. A 2% solution of anthrone in ethyl acetate (0·5 ml) was added and thoroughly mixed. The green colour was developed by heating in a water bath at 100°c for exactly 10 min. Analar D (+) glucose was used as standard and the results are expressed in terms of apparent glucose. It was important to purify commercial samples of anthrone (Hopkin and Williams Ltd.) by dissolving 10 g in warm benzene (100 ml) and reprecipitating by addition of light petroleum ether (b.p. 60–80°c, 30–40 ml).[19]

Determination of starch

Starch was determined in the insoluble residue fraction, described above, by hydrolysis with perchloric acid. The insoluble residue (0·1 g), dispersed in water (5 ml), was extracted with 52% perchloric acid (6·5 ml); the extract was diluted to 100 ml and filtered.[20] The reducing sugars present in aliquot portions of the filtrate were determined by the anthrone colour reaction as described above and calculated as $C_6H_{10}O_5$. The limits of error in determination of carbohydrates by the anthrone method have been reported by Yemm & Willis.[21]

Results and discussion

Effect of potassium nutrition on leaf composition

Cabbage was grown at 7 levels of potassium concentration in the nutrient medium as stated in Table I. The plants were harvested after 41 days of growth in sand culture. Leaf laminae only were taken for analysis. The analytical data, on a dry weight basis, are presented in Table I. The term 'content' is used for components of heterogeneous structures such as leaves; it refers to quantities as g/100g of tissue. The 'protein' values were based upon total Kjeldahl nitrogen determinations multiplied by the factor 6·25. In addition to true protein, the 'protein' values include amino acids and other organic nitrogen compounds. Since sucrose was hydrolysed under the conditions used in the anthrone reaction, the values for sugars in the Table represent reducing sugars + sucrose expressed as apparent glucose. The insoluble residue fraction includes starch, cellulose, hemicelluloses and other 70% alcohol-insoluble carbohydrates and proteins. The presence of starch in this fraction was confirmed as follows. Insoluble residue from $K_{2·5}$ and $K_{5·0}$ leaves (0·1 g) was warmed with a little water and cooled. Addition of iodine gave positive reactions for starch. 'Other carbohydrate' values were obtained from the content of this fraction (insoluble residue) less the known values for starch and 'protein' (Kjeldahl nitrogen × 6·25.) Total organic acid contents expressed as dry weight percentages were based upon Freeman's values.[7] For conversion of the values, expressed as mequiv. of organic acid per 100 g dry matter, to percentages, the mean equivalent weight of the acids was assumed to be 64. This assumption was based on the proportions of the component acids[7] (Fig. 2). Malic acid (equiv. 67) accounted for about 28% of the total organic acids and citric acid (equiv. 64) for about 70% of the total; the other organic acids have lower equivalent weights.

Potassium and sodium values were characteristic of these components of cabbage seedlings grown at the stated potassium concentrations in the medium.[8] The effect of potassium nutrition on the ionic balance of potassium and sodium and of potassium and calcium plus magnesium has been reported and discussed in earlier papers in this series.[8,15] 'Protein' contents (total N × 6·25) varied slightly from K_0 to $K_{5\cdot0}$ but the variation was small compared with that of the corresponding values of K and Na and it was concluded that this component showed no definite trend with changes of leaf potassium content. A curve relating sugar content with potassium concentration in the growth medium (not reproduced here) rose steeply and almost linearly from $K_{0\cdot05}$ to $K_{0\cdot25}$ and then flattened out at the highest K^+ concentrations. The corresponding curve for starch content was almost parallel to the sugar curve but the starch contents were, in general, less than half of the corresponding sugar contents.

As reported by Freeman[7] (Fig. 3) total organic acids content rose to a maximum at $K_{0\cdot1}$ and then fell off to a minimum at $K_{2\cdot5}$. Within the limits of experimental error, the totals of leaf components determined (K, P, Ca, Mg, Na, 'protein', sugars, starch, 'other carbohydrates' and total organic acids) were constant over the range of potassium concentrations of 0·1–5·0 mequiv./l in the nutrient medium and averaged 90·7% (range 89·1–92·5%) of the dried leaf. The observed total at 0·05 mequiv./l was 7·5% lower than the mean and there was insufficient quantity of leaf tissue for determination of organic acids, etc. at the lowest potassium concentration (K_0) in the nutrient medium. On the basis of these values, it was concluded that over the range 0·1–5·0 mequiv./l of potassium in the nutrient medium, changes in total organic acid content were balanced by reciprocal changes of sugars, starch and 'other carbohydrates'. The content of 'other carbohydrates' fell slightly from $K_{0\cdot05}$ to $K_{0\cdot1}$ and then rose to a maximum at $K_{2\cdot5}$; the curve was approximately a mirror image of the total organic acids curve. These observations show the importance of the 'other carbohydrates' component in contributing to the balance of percentage composition. The sum of the cation (K, Ca, Mg, Na) contents plus that of phosphorus fell from K_0 to $K_{0\cdot25}$ and then remained essentially constant to $K_{5\cdot0}$. A curve relating the sum of these components to potassium concentration in the nutrient medium approximated very closely in shape to the corresponding curve relating dry residue (%) with nutrient medium K^+ concentration.

Although it is not concerned in the maintenance of the balance of percentage composition, it is interesting to note that dry matter content fell from 14·7 to 7·47% of fresh weight with increase of potassium concentration in the nutrient medium from 0 to 0·25 mequiv./l. A similar fall took place in cabbage seedlings grown in solution culture.[15]

Although it was found that total Kjeldahl nitrogen was essentially constant over the range K_0 to $K_{5\cdot0}$, it is known that in fact the nature of the leaf nitrogenous components changes with changes in the potassium nutrition of the plant. This was established by Smith & Richards,[22] who found that agmatine and putrescine increased in quantity, in cabbage and other leaves, with increasing potassium deficiency. As an example of a decrease, with potassium deficiency, in the content of a nitrogenous component, Freeman[7] showed that the nitrate content of cabbage leaves decreased from 0·47% at $K_{5\cdot0}$ to 0·16% at K_0.

Variation of composition with age of leaves

Potassium-deficient ($K_{0\cdot1}$) plants were harvested after 38 days' growth and divided into four fractions on the basis of leaf age as follows: apical leaves, young folded leaves at the apex of the plant with unelongated internodes, showing slight or no symptoms of potassium deficiency; intermediate leaves, the next 3–5 leaves, showing slightly more severe symptoms; oldest true leaves, the remainder of the true leaves, showing definite symptoms of potassium deficiency in the form of chlorosis and necrotic spots; cotyledons, dead and desiccated leaves, showing obvious symptoms of potassium deficiency. The relative proportions of these fractions in terms of the total leaves (fresh weight basis) are given in Table II. Corresponding plants which had received an adequate quantity of potassium ($K_{5\cdot0}$) were fractionated in a similar way except that the cotyledons were not separated from the oldest leaf fraction. There were no symptoms of

TABLE II

Analysis of young cabbage plants, divided into fractions according to leaf age at two levels of potassium in the nutrient medium

Values are expressed as dry weight percentages unless otherwise stated

	K^+ concn. of nutrient medium, mequiv./l						
	0·1				5·0		
	Apical leaves	Intermediate leaves	Oldest true leaves	Cotyledons	Apical leaves	Intermediate leaves	Oldest leaves
K	0·60	0·38	0·28	0·11	4·84	5·72	5·82
P	0·84	0·67	0·65	0·85	0·55	0·43	0·53
Ca	2·20	3·77	4·53	5·50	1·74	3·34	5·26
Mg	0·53	0·77	0·99	1·39	0·48	0·68	1·09
Na	2·83	4·10	6·93	8·11	0·45	0·56	0·81
'Protein'	39·4	31·4	22·4	n.d.	32·4	29·0	26·1
Sugars	5·13	8·89	6·47	n.d.	9·30	n.d.	n.d.
Starch	3·29	2·05	1·65	n.d.	5·75	4·10	2·85
Proportion of total, % of fresh weight	17·0	34·0	48·7	0·3	17·0	55·2	27·8
Dry matter, % of fresh weight	9·00	7·60	8·00	100	10·5	9·70	7·80

n.d. = not determined

potassium deficiency in these plant fractions. The mineral, sugars and starch contents of the fractions were determined (Table II).

In the $K_{0\cdot1}$ plants, the potassium content of the leaves fell from 0·60 to 0·11% with increase in leaf age, suggesting that potassium had migrated from the oldest to the youngest leaves; this observation is in agreement with earlier published reports. The corresponding sodium contents rose from 2·83 to 8·11%. Calcium and magnesium contents also rose with increasing leaf age but less steeply. Phosphorus content was essentially constant. In the case of the $K_{5\cdot0}$ plant fractions, there was no apparent migration of potassium to the youngest leaves because adequate amounts were present throughout the plant. It was concluded that potassium, sodium and phosphorus were virtually constant in these plant fractions whereas in the cases of calcium and magnesium there was a progressive increase with increase in leaf age.

In the $K_{0\cdot1}$ plants, 'protein' content decreased with increase in age of the leaves; the observation that the highest 'protein' content was present in the most actively metabolising (youngest) leaves is in accordance with expectation. There was a similar though smaller difference in the $K_{5\cdot0}$ fractions. The sugars content of the apical leaves at $K_{0\cdot1}$ was lower than would be predicted from the relationship between leaf potassium and sugars contents in Table I but it is probable that sugars initially formed in these leaves by photosynthesis may have undergone rapid oxidation to provide energy for their relatively high metabolic activities or may have undergone rapid conversion to the other components such as structural polysaccharides. In the potassium-deficient leaves ($K_{0\cdot1}$), starch content increased with increasing potassium content in agreement with the trends shown in Table I. In the well-nourished plants ($K_{5\cdot0}$), starch contents also decreased with increase in age of the plant fraction. The 'protein', sugars and starch contents found in the cotyledons ($K_{0\cdot1}$) were very low, probably owing to microbial attack following the death of these leaves.

Taken as a whole, the fractionation experiments showed that there were wide differences in metabolic activity in the leaf fractions at different ages as evidenced by differences in composition. The differences were relatively large in the potassium-deficient plant fractions ($K_{0\cdot1}$) and were probably related to local potassium concentration. The smaller differences observed in the well-nourished plant fractions ($K_{5\cdot0}$) were related to leaf age but were apparently unrelated to the local potassium concentrations.

There is little published information on the composition of vegetable leaves for comparison with the values reported here. Mineral composition and protein content given by Long[23] for the edible portions of cabbage are similar to those of the present work and carbohydrate content is stated to be 40·4%. McCance & Widdowson[24] reported that several varieties of cabbage (edible portion, raw inner leaves) contained sugar (as invert sugar) 3·3–3·8%, starch (as glucose) 0·0%, and unavailable carbohydrate 2·5–3·4%, on a fresh weight basis. No reports of changes of composition of sugars, starch or other carbohydrates in cabbage leaves at different levels of potassium nutrition have been found. There are, however, several references to changes in carbohydrate fractions in potassium deficiency in other plants which are of interest for comparison with the present observations on cabbage. Reviewing effects of potassium deficiency on metabolic processes, Evans & Sorger[25] stated that soluble carbohydrates accumulated in *Ananas comosus*, soluble carbohydrates accumulated initially, then decreased, in *Lycopersicon esculentum*, reducing sugars accumulated in *Lycopersicon esculentum*, *Saccharum officinarum*, *Parthenium argentatum*, *Helianthus annuus* and *Triticum vulgare*, starch content decreased in *Ananas comosus* and starch initially increased and then decreased in *Lycopersicon esculentum*.

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EFFECT OF STAGE OF MATURITY OF PERENNIAL RYEGRASS ON ITS CONTENT OF SOME ORGANIC ACIDS AND PHENOLIC COMPOUNDS

By A. K. MARTIN

S24 perennial ryegrass was harvested at six stages of growth ranging from young leafy grass with no stem tissue to mature herbage cut when the seed was ripe. The contents of crude protein, lignin, total *o*-dihydroxyphenolic compounds and of chlorogenic, shikimic, quinic, succinic, malic and citric acids were determined in the grass samples from each cut and their distribution between leaf, stem and seed head tissue was examined. The decreases observed in the contents of malic, quinic and chlorogenic acids and of total *o*-dihydroxyphenolic compounds with increasing maturity of the herbage were linearly related to both the decrease in the proportion of leaf tissue and the increase in the lignin content of the herbage. The significance of the alicyclic acids, shikimic and quinic acids, and of the phenolic acids as dietary precursors of urinary aromatic acids in ruminants is discussed.

Introduction

The alicyclic acids, shikimic and quinic acids, and phenolic acids, particularly the frequently occurring phenolic cinnamic acid esters such as chlorogenic acid (3-*O*-caffeoyl quinic acid), may be significant as dietary precursors of the urinary aromatic acids excreted by ruminants.[1] These animals excrete considerable quantities of benzoic and phenylacetic acid conjugates in their urine[2] and almost all of these acids are derived from dietary sources.[3] It has been suggested that intermediates of lignin synthesis in plants may be the precursors of aromatic acids excreted by herbivorous animals.[4] These intermediates would be expected to be present in greatest concentration in immature plants, which may explain the observations made with cows[5] and sheep[6] that their urinary outputs of aromatic acids were greater when they consumed young leafy forage than when they were given mature herbage.

A pathway similar to the shikimic acid pathway, which is responsible for the synthesis of aromatic compounds in micro-organisms, is known to operate in higher plants.[7] Quinic acid, which is not an intermediate in the microbial pathway, accumulates in many tissues of higher plants, notably in fruits.[8,9] Chlorogenic acid is widely distributed in the plant kingdom[10] and concentrations of up to 7·5% of the dry tissue in coffee beans[11] and of up to 2·8% of the fresh tissue of some fruits[12] have been reported.

Some information is available on the quinic and shikimic acid content of grasses[13–15] but less is known on the quantitative occurrence of chlorogenic acid, although its presence in meadow foxtail (*Alopecurus pratensis* L.) and meadow fescue (*Festuca pratensis* Huds) has been noted.[16] The purpose of this paper is to report the content of possible precursors of urinary aromatic acids in perennial ryegrass (*Lolium perenne* L., cv. S24) harvested at six stages of growth. The opportunity was also taken to study the non-volatile organic acid, crude protein and lignin contents of the grass at each stage of growth.

Experimental

Harvesting of grass samples

A pure stand of S24 perennial ryegrass which had received a dressing of Nitrochalk (21% N) at the rate of 5 cwt/acre on 14th April was cut on six dates (see Table II). After the fifth cut, the field was mown and 2 cwt/acre of a fertiliser containing 25% N, 10% P_2O_5 and 10% K_2O was applied. On each occasion the grass was cut at a height of 2·5 cm with a mowing machine equipped with a cutter bar.

The proportion of leaf, stem and seed head tissues present in the different cuts was determined by dissecting six 100g fresh weight samples of each cut and determining the dry matter content of each tissue. In cuts 4 and 5, a proportion of the herbage cut consisted of young stem and leaf tissue which had developed from new tillers. This is referred to as secondary growth. Samples from cuts 1 to 5 inclusive were freeze-dried immediately after harvesting. Samples from cut 6 were dried in a forced draught oven at 105°c for 3 h.

Total *o*-dihydroxy phenolic compounds

The grass samples (2–4 g) were extracted in a Soxhlet apparatus with chloroform–cyclohexane (3:1 by vol.) until the solvent was colourless. The solvent was removed from the sample which was then extracted with methanol for 3 h. The total concentration of *o*-dihydroxy phenolic compounds present in the methanol extracts was determined by the method of Zucker & Ahrens.[17] The optical density of the final solution was determined at 520 nm (the absorption maximum for caffeoyl esters) and at 460 nm. Various pure phenolic compounds were subjected to this procedure and their molecular extinctions at 460 and 520 nm are recorded in Table I. Compounds not containing vicinal hydroxy groups (e.g., 3,5-dihydroxybenzoic acids, ferulic acid, vanillic acid and naringenin) or those containing three hydroxy groups (e.g., 3,4,5-trihydroxybenzoic acid) did not react; neither did quinic acid.

Chlorogenic acid

The volume of the methanolic extracts was reduced to give a concentration of between 1 and 2 mg/ml total *o*-dihydroxyphenolic compounds (expressed as chlorogenic acid). The concentrated extract (250 μl) was applied as a band (2 mm wide × 6 cm long) to strips of chromatography paper (Whatman 3 MM). Papers were developed by descending chromatography for 16 h using the upper phase of an n-butylacetate–acetic acid–water (4:1:5 by vol.) solvent. Chromatograms of grass extracts always showed two fluorescent bands with properties similar to those of chlorogenic

TABLE I

Molecular extinctions of phenolic compounds assayed by the procedure of Zucker & Ahrens[17] for total *o*-dihydroxyphenolic compounds

Compound	Molecular extinction × 10^{-3}			Colour of eluate	Eluate absorption maximum, nm
	460 nm	520 nm	Ratio 460:520 nm		
Chlorogenic acid	3·74	6·72	0·56	Red	520
Caffeic acid	3·39	4·18	0·81	Red	505
3,4-dihydroxybenzoic acid	5·01	7·45	0·67	Red	510
2,3-dihydroxybenzoic acid	3·63	6·76	0·54	Red	520
3,4,5-trihydroxybenzoic acid	0·45	0·27	1·67	Yellow	<350
3,4-dihydroxyphenylpropionic acid	5·42	6·56	0·83	Red	500
Pyrocatechol*	4·03	6·48	0·62	Red	510
Aesculetin*	0·66	0·29	2·28	Green-yellow	400
Quercetin*	3·38	2·28	1·48	Pale pink	<450
Naringenin*	5·72	0·55	1·04	Yellow	<450

* These compounds were only partly retained on the alumina column on reaction with nitrous acid and subsequent washing so molecular extinctions reported for them are only approximate

TABLE II

Dates of cutting, lengths of cut stems and anatomical composition (% of dry matter) of S24 perennial ryegrass cut at six stages of growth

Description	Cut	Date	Average height of cut stem, cm	Dry matter, %			
				Leaf	Stem	Seed heads	Secondary growth
Leafy grass—some stem	1	4th May	6	80·3	19·7	—	—
Seed heads about to emerge from stem	2	18th May	11	67·9	32·1	—	—
Seed heads fully emerged	3	8th June	31	27·2	50·4	22·4	—
Seed set	4	29th June	53	21·3	57·9	20·8	21·5*
Seed ripe	5	3rd August	74	—	67·2	23·3	9·5*
Leafy grass—no stem	6	5th September	0	100·0	—	—	—

* The anatomical composition of cut 4 herbage was determined prior to removal of secondary growth tissue (see experimental section). Thus, the leaf, stem and seed head distribution includes those tissues present in the secondary growth. In cut 5 the secondary growth was the only green tissue present

acid. Under u.v. light they exhibited a blue fluorescence which turned green on exposure to NH_3; they showed a positive Hoepfner reaction, i.e., a yellow colour on spraying with 1% wt./vol. $NaNO_2$ in 10% wt./vol. acetic acid followed by a red colour on spraying with 1 N-NaOH. When the fluorescent areas were eluted with 3:1 by vol. methanol–water the eluates exhibited the absorption spectrum of authentic chlorogenic acid.[18] The most mobile band ('Band 3') moved in a manner identical with pure chlorogenic acid and the least mobile band ('Band 1') had a flow rate, relative to Band 3, of 0·30. The chlorogenic acid content was determined from the optical densities of the eluates of 'Band 1' and 'Band 3' at 330 nm.

Another fluorescent band ('Band 2') which did not show the reactions of chlorogenic acid was present in small amounts in all samples. It had a mobility of 0·56 with respect to 'Band 3'.

Thin-layer chromatography of methanolic grass extracts

Extracts spotted on cellulose layers (Whatman CC41) were developed in n-butylacetate–acetic acid–water (1:1:1 by vol.). Plates were examined under u.v. light, after spraying with the Hoepfner[18] or Arnow reagents[19] or with an ethanolic solution of $AlCl_3$.[20]

Non-volatile organic acids of grass

Aqueous extracts of grass were decolorised with partly deactivated charcoal[21] and portions of the extracts were used for the determination of total non-volatile organic acids.[22] The remainder of the extract was freeze-dried; the residue was taken up in a little water and the individual acids were determined by gradient elution from an anion exchange column.[23] The identity and purity of the eluted peaks were determined by freeze-drying the appropriate fractions, taking up in a small quantity of water, and applying 0·05 μequiv. to cellulose thin layers (Whatman CC41) using two solvent systems.[24] After drying the developed chromatograms, the acids were located by spraying with 0·04% bromocresol purple (pH 7·0) or (for shikimic and quinic acids) with the Cartwright–Roberts spray reagent.[18]

Lignin

The method of Czerkawski[25] was used.

Results

Composition of grass cuts

A description of the stage of growth of each of the six cuts of S24 ryegrass, the dates of cutting, the heights of cut stems and anatomical composition of the cut grass are recorded in Table II.

Herbage of cut 6 was the least mature, consisting only of leafy tissue of the regrowth of the sward after mowing. Cuts 1 to 5 were successively more mature, the proportions of leaf tissue decreasing with increasing maturity.

Total *o*-dihydroxy phenolic compounds and chlorogenic acid

The results are presented in Table III. There was no direct relationship between either of these fractions and the anatomical composition of the herbage. As the proportion of leaf decreased from 100% in cut 6 to 80% in cut 1, the concentration of both fractions in the herbage increased. With increasing maturity and development of the seed heads, the content of both fractions in the herbage declined. Stem and leaf tissues from cuts 1 and 2 were not analysed separately but analyses of the tissues of cuts 3, 4 and 5 showed the stem to contain 0·1% or less of both fractions in all samples. Only three samples of leaf tissue were analysed for chlorogenic acid (cut 6 regrowth herbage which was 100% leaf and cuts 3 and 4). Again, there was a smaller chlorogenic acid concentration in leaf tissues from mature herbage (0·43% in cut 4) than was found in young leaf tissue (1·27% in cut 3 and 1·22% in cut 6). Seed heads contained between 3 and 5 times the amounts of both fractions as compared with those found in stem tissue. Stage of maturity did not have a noticeable effect on the quantities present in stem or seed head tissue.

In addition to the decline in concentration of total *o*-dihydroxyphenolic compounds with increasing maturity of the herbage, the proportion of this fraction present as chlorogenic acid also declined with increasing maturity (see Table III). Thin-layer chromatography of methanolic extracts showed that there was an increasing content of flavonoid reacting material with increasing maturity of the herbage. Flavonoids had R_f values of 0·14 and 0·57 in the solvent system described and were present in particularly large amounts in cuts 4 and 5. No free phenolic or neochlorogenic acids were present in any of the extracts. In addition to a spot with the same R_f as chlorogenic acid, a spot with an R_f of 0·08 gave a positive reaction with both Hoepfner and Arnow sprays. It showed a yellow fluorescence in u.v. light and again was particularly abundant in extracts of herbage from cuts 4 and 5.

An increasing proportion of flavonoids in the more mature grass may have been responsible for the decline in the proportion of total *o*-dihydroxy phenolic compounds found to be present as chlorogenic acid. The increase observed in the ratio, absorbance at 460 nm : absorbance at 520 nm, when applying the procedure of Zucker & Ahrens[17] to grasses of increasing maturity, may also have reflected the presence of larger amounts of flavonoids in the more mature herbage. For some flavonoids this ratio varied between 1·04 and 2·28, whereas that for chlorogenic acid was only 0·56 (Table I). The ratios found for eluates from alumina columns to which grass extracts were applied were, for cuts 6, 1, 2, 3, 4 and 5 respectively, 0·69, 0·76, 0·74, 0·76, 0·86 and 0·93. The

TABLE III

Contents of total *o*-dihydroxyphenolic compounds (calculated as chlorogenic acid) and chlorogenic acid in perennial ryegrass cut at six stages of maturity

Sample	Total *o*-dihydroxy phenolic compounds†, g/100 g dry matter	Chlorogenic acids, g/100 g dry matter			Proportion of chlorogenic acids (Bands 1 + 3) present as Band 3, %	Proportion of chlorogenic acid (Bands 1 + 3) as *o*-dihydroxy phenolic compounds, %
		Band* 1	Band* 3	Bands* 1 + 3		
Whole grass:						
6	1·22	0·13	0·54	0·67	80·6	54·9
1	1·54	0·21	0·83	1·04	79·9	67·5
2	0·85	0·09	0·45	0·54	83·3	63·5
3	0·76	0·07	0·32	0·39	82·1	51·3
4	0·35	0·02	0·10	0·12	83·3	34·3
5	0·29	0·01	0·05	0·06	83·3	20·7
Stem tissue:						
3	0·09	0·01	0·05	0·06	83·3	66·6
4	0·07	0·02	0·04	0·06	66·6	85·7
5	0·10	0·01	0·06	0·07	85·7	70·0
Leaf tissue:						
3	1·27	0·16	0·64	0·80	80·0	62·9
4	0·43	0·06	0·19	0·25	76·0	58·1
Seed heads:						
3	0·30	0·03	0·13	0·16	81·3	53·3
4	0·38	0·03	0·14	0·17	82·4	44·7
5	0·53	0·01	0·09	0·10	90·0	30·3
Secondary growth:						
4	0·29	0·04	0·13	0·17	76·5	58·6
5	0·78	0·14	0·50	0·64	78·1	82·1

* Areas of paper chromatograms giving positive tests for caffeoyl esters—see experimental section
† Total *o*-dihydroxyphenolic compounds determined by method of Zucker & Ahrens[17] and expressed as chlorogenic acid

TABLE IV

Total non-volatile organic acids of perennial ryegrass cut at various stages of growth and the major components of these acids, g/100 g dry matter

Cut	Peak						Total acids*	
	1 Shikimic acid	2 Quinic acid	3 Unknown acids	4 Succinic acid	5 Malic acid	6 Citric acid	Sum of peaks 1–6	% of separately determined total acids
6	0·30	0·61	0·00	0·05	0·82	0·50	2·28	100·0
1	0·06	0·53	0·04	0·06	0·73	0·59	2·01	98·3
2	0·08	0·49	0·05	0·05	0·41	0·56	1·64	84·0
3	0·07	0·25	0·03	0·06	0·49	0·50	1·40	89·6
4	0·01	0·12	0·01	0·03	0·22	0·34	0·73	85·7
5	0·00	0·05	0·01	0·00	0·07	0·04	0·17	65·2
3 Leaf	0·07	0·40	0·03	0·03	1·32	0·65	2·50	98·2
3 Stem	0·01	0·10	0·01	0·01	0·57	0·38	1·08	97·2
3 Seed heads	0·00	0·20	0·03	0·01	0·56	0·35	1·15	100·0

* The total non-volatile organic acids were determined separately (see experimental section). In the first total acid column, the sum of the individual acids (unknown acids expressed as malic acid) are recorded. In the second column, the sums of the malic acid equivalents of each peak are expressed as a % of the separately determined total non-volatile acids, also expressed as malic acid equivalent

average ratios for leaf, stem and seed head tissues, which were largely unaffected by stage of maturity, were for leaf 0·65, for stem 0·84 and for seed heads 0·83.

Non-volatile organic acids

The contents of non-volatile acids determined by ion-exchange chromatography of extracts of each cut are recorded in Table IV. The total content of non-volatile acids was also determined separately and expressed in terms of acidity equivalent to malic acid. The sums of the individual acids, also expressed in terms of malic acid, are expressed as a percentage of the total non-volatile acids in the last column of Table IV.

As the grass matured and the proportion of leaf declined, the content of total acids fell in almost direct proportion to the decrease in leaf content of the herbage (see Fig. 1). The greatest departure from a linear relationship occurred with the cut 3 herbage. Between cuts 2 and 3 a rapid stem elongation took place and the results indicate that the young stem tissue contained significant amounts of non-volatile organic acids. This was confirmed by analysis of the stem tissue from cut 3 herbage, recorded in Table IV, which showed a total of non-volatile acid content of 1·08%.

The relationship of malic acid and quinic acid to the proportion of leaf tissue in the herbage was similar to that for the total acids. Citric acid content varied little until the herbage had flowered (cut 3), after which it decreased to a very low level in the most mature grass. Shikimic acid was present in appreciable concentration only in young leafy regrowth tissue (cut 6); small concentrations were present until the flowering stage (cut 3) but thereafter only traces were found. The contents of the incompletely resolved peak 3 [which contained glyceric acid (2,3-dihydroxypropionic acid) and at least two unidentified acids] and of succinic acid changed little with stage of growth.

Crude protein (N × 6·25)

The crude protein content of the grass was greatest in cut 1 (19·7%), rather less in the younger cut 6 and declined rapidly with increasing maturity of the herbage following cut 1. The results are recorded in Table V.

TABLE V

Crude protein (N × 6·25) content of perennial ryegrass cut at six stages of growth together with the lignin (protein corrected) contents of the herbage

Cut	Crude protein, %	Lignin,* %
6	15·7	2·76 (9·3)
1	19·7	2·70 (14·0)
2	13·0	3·41 (4·5)
3	8·0	5·02 (11·6)
4	5·4	6·83 (6·4)
5	4·7	7·58 (10·0)

* The protein content (N × 6·25) as a % of the crude lignin preparation is given in parenthesis

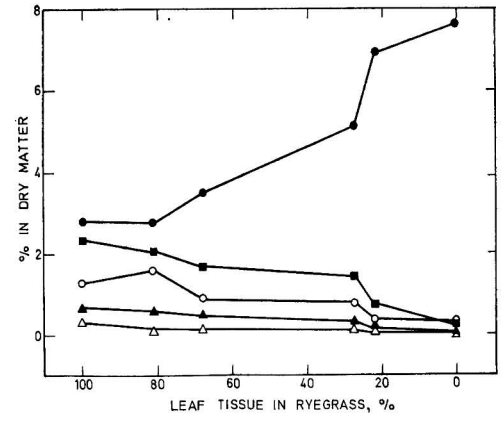

FIG. 1. *Relationship of lignin, total non-volatile acids, total o-dihydroxyphenolic compounds, quinic acid and shikimic acid to the % of leaf tissue of each of the six cuts of S24 perennial ryegrass*

● Lignin; ■ total non-volatile acids; ○ total *o*-dihydroxyphenolic compounds; ▲ quinic acid; △ shikimic acid

Lignin

The lignin content of the herbage was inversely related to its total non-volatile organic acid content. Lignification was most rapid between cuts 3 and 4 (Fig. 1) when the lignin content increased from 5·02 to 6·83% with only a small change in the proportion of leaf tissue in the herbage. In Table V, the lignin content of each cut is given together with the crude protein contents of the crude lignin preparations. The corrections for crude protein content ranged between 4·5 and 14·0% of the crude lignin, which is rather larger than the range of 5 to 8% reported by Czerkawski.[25]

Discussion

The relationship between chemical composition and the proportion of leaf tissue in the whole herbage, illustrated in Fig. 1 for the various cuts of ryegrass, may be due to the fact that the precursors of lignin, quinic acid, shikimic acid and chlorogenic acid are synthesised predominantly in leaf tissue. Radio-active tracer studies have shown that shikimic acid is incorporated into lignin.[26] In rose blooms, quinic acid can be metabolised to shikimic acid and the enzymes responsible have been detected in cell cultures of the Mung bean.[28] Quinic acid presumably could be converted to lignin in plant tissues, though whether or not this acid is in the direct pathway of lignin synthesis is yet to be established.[29] The position of chlorogenic acid with regard to lignin synthesis is similar to that of quinic acid. Basyouni & Neisch[30] define two classes of cinnamic acid esters in wheat tissues, those directly soluble in acetone and those released from the insoluble residue on autolysis. They consider the insoluble esters to be the precursors of lignin in wheat, with compounds such as flavonoids and quinic acid esters produced at branching points on the lignin pathways. While it has been shown that the metabolic turnover rate of chlorogenic acid in leaf tissue is high,[31] migration of chlorogenic acid out of plant leaves does not appear to be extensive. Chlorogenic acid has been noted to be present in greatest concentration in leaf tissue in other plants, e.g., in the tips of tobacco leaves, a large concentration which decreased continuously towards the stem has been found.[17] In some species, large quantities of chlorogenic acid accumulate in seed tissue: 2% has been found in lettuce seed.[32] No accumulation of this magnitude was found in ryegrass seed heads.

The preceding discussion suggests that the contents of quinic, shikimic and chlorogenic acids might be related to the lignin contents of the herbages. Linear regression equations were calculated for each of the compounds analysed (Tables III and IV) in the six cuts of herbage on the lignin content (Table V) of these cuts of herbage. The most significant regressions were those of quinic acid concentration on lignin ($P < 0{\cdot}001$), total non-volatile acid concentration on lignin ($P < 0{\cdot}001$) and the concentration of the total *o*-dihydroxyphenolic compounds on lignin ($0{\cdot}001 < P < 0{\cdot}01$). These relationships and their equations are illustrated in Fig. 2. Significant linear regressions of shikimic acid concentration (Y) on lignin content (X) [$Y = 0{\cdot}25–0{\cdot}03X$, $0{\cdot}001 < P < 0{\cdot}01$] and chlorogenic acid concentration (Y) on lignin content (X) [$Y = 1{\cdot}22–0{\cdot}16X$, $0{\cdot}001 < P < 0{\cdot}01$] were also found. All these regression equations have a negative slope, which is to be expected if these substances are precursors of lignin synthesised in the plants.

Comparison of the amounts of total non-volatile organic acids found with those found by Jones & Barnes in the same strain of ryegrass[15] show that considerably smaller quantities were found in the present experiments, the maximum being 2·3% in cut 6, whereas Jones & Barnes[15] found 4·1% in what (as far as can be assessed from data they give) was equivalent to cut 2 of the present experiments. With regard to distribution of the acids, Jones & Barnes[15] found very much greater proportions of succinic acid (6–15%) in the total acids than were found in the present work (0–4%). The ratio of malic to citric acid also differed: Jones & Barnes[15] reported ratios between 1·3 and 2·4, but in the present experiments the range was between 0·7 and 1·6. The reason for these differences is difficult to assess. Factors such as climate, fertiliser treatment[13] or the method of drying the grass may be important.

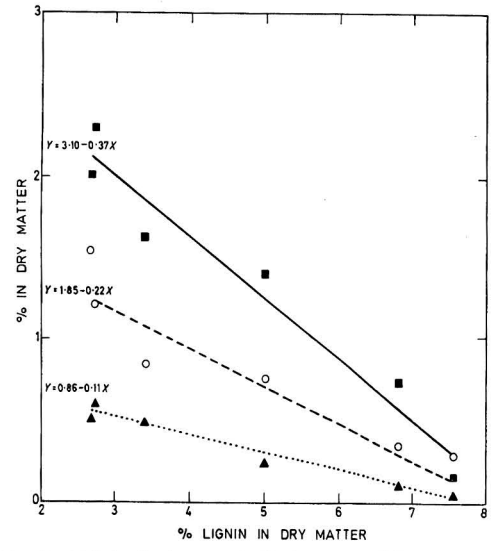

FIG. 2. *Relationship between the lignin content of the six cuts of S24 perennial ryegrass and their contents of total non-volatile acids* (■), *total o-dihydroxyphenolic compounds* (○) *and quinic acid* (▲)
In the regression equations X is the % of lignin in the dry matter and Y the % of total non-volatile acids, total *o*-dihydroxyphenolic compounds or quinic acid

The increase in lignin content of the ryegrass with increasing maturity occurred at a rate similar to that reported by other workers in this strain of perennial ryegrass.[33] The greatest departure from a linear relationship between lignin content and proportion of leaf in the plant was in cut 3. This corresponded with the period in which it has been reported that extensive lignification of the stem tissue takes place.[33] Consequently, the lignin content of stem tissue, comprising 50% of the herbage at this stage, was lower than in the more mature grass, which would cause a departure from this relationship.

As expected, the concentrations of the possible urinary aromatic acid precursors in S24 ryegrass declined as the maturity of the grass increased. The relationship between the intake of these precursors by sheep consuming the forages described here and their urinary output of aromatic acids are discussed elsewhere.[1]

Acknowledgments

The author is grateful to his colleagues in the Nutrition Department for helpful discussion and in particular to Dr. J. H. Moore for his encouragement. Thanks are also due to Dr. M. E. Castle of the Grass & Dairy Husbandry Department for making available the pure stand of S24 ryegrass and to Dr. D. Reid for advice in the management of the sward. Analytical assistance was rendered by Miss J. T. Begg and Miss G. Falconer.

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LUCERNE SAPONINS

II.*—Purification and fractionation of saponins from lucerne tops and roots and characterisation of the isolated fractions

By B. GESTETNER, S. SHANY, Y. TENCER, YEHUDITH BIRK and A. BONDI

Continuous flow paper electrophoresis or column chromatography on alumina of saponin extracts prepared from lucerne tops or roots resulted in the isolation of a saponin fraction. This fraction, which is the main component of root saponin extract but is present only in small amounts in saponins isolated from lucerne tops, consists of glucose, arabinose and xylose as carbohydrate constituents and medicagenic acid, soyasapogenol A and a new, hitherto unidentified sapogenin as aglycones. It is shown that this saponin fraction is the main haemolytic agent and contains almost all of the growth-impairing activity towards *Tribolium castaneum* larvae. Larval growth can, however, be restored to its normal level by the addition of cholesterol to the diet.

Further separation of non-homogeneous fractions obtained on alumina columns resulted in the isolation of individual saponins; examination of their haemolytic activities indicates that both the nature of the sapogenin, i.e., medicagenic acid, as well as the sapogenin/sugar ratio contribute to the extent of this activity.

Introduction

The presence of saponins in lucerne has been known for some time and their detrimental effects on ruminants, poultry, mice, rats, insects, fungi and germinating seeds have been reviewed.[1,2] Very little is known, however, about the exact nature and potency of these activities, whether and to what extent they are possessed by the different lucerne saponins. It has been shown earlier that lucerne root saponins are stronger haemolytic agents and are more toxic to *Tribolium castaneum* larvae than lucerne tops saponins, although both preparations are similar in overall composition.[3] The separation, purification and isolation of individual lucerne saponins from their complex mixtures is a prerequisite for study and elucidation of their composition–structure–activity relationship. The aim of this work was to elaborate suitable methods for the separation of individual saponins from lucerne tops and roots and study their chemical composition and some of their properties.

Experimental

Preparation of saponin extract

Saponin extracts (SE) were prepared either from commercially dehydrated lucerne (*Medicago sativa* cv. Hairy Peruvian) tops meal (SE_t), or from its roots (SE_r) by the combined methods of Thompson *et al.*[4] and of Shaw & Jackson[5] as described by Shany *et al.*[3]

Hydrolysis of SE and isolation of sapogenins and sugars

These were performed according to the method of Shany *et al.*[3] The amount of sugars liberated by acid hydrolysis of SE was determined with the 3,5-dinitrosalicylic acid reagent.[6]

Separation and identification of SE components, sapogenins and sugars

SE components were separated by thin-layer chromatography (t.l.c.) on Kieselgel G plates with the following solvent mixtures: (a) ethyl acetate–acetic acid–water (7:2:2 by vol.); (b) n-propanol–ethyl acetate–water (7:2:2 by vol.). Lucerne sapogenins were chromatographed on Kieselgel G plates with the solvents; (c) petroleum ether–chloroform–acetic acid (7:2:1 by vol.); or (d) di-isopropyl ether–acetone (5:2 by vol),[7] acidified with two drops of acetic acid. The sugars of the carbohydrate moiety were chromatographed on Whatman 3MM paper using the upper phase of benzene–n-butanol–pyridine–water (1:5:3:3 by vol.)[8] as solvent. Saponins and sapogenins were detected by staining the chromatoplates with conc. H_2SO_4 and heating the plates at 110°C for 10 min; sugars were identified with $AgNO_3$–NaOH reagent.[9]

Determination of haemolytic activity

This was performed with washed red blood cells (ram) in isotonic phosphate buffer, pH 7·4. Haemolytic activity was expressed as haemolytic index after 20 h interaction between lucerne saponins and the red blood cells as described by Shany *et al.*[3]

Fractionation of SE_t and SE_r

Continuous flow paper electrophoresis

Continuous flow paper electrophoresis (c.f.p.e.) of SE_t and SE_r was performed in a refrigerated Beckman Model CP continuous flow paper electrophoresis cell equipped with an automatic fraction collector. 40 mg/ml solutions of SE_t or SE_r in a buffer ($NaOH$–H_3BO_3; pH 8) were applied to a curtain of Schleicher and Schüll grade 470 paper (30 × 35 cm) at a flow rate of 0·2 ml/h; left and right capillaries and overflow were set at 4·0. The cell was operated at a constant voltage of 800 V, the current being 20–22 mA. After termination of the electrophoretic process (36–48 h) the curtain was dried and the zones were located by staining with the Carr–Price reagent.[10] The fractions were numbered consecutively from cathode (1) to anode (32) and were collected and combined according to the stained zones on the curtain. The fractions, after de-ionisation with Amberlite IR 120 (H^+ form), evaporation to dryness, and removal of boric acid as a volatile methyl ester, were dispersed in water, freeze-dried and analysed.

* Part I: *J. Sci. Fd Agric.*, 1970, **21**, 131

Al_2O_3 column

Acid-washed Al_2O_3 (Merck Co.) was equilibrated with benzene and poured into a 2·2 × 15 cm column. 1 g of SE_t or SE_r was suspended in 10 ml benzene, mixed with a small amount of Al_2O_3 and the mixture was applied to the column. Elution was performed with benzene, chloroform, mixtures of chloroform and absolute ethanol and aqueous solutions of ethanol successively, and fractions of different volumes were collected. The effective volume of each eluting agent was determined by t.l.c. of aliquots of effluents collected. The rate of flow was adjusted to ~200 ml/h. The eluates were concentrated under reduced pressure in a rotary vacuum evaporator, dispersed in a small amount of water, freeze-dried and weighed. Samples of each fraction were submitted to t.l.c. and those which were heterogeneous on the chromatoplate were further fractionated by semi-preparative t.l.c.

Semi-preparative thin-layer chromatography

Further fractionation of the heterogeneous fractions eluted from the Al_2O_3 column was carried out on Kieselgel HR (Desaga Co.) plates (20 × 40 cm, 500 μm thick). 1% solutions of the SE fractions were prepared in 90% ethanol and a line along the longer edge of the plate was applied with a Desaga sample applicator, each plate accommodating ~20 mg SE fraction. Chromatography was carried out with the solvent mixture ethyl acetate–acetic acid–water (7:2:2 by vol.). The fractions were located on the dried chromatogram by staining a narrow strip on each edge of the plate with conc. H_2SO_4. The separated zones were collected with a vacuum zone collector from the unstained part of the plate. The saponin fractions were eluted by shaking the collected powder with 90% ethanol, filtering and concentrating the filtrate to an aqueous solution, which was then freeze-dried and subjected to acid hydrolysis. The acid hydrolysates were analysed for sugars and sapogenins.

Effect of SE and of its various fractions on the growth of *Tribolium castaneum* larvae

Growth experiments with the larvae were carried out according to the method of Shany *et al.*[3] The cholesterol (Nutritional Biochemicals Corp.) added to the diets was crystallised from absolute ethanol.

Results

Continuous flow paper electrophoresis

By this method both SE_t and SE_r gave four distinct zones (I, II, III and IV), each of them consisting of several saponins (Fig. 1). Although the electrophoretic patterns of SE_t and SE_r seemed identical, chromatographic examination of the different zones revealed qualitative and quantitative differences between the two saponin extracts. As shown in Fig. 1, t.l.c. of zone I of SE_r gave three distinct spots at R_f 0·6–0·75 and a fourth at R_f 0·85 whereas the t.l.c. of the same zone of SE_t showed mainly impurities and only traces of spots at R_f 0·6–0·75, the fourth spot being absent altogether. Zones II and III consisted of the bulk of SE_t and SE_r. The t.l.c. pattern of these zones was similar in both saponin extracts, except for some spots which, in addition to those originating from SE_t, appeared in SE_r only. The saponins appearing in zone I of SE_r have been found to contain mainly glucose, and small amounts of arabinose and xylose, as well as medicagenic acid, soyasapogenol A and an unidentified sapogenin-like material (Table I). The possibility of lucerne saponin extracts

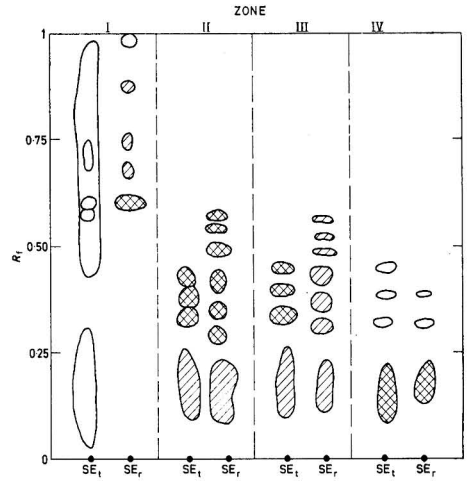

FIG. 1. *Thin-layer chromatographic separation of the saponins found in the various zones (obtained by continuous flow paper electrophoresis), on Kieselgel G plates with ethyl acetate–acetic acid–water (7:2:2 by vol.) as solvent*

Staining: conc. H_2SO_4
Intensity of colour is marked as following: ☒ > ▩ > □

containing an additional, hitherto unidentified sapogenin has already been reported,[3] but its presence could not be ascertained, since it was almost inseparable from the other sapogenins in the solvent used. It can, however, be clearly separated when the mixture of sapogenins obtained by acid hydrolysis of SE_t and SE_r is chromatographed with isopropyl ether–acetone. It gave a bright red colour with conc. H_2SO_4 and it was located at R_f ~0·55, between the slower-moving medicagenic acid and the fast-moving soyasapogenols. The chemical nature of this material has not been established as yet, and a relationship to lucernic acid isolated by Livingston[11] should not be excluded.

T.l.c. of zone IV obtained by c.f.p.e. showed the presence of a main spot in SE_r, which was also the dominant component in SE_t. Both in SE_t and SE_r, this zone contained several sugars and medicagenic acid as its main aglycone, indicating that SE_t and SE_r contain a saponin of the same or very similar composition. Zones II and III contained a variety of saponins, which had a similar chemical composition—containing all the soyasapogenols and also medicagenic acid as their aglycones—and could not be separated by c.f.p.e. It was found that haemolytic activity was exhibited mainly by the components of zone I from SE_r, the other zones from SE_t as well as from SE_r being much less haemolytic.

Column chromatography on Al_2O_3

The results of fractionation of SE_t and SE_r on Al_2O_3, and the t.l.c. pattern of the various fractions eluted from the column are given in Tables II and III and Fig. 2. It can be seen from Table II that the bulk of SE_t (~ 70%) was eluted in fractions IX and X with 80% and 70% ethanol, whereas in the case of SE_r, the main part of the extract emerged in fraction VIII (with 90% ethanol). This fraction had the highest haemolytic activity, both in SE_t and SE_r, the activity

of the latter being much higher. This difference in the degree of haemolytic activity may arise from the presence of accompanying non-haemolytic impurities in SE_t (Fig. 2), which could not be separated from the saponins by chromatography. On the other hand, the main component of fraction VIII (spot No. 8, R_f 0·65) was accompanied in SE_r by other saponins from the adjacent fractions, which were also haemolytic, though to a lesser extent than that eluted with 90% ethanol. With regard to composition (Table III), the only significant difference between the SE_t and SE_r fractions seems to be the abundance of medicagenic acid in the latter, especially in fraction VIII. In general, the total amount of reducing sugars is less in the SE_r fractions, which is in agreement with the originally lower sugar content of whole SE_r. Further separation between the components of the non-homogeneous Al_2O_3 fractions was attempted by semi-preparative t.l.c. A number of saponins, characteristic to SE_r only, have been isolated from fractions VII and VIII (eluted with 96% and 90% ethanol from the Al_2O_3 column). One of these was composed of glucose, arabinose and an unidentified sapogenin and two others, which contain glucose and medicagenic acid only (Table IV). Although these two sapogenins contain the same sugars and aglycones, they have different chromatographic mobilities (Fig. 2, spots 8 and 10), possibly owing to structural differences. The saponins of t.l.c. fractions 5 and 6 (Fig. 2), which have been analysed together, consist of soyasapogenol A and the unknown sapogenin as their aglycones, but because of the mixed nature of the eluted material the composition of their carbohydrate moieties has not been determined.

TABLE I

Chromatographic identification of sugars and sapogenins present in saponin fractions of the various zones obtained by continuous flow paper electrophoresis of SE_t and SE_r

Zone	Sugars identified*											
	Glucuronic acid		Galactose		Glucose		Arabinose		Xylose		Rhamnose	
	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r
I	tr.	—	tr.	—	+	+++	+	+	+	+	—	—
II	+	+	+	+	++	++	+	+	+	+	+	+
III	++	++	++	++	+++	+++	++	++	++	++	++	++
IV	+	+	—	—	+	++	+	+	+	+	+	+

Zone	Sapogenins identified*													
	Soyasapogenols										Medicagenic acid		Unidentified sapogenin	
	A		B		C		D		E					
	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_r	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r
I	+	+	tr.	--	—	—	—	—	—	—	+	+	tr.	+
II	+	+	+	+	+	+	+	+	+	+	—	++	—	+
III	++	++	++	++	++	++	++	++	++	++	+	+	+	++
IV	tr.	tr.	tr.	tr.	tr.	tr.	—	—	—	—	++	++	—	—

* Evaluation of spot intensity: + medium; ++ strong; +++ very strong

TABLE II

Yield and haemolytic activity of the different fractions obtained from 1 g SE_t or SE_r fractionated on an Al_2O_3 column

Fraction	Eluting agent	Volume of effluent, ml	Material eluted, mg**		Haemolytic index†	
			SE_t	SE_r	SE_t	SE_r
I–VI	Benzene Chloroform* Absolute ethanol	6000	19·7	26·8	None	None
VII	Ethanol 96%	3000	40·5	159·2	None	1500
VIII	Ethanol 90%	7000	100·5	559·2	4500	12500
IX	Ethanol 80%	7000	236·3	155·3	2500	2500
X	Ethanol 70%	7000	460·7	46·8	2500	< 500
XI	Ethanol 50%	7000	81·5	24·6	< 500	None

* Fractions I–VI contain materials eluted with benzene, followed by chloroform, chloroform + 5% absolute ethanol, chloroform + 20% absolute ethanol, chloroform + absolute ethanol (1:1 by vol.) and absolute ethanol, 1000 ml each. Because of their small amounts and identical chromatographic pattern (Fig. 2) they were pooled together for analysis

** 93·9% (SE_t) and 97·1% (SE_r) recovered from the columns

† Haemolytic indices of materials applied to the columns: SE_t 2200; SE_r 5400

TABLE III

Total amount of reducing sugars and chromatographic identification of sugar and sapogenin components of SE_t and SE_r fractions eluted from an Al_2O_3 column

Fraction	Sugars identified**													
	Reducing sugars,* mg/g		Glucuronic acid		Galactose		Glucose		Arabinose		Xylose		Rhamnose	
	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r
I–VI	none		none		none		none		none		none		none	
VII	305	150	—	—	—	—	+++†	++	—	+	—	—	—	—
VIII	250	275	—	—	—	—	++	++	+	+	—	+	—	—
IX	433	316	+	+	+	++	+++	+++	+	++	+	++	tr.	++
X	501	310	+	+	++	++	+++	+++	+	++	+	++	+	++
XI	428	180	+	+	++	++	++	++	+	+	+	+	+	+

Fraction	Sapogenins identified**													
	Soyasapogenols										Medicagenic acid		Unidentified sapogenin	
	A		B		C		D		E					
	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r
I–VI	none		none		none		none		none		none		none	
VII	—	+	—	—	—	—	—	—	—	—	—	++	—	+
VIII	+	+	—	—	—	—	—	—	—	—	+	+++	+	+
IX	++	+	++	+	++	+	+	+	+	+	+	++	tr.	+
X	++	+	++	+	++	+	+	+	+	+	++	++	+	+
XI	+	+	++	++	++	++	+	+	+	+	++	+	—	—

* Total amount of reducing sugars: SE_t, 550 mg/g; SE_r, 270 mg/g
** Evaluation of spot intensity: + medium; ++ strong; +++ very strong
† ~ 60% present also before acid hydrolysis

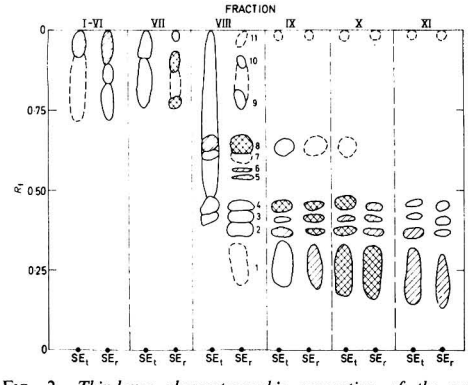

FIG. 2. *Thin-layer chromatographic separation of the various saponin fractions (obtained by column chromatography on Al_2O_3 of SE_t and SE_r), on Kieselgel HR plates with ethyl acetate–acetic acid–water (7:2:2 by vol.) as solvent*

Staining: conc. H_2SO_4
Intensity of colour is marked as following: ⊠ > ■ > □ > □

Semi-preparative thin-layer chromatography

Fractions IX, X and XI of SE_r, which had similar t.l.c. patterns (Fig. 2), were pooled together and then subjected to further separation. Because of large amounts of accompanying materials, further separation of fractions VII and VIII of SE_t could not be effected and only the combined fractions IX, X and XI were subjected to semi-preparative t.l.c. (Table V). From these fractions, two saponins, one of which contained medicagenic acid and a number of sugars, and another containing soyasapogenol A in association with all sugars (except for the pentoses) present in whole SE, were isolated both from SE_t and SE_r (Table V). Visual comparison of the chromatograms indicates that both saponins are found in larger amounts in SE_t than in SE_r. The composition of the so far non-homogeneous fractions (t.l.c. fractions 3 and 4) was also similar in both extracts.

It can be seen from Tables IV and V that t.l.c. fraction 8 from SE_r has a very high haemolytic index, whereas the indices of the other fractions are much lower and similar to each other. According to their electrophoretic and chromatographic behaviour, fraction 8 and the main component of zone I obtained by c.f.p.e. seemed to be identical saponins C.f.p.e. of fraction 8 and its mixture with zone I, resulted in the same electrophoretic pattern; when subjected to t.l.c., they had an R_f value of 0·65 in solvent (a) (Figs 1 and 2); in solvent (b) their common R_f was 0·52 and in both solvents their mixture yielded only one, undivided spot. The saponins common to both SE_t and SE_r (t.l.c. fractions 1 and 2) were chromatographically similar to the materials of zone IV obtained by c.f.p.e., having R_f values of 0·25–0·40.

Growth experiments with *Tribolium castaneum* larvae

The effect of the different SE fractions on the growth of the larvae is shown in Fig. 3. The generally more toxic effect of SE_r on larval growth[3] is due solely to fraction VIII, whereas the other fractions do not seem to exert any negative influence at all. The growth impairment caused by fraction VIII can be fully counteracted by the addition of 1% cholesterol to the diet.

TABLE IV

Chemical composition of the different fractions obtained by semi-preparative t.l.c. of SE_r fractions VII and VIII eluted from an Al_2O_3 column

Al_2O_3 fraction	T.l.c.* fraction	Sugars identified**						Sapogenins**							
								Soyasapogenols					Medicagenic acid	Unidentified sapogenin	Haemolytic index
		Glucuronic acid	Galactose	Glucose	Arabinose	Xylose	Rhamnose	A	B	C	D	E			
VII	10	—	—	+	—	—	—	—	—	—	—	—	+	—	24000
	9	—	—	+	+	—	—	—	—	—	—	—	—	+	10000
VIII	8	—	—	+++	—	—	—	—	—	—	—	—	++	—	40000
	5·6†	—	—	++	+	+	+	+	—	—	—	—	—	+	—

* For numbering see Fig. 2

** Evaluation of spot intensity: + medium; ++ strong; +++ very strong

† Because of their closeness on the chromatogram these fractions were eluted together and thus analysed. Fraction 7 appears only in trace amounts and was not further investigated.

TABLE V

Chemical composition of the different fractions obtained by semi-preparative t.l.c. of the combined SE_t fractions (IX, X and XI) and of the combined SE_r fractions (IX, X and XI) eluted from an Al_2O_3 column

Al_2O_3 fraction	T.l.c.** fraction	Sugars identified*											
		Glucuronic acid		Galactose		Glucose		Arabinose		Xylose		Rhamnose	
		SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r
IX–X–XI	8	—	—	—	—	++	+++	—	—	—	—	—	—
	4	+	+	+	+	++	++	+	+	+	+	+	++
	3	+	+	+	+	+	++	—	—	—	—	+	+
	2	+	+	+	+	++	++	—	—	—	—	+	+
	1	+	+	—	—	++	++	++	++	++	++	+	+

Al_2O_3 fraction	T.l.c.** fraction	Sapogenins identified*														Haemolytic index†
		Soyasapogenols										Medicagenic acid		Unidentified sapogenin		
		A		B		C		D		E						
		SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	SE_t	SE_r	
IX–X–XI	8	—	—	—	—	—	—	—	—	—	—	+	++	—	—	—
	4	—	—	+	+	++	+	+	+	+	+	+	+	+	+	—
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
	2	+	+	—	—	—	—	—	—	—	—	—	—	—	—	3200
	1	—	—	—	—	—	—	—	—	—	—	+	+	—	—	3500

* Evaluation of spot intensity: + medium; ++ strong; +++ very strong

** For numbering see Fig. 2

† For haemolytic index of Fraction VIII, see Table IV

Discussion

The fractionation of saponins isolated from lucerne tops and roots has been attempted by methods based on various principles. The results presented in this work indicate that the fraction from lucerne saponins which exhibits the strongest antibiological activities can be separated by column chromatography on Al_2O_3. Fraction VIII, which is eluted from the Al_2O_3 column with 90% ethanol, exhibits a much stronger haemolytic activity than the others, and almost all the negative activity on larval growth is concentrated in this fraction. Since more than 50% of SE_r and only 10% of SE_t consist of fraction VIII, its higher concentration in SE_r is the obvious reason for the greater toxicity of SE_r than of SE_t towards *Tribolium castaneum* larvae. The growth impairment of the larvae caused by this fraction can, however, be successfully counteracted by the addition of cholesterol to their diet, thus indicating a possible means of detoxification either by addition to the diet, or even by the presence *in situ* of other sterols in the plant.

Fraction VIII differs greatly in its chemical composition from the other fractions. It is generally accepted that carbohydrate moieties of saponins do not exert any detrimental activities; therefore, it can be assumed that the antibiological effects of this fraction are caused mainly by the presence of considerable amounts of medicagenic acid. This assumption may be supported by the fact that the other fractions which contain mainly the various soyasapogenols as their aglycones, do not cause any growth impairment; and also by previous findings,[12,13] that soyabean saponins, which resemble lucerne saponins in their composition, except that they lack medicagenic acid, are almost harmless to various test organisms.

On the other hand, contrary to findings for soyabean saponins,[13] in the case of lucerne saponins very good agreement was observed between the extent of haemolytic and

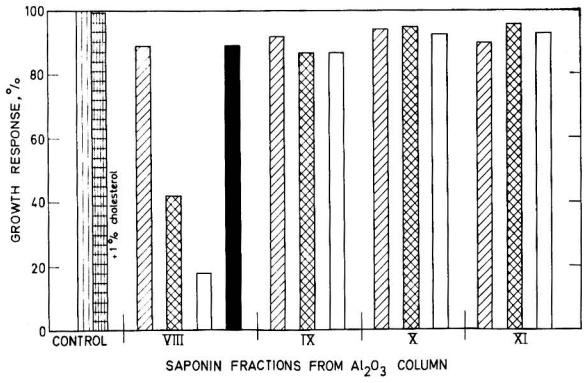

FIG. 3. *Effect of different concentrations of lucerne root saponin (SE_T) fractions and cholesterol on the growth response of* Tribolium castaneum *larvae*

▨ 0·1% of saponin fraction; ⊠ 0·25% of saponin fraction; □ 0·5% of saponin fraction; ■ 0·5% of saponin fraction + 1% cholesterol

larval growth impairing activities. Fraction VIII is the only fraction, isolated by column chromatography, which exhibits high haemolytic and growth-impairing activities, whereas the other fractions are only slightly active in both respects. The examination of the haemolytic activities of the various saponin fractions obtained by t.l.c. shows that those saponins, which have been isolated from SE_T and contain medicagenic acid and the unidentified sapogenin, have high haemolytic indices (Table IV), whereas the haemolytic indices of t.l.c. fractions 1 and 2, isolated both from SE_t and SE_T, are much lower and similar to each other (Table V). It appears that saponins containing medicagenic acid are more haemolytic than those which have one of the soyasapogenols as their aglycone, but the rather low activity of t.l.c. fraction 1 (Table V), which also contains medicagenic acid as its only aglycone, indicates that the constitution of the saponin is also relevant in this respect. Although no saponin containing one of the soyasapogenols attached to only one sugar molecule has yet been isolated from lucerne saponin extracts, the assumption that the sapogenin : sugar ratio is also a factor in determining the extent of haemolytic activity may be substantiated by previous findings;[12] it has been shown that a soyabean saponin, which consists of soyasapogenol A and glucose only, has a high haemolytic index (20,000), whereas other soyabean saponins, which have low sapogenin:sugar ratios exert lesser haemolytic activities, of an order of magnitude similar to t.l.c. fraction 1 and 2 isolated from lucerne.

The abundance of medicagenic acid in SE_T and its attachment to short carbohydrate moieties composed mainly of glucose is of special interest. Lucerne roots are known to be a richer source of saponins than lucerne tops.[3,14] This could be due to quantitative differences occurring during biosynthesis or to partial degradation of saponins during circulation in the plant, or to both; their subsequently reduced solubility in the plant sap would result in accumulation of saponins in roots. Since mainly medicagenic acid-containing saponins seem to be involved, the role of its special structural features, i.e., the presence of two COOH groups,[15] should be taken into account as well.

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LUCERNE SAPONINS

III.*—Effect of lucerne saponins on larval growth and their detoxification by various sterols

By S. SHANY, B. GESTETNER, YEHUDITH BIRK and A. BONDI

The role of sapogenins in the antibiological effects exerted by lucerne saponins has been studied. Data are presented, showing that lucerne sapogenins are more toxic towards *Tribolium castaneum* larvae than their respective saponins, from which they are isolated. Medicagenic acid and to a lesser extent a new, hitherto unidentified sapogenin are the main growth-depressing factors, the soyasapogenols being almost harmless in this respect. When cholesterol is also included in the diet, the negative influence of the sapogenins is completely abolished. The counteraction found with cholesterol, as well as with various plant sterols, of the growth inhibition of the larvae caused by dietary saponin extracts (SE) was studied quantitatively with different SE: sterol ratios. It is shown that the amount of cholesterol needed to counteract fully the growth impairment depends on the toxicity of the SE preparation and its concentration in the diet. The counteracting property of plant sterols on larval growth depression leads to the conclusion that a built-in defence mechanism exists in lucerne against the antibiological effects of saponins.

Introduction

In previous papers,[1,2] it has been shown that lucerne root saponins exert more pronounced detrimental activities than lucerne tops saponins and some of these activities, such as growth impairment of *Tribolium castaneum* larvae, can be successfully counteracted by addition of cholesterol to the diet. It was also found[2] that the toxicity of lucerne saponins is due mainly to one saponin fraction, which differs from the other harmless fractions by its aglycone moiety being composed chiefly of medicagenic acid.[1] In view of these findings additional experiments have been carried out to provide further evidence for the major influence exerted by medicagenic acid on larval growth impairment caused by lucerne saponins, and to investigate the possibilities of abolishing the growth-impairing effects of lucerne saponins by different sterols.

Experimental

Preparation of saponin extracts

Saponin extracts (SE) from lucerne tops and roots were prepared either by the method developed by Morris *et al.*[1] and designated SE_t1 and SE_r1, or according to the procedure described previously,[2] designated SE_t2 and SE_r2, respectively.

Hydrolysis of SE and isolation of sapogenins

SE was hydrolysed in 1 N-H_2SO_4 in dioxane–water (1:3 by vol.);[2] after termination of the hydrolysis, water was added to the hydrolysate and the precipitated sapogenins were collected by filtration. The mixture of sapogenins was subjected to semi-preparative thin-layer chromatography (t.l.c.) according to the procedure described for the fractionation of lucerne saponins,[3] but using di-isopropyl ether–acetone (75:30 by vol.),[4] acidified with a few drops of acetic acid, as solvent.

Growth experiments with *Tribolium castaneum* larvae

These were performed as described earlier.[2] The larval diets were supplemented with SE, or its sapogenins and/or with one of the following sterols; cholesterol crystallised from absolute ethanol, β-sitosterol and stigmasterol (all from Nutritional Biochemicals Corp.), and campesterol (donated by M. Katz).

Results

In view of previous results[3] it was of interest to investigate whether free sapogenins, which can be released by acid hydrolysis of SE_t2 and SE_r2, also possess growth-impairing activity when added to diets of *Tribolium castaneum* larvae. As shown in Fig. 1, lucerne sapogenins are toxic to the larvae; the extent of their toxicity considerably exceeds that observed previously[2] in case of the respective intact saponins from which they have been obtained. In each case, however, the toxic effect can be fully counteracted by the addition of cholesterol to the diet.

Attempts have also been made to elucidate the role of the individual sapogenins in causing larval growth impairment. According to the chromatographic pattern given in Fig. 2, the mixture of sapogenins was separated into three groups: (a) medicagenic acid; (b) unidentified sapogenin, (c) mixture of soyasapogenols.

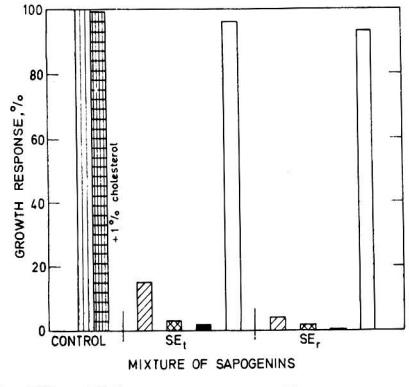


FIG. 1. *Effect of different concentrations of lucerne sapogenins and cholesterol in the diet on the growth response of* Tribolium castaneum *larvae*

▒ control + 0·1% sapogenin mixture; ▨ control + 0·25% sapogenin mixture; ■ control + 0·5% sapogenin mixture; □ control + 0·5% sapogenin mixture + 1% cholesterol

* Part II: Preceding paper.

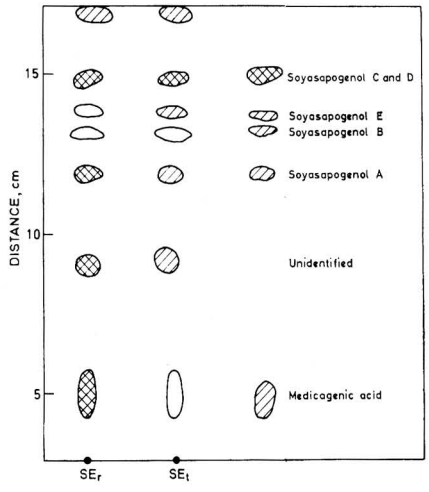


FIG. 2. *Thin-layer chromatographic separation of sapogenins found in acid hydrolysates of* SE_{r2} *and* SE_{r2}
Intensity of colour is marked as following: ⊗ > ▨ > □

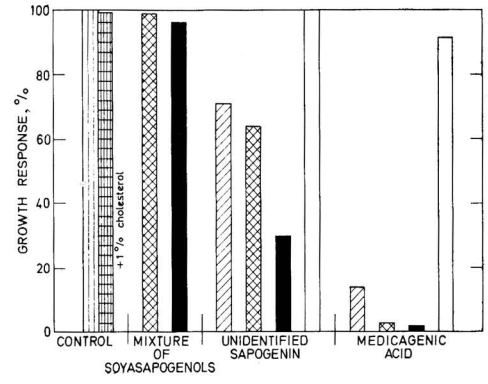
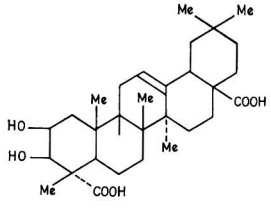


FIG. 3. *Effect of various concentrations of different lucerne sapogenins and cholesterol on the growth response of* Tribolium castaneum *larvae*
▨ control + 0·1% sapogenin; ⊠ control + 0·25% sapogenin; ■ control + 0·5% sapogenin; □ control + 0·5% sapogenin + 1% cholesterol

It can be seen from Fig. 3 that the strongest growth-impairing activity is exerted mainly by medicagenic acid and to a lesser extent by the unidentified sapogenin, whereas the soyasapogenols have no detrimental effect at all on larval growth. Here also, addition of cholesterol can, however, counteract the most drastic growth impairment, such as is caused by free medicagenic acid (I). With regard to the unidentified sapogenin, according to its infra-red and nuclear magnetic resonance spectra it consists of a triterpene skeleton, similar to the other lucerne sapogenins; investigations on the nature of the various functional groups and their structural arrangements have not yet been accomplished.



(I)

The beneficial effect of cholesterol and various plant sterols on the growth response of *Tribolium castaneum* larvae was examined with diets containing SE_t1 or SE_r1. It was shown[2] that the use of hot HCl when preparing these extracts results in a partial degradation of the saponins by shortening their carbohydrate moieties and thus increasing the proportion of sapogenins in the extracts as a whole. Consequently, they exhibit much stronger toxicity towards larval growth than other SE preparations prepared by milder methods,[2] and thus SE_t1 and SE_r1 can be used as convenient models for the examination of the counteracting potency of various sterols. Biological titration curves of these highly toxic saponin extracts with different amounts of sterols in the diet are given

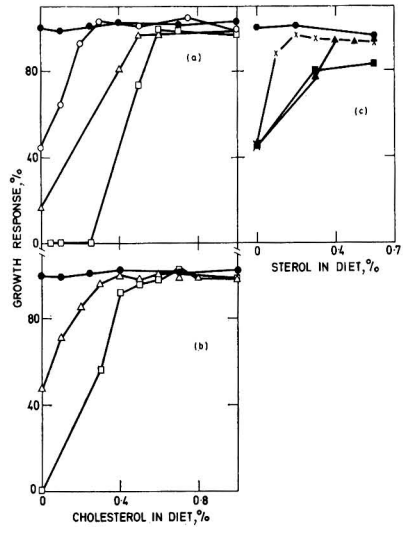


FIG. 4. *Effect of increasing concentrations of various sterols on the growth response of* Tribolium castaneum *larvae kept on diets supplemented with different SE preparations*
● control (contains one of the sterols)
(a) SE_r1 + cholesterol; ○ 0·1%; △ 0·25%; □ 0·5% SE_r1;
(b) SE_t1 + cholesterol; △ 0·25%; □ 0·5% SE_t1;
(c) 0·1% SE_r1; × plus camperterol; ▲ plus β-sitosterol; ■ plus stigmasterol

in Fig. 4. The amount of cholesterol needed to counteract the growth impairment caused by SE_t1 and SE_r1 depends on the toxicity of the SE preparations and on their concentrations in the diet. SE_r1 is considerably more toxic than SE_t1 and this is also revealed by the amounts of cholesterol 'consumed'. Nevertheless, even the strongest effect, caused by 0·5% SE_r1 (namely, total growth depression), can be fully overcome by cholesterol supplementation. The beneficial effect of plant

sterols was examined only with diets containing SE_r1, the preparation which has the highest growth-impairing activity. As shown in Fig. 4, campesterol and β-sitosterol could counteract fully the toxic effect of SE_r1, although higher concentrations of β-sitosterol were needed to abolish growth inhibition completely. Stigmasterol exhibited a similar beneficial trend, but in the concentrations used in this work, a plateau was reached at 80% reversal.

Discussion

The negative effects of lucerne saponins as expressed by the growth response of *Tribolium castaneum* larvae seem to be due mainly to the action of their aglycone part. The findings that partly degraded saponins (SE_r1 and SE_t1) are more toxic than the intact extracts (SE_t2 and SE_r2), and that the isolated lucerne sapogenins cause stronger growth depression than the extracts from which they are derived, clearly indicate such trends. Clearly, medicagenic acid is the sapogenin mainly responsible for this biological effect, which is also exhibited to a lesser extent by the hitherto unidentified sapogenin. The finding that soyabean sapogenins do not affect larval growth is concomitant with previous observations[3] that those lucerne saponin fractions, which contain mainly soyasapogenols as their aglycones, are also harmless to *Tribolium castaneum* larvae. The fact that, in the case of soyabean saponins,[5] as well as in other instances,[6] good agreement was found between the growth responses of *Tribolium castaneum* larvae and that of chicks, rats and mice, would indicate that such a correlation could be valid in the case of lucerne saponins as well. Since the main difference in the composition of soyabean and lucerne saponins is the presence in the latter of medicagenic acid and an unidentified sapogenin, it can be tentatively assumed that the growth impairment of chicks, rats and mice[5] caused by lucerne saponins may be attributed mainly to those saponins, which contain the respective sapogenins as their aglycones, whereas soyabean saponins lacking medicagenic acid and the unidentified sapogenin are almost harmless when added to the diets of chicks.

The analogy between larval growth and that of chicks, rats and mice has been extended also to the beneficial effect of cholesterol, when added to diets containing lucerne saponins.[2,5] With regard to larval growth, the data presented in Fig. 4 clearly indicate that, similarly to cholesterol, plant sterols are also able to counteract fully the harmful effects of lucerne saponins. The reversal of these effects by plant sterols and the fact that lucerne meals have such adverse effects only when added to the diets of chicks or laying hens at levels surpassing the usual percentages of lucerne meal in such diets,[7,8] lead to the conclusion that plant sterols, which are also present in lucerne, could provide a built-in defence mechanism against the harmful influences exerted by lucerne saponins.

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ALGERIAN AND FRENCH RAPESEED MEALS AS A PROTEIN SOURCE FOR CAGED LAYING HENS, WITH OBSERVATIONS ON THEIR TOXIC EFFECTS

By N. JACKSON

Algerian and French rapeseed meals have been examined as protein sources in layers diets also containing Peruvian fishmeal and soyabean meal. The eleven experimental diets used were designed to be isonitrogenous and isocaloric with respect to metabolisable energy.

Egg production on the Algerian meal was satisfactory up to the maximum level (8%) used. The French meal was satisfactory up to 6·9% but at a level of 8·6% egg, production and feed utilisation for egg production were significantly decreased.

The amino acid analysis of the diets showed these to be adequate in the light of known requirements.

Investigations of the thyroid, liver, kidney, adrenals and pituitary were carried out in an attempt to assess the nature of the toxic effects associated with rapeseed meal.

Introduction

The chemical composition of rapeseed meals and their use in animal feedstuffs has been the subject of several reviews.[1-4] Studies have been carried out on the value of rapeseed meal in the diet of growing birds and most of these have also included observations on the thyrotoxic effect of the rapeseed.[5-7]

Very few of the experiments carried out with rapeseed meal have been on its use and toxicity for mature birds fed over a laying year.

Fangauf & Haensel[8] concluded that not more than 10% of rapeseed meal should be used in layers diets while Frölich[9] found that fairly low levels of rapeseed meal in the diet of hens caused a decrease in the laying performance. O'Neill[10] found that when expeller rapeseed meal replaced all of the soyabean meal protein in layers diets which also contained some animal protein, there was no significant difference between egg production and the feed required per dozen eggs produced. Clandinin[11] concluded that 10% rapeseed meal in the diet of laying and breeding chicken and turkey rations would result in as satisfactory production, feed conversion, fertility and hatchability as corresponding amounts of protein from soyabean meal.

At the University of Manitoba[12] it was found that diets containing 10, 12, and 14% rapeseed meal caused a significant decrease in egg size and that egg production was lower than for control birds. In addition, this study showed that there was a sudden decrease in production following the introduction of rapeseed meal into the diet. In another recent Canadian report[13] rapeseed meal was used to replace soyabean meal as the principal source of protein in a layers diet when fed in a phase-feeding programme. None of the diets contained animal protein although DL-methionine was added. The groups receiving rapeseed meal produced slightly fewer eggs, smaller eggs and had poorer body weight gains than birds on soyabean diets. The fact that the rapeseed meal diets were lower in energy than the soyabean diets may have accounted for the lower egg production, egg size and poorer body weight gains. The authors concluded that since the birds on the rapeseed diets failed to increase their food consumption to compensate for the lower energy content it might be that some effect other than dietary energy was responsible and that dietary amino acid balance might be a factor involved.

A recent experiment carried out in this Department[14] showed that when Algerian rapeseed meal was fed at a level of up to 20% of the diet to light and medium body-weight hybrid strains of caged pullets the light body-weight strain was much more susceptible to the toxic effects of the rapeseed than the medium body-weight strain, having a 50% mortality at the highest level of dietary rapeseed, while the medium strain showed only the mortality normally associated with a group of caged layers over a laying year. Dietary treatment affected production, that by the survivors fed 20% rapeseed meal being significantly lower than by those fed up to 16% rapeseed meal in the diet. There was no effect of treatment on mean egg weight, although, in general, this was greater for the rapeseed diets than for the control diet. The best food conversion efficiency was obtained at the 8% level of rapeseed in the diet. With regard to thyrotoxicity, this was more marked in the light than in the medium strain when assessed by thyroid weight per unit of body weight. Liver lipid content was high in all treatments, but was unaffected by treatment. The Fe content of liver dry matter was significantly increased, the trend being for liver Fe content to increase as the level of dietary rapeseed increased. The blood haemoglobin was significantly greater for the control birds than for rapeseed-treated birds.

A further study is described in this paper in which rapeseed meals from two sources (Algeria and France) have been examined as protein sources for egg production by one breed of caged layer when fed in the diet at levels which it was believed, on the basis of previous experience,[14] would not cause mortality. The thyrotoxic and other possible toxic effects were also examined.

Experimental

The experiment, started on 6 August, 1968, extended over eleven 28-day periods until June 1969. The birds were housed in individual cages at 17 weeks of age and commenced laying at 20 weeks of age. They were introduced to the experimental diets at 28 weeks of age and recording began at that time.

194 White Leghorn layers were housed in individual cages equipped with individual feed troughs and communal drinkers, and were maintained on a lighting programme of 17 h light and 7 h darkness. The pullets were randomised into 11 treatment groups and each group was fed one of the 11 experimental diets. The diets were: a control diet in which the main protein source was Peruvian fishmeal; five diets in which the fishmeal was replaced by 1·6, 3·2, 4·8, 6·4 and 8·0% of Algerian rapeseed meal; and five diets in which the

fishmeal was replaced by 1·7, 3·4, 5·2, 6·9 and 8·6% of French rapeseed meal. At the highest level of rapeseed meal inclusion, the Peruvian fishmeal content of the diet was reduced to 56% of the level in the control diet in the case of the Algerian meal, and to 59% in the case of the French meal. The diets were designed to be isonitrogenous and to have a fairly small range of metabolisable energy (*ME*) values.

TABLE I

Composition and metabolisable energy content (*ME*) of the Algerian and French rapeseed meals*

	Algerian rapeseed meal	French rapeseed meal
Standard *ME*, kcal/kg	1880	1680
N-corrected *ME*, kcal/kg	1974	1827
Dry matter, %	90·6	89·3
Crude protein, %	39·6	37·6
Crude fibre, %	11·6	13·8
Ether extract, %	2·31	1·77
Ca, %	0·71	0·79
P, %	0·98	0·89
Oxazolidinethione, %	0·81	0·82
Isothiocyanate, %	0·19	0·15
Alanine, %	1·53	1·46
Arginine, %	2·45	2·52
Aspartic acid, %	2·45	2·21
Cystine, %	1·86	2·08
Glutamic acid, %	6·58	6·15
Glycine, %	1·87	1·72
Histidine, %	1·30	1·06
Isoleucine, %	1·51	1·31
Leucine, %	2·57	2·37
Lysine, %	2·13	2·33
Methionine, %	0·53	0·72
Phenylalanine, %	1·47	1·30
Proline, %	2·32	2·08
Serine, %	1·46	1·46
Threonine, %	1·49	1·58
Tyrosine, %	1·18	1·17
Valine, %	1·76	1·71

* Amino acids uncorrected for losses during acid hydrolysis

The control diet had a calculated *ME* value of 3000 kcal/kg, and this calculated value fell to 2920 kcal/kg at the top levels of rapeseed meal. The calculated Ca content was fixed at 3% and the calculated P content at 0·72–0·75%.

Throughout the experiment individual records were kept of egg weight, egg numbers and feed intake for each hen. Egg production was recorded daily, and egg weight was recorded three times per week. Body weight was recorded initially and at the end of each 28-day period. The compositions of Algerian and French rapeseed meals are presented in Table I. The amino acid contents of the rapeseed meals and of the 11 experimental diets were determined as acid hydrolysates, using a Technicon Auto-Analyzer. No correction was applied for the loss of amino acids during the acid hydrolysis. The composition, determined metabolisable energy, crude protein, calcium and phosphorus contents of the 11 diets are given in Table II.

ME determinations were carried out for each diet by the total collection method, using four hens taken from the relevant dietary treatment at the end of the laying experiment. The *ME* contents of the rapeseed meals were determined using birds which had previously been on the highest level of dietary rapeseed meal. The determined amino acid contents of the diets are presented in Table III.

At the end of the laying period, 6 birds from each of the 11 treatments were slaughtered by decapitation. Blood haemoglobin, packed cell volume, glutathione and methaemoglobin were determined, and cell counts were made. In addition, smears were prepared and examined for any possible haematologic abnormality. The livers were removed, weighed and the lipid content was determined by the method of Folch *et al.*,[16] and the total Fe content was determined by atomic absorption spectroscopy subsequent to dry ashing and solution in dilute HCl. The livers were also examined histologically. A portion of kidney was removed and also examined quantitatively for Fe content. The blood data and the Fe contents of liver and kidney dry matter are presented in Table IV.

With regard to the endocrine system, the thyroid glands were removed and weighed, a histological examination was carried out and the number of follicles in a standard microscope field was observed. The pituitary and adrenals

TABLE II

Composition of the basal diet and the rapeseed meal diets in treatments 1–11

	Control	Algerian rapeseed meal diets					French rapeseed meal diets				
	1	2	3	4	5	6	7	8	9	10	11
Maize meal (9·1% crude protein)	76·8	76·4	75·8	74·8	73·5	73·1	76·3	75·4	74·3	73·0	72·2
Soyabean meal (43·5% crude protein)	3·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0
Fishmeal (61·7% crude protein)	10·2	9·0	8·0	7·4	6·9	5·7	9·0	8·2	7·5	6·9	6·0
Rapeseed meal:											
Algerian (39·6% crude protein)	0·0	1·6	3·2	4·8	6·4	8·0	0·0	0·0	0·0	0·0	0·0
French (37·6% crude protein)	0·0	0·0	0·0	0·0	0·0	0·0	1·7	3·4	5·2	6·9	8·6
Dried grass meal (14·9% crude protein)	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0
Ground limestone	6·0	6·0	6·0	6·0	6·1	6·1	6·0	6·0	6·0	6·1	6·1
Dicalcium phosphate	1·5	1·5	1·5	1·5	1·6	1·6	1·5	1·5	1·5	1·6	1·6
Common salt	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Total	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0
Standard *ME*, kcal/kg	3010	3070	2990	2920	2890	2940	3000	2900	3010	2920	2930
N-corrected *ME*, kcal/kg	2910	2970	2900	2860	2800	2850	2920	2820	2930	2840	2850
Crude protein, %	14·48	14·62	14·47	14·47	14·44	14·38	14·42	14·45	14·51	14·46	15·40
Calorie: protein ratio	208	210	207	202	200	204	208	201	207	202	190
Calcium, %	3·50	3·07	3·24	3·40	3·26	3·42	3·51	3·38	3·25	3·27	3·43
Phosphorus, %	0·62	0·64	0·62	0·61	0·65	0·63	0·63	0·62	0·62	0·61	0·62
Dry matter, %	88·85	88·68	88·15	88·81	88·78	88·68	88·67	88·84	89·02	89·03	88·54

TABLE III

Percentage amino acid composition of the basal diet and the rapeseed meal diets in treatments 1–11*

	Control	Algerian rapeseed meal diets					French rapeseed meal diets				
	1	2	3	4	5	6	7	8	9	10	11
Rapeseed meal in diet, %	0·0	1·6	3·2	4·8	6·4	8·0	1·7	3·4	5·2	6·9	8·6
Alanine, %	0·99	0·91	0·86	0·97	0·96	0·91	1·01	1·02	0·93	0·82	0·78
Arginine, %	0·94	1·06	0·99	0·99	0·73	0·99	0·99	0·95	0·91	1·17	0·84
Aspartic acid, %	1·20	1·15	1·00	1·22	1·18	1·18	1·08	1·17	1·21	1·13	1·13
Cystine, %	0·82	0·74	0·70	0·84	0·74	0·65	0·73	0·65	0·68	0·82	0·86
Glutamic acid, %	2·32	2·37	2·15	2·49	2·41	2·45	2·22	2·39	2·83	2·40	2·30
Glycine, %	0·86	0·78	0·70	0·80	0·90	0·80	0·84	0·85	0·85	0·74	0·75
Histidine, %	0·71	0·60	1·27	0·56	0·39	0·45	0·52	0·49	0·41	0·61	0·31
Isoleucine, %	0·62	0·59	0·59	0·68	0·64	0·64	0·63	0·86	0·64	0·72	0·65
Leucine, %	1·47	1·40	1·40	1·43	1·34	1·34	1·32	1·47	1·39	1·51	1·35
Lysine, %	0·85	0·98	0·80	0·90	0·74	0·77	0·85	0·79	0·75	1·00	0·64
Methionine, %	0·37	0·30	0·31	0·41	0·34	0·28	0·33	0·36	0·32	0·39	0·31
Phenylalanine, %	0·72	0·77	0·73	0·82	0·82	0·81	0·87	0·90	0·80	0·87	0·72
Proline, %	1·04	0·95	0·99	0·98	1·13	1·26	1·00	1·20	1·21	1·08	1·04
Serine, %	0·69	0·70	0·66	0·68	0·71	0·72	0·71	0·77	0·85	0·67	0·70
Threonine, %	0·58	0·56	0·47	0·57	0·56	0·54	0·49	0·55	0·67	0·56	0·58
Tyrosine, %	0·68	0·72	0·67	0·76	0·71	0·62	0·63	0·66	0·57	0·68	0·64
Valine, %	0·83	0·81	0·75	0·84	0·83	0·82	0·84	0·84	0·86	0·86	0·81

* Amino acids uncorrected for losses during acid hydrolysis

TABLE IV

Blood data and liver and kidney Fe contents in treatments 1–11

No. of observations per treatment = 6

	Control	Algerian rapeseed meal diets					French rapeseed meal diets					S.E. of a mean
	1	2	3	4	5	6	7	8	9	10	11	
Red cells, $\times 10^6$/mm^3	3·33	3·50	3·43	3·27	3·40	3·44	3·08	3·44	3·43	3·40	3·32	0·123
Hb, g/100 ml	10·0	10·6	9·9	9·1	9·4	9·9	9·00	10·2	9·8	9·7	9·3	0·398
MCHC, %	32	31	33	31	32	31	31	31	32	31	30	0·780
White cells, $\times 10^3$/mm^3	17·3	12·3	14·9	15·8	19·0	20·1	17·6	14·8	15·8	19·4	18·4	3·67
Glutathione (μmole/g Hb)	15·0	15·2	14·9	12·4	13·6	13·7	14·1	13·5	14·6	16·0	15·2	1·52
Liver Fe, ppm in dry matter	386	364	305	356	284	314	288	244	287	352	293	78·5
Kidney Fe, ppm in dry matter	230	235	417	236	199	223	227	279	197	267	255	56·9

TABLE V

Thyroid, liver and adrenal and pituitary data for treatments 1–11

	Control	Algerian rapeseed meal diets					French rapeseed meal diets					S.E. of a mean
	1	2	3	4	5	6	7	8	9	10	11	
Rapeseed meal in diet, %	0	1·6	3·2	4·8	6·4	8·0	1·7	3·4	5·2	6·9	8·6	
No. of observations	6	6	6	6	6	6	6	6	6	6	6	
Thyroid weight, g	0·171	0·616	0·949	1·624	4·969	1·877	0·535	1·547	2·118	2·378	2·534	0·634
Thyroid weight, g/kg bodyweight	0·083	0·319	0·451	0·892	2·176	0·918	0·268	0·799	1·082	1·102	1·262	0·301
Thyroid—no. of follicles in unit microscope field	13	5	4	3	2	2	5	3	3	3	2	
Fresh liver weight, g	43·9	32·1	43·1	37·5	43·1	31·9	31·4	33·6	32·9	36·4	35·7	4·02
Fresh liver weight, g/kg bodyweight	20·4	16·3	19·5	20·5	19·3	16·2	16·2	17·0	17·2	17·9	18·6	1·387
Liver dry matter, %	41·2	33·9	40·9	37·8	38·3	34·3	35·5	35·2	36·6	36·1	32·4	3·273
Fresh liver lipid, %	18·6	11·8	19·9	16·1	15·9	11·7	15·1	12·2	12·7	13·6	11·5	3·390
Adrenal weight, mg	135	154	143	151	126	148	120	133	149	126	147	11·62
Pituitary weight, mg	9·56	10·30	8·45	7·71	9·33	11·40	8·53	9·11	10·82	8·50	9·58	1·086

were also removed and weighed. The pituitary was subjected to histological examination. The thyroid, liver weight and lipid data, and the adrenal and pituitary weights are presented in Table V.

Results

The oxazolidinethione and isothiocyanate contents of the rapeseed meals (Table I) were not very dissimilar but were higher than those found previously in this laboratory for Algerian rapeseed meal.[14]

The average mortality over the experimental period was 11·8% for the control group, 5·5% for the birds fed the Algerian rapeseed meal diets and 7·1% for birds fed the French rapeseed meal diets. The correlation between treatments and mortality was tested using the χ^2 test and showed

that there was no significant correlation. In addition, treatment did not have any effect on the survival times of the birds which did die. The blood data (Table IV) show no effects of treatment nor do the liver and kidney Fe contents. No methaemoglobin was detected in any of the blood samples examined.

Dietary treatment had a highly significant effect on thyroid weight. The heaviest total weight of the two glands for one bird was 10·95 g, this being obtained on the 6·4% Algerian rapeseed meal diet. This bird also had the heaviest weight for a single thyroid, this being 7·65 g. In general, the mean thyroid weights were heavier for birds fed the French rapeseed diets than for those fed the Algerian rapeseed diets, the exception being at the 6·4% level of Algerian rapeseed meal. Histological examination of the thyroids showed follicular enlargement with no cellular infiltration. The degree of enlargement was assessed both by weight and by the number of follicles present in a standard microscope field, which decreased from 13 in the control thyroids to 2 for the highest level of both types of rapeseed.

Total fresh and dry liver weights were not affected by dietary treatment, neither were the percentage dry matter in the liver nor the lipid content.

Neither adrenal nor pituitary weights were significantly affected by treatment. Microscopic examination of the pituitaries failed to show any sign of cytological changes due to treatment.

The results for egg production, egg weight, food intake and food conversion of the survivors, expressed as bird means for the various treatments, are given in Table VI. In the case of the birds fed the Algerian rapeseed diets, food intake was not significantly affected by dietary treatment and the group with the highest intake was treatment 6 (8·0% Algerian rapeseed meal). In the case of birds fed the French rapeseed diets, the lowest intake was by the hens in treatment 11 (8·6% addition of rapeseed meal) although this was not statistically significantly different from the intakes by the control group. It was, however, significantly lower than the intake by the hens on the 6·9% French rapeseed meal diet.

The inclusion of Algerian rapeseed meal in the diets did not have an effect on egg production although on all of treatments 2–6 production was slightly lower than for the control diet. When fed the French rapeseed meal diets, egg production was slightly lower than for the control group, except in the case of the 8·6% level of inclusion which caused a marked ($P < 0·01$) depression in egg production compared with the control, and a significant depression ($P < 0·05$) when compared with the other four groups fed the diets containing French rapeseed meal.

Total egg weight was lower in all the rapeseed diet groups compared with the control diet, but in the case of the Algerian rapeseed diets, none of these differences was significant. The most marked depression of total egg weight was in the case of treatment 11 (8·6% French rapeseed) and this effect was statistically significant ($P < 0·01$) when compared with the control and with treatment 8 (3·4% French rapeseed) and when compared with the other 3 levels of dietary French rapeseed ($P < 0·05$). Lower total egg weight is due to both lower mean egg weight and lower production, especially the latter. Dietary treatment had no statistically significant effect on mean period mean egg weight:

$$\left(\begin{matrix}\text{Period mean}\\ \text{egg weight}\end{matrix} = \frac{\text{total weight of eggs weighed per period}}{\text{number of eggs weighed per period}}\right)$$

in the case of the Algerian rapeseed, but in the case of the maximum level of French rapeseed meal in the diet, mean period egg weight was significantly depressed ($P < 0·05$) below the values for the control and for the 1·7, 3·4 and 6·9% levels of addition. This effect is not so marked when the mean egg weight is expressed as total egg weight divided by the number of eggs laid.

With regard to food conversion efficiency, the best value was obtained at the 3·2% addition of Algerian meal. At this level, and at the 1·7% and 3·4% levels of addition of French rapeseed meal, food conversion efficiency was better than for the control. This effect was statistically significant ($P < 0·05$) in the case of the 3·2% addition of Algerian rapeseed meal. None of the other differences was statistically significant in the case of the Algerian rapeseed meal addition. A marked deterioration of the value for the efficiency of food conversion to egg product occurred at the highest level of addition of French rapeseed meal, this value being significantly poorer than the value obtained for birds fed the control diet or diets containing 5·2% and 6·9% French rapeseed meal ($P < 0·05$) and poorer ($P < 0·001$) than the value at the 1·7% and 3·4% levels of addition. The poor food conversion figure for treatment 11 was associated with a continuous but rather erratic rate of egg production rather than with a cessation of egg production by any of the birds on this treatment. There

TABLE VI

Mean egg production, egg weight, food intake and food conversion data for treatments 1–11

	Control	Algerian rapeseed meal diets					French rapeseed meal diets					S.E. of a mean
	1	2	3	4	5	6	7	8	9	10	11	
Rapeseed meal in diet, %	0·0	1·6	3·2	4·8	6·4	8·0	1·7	3·4	5·2	6·9	8·6	
No. of observations	14	14	14	14	14	14	14	14	14	14	14	
Mean eggs per bird	220	208	219	210	219	211	216	218	214	214	182	11·034
Mean egg production, %	71·45	67·67	71·24	68·13	71·22	68·67	70·27	70·71	69·62	69·59	58·97	3·582
Mean total egg weight, kg	12·56	11·85	12·44	11·61	12·31	12·03	12·26	12·48	12·21	12·22	10·09	0·634
Mean period mean egg weight, g	55·67	55·30	56·10	56·15	54·90	55·44	56·47	55·77	53·59	56·53	52·24	1·220
Mean total egg wt. Mean eggs per bird, g	57·07	56·84	56·68	55·32	56·12	56·90	56·64	57·31	56·96	56·99	55·55	
Mean total food intake, kg	33·61	32·99	33·13	32·35	33·74	34·38	33·24	33·75	32·92	34·08	31·69	0·760
Mean daily food intake, g	109·1	107·1	107·6	105·0	109·5	111·6	107·9	109·6	106·9	110·7	102·9	2·4678
Mean food conversion, kg meal/kg eggs	2·787	2·912	2·697	2·876	2·796	3·027	2·773	2·753	2·861	2·837	3·399	0·1672

were no other statistically significant differences in food conversion in the French rapeseed dietary groups.

The mean initial and final body weight, *ME* intake, crude protein intake and *ME* conversion data are presented in Table VII, together with the average body weights over the experimental period. These last mentioned figures were obtained from a regression equation for the body weights attained at 28-day intervals over the experimental period.

The daily *ME* intakes of the birds were fairly similar for all treatments, with the exception of the birds receiving the highest level of French rapeseed meal, where there was a marked fall (8·2%) in daily *ME* intake.

Discussion

The birds showed very marked enlargement of the thyroid glands at the end of the 308 days on the diets, this effect being of the order observed previously in this department[14] when the birds were fed up to 20% Algerian rapeseed meal in the diet for a period of 252 days. The weight of the thyroid gland, expressed on a body weight basis, increased 26-fold at the 6·4% level of dietary Algerian rapeseed, while for the French rapeseed the maximum increase was 15-fold and occurred at the 8·6% level of addition.

The degree of thyroid enlargement in this experiment is greater than that reported in most other experiments with fowl.[2-4,6,7,17-19] Frölich[9] fed 5% and 10% of rapeseed meal in the meal portion of the feed of laying hens for a period of 112 days and found no significant differences in thyroid weight between these and control birds fed no rapeseed. The effect on the thyroid appears to be irreversible or perhaps slowly reversible, since even after restoring the birds to a diet containing no rapeseed meal the thyroid hypertrophy is still very marked after fifteen months.

The haematological data showed no evidence of toxic effects, nor did the liver or kidney analyses indicate any effect of treatment. Thus they did not substantiate the view expressed previously[14] that dietary rapeseed may lead to an increased liver Fe content, although attention must be paid to the fact that in the previous experiment[14] the levels of rapeseed meal used were much higher.

The egg production data show that, despite the obvious thyrotoxic effect of the rapeseed, birds fed the Algerian rapeseed up to 8·0% in the diet and French rapeseed to a level of 6·9% in the diet had a satisfactory egg production, and only at the highest level of French rapeseed meal was any adverse effect on production noted.

The statistically significant effect on food conversion at the 3·2% level of Algerian meal, and the better, although not statistically significant, improvement for the two lowest levels of French rapeseed agree with other studies[12,14] where up to 10% rapeseed gave improved feed utilisation compared with controls or birds receiving higher levels of dietary rapeseed.

The absence of any definite effect of treatment on mean egg weight is in agreement with previous results.[14] Although studies at the University of Manitoba[12] and at Guelph[13] have shown dietary rapeseed meal to decrease egg size, this effect was noted at much higher levels of rapeseed meal inclusion.

A significant observation was the difference (Table I) between the *ME* content of the Algerian (1880 kcal/kg) and the French (1680 kcal/kg) rapeseed meals, indicating the importance of using a determined value for *ME* before computing rations. The value obtained for the Algerian meal was similar to the value of 1820 kcal/kg obtained previously[41] and the value of 1880 kcal/kg reported by Summers *et al.*[13] A much lower value of 1530 kcal/kg was reported by Sibbald & Slinger[20] who used chicks for the assay, and a higher value of 2295 kcal/kg was reported by Sell,[21] using laying hens. The variability of the data, and the lack of published information on the *ME* of rapeseed meals further emphasises the necessity of obtaining accurate data for the *ME* of this feed ingredient. As in the previous study[14] the *ME* of the rapeseed meal was obtained by feeding the meal alone to birds which had previously been on the highest levels of rapeseed meal in the diets. The birds were in a state of negative nitrogen balance when fed the rapeseed meals, so that the N-corrected *ME* values are considerably larger than the uncorrected values. The lower value for the *ME* of the French rapeseed meal was not reflected in the *ME* of the diets. The final body weights (Table VII) of the birds show no significant differences, all the birds gaining some weight over the experimental period.

The amino acid data for the rapeseed meals were fairly similar to existing data,[1,3] but the Algerian and French rapeseeds used in the present work did have a higher cystine content than those previously reported. The amino acid contents of the diets were adequate by the A.R.C. standards[22] for layers diets, assuming satisfactory availability, and the

TABLE VII

Mean bodyweight, *ME* and crude protein intake and utilisation data for treatments 1–11

	Control	Algerian rapeseed meal diets					French rapeseed meal diets					S.E. of a mean
	1	2	3	4	5	6	7	8	9	10	11	
Rapeseed meal in diet, %	0·0	1·6	3·2	4·8	6·4	8·0	1·7	3·4	5·2	6·9	8·6	
Bodyweight (survivors), kg												
Initial	1·72	1·71	1·72	1·55	1·64	1·63	1·68	1·74	1·66	1·78	1·70	0·0515
Final	2·05	2·05	2·05	1·83	1·99	1·89	2·04	2·01	1·93	1·98	1·97	0·0743
Average bodyweight increase of survivors, g	324	342	334	274	354	256	360	276	274	201	272	
Average bodyweight (survivors) over the experimental period, kg	1·94	1·95	1·93	1·78	1·90	1·80	1·90	1·92	1·84	1·93	1·84	
Meal daily *ME* intake, kcal	328	329	322	307	316	328	324	318	322	323	301	
Daily crude protein intake, g	15·80	15·66	15·57	15·19	15·81	16·05	15·56	15·84	15·51	16·01	15·85	
Mean *ME* conversion, Mcal/kg eggs	8·043	8·553	7·974	8·144	7·906	8·394	8·140	7·847	8·120	8·143	9·188	

results of the chemical analyses are supported by the satisfactory production in all but the diet containing 8·6% of French rapeseed meal.

The experiment has shown that the Algerian rapeseed meal was used successfully up to a level of 8% of the diet, when 22% of the total dietary protein was supplied by the rapeseed and 24·5% by fishmeal and that successful production and utilisation was obtained with the French meal up to a level of 6·9% of the diet. At this level, 18% of the protein was provided by the rapeseed and 29·5% by fishmeal.

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LIPIDS OF BERSEEM (*TRIFOLIUM ALEXANDRINUM*) SEED

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Berseem seed oil was fractionated into non-polar and polar components. Tentative identification was made of hydrocarbons, sterol esters, triglycerides, free fatty acids and partial glycerides in the non-polar fraction and of lysophosphatidyl choline, phosphatidyl inositol, lysophosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl ethanolamine, digalactosyl diglycerides, sterol glycosides, phosphatidic acid, monogalactosyl diglycerides, cerebrosides and esterified sterol glycosides in the polar fraction. The fatty acid constituents of the major polar and of all the non-polar lipid components are also reported.

Introduction

The seed of berseem (*Trifolium alexandrinum*), also known as Egyptian clover, is used for growing the winter green fodder in India. Earlier studies by El-Arwady & Motawi[1] revealed that this seed is a good source of protein, oil and minerals. Salah & El-Arwady[2] reported that berseem seed oil is semi-drying in nature and exhibits antioxidant properties. There is no other report on the detailed composition of the oil from this seed. The present study was, therefore, undertaken to identify various polar and non-polar lipid components and to study their fatty acid constituents.

Experimental

Extraction and fractionation of lipids

The seeds of a local variety of berseem were procured from the Plant Breeding Department, Punjab Agricultural University, Ludhiana. The total lipids were extracted and purified by the method of Folch *et al.*,[3] the free lipids by using deoxygenated hexane by the method of McKillican,[4] and the bound lipids with water-saturated n-butanol by the procedure described by Pomeranz *et al.*[5] Separation into polar and non-polar lipid fractions was achieved as recommended by Galanos & Kapoulas.[6]

The non-polar lipids were further fractionated into individual components, by thin-layer chromatography (t.l.c.) as described by Malins & Mangold.[7] The polar lipids were separated by t.l.c. using chloroform–methanol–acetic acid–water (85:15:10:4 by vol.) solvent system. Lysophosphatidyl choline, lysophosphatidyl ethanolamine, phosphatidyl ethanolamine, phosphatidyl choline and phosphatidic acid, procured from V.P. Chest Institute, University of Delhi, were used as reference standards. The spots of phosphatides were identified by spraying with modified spray reagent for detection of phospholipids,[8] while the glycolipids were identified with diphenylamine reagent.[9] Various spray reagents given by Lepage[10] were also used for the tentative identification of different classes of polar lipids.

Quantitative evaluation of individual non-polar lipids was done by the densitometric method of Privett *et al.*[11] using a Densicord densitometer (Photo-volt Corporation, New York). This method gave reproducible results within $\pm$ 5% in the concentration range over which it was applied. The calculated peak areas bore a nearly linear relationship to the applied concentrations.

Fatty acid analysis by gas–liquid chromatography (g.l.c.)

The methyl esters were prepared by the method recommended by Instrumental Techniques Committee.[12] These were fractionated by g.l.c. on an Aerograph Hy-Fi GLC apparatus (Model 600-C) using a 10 ft $\times$ 1/6 in stainless-steel column packed with 20% diethylene glycol succinate (DEGS), on 60–80 mesh Chromosorb-W. Tentative identification of the peaks was made by comparison of their retention times with those of known fatty acids. Relative percentages of the fatty acids were calculated directly from the peak areas determined by a planimeter.

Results and Discussion

Berseem seed contained on an average 9·7% total lipids of which the free lipid fraction (6·13 g) was almost double the amount of the bound lipids (3·53 g). Similarly, the polar lipid content (2·31 g) was nearly double that of non-polar lipids (1·22 g) in the bound fraction while in the free lipid fraction, the non-polar lipids comprised about 99%. This indicates that the phospho- and glyco-lipids (polar lipids) of the seed are predominantly present in the bound form, and the non-polar lipids exist primarily in the free form.

For identification of non-polar lipids, palmitic acid, cholesterol acetate, liquid paraffin and tripalmitin were used as reference standards. This fraction consisted of hydrocarbons, sterol esters, triglycerides, free fatty acids and partial glycerides. Quantitative thin-layer chromatography revealed that triglycerides were the predominant components in the non-polar lipid fractions of both free and bound oils (Table I). The concentration of free fatty acids in the bound lipids was higher than in the free lipids. Because the amount of polar lipids (1%) in free oil was very small, they could not

TABLE I

Composition (relative percentage) of non-polar lipids of berseem seed oil

Values are determined densitometrically

Lipid components	Lipid fraction, %	
	Free	Bound
Partial glycerides	2·08	1·47
Free sterols	11·50	9·19
Free fatty acids	9·57	13·08
Triglycerides	73·44	72·58
Sterol esters + hydrocarbons	3·30	3·67

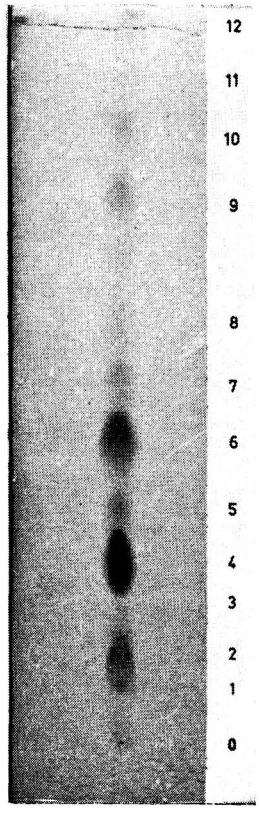

FIG. 1. *Thin-layer chromatogram of the polar lipids (bound fraction) of* Trifolium alexandrinum *seed*

Mobile phase: chloroform–methanol–acetic acid–water (85:15:10:4 by vol.) Identity of spots: 0, origin; 1, lysophosphatidyl choline; 2, phosphatidyl inositol; 3, lysophosphatidyl ethanolamine; 4, phosphatidyl choline; 5, phosphatidyl glycerol; 6, phosphatidyl ethanolamine + digalactosyl diglycerides; 7, cerebrosides; 8, sterol glycosides; 9, phosphatidic acid; 10, monogalactosyl diglycerides; 11, esterified sterolglycosides; 12, neutral lipids

be further fractionated into various components. However, the bound polar fraction revealed the presence of lysophosphatidyl choline, phosphatidyl inositol, lysophosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl ethanolamine + digalactosyl diglycerides, cerebrosides, sterol glycosides, phosphatidic acid, monogalactosyl diglycerides and esterified sterol glycosides (Fig. 1 and Table II). The identity of spots 1, 3, 4 and 9 as lysophosphatidyl choline, lysophosphatidyl ethanolamine, phosphatidyl choline and phosphatidic acid, respectively, was confirmed by co-chromatography with authentic standards. Spots 8 and 11 gave a typical Lieberman–Burchard colour reaction on heating the sulphuric acid-sprayed t.l.c. plates, indicating the presence of sterols in them, and were identified as sterol glycosides and esterified glycosides, respectively. Their relative positions on the thin-layer chromatogram were similar to those observed by Nichols[13] for these compounds. A Dragendorffs positive spot (spot 7) lying between phosphatidyl ethanolamine and sterol glycosides was observed, which, on the basis of relative R_f value, was tentatively identified as cerebrosides. Cerebrosides, though normally of animal origin have, nevertheless, been reported by Nichols[13] in groundnuts, another leguminous plant, and in wheat by Carter.[14]

From a visual comparison of the spot intensities, it was observed that in the polar fraction of the bound oil, phosphatidyl choline was the principal component, followed by phosphatidyl ethanolamine + digalactosyl diglycerides. Phosphatidyl inositol was also present in fairly good concentration. The other fractions were present as minor components (Fig. 1).

In the berseem seed oil only five fatty acids, i.e., palmitic, stearic, oleic, linoleic and linolenic acid, were detected. Linoleic acid is the principal acid of this oil and occurs in maximum concentration in phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl choline fractions of bound oil and triglycerides, partial glycerides and sterol esters fractions of both the free and bound oils (Table III). Palmitic acid was the major constituent in the free fatty acids and glycolipids of bound oil. Stearic acid occurred in lowest concentration in all fractions. In general, the fatty acid constituents of the non-polar fraction were similar in the free and bound oil.

TABLE II

Tentative identification of various polar lipid classes of berseem seed oil

Spot No.	Spray reagents						R_f value	Tentative identity
	50% Sulphuric acid	Iodine vapours	Ninhydrin	Dragendorffs' reagent	Molybdenum	Diphenylamine		
1.	+	+	—	+	+	—	0·07	Lysophosphatidyl choline
2.	+	+	—	—	+	—	0·10	Phosphatidyl inositol
3.	+	—	+	—	+	—	0·19	Lysophosphatidyl ethanolamine
4.	+	+	—	+	+	—	0·24	Phosphatidyl choline
5.	+	+	—	—	+	—	0·31	Phosphatidyl glycerol
6.	+	+	+	+	+	+	0·40	Phosphatidyl ethanolamine + digalactosyl diglycerides
7.	+	+	—	+	—	+	0·49	Cerebrosides
8.	+	—	—	—	—	+	0·57	Sterol glycosides
9.	+	+	—	—	+	—	0·74	Phosphatidic acid
10.	+	—	—	+	—	+	0·83	Monogalactosyl diglycerides
11.	+	+	—	+	—	+	0·92	Esterified sterol glycosides
12.	+	+	—	—	—	—	0·98	Neutral lipids

+ Indicates a positive reaction with the spray reagent
— Indicates a negative reaction with the spray reagent

TABLE III

Fatty acid constituents of different lipid classes of berseem seed oil

Areas of peaks for different fatty acids obtained by g.l.c. were determined by planimeter and the % areas converted directly into wt.-% of the fatty acids

Fatty acids	Bound oil										Free oil					
	Non-polar fraction	Polar fraction	Glyco-lipids	PE	PG	PC	Partial glycerides	FFA	Tri.	SE	Non-polar fraction	Polar fraction	Partial glycerides	FFA	Tri.	SE
Palmitic	17·09	21·91	42·11	32·17	17·33	20·71	20·00	55·55	19·22	24·30	15·90	15·92	11·60	24·30	15·73	19·64
Stearic	1·72	1·37	5·26	tr.	0·72	tr.	16·67	7·42	7·69	12·95	3·40	2·49	4·35	8·41	3·74	8·93
Oleic	17·10	11·68	21·05	10·34	20·21	14·79	20·66	11·11	15·38	14·15	16·47	15·45	21·74	17·75	18·73	32·14
Linoleic	47·00	51·35	21·05	48·28	50·55	50·30	30·00	14·81	40·38	28·04	41·51	44·93	53·61	37·38	44·94	26·78
Linolenic	17·09	13·69	10·53	9·20	11·19	14·20	12·67	11·11	17·32	20·56	22·72	21·21	8·70	12·15	16·85	12·50

PE = Phosphatidyl ethanolamine
PC = Phosphatidyl choline
Tri. = Triglycerides
PG = Phosphatidyl glycerol
FFA = Free fatty acids
SE = Sterol esters

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COMPOSITION OF WHEAT-FLOUR LIPIDS

By T. A. MACMURRAY* and W. R. MORRISON

Lipids were extracted from a single sample of wheat flour using three solvent systems: ethanol–diethyl ether–water (2:2:1 by vol.); chloroform–methanol (2:1 by vol.); and water-saturated n-butanol. Analysis of the extracts and of residual lipid in the extracted flour showed that water-saturated n-butanol was the most efficient solvent.

Wheat-flour lipids were extracted with water-saturated n-butanol and separated by chromatographic procedures into individual components. The lipid classes which were isolated and studied were steryl ester, free sterol, 6-*O*-acyl steryl glucoside, steryl glucoside, triglyceride, diglyceride, monoglyceride, free fatty acid, monogalactosyl diglyceride, 6-*O*-acyl monogalactosyl diglyceride, digalactosyl diglyceride, digalactosyl monoglyceride, monoglycosyl ceramide, diglycosyl ceramide, *N*-acyl phosphatidyl ethanolamine, *N*-acyl lysophosphatidyl ethanolamine, phosphatidyl ethanolamine, lysophosphatidyl ethanolamine, phosphatidyl choline, lysophosphatidyl choline, phosphatidyl serine and phosphatidyl inositol. Monogalactosyl monoglyceride was also tentatively identified. The quantitative distributions of the lipid classes were determined.

Monoglycosyl ceramide contained small amounts of normal fatty acids (12:0–24:0) and large amounts of 2-hydroxy fatty acids (principally 16:0 and 20:0), with similar amounts of dihydroxy long-chain bases (18:0 and 18:1) and trihydroxy long-chain bases (18:0, 18:1, 19:0, 19:1, 20:0, 22:0). The principal sterols were identified as β-sitosterol, campesterol, and C_{28} and C_{29} saturated sterols.

The fatty acids in the sterol lipids were principally 16:0 (50–60%) and 18:2 (28–30%) with small amounts of 16:1, 18:0, 18:1 and 18:3. The fatty acids in all the glycerides were principally 18:2 (51–84%) with lesser amounts of 16:0, 18:0, 18:1 and 18:3.

Introduction

The importance of wheat lipids in baking technology has been recognised for many years, and has been the subject of several recent reviews.[1–9] There are many lipid classes in wheat, most of which have been isolated and identified individually. Nevertheless, in papers on the analysis of wheat-flour lipids identification of the lipids is often incomplete. In this paper the classes of lipids in wheat flour are reviewed, and a complete analysis of the lipids in a single sample of flour is described.

Nonsaponifiable matter, sterols

Flour contains 0·5–3·0% lipids, of which 4·9–6·7% is nonsaponifiable matter,[1,4,10] consisting of hydrocarbons, tocopherols, carotenoid pigments and sterols. Normal C_8–C_{38}, iso-C_{18}–C_{33}, and cyclohexyl C_{16}–C_{31} hydrocarbons have been identified by gas–liquid chromatography (g.l.c.), and squalene has been tentatively identified.[11,12] Wheat tocopherols have been analysed by g.l.c.[13–15] and the major tocopherols in order of abundance are 5,8-dimethyltocotrienol (β-T-3), 5,7,8-trimethyltocol (α-tocopherol, α-T), 5,8-dimethyltocol (β-tocopherol, β-T), 5,7,8-trimethyltocotrienol (α-T-3).

The carotenoid pigments are principally lutein (xanthophyll) and lutein esters.[17] LePage & Sims[18] found greatly differing proportions of lutein (84·8%, 21·6%), lutein monoesters (9·8%, 46·5%), and lutein diesters (5·3%, 31·9%) in Mindum and Thatcher wheats. The esterified fatty acids in the lutein esters were 16:0 (22·7%), 18:0 (4·4%), 18:1 (33·9%), 18:2 (32·2%) and 18:3 (2·1%).

The principal sterol in wheat is β-sitosterol. According to Fisher[1] α-sitosterol, γ-sitosterol, dihydrositosterol and stigmasterol have all been reported in wheat. It now seems likely[19] that the substance identified as γ-sitosterol is campesterol. Berry *et al.*[20] analysed wheat sterols by g.l.c. and, on the basis of g.l.c. retention data, identified four components as β-sitosterol, campesterol, cholesterol and brassicasterol. Knights[21] has analysed wheat sterols by combined gas chromatography–mass spectrometry and he has shown that they consist of 55% β-sitosterol, 21% campesterol, a trace of cholesterol, no stigmasterol, 8% saturated C_{28} sterol, 10% saturated C_{29} sterol, and small amounts of other sterols.

Neutral lipids

The triglyceride fraction constitutes about 30–36% of wheat flour lipids.[22–24] Triglycerides have been subfractionated by counter-current distribution into groups of which about 80% consisted of 16:0–18:2–18:2, 18:1–18:2–18:2 and 18:2–18:2–18:2 molecular species.[23]

The neutral lipids contain small amounts of 1,2- and 1,3-diglycerides and 1- and 2-monoglycerides,[10,22,25] but it is not known whether the diglyceride and monoglyceride isomers exist as such *in vivo* or whether the two forms of each arise by acyl migration during milling, flour storage, or lipid isolation procedures.

Freshly milled flour lipids contain 5–10% free fatty acids[26,27] and their level rises to 30–78% during prolonged storage.[28,29] The nature of the hydrolytic processes has not been elucidated, but there is some degree of selectivity in the types of esterified fatty acids which are hydrolysed.[27] The fatty acid compositions of the free and esterified acids are generally similar, the principal acids[22] being 16:0 (17–24%), 18:0 (0·3–1·2%), 18:1 (7–14·5%), 18:2 (60–70%) and 18:3 (2·5%), but there are variations between individual classes of lipids.[1,10,25–27,29] The minor fatty acids[29] include 11:0, 12:0, 13:0, 14:1, 15:0, 20:4, 21:0 (<0·1%) and 14:0, 16:1, 17:0, 20:0 and 22:0 (0·1–0·25%). The major unsaturated acids are 9-*cis*-18:1, 9-*cis*,12-*cis*-18:2, and 9-*cis*,12-*cis*, 15-*cis*-18:3, and there appear to be no *trans* or positional isomers (Hay, J. D., & Morrison, W. R., unpublished results).

Phosphoglycerides

Phosphatidyl choline and phosphatidyl ethanolamine have been identified by several analytical techniques, and are major components of the phosphoglycerides.[10,24,25] Lysophosphatidyl choline is the major lipid in wheat starch and is present in smaller amounts in flour,[30–33] together with a small proportion of lysophosphatidyl ethanolamine.[25]

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Early reports state that phosphatidic acid is a major phospholipid in flour,[4,34,35] but Mason & Johnson[30] claim that it does not exceed 10% of the lipids and others have found it to be a minor phospholipid.[25,34] Inositol is present in wheat lipids, and Carter *et al.*[36] isolated lipophytin (a mixture of inositol polyphosphates) from wheat germ. Phosphatidyl inositol has now been identified and isolated in pure form from wheat flour.[35,37]

Fisher *et al.*[10] tentatively identified phosphatidyl glycerol by the product, glycerylphosphoryl glycerol (GPG), obtained after mild alkaline de-acylation, and Wren & Szczepanowska[34] have found it in a fraction eluted from a silicic acid column together with phosphatidyl ethanolamine (PE) and sulpholipid. Phosphatidyl serine is also present in small amounts.[10,25,38,39]

Bomstein[40] found two novel phospholipids in wheat flour which he identified as *N*-acyl phosphatidyl ethanolamine and *N*-acyl lysophosphatidyl ethanolamine. These lipids have since been found in other plant tissues.[41–44] *N*-acyl derivatives of phosphatidyl ethanolamine are known to be formed as artifacts during saponification or alcoholysis of lipids,[45] but there is no indication that the *N*-acyl lipids in wheat flour are artifacts.

Glycolipids

The principal glycolipids in wheat are monogalactosyl and digalactosyl diglycerides. These lipids were first isolated and characterised from wheat flour by Carter *et al.*,[46–48] and are now known to occur widely in plant tissue and to a much smaller extent in animal tissues. 6-*O*-Acylgalactosyl diglyceride is an artifact formed during the isolation of lipids from leaf homogenates,[49] and it has now been reported in wheat flour.[50]

Sulpholipid is generally found in lipids from photosynthetic tissue, and it may be present in small amounts in wheat flour lipids.[34] Steryl glucosides and 6-*O*-acyl steryl glucosides (esterified steryl glucosides) have been identified in wheat flour.[34,47,50,51] The principal sterols in the glucosides are β-sitosterol and campesterol, as in the steryl esters and free sterols.[51]

Sphingolipids

Sphingomyelin has been tentatively identified in wheat flour,[25,39] but in the absence of firm evidence of its identity there must be some doubt as to whether this typically mammalian lipid is present in wheat at all.

Ceramide monoglycoside was isolated by Carter *et al.*[47,52] during the course of work on the galactosyl diglycerides. The long-chain bases were shown to be 4-D-hydroxysphinganine (phytosphingosine), sphinganine, an isomer of sphingenine, and 4-D-hydroxy-Δ^8-sphingenine.[52] No ceramide diglycoside has been reported, but there may be some ceramide trimannoside.[47] The principal fatty acid in wheat ceramide monoglycoside is 2-hydroxy-stearic acid.[52]

Carter *et al.*[36] isolated a phytoglycolipid complex containing phytosphingosine, fatty acid, phosphate, glucuronic acid, glucosamine, mannose, galactose and arabinose from a variety of plant seeds and from wheat germ oil. Further work has been largely confined to corn and flax phytoglycolipid and the basic structure has now been elucidated.[53] Phytoglycolipid preparations consist of a series of lipids containing phytoglycolipid trisaccharide and phytoglycolipid tetrasaccharide of known structure, and penta- to octasaccharides in which the configurations of the additional saccharides have not yet been determined. Wren & Szczepanowska[34] found no evidence for phytoglycolipid in flour, but it seems likely that, because of its high carbohydrate content, it will have solubility properties analogous to the gangliosides, and it will therefore be readily lost during normal lipid extraction and purification procedures.

Experimental

Materials

A single sample of unbleached, untreated high grade spring wheat flour was supplied by J. & R. Snodgrass Ltd., Glasgow. Solvents were freshly redistilled before use. Column chromatography was performed with 20 cm × 5 cm dia. columns of 100 mesh Mallinckrodt silicic acid (activated for 1 h at 120°C), or with 30 cm × 3·5 cm dia. columns of Whatman DE1 DEAE cellulose prepared according to the method of Rouser.[54]

Thin-layer chromatography (t.l.c.) plates were made in the usual way with 250 μm layers of silica gel G (E. Merck, Darmstadt). Sodium tetraborate (2% wt./vol.) or silver nitrate (2·5% wt./vol.) solutions were used instead of water for preparation of 4% borate- or 5% silver nitrate-impregnated t.l.c. plates.

Methods

The t.l.c. solvent systems which were found most useful were: I, diethyl ether–benzene–ethanol–acetic acid (40:5:2:0·2 by vol.);[55] II, chloroform–acetone–methanol–acetic acid (80:18:1·5:0·5 by vol.);[56] III, chloroform–acetone–water (15:30:1·5 by vol.); IV, chloroform–methanol–ammonia (30% wt./vol.)–water (65:35:5:2·5 by vol.);[57] V, chloroform–methanol–acetic acid–water (65:25:8:4 by vol.).[58] Details of the t.l.c. analysis of wheat flour lipids have been published elsewhere.[16] Lipids were detected on t.l.c. plates by charring with 50% sulphuric acid; or specific groups in lipids were detected with ninhydrin (–NH_2),[57] modified Zinzadze reagent (phosphate),[59] or α-naphthol reagent (sugars).[60]

Lipids separated by t.l.c. were quantitated by the sulphuric acid–dichromate method of Amenta,[61] and standard curves were prepared with known amounts of pure lipids. Lipids were also quantitated by charring on t.l.c. plates with 50% sulphuric acid,[62] and measuring spot density with a Chromscan densitometer (Joyce, Loebel & Co. Ltd.). Gravimetric analyses were made on aliquots of lipid solution (typically 10–15 μl of solution containing 10–100 μg lipid) with a Cahn 'Gram' microbalance.

Nitrogen in the range 1–10 μg was determined by the ninhydrin method of Jacobs.[63] The method is susceptible to high blank values through deterioration of reagents, and blank and standard samples were always determined concurrently with unknown samples. Latterly a change was made to the phenol–hypochlorite method of Sloane-Stanley[64] since the reagents are more stable and the method can be used to measure as little as 0·5–3·0 μg nitrogen. Hexose was determined by the phenol–sulphuric acid method of Dubois *et al.*[65] as modified by Galanos & Kapoulas.[66] Phosphorus was determined by the method of Morrison.[67]

Since the above determinations were sometimes carried out on lipids scraped from t.l.c. plates together with silica gel, a brief study of the effects of silica gel was made. Total organic matter (Amenta method), nitrogen, and phosphorus determinations were substantially unaffected when suitable silica gel blanks were used, but a small increase in colour

development was found in the hexose determination and corrections were made by use of appropriate standards.

Glycerol was determined by the periodate–chromotropic acid method of Butler *et al.*,[68] using tripalmitin for the preparation of a standard curve. Glycerol in phosphoglycerides was not satisfactorily measured by this method, and the method of Body & Gray[69] was used instead. Esters were determined by the method of Walsh *et al.*,[70] or by methanolysis with BF_3-methanol[71] followed by g.l.c., using heptadecanoic acid (17:0) as internal standard. Ethanolamine was determined by the method of Axelrod *et al.*[72]

Fatty acid methyl esters were analysed by g.l.c. with a Varian 600 C gas chromatograph equipped with flame ionisation detector and 5 ft × ⅛ in o.d. stainless-steel columns packed with 15% EGSS-X on 100–120 mesh Gas Chrom P (Applied Science Labs.). The column temperature was 175°c and the carrier gas was high purity argon used at a flow rate of 20 ml/min.

Sterol trimethylsilyl ethers were analysed by g.l.c. with a Pye Argon gas chromatograph fitted with a 4 ft × 4 mm i.d. silanised glass column packed with 1% SE 30 on 80–100 mesh Gas Chrom Q (Applied Science Labs.), operated at 235°c and 40 ml/min gas flow rate.

Infra-red spectra were obtained from thin films of lipids on NaCl discs, using a Unicam SP200 infra-red spectrophotometer and beam condenser unit.

Errors in the above determinations in calibration experiments were found to be within ± 3% of average values. Experimental values given are averages of triplicate analyses, and were not evaluated statistically.

Results and Discussion

Extraction of lipids

Few solvent systems extract wheat-flour lipids efficiently, and it is possible to extract traces of lipids from flour even after 4 or 5 extractions with a relatively efficient solvent such as water-saturated n-butanol. Since lipid solvents extract varying amounts of non-lipid impurities it was considered necessary to compare solvent efficiencies on a common basis. Residual lipid in extracted flour was determined by the A.O.A.C. acid hydrolysis method[73] after extracting 60 g of flour with (i) 1·75 l water-saturated n-butanol, or (ii) 240 ml methanol and 1·35 l chloroform–methanol (2:1 by vol.), or (iii) 1·75 l ethanol–ether–water (2:2:1 by vol.) Residual flour lipid decreased in the order (iii) > (ii) > (i), and it was concluded that water-saturated n-butanol was the best extraction solvent system. This is contrary to Wooton's findings,[74] but is in agreement with results in several other papers.[31,32,75–77]

The same conclusion was reached by comparing the phosphoglycerides extracted by these solvents (see phosphoglycerides section). The yield of lysophosphatidyl choline (LPC) was taken as indicative of the thoroughness of extraction, since it is the most difficult lipid to remove from wheat starch or flour.[31,32] The results (Table I) show that water-saturated n-butanol was a much superior solvent in that it extracted much more LPC. The comparatively constant yields of the other phosphoglycerides (on a flour wt. basis) indicate that they were more readily extracted, and that LPC did not arise by hydrolysis of phosphatidyl choline (PC).

Preliminary fractionation of flour lipids

It soon became evident that an effective analysis of flour lipids could only be achieved after removal of non-lipid impurities and a preliminary fractionation of the total lipids. Purification of butanol-extracted lipids by the Sephadex method of Wuthier[78] was most effective, but for handling larger amounts of lipids a simple Folch washing procedure[79] was more convenient and was almost as effective.

Total purified lipids were separated by DEAE cellulose column chromatography,[80] and the lipid fractions eluted with various solvents were examined by t.l.c. The neutral lipids and glycolipids were readily eluted as pure fractions, but the phosphoglycerides presented difficulties since *N*-acyl phosphatidyl ethanolamine (*N*-acyl PE) appeared in all the later phosphoglyceride fractions. This technique was eventually abandoned, and a preliminary separation by silicic acid column chromatography was developed (Fig. 1). The hexane, ether–hexane, ether and chloroform eluates were normally combined and termed non-polar lipids. Small amounts of esterified (6-*O*-acyl) steryl glucoside (ESG), esterified (6-*O*-acyl) monogalactosyl diglyceride (EMGDG), monogalactosyl diglyceride (MGDG), and a trace of *N*-acyl PE appeared in this fraction. Acetone-based solvents were used to elute the glycolipids contaminated with a little *N*-acyl PE, and the phosphoglycerides were then eluted with chloroform–methanol and methanol. The three combined fractions were concentrated by rotary vacuum evaporation under nitrogen, and re-dissolved in small volumes of chloroform for further analysis.

TABLE I

Composition of phosphoglycerides (wt. basis) extracted from wheat flour by three solvent systems

Phosphoglyceride	WSB*		CM†		EEW**	
	% of phospholipids	mg/100 g flour	% of phospholipids	mg/100 g flour	% of phospholipids	mg/100 g flour
N-acyl PE	21·8	6·9	32·5	8·0	56·5	8·6
N-acyl LPE	12·8	4·1	15·7	3·8		
PE	3·5	1·1	3·6	0·9	4·4	0·7
LPE	4·0	1·3	1·5	0·4	2·8	0·4
PC	25·4	8·0	33·4	8·2	32·6	5·0
LPC	29·6	9·4	12·7	3·1	3·4	0·5
Others	2·9	0·9	0·6	0·1	0·4	0·1

* WSB = water-saturated n-butanol
† CM = chloroform–methanol (2:1 by vol.)
** EEW = ethanol–diethyl ether–water (2:2:1 by vol.)

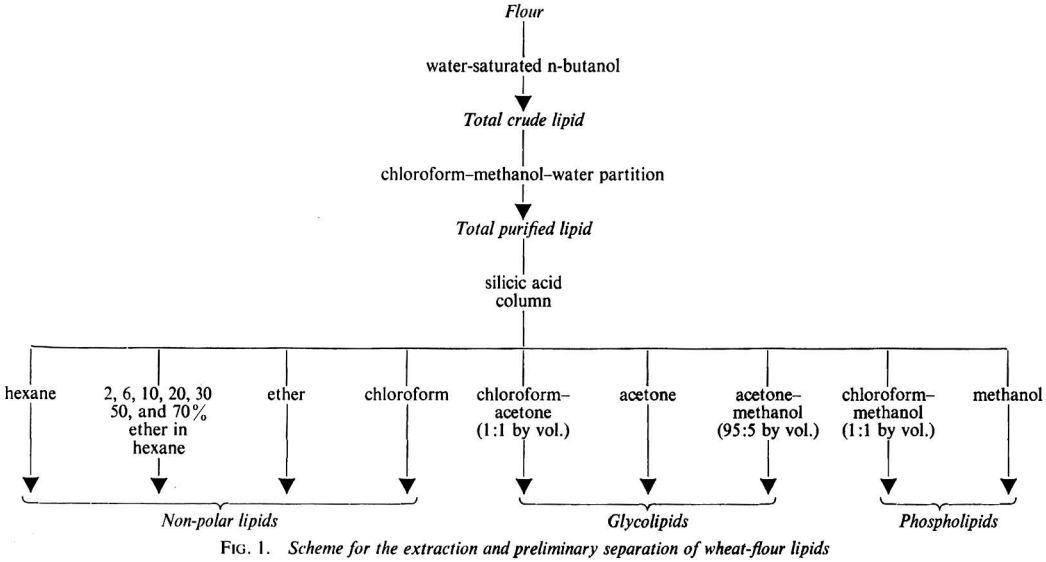

FIG. 1. *Scheme for the extraction and preliminary separation of wheat-flour lipids*

TABLE II

Fatty acid composition (wt.-%) of wheat-flour non-polar lipids

Fatty acid	TG	1,3-DG	1,2-DG	1-MG, 2-MG	FFA	SE	ESG	EMGDG
12:0					0·2		0·4	0·1
14:0	0·1	0·3	0·2	0·2	0·3	2·5	0·7	0·1
16:0	16·7	17·1	14·8	22·0	13·5	58·2	52·0	24·1
16:1					0·3		1·5	0·4
18:0	1·2	2·7	1·4	1·3	1·4	1·8	5·1	1·8
18:1	13·9	12·2	12·9	8·7	9·3	5·8	6·1	7·2
19:0							1·7	0·6
18:2	63·7	64·4	64·4	62·4	68·0	27·7	28·2	61·6
20:0				1·2				
18:3	4·3	3·3	6·3	4·2	6·5	2·6	3·0	3·6

Analysis of the non-polar lipid fraction

The non-polar lipid fraction was separated by t.l.c. (solvent I) together with authentic lipid standards. The lipids identified in this way were steryl ester (SE), triglyceride (TG), diglyceride (DG) (1,2-and 1,3-isomers), monoglyceride (MG) (1-and 2-isomers), free fatty acid (FFA), ESG, and free sterols. Further detailed work in this laboratory has confirmed these identifications, and will be published later. The lipids were isolated by preparative t.l.c., and their fatty acid compositions were determined by g.l.c. (Table II). The fatty acid composition of ESG differed considerably from that given by Myhre,[50] although the values for EMGDG were very similar. The fatty acid compositions of the non-polar lipids were otherwise in good agreement with previous analyses.[1,10,22,25–29]

The portion of the neutral lipids eluted with 50–70% ether in hexane contained ESG and an unidentified lipid, which chromatographed with MG in solvent I, but which separated from MG in solvent II. The unknown lipid gave a positive hexose (α-naphthol) test on t.l.c. plates. The infra-red spectrum of the unknown showed the presence of hydroxyl and ester groups and was similar to that of EMGDG and MGDG.[49] The fatty acid content determined by g.l.c., using an internal standard, was found to be 75·7% (calc. for EMGDG = 72%, ESG = 32%, triply-esterified ESG = 60%). On the basis of the evidence above, the lipid was tentatively identified as EMGDG. Its fatty acid composition (Table II) corresponded to that of MGDG with additional fatty acids on the galactose having the same composition as those in the 6-position of the glucose in ESG. Since these are different from the FFA of wheat flour it would appear that the lipid may not be an artifact. Heinz[49] has shown that in leaf homogenates EMGDG is an artifact produced by enzymic acyl transfer from digalactosyl diglyceride (DGDG).

TABLE III

Composition of wheat-flour non-polar lipids

Lipid	Wt.-%
Steryl ester	14·7
Triglyceride	40·9
1,2-Diglyceride	12·2
1,3-Diglyceride	11·8
Free fatty acid	13·7
Free sterol	4·1
Monoglyceride	2·6

The neutral lipid fraction was separated by t.l.c. (solvent I) and the spots were visualised by charring with 50% sulphuric acid. The spot densities were then measured with a Joyce-Loebl Chromoscan densitometer, and the quantitative distribution of the lipids was calculated (Table III) using correction factors obtained from runs with known amounts of these lipids isolated by preparative t.l.c. The FFA and free sterol spots overlapped, and an independent measure of the FFA was obtained by g.l.c. using an internal standard.

Analysis of the sterols

The sterols in the various sterol lipids were isolated for detailed analysis. SE was refluxed under nitrogen with 0·2N potassium methoxide in methanol for 20 min, water was added, and the free sterols were extracted with diethyl ether. Sterols were liberated from ESG and sterylglucoside (SG) by hydrolysis with 5% hydrochloric acid in anhydrous methanol at 100°C for 4 h and the free sterols were isolated by preparative t.l.c. on plates developed with ether–hexane–acetic acid (10:90:1 by vol.).

The sterol preparations were then acetylated, and separated into saturated and unsaturated fractions by argentation t.l.c.[21] The steryl acetates were next saponified as above, converted to trimethylsilyl ethers,[81] and analysed by g.l.c. and by combined g.l.c.–mass spectrometry.

G.l.c. analysis showed that both saturated and unsaturated fractions consisted of two components which overlapped in the total sample to appear as two peaks instead of four.

TABLE IV

Composition (wt.-%) of sterols in wheat flour lipids

Sterol	SE	Free sterol	ESG	SG
C_{28} saturated	5·9	6·4	5·7	6·0
C_{29} saturated	14·1	13·6	14·3	14·0
Campesterol	4·8	16·8	17·2	14·4
β-Sitosterol	75·2	63·2	63·8	65·6

Combined g.l.c.–mass spectrometry showed that the sterols were the same as those identified in wheat germ oil by Knights[21]—namely C_{28} and C_{29} saturated sterols, β-sitosterol and campesterol. The composition of the sterols in the various lipids is given in Table IV. The results were in agreement with those of Knights,[21] but differed from those of Berry *et al.*[20] in that no brassicasterol was detected. McKillican[51] has also reported that β-sitosterol accounts for 70% of the sterols in the sterol, ESG, SE and SG fractions of three varieties of wheat.

Analysis of the glycosphingolipids

A portion of the total glycolipid fraction was saponified (0·2 N methanolic KOH, 10 min at 60°C) under nitrogen and was examined by t.l.c. (solvent IV) using pure ceramide monohexoside (CMH) and ceramide dihexoside (CDH) from bovine milk[82] as reference compounds. Two compounds with similar t.l.c. mobilities were tentatively identified as CMH and SG, and a third component as CDH. The saponified glycolipids were applied in chloroform to a DEAE cellulose column, and eluted with 500 ml chloroform–methanol (99:1 by vol.), 2·5 l chloroform–methanol (95:5 by vol.), 500 ml chloroform–methanol (9:1 by vol.) and 500 ml chloroform–methanol (2:1 by vol.). The eluate was collected in 25 ml fractions for examination by t.l.c. Fractions prior to 22 contained pure SG, fractions 22–26 contained small amounts of SG and CMH, fractions 27–35 contained pure CMH, and fractions 38 onwards contained pure CDH. The CMH and CDH were recrystallised from methanol at −20°C.

The infra-red spectra of wheat CMH and CDH agreed with published spectra,[83] and the molar nitrogen:hexose ratios were 1:1·06 and 1:1·7, respectively. Lack of material prevented satisfactory detailed analysis of CDH, but CMH was analysed according to the scheme in Fig. 2.

CMH contained trihydroxy and dihydroxy bases combined with hydroxy acids and small amounts of normal acids. Periodate oxidation of intact CMH (Fig. 2) liberated alde-

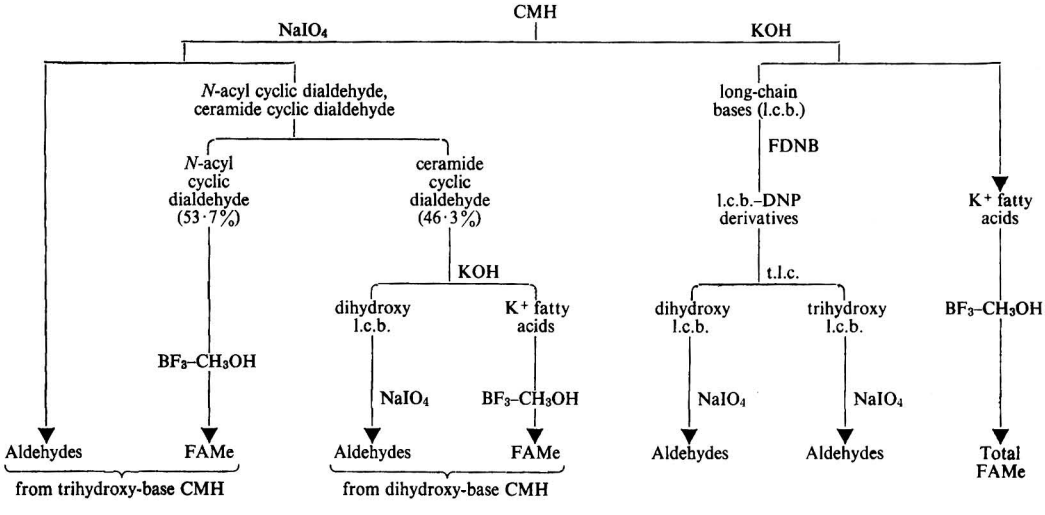

FIG. 2. *Scheme for the analysis of wheat-flour CMH*

hydes (from the trihydroxy bases) which were extracted with pentane and analysed by g.l.c.[82] The residual material in the aqueous methanol phase was extracted with chloroform and separated by preparative t.l.c. (developed with chloroform–methanol, 9:1 by vol.) into normal- and hydroxy-acyl cyclic dialdehyde residues[84] from the dihydroxy-base CMH. The relative proportions of these residues, determined by measuring the nitrogen content of material separated by t.l.c., corresponded to 53·7% trihydroxy-base CMH and 46·3% dihydroxy-base CMH. Alkaline hydrolysis was used to de-acylate the dihydroxy-base cyclic dialdehyde derivative, and periodate oxidation yielded aldehydes from the long-chain bases for g.l.c. analysis.[82] The dihydroxy long-chain base moiety was also oxidised with permanganate–periodate to carboxylic acids, which were converted to methyl esters and analysed by g.l.c.[85]

An alternative approach to the analysis of the long-chain bases (Fig. 2) was made by subjecting CMH to vigorous alkaline hydrolysis (0·2 N methanolic KOH, 18 h at 110°), and converting the mixture of free long-chain bases and base hexosides to dinitrophenyl derivatives.[86] Preparative t.l.c. on borate-impregnated plates[82] gave saturated dihydroxy bases, unsaturated dihydroxy bases and trihydroxy bases. Base-hexosides remained at the origin and were not recovered. Periodate oxidation of the various base fractions gave aldehydes which were analysed by g.l.c.

The results of these analyses are summarised in Table V. The CDH figures do not represent a complete analysis because of insufficient working material, but they indicate that there is a different pattern from the bases in CMH. The dihydroxy bases in CMH, when oxidised with permanganate–periodate, yielded a C_{16} monocarboxylic acid from the dihydroxy 18:0 base, and a C_{10} monocarboxylic acid and a C_6 dicarboxylic acid from the dihydroxy 18:1 base. The double bond was therefore in the 8-position of the dihydroxy 18:1 base. The double bond positions in the trihydroxy bases were not determined, but Carter *et al.* have established the structure of the major unsaturated base as 4-D-hydroxy-Δ^8-sphingenine.[52]

Fatty acids were recovered from alkaline hydrolysates and converted to methyl esters. Alternatively, the esters were prepared by direct methanolysis[71] of CMH or its various subfractions, and were then separated by preparative t.l.c.[71] into normal and hydroxy esters for g.l.c. analysis (Table VI).

CMH contained very small amounts of normal acids which were principally 16:0, 18:0 and 18:1. The major acids were 16:0 and 20:0 hydroxy acids with lesser amounts of 18:0 and 22:0 hydroxy acids. This analysis differs from that of Carter *et al.*,[52] who found 2-hydroxy stearic acid to be the principal fatty acid in wheat CMH.

Analysis of the galactosyl glycerides

The fraction of total lipids eluted from the silicic acid column (Fig. 1) with chloroform–acetone (1:1 by vol.) consisted largely of MGDG, which was purified by preparative t.l.c. using chloroform–acetone (1:1 by vol.) as developing solvent. Phospholipid impurities remained at the origin. The fraction, eluted with acetone (Fig. 1), was almost pure DGDG, and was further purified by chromatography on a silicic acid column. A minor lipid, digalactosyl monoglyceride (DGMG), with a t.l.c. mobility about half that of DGDG, which was present in the original acetone eluate, was isolated by preparative t.l.c. using chloroform–methanol–water (65:25:4 by vol.) as developing solvent.

The infra-red spectra of the galactosyl diglycerides agreed with published spectra of MGDG and DGDG.[80] The DGMG was originally thought to be sulpholipid on account of its t.l.c. mobility, but its infra-red spectrum resembled that of DGDG rather than that of sulpholipid.[80] The hexose:ester molar ratios of the galactosyl glycerides were MGDG = 0·55:1, DGDG = 1·20:1, and suspected DGMG = 2·1:1.

Authentic DGMG, prepared from DGDG by digestion with pancreatic lipase,[87] was co-chromatographed with the suspected DGMG (solvent IV). DGDG and the suspected DGMG were also acetylated and compared. Their t.l.c. mobilities were identical, and their infra-red spectra were identical except for slightly greater CH_2 absorption in the acetylated DGDG, attributed to the presence of a second long-chain esterified fatty acid. On the above evidence, it was concluded that the new lipid was DGMG. In the t.l.c. system used by Nichols,[58] sulpholipid and DGMG would migrate between PC and phosphatidyl inositol (PI). There was no evidence of any lipid other than DGMG in the present study which would have such a t.l.c. mobility, and it was presumed that there was no sulpholipid in the sample of flour which was studied.

TABLE V

Composition of the long-chain bases (l.c.b.) in wheat-flour glycosylceramides

*ECL** of aldehyde†	CMH				CDH	
	L.c.b.**	Wt.-%	L.c.b.**	Wt.-%	L.c.b.**	Wt.-%
15·0			t-18:0	14·3	t-18:0	21·1
15·5			t-18:1	11·6	t-18:1	60·0
16·0	d-18:0	11·4	t-19:0	0·8	t-19:0	14·8
16·5	Δ^8-d-18:1	34·9	t-19:1	5·7	t-19:1	2·4
17·0			t-20:0	2·9		
19·0			t-22:0	18·7	t-22:0	1·7
Total		46·3		53·7		100·0

* *ECL* = equivalent chain length

† C_n dihydroxy l.c.b. + $NaIO_4$ ⟶ C_{n-2} aldehyde;
C_n trihydroxy l.c.b. + $NaIO_4$ ⟶ C_{n-3} aldehyde

** The shorthand nomenclature for the bases indicates the carbon skeleton and double bond content in the usual way, with the prefixes d- or t- to indicate whether they are dihydroxy or trihydroxy bases

TABLE VI

Composition (wt.-%) of normal and hydroxy fatty acids in wheat flour CMH

Fatty acid	Normal acids	Hydroxy acids	
	Total CMH	Total CMH	Trihydroxy-base CMH
12:0	1·3		
14:0	1·5		1·1
16:0	29·3	38·2	41·7
16:1	6·0		
17:0	3·7		
17:1	1·1		
18:0	18·6	7·6	9·5
18:1	15·1		
19:0	1·5	1·8	
19:1	2·7		
20:0	1·7	46·2	44·7
21:0		1·4	
22:0	5·4	4·8	3·0
23:0	5·5		
24:0	6·4		

TABLE VII

Fatty acid composition (wt.-%) of wheat-flour galactosyl glycerides

Fatty acid	EMGDG	MGDG	DGDG	DGMG	2-Acyl DGMG*
14:0	0·1	0·2	0·1	0·2	
16:0	24·1	5·5	10·6	11·4	1·7
16:1	0·4	0·2	0·2	0·3	0·1
18:0	1·8	0·6	1·4	1·8	0·3
18:1	7·2	8·2	7·3	7·8	7·3
18:2	61·6	79·0	73·1	71·9	83·6
18:3	3·6	6·3	7·3	6·5	7·0

* DGMG produced by pancreatic lipolysis of DGDG

TABLE VIII

Composition of wheat-flour glycolipids

Lipid	Wt.-%
6-*O*-Acyl steryl glucoside	6·1
6-*O*-Acyl monogalactosyldiglyceride	13·6
Monogalactosyl diglyceride	18·5
Steryl glucoside + ceramide monoglycoside	6·8
Monogalactosyl monoglyceride	1·5
Digalactosyl diglyceride	51·1
Ceramide diglycoside	0·1
Digalactosyl monoglyceride	2·3

The fatty acid composition of the galactosyl glycerides is shown in Table VII. The close similarity in fatty acid composition between DGDG and DGMG indicates a random hydrolysis of DGDG, which seems to be confirmed by the fact that 2-acyl DGMG, produced by pancreatic lipolysis of DGDG, has a different fatty acid composition. The DGMG is not thought to be an artifact since t.l.c. analyses of fresh lipid extracts (Clayton, T. A., unpublished) show that DGMG is present in small amounts in fresh flour, and in much larger amounts in stored flour.

Quantitation of the glycolipids

The glycolipids were present in several of the fractions eluted after the preliminary silicic acid column fractionation (Fig. 1). Since it was impracticable to attempt to separate the complete range from ESG to DGMG on one t.l.c. plate, the glycolipids in each fraction were separated by t.l.c. and quantitated by determination of their hexose content. The total weight of each glycolipid in each fraction was obtained from these results, and the total glycolipid composition was then calculated (Table VIII).

Analysis of the phospholipid fraction

The phospholipid fraction was de-acylated, and the water-soluble glycerophosphate esters were examined by ion-exchange chromatography.[88] Very erratic results were obtained, principally due to variable amounts of a major unidentified component. When phospholipids free from *N*-acyl PE and *N*-acyl lysophosphatidyl ethanolamine (LPE) were used (isolated by preparative t.l.c.) this trouble was eliminated and analyses gave 94·2% glycerylphosphoryl choline (GPC) + glycerylphosphoryl ethanolamine (GPE), 1·3% glycerylphosphoryl inositol (GPI) and 4·5% glyceryl-phosphoryl serine (GPS). There was no evidence of significant amounts of glycerophosphoric acid (GP) or polyglycero-phosphoric acids. The effect of *N*-acyl PE was attributed to *N*-acyl GPE partitioning between the aqueous and organic phases with almost equal facility after de-acylation, so that small variations in experimental technique gave significant variations in partitioning of *N*-acyl GPE.

The phospholipid fraction was also separated by two-dimensional t.l.c.,[89] and detected with Zinzadze reagent. Spots corresponding to PE, phosphatidyl serine (PS), PI, PC, LPE and LPC were clearly resolved and spots corresponding to *N*-acyl PE and *N*-acyl LPE were found at the second solvent front. Exploratory t.l.c. analyses with solvent systems suitable for resolution of acidic phospholipids[90–92] failed to reveal significant amounts of these lipids.

Preparative t.l.c. (solvent IV) was used to isolate pure phospholipids. The slowest moving band, which consisted of PS + PI + LPC, was separated by re-chromatographing with solvent V. Satisfactory infra-red spectra were obtained for each lipid. The N:P:ester molar ratios of each phosphoglyceride were determined (Table IX), and the fatty acid methyl esters were prepared for g.l.c. analysis (Table X). In the case of *N*-acyl PE and *N*-acyl LPE, esterified fatty acids were obtained by mild alkaline methanolysis[88] and separated from the *N*-acyl GPE and purified by preparative t.l.c. The *N*-acyl fatty acids of the *N*-acyl GPE were then obtained as fatty acid methyl esters (FAMe) by BF_3-catalysed[71] methanolysis (90 min at 100°).

The identities of the *N*-acyl PE and *N*-acyl LPE were confirmed by more thorough investigations using material from the DEAE cellulose column (in preliminary work) purified by preparative t.l.c. or (latterly) using material obtained directly from the total phospholipids by preparative t.l.c. (solvents III and IV). The infra-red spectra of both lipids showed typical ester absorption at 1740 cm^{-1} and amide absorption at 1650 and 1560 cm^{-1}. Mild alkaline hydrolysis of both gave the same product (*N*-acyl GPE) which migrated on t.l.c. plates to the same position as sphingomyelin (solvents IV, V). The products from acetolysis[70] of the *N*-acyl lipids were compared with tripalmitin, acetodiglyceride from acetolysis of PC, and diacetomonoglyceride from acetolysis of LPC. The product from the lipid believed to be *N*-acyl PE was a monoacetodiglyceride, showing that the parent lipid contained a glycerol residue with two esterified fatty acids, and the product from the lipid believed to be *N*-acyl LPE was a diacetomonoglyceride, showing that it was the corresponding mono-acyl or lyso compound. Since the compositions of the *N*-acyl fatty acids (Table X) are different from those of any other lipid in wheat, it seems unlikely that these lipids are artifacts[45] of the extraction and isolation procedures.

Quantitation of phospholipids

Two-dimensional t.l.c., using the solvent systems described by Morrison[89] or Gray,[91,92] resolved most of the phosphoglycerides, but no single system was adequate for the complete range of phosphoglycerides. The total phosphoglyceride fraction (or total purified lipid) was therefore separated by one-dimensional t.l.c. (solvent IV), the phosphoglycerides were detected with Zinzadze reagent, and the phosphorus content of the spots was determined (recoveries were 100 ± 4%). A combined figure for LPC + PS + PI was resolved by re-chromatographing some of these lipids on plates developed with solvent V. The results are given in Table IX.

TABLE IX
Composition of wheat-flour phosphoglycerides

Phospholipid	Wt.-%	Molar ratios			
		N	P	Fatty acids	NH_2
N-Acyl phosphatidylethanolamine	21·8	1·06	1	3·2**	
N-Acyl lysophosphatidylethanolamine	12·8	1·10	1	1·9**	
Phosphatidyl ethanolamine	3·5	0·97	1	2·3	1·08
Phosphatidyl choline	25·4	0·93	1	2·1	
Lysophosphatidyl ethanolamine	4·0	1·10	1		
Lysophosphatidyl choline	29·6		1	1·3	
Phosphatidyl inositol	0·6		1	2·1	
Phosphatidyl serine	0·8				
Streak*	1·5				

* Material extended from origin to PS, possibly degraded PS
** Obtained by BF_3-catalysed methanolysis with $C_{17:0}$ internal standard, so that *N*-acyl fatty acids are included in the fatty acid figure

TABLE X
Fatty acid composition (wt.-%) of wheat-flour phosphoglycerides

Fatty acid	*N*-acyl		Ester		PE	LPE	PC	LPC
	N-acyl PE	*N*-acyl LPE	*N*-acyl PE	*N*-acyl LPE				
16:0	8·5	6·5	27·4	28·8	22·2	31·6	21·2	38·7
16:1	0·8	0·5	0·3	0·2	1·3			
18:0	1·1	0·9	1·0	0·9	1·3	2·1	1·4	2·0
18:1	10·6	9·7	9·9	6·0	5·3	7·7	11·7	5·7
18:2	70·6	75·0	58·0	60·6	61·9	52·4	62·5	50·6
18:3	7·6	6·6	1·8	3·0	2·6	4·4	1·9	2·4
Others	0·8	0·8	1·6	0·5	5·4	1·8	1·3	0·6

TABLE XI
Composition of wheat-flour lipids

Lipid	Wt.-%	
Steryl ester	7·5	
Triglyceride	20·8	
1,2-Diglyceride	6·2	
1,3-Diglyceride	6·0	50·9
Free sterol	2·1	
Free fatty acid	7·0	
Monoglyceride	1·3	
6-*O*-Acyl monogalactosyl diglyceride	3·6	
6-*O*-Acyl steryl glucoside	1·6	
Monogalactosyl diglyceride	4·9	
Steryl glucoside + ceramide monoglycoside	1·8	26·4
Monogalactosyl monoglyceride	0·4	
Digalactosyl diglyceride	13·5	
Ceramide diglycoside	0·03	
Digalactosyl monoglyceride	0·6	
N-Acyl phosphatidyl ethanolamine	4·9	
N-Acyl lysophosphatidyl ethanolamine	2·9	
Phosphatidyl ethanolamine	0·8	
Phosphatidyl choline	5·8	
Lysophosphatidyl ethanolamine	0·9	22·7
Lysophosphatidyl choline	7·1	
Phosphatidyl inositol	0·1	
Phosphatidyl serine	0·2	

Conclusions

Wheat flour contains a complex mixture of lipids which cannot be separated by any single analytical procedure. The t.l.c. analyses of Graveland[25] come nearest to separating all the neutral and polar lipids on a single plate, but it is clear that in several cases two or more lipids were present in spots detected as one component. It is not easy to identify all these lipids even with two or three complementary t.l.c. systems[16] and the mobilities of lipids relative to each other sometimes vary with different batches of silica gel.

In the present work, a complete analysis of the lipid classes (Table XI) was obtained by combining gravimetric analyses of neutral lipid, glycolipid, and phospholipid fractions with the analyses in Tables III, VIII, and IX. A simplified procedure will be published later (Clayton, T. A., & Morrison, W. R.). In the present work, 23 classes of lipids were identified, compared with 19 classes of lipid (plus 6 unknowns) found by Graveland,[25] and 12 classes of lipid (plus 6 unknowns) found by McKillican.[51] The points of difference between the present and previous analyses are that in this work no sphingomyelin or sulpholipid was found but *N*-acyl PE, *N*-acyl LPE, EMGDG, monogalactosyl monoglyceride (MGMG), DGMG, and CDH were identified. Most of the latter group have been identified at one time or another in flour, but have not so far been included in a comprehensive analysis of wheat-flour lipids.

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PROPERTIES OF α-AMYLASE PRODUCED BY DIFFERENT BARLEY LINES AND THEIR HYBRIDS DURING GERMINATION

By B. J. MIFLIN and O. A. ATANDA*

The amount of activity, the thermal stability and the calcium retaining ability of α-amylases produced by different barley varieties and their reciprocal hybrids have been investigated. α-Amylase was produced by seeds germinated normally and by de-embryonated seeds incubated with gibberellic acid. The activity of the enzyme has been compared between lines on various bases and the validity of these comparisons is discussed. The inactivation constants of the partly purified enzymes dialysed against EDTA in the presence of trypsin have been calculated and compared. Similarly the rate of thermal inactivation of the enzymes has been investigated. In general, little evidence was found to suggest that heterosis existed in terms of any of the characteristics studied.

Introduction

Many investigations have been made into the possibility that heterosis in terms of yield may be the result of the superior activity of the hybrid at the biochemical level. Sarkissian and his co-workers have related heterosis to increased levels of respiration and α-amylase activity in maize,[1] to increased rates of carbon dioxide fixation in barley[2] and to complementation of maize mitochondria[3,4] in the hybrids in various cases. In contrast, the studies of Hageman's group of workers with enzymes from maize involved in energy conversion,[5] with nitrate reductase,[6,7] and with chloroplast activity,[8] have shown that, with the exception of crosses involving inbreds having very low levels of nitrate reductase activity,[7] the levels of activity in the hybrid are generally intermediate between those of the parents. Similarly, in contrast to the above reports, other workers have failed to find heterotic levels of α-amylase in either maize[9] or barley.[10]

Besides the possibility that the hybrid enzymes may be more active than those of the parents, they may also be more adaptable, in the sense that they may have wider temperature and pH optima and be generally more able to withstand unfavourable conditions. Schwartz and co-workers[11,12] have shown, by means of electrophoresis, that maize hybrids contain not only the esterases present in the parents, but also some extra hybrid enzymes as well. Similarly, in maize, hybrid alcohol dehydrogenases exist. In one case, Schwartz & Laughner[13] have demonstrated that the hybrid enzyme (an allodimer) is more resistant to extremes of pH than one autodimer parent and more active than the other.

The hypothesis has also been put forward that heterotic properties in germinating seedlings may result from increased levels of plant hormones such as gibberellic acid (GA).[1] Higher concentrations of growth-stimulating substances have been shown to be present in hybrid maize,[14] but there is little evidence on GA production. Since the production of α-amylase in the barley endosperm is directly related to GA concentration over a certain range,[15] heterotic production of GA should result in heterotic levels of α-amylase. Thus, by comparing the amount of α-amylase produced under normal germination with that produced in response to a standard amount of exogenously supplied GA, the above hypothesis could be tested. This has been done and, in the course of these studies, the properties of the α-amylase produced by the inbreds and hybrids have also been investigated. In particular, the ability of the enzyme to retain its calcium, a property that varies widely between amylases of different species,[6] and the thermal stability of the enzymes have been investigated.

Experimental

The general techniques used in this study have been described previously.[17] Whole seeds were dehusked and germinated at 21°C. Embryos were removed from seeds and the sterilised dehusked endosperms were incubated first with water for 10–12 h and then with 2 ml of GA (1 mg/l), with constant agitation at 21°C until the times indicated. All incubations were done with maximum precautions to maintain sterility. Infected tubes were discarded.

Purification of the enzyme

The enzyme was assayed by its action on β-limit dextrin, determined colorimetrically.[17] The enzyme was purified for calcium retention and thermal stability studies by a modification of the method of Loyter & Schramm.[18] The method involves the selective precipitation of α-amylase in the form of a glycogen complex. The glycogen reagent was prepared by making a 2% aqueous solution and centrifuging at 2,700 rev/min for 10 min. The supernatant was then treated with 5 g of Amberlite IRA-400 (CO_3^{2-} form) and 5 g of Dowex-50 (H^+ form) per 100 ml of solution. The mixture was stirred for 50 min and the resins were removed by centrifugation. The process was repeated with addition of fresh amounts of resin. The final glycogen solution was stored frozen. Preliminary work indicated that a ratio of 1–4 mg glycogen to 7·0–8·0 amylase units was optimum for enzyme precipitation. The following method was, therefore, generally used. Enzyme extract was made 40% with respect to ethanol and centrifuged at 2,700 rev/min for 10 min. The activity of the supernatant was assayed and diluted accordingly (1–5 amylase units/ml). To each 2·5 ml of such supernatant were added 0·125 ml of 0·2 M phosphate buffer (pH 7·5), 0·60 ml (=1 mg) of glycogen reagent and 0·40 ml of ethanol, in that order. The mixture was shaken for 5 min, left to stand at 0°C for a few minutes and then centrifuged for 30 min at 2,700 rev/min

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at 0–2°C. The precipitate was washed twice with cold 40% ethanol. The washed precipitate was then dissolved in 5 ml of 0·02 M citrate buffer (pH 5·7) containing 0·01 M-$CaCl_2$ and used. With this method a twentyfold purification with an 80% recovery of activity can be obtained.

For calcium retention studies the glycogen-precipitated α-amylase fraction was pre-incubated at 35°C for 30 min. Dilutions of approximately equal activity were then enclosed in dialysis tubing which had been previously treated with boiling water for 10 min. The dialysis bags, each containing 20 ml of extract and 2 mg trypsin, were suspended in 6 l of 0·01 M EDTA/0·02 M citrate buffer, pH 6·0. The open end of the bags was closed to prevent evaporation. The EDTA solution was continually stirred and maintained at 20°C. Triplicate samples were taken at intervals up to 9 h and assayed in the usual way, except that the reaction medium did not contain calcium.

Temperature stability determinations were also carried out on the partly purified enzymes in 0·02 M citrate buffer, pH 5·7, 0·003 M-$CaCl_2$, diluted to give equivalent levels of activity for each line. The extract was incubated at 50°C with continual stirring and triplicate samples were taken and assayed as above at intervals up to 4 h.

Seed treatments

Inbred and hybrid seed was obtained from plants grown in a glasshouse under identical conditions and at the same time. Hybridisation was achieved by removing the anthers of selected ears and pollinating the ovaries within 24 h of this emasculation. The ears were covered with partly transparent paper bags loosely tied around cotton, wound onto the supporting stakes. These bags remained on the seed from emasculation until the developing seed was visually recognisable. Harvesting of the seed was done as soon as the seeds were fully mature and dry. The efficiency of hybridisation was tested by means of tillering ability, anthocyanin pigmentation of the basal sheath, width of leaf blade, early growth habit, and hullness of F_2 seeds. No evidence was obtained to suggest that any self-fertilisation had taken place. The efficiency of hybridisation was observed by planting out the hybrids and their parents in 5-in pots (one plant/pot) in the glasshouse and observing their growth characteristics over a period of time. The number of visible tillers formed by the 18th, 25th, 38th and 60th days of growth were recorded. Anthocyanin pigmentation of the basal sheath was recorded on 25-, 38- and 60-day old seedlings. Maximum widths of leaf blade of randomly selected leaves were averaged to give a value for each plant at 4 sampling dates. Semi-spreading early growth habit was observed during the first 18 days. A summary of the results is given in Table I. Obviously all tests are not applicable to all crosses and, where this is the case, the column is left blank. There were two questionable results in the tiller numbers experiment in which the hybrid did not exceed its parents in tiller number, but otherwise there were no negative results to suggest that self-fertilisation had taken place. All but the Kenia and Domen crosses shared hybrid vigour in terms of tiller production. The absence of two of the reciprocal crosses from the Table was due to lack of sufficient seed remaining after the biochemical tests to give a reasonable sample test. The extraction and estimation of DNA was carried out using a modification[19] of the method of Burton.[20] The standard DNA curve was obtained using highly polymerised DNA (Type V) from B.D.H. Ltd.

TABLE I
Summary of the results of the hybridisation tests

Reciprocal crosses*	Tillering† ability	Anthocyanin pigmentation	Leaf blade widths	Early growth habit	Hullness of seed
Nudum × Cambrinus	+ (4)				
Cambrinus × Nudum	+ (3)		+		+
Domen × Cambrinus	+ (4)		+	+	
Cambrinus × Domen	+ (4)	+			
Domen × Nudum	? (2)		+	+	
Nudum × Domen	+ (4)	+			+
Nudum × Kenia	+ (4)	+			+
Domen × Archer	+ (4)				
Archer × Domen	+ (4)				
Nutans × Domen	+ (3)				
Domen × Nutans	+ (3)				
Kenia × Domen	?				
Domen × Kenia	?		+		
Archer × Nutans	+ (4)				
Nutans × Nudum	+ (4)		+	+	
Nudum × Nutans	+ (4)	+			+

* The female parent is given first
† Positive evidence for hybridisation is given as +, negative as —, indecisive evidence is marked ?. The numbers in brackets indicate the number of sampling dates on which the hybrids had more tillers than both parents

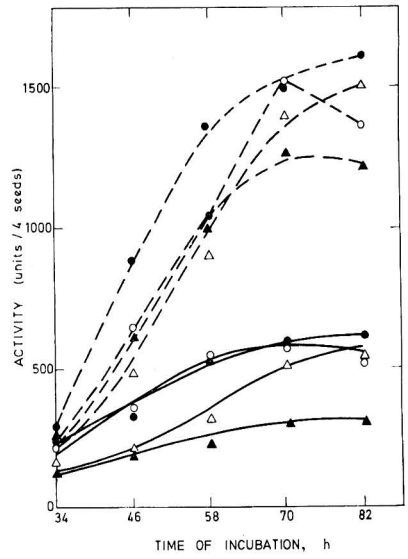

FIG. 1. *α-Amylase production by intact (———) and de-embryonated (– – –) seeds of Domen, Nudum and their reciprocal hybrids*

Each point is the mean of triplicate assays of extracts from replicates
● Domen; ▲ Nudum; ○ Domen ♀ × Nudum ♂; △ Nudum ♀ × Domen ♂

Results

α-Amylase activity

Previous studies[17] demonstrated that a range of activities existed in the inbreds and various crosses were made within this range. These crosses were harvested and, after a period of storage, were incubated and assayed. Preliminary work indicated that maximum activity was produced 70–84 h after the onset of water imbibition. This was verified for Domen, Nudum and their reciprocal hybrids (Fig. 1). A series of studies of the various crosses and their parents were then made by assaying activity after 70 h incubation, both from normally germinated seeds and from de-embryonated seed supplied with GA. The results (Table II) are presented in terms of activity per seed and per gram original unimbibed weight. The following generalisations may be made for the activities of intact seed on a per seed basis. First, the mean of the hybrids is not significantly different from the mean of the parents. Secondly, although the reciprocal crosses do not always differ significantly, there is a trend in which the hybrid with the most active maternal parent is higher. This is only to be expected if the triploid nucleus of the aleurone layer is the major factor in determining the activity.

When the activity is calculated on the basis of original unimbibed weight then the hybrids in general have higher activity than the inbred parents, although only in two cases is this significantly ($P = 0{\cdot}01$) greater than the most active parent. However, owing to interference with grain filling during the hybridisation of the seed, this basis of comparison is misleading. The results with the de-embryonated seed supplied with exogenous GA do not differ greatly in overall pattern from those of the intact seed.

TABLE II

α-Amylase activity of various barley inbreds and their reciprocal hybrids

All values based on the mean of duplicate experiments; two replicates for each variety per experiment

Line	Enzyme activity			
	Intact		De-embryonated	
	units/4 seeds	units/g wt.	units/4 seeds	units/g wt.
Domen	656	2655	1381	5600
Nudum	343	1816	2143	6114
Nudum × Domen*	537	2726	1281	6440
Domen × Nudum	350	2156	1231	6348
L.S.D. $P = 0{\cdot}05$	171	417	235	986
Kenia	289	1524	675	3411
Domen	450	1877	888	3343
Kenia × Domen	401	2638	688	4146
Domen × Kenia	451	2520	782	4036
L.S.D. $P = 0{\cdot}01$	98	861	146	955
Nutans	437	2232	975	4975
Domen	625	2520	1262	5091
Nutans × Domen	500	2910	850	5822
Domen × Nutans	556	3020	975	5478
L.S.D. $P = 0{\cdot}01$	89	591	175	628
Kenia	325	1867	737	4238
Nutans	343	1807	662	3486
Kenia × Nutans	312	1953	625	3906
Nutans × Kenia	325	2389	712	5239
L.S.D. $P = 0{\cdot}01$	77	429	265	1217
Nutans	343	1808	662	3487
Nudum	400	2020	850	4293
Nutans × Nudum	275	2222	675	4963
Nudum × Nutans	287	1843	725	4647
L.S.D. $P = 0{\cdot}01$	72	309	184	1033

* In each cross the female parent is given first

TABLE III

α-Amylase activity and DNA content of GA-treated aleurone layers of Kenia, Domen and their reciprocal hybrids

Seeds soaked and aleurone layers removed and incubated with 1 mg/l GA for 60 h. Each figure is the mean of triplicate assays of four replicates, 5 seeds per replicate

Line	Activity units/5 aleurone layers	Total DNA, μg	Activity/μg DNA
Kenia	267·5	86·25	3·10
Domen	1150·0	106·9	10·76
Kenia × Domen	712·5	97·5	7·31
Domen × Kenia	742·5	101·3	7·33
L.S.D. $P = 0{\cdot}05$	291·3	18·0	6·2

Because the unsatisfactory nature of both of the bases became apparent during the course of this work, an attempt was made to find an alternative base for the comparison of activities. The aleurone layer was removed by the technique of Chrispeels & Varner[21] and both α-amylase and DNA content were measured after 72 h incubation (Table III).

The experiment suffered in that dissection proved more difficult in the case of Kenia than for the other seeds and activity in this variety was low, probably because of mechanical damage further accentuated by sterilisation. Such damage would not affect the DNA determinations. In this one experiment, in which α-amylase activity is expressed in terms of DNA content, there is no evidence for heterotic levels of α-amylase activity. This is in contrast to the results for this cross expressed on the basis of gram unimbibed weight, in which the hybrids are significantly different from the parents at the P = 0·05 level, but is in agreement with the activity per seed results.

Calcium retention

The removal of calcium from α-amylase by dialysis against EDTA leads to a reversible loss of activity.[22] This loss is made irreversible in the presence of denaturing agents or proteases such as trypsin. Trypsin is active only when the calcium has been removed.[23] To ensure that there was no reactivation in these experiments, trypsin was routinely added to the α-amylase preparations for dialysis. The time course of inactivation for Domen, Nudum and their reciprocal hybrids is shown in Fig. 2. In an inactivation experiment such as this the rate of inactivation may be considered to be constant (k) and the amount of activity (A) remaining at any given time will depend on this constant and on the amount of activity originally present (A_0). The rate of loss of activity will be:

$$-\frac{dA}{dt} = kA_0$$

Integrating this equation from t_0 to t yields:

$$\log_n \frac{A}{A_0} = -kt$$

$$\text{or } \log_{10} A = \log_{10} A_0 - 0{\cdot}4343\, kt$$

Thus a plot of $\log_{10} A$ against time should yield a straight line of a slope $b = -0{\cdot}4343\, k$. Fig. 2 shows that the data for this particular experiment fit such a line fairly closely and this was true for the other experiments carried out. From the results, a value for k, the inactivation constant, can be calculated. This value has been used to compare the calcium retaining ability of the various inbreds and hybrids (Table IV). Each line in this Table is the result of two experiments carried out separately. Each experiment used amylase formed at the same time, stored and purified under identical conditions. The preparations used in different comparisons were stored for different times and thus the values for one variety may differ from one experiment to another as the time of storage was found to influence k. The concentration of the enzymes was so adjusted that the initial activities for all four lines in an experiment were as near as possible the same. Differences in enzyme concentration, if very large, also affect k. When all of these variables were controlled there was very little difference between varieties and in only one case was this significant. There was no evidence for heterotic effects and, in general, values tended to fall in between those of the parents.

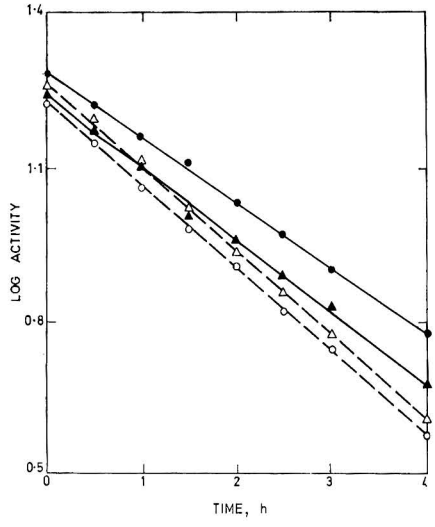

FIG. 2. *Rate of inactivation of α-amylase by dialysis against EDTA*

Values are derived from the mean of two experiments in which triplicate assays were averaged to give a value for each sampling
● Domen; ▲ Nudum; △ Domen ♀ × Nudum ♂; ○ Nudum ♀ × Domen ♂

Heat inactivation

The results were again plotted as $\log_{10}$ activity against time and a value for k for the enzyme was determined (Table V).

TABLE IV

Rates of inactivation, by dialysis against EDTA, of α-amylase from different barley inbreds and their hybrids

Parents	1st Parent	2nd Parent	1 ♀ × 2 ♂	2 ♀ × 1 ♂	L.S.D. P = 0·01
Domen, Cambrinus	0·202*	0·171	0·171	0·194	0·047
Domen, Nudum	0·192	0·167	0·168	0·166	0·029
Nutans, Domen	0·160	0·179	0·164	0·155	0·038
Kenia, Nudum	0·216	0·205	0·207	0·202	0·048
Domen, Archer	0·136	0·123	0·138	0·148	0·025
Kenia, Domen	0·136	0·129	0·132	0·146	0·025

* k values in $\log_{10} A$/h calculated, as described in the text, from assays of activity taken after 0, 1, 2, 3, 4, 5, 7 and 9 h of dialysis against EDTA. Values are derived from the mean of two experiments in which triplicate assays were averaged to give a value for each sampling

TABLE V

Rates of temperature inactivation of different barley inbreds and their hybrids

Parents	1st Parent	2nd Parent	1 ♀ × 2 ♂	2 ♀ × 1 ♂	L.S.D. P = 0·01
Nudum, Cambrinus	0·332*	0·298	0·378	0·378	0·037
Nutans, Archer	0·376	0·457	0·492	0·513	0·037
Domen, Cambrinus	0·445	0·335	0·389	0·411	0·030
Domen, Nudum	0·605	0·318	0·378	0·342	0·063
Archer, Domen	0·397	0·455	0·371	0·332	0·069

* k values in $\log_{10} A/h$ calculated, as described in the text, from assays of activity taken after 0, 0·5, 1, 1·5, 2, 2·5, 3 and 4 h incubation at 50°C. Triplicate assays taken at each time and averaged to give the value shown

As in the calcium retention experiments there is a good fit of the data to the predicted straight line (Fig. 3). The enzymes compared in any one experiment were treated in the same uniform manner as for the dialysis experiments. The differences between the inbreds were generally statistically significant. The hybrids showed no consistent pattern and, in some cases, the inactivation rate tended to be faster than that of the parents. In no case was there any evidence for any hybrid enzyme being more stable than that of its parents.

Discussion

The results presented here confirm the previous conclusion[17] that the production of α-amylase in the aleurone cells is limited largely by the supply of GA to all of the cells potentially capable of synthesising the enzyme. Because of this it might be expected that, if hybrids were heterotic with respect to GA production, heterotic levels of amylase activity per seed would have been observed in the intact hybrid seed. Since this was not so, there is little evidence from this study to support the hypothesis[1] that heterosis may be a result of greater production of growth regulators.

In the course of this work it became apparent that the choice of a base by which to compare enzyme activities from different plants presents a considerable problem. For any proper comparison between activities reference should be made to a base which is constant and independent of the effects of the enzyme. Any base which itself may change together with, or be affected by, a change in the activity of the enzyme under consideration, should not be used. Two examples will suffice to make this point. First, in the present study, where GA will also cause the release of proteases[24] which break down the reserve seed protein, thereby changing the amount of extractable protein, expressing activity on a per protein basis is obviously no use and probably will reflect only the relative activities of the two enzymes. Secondly, in growing tissue, expressing activity per unit of size (e.g., gram fresh weight or per unit cell volume) is self defeating, since by definition a more efficient enzyme system should produce more plant material per unit of enzyme than an inefficient one and thus both the activity and the base will increase in a related manner.

These difficulties do not seem to have been appreciated in many previous studies and the aim of such studies on the role of enzymes in hybrid vigour is not always clearly stated. Presumably it is to study the levels of enzyme activity in the component cells of the plant. These levels may vary at any

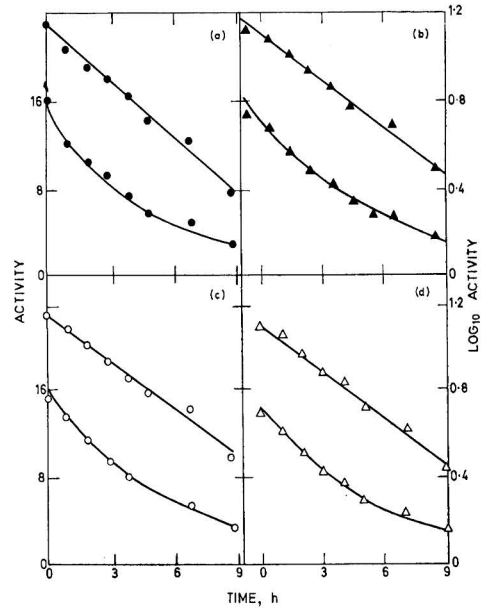

FIG. 3. *Rate of thermal inactivation of α-amylase*

Values are derived from the mean of triplicate assays. The bottom curve in (a), (b), (c) and (d) is a plot of A against time and the upper curve of $\log_{10} A$ against time ● Cambrinus ($b = -0·082$, $k = 0·982$); ▲ Nudum ($b = -0·072$, $k = 0·979$); ○ Cambrinus ♀ × Nudum ♂ ($b = 0·073$, $k = 0·975$); △ Nudum ♀ × Cambrinus ♂ ($b = -0·072$, $k = 0·983$)

fixed time of comparison, due either to more enzyme molecules being present in the cell, or because of the differential efficiency of a given enzyme molecule. Differences in these two properties may arise from various causes, for example, the number of enzyme molecules is liable to be a function of the number of structural genes specifying the enzyme, the rate of their transcription, the stability of the mRNA and its rate and amount of translation and the rate at which the enzyme is broken down. This has recently been elegantly demonstrated by Warner *et al.*[25] Differences in efficiency can be measured by looking at the characteristics of the enzyme such as cofactor and substrate binding or expressing activity per mg of enzyme protein. The latter implies

that the protein should only refer to the enzyme and that the extract has thus been purified. Differences in the number of enzyme molecules should be reflected in comparisons of activity with unit genetic material, provided that the species does not contain large and variable quantities of reiterated DNA. The difficulties arise in getting bases which can be easily measured and related to these properties. Of the two main bases used in this study, activity per unit weight is not proportional to the functional material since the aleurone layer forms a small and variable proportion of the total endosperm. Particularly in this study, the techniques of hybridisation have interfered with grain filling, but do not seem to have produced a great variation in the size of the aleurone. This is verified in Table III which shows that the amount of aleurone DNA per seed in the hybrid is only slightly less than that of Domen, although the latter seed was 1·4 times as heavy as the Domen × Kenia seed. Thus activity per gram dry weight is not considered a valid basis for comparison. Since the variation in aleurone layers appeared to be small, and as the tissue was non-dividing and thus not varying with time, activity per seed was considered to provide a reasonable, but not entirely satisfactory, base. A theoretically better base would have been unit DNA and, although one experiment was done, the procedure is somewhat difficult and introduces errors in the quantitative direction of the aleurone layer. Also, unfortunately, insufficient material was obtained to be able to repeat all of the crosses on a DNA basis. However, taking all of the factors into account, there seems to be little evidence from these results to suggest that barley hybrids contain higher levels of α-amylase activity than their parents. This study also showed that in the lines tested there was no evidence for the superiority of the enzyme from the hybrid in calcium retention or thermal stability.

The lack of heterosis in any of the enzymic characteristics tested may be related to the fact that α-amylase is probably a monomeric protein, i.e., it is not an enzyme that can be irreversibly dissociated into component parts. Thus it is unlikely that the hybrid contains new configurations of proteins (formed by association of one protein chain specified by one parent and one chain specified by the other parent) that did not exist in the parents. Such new configurations, where they exist, have the possibility of producing more active and more diverse enzymes than existed in the parents and thus contributing to the overall heterotic ability of the hybrid.[13] However, as has been discussed previously[5,8] it is possible to envisage heterotic growth without the need for heterotic levels of individual enzymes.

Acknowledgments

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DETERMINATION OF COPPER IN FOODS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

By A. G. CAMERON and D. R. HACKETT

A method for determining copper in a wide range of foods by atomic absorption spectrophotometry is described and shown to be reliable and sensitive. It is shown that, when copper is chelated and extracted into ketone, very high sensitivity may be achieved; copper in aqueous extracts can be determined at a level of 0·003 ppm. The main problem of the method is found to be sample preparation which for many foods is lengthy. Direct sampling, dry ashing and wet oxidation are described and the method of atomic absorption is compared with a colorimetric procedure.

Introduction

Although the determination of copper by atomic absorption spectrophotometry has been thoroughly investigated, relatively few applications to food have been reported.[1-3] The particular merits of the method are that it is potentially accurate, quick and free from interferences from other elements. The main problem is associated with sampling procedure rather than instrumental measurement.

The determination of traces of copper is important in many foods both to ensure compliance with legal or recommended standards and to minimise undesirable reactions in the product. In the U.K., legal maxima are in force for gelatine (30 ppm) and some tomato products (20 ppm), recommended limits are applied to a variety of foods (2–300 ppm) and there is a general recommended limit for all other foods (20 ppm). The undesirable effects of traces of copper in food include catalytic acceleration of oxidative rancidity in fats and fatty foods and the rapid destruction of vitamin C in fruits, vegetables and other products.

The colorimetric determination of copper in food is well established and the procedure of Cheng & Bray[4] using sodium diethyldithiocarbamate has been shown to be reliable. It is, however, rather tedious as it is necessary to prevent interferences by elements other than copper present in the sample and it was considered that the atomic absorption technique might offer advantages of speed and simplicity, as it is essentially interference-free. It was also thought that the use of chelating agents[5] and extraction into organic solvents[6] could be used to give the atomic absorption technique a sensitivity superior to that of the colorimetric procedure.

Experimental

Apparatus and reagents

A Perkin-Elmer 290 atomic absorption spectrophotometer was used in conjunction with an Aerostyle model A air compressor and a Servoscribe Potentiometric recorder, together with a Perkin-Elmer copper lamp. Colorimetric measurements were made with a Unicam SP800 spectrophotometer.

A stock solution containing 500 ppm copper was made by dissolving 0·5 g high purity copper in a minimum of concentrated nitric acid and diluting to 1 litre with de-ionised water. Aqueous standard copper solutions in the range 0·5–10 ppm were prepared from a freshly made intermediate standard containing 50 ppm of copper. Standard solutions containing 0·5–2 ppm of copper in methyl isobutyl ketone were prepared from suitable quantities of the 50 ppm aqueous standard by extracting into 20 ml ketone with aqueous ammonium pyrrolidine dithiocarbamate.

Sample preparation

Ashing was carried out using 5 or 10 g of sample. Some samples, notably chocolate, showed a tendency to catch fire during the initial heating over a bunsen flame, but this risk was minimised by using a shield on the burner. Ashing was completed in a muffle furnace at 550°C, and the ash was extracted into aqua regia as described by Middleton.[7] When the sample was required in methyl isobutyl ketone, the copper was chelated with 1 ml of 1% aqueous ammonium pyrrolidine dithiocarbamate and was extracted into the ketone, sufficient being used to give the desired order of copper in the organic phase.

Wet oxidation was performed by adding 20 ml concentrated nitric acid and 10 ml water to 5 or 10 g of sample in a digestion flask. After boiling for several minutes, the flask was cooled and 10 ml of concentrated sulphuric acid was added. Boiling was continued, concentrated nitric acid being added when the liquid darkened. Heating and addition of further amounts of nitric acid were continued until a clear liquid was obtained. The liquid was heated for a further 30 min until white fumes appeared, after which the liquid was cooled and 5 ml of saturated ammonium oxalate was added. The flask was again heated until white fumes appeared, after which it was cooled and sufficient water was added to prevent solidification. Copper was chelated and extracted by diluting the solution to about 50 ml with water and adding 1 ml of a 1% solution of ammonium pyrrolidine dithiocarbamate followed by 10 or 50 ml of methyl isobutyl ketone.

Samples for colorimetric determination of copper were prepared from aqueous extracts of ashed foods using EDTA and sodium diethyldithiocarbamate according to the method of Cheng & Bray.[4] Standard solutions were prepared in the same way using an appropriate amount of the 50 ppm stock solution of copper instead of the extract from the ashed sample.

Instrumental conditions

All measurements with the atomic absorption spectrophotometer were made under the following conditions: wavelength, 324·7 nm; spectrum band width, 0·7 nm; oxidant, air; fuel, acetylene; lamp current, 5 mA. The recorder was operated to give a scale expansion of 4.

Measurements with the SP800 were made at 435 nm, which was found to give maximum absorbance.

Results

The atomic absorption spectrophotometer gave linear calibration graphs for both aqueous and organic solutions.

TABLE I

Comparison of atomic absorption and colorimetric methods for determining copper in plain chocolate

Method	Sample weight, g	Sampling	Extract	Cu, ppm	Mean
Atomic absorption	5	Ashing	Water	7·4, 6·9, 7·6, 7·7, 7·5	7·4
Colorimetric	10	Ashing	CCl_4	6·9, 5·7	7·1
Colorimetric	5	Ashing	CCl_4	8·4, 7·2	

TABLE II

Copper in a variety of food samples

Food	Method	Sample weight or volume	Sampling	Extract	Copper, ppm
Plain chocolate	Atomic absorption	10 g	Ashing	Ketone (20 ml)	7·2
Plain chocolate	Atomic absorption	5 g	Wet oxidation	Ketone (50 ml)	7·5
Tomato purée	Atomic absorption	5 g	Ashing	Aqueous (10 ml)	3·2
Tomato purée	Colorimetric	10 g	Ashing	CCl_4 (15 ml)	2·2
Tomato ketchup	Atomic absorption	10 g	Ashing	Aqueous (10 ml)	3·9
Tomato ketchup	Atomic absorption	10 g	Wet oxidation	Ketone (10 ml)	3·8
Low-calorie apple drink	Atomic absorption	25 ml	Direct	Ketone (25 ml)	0·25

Using aqueous standard copper solutions and no scale expansion, full-scale deflection was obtained for 40 ppm of copper, whereas with maximum scale expansion, full-scale deflection was obtained for 8 ppm of copper, thus giving a five-fold increase in sensitivity. Using solutions in methyl isobutyl ketone, full-scale deflection was obtained with 7·5 ppm of copper (no scale expansion) and 1·5 ppm of copper (maximum scale expansion). Thus, sensitivity in methyl isobutyl ketone is slightly more than five times that in aqueous solution.

A comparison of results of atomic absorption and colorimetric methods for the determination of copper in plain chocolate is shown in Table I, while further results for plain chocolate, tomato purée, tomato ketchup and a low-calorie apple drink are shown in Table II.

Discussion

The atomic absorption spectrophotometer method proved to be quick, reliable, interference-free and sensitive. The chelation of copper with ammonium pyrrolidine dithiocarbamate and subsequent extraction into methyl isobutyl ketone gave a notable improvement in sensitivity and it was found that, within the limits of measurement, all the copper was extracted. The best concentration ratio that could be achieved was 1:20 and the enhancement achieved by aspirating ketone instead of water was 5 giving an overall improvement in sensitivity of 100. Under these conditions, copper in aqueous extracts could be determined at a level of 0·003 ppm.

The main problem of the atomic absorption technique as applied to food concerned sampling. It was found that low-calorie fruit drinks could be aspirated directly into the spectrophotometer and, if the copper was chelated and extracted into ketone, this method was extremely quick, highly sensitive and free from interferences. It was found that drinks containing sugar could not be aspirated into the flame directly nor successfully extracted into ketone and these, and most other foods, required preliminary treatment to destroy all organic matter. It was this preliminary treatment that proved to be slow.

Both dry ashing and wet oxidation were used to destroy organic matter and both proved to be satisfactory. Dry ashing was straightforward but lengthy, requiring about 4 h for ashing and a further 2–3 h for digesting the ash with acid and preparing a solution. Although wet oxidation was quicker than dry ashing—taking between 1½ h for tomato ketchup and 3 h for chocolate—it required more attention. It proved to be particularly suitable, however, when the copper was to be extracted with ketone, though extracting with nitric acid after ashing did not interfere with the chelation process as might have been expected. Complete determination of copper in chocolate, using wet oxidation and atomic absorption spectrophotometry, was performed in 4 h or less and, as chocolate is one of the more difficult foods to oxidise, the time involved for most other foods is less.

The results obtained by atomic absorption spectrophotometry were consistent with those obtained by colorimetry, the former technique being simpler and somewhat quicker. Where relatively rapid wet oxidation was achieved, as with tomato purée, the atomic absorption method was significantly quicker than the colorimetric one. As the atomic absorption technique is free of interferences from other metals, the preparation of samples for aspirating was simpler and quicker than preparation of carbon tetrachloride extracts for the method of Cheng & Bray.[4]

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BROWNING REACTION IN DRYING CACAO

By V. C. QUESNEL and K. JUGMOHUNSINGH

The polyphenol oxidases of fermented and unfermented cacao have the same pH optimum and the same response to the inhibitors diethyldithiocarbamate, cyanide and ethylenediaminetetra-acetic acid. Temperature optima and activation energies differ, the oxidase of fermented cacao having a higher temperature optimum and a lower activation energy than that of unfermented cacao. Activity is unrelated to total copper content and the conclusion is drawn that the activity of the fermented seed is not due to inorganic copper or unspecific copper–protein complexes but to a modified form of the original enzyme. Some data on heat inactivation are given and the relevance of the findings to drying temperatures for cacao is discussed.

Introduction

In preparation for the market, cacao undergoes a two-phase curing process in which the first phase is fermentation and the second is drying.[1] During drying, the brown colour characteristic of fermented cacao and of chocolate develops. It is well known that the unfermented seed contains a polyphenol oxidase[2,3] and oxidase activity has been shown to decrease during fermentation.[4] Because oxidase activity at the end of fermentation can still be inhibited with copper–enzyme poisons such as diethyldithiocarbamate it was concluded that the browning reaction during drying is enzymic.[4] However, inorganic copper can catalyse the oxidation of phenols[5,6] and so can combinations of inorganic copper and protein or polypeptide.[6–8] Thus, the possibility remained that the browning reaction in drying cacao was due to such unspecific copper–protein complexes or even to inorganic copper. An important practical matter hinges upon the distinction between these two possibilities. If the reaction is enzymic it will undoubtedly have an optimum temperature and will be completely inhibited by temperatures much in excess of this. If, on the other hand, the reaction is not enzymic there may be no optimum temperature or inactivation temperatures within the range that could be used in drying cacao. In the first case, a limit would be set to the temperature that could be used in the artificial drying of cacao whereas in the second case, the limit, if any, would be set by other considerations.

In view of these practical aspects an investigation of the browning reaction in drying cacao was undertaken. The oxidase activity of fresh and dry unfermented seeds was compared with that of fermented seeds for such characteristics as pH and temperature optima, heat inactivation and inhibition by copper–enzymes poisons.

Experimental

Enzyme preparation

Oxidase activity in the fermented seed is associated with matter that is insoluble in water or buffer solutions. Therefore, acetone powders were prepared and the activity was determined in suspensions of the powders in buffer solutions. The powders were prepared by a standard procedure from fresh seeds newly removed from the pods, from dried unfermented seeds and from fermented seeds taken from sweatboxes at the end of 5–7 day fermentations. Twenty-five seeds were peeled and macerated in a blender with 250 ml acetone. The suspension was filtered on a Buchner funnel and the residue in the funnel was extracted with 500 ml 80% aqueous acetone and then with 200 ml anhydrous acetone. It was dried in a vacuum oven at about 30°C and stored in a vacuum desiccator. Before use it was sieved through an 80-mesh sieve.

Determination of activity

In the first experiment, with powders from unfermented seeds, the method of Dawson & Magee[9] was used. When this method was tried with powders from fermented seeds difficulties arose because of the brown colour of the suspension and the low level of activity which necessitated relatively large amounts of suspended material. Both conditions helped to obscure the end-point and results were so inconsistent that the method was temporarily abandoned. Other methods tried were equally unsatisfactory so the method of Dawson & Magee[9] was modified.

Normally, substrate is added to a mixture of enzyme and a known quantity of ascorbic acid at zero time and the mixture is siphoned out dropwise into an indicator solution of starch, acid and potassium iodide in a glass dish. When the ascorbic acid has been consumed the quinone of the substrate oxidises the iodide to iodine which reacts with the starch to give a blue colour.

The major modification the present authors introduced concerns the mixing of reaction mixture and indicator solution. Instead of allowing the reaction mixture to drip into a fixed quantity of indicator, both the reaction mixture and the indicator dripped into a siphoning funnel (Fig. 1). With this arrangement, end-points can be determined very accurately and the rate of dripping of the reactants is not as critical

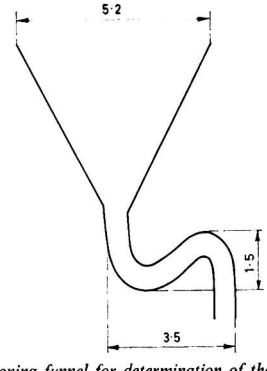

FIG. 1. *Siphoning funnel for determination of the end-point of the polyphenol oxidase reaction*

The dimensions given are in centimetres and are external dimensions. The bore of the siphon is 0·5 cm i.d.

as it is in the original method. Three funnels were tried and the best had the dimensions shown.

The second modification concerns the indicator solution itself. The authors omitted the pyrogallol. Without it, the solution turned a deep blue colour in an hour or two. Before each determination 0·001N sodium thiosulphate was added dropwise until the colour was reduced to a pale pinkish haze. In this condition the indicator was very sensitive.

Other minor modifications were the addition of aerosol 1B at 0·1% to the buffers and the use of oxygen instead of air to agitate the reaction mixture. The oxygen was first passed through water at the same temperature as the reaction mixture to avoid cooling the mixture. 4-Methyl catechol was tried as a substrate but solutions oxidised so rapidly in air that this was abandoned in favour of catechol. The catechol solution was made up in the recommended concentration, treated with charcoal and filtered. Preliminary experiments showed that the optimum concentration was 20 ml in a total reaction volume of 100 ml for enzyme from unfermented seeds and 15 ml for enzyme from fermented seeds. Ascorbic acid was used in amounts of 1–4 mg per determination, as recommended. Except as mentioned above, reagents and inhibitors were used without purification and were those commercially available. All determinations were made in duplicate.

Results

pH optimum

Activity at various pH values in McIlvaine's buffer was determined at 30°C for 6 enzyme powders, 2 prepared from fresh unfermented seeds, 2 from dry unfermented seeds, and 2 from fermented seeds. Each preparation showed a definite optimum at pH 6·0, the optimum for the fermented seed being the same as that for the unfermented seed although the shapes of the curves differed slightly. This result differs from previous results which have shown a pH optimum of 5[2,10] for the enzyme from unfermented cacao. No explanation can be advanced for the discrepancy.

Temperature optimum

The activities of 5 preparations (3 unfermented and 2 fermented) were determined at various temperatures in buffer at pH 6·0 with the results illustrated in Fig. 2. There is a well marked optimum temperature for the unfermented seed at 31·5°C and a feebly expressed optimum at 34·5°C for the fermented seed. The different shapes of curves in Fig. 2 are presumably a reflection of the different activation energies of the catalysts in fermented and unfermented cacao. Before calculation of the activation energies from these results it was shown experimentally that reaction inactivation was not unduly affecting them. Reaction times for two different preparations each of fermented and unfermented seeds were obtained at 30°C and pH 6·0 with increasing amounts of ascorbic acid. When reaction time was plotted against ascorbic acid content, linear graphs were obtained for reaction times up to 60 sec for three preparations and up to about 40 sec for the remaining preparation from fermented cacao. Thus it was concluded that all reaction times within 40 sec would measure true initial velocity, whereas those beyond this might be influenced to some extent by reaction inactivation.

In the temperature-optimum experiments all the reaction times were within 40 sec. When the logarithm of rate was plotted against the reciprocal of absolute temperature straight lines were obtained (Fig. 3) which gave the activation energies (μ) from the slopes.[11] These were 1140 cal/mole and 390 cal/mole for unfermented and fermented cacao, respectively. Both of these values are well below the activation energy (2,700 cal/mole) recorded for polyphenol oxidases from mealworms, mushrooms and potatoes.[11]

Inactivation

Reaction inactivation has already been mentioned. This factor enters those determinations lasting more than 40 sec and the results indicate that fermented seed tissue may be slightly more susceptible to this than unfermented seed tissue.

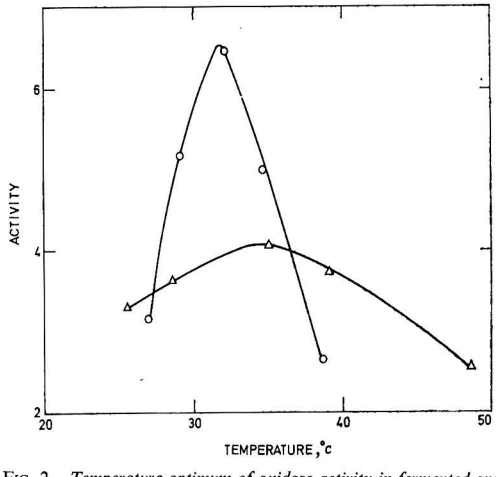

FIG. 2. *Temperature optimum of oxidase activity in fermented and unfermented cacao*

Activity is measured in arbitrary units as $(1/t) \times 100$ where t is the time taken in seconds to oxidise 4 mg ascorbic acid

○ unfermented (100 mg); △ fermented (400 mg)

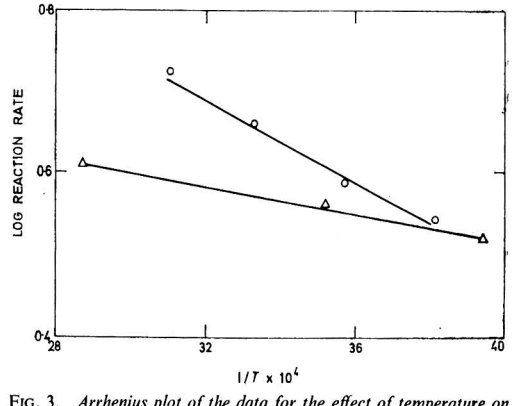

FIG. 3. *Arrhenius plot of the data for the effect of temperature on reaction rate* $(\frac{1}{t} \times 100)$ *where* t *is in seconds and* T *is absolute temperature*

The activation energy, $\mu = 4{\cdot}58 \frac{(\log k_2 - \log k_1)}{\frac{1}{T_1} - \frac{1}{T_2}}$ where $k = \frac{1}{t} \times 100$

○ unfermented; △ fermented

Heat inactivation was studied by immersing flasks, which contained suspensions of enzyme powder (200 mg or 250 mg) in buffer at pH 6·0 (10 ml), in a water bath at 45° or 60°C, removing the flasks at known intervals and allowing them to remain at room temperature subsequently for such a period that the total time before determination of activity was 5 h. Results for three experiments, Fig. 4, show a 50% decrease in activity in about 4 h for all preparations, both at 45° and 60°C. The similarity in rate of inactivation at 45° and 60°C is surprising and unexplained. Earlier results[3] cannot be easily compared but are not incompatible.

Of more importance than the heat inactivation of enzyme preparations is the heat inactivation of the enzyme within the seed under conditions similar to those obtained during the curing process. Fresh seeds were washed free from pulp and immersed in 2% acetic acid at 43°C for 18 h. This treatment kills the seeds[12] and brings them to the condition of seeds at about the end of the second day in a typical sweat-box fermentation. Thereafter three lots of seeds were placed in stoppered flasks at each of the temperatures 33·5°, 42° and 50°C. A flask was removed from each incubator after 3, 6 and 10 days and acetone powders were prepared from the seeds by the standard procedure. The powders were tested for activity the following day. To obtain consistent results it was found necessary to use seeds from the same tree. Fig. 5 shows that the rate of inactivation depends upon temperature and that the time taken for 50% inactivation ranges from 10 h at 50° to 3¼ days at 33·5°C, rather longer than the time required for 50% inactivation in enzyme preparations. The curves for 42° and 50°C are in fact not unlike that found previously[3] for a batch of seeds fermenting in a sweat-box in which, after 7 days, only 2% of the activity was left.

Because oxidase activity appears to decline almost exponentially during heat treatment (Fig. 5) it was relevant to determine whether the little activity remaining after a normal fermentation could be eliminated completely by temperatures that have been used for drying cacao. Two temperatures were chosen for study, 55° and 65°C. Fermented seeds were removed from a sweat-box and placed in stoppered containers held at these two temperatures. Samples were removed after 24 and 48 h and acetone powders were prepared and tested for activity. The results (Table I) show that at 65° the enzyme was completely inactivated within 24 h and that at 55°C more than 80% of the activity was gone in 24 h and over 95% in 48 h.

After preparation, enzyme powders were normally stored in desiccators at room temperature. It had been noticed repeatedly that powders from fermented seeds lost activity more quickly than those from unfermented seeds. To confirm this, portions of enzyme powders of both unfermented

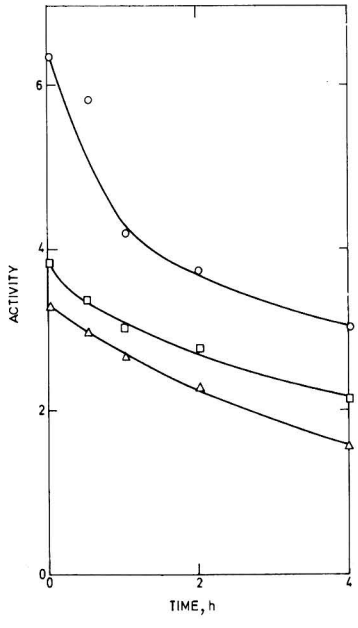

FIG. 4. *Heat inactivation of polyphenol oxidase in acetone powders from fermented and unfermented cacao*

Activity is measured in arbitrary units as $\frac{1}{t} \times 100$ where t is the time taken in seconds to oxidise 4 mg ascorbic acid

○ unfermented dried seeds (250 mg), 45°C; □ fresh unfermented seeds (200 mg), 60°C; △ fermented seeds (250 mg), 45°C

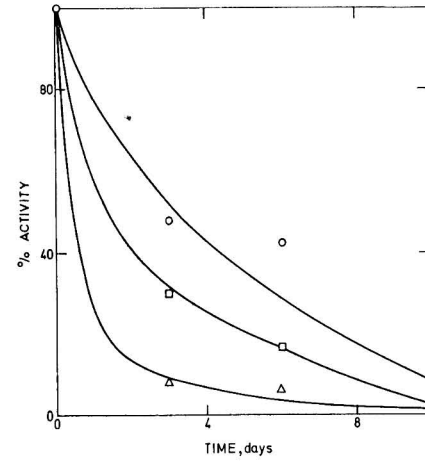

FIG. 5. *Inactivation of polyphenol oxidase in killed cacao seeds during treatment at different temperatures*

○ 33·5°C; □ 42°C; △ 50°C

TABLE I

Loss of polyphenol oxidase activity in acetone powders prepared from fermented cacao held at different temperatures

Time, h	Activity	
	55°C	65°C
0	11·0	11·0
24	1·9	0
48	<0·5	0

Activity is measured in arbitrary units as $\frac{1}{t} \times 100$ for the oxidation of 1 mg of ascorbic acid by 100 mg of acetone powder when t is measured in seconds; 0 = no detectable activity

and fermented seeds were placed in closed containers in atmospheres of known humidity, 71%, 51% and 1·5%.[13] Samples were withdrawn periodically and tested for activity. Powder from unfermented seeds lost almost no activity over a period of 19 days whereas powder from fermented seeds lost slightly more than half its activity in 12 days at all relative humidities.

Another preparation from fermented cacao lost 68% of its activity after 11 days on the laboratory bench while the same powder lost 60% of its activity in a vacuum desiccator containing alkaline pyrogallol as well as desiccant. Thus the rate of inactivation of powders seems to be unrelated to oxygen or water vapour content of the surrounding air. Since the rate of inactivation is dependent on temperature (see above) the storage life of powders could presumably be extended at low temperatures but this was not investigated.

Inhibitor studies

Cacao polyphenol oxidase is known to be inhibited by copper poisons.[2,4,14] More detailed comparisons between fermented and unfermented seeds are now described. Two powders from fresh unfermented seeds, one from dry unfermented seeds and two from undried fermented seeds were treated with various concentrations of diethyldithiocarbamate. The inhibition curves differed slightly but there was no outstanding difference between unfermented and fermented seed powder; 50% inhibition was obtained in the range $1 \cdot 5$–$4 \cdot 5 \times 10^{-3}$M.

With potassium cyanide also, no marked differences were observed; 50% inhibition in both fermented and unfermented seed powder was obtained in the range $1 \cdot 5$–$2 \cdot 0 \times 10^{-4}$M. No inhibition in either powder was caused by EDTA at concentrations up to 2×10^{-2}M. Sodium azide at high concentrations interfered with the end point but appeared to cause no inhibition in concentrations up to 2×10^{-3}M.

Copper content

The powders used in the study of inactivation under different conditions of humidity were analysed in triplicate for copper with the following results: unfermented, 60 ppm; fermented, 56 ppm. The difference is not significant and the total copper content is obviously unrelated to activity since the unfermented powder was approximately 5 times as active as the fermented powder.

Inorganic copper oxidised catechol too slowly to be measured by the method described and gave a perceptible darkening of colour after 1 h only in concentrations of $\sim 10^{-2}$M and above. This agrees with the results of Baghvat & Richter[5] who measured an uptake of 45 μl/h (4 μmole catechol) at pH 7·0 and 30°C for the oxidation of catechol by 2 mg cupric ions in 2 ml buffer. Thus, the difference in activity of even the fermented seed powder and that of inorganic copper is striking. Of the powder containing 56 ppm of copper, 500 mg were used for each determination of activity, which corresponds to 28 μg copper. This oxidised 4 mg ascorbic acid in 18·8 sec, equivalent to 4·33 m mole of catechol per hour. Thus, the powder was approximately 5×10^6 times more active than inorganic copper.

Discussion

It is clear from the difference in activity between the seed powders and inorganic copper that polyphenol oxidation in fermented cacao cannot be due to inorganic copper. This is further supported by the definite pH optimum obtained for the fermented powder as against the increasing oxidation observed with increasing pH for inorganic copper and by the lack of correlation between activity and copper content of the powders.

The possibility that the activity could be due to an unspecific copper–protein complex must now be examined. Synthetic copper–polypeptide complexes have oxidase activity for various substrates[6–8] and ceruloplasmin, whose physiological function is elusive but is probably related to copper balance,[15] is enzymically active.[15] Cu-poly-L-histidine shows typical Michaelis–Menten behaviour in the oxidation of ascorbate,[16] though not in the oxidation of *N,N,N′,N′*-tetramethylphenylenediamine,[6] but does not oxidise catechol at a rate that is faster than oxidation by cupric copper.[6] A complex of copper and plasma protein oxidised dopa at a rate that increased when pH increased from 6·5 to 11·0,[7] and its activation energy was 23·7 kcal/mole[7] compared with 2700 cal/mole for tyrosinase.[11] Thus, ceruloplasmin and the artificial copper–polypeptide complexes have some of the features of enzymes.

Most copper enzymes are readily inhibited with copper-complexing agents. Thus, diethyldithiocarbamate inhibits polyphenol oxidase,[17] laccase,[17] ascorbic acid oxidase,[17,18] dopamine-β-hydroxylase[19] and beef liver mitochondrial amine oxidase[20] but not ceruloplasmin.[21] Cyanide inhibits ceruloplasmin,[21,22] polyphenol oxidase,[23] laccase,[24] ascorbic acid oxidase[23] and many other enzymes[17] including one monoamine oxidase[23] but not beef liver mitochondrial amine oxidase.[20] EDTA does not inhibit beef liver mitochondrial amine oxidase[20] or ceruloplasmin,[15] hardly inhibits laccase[24] but inhibits some ascorbic acid oxidases[18] and dopamine-β-hydroxylase.[19]

In all the aspects of its behaviour that were investigated the polyphenol oxidase of fermented cacao behaved like an enzyme. It had definite pH and temperature optima, and it was inhibited by diethyldithiocarbamate and cyanide though not by EDTA which was, however, also inactive against the enzyme of the unfermented seed. It even had a lower activation energy than the enzyme of the unfermented seed. But its behaviour was not identical with that of the enzyme from unfermented cacao. Thus, the temperature optima, the activation energies and the inactivation rates during storage of the two differed. Also the solubilities differed; the enzyme of the unfermented seed was soluble in buffered aqueous solutions whereas that of the fermented seed was not. There was some evidence that buffered solutions containing 5% dimethyl sulphoxide extract about 10% of the bound enzyme but experiments along this line were not pursued. Dimethyl sulphoxide itself causes no inhibition of activity in concentrations below 20%.

The conclusion seems inescapable that the enzyme of the fermented seed is a modified form of the original enzyme and that the modification arises by phenolic tanning which is known to occur during fermentation.[4] It is suprising that the modification is accompanied by a lowering of activation energy. It was considered possible that this was a result of a reduced reaction inactivation but experiments give no support to this idea. Reaction inactivation is, if anything, slightly more pronounced with enzyme from the fermented seed than from the unfermented seed. The cause of the lower activation energy remains unknown.

The optimum temperature for the enzyme of fermented seeds is fairly low and during the commercial sun drying of fermented cacao the actual temperature of the seeds, although variable, must be close to the optimum for much of the time

However, during artificial drying air temperatures as high as 90° have been used.[1,25] The temperature of the cotyledons will be below the air temperature as long as water is being evaporated from the seeds but it seems possible that such high air temperatures could produce seed temperatures, not only above the optimum but well into the range where inactivation is rapid. De Vos[25] did indeed find that under certain conditions air temperatures between 60° and 95° gave an unacceptable product. In the method he finally developed, the cacao is predried in a rotary dryer for $2\frac{1}{2}$ h at an air temperature of 90° and then given a final drying on trays in a tunnel dryer at an air temperature of 70°. According to his measurements the seed temperature does not rise above 54° during predrying nor above 66° at the end of the final drying. This method is stated to give a product with a good flavour and colour.

De Vos[25] does not record seed temperatures for the early experiments that resulted in unacceptable products but from his descriptions it is possible to conclude that they were much higher in these experiments. Thus his experiments and those described here lead to the conclusion that seed temperatures of 65° and over are to be avoided in the artificial drying of cacao. In a personal communication to Rohan,[1] Roelofsen stated that seed temperatures should never exceed 60° since this changes the colour and flavour of the seeds and Phillips[26] showed that methanol extracts far less polyphenolic material from seeds dried at 60° or 105° than from sun-dried seeds. All the evidence indicates therefore that air temperatures can be high only if seed temperatures remain below the range of 60–65°.

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CHANGES IN THE LEVELS OF CATECHOL OXIDASE AND LACCASE ACTIVITY IN DEVELOPING PEACHES

By E. HAREL, A. M. MAYER and H. R. LERNER

Catechol oxidase and laccase activity and the content of *o*-diphenols were followed in developing peaches from 2 weeks after fruit set to harvest time. Catechol oxidase activity in the soluble fraction drops sharply during the first few weeks and remains low until harvest time. Laccase activity rises from a very low level in the first 3 months to a level higher than that of catechol oxidase at harvest time. Catechol oxidase activity in a particulate fraction and *o*-diphenols content per fruit, each show a peak during fruit development. The peak in *o*-diphenols content precedes that of catechol oxidase activity.

Introduction

Peaches contain two enzymes capable of oxidising phenolic substrates.[1-3] A particulate fraction of the cell (1000 × *g* precipitate) contains a catechol oxidase (E.C. 1.10.3.1) which oxidises *o*-diphenols to the corresponding quinones and can also hydroxylate monophenols to *o*-diphenols. The soluble fraction contains a laccase-like enzyme (*p*-diphenol oxygen-oxido-reductase E.C. 1.10.3.2), capable of oxidising both *o*-and *p*-diphenols. The two enzymes differ in their pH optima, sensitivity to inhibitors and their behaviour in column chromatography and gel electrophoresis.[1-3]

The laccase-like enzyme was present only in part of the varieties tested and it seemed likely that the level of enzyme activity varied with the degree of ripening of the fruit.

In view of the importance of these enzymes in the browning of fruits and in fruit processing, it was decided to follow the changes in the levels of the two enzymes during fruit development.

Experimental

Materials

Peaches (var. Salvey) were picked from selected trees in an orchard near Jerusalem, at intervals from fruit set until the time of commercial harvest. The samples of fruit were examined immediately after picking.

Methods

Fresh weight of fruit, including the stone, was determined by weighing. *o*-Diphenols were determined according to Mapson *et al.*,[4] using the molybdate method. *o*-Diphenols content was expressed as mg catechol.

De-stoned fruit sections were homogenised in 0·4M sucrose–0·1M phosphate buffer, pH 7·3, containing 0·01M sodium ascorbate. The homogenate was filtered through gauze and centrifuged at 20,000 × *g* for 30 min. The precipitate was suspended in 0·4M phosphate–citrate buffer, pH 6·0 or 5·1. The supernatant was brought to the appropriate pH (6·0 or 5·1) by addition of 2M citric acid. Phenol oxidase activity was determined using a polarographic oxygen electrode as previously described.[5,6] Catechol oxidase activity was measured in 0·1M phosphate–citrate buffer, pH 5·1, with 5mM of either 4-methylcatechol or *p*-cresol as substrate. Laccase activity was determined in 0·1M phosphate–citrate buffer, pH 6·0, with 10mM quinol as substrate. Results were expressed as scale units/min (1 scale unit = 5·5 μl O_2).

Protein was determined according to the method of Lowry *et al.*,[7] using the Folin–Ciocalteu reagent. This method is based on a reaction with the tyrosine residues in the protein.

Results and Discussion

Catechol oxidase and laccase activity were followed during fruit development from 2 weeks after fruit set to the time of commercial harvest. Enzyme activities were determined in both the particulate and soluble fractions. The content of *o*-diphenols was also determined.

The soluble fraction contained an extremely high level of catechol oxidase activity, which declined sharply during the first few weeks of fruit development (Fig. 1). The decline continued more slowly until harvest time. Laccase activity was almost absent during the first 3 months of fruit development. Activity rose towards fruit maturation, reaching a level slightly higher than that of the residual activity of catechol oxidase in the soluble fraction at harvest time (Fig. 1). A low level of laccase activity appeared also in the particulate fraction (Fig. 3). However, this could be due to artifacts arising from the isolation method.

The changes in catechol oxidase activity in the particulate fraction, as calculated per unit fruit, differed considerably from those observed in the soluble fraction. Activity rose at an uneven rate until 6 weeks before harvest and then declined rapidly to a very low level (Fig. 2). The activity towards *p*-cresol, though relatively low (1–2% of the rate of oxidation of 4-methyl-catechol), closely followed the activity observed for 4-methyl-catechol (Fig. 2). This would be expected if the

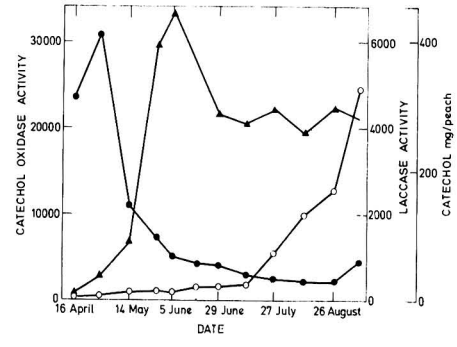

FIG. 1. *Changes in* o-*diphenols and in catechol oxidase and laccase activity in the soluble fraction of peaches during fruit development*

Enzyme activity as scale units/min

▲ *o*-Diphenols/peach; ● catechol oxidase activity (substrate: 4-methylcatechol); ○ laccase activity

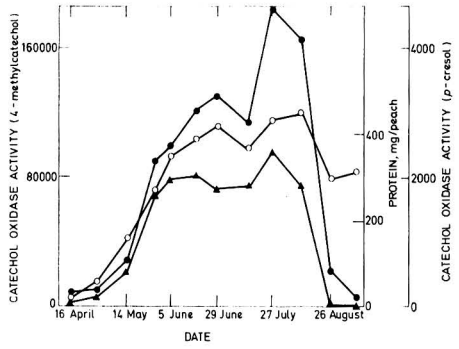

FIG. 2. *Catechol oxidase activity and protein content in the particulate fraction of peaches during fruit development*

Enzyme activity as scale units/min
● Catechol oxidase (substrate: 4-methylcatechol); ▲ catechol oxidase (substrate: *p*-cresol); ○ protein

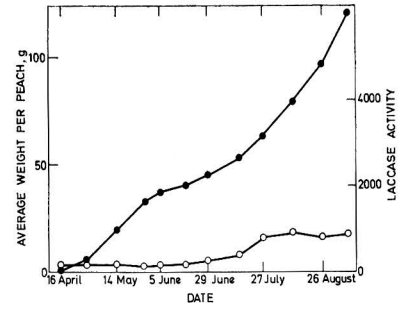

FIG. 3. *Changes in fresh weight of peaches and in the activity of laccase in the particulate fraction during fruit development*

● Fresh weight; ○ laccase activity (scale units/min)

same enzyme is responsible for both activities. No activity towards *p*-cresol was observed in the soluble fraction. The changes in catechol oxidase activity in the particulate fraction corresponded to the changes in the protein content of the fraction (Fig. 2).

Reeve[8,9] reported semi-quantitative histochemical observations on the content of polyphenols in peaches during fruit development. He observed a rise in the content of catechol derivatives in the tissue until pit hardening, with a decline thereafter. This is in agreement with the present results, as pit hardening coincided with a peak in *o*-diphenols content when this is calculated per g fresh weight.

Results which were similar to those reported in this paper were reported for developing apples,[10] where both *o*-diphenols content and catechol oxidase activity per unit fruit rose to a peak and afterwards declined towards fruit ripening. In apples, the peak in *o*-diphenols content also precedes the peak in catechol oxidase activity. Differences between results obtained by various methods for the determination of phenol oxidase activity should be borne in mind when data, expressed as μl O_2, are compared.[6]

It could be suggested that the activities of catechol oxidase observed in the soluble and particulate fractions originate in the same subcellular fraction. Soluble catechol oxidase could co-precipitate with the particulate fractions as a result of interaction with phenolic compounds.[11] However, this seems unlikely, as fractionation in the presence of insoluble polyvinyl pyrrolidone (Polyclar AT)[12] did not affect the distribution of the enzyme between the soluble and particulate fraction. It could also be argued that the soluble catechol oxidase originated in the particulate fraction. This phenomenon has been observed in apples[10] and apricots, where leakage from subcellular particles or solubilisation of catechol oxidase by detergents is accompanied by an almost complete loss of activity towards *p*-cresol.[13] However, the peak in activity in the soluble fraction occurs early on in fruit development while that in the particulate fraction occurs much later. Moreover, activity in the soluble fraction is considerably higher than in the particulate one during the early stages of fruit development while the opposite is true later on (Figs 1 and 2). This seems to exclude the possibility that the two activities originate in the same cell fraction.

Singer & Kearney[14] pointed to the close relationship between catechol oxidases and laccase. They suggested that the laccases are catechol oxidases which differ in their substrate specificity and are therefore incorrectly regarded as separate entities. Bonner[15] went even further to suggest a possible interconversion between the two types of enzymes. The present results provide no evidence to support this view. The laccase in peaches seems to be formed (or activated) independently of the catechol oxidase in the soluble fraction. It could be argued that the catechol oxidase in the particulate fraction is converted to laccase which appears in the soluble fraction. However, this seems to be most unlikely, particularly because of the quite different properties of the enzymes in the two fractions. In this respect, it is important to recall that the two enzymes differ not only in their substrate specificity, pH optima and sensitivity to inhibitors but also in molecular weight, electrophoretic mobility and absorption on ion-exchange columns.[1-3]

Finally, the existence in fruits of more than one type of enzyme oxidising phenols should be borne in mind when ways to prevent or inhibit browning during fruit processing are contemplated. Laccase and catechol oxidase, for example, differ considerably in their sensitivity to inhibitors[1,2] and no compound is known which inhibits both satisfactorily. The prevention of browning in peaches during processing must take into account the relative levels of the two enzymes at the time of harvest. Either combination of inhibitors should be attempted or the products of phenol oxidation must be reduced or removed.

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FOOD AND AGRICULTURE

ABSTRACTS

OCTOBER, 1970

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

OCTOBER, 1970

1.—AGRICULTURE AND HORTICULTURE

Soils and Fertilisers

Soil Formation, Classification, Constituents

Morphology and geochemistry of three clay soils of a tropical coastal plain (Surinam). S. SLAGER and J. VAN SCHUYLENBORGH (*Versl. landbouwk. Onderz. Ned.*, 1970, (734), 34 pp.).— P.P.R.

Free iron and manganese oxide content of reference clays. B. J. ANDERSON and E. A. JENNE (*Soil Sci.*, 1970, **109** (3), 163–169, 18 ref.).—Free Fe oxides, in notable amt., occur in reference clay minerals and may cause difficulties in the identification of these in soils and sediments. A. G. Pollard.

Chemical properties of allophane from Hawaiian and Japanese soils. SUNG-HO LAI and L. D. SWINDALE (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 804–808. 18 ref.).—Chem. and clay mineralog. properties of allophane separated from volcanic ash soils are reported. *CEC* of the separated clay fractions increased with pH, and was correlated with $Al_2O_3/(Al_2O_3 + SiO_2)$ ratio. A. H. Cornfield.

Reductive cleavage of humic acids of chernozemic soils. J. F. DORMAAR (*Pl. Soil*, 1969, **31** (1), 182–184. 7 ref.).—The Na amalgam reduction method showed no qual. differences between the nuclei of the humic acids of the Ah and Bm horizons of chernozemic soils. The method differentiated between soil zones and parent materials. A. H. Cornfield.

Physical Properties of Soils

Sodium hazard of bicarbonate irrigation waters. P. F. PRATT and F. L. BAIR (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 880–883. 13 ref.).—Lysimeter tests over 6 yr showed that water quality value based on the pptn. index (*Soil Sci.*, 1968, **106**, 29) for $CaCO_3$ satisfactorily estimated the effect of the water on composition of saturation extracts of soil samples, except where pptn. of Na^+ occurred, presumably as a zeolite. Essential conditions for Na^+ pptn. were pH > 8·6 and time for dissolution of reactants from the soil and/or formation of the product. Growth of lemon trees and evapotranspiration were correlated with water quality values based on the pptn. index. A. H. Cornfield.

Predicting specific conductance from electrolytic properties and ion association in some aqueous solutions. K. K. TANJI (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 887–890. 16 ref.).—Sp. conductances in aq. soln. of electrolytes were predicted with an extended form of the Onsager limiting equation and compared with measured values. Results are discussed in relation to the use of electrical conductance for determining salt concn. in water and soil soln. A. H. Cornfield.

Effect of entrapped air on the hysteresis curves of a porous body and on its hydraulic conductivity. A. POULOVASSILIS (*Soil Sci.*, 1970, **109** (3), 154–162. 3 ref.).— A. G. Pollard.

Measurement of unsaturated conductivity and diffusivity by infiltration through an impeding layer. D. HILLEL and W. R. GARDNER (*Soil Sci.*, 1970, **109** (3), 149–153. 10 ref.).—The method described measures the hydraulic condition in a soil column in relation to water content or suction and involves a series of infiltration tests through capping plates (crusts) of varied resistance, the resistance serving as suction at the infiltration surface. The infiltration is allowed to proceed to the steady stage as a measure of the capillary conductivity. A. G. Pollard.

Movement of salt and water near crystalline salt in relatively dry soil. D. R. SCOTTER and P. A. C. RAATS (*Soil Sci.*, 1970, **109** (3), 170–178. 17 ref.).—The mechanics of the movement of water through soil towards cryst. salts is examined in columns of initially dry soil in which salt was placed at one end. After various periods (t), the distribution of salt and of water in the column was detd. Both water and salt profiles were functions of the distance from the salt–soil interface $\times \frac{1}{\sqrt{t}}$. The prepn. of a mathematical model is described. A. G. Pollard.

Infiltration, redistribution, and subsequent evaporation of water from soil as affected by wetting rate and hysteresis. E. BRESLER, W. D. KEMPER and R. J. HANKS (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 832–840. 15 ref.).—Evaluation of the relationships of wetting rates to water retention in soil and their effects on subsequent evaporation was based on data obtained from a numerical solution to the isothermal water flow equation and expl. results. A. H. Cornfield.

Interlamellar adsorption of a black earth humic acid on Na montmorillonite. F. M. MARTINEZ and J. L. P. RODRIGUEZ (*Z. PflErnähr. Bodenk.*, 1969, **124** (1), 52–57. Engl., 11 ref.).— M. Long.

Soil properties affecting the sorption of atmospheric ammonia. R. B. HANAWALT (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 725–729. 19 ref.).—Sorption of NH_3 by soils was weakly correlated with increasing soil *CEC* and clay content, but was little affected by org. matter content or pH. NH_3 was sorbed by air-dry soils, but sorption increased with soil moisture content. The extent of sorption of NH_3 is influenced more by atm. NH_3 concn., air velocity across the soil surface, and air temp., than by the sorptive capacity of the soil. A. H. Cornfield.

Adsorption of nitrate in amorphous and kaolinitic Hawaiian soils. B. R. SINGH and Y. KENEHIRO (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 681–683. 16 ref.).—The extent of adsorption of NO_3^- (against extraction with N-KCl or N-K_2SO_4) by an amorphous and a kaolinitic silty clay increased with concn. of NO_3^- used initially for equilibration. Adsorption followed the Freundlich-type isotherm; it decreased with increasing pH (3·5–9·0). A. H. Cornfield.

Stability of saturated soil aggregates in certain tropical soils as affected by solution composition. S. A. EL-SWAIFY (*Soil Sci.*, 1970, **109** (3), 197–202. 12 ref.).—Changes in size-distribution of a Low Humic Latosol and a Red Desert loam soil after treatment with soln. of electrolytes were detd. by wet sieving. Subsurface soils showed no structural changes after treatment with sea-water or chlorides of Na, Ca or Mg in concn. up to 0·6 N. The structure of surface soils known to be less stable than that of subsurface soils was unaffected by the chem. treatments. A. G. Pollard.

Influence of various treatments on the dissolution of dicalcium phosphate in soils. U. S. SREE RAMULU and P. F. PRATT (*Soil Sci.*, 1970, **109** (3), 186–189. 15 ref.).—Suspensions of soils mixed with various proportions of dicalcium phosphate dihydrate (DCPD) were stirred together daily for periods of up to 45 days and the sol. Ca and P in the extracts were detd. Preheating the soil at 600°c for 2 h or the addn. of org. matter to anaerobic samples increased the rate of soln. of DCPD; wetting and drying had a similar though smaller effect. Anaerobic conditions without org. matter had no measurable result. A. G. Pollard.

Concentration of K, Ca and Mg in the saturation extract in relation to exchangeable K, Ca, and Mg. K. NÉMETH, K. MENGEL and H. GRIMME (*Soil Sci.*, 1970, **109** (3), 179–185. 15 ref.).—In samples of 72 soils of differing location and texture, the concn. of K, Ca and Mg in the soil soln. (saturation extract) is compared with other soil properties. The concn. of the 3 bases in the soil soln. varied widely; the $[Ca^{2+}]/[Mg^{2+}]$ increased with exchangeable Ca^{2+} and Mg^{2+} regardless of soil texture. Exchangeable K and the [K] of the soil soln. were correlated only if the soils were classified according to their clay and silt contents. The Gapon coeff., calc. from the exchangeable K and Ca, and the corresponding concn. in the soil soln. differ considerably; both increase with

diminution of [K] in the soil soln. and are higher for sands than for silty soils. This variation in Gapon coeff. may result from differences in the kinds of K adsorption sites. A. G. Pollard.

Retention of strontium by soils as influenced by pH, organic matter and saturation cations. A. S. R. Juo and S. A. Barber (*Soil Sci.*, 1970, **109** (3), 143–148. 9 ref.).—The sorption of Sr by soils and by bentonite from 3×10^{-3} M $SrCl_2$ labelled with ^{89}Sr is examined. Sorption increased with the pH (4–8) of the system. The effect of other saturating cations was in the order Na > K > Mg > Ca > Ba > H. With increase in pH, an increasing proportion of absorbed Sr became non-exchangeable and could remain, presumably 'fixed', under appropriate conditions. Increasing the alkalinity of an org. soil may cause a fraction of the added Sr to be present as sol. Sr, e.g. chelates. A. G. Pollard.

Reactions of ^{144}cerium in solution and in suspensions of soil humic acid and bentonite. R. E. Brown, R. E. Franklin and R. H. Miller (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 677–681. 13 ref.).—When equilibrated with humic acid (*HA*) and bentonite (*B*), alone or in mixtures at various base saturations, the ratio of radiocolloidal (polymerised) to monomeric Ce^{3+} increased with concn. of Ce^{3+} and pH. Above pH 6 most of the Ce^{3+} was present in radiocolloidal form. *HA* adsorbed much greater amt. of Ce^{3+} and restricted its uptake by barley and soyabean roots more than did Ca^{2+}–H^{+}–*B*. In K^{+}–H^{+}-satd. systems *HA* and *B* adsorbed similar amounts of Ce^{3+}. Mixtures of *B* and *HA* were synergistic for Ce^{3+} adsorption. A. H. Cornfield.

Determination of cation exchange capacity over a wide range of pH using various index cations. E. P. Papanicolaou and R. Overstreet (*Z. PflErnähr. Bodenk.*, 1969, **123** (3), 205–212. Engl., 16 ref.).—A method is described for the detn. of *CEC* over a pH range 4–11. The index cations were Na^{+}, K^{+}, Ca^{2+} and Mg^{2+}; materials were Wyoming bentonite and Yolo loam. Except for Ca, the amt. of cation adsorbed at pH < 7 was nearly the same for all cations; at pH > 7 this remained the case only for monovalent cations. Polyvalent cations gave much higher values at pH > 7. The amt. of adsorbed Ca was greater than the corresponding *CEC* of the Yolo loam for the whole of the pH range investigated. Evidence showed that this was due to solid phase Ca compd., sol. in acetates. The higher values for polyvalent ions is explained on the basis of preferential adsorption or pptn. of hydroxides or carbonates at high pH. M. Long.

Biological Aspects, Available Nutrients, Soil Analysis

Effect of anionic and non-ionic detergents on soil microfungi. B. K. H. Lee (*Can. J. Bot.*, 1970, **48** (3), 583–589. 17 ref.).—Three *Aspergillus* spp., 4 *Penicillium* spp., 1 *Chaetophoma*, 1 *Sporonema* and 1 *Actinomucor* were tested for the effects of Tergitol anionic 15-S-3A (**I**) and non-ionic 15-S-9 (**II**), detergents. Both inhibited growth at low concn., but individual spp. reacted differently, at higher concn. Both caused morphological differences; sporulation was affected most in *Penicillium* spp. Some spp. used **I** as a C source, while others used **II**. In general **I** was more available. M. T. Rawnsley.

Influence of acidic gas absorption on certain metabolic processes occurring in anaerobically incubated soils. J. T. Moraghan (*Pl. Soil*, 1969, **31** (1), 1–10. 25 ref.).—Accumulation of CH_4 was decreased and that of acetate increased during anaerobic incubation (80% saturation) of soil in He atm. when a vial of aq. NaOH was placed in the flask. The presence of the vial during anaerobic incubation decreased soil pH and extractable Fe^{2+} and S^{2-} and, in an acid soil, decreased net ammonification. Addn. of $CaCO_3$ to the acid soil before incubation increased CO_2, CH_4, acetate and S^{2-} accumulation; the presence of the vial suppressed accumulation of CH_4, acetate and S^{2-} but ammonification was little affected. A. H. Cornfield.

Locus of urease activity in soil. K. N. Paulson and L. T. Kurtz (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 897–901. 16 ref.).—Under steady state conditions 79–89% of the urease activity of a silty clay loam was due to urease adsorbed on soil colloids. Increasing the microbial population by adding dextrose reduced this % only temporarily. Addn. of 13 ppm urea-N to the soil induced micro-organisms to produce more urease. A. H. Cornfield.

Reduction of sulphate to sulphide in waterlogged soil. W. E. Connell and W. H. Patrick, jun. (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 711–715. 9 ref.).—Sulphide was detected within 2 days of addn. of SO_4^{2-} to a soil which had been treated with rice straw and waterlogged. S^{2-} appeared more slowly in subsoil than in top soil. The amount of S^{2-} pptd. from added H_2S was approx. equiv. to the amt. of Fe^{2+} released by waterlogging. Exposure of a highly reduced soil to air resulted in complete disappearance of S^{2-} within 8 h. Where NO_3^{-} and SO_4^{2-} were added to a waterlogged soil S^{2-} did not appear until all the NO_3^{-} had disappeared. A. H. Cornfield.

Interaction between the humic acid fractions of soils and metallic cations. S. U. Khan (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 851–854. 14 ref.).—Potentiometric titrations of extracted humic acids (*HA*) in presence of cations showed the formation of metal–humic acid complexes. The effectiveness of added cations in coagulating *HA* increased in the order Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Al^{3+}. This order was similar for *HA* extracted from a Black Chernozem and from two Gray Wooded soils. A. H. Cornfield.

Gaseous products produced by anaerobic reaction of sodium nitrite with oxime compounds and oximes synthesised from organic matter. L. K. Porter (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 696–702. 29 ref.).—N_2O was the major gaseous product arising from anaerobic reaction between $NaNO_2$ and all pure oxime compd. tested, except for dimethylglyoxime (**I**) and quinone oximes (**II**), which gave NO. Org. matter (soil colloids), org. matter oximes and aromatic compd. when reacted with $NaNO_2$ gave NO, so that if soil org. matter reacts with NH_2OH to give oximes, these must behave like types **I** and **II**. Since very little N_2O is evolved from reaction of $NaNO_2$ with org. matter oximes, this is probably not a major pathway for loss of N_2O from soils. A. H. Cornfield.

Measurement of nitrogen and nitrous oxide evolution from soil–plant systems using sealed growth chambers. R. C. Stefanson and D. J. Greenland (*Soil Sci.*, 1970, **109** (3), 203–206. 6 ref.).—The apparatus used is described. The sealed growth chamber permits removal of 3-ml samples of the atm. in the chamber. Means of flushing the chamber with Ar are provided. A. G. Pollard.

Extraction of soil organic matter by autoclaving in water. III. Diffusible ammonia as an index of nitrogen availability. G. Stanford and W. H. Demar (*Soil Sci.*, 1970, **109** (3), 190–196. 11 ref.).—Soil samples, after removal of NH_4^{+}- and NO_3^{-}-N by extraction with aq. KCl, were autoclaved at 121°c for 16 h with 25 ml of 0·01 M-$CaCl_2$. NaOH-distillable N was detd. in the extract and diffusible N by modified (Conway) diffusion. The amino sugar fraction was calc. as the total distillable N (boiling) minus the diffusible N. Data for the N-mineralisation capacity (by autoclaving with $CaCl_2$) and for the diffusible NH_3 in the soil extracts are shown; the latter was closely correlated with the alkali-distillable NH_3-N (from the soil extracts by boiling). Glucosamine and chitin are unstable during autoclaving. Hexose sugars in the soil extracts expressed as 'glucose equiv.' correlated well with 'non-distillable' or total N (i.e., distillable + non-distillable N) but less closely with N-mineralisation than did the 'alkali-diffusible' or distillable NH_4^{+}-N. A. G. Pollard.

Nitrate accumulation, distribution and utilisation during fallow–wheat culture in Turkish soils. R. L. Fox, Burhan Kacar, Akgun Aydeniz and Sevin Zabunoglu (*Soil Sci.*, 1970, **109** (1), 60–65. 5 ref.).—Leaching of NO_3^{-} from the soils was considerable even with relatively low rainfall (10 cm). The depth of max. accumulation of NO_3^{-} could be predicted from the water-holding capacity of the soil and the rainfall. Wheat roots in these soils were generally less extensive than those reported in Nebraska, probably because wheat removes relatively more water than NO_3^{-} from the soil. Although the Turkish soils, after long cultivation, contain little org. matter, responses to N fertilisers were low or negative. Many soils of Thrace in which clays are poorly aggregated, show restricted nitrification. Total N and org. matter content are closely correlated with rate of nitrification. Accumulation of NO_3^{-} during the fallow season may indicate which of the soils can supply N to winter wheat, provided the plants can be encouraged to develop a sufficient root system in autumn, e.g., by use of P fertilisers, or mechanically. A. G. Pollard.

Nitrogen distribution and accretion in an alder *(Alnus rugosa)* ecosystem. G. K. Voigt and G. L. Steucek (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 946–949. 19 ref.).—Soil under alder (N-fixer) was significantly higher in total N than under adjacent hardwood–hemlock. Total soil N under alder increased with soil moisture content. The distribution of N in various parts of the plant is presented. The ecosystem showed an increase in total N of approx. 85 kg per ha due to N fixation by alder. A. H. Cornfield.

Increased availability of nitrogen and phosphorus in the root zone of conifers. R. F. FISHER and E. L. STONE (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 955–961. 21 ref.).—Herbaceous plants growing beneath or near pine or larch plantations were higher in N% and P% than those growing on nearby unforested land. Soil NO_3^-- and NH_4^+-N and extractable P were also higher under the forest canopy. There were no consistent differences in soil total N between canopy and open sites. Conifers probably extract or mineralise N and P, thus making these nutrients more available to the forest floor vegetation. A. H. Cornfield.

Native inorganic phosphorus forms and their relation to some chemical indices of phosphate availability for soils of Agra District, India. B. R. TRIPATHI, H. L. S. TANDON and E. H. TYNER (*Soil Sci.*, 1970, **109** (2), 93–100. 43 ref.).—The relative proportions of the inorg. P in the Al-, Fe-, reductant-sol., and Ca-P fractions in surface soils in the District averaged 1 : 1 : 5 : 10 resp. The Ca–P fraction (41% of total P) contributed little labile-P as detd. by Olsen's method, by Bray's (NH_4F–HCl) method or by isotopic exchange techniques. Most of the Ca-P fraction is regarded as occluded within $CaCO_3$ matrices. Variations in Olsen-P and Bray-P may be accounted for by Al-P and those in isotopically-exchangeable P were probably due to Fe-P. For determining the P needs of crops for growth on Agra soils, Olsen's method is probably superior to Bray's method. A. G. Pollard.

Exchangeable potassium and its selectivity by soils as quantity–intensity parameters for soil potassium. A. I. REZK and F. AMER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 876–880. 22 ref.).—The selectivity of 12 calcareous soils for K^+ *versus* $Ca^{2+} + Mg^{2+}$ was measured by determining the Gapon selectivity coeff. The ratio of exchangeable K to selectivity coeff. was superior to exchangeable K alone in assessing the K supply to barley in pot tests with the 12 soils. A. H. Cornfield.

Content of easily-soluble boron in French arable earths. D. BERTRAND (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (18), 1253–1257. Fr., 10 ref.).—Of 144 samples from different sources, 97 had < 1 mg B/kg, i.e., less than the limit for trace elements. Twelve had > 2 mg. It is suggested that more research should be done on the amount of B required. The discussion questions the correctness of the sampling methods used. M. T. Rawnsley.

Automatic methods for determining nitrate and nitrite in water and soil extracts. A. HENRIKSEN and A. R. SELMER-OLSEN (*Analyst, Lond.*, 1970, **95** (1130), 514–518. 17 ref.).—Using a Technicon AutoAnalyzer, the water/soil extract is treated with copperised-Cd in the presence of NH_4Cl and the NO_2^- produced is used to diazotise sulphanilamide which is then coupled with *N*-1-naphthylethylenediamine. The intensity of the resulting colour is measured at 520 nm to give the sum of the $[NO_2^-]$ and $[NO_3^-]$; the $[NO_2^-]$ alone is detd. without Cu–Cd reduction and the $[NO_3^-]$ obtained by difference. 20 samples/h can be analysed and the useful range is 0·01–1·0 mg nitrate-N/l. A dialyser is used only for turbid or highly coloured soln. S. S. Chissick.

Sulphate determination in soil extracts by an indirect atomic absorption spectrophotometric method. G. G. GALINDO, H. APPELT and E. B. SCHALSCHA (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 974–975. 7 ref.).—Sulphate is extracted from soil by shaking with 500 ppm KH_2PO_4, and org. matter in the extract is destroyed with 30% H_2O_2. The hot soln. is treated with $BaCl_2$ and the centrifuged ppt. of $BaSO_4$, after washing, is dissolved in $(NH_4)_2$-EDTA and Ba detd. by atomic absorption spectrophotometry. A. H. Cornfield.

***X*-ray fluorescence technique for determination of iron and manganese in soils and concretions.** R. I. BARNISHEL, W. R. PHILLIPPE and R. L. BLEVINS (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 811–813. 7 ref.).—A 0·025-g sample is suspended in glue–MeOH and deposited uniformly on filter paper, which is then subjected to *X*-ray fluorescence analysis. Accurate and reproducible results were obtained over a wide range of Fe and Mn oxide contents. A. H. Cornfield.

Total phosphorus contents of soils by perchloric acid digestion and sodium carbonate fusion. G. E. G. MATTINGLY (*J. agric. Sci., Camb.*, 1970, **74** (1), 79–82. 11 ref.).—The total P content of soils, as detd. by Na_2CO_3 fusion, was higher than when detd. by $HClO_4$ digestion. Fusion values (P_f) were related to perchloric acid digestion (P_p) by the equation: P_f (ppm) = 38·8 (+ 6·86) + 10021 (+ 0·01176) P_p. Recovery of P, added as superphosphate, was the same by both methods. M. Long.

Spectrographic analysis of the Cu, Mn and Fe content of some rock phosphates and superphosphates. M. VARJU (*Z. PflErnähr. Bodenk.*, 1969, **124** (1), 12–18. Ger., 13 ref.).—The Cu, Mn and Fe contents of H_2SO_4, citric acid and water extracts of some rock phosphate and superphosphate samples were estimated. The elements were extracted with Na pyrrolidinedithiocarbamate. After extraction, the complexes were destroyed by H_2SO_4 digestion and estimated by rotating disc spectrography. The micro-elements were mostly sol. in acidified rock phosphate with a positive correlation between free acid content and their solubility. The amt. of micro-element released from superphosphate may help to replace losses from the soil. M. Long.

Evaluation of atomic absorption methods for determinations of aluminium, iron and silicon in clay and soil extracts. T. L. YUAN and H. L. BRELAND (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 868–872. 12 ref.).—The direct detn. of Al, Si (N_2O–C_2H_2 flame), and Fe (O_2–C_2H_2 flame) by atomic absorption spectroscopy in a no. of extractants gave results very similar to those obtained by colorimetry. The extractants studied were N-NH_4OAc (pH 4·8), 0·1N-HCl, 0·2M-$(NH_4)_2C_2O_4$ (pH 3·0), 0·5N-NaOH, and buffered $Na_2S_2O_4$. Recovery tests for Fe from 0·5N-NaOH, 0·2N-$(NH_4)_2C_2O_4$, and $Na_2S_2O_4$ extracts of soils gave high results. A. H. Cornfield.

Fertilisers

Covering soil with a black polyethylene film in banana plantations in the Ivory Coast. J.-M. CHARPENTIER, J. GODEFROY and Y. MENILLET (*Fruits d'outre mer*, 1970, **25** (2), 77–85. Fr., 3 ref.).—Four expt. using (*i*) control (mulch + mineral fertiliser), (*ii*) bare soil + standard herbicide + fertiliser, (*iii*) partial covering with black polyethylene + herbicide on uncovered soil + herbicide and fertiliser under cover, (*iv*) as (*iii*) but with less fertiliser, over a period of months, showed that (*i*) gave best results. Exchangeable cations etc. are increased, and humidity in the dry season is better. For (*ii*) and (*iii*) results were similar except that in (*iii*) acidity increased. M. T. Rawnsley.

Controlled release of magnesium, zinc, boron and nitrogen from polyethylene capsules. D. L. ASHLEY (*Can. J. Pl. Sci.*, 1969, **49** (5), 555–566. 6 ref.).—Salts enclosed in perforated polyethylene capsules were placed in soil cropped with perennial ryegrass, comparison being made with similar amounts of the salts mixed with the soil. In general, encapsulation gave better results, particularly for N. Mg with N in capsules perforated on the upper or both surfaces was released more rapidly than from capsules perforated only on the lower surface. Movement of Mg from capsules placed in a column of soil remained substantially const. over a period of 36 weeks. Antagonistic effects are recorded between the release from capsules of Zn and B and between N and Mg. Movement of salts from capsules perforated on both sides was mainly downwards. A. G. Pollard.

Retention of zinc by some arid zone soils treated with zinc sulphate. R. G. SHARPLESS, E. F. WALLIHAN and F. F. PETERSON (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 901–904. 12 ref.).—Application of $ZnSO_4$ in aq. soln. to soils resulted in rapid conversion of most of the Zn to exchangeable form. The subsequent rate of conversion of Zn from exchangeable to 0·1N-HCl-extractable form varied widely among soils. Retention of added Zn increased with pH and with *CEC* but was not related to extractable soil phosphate. A. H. Cornfield.

Effect and interaction of molybdenum and limestone on growth and molybdenum content of cauliflower, lucerne, and bromegrass on acid soils. U. C. GUPTA (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 929–932. 14 ref.).—High yields of cauliflower on 3 acid soils (pH ~ 5) were obtained only when Mo + $CaCO_3$ was applied. Lucerne responded in two of the soils. Bromegrass responded to $CaCO_3$, but not to Mo, applications. Significant Mo × $CaCO_3$ interactions occurred in two of the soils. Application of $CaCO_3$ increased plant Mo content, particularly of cauliflower and lucerne. Mo uptake by all crops increased with level of applied Mo. A. H. Cornfield.

Nutrient cycling in grazed pastures. II. Further observations with [^{35}S]-gypsum. A. R. TILL and P. F. MAY (*Aust. J. agric. Res.*, 1970, **21** (2), 253–260. 7 ref.).—Wool samples were taken from 3 groups of sheep, which had been put to graze on pastures enriched with [^{35}S]-gypsum. Radioactivity of soil and wool was detd. by liquid scintillation counting. After 3 yr, S for the original gypsum was still being turned over in the soil–plant–animal system. M. J. Rawlins.

Composted municipal refuse: its effects on carbon dioxide, nitrate, fungi, and bacteria in Arredendo fine sand. D. F. ROTHWELL

and C. C. HORTENSTINE (*Agron. J.*, 1969, **61** (6), 837–840. 11 ref.).—Nitrate production during aerobic incubation of a fine sand treated with cow manure increased with level of manure applied (2–10 g, dry wt., per 100 g soil). Where sewage sludge and chicken manure were applied, NO_3^- production increased with application of up to about 2 g material per 100 g of soil, but decreased with further increasing rate of application. There was no NO_3^- production from application of any level of composted municipal refuse. CO_2 evolution increased with rate of application of all materials, sewage sludge and chicken manure having the greatest effects. The no. of bacteria + actinomyces were high during the first few days of incubation, but then fell rapidly to low levels. The no. of fungi increased with time of incubation (up to 26 days).
A. H. Cornfield.

Plant Physiology, Nutrition and Biochemistry

Light, Air and Water Relationships

Photosynthetic activity of fruits. C.-T. PHAN (*Fruits d'outre mer*, 1970, **25** (2), 109–111. Fr., 8 ref.).—Tests on apples, in the Warburg apparatus, showed that fruits not yet at the climacteric have a photosynthetic activity. Although previously thought to occur only in the skin, it is shown that tissues near the carpellar area are active. This is however, dependent on the absence of chlorophyll in the skin, and decreases with maturity. The 'aeration' of the internal tissues of the fruit could thus be explained.
M. T. Rawnsley.

Carbon dioxide transfer resistance as a factor in shade tolerance of tree seedlings. J. E. WUENSCHER and T. T. KOZLOWSKI (*Can. J. Bot.*, 1970, **48** (3), 453–456. 9 ref.).—Work on single leaves of two *Quercus* spp. and *Acer saccharum* Marsh, at light intensities of 0·03 to 0·24 cal cm $^{-2}$min $^{-1}$ (400–700 nm) showed that variations in photosynthesis between shade-tolerant spp. could be partly explained by CO_2 transfer resistance. M. T. Rawnsley.

Soil oxygen and the growth of white clover. M. W. GRADWELL (*N.Z. Jl agric. Res.*, 1969, **12** (4), 615–629. 15 ref.).—The growth of mature plants can be substantially reduced when soil oxygen flow is low. However, this must be extremely low ($< 10^{-7}$ g/cm^2/min) for any noticeable reduction. M. T. Rawnsley.

Evapotranspiration and water stress of barley with increased nitrogen. R. E. LUEBS and A. E. LANG (*Agron. J.*, 1969, **61** (6), 921–924. 16 ref.).—Leaf area index, before stem elongation, and evapotranspiration, before and during tillering, increased with level of N (45–90 kg per ha) applied at emergence. During a subsequent 26-day period, depletion of available soil water increased with level of applied N. Relative turgidity of barley leaves 2 weeks before heading decreased with rate of N. A. H. Cornfield.

Osmotic and matric suction effects on relative turgidity, temperature and growth of cotton leaves. J. R. THOMAS and C. L. WIEGAND (*Soil Sci.*, 1970, **109** (2), 85–92. 14 ref.).—Growth of cotton plants and yields of lint decreased as the total soil water suction (*TSWS*) and osmotic suction (*OS*) increased. Matric suction (*MS*) and *OS* were inversely related. In saline soils *OS* was the major component of the *TSWS*. Cotton plants appeared to be adjusted to saline conditions and could obtain water in which the *OS* was > 14 bars. The suction in the root zone of the soil was not completely characterised by the (*MS* + *OS*). The relative turgidity of leaves decreased and leaf temp. increased with increase in *TSWS*. (*MS* + *OS*) constituted 35–76% of the variation in relative turgidity. *MS* never accounted for > 3% of the total variation. A. G. Pollard.

Plant Nutrition and Metabolism

Ionic interaction in diffusion. M. H. FRERE (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 883–886. 8 ref.).—A numerical analysis of diffusion of ions to plant roots showed that the assumption of const. diffusion coeff. is not always valid. The apparent diffusion coeff. changed to the greatest extent with distance of the ion from the root, followed in decreasing order by changes in soln. concn., rate of uptake and time. A. H. Cornfield.

Supply of nutrient ions by diffusion to plant roots in soil. II. Effect or root hairs on the uptake of potassium by roots of ryegrass. M. C. DREW and O. H. NYE (*Pl. Soil*, 1969, **31** (3), 407–424. 22 ref.).—The absorption of K by ryegrass grown in two soils with different diffusion characteristics, and at a range of concn. of soln. and labile soil K, was studied. The significance of root hairs in K absorption was examined. A. H. Cornfield.

Accumulation of salts in the sub-irrigation of pot plants. G. GUTTORMSEN (*Pl. Soil*, 1969, **31** (3), 425–438. 5 ref.).—Even moderate amounts of N and K salts supplied to plastic pots through sub-irrigation on sand trays led to considerable salt accumulation, particularly in the upper layers of the pots. Water-sol. Ca and Mg concn. were also affected, and there was some reduction in pH.
A. H. Cornfield.

Influence of fertiliser treatment on apple fruit composition and physiology. III. Influence on contents of phosphorus, magnesium, calcium and potassium. D. S. LETHAM and H. J. W. MCGRATH (*N.Z. Jl agric. Res.*, 1969, **12** (4), 642–649. 9 ref.).—P (ethanol-sol. and -insol.), Mg, Ca, and K were detd. and detailed results are given. P is shown to be an important factor in storage of Sturmer apples. M. T. Rawnsley.

Micronutrient availability on three soils as affected by applications of zinc, lime, and sulphur. R. G. HOEFT and R. C. SORENSEN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 924–928. 21 ref.).—The effects of application of elementary S, $CaCO_3$ and $ZnSO_4$ to soils of varying texture and pH on performance of maize, and uptake of Zn, Fe, and Mn, were studied in pot tests. Soil type had the greatest influence. A. H. Cornfield.

Iron metabolism of grasses. I. Effect of iron supply on some inorganic and organic constituents. M. O'SULLIVAN (*Pl. Soil*, 1969, **31** (3), 451–462. 31 ref.).—Effects of Fe supply (0·04–10·24 ppm in the nutrient in sand cultures) on tissue concn. of total and N-HCl-sol. Fe, total N, P, K, Ca, Cu, and Mn and chlorophyll, catalase and peroxidase in timothy (*Phleum pratense*) and Yorkshire fog (*Holcus lanatus*) are reported.
A. H. Cornfield.

Effect of chelating agents on availability to plants of carrier-free ^{59}Fe and ^{65}Zn added to soils to simulate contamination from fallout. A. WALLACE and R. T. MUELLER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 912–914. 9 ref.).—The effects of Na_2EDTA and Na_2EDDHA on uptake of total and labelled Fe and Zn varied with crop species and soil type. A. H. Cornfield.

Effect of zinc sources on micronutrient contents of Golden Cross Bantam corn (maize). A. WALLACE and E. M. ROMNEY (*Soil Sci.*, 1970, **109** (1), 66–67. 5 ref.).—Various chelated Zn compd., e.g., ZnNTA (**I**), ZnEDTA (**II**) and other Zn sources were applied to pot-cultured maize and their effects on growth (wt.) and contents of micronutrients were compared. In general, chelated forms of Zn were available to the plants, the more stable forms producing the higher levels of plant-Zn. A. G. Pollard.

Sensitivity of annual *Medicago* species to manganese toxicity as affected by calcium and pH. A. D. ROBSON and J. F. LONERAGAN (*Aust. J. agric. Res.*, 1970, **21** (2), 223–232. 25 ref.).—The effect of Ca concn. and pH on the absorption of Mn from soln. by 4 annual legumes was studied in a flowing culture system. Increasing Ca concn. to 2500 μM or decreasing soln. pH to 5·4 eliminated severe Mn toxicity symptoms at a low Mn concn. in complete nutrient soln. It was considered that sensitivity to Mn toxicity may contribute to the poor growth of *Medicago* species on acid and water logged soils. M. J. Rawlins.

Influence of fat–sugar-derived surfactants on phosphorus absorption through leaf surfaces. D. J. CANTLIFFE and G. E. WILCOX (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 141–143. 9 ref.).—Several of the fat–sugar-derived surfactants added to foliar sprays at 0·1–1·0% increased PO_4^{3-} absorption by bean plants. Most materials increased translocation into apical tissue, but had little effect on that into cotyledonary leaves. A. H. Cornfield.

Changes in nitrogen metabolism in the wheat root tip following growth and vernalisation. G. STE-MARIE and P. WEINBERGER (*Can. J. Bot.*, 1970, **48** (4), 671–681. 34 ref.).—The free amino acid content and its distribution in Rideau and Marquis wheat root tips (3·5 mm) was detd. The dominant acids are glutamic and aspartic, as in maize, contrasting with homoserine in peas. The metabolism, protein synthesis, etc. are discussed in the light of these results. M. T. Rawnsley.

Effect of a short term inorganic nitrogen fertilising on the soluble amino acid composition of wheat seedling roots and shoots. K. MENGEL and M. HELAL (*Z. PflErnähr. Bodenk.*, 1969, **123** (3), 196–204. Ger., 10 ref.).—Wheat seedlings were grown in water culture with all nutrients, except N, present. At the start of the expt., NH_4NO_3 was added. Fresh root and shoot samples were taken at the time of adding N, and 1 h and 4 h later. Sol. amino acid content increased by 50% in the roots and by 25% in the shoots after 1 h compared to the content at the time of adding N. These contents had fallen to a 12% increase for the roots and

74% of the initial value for the shoots after 4 h. In the first h the content of all amino acids, except asparagine and aspartic acid, increased. Initially asparagine increased in the shoots and then fell with a corresponding increase in alanine. M. Long.

Modification of the metabolism of proteins during cold hardening of winter wheat. M. V. BRIQUET, H. P. THERRIEN and E. A. ROCHAT (*Naturaliste can.*, 1969, **96** (6), 895–901. Fr., 7 ref.).—In the pre-tillering stage, tracer studies showed a marked decrease of rate of incorporation of L-leucine-^{14}C in sol. and in ribosomal proteins, this being a function of time. M. T. Rawnsley.

Effects of aflatoxin B_1 on protein synthesis by cucumber cotyledon discs. B. TRUELOVE, D. E. DAVIS and O. C. THOMPSON (*Can. J. Bot.*, 1970, **48** (3), 485–491. 28 ref.).—Tracer studies showed that the amt. of ^{14}C-leucine absorbed was reduced when the discs were incubated with small amt. of aflatoxin. Incorporation into protein was not affected. M. T. Rawnsley.

Use of acetylene reduction to study the effect of nitrogen fertiliser and defoliation on nitrogen fixation by field-grown white clover. E. MOUSTAFA, R. BALL and T. R. O. FIELD (*N.Z. Jl agric. Res.*, 1969, **12** (4), 691–696. 13 ref.).—Addn. of fertiliser (80 lb/acre) to established clover reduced the N fixation rate to 23–30% of the rate without N fertiliser. Defoliation caused reduction within 24 h but plants returned to normal after 21 days. M. T. Rawnsley.

Germination, Growth Regulation, Senescence

Some factors influencing the germination and early seedling development of pasture plants. J. R. MCWILLIAM, R. J. CLEMENTS and P. M. DOWLING (*Aust. J. agric. Res.*, 1970, **21** (1), 19–32. 45 ref.).—Seven important commercial pasture species of legumes and grasses were examined. Imbibition and germination, uptake of nutrients, CO_2 exchange, root extension, utilisation of reserves and development of photosynthetic activity were studied. Under good conditions, the onset of autotrophic growth occurred within 5 days of imbibition, well before the exhaustion of endogenous reserves. The results are discussed in relation to breeding and field problems. M. J. Rawlins.

Effects of a growth retardant (Alar) on shoot meristems of apple. M. H. WILDE and L. J. EDGERTON (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 118–122. 11 ref.).—The effects of spraying apple seedlings (10–15 cm high) with 1000 ppm Alar [succinic acid mono (2,2-dimethyl hydrazide)] on cytohistology of shoot meristems 2 h to 15 weeks after treatment are reported. A. H. Cornfield.

Chemical control of flower development in sweet orange *(Citrus sinensis)*. G. I. MOSS (*Aust. J. agric. Res.*, 1970, **21** (2), 233–242. 23 ref.).—No stimulation of flowering was produced by applications of several growth retardants. Gibberellic acid (GA) provoked response; in two expt., it decreased flowering but increased the proportion of the more fertile inflorescences. Increase in % fruit set by some GA treatments appeared to be the result of fewer inflorescences, thereby reducing competition. Effects of GA were greatest when applications were made in June/July. M. J. Rawlins.

Effects of ethylene on morphology and flowering of *Chrysanthemum morifolium* Ramat. B. O. S. TJIA, M. N. ROGERS and D. E. HARTLEY (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 35–39. 16 ref.).—Subjecting chrysanthemum plants to atm. containing 1–4 ppm C_2H_4 (to simulate winter glasshouse conditions where open-flame burners are used) resulted in failure to initiate and develop flower buds under short day conditions. Typical epinastic symptoms developed and many short auxillary shoots were formed. Endogeneous auxins were maintained at high levels in C_2H_4-treated plants and the polar auxin transport system of the plant was also affected. A. H. Cornfield.

Effects of ripening processes on chemistry of tomato volatiles. B. M. SHAH, D. K. SALUNKHE and L. E. OLSON (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 171–176. 22 ref.).—Several alcohols, carbonyls, and esters were identified in the volatiles of tomato fruit. The concn. of short-chain (C_4–C_6) compd. were higher in artificially ripened fruit, whilst the long-chain (C_9–C_{12}) carbonyls (**I**) and the terpene esters (**II**) were predominant in field-ripe fruit. It was indicated that **I** and **II** contributed to ripe tomato odour. A mechanism for the biogenesis of these compd. and changes in chem. compn. during senescence of fruit are also presented. A. H. Cornfield.

Influence of oestrone on growth and endogenous gibberellins content in dwarf pea. J. KOPCEWICZ (*Bull. Acad. Pol. Sci., Sér. biol.*, 1970, **17** (11–12), 727–731. Engl., 17 ref.).—Pea seeds were germinated and cultivated in sterile sawdust in red light at 20–22°C; after six days, seedlings were selected and treated with 10 μl aliquots of oestrone or gibberellic acid (doses of 0·1 and 0·001 μg of hormone per plant, resp.) and growth and changes in gibberellins content were followed over 96 h. Physiol. effects of both hormones were identical, and in both cases growth stimulation paralleled endogenous gibberellins content. Results suggest that growth stimulation by oestrone is due to its effect on biosynthesis of gibberellins. E. C. Apling.

Growth promoting effect on plants of 3,4-benzpyrene and benzfluoranthrene. K. H. WAGNER, E. WAGNER-HERRING and K. BUCHHAUPT (*Z. PflErnähr. Bodenk.*, 1969, **123** (3), 186–196. Ger., 16 ref.).—The effect of 3,4-benzpyrene and 3,4-benzfluoranthrene on rye, summer wheat and maize was investigated in pot trials. The two compd. were mixed with the soil at the rates of 0·1 and 1 mg each in the case of rye and 1 and 10 mg per pot (100 g dry soil) for the other two crops. Compared with the controls, neither level of the two compd. had any effect on growth of the test plants. It is concluded that the effect of the compd. on plant cells differs from that on animal cells. M. Long.

Other Aspects

Effect of fluoride on ribosomes and ribonuclease from corn roots. C. W. CHANG (*Can. J. Biochem.*, 1970, **48** (4), 450–454. 17 ref.).—In the roots of corn seedlings aged by F^-, a reduction of ribosome content was noted in which a decrease in polysome level was accompanied by an increase in no. and concn. of monosomes and subunits combined. F^- also increased the specific activity of ribonuclease, probably associated with ribosomal protein. E. G. Brickell.

Root cation exchange capacity measurement by lithium-exchange. T. ANDO, J. H. BAKER and M. DRAKE (*Pl. Soil*, 1969, **31** (3), 473–485. 29 ref.).—Root material is shaken with 0·1N-LiCl in a stream of N_2 for 30 min and then washed with water. The residue is dried and ashed and Li detd. by atomic absorption spectroscopy. A. H. Cornfield.

Bicarbonate accumulation and pH changes at the soyabean root-soil interface. D. RILEY and S. A. BARBER (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 905–908. 12 ref.).—When soyabeans were grown in a silt loam, the soil near the roots had pH up to 1 unit higher than that of the original soil and also had high concn. of HCO_3^-. Accumulation of HCO_3^- in the soil increased with level of applied NO_3^-. A. H. Cornfield.

Crops and Cropping

Field crops

Effect of nitrogenous fertiliser on wheat yield and baking quality. G. M. WRIGHT (*N.Z. Jl agric. Res.*, 1969, **12** (4), 727–746. 31 ref.).—Time of N application and time of sowing were the most important factors in yield responses. Cycle of crops was important. Baking quality was improved in all cases, but again the crop cycle was critical. M. T. Rawnsley.

Grain yield of maize in relation to nitrogen, phosphorus, sulphate, chloride, zinc, boron, manganese and plant population. H. D. FUEHRING, H. F. MIRREH, N. AHMAD and P. N. SOLTANPOUR (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 721–724. 8 ref.).—High yields of maize on an irrigated calcareous clay required high levels of applied N and also relatively high levels of P, Zn, B and plant population. Application of SO_4^{2-} and Cl^- tended to decrease yields, whilst Mn had no effect. There were many significant first and second order interactions among major and most trace elements. A. H. Cornfield.

Drainage and nutrient effects in a field lysimeter study. I. Maize yield and soil conditions. R. LAL and G. S. TAYLOR (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 937–941. 13 ref.).—Lysimeter studies with a silt loam showed that application of N, Zn or Cu did not overcome the detrimental effects of inadequate drainage (due to excessive irrigation) on maize yields. Ear leaf N and Zn levels were better correlated with grain yields at low than at high levels of application of either nutrient. A shallow water table decreased soil O_2 and increased soil CO_2. A. H. Cornfield.

Effect of oat seed treatment with microelements. T. SARIC and B. SACIRAGIC (*Pl. Soil*, 1969, **31** (1), 185–187. 7 ref.).—Treatment of oat seed with Cu^{2+}, Co^{2+}, or BO_3^{3-} before sowing had no effect on germination. The Co and B treatments increased tillering,

B and Co increased panicle length, and Co increased plant height. B and Co increased seed wt., whilst Co and Cu increased grain yields per acre. A. H. Cornfield.

Growth of crops in sand dune soil. IV. Effect of nitrogen top dressing at time of maximum tillering on the growth of upland rice. M. YAMANOUCHI and S. SAWADA (*Sand Dune Res.*, 1968, **14** (2), 22–31. Jap.).—(Engl. summ.). C. V.

Effects of plant density and frequency of cutting on the growth of cocksfoot (*Dactylis glomerata* L.). I. Production of vegetative and reproductive tillers. R. KNIGHT (*Aust. J. agric. Res.*, 1970, **21** (1), 9–17. 8 ref.).—The no. of vegetative tillers per plant was reduced by high *d* in all treatments. A change in cutting frequency from 8 to 4 weeks had little effect on numbers, but cutting every 2 weeks reduced numbers. Reproductive tillers were affected by the treatments much more than vegetative tillers.
M. J. Rawlins.

Row spacing and fertilisation influences on forage and seed yields of intermediate wheatgrass, Russian wild rye, and green needlegrass on dryland. A. L. BLACK and L. L. REITZ (*Agron. J.*, 1969, **61** (5), 801–805. 14 ref.).—The effects of contour row spacing (76–152 cm) and application of N or N + P on forage and seed production of and water use efficiency by *Agropyron intermedium*, *Elymus junceus*, and *Stipa viridula* are reported over 5 yr.
A. H. Cornfield.

Nutrient interacting effects on sucrose yield of sugar-beet on a calcareous soil. H. D. FUEHRING, M. A. HASHIMI, K. S. HADDAD *et al.* (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 718–721. 6 ref.).—High yields of sucrose from sugar-beet on an irrigated calcareous clay required application of high rates of N, P, and Na^+ and there were no responses to K^+, Mg^{2+}, S, Cl^-, B and Zn. Yields were decreased when Zn and S were applied with high N and/or high Na^+. Response to Na^+ was best where Mg^{2+}, S and Cl^- were not applied. A. H. Cornfield.

Horticultural Crops

Regulation of growth, flowering and fruit abscission with 2-chloroethylphosphonic acid (Ethrel). L. J. EDGERTON and W. J. GREENHALGH (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 11–12. 1 ref.).—Spray application of 1000–2000 ppm Ethrel to branches of young apple trees during pre-bloom to early post-bloom stages virtually eliminated all fruit with little or no phytotoxicity. Treatment of peach checked vegetative growth and promoted flower bud formation in some cases. Fruit set was reduced by treatment 1–4 weeks after bloom, but phytotoxicity sometimes occurred. The treatments promoted abscission at maturity. The compd. does not appear promising as a thinning agent.
A. H. Cornfield.

Effect of the timing of urea sprays on apple trees. I. The effect on vegetative growth. P. LÜDDERS and G. BÜNEMANN (*Z. PflErnähr. Bodenk.*, 1969, **124** (2), 157–172. Ger., 32 ref.).—Cox's Orange Pippin trees were grown in pots with sand as the medium. All trees received a basic N supply of 2 mequiv./l in the nutrient soln. The growing season was divided into 4 periods of 2 months each. The sprays were applied either in one, two or three periods. Best growth was obtained with sprays evenly distributed throughout the growing period. Little growth was obtained if all the sprays were applied in one period. Net assimilation was max. when the sprays were applied during the summer, although the dry %-wt. of root on total dry matter of the tree was reduced. Trees receiving only the basic N supply in June and July showed necrotic spots on basal leaves resembling 'Cox leaf spots'. M. Long.

Degreening of citrus fruits in response to varying levels of oxygen and ethylene. O. L. JAHN, W. G. CHACE, JUN. and R. H. CUBBEDGE (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 123–125. 15 ref.).—Exposure of autumn-harvested oranges to an atm. containing 5–10 ppm C_2H_4 resulted in rapid degreening. A 50% O_2 atm. also increased degreening rate, but high $O_2 + C_2H_4$ were not additive in this respect. A low-O_2 (10%) atm. reduced the degreening response to C_2H_4. A. H. Cornfield.

Regulation of sweet cherry maturity with succinic acid mono (2,2-dimethylhydrazide) (Alar) and 2-chloroethylphosphonic acid (Ethrel). N. E. LOONEY (*Can. J. Pl. Sci.*, 1969, **49** (5), 625–627. 8 ref.).—Treatment of cherry trees, 2 weeks after flowering, with Alar (to 2000 ppm) permitted mechanical harvesting of the fruit a week prior to commercial maturity. Application of Ethrel (to 1000 ppm) alone or with Alar (2000 ppm) delayed harvesting.
A. G. Pollard.

Response of garden crop plants to low-dose gamma-irradiation of seeds. V. W. NUTTALL, L. H. LYALL, D. H. LEES and H. A. HAMILTON (*Can. J. Pl. Sci.*, 1968, **48** (4), 409–410).—Exploratory trials with sweetcorn, cucumber, egg plant, lettuce, pea, pumpkin and tomato gave indications of earlier maturity and increased yields following irradiation of the seed. A. G. Pollard.

Plantation Crops

Search for a K/N equilibrium in the production of fresh pineapple in the Cameroun. II. Foliar analyses. J. MARCHAL, P. MARTIN-PREVEL, J.-J. LACOEUILHE and P. LOSSOIS (*Fruits d'outre mer*, 1970, **25** (2), 87–95. Fr., 5 ref.).—'D' leaves were analysed, according to normal practice. Detn. of N, P, Ca and Mg were made. It is shown that the potential growth of pineapple is detd. first by climatic factors, then by N fertiliser. The action of N determines the need for K, while it can stimulate the absorption of K. This could explain the rapid exhaustion of pineapple-growing soils. Growth and absorption factors for Ca, P and Mg are also discussed. M. T. Rawnsley.

Field observations on the efficacy of foliar sprays of zinc salts for correcting zinc deficiency in Arabica coffee. B. B. ANANTH and H. HANUMANTHA RAO (*Indian Coff.*, 1970, **34** (1), 15–17. Engl., 4 ref.).—Results showed that foliar sprays of NuZ (a proprietary product contg. Zn oxysulphate with 55% metallic Zn) at 2 lb/100 gal of water gave max. response, followed closely by $ZnSO_4$ at 2·5 lb/100 gal water. Both NuZ and $ZnSO_4$ were superior to $ZnCl_2$ and $ZnNO_3$. W. J. G.

Forest Crops

No abstracts

Plant-growth regulants. FMC CORP. (Inventor: K. L. Hill) (Br. Pat. 1,190,312, 23.7.68).—Phenylfuroxan is claimed as a plant-growth regulant, e.g., early development of fruit, early maturation, early break of dormancy, etc. F. R. Basford.

Animal Husbandry

Feedstuffs

Effect of phosphate and potash fertilisers on cut and grazed grassland. J. S. BROCKMAN, P. G. SHAW and K. M. WOLTON (*J. agric. Sci., Camb.*, 1970, **74** (2), 397–407. 25 ref.).—The effects of cutting with removal of herbage, grazing with animals previously on pasture similarly fertilized and grazing with no such preconditioning were compared on a grass/clover sward at the same time as the effects of P and K at the rates of 22 lb and 56 lb/acre respectively. P increased grass yields in the first 4 yr and decreased clover in the 4th and 5th; both grazed swards and cut sward responded similarly to P. K had no effect on grass yield, but markedly increased clover and the K content of both grass and clover herbage. Grazing led to higher herbage K content than cut swards, especially in plots grazed by preconditioned animals. Thus, while cutting management is acceptable in short term trials, different results will be obtained in the long term. Preconditioned animals must also be used to graze plots so as to avoid transfer of nutrients.
M. Long.

Relationship between the soluble constituents of herbage and their dry-matter digestibility. J. M. A. TILLEY and R. A. TERRY (*J. Br. Grassld Soc.*, 1969, **24** (4), 290–295. 29 ref.).—The relationships between the amounts of dry matter, carbohydrates, N extracted by acid-pepsin and hot water, crude and true protein and sol. carbohydrates are discussed, particularly with respect to herbage digestibility. These simple extractants may be more useful than conventional chem. techniques for assessing the comparative nutritive value of herbages. A. H. Cornfield.

Behaviour of certain varieties of white clover under simulated grazing conditions. P. GERVAIS (*Naturaliste can.*, 1969, **96** (6), 903–912. Fr., 26 ref.).—Seven varieties were grown in association with timothy grass over 3 yr. The Ladino variety was superior to all others in dry matter yield, protein yield, competitive ability and persistency. M. T. Rawnsley.

Sensory evaluation of the feeding value of hay. J. E. TROELSEN, P. I. MYHR, R. W. LODGE and M. R. KILCHER (*Can. J. Anim. Sci.*, 1968, **48** (3), 373–381. 11 ref.).—Hays of 4 grasses and 2 lucernes were each harvested at six different stages of maturity and subjected to sensory tests based on phys. compn., growth stage, colour, texture, freshness, odour and cleanness. Each sample was then

fed to sheep and graded according to the amt. voluntarily consumed. In general, correlation between voluntary consumption and gradings by sensory tests were significant ($P < 0.01$) except for freshness, odour or cleanness. The variability in intake 'accounted for' by the sensory grading ranged from 59 to 90% for the different hays and 65% if the hays were pooled.
A. G. Pollard.

Yield, chemical composition and nutritive value of residual maize fodders (*Zea mays*)—when taken as dual purpose crop. S. K. Ranjan and R. C. Kariyar (*Indian J. Dairy Sci.*, 1969, **22** (3), 136–139. 4 ref.).—Detn. of fodder and nutrient yields from Ganga 101, Bixcuba, Doeta Comb and Ranjit maize varieties showed the latter to be superior to the others, both qual. and quant. Nutritive value of residual fodder, after removal of the cobs of mature Ganga 101, was detd. The digestibility coeff. of dry matter, crude protein, ether extract, crude fibre and N-free extract were 55·5, 39·8, 59·6, 58·3 and 65·1, resp. Digestible crude protein, total digestible nutrients and starch equiv. were 2·82, 58·57 and 45·43 kg, resp., per 400 kg fodder (dry matter basis). M. O'Leary.

The nutritive value for ruminants of a complete processed diet based on barley straw. F. W. Wainman, K. L. Blaxter and J. D. Pullar (*J. agric. Sci., Camb.*, 1970, **74** (2), 311–314. 5 ref.).—Calorimetric trials were carried out on a complete extruded diet for ruminants containing 13·5% *CP* and 19% *CF*, with a starch equivalent (dry basis) of 38·3%. The diet had a *ME* value of 2·32 kcal/g org. matter with net availability of *ME* of 42·7% for fattening and 68% for maintenance. These values agreed well with values predicted from equations published by the Agricultural Research Council. M. Long.

Yield, chemical composition and out-turn of nutrients of various varieties of cow-pea fodder. M. L. Maheshwari and S. K. Ranjhan (*Indian J. Dairy Sci.*, 1969, **22** (3), 200–201. 3 ref.).—Five varieties of cow-pea, namely: Russian Giant (U.S.S.R.), Cheena Gram (I.A.R.I.), F.A.O. 12024, E.C. 4216 (I.A.R.I.) and Black Eye (U.S.A.), were tested. Complete randomized block design with twenty replications was followed. There was a significant difference between the dry matter (*DM*) yield per hectare amongst the different varieties ($P < 0.05$). Black Eye variety gave the highest *DM* yield (38·4 quintales/ha) as well as out-turn of nutrients.
M. O'Leary.

Effect of 50 and 100 per cent replacement of oats by tapioca in the concentrate mixture of dairy cows. M. L. Mathur, S. R. Sampath and S. N. Ghosh (*Indian J. Dairy Sci.*, 1969, **22** (3), 193–199. 5 ref.).—Results of trials with 18 crossbred lactating cows indicate that the cereal portion of the concentrate ration may be replaced by tapioca chips without any adverse effect on animal performance. M. O'Leary.

Growth and digestibility studies to evaluate rapeseed meal as a protein supplement for swine. H. S. Bayley, C. Y. Cho and J. D. Summers (*Can. J. Anim. Sci.*, 1969, **49** (3), 367–373. Engl., 8 ref.).—Pre-pressed, solvent-extracted rapeseed meal can be used at the 11% level in maize–soya–rapeseed diets with lysine and methionine supplements, for finishing swine (> 45 kg).
M. T. Rawnsley.

Evidence that taurine may be one of the elusive unidentified factors. W. J. Monson (*Poult. Sci.*, 1969, **48** (6), 2069–2074. 7 ref.).—Taurine appeared to be an essential amino acid for the chick, but did not express itself unless a fermentation factor was also added to the diet. Taurine % in various diets and fish and animal meals are presented. A. H. Cornfield.

Methods for determining amino acid availability of feeds. D. B. Bragg, C. A. Ivy and E. L. Stephenson (*Poult. Sci.*, 1969, **48** (6), 2135–2137. 5 ref.).—Availability values of amino acids from grain sorghum obtained with normal chicks were slightly lower than those obtained with surgically modified (artificial anus) chicks. Since individual chicks of the surgically modified groups sometimes gave values > 100%, the method is considered inferior to the use of normal chicks. A. H. Cornfield.

Effects of water treatment of components of hard red winter wheat on growth and energy utilisation by the chick. E. C. Naber and S. P. Touchburn (*Poult. Sci.*, 1969, **48** (6), 2052–2058. 7 ref.).—Water treatment of ground grain, its long patent flour milling component or the starch fraction of the latter component increased chick growth, % dry matter retention and/or *ME* value of diets. Water treatment probably increases the susceptibility of starch to enzymic degradation, thus increasing energy utilisation by the chick. A. H. Cornfield.

Comparison of sprouted *versus* normal wheat when fed to White Leghorn chicks. L. F. Falen and C. F. Petersen (*Poult. Sci.*, 1969, **48** (5), 1772–1773. 3 ref.).—Wt. gains and feed efficiency of chicks to 4 weeks of age when fed a diet containing 61% wheat were no different between using normal wheat and replacing 25–100% of this with sprouted wheat. The metabolisable energy values of mixtures of the two types of wheat were significantly higher than those of each wheat fed alone. A. H. Cornfield.

Computer programmed least-cost diets limited to arginine, glycine, methionine, methionine + cystine, lysine and tryptophan for turkey poults from 3–8 weeks. J. H. Trammell, J. R. Couch and C. R. Creger (*Poult. Sci.*, 1969, **48** (5), 1563–1566. 4 ref.).—When soyabean meal was used as the protein source in a 2145 kcal per kg diet, the estimated requirement of amino acids during the 3–8 week growth period were 1·46% arginine, 0·45% methionine, 1·30% lysine, 1·29% glycine, 0·81% methionine + cystine, and 0·27% tryptophan. A. H. Cornfield.

Effects of Diet and Environment on Livestock

Performance of steers fed rations containing urea and chlortetracycline alone and in combination. L. J. Sutherland and T. D. Burgess (*Can. J. Anim. Sci.*, 1968, **48** (3), 443–447. 9 ref.).—In trials with steers, whether individually- or group-fed, growth rates and feed efficiency showed no interaction between urea and chlortetracycline in the diet. Replacement of soyabean meal by urea as a supplement reduced wt. gains. Inclusion of chlortetracycline had no effect on wt. gain. A. G. Pollard.

Utilisation by beef cattle of concentrate diets containing different levels of milled barley straw and of protein. A. M. Raven, T. J. Forbes and J. H. D. Irwin (*J. agric. Sci., Camb.*, 1969, **73** (3), 355–363. 6 ref.).—Groups of Friesian steers were allocated to 8 dietary treatments, divided into two groups in each of which concentrate (*C*) was fed alone or with 10, 20 or 30% milled barley straw. In one group the same *C* was used, whilst in the other the % protein of the *C* was increased with increasing straw so as to keep dietary protein at the same relative level. With 20 and 30% straw diets, live wt. gains were less than with the *C* only and C+10% straw diets. Total dry matter intakes were highest on the 10 and 20% straw diets. Food conversion ratio increased with increasing straw content of the diet. Org. matter digestibility fell sharply with inclusion of straw. The molar proportion of HOAc in volatile fatty acids of the rumen liquor increased and that of propionic acid decreased with inclusion of straw.
M. Long.

Effect of composition and processing of diets on performance of Holstein steers. D. A. Christensen, B. A. Duck and H. H. Nicholson (*Can. J. Anim. Sci.*, 1968, **48** (3), 263–267. 9 ref.).—The expt. show that satisfactory performance is achieved by feeding mainly barley rations contg. ± 13·2% crude protein to Holstein steers (wt. 90–450 kg). Addn. of urea, vitamin E, Mn, Zn, Cu and S did not improve rate of gain or feed efficiency. Implanting 24 mg diethylstilboestrol increased rate of gain at 240 kg. A. G. Pollard.

Comparison of alfalfa [lucerne]–orchardgrass and sorghum–Sudangrass hybrid low-moisture silages with corn silage for lactating dairy cattle. M. J. Montgomery, B. J. Bearden, J. T. Miles and J. W. High (*J. Dairy Sci.*, 1970, **53** (4), 446–448. 5 ref.).—The results of a 2-yr trial with 24 cows showed that cows fed lucerne–orchardgrass low-moisture silage produced more milk than animals fed either low-moisture sorghum–Sudangrass or maize silage. Dry matter intake was significantly ($P < 0.05$) higher for cows fed lucerne–orchardgrass. M. O'Leary.

Milk production in range beef cows and its relationship to calf gains. V. M. Gleddie and R. T. Berg (*Can. J. Anim. Sci.*, 1968, **48** (3), 323–333. 22 ref.).—Milk samples were taken at monthly intervals following oxytocin injections. The 24-h milk yields, classified by breed and age, ranged from 3·7 to 8·4 kg (average 6·4 kg). Of the variance in yields, 82·5% was attributed to breed. The decline in daily milk yield averaged 0·02 kg per day of lactation. The correlation between milk yield and calf consumption was 0·58, av. milk compn. being: butterfat 3·9%, protein 3·5%, solids-not-fat 9·1% and total solids 13·0%. The compn. was not greatly affected by breed or age of cows. Single samples afforded a good estimate of yield but not of compn. of milk. Close relationship is shown between estimated yield for any given month and av. daily gain in wt. of calves from birth to weaning. Milk solids (%) and calf gains were not closely related. A. G. Pollard.

Effect of rapeseed meal and urea on *ad libitum* consumption of grain rations by dairy cows. J. R. INGALLS, M. E. SEALE and J. A. MCKIRDY (*Can. J. Anim. Sci.*, 1968, **48** (3), 437–442. 9 ref.).—Grain rations for lactating cows (maize, maize + barley or barley + oats) were modified by replacement of soyabean meal by rapeseed meal (*RM*). *RM* (12–13%) lowered the grain intake as also did urea (equiv. to 22 or 19% of the total intake of crude protein). *RM* (6%) + 0·8% urea in the ration had an effect comparable with that of 12% *RM*, but less than that of 1·6% urea, on consumption. *RM* did not significantly effect milk compn. or production.
A. G. Pollard.

Chromic oxide in digestibility studies with lactating dairy cows. A. L. HOOGENDORN and C. M. GRIEVE (*Can. J. Anim. Sci.*, 1969, **49** (3), 383–388. Engl., 13 ref.).—Two cows had 3·46 g and two had 10·38 g of Cr_2O_3 fed daily for 8 days. These variations affected recovery from faeces, the latter being more consistent. It was shown that digestibility of normal diets could be assessed by this method. No definite correlation was found between protein, P and Ca detn. and Cr_2O_3 excretion. M. T. Rawnsley.

Energy requirements for wintering mature pregnant beef cows. R. HIRONAKA and H. F. PETERS (*Can. J. Anim. Sci.*, 1969, **49** (3), 323–330. Engl., 9 ref.).—Expt. over three winters using high, medium and low energy rations showed that weather variations are an important factor. It is recommended that these cows should be fed the level recommended by the U.S. National Research Council (18,000 kcal digestible energy/day), but that in mild weather cows could be given less, esp. if they have good body reserves.
M. T. Rawnsley.

Growth, physiological responses, and evidence of toxicity in yearling dairy cattle grazing different grasses. D. R. JACOBSON S. B. CARR, R. H. HATTON *et al.* (*J. Dairy Sci.*, 1970, **53** (5), 575–587. 15 ref.).—Results are presented of a study of the nutritive worth of new tall fescue strains and varieties in comparison to economically important grass sp. for grazing. Observations on the incidence, clinical symptoms and physiol. effects of fescue toxicity are made. M. O'Leary.

Coarsely milled barley straw in finishing diets for young beef cattle. T. J. FORBES, A. M. RAVEN and J. H. D. IRWIN (*J. agric. Sci., Camb.*, 1969, **73** (3), 365–372. 13 ref.).—Young beef cattle were fed diets contg. 0, 10, 20, 30, 40 and 50% of barley straw. For each 10% increase in straw content, live-wt. gain fell 0·62 ± 0·133 kg per week, whilst dry matter (*DM*) conversion ratio increased at the rate of 0·65 ± 0·186 units. *DM* intake reached a max. with 18·8% straw in the diet, although there was no increase in digestible org. matter. Chilled carcass wt. fell at the rate of 5·7 ± 0·096 kg for each 10% increase in straw. Killing-out % fell significantly as the straw content of the diet increased from 20 to 30%. M. Long.

Growth of Holstein calves fed alfalfa [lucerne] pasture, alfalfa greenchop, or alfalfa hay. D. A. STILES, E. E. BARTLEY, A. D. DAYTON *et al.* (*J. Dairy Sci.*, 1970, **53** (4), 489–492. 5 ref.).—Results of feeding trials with Holstein calves showed that, after 12 weeks, animals fed lucerne pasture or greenchop gained significantly more wt. than those fed lucerne hay. Calves on lucerne pasture gained significantly more in height than did those fed greenchop or hay. M. O'Leary.

Effects of fibre and ratio of starch to sugar on performance of ruminating calves. E. JAHN, P. T. CHANDLER and C. E. POLAN (*J. Dairy Sci.*, 1970, **53** (4), 466–474. 19 ref.).—Results of trials with 40 Holstein male calves showed that variation of the starch : sugar ratio from 1 : 1 to 3 : 1 had no effect on performance. A study of rations contg. from 5 to 60% straw showed that live wt. gains from 8 to 20 weeks of age were satisfactory with rations contg. up to 23% acid detergent fibre. Feed intake was max. at this level of fibre, with dry matter digestibility being 30%. Fill and fibre digestibility increased and dry matter and crude protein digestibility decreased with increase in fibre content of the ration.
M. O'Leary.

Performance of beef steer calves during winter and on pasture as affected by feeding a wintering ration at different levels with and without added fat. C. D. T. CAMERON (*Can. J. Anim. Sci.*, 1968, **48** (3), 397–402. 13 ref.).—Hereford and Angus steer calves were overwintered on rations having a total digestible nutrient (*TDN*) level exceeding the U.S. Nat. Res. Council standard by 14%. After 100 days grazing, their gains exceeded those of similar calves wintered on 2–22% lower levels of intake. The ratio, *TDN* : wt. gain for cattle at the lowest level of intake (1·13 kg *TDN* daily/100 kg) was significantly greater than that for calves fed at higher levels. Mean gains by calves given added fat in winter and at pasture were greater, during both periods, than when similar rations without added fat were supplied, as feed utilisation was more efficient. Apparent digestibilities of feed components other than crude fibre were not affected by the level of intake. Fibre digestion coeff. for calves fed at the higher levels of intake exceeded those for calves fed at lower levels. Both winter and summer gains were greater for Hereford than for Angus calves. A. G. Pollard.

Growth, digestibility and nitrogen retention by calves fed milk replacers containing milk and soyabean proteins, supplemented with methionine. A. D. L. GORRILL and J. W. G. NICHOLSON (*Can. J. Anim. Sci.*, 1969, **49** (3), 315–321. Engl., 13 ref.).—Protein from soyabean protein concentrates, or fully cooked flour predigested with acid or alkali, can replace all or most of the milk protein for both bull and heifer calves. The methionine factor may become limiting if high energy rations are required.
M. T. Rawnsley.

Nutrient digestibility by new-born calves fed milk replacer. O. M. RADOSTITS and J. M. BELL (*Can. J. Anim. Sci.*, 1968, **48** (3), 293–302. 20 ref.).—Using Holstein-Friesian calves (6–24 days old), the digestibility of nutrients from a milk replacer contg. dried skim milk with various animal and vegetable fats, fine oatmeal, vitamins and minerals was recorded. All nutrients showed low fat-digestibility levels at six days (~ 29%) but this increased to 82% with calves 24-days old. Over this period, the digestibility of energy rose from 72 to 88% and that of calc. non-fat, non-protein energy from 54 to 77%. Oatmeal energy rose from 0 at 6 days to 26% at 24 days. The digestibilities of the fatty acids caprylic, capric and lauric all reached 90–100% while those of palmitoleic, oleic and linoleic were 89, 75 and 79% resp., palmitic acid, 42%, and stearic acid, 26%. Amino acids showed some low digestibility coeff. with calves up to 1 week old (e.g., methionine 11%) but later averaged 70–86%, except for cystine which reached only moderate values. A. G. Pollard.

Effect of added bulk on growth, nutrient utilisation, digestive system, and diarrhoea in calves fed milk replacer. A. D. L. GORRILL and J. W. G. NICHOLSON (*Can. J. Anim. Sci.*, 1969, **49** (3), 305–313. Engl., 11 ref.).—Bull calves were fed whole milk, or liquid milk replacer, with oat hulls, sawdust or purified wood cellulose. Some calves ate bedding to control diarrhoea, and it is considered that calves without bedding could be fed sawdust, etc., preferably so that it will enter the rumen as directly as possible.
M. T. Rawnsley.

Evaluation of corn [maize] and sorghum–Sudan [grass] silages on the bases of dry matter intake, digestibility and milk production. L. J. FISHER (*Can. J. Anim. Sci.*, 1968, **48** (3), 431–435. 11 ref.).—The silages were fed to lactating cows to determine milk yields and to wether lambs for digestibility trials. The sorghum–Sudan grass (*SS*) silage contained more dry matter than did the maize silage (*M*) but the av. daily dry-matter intakes per cow were similar. Milk production and body-wt. changes were smaller with *SS* feeding. In samples of rumen fluid from cows fed *M*, the molar % AcOH was lower and that of butyric acid was higher than in samples from cows fed *SS*. Digestibility coeff. were greater for *M* dry matter, N-free extract, fibre and energy but less for protein, than for *SS*. A. G. Pollard.

Comparison between rations of different protein : energy ratio for lambs weaned at 3 or at 15 days of age. G. J. BRISSON J. P. LEMAY (*Can. J. Anim. Sci.*, 1968, **48** (3), 307–313. 12 ref.).—The 2 groups of lambs were fed milk-substitutes up to live-wt. of 25 kg, the rations having protein : calorie ratios of 38·9 (*A*) and 34·5 (*B*) and grass energies of 4·7 (*A*) and 5·3 kcal/g (*B*) resp. Age at weaning did not affect growth-rate up to 8 weeks, or the efficiency of feed conversion. The protein : energy ratio appeared to have a considerable effect on the quality of lamb carcasses. A. G. Pollard.

Effect of fermentation time in the artificial rumen on the relationship of *in vitro* digestibility to digestibility and intake of hay by sheep. J. E. TROELSEN and J. M. BELL (*Can. J. Anim. Sci.*, 1968, **48** (3), 361–372. 12 ref.).—Comparison is made of the digestibility and intake of hays at various stages of growth in sheep. The *in vitro* digestibility, (over 6 to 96 h), correlated significantly with the *in vivo* digestibility for each period. Optimal digestibility and intake were found after the same fermentation periods. Among different kinds and ages of hay, a change of 1 unit of *in vitro* digestibility was associated with an 18% greater change in intake of grass than of lucerne hay but at 70% digestibility,

consumption of both hays was similar. The preferred optimal period for assays of digestibility and intake was 48 h.
A. G. Pollard.

Water economy and food intake of sheep when watered intermittently. A. D. WILSON (*Aust. J. agric. Res.*, 1970, **21** (2), 273–281. 14 ref.).—Penned medium-wool and fine-wool Merino and Border Leicester wethers were fed 2 diets at low and high temp (max. 12 and 36°C resp.). H_2O was given every 3–4 days. Urine and faeces were collected, and samples of food and faeces were dried to determine H_2O contnt. Results suggested that although H_2O economy assisted survival when H_2O was unavailable, it was inversely related to food intake, and therefore of limited value in selecting sheep for arid climates. M. J. Rawlins.

Observations on endogenous loss of calcium in the sheep. A. C. FIELD and N. F. SUTTLE (*J. agric. Sci., Camb.*, 1969, **73** (3), 507–509. 6 ref.).—Balance studies were conducted on lambs and adult sheep with diets deficient and adequate with respect to Ca and P. Estimates of endogenous losses (*EL*) of Ca were lower than those obtained from methods using radioactive Ca. *EL* were less than those quoted by the Agric. Res. Council's recommendations of 1965 and were close to those of cattle (16 mg/day). M. Long.

Lysine and protein requirements of 23- to 89-kg pigs. J. M. BELL and L. O. VOLDENG (*Can. J. Anim. Sci.*, 1968, **48** (3), 251–261. 15 ref.).—The lysine requirement of pigs of live-wt. 23–57 kg, given rations providing 3,330 kcal digestible energy per kg, appeared to be ~0·7%. The pigs after reaching 57 kg wt. were fed 14 or 16% finisher rations. A. G. Pollard.

Utilisation of fat by young pigs: fatty acid composition of ingesta in different regions of the digestive tract. W. E. CARLSON and H. S. BAYLEY (*Can. J. Anim. Sci.*, 1968, **48** (3), 315–322. 11 ref.).—Using pigs 13–22 days of age, weaned at 7 days, the mean corrected digestibilities were 86, 81 and 56%, resp., for miaze oil, lard and tallow. All animals were slaughtered at 22 days of age and the compn. of the fatty acids in samples from different regions of the digestive tract were examined by g.l.c., comparison being made with corresponding samples from a germ-free pig on the same diet. The low digestibility of stearic acid and the high digestibility of oleic and linoleic acids appeared to be due to microfloral activity rather than to differential ability of the animals to absorb the fatty acids. A. G. Pollard.

Digestibility of rye and its value in pelleted rations for pigs. D. W. FRIEND and J. M. MACINTYRE (*Can. J. Anim. Sci.*, 1969, **49** (3), 375–381. Engl., 18 ref.).—Gilts and barrows were fed pelleted and non-pelleted rye. Non-pelleted rye had a retarding effect on growth. Comparison of rye and barley showed that the former had higher dry matter and crude protein digestibility.
M. T. Rawnsley.

Effect of nitrogen utilisation in the sow of variation in dietary protein concentration and pattern of feeding in pregnancy. I. H. PIKE (*J. agric. Sci., Camb.*, 1970, **74** (2), 209–215. 26 ref.).—A factorial feeding trial at two protein levels with three patterns of feeding was carried out with Large White × Wessex sows in their second and third pregnancies. The high protein (*HP*) diet contained 19·5% *CP* and the low 10·5%; levels of feeding were 1·8 kg, 2·7 kg and 3·6 kg/day. Extra-uterine N deposition was significantly greater ($P < 0·001$) with the *HP* diet and subsequent lactation was enhanced while intra-uterine N deposition was unaffected by any treatment. Protein utilisation was more efficient in late than in early pregnancy so feeding of higher protein levels during late pregnancy should increase efficiency throughout pregnancy providing energy intake is adequate. M. Long.

Supplemental copper for swine: growth, digestibility and carcass measurements. A. G. CASTELL and J. P. BOWLAND (*Can. J. Anim. Sci.*, 1968, **48** (3), 403–411. 15 ref.).—Pigs were fed 14 or 17% protein rations *ad lib.* supplemented with 0·1% $CuSO_4 . 5H_2O$, from weaning to market wt. Cu increased with rate of gain in wt. by 6·2% and the feed efficiency by 3·8% up to 50 lb live-wt., particularly when fishmeal was used as protein source. In digestibility trials, use of Cr_2O_3 as indicator gave results comparable with those by the total collection method but the calc. digestion coeff. were higher with the latter method. Digestibility and retention of energy and N were not influenced consistently by Cu and metabolisable energy was unaltered. Carcasses of pigs fed *ad lib* on rations supplemented with Cu were unaffected by Cu, except in having less back-fat, larger cross-sectional areas of loin and higher Canadian Record of Performance scores than when no Cu was given. Comparable animals on a fishmeal ration given Cu yielded a larger proportion of soft-fat carcasses than when fishmeal was replaced by soyabean meal. The latter effect was not apparent in animals fed standard (restricted) rations until slaughter. A. G. Pollard.

Effect of Peruvian anchovy (*Engraulis ringens*) meal supplemented with Santoquin (ethoxyquin) on growth and fish flavour of broilers. S. W. ROJAS, A. B. LUNG and R. V. NIÑO DE GUZMÁN (*Poult. Sci.*, 1969, **48** (6), 2045–2052. 23 ref.).—Addn. to the diet of up to 20% Peruvian anchovy meal, replacing soyabean meal on protein basis, had no effect on wt. gains or feed efficiency of broilers to 8 weeks of age. Addn. of 800–1000 ppm Santoquin to the diets contg. anchovy meal increased shank pigmentation, particularly of males. Fishy or off-flavours of the broiler meat occurred only where > 8% anchovy meal was added to the diet. Santoquin had only a small effect in decreasing these flavours.
A. H. Cornfield.

Comparison of the feeding value for broilers of maize, grain sorghum, barley, wheat, and oats, and influence of the various grains on the composition and taste of broiler meat. V. E. PETERSEN (*Poult. Sci.*, 1969, **48** (6), 2006–2013. 7 ref.).—Use of diets contg. 50% of various grains showed that wt. gains of broilers to 53 days of age decreased in the order: maize, oats, sorghum low in tannin, wheat, sorghum high in tannin and barley. Wt. gains were highly correlated with energy intake. Grain type affected chem. compn. of the bird and edible quality of the meat; high-tannin sorghum imparted a significant off-flavour. A. H. Cornfield.

Comparative effect of herring, menhaden, and safflower oils on broiler tissue fatty acid composition and flavour. D. MILLER and P. ROBISCH (*Poult. Sci.*, 1969, **48** (6), 2146–2157. 20 ref.).—When fed at 1·5–2·5% levels, menhaden oil was slightly more effective than herring oil in increasing the deposition of the $\omega 3$ fatty acids in broiler tissues (liver, adipose, thigh, breast and heart), whilst the reverse was true for the $\omega 9$ fatty acids. Safflower oil substantially increased $\omega 6$ acids in all tissues. Off-flavour in broiler flesh was correlated with increased deposition of 20 : 5$\omega 3$, 22 : 5$\omega 3$, and 22 : 6$\omega 3$ acids in the tissues. A. H. Cornfield.

Effect of choline, methionine, and vitamin B_{12} on liver fat, egg production and egg weight in hens. M. GRIFFITH, A. J. OLINDE, R. SCHEXNAILDER *et al.* (*Poult. Sci.*, 1969, **48** (6), 2160–2172. 20 ref.).—Addn. of choline (0·85 g), methionine (1 g), or vitamin B_{12} (0·005 mg per kg of feed) to a low-methionine diet increased egg production and size. Choline decreased, whilst the two other additives had little effect on, liver fat levels. The combined use of all three additives increased egg production and size and decreased liver fat more than did the use of any one alone. Hens housed in cages had higher liver fat levels than those in floor pens.
A. H. Cornfield.

Nucleotide and amino acid rhythms in the chick under temperature stress. R. L. SQUIBB and C. H. REED (*Poult. Sci.*, 1969, **48** (6), 1996–2005. 19 ref.).—The effect of heat and cold stress on protein metabolism in chicks was studied by measuring nucleic and free amino acids in the liver and free amino acids in plasma over 24-h periods. A. H. Cornfield.

Dietary selenium and arsenic additions to diets for chickens over a life cycle. N. T. THAPAR, E. GUENTHNER, C. W. CARLSON and O. E. OLSON (*Poult. Sci.*, 1969, **48** (6), 1988–1993. 12 ref.).—Addn. of 2 ppm Se (H_2SeO_3) to the diet, commencing at hatch and continuing for a life cycle, had no effect on growth or reproductive performance; 8 ppm Se markedly reduced body wt., egg production and wt., hatchability of fertile eggs and progeny growth. Addn. of 15 ppm As ($NaAsO_2$) to the diet largely overcame these effects, except that egg production and size were not quite restored to normal. The As treatment increased Se deposition in livers to 4 weeks of age, but decreased it in liver and eggs by maturity. A. H. Cornfield.

Effect of supplementary biotin on performance of turkeys fed maize–soyabean meal type diets. P. E. WAIBEL, L. M. KRISTA, R. L. ARNOLD *et al.* (*Poult. Sci.*, 1969, **48** (6), 1979–1985. 16 ref.).—Addn. of 0·3 ppm biotin (*B*) to a turkey hen diet did not improve hen or progeny performance (the basal diet contained 0·105 ppm *B*). Addn. of *B* to a poult diet did not affect poult performance. High levels of antibiotics added to a poult diet did not produce any symptoms of *B* deficiency. A. H. Cornfield.

Effect of dietary energy source on racing performance in the pigeon. H. M. GOODMAN and P. GRIMINGER (*Poult. Sci.*, 1969, **48** (6), 2058–2063. 7 ref.).—Diets contg. 5% maize oil were superior to those contg. 5% glucose. A. H. Cornfield.

Analysis and Other Aspects

Effect of soil content of faeces on faecal organic matter values as determined by the loss on ignition technique. W. B. HEALY (*N.Z. Jl agric. Res.*, 1969, **12** (4), 639–641. 3 ref.).—This factor must be taken into consideration when determining feed intake by digestible organic matter detn. It is suggested that the loss on ignition value should be included in the calculation.
M. T. Rawnsley.

Simple method for measuring the foaming of plant proteins involved in bloat. D. E. WRIGHT (*N.Z. Jl agric. Res.*, 1969, **12** (4), 669–675. 17 ref.).—Samples of 10 ml in 24-ml bottles were foamed at room temp. in a homogeniser at 10,000 rev/min for 1 min. After standing for 5 min, they were centrifuged at 375 × *g* for 1 min and the vol. of foam was recorded. pH was maintained at 5·5. The prepn. and storage of the plant protein is described. Protein was detd. by use of the Folin–Ciocalteu reagent. The times and quantities are critical, and the results are compared with those of Mangan (*ibid.*, 1959, **2**, 47). Antifoaming compd. can be tested by this method. M. T. Rawnsley.

Effect of exogenous hormones on beef production from Holstein–Friesian steers and bulls reared from birth to 475 kg on all-concentrate rations. R. J. FORREST (*Can. J. Anim. Sci.*, 1968, **48** (3), 269–274. 8 ref.).—The bull calves grew, with greater feed efficiency, more rapidly but with less response to hormone implantation than did comparable steers. Bulls also had heavier heads and hides, lighter hindquarters, more 'cooler-shrink', and less abdominal and rib fat than the steers. Hormone implantations increased hide-wt. and rib-moisture in steers and decreased those in bulls. Liver-wt. were greater in bulls than in steers; hormone treatment increased liver-wt. in the latter. A. G. Pollard.

Influence of repeated oxytocin injections on composition of dairy cows' milk. G. T. LANE, C. W. DILL, B. C. ARMSTRONG and L. A. SWITZER (*J. Dairy Sci.*, 1970, **53** (4), 427–429. 13 ref.)—The solids-not-fat, protein and lactose of milk, obtained after each of four successive i.v. oxytocin injections at 20 min intervals, were shown to be significantly lower than those of milk obtained normally. Milk fat increased through the first oxytocin sample and then decreased to that of normally obtained milk. Milk yield approached a const. level with repeated injections of oxytocin, indicating a const. rate of secretion. Rate of secretion approached the rate needed to synthesise the milk produced daily by these cows. M. O'Leary.

Distribution in the sheep of selenium derived from ^{75}Se-labelled ruminal pellets. K. A. HANDRECK and K. O. GODWIN (*Aust. J. agric. Res.*, 1970, **21** (1), 71–84. 36 ref.).—Two groups of sheep were used, one of normal Se status and the other low in Se (proved by analysis of blood Se levels). Pellets of elemental Fe and Se, labelled with ^{75}Se, were administered to both groups one month before slaughter. The pellets released 0·5–1·3 mg Se/day, 30% of which was excreted in the urine and 1% in expired air. The analysis of body tissue and organs, after slaughter, showed no excessive accumulation. No toxic effects were observed. M. J. Rawlins.

Some aspects of selenium metabolism in normal and dystrophic sheep. M. HIDIROGLOU, K. J. JENKINS, R. B. CARSON and R. R. MACKAY (*Can. J. Anim. Sci.*, 1968, **48** (3), 335–346. 19 ref.).—Over a 2-yr period, ewes were fed hay, deficient in Se, from another area in which losses of calves and lambs from nutritional muscular dystrophy were high. Cases of dystrophic lambs were few in the 1st yr, but in the 2nd yr the incidence of muscle degeneration was high in 4-month-old lambs; tissue Se levels were low in both lambs and ewes. Supplementing the ewes' diet with Se and vitamin E during gestation only partially corrected the defect in the lambs. In Se-depleted sheep a single oral dose of $H_2{}^{75}SeO_3$ was followed by rapid incorporation of Se into the tissues, notably the kidneys, liver and adrenals, 80% of the dose being excreted in faeces within 3 days and the remainder in urine during a subsequent 10-day period. Lambs have a low Se-requirement which may explain the slow development of dystrophy. A. G. Pollard.

Ammonia and urea toxicoses in sheep and their relation to dietary nitrogen intake. J. G. MORRIS and E. PAYNE (*J. agric. Sci., Camb.*, 1970, **74** (2), 259–271. 22 ref.).—NH_4 acetate soln. infused i.v. into sheep produced a toxic condition showing the clinical signs, pathological findings and [NH_3] similar to those found in urea toxicosis, providing the induction period was similar. Comparison of NH_4Cl, NH_4 acetate and NH_4OH infusion effects showed some relationship to basicity of the compd. but alkalosis did not appear to be a necessary prerequisite for NH_3 toxicosis. The tolerance of sheep towards both orally administered urea and i.v. infusion of NH_4 salts was related to dietary N intake which influences concn. of urea-metabolising enzymes in the liver. There is a much higher rise in blood [NH_3] on a low rather than a high protein diet. I.v. admin. of arginine or of γ-aminobutyric acid plus glucose was not of practical value in the prevention of urea toxicosis. M. Long.

Supplemental copper for swine: effects on haemoglobin serum proteins and tissue copper levels. A. G. CASTELL and J. P. BOWLAND (*Can. J. Anim. Sci.*, 1968, **48** (3), 415–424. 27 ref.).—
A. G. Pollard.

Aortic rupture, body weight, and blood pressure in the turkey as influenced by strain, dietary fat, β-aminopropionitrile fumarate, and diethylstilboestrol. L. M. KRISTA, P. E. WAIBEL, J. H. SAUTTER and R. N. SHOFFNER (*Poult. Sci.*, 1969, **48** (6), 1954–1960. 14 ref.).—Addn. of 10% animal fat to the diet of male turkeys increased body wt., but did not increase blood pressure or provoke aortic rupture. 0·01% β-aminopropionitrile did not influence these factors, whilst 0·015% decreased body wt., increased incidence of aortic rupture but had no effect on blood pressure. Implanted diethylstilboestrol pellets had no effect on body wt., decreased blood pressure, and increased incidence of aortic rupture. Three strains of turkey differed somewhat in their response.
A. H. Cornfield.

Estimates of changes in plasma cholesterol and protein in relation to certain reproductive traits in female breeder turkeys. T. K. MUKHERJEE, G. W. FRIARS and J. D. SUMMERS (*Poult. Sci.*, 1969, **48** (6), 2081–2086. 10 ref.).—A study was made of the effect of an increased photoperiod, prior to sexual maturity, on plasma cholesterol and protein levels in female breeder turkeys, estimates of the heritabilities of these plasma components and the relationship of the changes to measures of reproductive performance.
A. H. Cornfield.

Protein supplements for ruminant feeding stuffs. IMPERIAL CHEMICAL INDUSTRIES LTD. (Inventors: N. WATCHORN and A. W. J. BROOME) (Br. Pat. 1,191,470, 29.12.67).—A ruminant feed compn. is described having one or more feeding stuffs and a non-protein N supplement comprising a mixture of urea auto-condensation products contg. a major proportion of cyanuric acid, untreated urea > 10% and < 5% of ammeline. The supplement is obtained by heating urea at 220–300°C until NH_3 evolution ceases. In an example, the gain in wt. with lambs on a 'cyanuric acid' diet (prep. by heating urea to 220°C) was greater than with other diets. Toxicity studies are discussed. A. Gordon.

Tannin treatment of oleaginous and proteineous material of vegetable origin, and products obtained. INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE and PRODUITS CHIMIQUES ET CELLULOSES REY (Br. Pat. 1,189,754, 8.3.68. Fr., 10.3.67).—The raw materials and products of the industrial treatment of oleaginous and proteineous vegetable substances may be improved by treatment with < 1 tannin-contg. substance followed by separation of the oily and greasy substances. E.g. decorticated groundnut grains and proteineous oil cake are crushed and tanning extract of chestnut wood is added. The mixture is heated by steam to 80°C and after 12 h is extracted with hexane. Improved oils and fats and oil-free vegetable cakes for animal feeds are obtained by the process. S. S. Chissick.

2.—FOODS AND CROP CONVERSION

Cereals, Flours, Starches, Baking

Effect of parboiling on some physicochemical properties of rice. S. N. RAGHAVENDRA RAO and B. O. JULIANO (*J. agric. Fd Chem.*, 1970, **18** (2), 289–294. 43 ref.).—Seven varieties of rough rice, ranging from 2–27% in brown rice amylose content were studied. The rice was soaked, drained, steamed and dried. The range and mean of some properties of the raw and parboiled rice are tabulated. With parboiling, grain translucency and hardness were improved, and there was a disruption of protein bodies, and gelatinisation of the starch granules. Changes in amylograph characteristics on parboiling were influenced by amylose content of the samples.
M. J. Rawlins.

Rheological properties of dough and their significance in the breadmaking process. I. HLYNKA (*Baker's Dig.*, 1970, **44** (2), 40–46, 57).—Rheological models are presented that (*a*) represent the behaviour of dough showing yield value, instantaneous elasticity,

retarded elasticity, damping viscosity and flow viscosity, (*b*) viscosity, elasticity and yield value, (*c*) Maxwell model consisting of a viscous and an elastic element linked in series, and (*d*) a Voigt or Kelvin model in which an elastic and a viscous element are connected in parallel. Factors influencing rheological properties such as mixing, extrusion, water content, salt content, flour improvers and leavening are discussed, and also the significance of rheological properties of dough at each particular step of the bread-making process. I. Dickinson.

Nature of the San Francisco sour dough French bread process. I. Mechanics of the process. L. KLINE, T. F. SUGIHARA and L. BELE MCCREADY. **II. Microbiological aspects.** T. F. SUGIHARA, L. KLINE and L. BELE MCCREADY (*Baker's Dig.*, 1970, **44** (2), 48–50, 51–57).—Preparation and handling of the starter sponge and bread dough are described, taking into account the nature of the acidity and the subsequent baking processes. The nature of the micro-organisms involved in the leavening and souring actions of the dough is discussed. I. Dickinson.

Factors affecting the quality of pie dough and pie crust. B. S. MILLER and H. B. TRIMBO (*Baker's Dig.*, 1970, **44** (1), 46–55. 25 ref.).—The effect of water level, type and level of shortening, type and degree of mixing, and protein content of the flour on tensile strength, stickiness and stretchability of the dough and tenderness, shrinkage and flakiness of the baked crust, were investigated by objective measuring techniques. I. Dickinson.

Practical aspects of raisin utilisation. I. J. MATHASON (*Baker's Dig.*, 1969, **43** (6), 40–44. 11 ref.).—Optimum results with yeast-raised products are obtained by min. soaking, full dough development during mixing, use of monoglycerides and dough improvers, increase in the yeast level and accurate control of dough absorption. Chem. leavened products require more soaking and tenderising than yeasted doughs. Uniform raisin suspension in cake batters during the baking stage is promoted by the use of pregelatinised starch and of $CaCl_2$. Raisin pastes are available for automated Danish and coffee cake make-up. I. Dickinson.

Sugars, Syrups, Confectionery

Production of laevoglucosan by pyrolysis of carbohydrates. Pyrolysis in hot inert gas stream. C. M. LAKSHMANAN and H. E. HOELSCHER (*Ind. Engng Chem. Prod. Res. Dev.*, 1970, **9** (1), 57–59. 4 ref.).—Optimum conditions for production of laevoglucosan by starch pyrolysis were detd. Steam was found to be more effective and convenient than He or N_2 as inert gas because of its high heat capacity and ease of condensation; yields were higher than those given by the vac. pyrolysis process, but product vapours must be removed immediately from the high-temp. zone to avoid subsequent decomp. Pre-treatment of the starch with 10%-HOAc, followed by drying before pyrolysis, gave significantly increased yields (up to 44·5%); low concn. of NH_3 in any one of the gases used significantly reduced the yield. L. MacQuisten-Wallace.

Problems of flavouring in continuous candy production. P. CABELLA (*Industrie aliment., Pinerolo*, 1970, **9** (5), 62–65. Ital.).— P. P. R.

Malting, Brewing and Alcoholic Beverages

2-Phenylethanol: the most interesting aromatic alcohol in beer. S. ENGAN (*Brygmesteren*, 1969, **26** (1), 23–31. 33 ref.).—A review. G.c. methods of detn. are described. Temp. of fermentation has a much greater influence on formation of 2-phenylethanol than formation of aliphatic higher alcohols. Concn. in Pilsener beer from 7 Norwegian breweries varied between 5·6 and 27 ppm and that for other beers produced in other countries is also recorded. C. V.

Continuous fractionation of foam from beer. P. GJERTSEN (*Brygmesteren*, 1969, **26** (4), 106–111).—Construction of a 'foam tower' is described and its operation outlined. From 17 kg beer, 663 g foam was isolated in 12 h. The foam fraction and 250 ml of defoamed beer were freeze-dried. 3·36% dry matter was found in the beer and 3·19% in the defoamed fraction. The two fractions contained approx. the same amt. of carbohydrate. Kjeldahl analysis showed the foam to contain 5% more protein than the defoamed beer; the bitter substances are conc. ~ 10-fold in the foam. N compd. were examined by gel filtration on Sephadex; 10% of N compd. in the foam remained undissolved while the fraction in the defoamed beer was fully dissolved. C. V.

Influence of various methods of winemaking, particularly heating the grapes, in the characteristics of wines. C. FLANZY and P. BÉNARD (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (18), 1266–1274. Fr., 7 ref.).—Heating grapes, esp. to 70–80°C, gives better results than conventional pressing. Wines from CO_2 mashing, without SO_2 present, are always better than those from pressed grapes, according to a taste panel. It is suggested that both methods could be used to treat grapes in a bad year. M. T. Rawnsley.

Treatment by steam of grapes with grey rot. P. MARTINIÈRE and P. SUDRAUD (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (17), 1217–1224. Fr., 16 ref.).—Grapes were treated with a steam jet for 3 min, followed by cooling with a strong draught. With sound grapes, a wine of more than normal colour strength was obtained. With partially grey and very grey grapes, a colour much more intense than would normally be expected was obtained. Wines had good taste, etc. This treatment is therefore recommended for red wine production in bad years. The mechanisms of destruction of oxidising diastases, conservation of pectolytic enzymes, movement of colour, etc., are discussed. M. T. Rawnsley.

Preservation of wines under an inert gas. E. PEYNAUD (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (17), 1213–1216. Fr., 3 ref.).—An installation consisting of metallic cylindrical vessels, with only two top openings, is recommended. Pressure of N_2 is maintained at 0·1 or 0·2 atm, and each vessel is hermetically sealed whilst still connected to the others. Problems involved are discussed. M. T. Rawnsley.

Yield of yeast from juices of grapes placed in a carbon dioxide atmosphere. P. BARRE (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (18), 1274–1277. Fr., 4 ref.).—When grapes were placed in CO_2 at 35°C, the nutritional ability of the liquid phase of the musts increased vis-à-vis the various species of yeast. The evolution of N_2, its source, production of other yeasts and musts, etc., are discussed. M. T. Rawnsley.

Production of yeast or yeast foods from waste materials of the fruit industry. L. LEFRANCOIS (*Fruits d'outre mer*, 1970, **25** (2), 112–114. Fr.).—Unsaleable bananas are skinned, crushed, and divided into two parts. The first is fermented and the cream produced mixed with the second part for drying. Av. compn. of the dried product is water 6, glucides 50, yeast 25, misc. proteins 4·5, starch 3·5, lipids 3·5, misc. 7·5%. Variations of this process can produce a yeast suitable for all purposes. Water pollution, cost, possible profit etc. are discussed M. T. Rawnsley.

Improvement of beer flavour. ALFRED JØRGENSEN, GAERINGSFYSIOLOGISK LABORATORIUM A/S (Inventors: E. J. HELM and R. S. W. THORNE) (Br. Pat. 1,188,291, 10.8.66).—The H_2S content is reduced by dissolving Cu in the beer by d.c. electrolysis using Cu electrode(s), prior to final filtration (2 diag.). S. S. Chissick.

Fruits, Vegetables and Their Products

Analytical problems with fruit products. J. F. KEFFORD (*Fd Preserv. Q., C.S.I.R.O. Aust.*, 1969, **29** (4), 65–71. 38 ref.).—Difficulties of detecting adulteration of fruit products are reviewed and methods for the detn. of various fruit constituents which can be used as indicators of the level of fruit content are surveyed, with special reference to citrus fruits. E. C. Apling.

Laboratory studies on the effects of chemicals on the coloration of apples. R. M. SMOCK (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 49–51. 10 ref.).—Apples were dipped in soln. contg. the test compd. and anthocyanins were detd. after 36–72 h. Treatment with K_2CO_3–$KHCO_3$, ethylene carbonate and diethyl pyrocarbonate increased coloration. Diuron at 10 ppm increased coloration but higher concn. decreased it. A. H. Cornfield.

Peculiarities [found] in film packed pears upon long storage. A. A. KOLESNIK and P. F. PONOMAR'YOVA (*Pishch. Tekhnol.*, 1970, [1 (74)], 18. Russ.).— C. V.

Effect of certain chemicals on the colour and polysaccharides of strawberry purée. W. A. SISTRUNK and J. N. CASH (*Fd Technol., Champaign*, 1970, **24** (4), 473–477. 17 ref.).—Purée was adjusted to pH 3·0, 3·4 and 3·8 with 50% citric acid or Na citrate. Ascorbic acid was added to half of each sample at the rate of 40 mg/100 g and soln. of $AlCl_3$, $SnCl_2$ and $SnCl_4$ were added. pH had the greatest effect on colour. Ascorbic acid oxidn. was more rapid at pH 3·0 than 3·8 in the presence of Sn salts whilst colour was more stable at lower pH. Increase in H_2O, sol. pectin, hemicellulose and cellulose occurred during holding of the purée at 50°C. M. J. Rawlins.

Storage of loquats (*Eriobotrya japonica*). S. GUELFAT-REICH (*Fruits d'outre mer*, 1970, **25** (3), 169–173. Fr., 5 ref.).—Detailed tests at 0, 6 and 8°c of 4 varieties of medlar, showed that Acco 13 and Tsrifin are suitable for export, esp. at 0°c in polyethylene. Tanaka can be stored, but the taste deteriorates. Acco 1 cannot be stored. The fresh appearance of the fruit is preserved in polyethylene, but decay and browning set in. The use of perforated sheet may prevent this. Tests on respiration of fruit show that it must be ripened on the tree before eating. These are the first known tests for storage on a fruit normally considered to be for immediate consumption. M. T. Rawnsley.

Provitamin A and vitamin C contents of several varieties of mango (*Manganifera indica*) grown in Puerto Rico. L. M. IGUINA DE GEORGE, A. L. COLLAZO DE RIVERA, J. R. BENERO and W. PENNOCK (*J. Agric. Univ. P. Rico*, 1969, **53** (2), 100–105. 11 ref.).—The β-carotene, vitamin C, total and sol. solids, total and reducing sugars, pH and total titratable acidity of the pulp of 30 varieties of mango are reported. A. H. Cornfield.

Effects of a light treatment on the ripening of detached tomato fruits. A. L. SHEWFELT (*Fd Technol., Champaign*, 1970, **24** (5), 609–613. 11 ref.).—Two sources of tomato fruit were harvested at a mature green stage, and sorted to achieve size uniformity. The selected fruits were placed in identical temp.-controlled cabinets, one providing light and the other darkness. Daily measurements of total acidity, ascorbic acid content, shear press firmness, and colour were made. The rate of colour development was substantially increased by light treatment. Other factors were little affected. M. J. Rawlins.

Improved chlorophyll retention in green beans held on a steam table. J. P. SWEENEY (*Fd Technol., Champaign*, 1970, **24** (4), 490–493. 13 ref.).—1000 g of beans/400 ml of H_2O were boiled and held at 100°c by steam. Measurements were made of colour and chlorophyll retention, shear force, ascorbic acid content and palatability. Chlorophyll retention was improved 20% by this method, when the final pH of the beans after cooking was increased by 0·3 units over that of control samples. M. J. Rawlins.

Comparison of microwave, conventional and combination blanching of Brussels sprouts for frozen storage. W. C. DIETRICH, C. C. HUXSOLL and D. G. GUADAGNI (*Fd Technol., Champaign*, 1970, **24** (5), 613–617. 11 ref.).—Brussels sprouts on a moving belt were heated by microwave energy for 1–6 min. They lost considerable moisture but retained residual peroxidase enzyme in the outer leaves. A combination blanch of microwave energy, after steam or before water blanching, inactivated the peroxidase and the sprouts were as flavour stable at −20, 0 and 20°F and has as good or better chlorophyll and ascorbic acid retention as conventionally processed samples. M. J. Rawlins.

Fruit-flavoured spread. UNILEVER LTD. (Inventors: K. E. ELDRIDGE and L. L. LINTERIS) (Br. Pat. 1,189,854, 31.5.67. U.S., 2.6.66).—A liquid fruit preserve stock (I) and edible fat (e.g., butter, vegetable oil, etc.) are blended and the I is then allowed to gel. E.g., Diluted strawberry jam stock is blended with a margarine emulsion (15 min. at 60°c), then aq. citric acid is added and, after mixing (5 min), the product is transferred to containers and allowed to gel. S. S. Chissick.

Non-alcoholic Beverages

No abstracts

Milk, Butter, Other Dairy Products, Eggs

Rapid neutron activation method for determination of iodine in milk. O. JOHANSEN and E. STEINNES (*J. Dairy Sci.*, 1970, **53** (4), 420–422. 11 ref.).—A simple method for detn. of total I in milk, based on neutron activation analysis, is described. After the irradiation, I is separated from the milk samples by a radiochem. procedure based on solvent extraction and pptn., and ^{128}I activity is subsequently measured with a γ-ray spectrometer. (From summ.) M. O'Leary.

Comparison of rennet curd tension with undenatured whey protein as a measure of heat treatment. U. S. ASHWORTH and J. NEBE (*J. Dairy Sci.*, 1970, **53** (4), 415–419. 6 ref.).—Studies with unheated milks showed that natural variations in rennet curd tension are almost twice as great as variations in whey protein N. Consequently, the latter parameter is more useful as a measure of heat treatment, though both are correlated with the effective heat applied, as calc. from time and temp. measurements. Condensing and drying operations were shown to have little effect on either whey protein N or curd tension. M. O'Leary.

Direct production of lactic acid from whey by ion exchange. A. SURAZYNSKI, S. POZNAŃSKI, J. BUDSLAWSKI and A. POLKOWSKA *Milchwissenschaft*, 1969, **24** (6), 352–354. Engl., 11 ref.).—The 50% lactic acid soln., obtained from whey by use of the Polish resin SD, contained 0·06% ash, < 0·1% volatile acids and was free of HCN, Zn, Cu, Pb, Fe, As, etc. About 80 g of acid were obtained from 1 l of whey. P. P. R.

Continuous determination of water in mechanically manufactured butter (Fritz-butter). K. KOENEN (*Fette Seifen AnstrMittel*, 1970, **72** (4), 296–299. Ger., 8 ref.).—An apparatus for the continuous detn. of moisture in butter determines changes in the dielectric const. of a test material placed between the plates of a condenser operating at a frequency of 13 MHz. Visual indicators are fitted to show when the moisture content is out of the desired range. Accuracy of the method is ± 0·1% H_2O. G. R. Whalley.

Partition coefficients of some antioxidants in butteroil–water model systems. D. G. CORNELL, E. D. DE VILBISS and M. J. PALLANSCH (*J. Dairy Sci.*, 1970, **53** (5), 529–532. 9 ref.).—The butteroil/water partition coeff. of the esters of gallic acid (I), BHA and nordihydroguaiaretic acid were detd. BHA was found to have a partition coeff. > 800 and the coeff. of I esters increased with increasing chain length of the alcoholic substituent (0·24 for Et gallate; 71 for hexyl gallate). The gallate coeff. decreased with increase in temp. Milk salts caused a decrease of partitioning of the gallates but had no effect on the other esters. M. O'Leary.

Growth stimulation of *Lactobacillus casei* by sodium citrate. A. L. BRANEN and T. W. KEENAN (*J. Dairy Sci.*, 1970, **53** (5), 593–597. 14 ref.).—Addn. of sodium citrate to a broth medium resulted in significant stimulation of *Lactobacillus casei*. Optimum concn. for stimulation was 12–18 μM/ml of broth; 40 μM/ml completely inhibited growth. Both $MgSO_4$ and $CaCl_2$ were found to be stimulatory and to have an enhancing effect on citrate stimulation. Shaking of cultures and the addn. of diacetyl had no significant effect on citrate-induced growth stimulation. M. O'Leary.

Factors affecting flavour development in Cheddar cheese slurries. S. SINGH and T. KRISTOFFERSEN (*J. Dairy Sci.*, 1970, **53** (5), 533–536. 11 ref.).—Flavour development in Cheddar cheese slurries was enhanced by the addn. of 100 ppm of reduced glutathione, 3%-NaCl, storage at 30–35°c, daily agitation and the addn. of Na citrate, Mn, riboflavin, or Co. The adverse effect of prolonged frozen storage on subsequent flavour intensity was prevented by adding lyophilised lactic cultures to the slurries. A relationship between the formation of active-SH groups, free fatty acids, and flavour development was indicated. M. O'Leary.

Simplified pouch method for enumeration of 'stinker' Swiss cheese organisms. G. H. RICHARDSON and G. A. FOWLER (*J. Dairy Sci.*, 1970, **53** (5), 599–601. 11 ref.).—*Clostridium* spp. were successfully enumerated in a rectangular, commercially available boil-in-bag pouch. One ml of raw milk and 45 ml of Fe^{2+}-contg., dextrose-free thioglycollate medium are sealed in the pouch, heated for 10 min at 80°c, and allowed to solidify. H_2S-producing colonies are enumerated after 48 h at 37°c. (From summ.) M. O'Leary.

Ice-cream technology and manufacture. I. Gross composition of plain ice cream. II. A study on standardisation of plain ice cream manufacturing processes. S. C. JAIN and I. S. VERMA (*Indian J. Dairy Sci.*, 1969, **22** (3), 140–146. 4 ref.; 147–154. 16 ref.).—Results are presented of an investigation to find the most suitable mix and manufacturing procedure for ice-cream for sale in India. M. O'Leary.

Relationship of β-backscatter counts to egg shell thickness and calcium content. J. BITMAN, H. C. CECIL, S. J. HARRIS and P. E. JAMES (*Poult. Sci.*, 1969, **48** (6), 2184–2185. 1 ref.).—Counts from eggs of the Japanese quail were highly correlated with shell thickness and shell Ca, expressed as % of total egg wt. A. H. Cornfield.

Preparing cheese. MONSANTO CO. (Br. Pat. 1,191,074, 17.5.68. U.S., 18.5.67).—A mixture of cheese, water, a fat–casein emulsifying agent, e.g., $Na_2HPO_4.2H_2O$ and a proteolytic enzyme (I), e.g., papain, trypsin, is held at a temp. at which I is active and then heat pasteurised and cooled to obtain a process cheese. S. S. Chissick.

Improved process cheese. STAUFFER CHEMICAL CO. (Br. Pat. 1,189,003, 23.5.67. U.S., 31.5.66).—At least 2 cheeses (⩽ 1 being rework cheese) are comminuted and mixed, with the aid of heat and water, in presence of an emulsifier and a surface active agent. E.g., 3 different (pH) Cheddar cheeses plus water are ground in presence of polyoxyethylene monostearate and sodium aluminium phosphate, NaCl and water added at 80°c; after mixing, the whole is cooked (3 min). The product after cooling has a 'Bloom' reading of 180. S. S. Chissick.

Edible Oils and Fats

Aromatic hydrocarbon content of olive oils. W. CIUSA, V. D'ARRIGO and G. MARCHESINI (*Riv. ital. Sostanze grasse*, 1970, **47** (3), 114–119. It., 13 ref.).—Chromatographic and extraction techniques were used to separate the hydrocarbons. In the former, the oil (250 g) is passed through an activated alumina column (7 cm dia.) and eluted with cyclohexane followed by cyclohexane + 15% benzene. The pigment and part of the hydrocarbon content remain on the column. The eluate containing 90–95% of the oil is concentrated and saponified, and the unsaponifiable matter is separated chromatographically on activated alumina. The original chromatographic column is subdivided and extracted with benzene and the extracts are rechromatographed. Finally, all the fractions (400–500 for each sample) are examined spectrophotometrically and spectrofluorimetrically. In the extraction technique, the oil dissolved in cyclohexane is extracted with dimethyl formamide: H_2O (9 : 1), the extract is diluted with H_2O, extracted with cyclohexane, and the extract is concentrated and chromatographed on alumina and the fractions examined as above. This technique is less satisfactory. The total aromatic hydrocarbon content of expressed, sansa and rectified sansa oils ranged from 200 to 700 μg/kg. Decolorisation with charcoal reduced the content only slightly. The hydrocarbon present in the highest amount was usually phenanthrene. 3,4-Benzopyrene contents were highest in sansa oils (up to 25 μg/kg). L. A. O'Neill.

Analysis of peach kernel oil. G. MARTINEZ and R. TORLASCHI (*Revta Inst. argent. Grasas Aceit.*, 1969, **11** (1), 9. Span., 4 ref.).—The characteristics of the oil, determined by standard methods, are: d_4^{25} 0·9116, n^{25} 1·4690, acidity 1·8%, I_2 value 96·5 and saponification value 190. The main component acid is oleic acid. L. A. O'Neill.

Kinetic antioxidant properties of amino acids. J. SLIWIOK and J. SIECHOWSKI (*Riv. ital. Sostanze grasse*, 1970, **47** (2), 73–75. Engl., 8 ref.).—The effect of some amino acids (0·05–0·2%) on the rate of oxidation of soyabean oil at 60°c measured iodometrically was examined. Methionine and to a lesser extent cystine were antioxidants but cysteine was a pro-oxidant. Results with olive oil were similar. L. A. O'Neill.

Composition of beechnut oil. G. BIONDA, E. TASSARA and E. CARLISI (*Riv. ital. Sostanze grasse*, 1970, **47** (2), 76–79. It., 4 ref.).—Beechnut oil is a semidrying oil of possible value for food or pharmacological use. Characteristics are I_2 value 109, saponification value 193 and n_D^{25} 1·475. Fatty acid composition (g.l.c.) is palmitic 9·1–9·2, stearic 1·4–1·7, oleic 40·7–42·3, linoleic 40·0–40·5, arachidic 3·5–3·7 and linolenic acid 3·8–4·0%. The u.v. and i.r. spectra are recorded. L. A. O'Neill.

Correlations involving oil and fatty acids in rapeseed. B. R. STEFANSSON and A. K. STORGAARD (*Can. J. Pl. Sci.*, 1969, **49** (5), 573–580. 11 ref.).—Fatty acid components (%) of the seed oil from groups of plants and % total fatty acids (*TFA*) per dry wt. of seed were compared. When the acids (palmitic, oleic, linoleic, linolenic and eicosenoic) were expressed as % of *TFA*, −ve correlations between oleic and other fatty acids and +ve correlations between linoleic and linolenic acids were observed. With fatty acids expressed as %*TFA* and oil as % of seed several consistently high −ve correlations were found. When both oil and *TFA* were expressed as % of seed, correlations between the two sets of values were mainly positive. A. G. Pollard.

Chemical studies on animal and vegetable fats. I. A trial to differentiate between cow- and buffalo-butter-fat (Samn). II. Identification of animal and vegetable fats and oils in shortenings. A. LATIF and U. MAZLOUM (*Indian. J. Dairy Sci.*, 1969, **22** (3), 162–167. 14 ref.; 168–172. 5 ref.).—I. A method, based on the acetone crystallisation of buffalo- and cow-butter-fat, for distinguishing between these two fats is described.

II. Application of the method and also a method based on the glyceride structure of butter-fat in the identification of animal and vegetable fats and oils in shortenings, is described. M. O'Leary.

Fats. UNILEVER LTD. (Inventor: H. A. GRAFFELMAN) (Br. Pat. 1,190,553, 28.6.66).—Hardened palm kernel oil and hardened vegetable oil of slip m.p. ⩽ 50°, are interesterified (e.g., at 80°c in presence of NaOEt) and blended with a liquid vegetable oil containing ⩽ 40% linoleic acid and in which the ratio of satd. to unsatd. acid is ⩾ 1 : 2, to yield an edible fat product (margarine). S. S. Chissick.

Meat, Poultry, Fish

Some sources of variation in tenderness and cooking losses in lamb and mutton. R. W. PURCHAS, O. WILLIAMSON, R. A. BARTON and A. L. RAE (*N.Z. Jl agric. Res.*, 1969, **12** (4), 676–690. 43 ref.).—Although tenderness was affected by sire and selection for this could be important, other factors such as pasture type and slaughter reaction could not be correlated. Cooking loss was due mainly to dripping loss. M. T. Rawnsley.

Fat analysis of boned meat by the specific gravity method. R. C. WHITEHEAD (*Fd Technol., Champaign*, 1970, **24** (4), 469–473).—Accurate measurements of wt., compacted vol. and temp. of a boned meat sample were made. Sp. gr. varied linearly with the % fat in the sample, as determined by ether extraction. An instrument was developed capable of measuring the fat content of a 750-g meat sample in less than 1 min. M. J. Rawlins.

Phosphate corrosion in canned meat. P. W. BOARD (*Fd Preserv. Q., C.S.I.R.O. Aust.*, 1969, **29** (4), 72–75).—Lab. trials confirm that addn. of polyphosphate increase the intensity of S-staining and rate of corrosion of tin coating, with formation of H_2; deleterious effects tend to be most pronounced in packs having the max. permitted level of additive (0·3% equiv. P_2O_5). Electrolytic tinplate was more resistant to S-staining, but subject to more rapid de-tinning, than hot-dipped tinplate; dark detinned areas might be as objectionable to consumers as heavy staining. Canners who add polyphosphates are advised that storage life is shortened and that the marketing of such products should be carefully supervised. E. C. Apling.

Fluorescent dye tracing of water entry and retention in chilling of broiler chicken carcasses. D. H. SANDERS (*Poult. Sci.*, 1969, **48** (6), 2032–2037. 21 ref.).—A Rhodamine B dye tracer method is described. Although dye binding and retention were related to agitation and water penetration, the technique was not suitable for quant. studies of water uptake and retention during subsequent storage. A. H. Cornfield.

Chilling and freezing of poultry with liquid nitrogen. D. KUSCHFELDT and W. THIEL (*Fleischwirtschaft*, 1969, **49** (9), 1153–1158. Ger., 11 ref.).—The advantages of the 'rapid cryogen method' (freezing of freshly killed, vac. packed birds in liquid N_2) are discussed. Pre-chilling with iced water is undesirable both for hygienic reasons, and because it leads to uptake of up to 15% of extraneous water. P. P. R.

Flavour comparisons between various chicken and turkey preparations. K. N. HALL and J. V. SPENCER (*Poult. Sci.*, 1969, **48** (5), 1575–1579. 13 ref.).—A study was made to select terms to describe the aroma and flavour of chicken and turkey and to establish whether the primary aroma and flavour distinction between the species was due to quant. or qual. differences. A. H. Cornfield.

Processing factors affecting pheasant meat quality. S. T. MCCREADY and J. D. MITCHELL (*Poult. Sci.*, 1969, **48** (6), 2018–2022. 11 ref.).—The effect of scalding temp. and ageing water temp. on quality of cooked breast meat and pH, lactate dehydrogenase activity and total protein of uncooked meat of male and female pheasants is reported. A. H. Cornfield.

Handling fish before canning. R. MCLAY (*Torry advis. Note*, 1970, (44), 6 pp.).—Advice is given on chilling, freezing, storing and thawing fatty fish, particularly herring, sprats, pilchards and mackerel. E. G. Brickell.

Solubilisation of fish protein concentrate [*FPC*]. II. Utilisation of the alkaline-process product. S. R. TANNENBAUM, R. P. BATES and L. BRODFELD (*Fd Technol., Champaign*, 1970, **24** (5), 607–609. 5 ref.).—Neutralised alk. *FPC* was mixed with soya protein isolate, dried in thick films at 110°c and rehydrated in an excess of H_2O. The resulting gel product had interesting texture and no off-flavour or after taste. Granular protein products, simulating ground beef, were prep. from *FPC* and gluten. Attempts to prep. milk substitute using *FPC* as the sole protein source were unsuccessful, but the product could be used directly to produce heat sterilised milk-like beverages. M. J. Rawlins.

Solubilisation of fish protein concentrate. I. An alkaline process. S. R. TANNENBAUM, M. AHERN and R. P. BATES (*Fd Technol., Champaign*, 1970, **24** (5), 604–607. 13 ref.).—Fish protein concentrate was slurried with aq. NaOH and agitated for 20 min at 95°C. Precipitable protein was detd. by adjusting the pH to 4·5 with 6N-HCl and recovering the protein by centrifugation. Some depolymerisation of the protein occurred during the process but ~ 50% of the protein was still acid precipitable.
M. J. Rawlins.

Inhibition of *Pseudomonas* species by hydrogen peroxide-producing lactobacilli. R. J. PRICE and J. S. LEE (*J. Milk Fd Technol.*, 1970, **33** (1), 13–18. 37 ref.).—*Lactobacillus* spp. from Pacific oysters and *Pseudomonas* spp. from marine sources were examined. Results showed that *Lactobacillus* spp. produced H_2O_2, reaching max. concn. in 4–5 days at 30°C. The H_2O_2 was inhibitory to *Pseudomonas*, *Bacillus* and *Proteus* spp. The possible application in foods is discussed. W. J. G.

Food Additives

Preservatives, Colouring Matter

Evaluation of colour additives using a differential scanning calorimeter. D. M. MARMION (*J. Ass. off. analyt. Chem.*, 1970, **53** (2), 244–249. 6 ref.).—This technique is used for detn. of m.p., moisture content, purity and heat of fusion of certifiable colours. Relative stabilities and identification of the colours could also be deduced from the thermograms. P. P. R.

Spices, Flavours, Other Additives

Routine examination of spices for volatile oils and other constituents. III. Nutmeg and mustard. IV. Ginger, cinnamon, garlic and onion. U. GERHARDT (*Fleischwirtschaft*, 1969, **49** (9), 1191–1193. Ger., 7 ref.; **49** (10), 1356–1358. Ger., 3 ref.).—Values for loss on drying, ash (at 1000°C), moisture, cold water extract, ether extract and volatile oil content are given. P. P. R.

Effect of natural spices, spice extracts, volatile oils, extraction residues and synthetic antioxidants on degradation of pork fat and model lipids. A. PALITZSCH, H. SCHULZE, F. METZL and H. BAAS (*Fleischwirtschaft*, 1969, **49** (10), 1349–1354. Ger., 16 ref.).—Marjoram, nutmeg, white pepper, rosemary, sage and their extracts, were added (singly or mixed) to lard, with or without ascorbic acid (**I**), tocopherol (**II**) and a synthetic antioxidant (BHA). Rosemary, sage and nutmeg were strongly antioxidant; some synergistic effects were also observed with certain combinations of spices/extracts with one another or with I or II. P. P. R.

Food Processing, Refrigeration, Packaging and Storage

Atmospheric fluidised bed freeze drying. G. J. MALECKI, P. SHINDE, A. I. MORGAN, JUN. and D. F. FARKAS (*Fd Technol., Champaign*, 1970, **24** (5), 601–603. 7 ref.).—Commercial apple juice, and rehydrated egg albumen were sprayed from an atomiser into a thin sheet of liquid N_2 flowing down the walls of a cylindrical vessel. Frozen powders were fluidised. Frozen droplets 30–60 mesh dia. were obtained. Egg albumen particles fluidised well at −20°C and the rate of H_2O loss was 6·5% per h. Apple juice fluidised at temp. < −34° and moisture loss was 0·6% per h.
M. J. Rawlins.

Packaging materials as barriers to piperonyl butoxide migration. H. A. HIGHLAND, M. SECREAST and P. H. MERRITT (*J. econ. Ent.*, 1970, **63** (1), 7–10. 9 ref.).—Of the 48 compd. tested, 13 prevented migration of piperonyl butoxide (into flour) during the 6 months exposure. Except for silicone-treated kraft, all compd. were partly effective. Among the completely effective materials were foil/PE and vinyl coated cellophane. Materials of the same generic type varied greatly in their effective results.
C. M. Hardwick.

Nutrition, Proteins, Amino Acids, Vitamins

Specialist periodical reports: amino acids, peptides and proteins. G. T. YOUNG (*Chem. Soc.*, 1969, **1**, 308 pp.).—**Amino acids.** J. H. JONES (1–30; 285 ref.); **Structure of peptides and proteins.** R. N. PERHAM, P. M. HARDY and C. C. F. BLAKE (31–173; 738 ref.). **Peptide synthesis.** J. H. JONES (174–210; 213 ref.). **Peptides of abnormal structure.** J. S. DAVIES (211–248; 132 ref.). **Structure and biological activity of some peptides and proteins (excluding enzymes).** D. G. SMYTH (249–261; 65 ref.). **Metal derivatives of amino acids, peptides and proteins.** R. D. GILLARD and S. H. LAURIE (262–293; 151 ref.). C. V.

Utilisation of hydrocarbons by micro-organisms. II. Use of n-paraffins and vitamin B_2 production by *Pichia guilliermondii* Wickerham (*J. Ferment. Technol., Osaka*, 1970, **48** (1), 1–7. Jap.).—63 micro-organisms were isolated from soils near to gas stations or a petrochemical factory; 13 were able to produce vitamin B_2 in a hydrocarbon medium, the most efficient being the yeast strains *P. guilliermondii* Wickerham. The optimum initial pH was 6·5 and the combined use of $NH_4H_2PO_4 + (NH_4)_2SO_4$ was the most effective source of N. The yeast used C_{10}–C_{15} n-alkanes but none with longer chains could be utilised under the conditions studied. n-Dodecane gave the best results and non-ionic surfactants (Tween-20, 60, etc.) stimulated vitamin production. (From Engl. summ.).
C. V.

Effect of certain additives on the photochemistry of riboflavin. CHUNG TECK SHIN, B. J. SCIARRONE and C. A. DISCHER (*J. pharm. Sci.*, 1970, **59** (3), 297–302. 27 ref.).—A micro-irradiation method was used to determine the quantum efficiency (*QE*) of riboflavin. The initial *QE* was const. and independent of intensity and wavelength of light and concn. Addn. of phenol, *p*-chlorophenol, *p*-methoxyphenol, resorcinol and hydroquinone decreased the *QE* and hence the rate of photodecomp. Benzyl alcohol and benzoic acid had little effect, while cinnamyl alcohol enhanced photodecomp. Effects of temp. and pH on the system were detd. and a reaction mechanism is proposed. G. W. Flinn.

Unclassified, Tobacco

Utilisation of hydrocarbons by micro-organisms. V. Production of α-ketoglutaric acid by fermentation. K. TANAKA, K. KIMURA, T. SUZUKI *et al.* (*J. Ferment. Technol., Osaka*, 1969, **47** (5), 291–296. Jap., 10 ref.).—*Corynebacterium* sp. (K74439) produced up to 40% of the acid from n-alkanes. The production ratio to consumed hydrocarbons (**I**) was increased by lowering the **I** concn. Adding 8·7% (v/v) of n-paraffins resulted in an acid yield as high as 85·5%. (From Engl. summ.) C. V.

New technique for the economic production of cellulase. A. CHANDRASEKARAN and M. S. SHANTHAMMA (*J. Fd Sci. Technol.*, 1969, **6** (1), 12–14. 12 ref.).—Three cellulolytic fungal cultures were grown on wheat bran medium and the crude enzyme was extracted with distilled water. The active cellulase present in the culture extract was pptd. by $(NH_4)_2SO_4$ (satn. 80%) and dissolved in distilled water; this was used as enzyme samples for the assays. The efficiency of the conc. crude enzyme samples to hydrolyse various pure cellulosic substrates was studied. The different components of the enzyme samples obtained from the second series showed increased activity compared with the other two series.
I. Dickinson.

Effect of proteins on the reversibility of the thermal inactivation of *Bacillus subtilis* α-amylase. G. BERTAGNOLIO, R. GOT and L. COLOBERT (*Experientia*, 1970, **26** (2), 140–141).—The pptn., occurring upon heat denaturation of protein, is probably due to non-covalent inter- and intra-chain associations; such interactions are believed to be dissociated by urea (**I**) or guanidine–HCl (**II**) and attempts are made to regain activity by dissolving the heat denatured pptd. enzyme in these reagents; they are removed by dilution. C. V.

Chip-type food product from ground nuts. IMPERIAL TOBACCO CO. OF CANADA LTD. (Br. Pat. 1,180,686, 17.7.68. Can., 18.7.67).—The product comprises 60–95% (by wt.) crushed nuts (max. particle size $\frac{1}{16}$ in), ≯ 30% binder (durum semolina), ≯ 20% corn and potato starch, and ≯ 10% vegetable gum and salt. The moisture content is < 2% and prepn. is by air-drying a slurry of the constituents in the form of a thin sheet, so as to form chips.
S. S. Chissick.

Producing a dry enzyme or protein preparation. FORSCHUNGSINSTITUT FUER DIE GAERUNGSINDUSTRIE, ENZYMOLOGIE UND TECHNISCHE MIKROBIOLOGIE (Inventors: D. SCHOEPFEL and J. HUBER) (Br. Pat. 1,181,484, 15.2.68).—The prepn., which has a low salt content, is obtained from an albumen-contg. culture soln./filtrate, by adding a precipitant (**I**), e.g., a synthetic org. tanning agent, and subsequently extracting inorg. salts and excess **I** with water and/or an org. solvent. E.g., a culture filtrate of *Bacillus subtilis* is adjusted to pH 7 with aq. Na_2CO_3 and a condensed phenolic compd. added to yield a ppt. which is separated and washed twice with acetone. S. S. Chissick.

Recovering compositions containing calcium sugar phosphates and inorganic phosphate. COLONIAL SUGAR REFINING CO. LTD. (Br. Pat. 1,178,509, 30.5.68. U.S., 8.6.67).—An aq. sucrose phos-

phorylation reaction liquor, contg. Ca sugar phosphates, inorg. Ca phosphate, $CaCl_2$ and unreacted sugar, is dehydrated (spray dried); the resulting finely divided material is treated with aq. EtOH to remove $CaCl_2$ and the total residue dried to give a product useful for preventing dental caries, as a plant and animal nutrient and for coating breakfast cereals. (1 diagram.) S. S. Chissick.

Chewing gum. TAKEDA YAKUHIN KOGYO K.K. (TAKEDA CHEMICAL INDUSTRIES LTD.) (Br. Pat. 1,179,473, 31.1.67. Jap., 1.2.66).—A low adhesion chewing gum is prep. by mixing sugar and an organopolysiloxane (mol. wt. 50,000–5,000,000) of formula $R_3SiO\cdot(R_2SiO)_n\cdot SiR_3$, where R is Ph or 1–4 C alkyl. S. S. Chissick.

Tobacco product. EASTMAN KODAK CO. (Inventors: E. G. MILLER, JUN. and W. D. KENNEDY) (Br. Pat. 1,189,880, 4.8.67. U.S., 5.8.66).—Leaf tobacco (**I**) is treated with $\leq$ 1 metal chlorate to reduce certain noxious constituents produced in smoking. E.g., **I** is blended with $KClO_3$ in water, then air dried. On burning, the amount of fluorescent material produced is 28% less than that of a control. S. S. Chissick.

3.—PEST AND DISEASE CONTROL, SANITATION

Plant Diseases, Pests and Weeds

Influence of caging and pesticide drenches on yield and persistence of red clover, *Trifolium pratense*. K. E. ZEIDERS, R. C. NEWTON and J. H. GRAHAM (*Agron. J.*, 1969, **61** (6), 952–953. 5 ref.).—Soil fumigation with MeBr before sowing and treatment of the plants with the fungicide Me arsinoxide had little effect on yields and persistence of red clover over 3 yr. Treatment with heptachlor + $C_6H_6Cl_6$ increased yields somewhat, and greater increases were obtained with a combination of insecticides and caging. Caging was more effective in decreasing the incidence of virus symptoms in plants than in increasing yields. A. H. Cornfield.

Annual review of phytopathology. J. G. HORSFALL, K. F. BAKER and D. C. HILDEBRAND (Eds.) (*A. Rev., Phytopathol.*, 1969, **7**, 477 pp.).—20 communications are presented and the following are specially noted: **Epidemiology and control of bacterial leaf blight of rice.** T. MIZUKAMI and S. WAKIMOTO (150 ref.). **Insect tissue cultures as tools in plant virus research.** L. M. BLACK (74 ref.). **Cellular responses of plants to nematode infections.** V. H. DROPKIN (92 ref.). **Culturing rust fungi.** K. J. SCOTT and D. J. MACLEAN (102 ref.). **Parasexuality in plant pathogenic fungi.** R. D. TINLING and B. H. MACNEILL (154 ref.). **Biochemistry of the cell wall in relation to infective processes.** P. ALBERSHEIM, T. M. JONES and P. D. ENGLISH (82 ref.). **Role of phenolics in host response to infection.** T. KOSUGE (183 ref.). **Modes of infection and spread of *Fomes annosus*.** C. S. HODGES (127 ref.). **Irrigation and plant diseases.** J. ROTEM and J. PALTI (114 ref.). **Soil water in the ecology of fungi.** D. M. GRIFFIN (136 ref.). **Effect of fungicides on protein and nucleic acid synthesis.** H. D. SISLER (110 ref.). **Heat therapy of virus diseases of perennial plants.** G. NYLAND and A. C. GOHEEN (209 ref.). **Development of disease-resistant rice.** S. H. OU and P. R. JENNINGS (166 ref.). **Use of plant pathogens in weed control.** C. L. WILSON (145 ref.). C. V.

Herbicides

Effects of surface area, exchange capacity and organic matter content on miscible displacement of atrazine in soils. K. W. SNELLING, J. A. HOBBS and W. L. POWERS (*Agron. J.*, 1969, **61** (6), 875–878. 9 ref.).—Retention of atrazine (5 ppm in 0·05N-$BaCl_2$) on soil columns increased with surface area, *CEC* and org. matter contents of the soils. A. H. Cornfield.

Decomposition in soil of the herbicide chloral hydrate. H. R. SHÜTTE and U. STEPHAN (*Z. PflErnähr. Bodenk.*, 1969, **123** (3), 212–219. Ger., 16 ref.).—Decomp. of chloral hydrate (**I**) to trichloroacetic acid was investigated in five slightly acid to neutral soils. Temp. and not soil type was found to be the controlling factor. In the decomp. of C^{14}-labelled **I**, $^{14}CO_2$ was detected; HCHO was also detected in the early stages. The process possibly may involve a combination of chem., photochem. and biol. steps. M. Long.

Persistence and movement of fluorodifen in soils and plants. J. P. WALTER, E. F. EASTIN and M. G. MERKLE (*Weed Res.*, 1969, **10** (2), 165–171. 5 ref.).—Results showed only limited translocation of fluorodifen following application to roots or leaves of soyabean seedlings. W. J. G.

Picloram residues and crop production. W. H. VANDEN BORN (*Can. J. Pl. Sci.*, 1969, **49** (5), 628–629. 5 ref.).—The possibility of prolonged effects of picloram (*P*), several years after application, is examined. An area densely infested with Canada thistle was treated with *P* at 1·62 or 3·24 kg/ha and subsequently sown with wheat. Treated areas showed 80% control of the thistle but the wheat plants showed severe injury by *P* (20–40% reduction in height). Barley and wheat were more sensitive to *P* than was oats. After 5 yr, lucerne and sunflower (sensitive to *P*) were readily established on the experimental area. A. G. Pollard.

Reaction of twelve *s*-triazines (herbicides) with soil clays. C. B. BROWN and J. L. WHITE (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 863–867. 16 ref.).—Infra-red and n.m.r. data, after reaction of soil clays and bentonite with *s*-triazines in aq. or $CHCl_3$ suspensions, were studied. A. H. Cornfield.

Effect of picloram, diuron, ametryne, and prometryne on nitrification in some tropical soils. H. D. DUBEY (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (6), 893–896. 16 ref.).—Addn. of 2 ppm picloram before incubation of tropical soils decreased nitrification slightly, but at 20–100 ppm inhibited nitrification completely, except in a soil of high nitrifying capacity. Diuron inhibited nitrification at 20 ppm or higher in soils of low capacity, but had no effect even at 100 ppm in a soil of high capacity. 100 ppm ametryne and prometryne resulted in NO_2^- accumulation, but only in soils of pH $\leq$ 6·8. A. H. Cornfield.

Translocation and metabolism of methanearsonic acid (MAA) in johnsongrass (*Sorghum halapense*) and cotton. M. M. SCKERL and R. E. FRANS (*Weed Sci.*, 1969, **17** (4), 421–427. 28 ref.).—The basis for selectivity of MAA between *S. halapense* (**I**) (susceptible) and cotton (**II**) (tolerant) was studied, using ^{14}C-labelled MAA. A ninhydrin-positive MAA metabolite was found in the MeOH extract of **I** but not of **II**; this metabolite may be a combination of MAA with histidine (or an analogue). MAA treatment caused a build-up of amino acids in **I** but not in **II**, indicating blocking of a protein synthesis pathway. A. H. Cornfield.

Pre-emergence weed control in young deciduous fruit trees. A. H. LANGE, J. C. CRANE, W. B. FISCHER *et al.* (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 57–60. 9 ref.).—Ten pre-emergence herbicides were tested at 2 locations on young peach, plum, cherry, pear and walnut rootstocks. Location had the greatest effect on extent of damage, but there were also varietal differences in light soils. Simazine was safe in the heavier soil, but gave variable weed control. Diuron was equally effective in weed control, but was safer than simazine. Of the commercial uracil herbicides tested, only Terbacil (5-chloro-3-t-butyl-6-methyluracil) was of sufficient interest for further study. A. H. Cornfield.

Use of mixtures of granular dalapon, birdsfoot trefoil seed and fertiliser for roughland pasture renovation. J. E. WINCH, E. M. WATKIN, G. W. ANDERSON and T. L. COLLINS (*J. Br. Grassld Soc.*, 1969, **24** (4), 302–307. 10 ref.).—When applied with trefoil seed, dalapon, at 4·4 kg a.i. per ha, controlled *Poa compressa* without deleterious effects on trefoil performance. Triple superphosphate (112 kg P_2O_5 per ha) applied simultaneously did not decrease trefoil establishment. Aircraft application of seed, dalapon and P resulted in 5–10-fold increases in pasture productivity. A. H. Cornfield.

Fungicides

Phytoalexin (pisatin) production by leaves of *Pisum sativum* in relation to senescence. J. A. BAILEY (*Ann. appl. Biol.*, 1969, **64** (2), 315–324. 25 ref.).—Results obtained are discussed in relation to the mechanisms of disease resistance. A. H. Cornfield.

Infection of the flowers and seed of parsnip by *Itersonilia pastinacae*. A. G. CHANNON (*Ann. appl. Biol.*, 1969, **64** (2), 281–288. 9 ref.).—Various symptoms are described. Seed infection was eliminated by soaking seed in aq. 0·2% thiram at 30° for 24 h. A. H. Cornfield.

Restriction of *Cladosporium fulvum* and *Botrytis cinerea*, attacking glasshouse tomatoes, by automatic humidity control. K. W. WINSPEAR, J. D. POSTLETHWAITE and R. F. COTTON (*Ann. appl. Biol.*, 1970, **65** (1), 75–83. 18 ref.).—Decreasing the periods of atm. humidity in the glasshouse to 90% and 75% r.h. decreased the incidence of *C. fulvum* and *B. cinerea* on foliage and fruit, resp., of tomato, and sometimes increased tomato yields. Humidity control was particularly effective at low atm. temp., which tend to favour moist conditions. A. H. Cornfield.

Tests of the action of fungicide 1991 [methyl ester of 1-butylcarbamyl-2-benzylimidazol carbamic acid] on the preservation of Valencia oranges, and comparative studies with the action of other fungicidal products. J. MACHO-QUEVADO BARON and J. CUQUERELLA CAYUELA (*An. Inst. nac. Invest. agron., Madrid*, 1969, **18** (3), 285–296. Span., 8 ref.).—Semi-industrial and lab. trials are reported. Treated fruits showed considerably improved resistance to attack by *Penicillium italicum* and *P. digitatum*. Immersion treatment with 1991 was considerably more effective than treatment with tribendazol [2-(4-thiazolyl)-benzimidazol] or sodium *o*-phenylphenate, but applications 10 days before harvest of each fungicide gave similar results. Best control was achieved by spray treatment with 1991 followed by sealing with wax; of 150 oranges treated at each level and stored for 12 weeks at 25°C, only one of those treated with 500 ppm and none with 1000 ppm was attacked by *Penicillium* compared with 64 of the controls. E. C. Apling.

New aspects of control of banana leaf spot. PH. MELIN (*Fruits d'outre mer*, 1970, **25** (3), 141–145. Fr.).—It was found that Benlate (= benomyl) (250 g a.m./ha) in the oil normally used for spraying, was successful in removing banana leaf spot. Field tests showed that the no. of applications, at least in Cameroun, could be reduced when Benlate was present. Benlate in water was less effective. M. T. Rawnsley.

Control of ripe fruit rots of bananas by the use of post-harvest fungicidal dips. O. J. BURDEN (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (41), 655–658. 14 ref.).—Benlate (benomyl), 3,4-dihydroxybenzaldehyde (3,4-DHB), thiabendazole (TBZ), Panacide and 2-aminobutane (2-AB) were tested. Benlate was found to control black end, crown rot and anthracnose, all caused by *Colletotrichum musae*. Panacide had no effect, while TBZ and 2-AB had some effect. 3,4-DHB is recommended for further tests. Residues must be investigated before commercial use. M. T. Rawnsley.

Possible side effects of fungicides on banana and coffee diseases. I. D. FIRMAN (*Nature, Lond.*, 1970, **225** (5238), 1161. 10 ref.).—The apparent favouring of a virulent pathogen at the expense of a related saprophytic or less virulent form can occur as a result of (*i*) replacement of *Mycosphaerella musicola* Leach (causing Sigatoka disease of bananas) by *Mycosphaerella* sp. causing the much more serious black leaf streak disease, in the Pacific region, and (*ii*) increased population of virulent *Colletotrichum coffeanum* Noack in coffee bark in Africa as a result of Cu spraying. Oil spraying to control Sigatoka disease seems to have favoured a strain of *Mycosphaerella* characterised by sparse conidiophores and abundant ascophores, whereas relative concn. of these are the reverse in *M. musicola*. W. J. Baker.

Ammonia as a necrotoxin in the hypersensitive reaction caused by bacteria in tobacco leaves. L. LOVREKOVICH, H. LOVREKOVICH and R. N. GOODMAN (*Can. J. Bot.*, 1970, **48** (1), 167–171. 13 ref.).—The wildfire disease caused by *Pseudomonas tabaci* involves NH_3 formation. Tissue necrosis also occurs when incompatible bacteria, e.g., *P. pisi*, *P. syringae* and *Erwinia amylovora*, are injected, and NH_3 is again evolved. Tests show that NH_3 is a major factor in tissue necrosis, although artificial induction gives symptoms more quickly. It is possible that wildfire disease is a slow-developing hypersentitive reaction but only undissociated NH_3 causes necrosis. M. T. Rawnsley.

Insecticides and others

Laboratory trials of the control of a strain of Colorado beetles, highly resistant to chlorinated insecticides, by new insecticides and mixtures of new and well-known insecticides. J. M. DEL RIVERO, J. J. TUSET, F. J. ROIG and M. LAFUENTE (*An. Inst. Nac. Invest. agron., Madrid*, 1969, **18** (2), 129–143. Span., 2 ref.).—Results of preliminary trials with 136 different formulations are reported. Best results (100% mortality of both adults and larvae) were obtained with Bayer 5,621, Bayer 5,691, N-4,543, Hercules 14,503, chlorfenvinphos (Birlane) and Trimidan (commercial mixture of carbophenothion and imidithion). E. C. Apling.

New experiments in control of the potato beetle (*Leptinotarsa decemlineata* Say) resistant to organochlorine insecticides. J. M. DEL RIVERO, E. MIQUEL and M. LAFUENTE (*Revta Agroquim. Tecnol. Aliment.*, 1969, **9** (4), 569–573. Span., 3 ref.).—Lab. trials of a large no. of formulations against both adults and larvae are reported, with 100% kill by HOE-2960, Azodrin, Kepone, PP-511, P-211 and C-8353. E. C. Apling.

A rapid colorimetric estimation of lindane. K. VISWESWARAIAH and M. JAYARAM (*J. Fd Sci. Technol.*, 1969, **6** (2), 111–112. 10 ref.).—Lindane and *o*-toluidine form a blue complex when mixed in optimum quantities and exposed to diffused daylight. Conditions were standardised for optimum exposure time to sunlight, the extraction of the complex and its stabilisation. It was found to have max. absorption at 620 nm and Beer's law was obeyed for concn. 2 μg–12 μg/ml. M. J. Rawlins.

Sex-pheromone of the false codling moth *Cryptophlebia leucotreta* Meyr. (formerly *Argyroploce leucotreta* Meyr.). Synthesis and bioassay of *trans*-dodec-7-en-1-yl acetate and related compounds. H. E. HENDERSON and F. L. WARREN (*J. S. Afr. chem. Inst.*, 1970, **23** (1), 9–12. Engl., 6 ref.).—This compd. was synthesised from 6-bromohexan-1-ol (**I**) by a procedure similar to that used by Berger (*J. econ. Ent.*, 1968, **61**, 452). Reaction of I with dihydropyran at > 30° yielded the 6-bromohexanyloxytetrahydropyran, which, on condensation with 1-hexyne in presence of Li in liquid NH_3, yielded (71%) dodecynyloxytetrahydropyran (**II**). Hydrogenation of II gave pure *trans*-dodecenyloxytetrahydropyran, which on hydrolysis and acetylation gave *trans*-dodecenyl acetate (**III**). The corresponding propionate and butyrate were prep. analogously. Tests showed that III elicited full sexual behaviour from the male moth, but that the two homologues in similar concn. stimulated only flight response. W. J. Baker.

Use of predatory coccinellids [ladybirds] in biological control. J. C. TOURNEUR (*Fruits d'outre mer*, 1970, **25** (2), 97–107. Fr., 27 ref.).—The protection of crops against scale insects and aphids is reviewed, and the feeding habits and efficiency of predatory coccinellids is examined. Specific cases, e.g., control of *Icerya purchasi* in California by *Rodolia cardinalis*, are discussed. It is concluded that feeding habits etc. of each predatory species, must be known before introducing it. M. T. Rawnsley.

Potential of *Chrysopa carnea* as a biological control agent of *Myzus persicae* on glasshouse chrysanthemums. N. E. A. SCOPES (*Ann. appl. Biol.*, 1969, **64** (3), 433–439. 7 ref.).—*M. persicae* were eliminated by introducing 1-day-old or third-instar larvae of *Chrysopa carnea* at aphid/chrysopid ratios of 50/1 or 200/1, respectively. Control was less effective at very low aphid densities. A method of predicting the extent of aphid control by *C. carnea* is described. A. H. Cornfield.

Pyrimidine derivatives. IMPERIAL CHEMICAL INDUSTRIES LTD. (Inventors: W. G. MOSS JONES, F. L. C. BARANYOVITS, R. GHOSH, N. D. BISHOP and P. F. H. FREEMAN) (Br. Pat. 1,181,657, 31.3.66) (36 pp., 42 claims).—Compd. effective against, e.g., *Aedes aegypti*, *Aphis fabae*, *Tetranychus telarius*, *Musca domestica*, *Puccinia recondita*, *Erysiphe graminis* have formula NR^IR^{II}·CX-YR wherein X and Y are O or S; R is 2-NR^VR^{VI}-4-R^{IV}-5-R^{III}-pyrimid-6-yl; R^I, R^{II}, R^V and R^{VI} are H or hydrocarbon radicals or NR^IR^{II} and NR^VR^{VI} are heterocyclyl; and R^{III}-R^{IV} are H, halogen, or optionally substituted hydrocarbon radicals. In an example, a mixture of 2-dimethylamino-4-hydroxypyrimidine, K_2CO_3, and NMe_2·COCl is boiled in acetone for 4 h, then worked up to give 2-dimethylaminopyrimidine, b.p. 98°/0·01 mm. A further 56 compd. are described and their activities tabulated. F. R. Basford.

3-(Cycloalkylmethyl and cycloalkenylmethyl)-5-phenyltetrahydro-4*H*-1,3,5-oxadiazin-4-ones. E. I. DU PONT DE NEMOURS & CO. (Inventor: R. W. LUCKENBAUGH) (Br. Pat. 1,176,180, 6.7.67).—The title compd. are herbicides, effective against *Digitaria* spp., *Setaria gaberii*, *Echinochloa crusgalli*, *Brassica* spp. As an example of method of prepn., a mixture of 3-phenyl-1-(cyclohexylmethyl)urea, paraformaldehyde, *p*-$C_6H_4Me·SO_3H·H_2O$, dioxane and benzene is boiled to remove water, then usual work-up affords 5-phenyl-3-(cyclohexylmethyl)-4-oxo-tetrahydro-1,3,5-oxadiazine b.p. 195–196°/0·5 mm, mp. 107–109°. F. R. Basford.

[Herbicidal] sulphamoylaryl ureas. FARBWERKE HOECHST A.-G. (Br. Pat. 1,178,283, 14.7.67. Ger., 16.7.66) (23 pp).—The ureas, of formula $SO_2NR^IR^{II}·R·NHCONR^{III}R^{IV}$, are active against a variety of weeds (e.g., *Sinapis arvensis*, *Stellaria media*, *Chenopodium album*, *Echinochloa crus-galli*, *Poa annua*) without appreciably affecting crops, such as potatoes, maize. R is benzene residue which may further contain alkyl, alkoxy, halogen; R^I is H or alkyl; R^{II} is alkyl, alkoxy, or dialkylamino, or NR^IR^{II} is heterocyclyl; R^{III} is H or alkyl; and R^{IV} is alkyl or alkoxy. In an example, 1-[*p*-(methylaminosulphonyl)phenyl)-3-methylurea, m.p. 173°, is prep. by reacting 1-(*p*-chlorosulphonyl)phenyl-3-methyl urea (obtained from 1-phenyl-3-methyl urea and $ClSO_3H$) with aq. NH_2Me. F. R. Basford.

[Herbicidal] benzimidazoles. ELI LILLY & CO. (Br. Pat. 1,179,461, 26.1.67. U.S., 26.1.66).—Compd. effective against, e.g., crabgrass, pigweed, foxtail, and velvet grass comprise 2-trifluoromethyl-

benzimidazoles substituted in the 5-position by SO_2Me, or in the 4-position by NO_2 and in the 6-position by CF_3 or SO_2Me. In an example, 2,6-dinitro-4-trifluoromethylaniline, m.p. 142–144° (prepn. described) is dissolved in EtOH and reacted with 20% aq. NH_4 polysulphide, contg. 5% free S, the temp. rising from 35° to 60°. The cooled mixture is poured into water and worked up to give 3-nitro-5-trifluoromethyl-*o*-phenylenediamine, m.p. 121–123°. This is converted into 4-nitro-2,6-di-(trifluoromethyl)benzimidazole, m.p. 95–97° (benzene–hexane), by boiling in aq. $CF_3 \cdot CO_2H$ for 4 h. F. R. Basford.

Bispyridinium compounds as [herbicides]. GULF RESEARCH AND DEVELOPMENT CO. (Br. Pat. 1,189,173, 21.8.67. U.S., 24.8.66).—The compd. have 2 pyridinium salt residues connected by a divalent conjugated group and are of formula $\bar{X}[R \cdot \overset{+}{N}C_5H_4 \cdot A \cdot C_5H_4\overset{+}{N} \cdot R]\bar{X}$ (I) in which $\bar{X}$ is a halide ion and R a hydrocarbon group of mol. wt. < 150. I is e.g., 4,4′-[2,5-(1,3,4-thiadiazole)ylene] bispyridinium halide, in which R = CH_3 and X^- is I^-, Cl^- or Br^-. A can also have a (1,2,4-thiadiazole) or (1,3,4-oxadiazole) moiety or the structure —CH:N·N:CH–. In an example, 2,5-bis (4-pyridyl)-1,3,4-thiadiazole in $HCONMe_2$ reacts with n-decyl-bromide at 100–105°c for 22 h and is cooled, to yield didecyl 4,4′-[2,5-(1,3,4-thiadiazol)ylene] bispyridinium bromide, m.p. 245–7°c (decomp.). A. Gordon.

Conjugated dipyridinium salts. B.P. CHEMICALS (U.K.) LTD. (Inventor: T. M. B. WILSON) (Br. Pat. 1,189,330, 11.11.67).—Herbicidal compd. of formula $[R^1 \cdot \overset{+}{N}C_5H_4 \cdot CH{:}CH \cdot R \cdot CH{:}CH \cdot C_5H_4\overset{+}{N} \cdot R^2]2X^-$ (in which R is a bond, or a divalent org. radical which is unsatd. to give a fully conjugated structure over the whole molecule, bonds to the heterocyclic rings being attached to either the 2- or 4- position, R^1 and R^2 are alkyl (Me, Et), and X^- is I^-, Cl^-, Br^- or *p*-toluene-sulphonate anion) are prep. by reacting a quaternary ammonium salt of α- or γ- picoline with glyoxal (or a conjugated, sterically unhindered dialdehyde) in MeOH under reflux in presence of a base, e.g. piperidine. A. Gordon.

[Pesticidal] phenylazothioformamides. N. V. PHILIP'S GLOEILAMPENFABRIEKEN (Br. Pat. 1,178,904, 6.4.67. Neth., 9.4.67).—The compd., in which the Ph ring is optionally substituted with ⩾ 3 groups chosen from Cl, MeO and NMe_2, are active against, e.g., mildews, such as *Botrytis allii*, and are prep. by oxidising the corresponding phenylthiosemicarbazide. E.g., phenylazothioformamide is prep. by reacting 1-phenylthiosemicarbazide and *p*-benzoquinone in MeOH for 1 h. S. S. Chissick.

[Fungicidal] α,α-diacyloxypentachlorotoluenes. SUMITOMO CHEMICAL CO. LTD. (Br. Pat. 1,177,442, 16.2.68. Jap., 22.2.67 and 1.3.67).—The compd., contg. acyl groups RCO·O, wherein R is optionally halogen substituted 1–4 C alkyl or alkenyl, are active against, e.g., rice blast, and can be prep. by reacting $(RCO)_2O$ with C_6Cl_5CHO (I). E.g., I (1 mole) is refluxed with Ac_2O (1·1 mole) in PhMe, in presence of H_2SO_4, for 5 h and the mixture worked up to give a product of m.p. 139°c (decomp.). S. S. Chissick.

Insecticidal oximes. RHÔNE-POULENC S.A. (Inventor: R. GIRAUDON) (Br. Pat. 1,184,858, 16.8.68. Fr., 16.8.67 and 27.5.68).—Oximes of formula $R^1 \cdot NO_2 \cdot C_6H_3 \cdot CH{:}N \cdot O \cdot CH_2 \cdot C_6H_{10} \cdot R^2$ where R^1 is H or alkyl of 1–3 C, R^2 is H or one or more alkyl groups of 1–4 C, e.g., *O*-(3-t-butyl-cyclohexylmethyl)-3-nitrobenzaldoxime (I), are prep. by reacting an oxime of formula $R^1 \cdot NO_2 \cdot C_6H_3 \cdot CH{:}NOH$ or its alkali metal salt with an ester of formula $R^2 \cdot C_6H_{10} \cdot CH_2X$ where X is a reactive ester residue. Powder prep. from I, kaolin, gum arabic and Ca lignosulphite can be used to protect plants against attack from mites at a rate of 250 g powder per 100 l of water. A. Gordon.

[Pesticidal] 1-carbamyl-*N*-(substituted carbamyloxy)thioformimidates. E. I. DU PONT DE NEMOURS & CO. (Br. Pat. 1,181,023, 7.6.68. U.S., 19.6.67, 13.5.68) (23 pp.).—The pesticides, characterised by outstanding activity against *Anthonomus grandis* and *Prodenia eridania*, have formula $NR^{II}R^{III} \cdot CO \cdot C(SR^I){:}N \cdot O \cdot C\text{-}O \cdot NR^{IV}R^V$ wherein R^I–R^{III} are alkyl, alkenyl or R^{II} is, e.g., H or OMe, or R^{III} is H, or R^{II}–R^{III} together represent alkylene; R^{IV} is alkyl, allyl, or propargyl; and R^V is H or Me. In an example, MeSH is added at 5° to a mixture of $CONH_2 \cdot CCl{:}NOH$, MeOH and water, followed by 50% aq. NaOH at −5° to 0°, to pH 8. Filtered liquor is evaporated to give Me 1-carbamyl-*N*-hydroxythioformimidate, m.p. 163–164°, which is suspended in acetone contg. MeNCO, then a trace of 1,4-diazabicyclo-[2.2.2.]-octane is added, temp. rising to 55°. After some hours Me 1-carbamyl-*N*-(methylcarbamoyloxy) thioformimidate, m.p. 162–164°c, is collected. F. R. Basford.

Alkanesulphonate and α-toluenesulphonate pesticides. INTERNATIONAL MINERALS AND CHEMICAL CORP. (Br. Pat. 1,184,876, 31.5.68. U.S., 14.6.67).—Compd. such as 3,5-dichloro-2-pyridylmethanesulphonate, 6-chloro-2-pyridyl α-toluene sulphonate are prep. by reacting halogenated pyridinol with alkanesulphonyl halide and α-toluenesulphonyl halide, resp., in presence of hydrogen halide accepter. The compd., in form of dispersion, emulsion or soln. at concn. approx. 20–10,000 ppm, are used for protecting plants from pests or applied to nematode-infested soil at a rate of 2–200 lb/acre. A. Gordon.

Diseases and Pests in Livestock; Veterinary Treatments

Control of Exogenous Pests

Samorin: Protection of 60 Nigerian cattle from trypanosomiasis. B. K. NA'ISA (*Bull. epizoot. Dis. Afr.*, 1969, **17** (1), 45–54).—A dosage of 0·25 mg/kg was as effective in preventing infection and maintaining wt. gain as 0·5 mg/kg. C. V.

Bovine trypanosomiasis control in Tanzania. Comparative field trial of available chemoprophylactics under ranching conditions. E. WEISENHÜTTER, D. B. TURNER and K. A. KRISTENSEN (*Bull. epizoot. Dis. Afr.*, 1968, **16** (4), 419–424).—An arbitrary 10% trypanosome breakthrough rate indicated a period of protection of 24 weeks for Samorin (I) at 0·5 mg/kg, while Antrycid Pro-Salt RF at 7·4 mg/kg gave 26 weeks, I at 1 mg/kg gave 30 weeks and Prolkidium at 2·0 mg/kg gave 35 weeks protection. C. V.

Tick control on domestic animals. R. D. SHAW (*Tropical Sci.*, 1969, **11** (2), 113–119. Engl., 12 ref.).—A review with emphasis on the economic significance of tick infestations. W. J. G.

Preliminary laboratory tests on the effect of herbicides on ixodid ticks. S. J. MCHINJA (*Bull. epizoot. Dis. Afr.*, 1969, **17** (4), 447–451. 17 ref.).—Herbicides, MCPA (I), dalapon (II), diuron (III), bromacil (IV) and paraquat (V), were studied to determine the effect on various stages of development of *Rhipicephalus appendiculatus* (*RA*), *R. evertsi*, *Amblyomma variegatum* and *Boophilus dicoloratus*. II and III lowered the hatching rate of eggs, specially with *RA*, I and IV gave high larval mortality, but V had only a negligible effect at all stages. C. V.

Other Treatments

Detection of antibiotics in animal tissues. W. W. WRIGHT (*J. Ass. off. analyt. Chem.*, 1970, **53** (2), 219–223. 35 ref.).—Methods of sample prepn., and organisms for assay of bacitracin, carbomycin, penicillin, erythromycin, neomycin, oleandomycin, tylosin, hygromycin, novobiocin, nystatin, polymyxin, spectinomycin, streptomycin and tetracyclines in these samples, are given in tables. P. P. R.

Gastric ulcers in swine. IV. Effects of dietary particle size and crude fibre content. B. BAUSTAD and I. NAFSTAD (*Pathologia Vet.*, 1969, **6** (6), 546–556. 16 ref.).—Coarsely ground soyabean (*SB*) meal (10%) in the diet gave better protection than finely ground meal. Isolated *SB* protein had no protective action. Coarsely ground barley straw (5–10%) included in the diet gave almost full protection but at 2·5% only partial protection was noted. C. V.

Pathology of locoweed poisoning in sheep. K. R. VAN KAMPEN and L. F. JAMES (*Pathologia Vet.*, 1969, **6** (5), 413–423. 23 ref.).—C. V.

Efficacy of parbendazole in treatment of lambs naturally infected with gastrointestinal nematodes. G. LUQUE, F. G. D. BORING and J. L. EHLER (*Vet. Med.*, 1969, **64** (11), 962, 964, 966).—30 lambs were marked, weighed and grouped according to egg count (4200–32,700). An aq. suspension was given in a single drench: group I control, II 7·5, III 10·0, IV 12·5, V 15·0 mg/kg. Details of pre- and post-treatment are given. Animals were slaughtered after 7 days and gastrointestinal tracts were examined. Treatment was 100% effective in elimination of adult stages of *Haemonchus*, *Trichostrongylus*, *Osteragia* and *Bunostomum* from the abomasum and small intestine and *Clabertia* was completely eliminated from the large intestine. The activity against *Trichuris* varied. At lower dosage level, there was almost complete elimination, but at 15 mg/kg only 70% efficiency was noted. C. V.

Drug resistance in *Eimeria tenella*. VIII. Influence of quinacrine hydrochloride on the development of resistance to glycarbylamide. D. K. McLoughlin and J. L. Gardiner (*J. Parasit.*, 1969, **55** (1), 208–210).—A strain not previously exposed to drugs was serially propagated in groups of chickens fed mash containing 0·003% of glycarbylamide (I) and similar groups to which quinacrine hydrochloride (II) had also been added. Exposure to II did not prevent the development of resistance similar to that found with I above. C. V.

Effect of Coyden coccidiostat on pullets and laying hens. O. C. Bucek (*Poult. Sci.*, 1969, **48** (6), 2173–2177. 5 ref.).—Addn. of Coyden coccidiostat containing clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) at 125–500 ppm to the diet of hens in a 3-generation life cycle and a 2-yr dietary study had no significant effect on egg production or size in the 1st or 2nd generation, or on fertility or hatchability in all generations. In the 3rd generation, egg production and size were altered slightly by the treatments.
A. H. Cornfield.

Histomonastatic activity of Nifursol in chickens. R. D. Vatne, R. R. Baron and N. F. Morehouse (*Poult. Sci.*, 1969, **48** (6), 2157–2160. 3 ref.).—Addn. of 0·0025–0·01% Nifursol (3,5-dinitrosalicylic acid, 5-nitrofurfurylidene hydrazide) to the diet of infected chicks from 2 to 5 weeks of age gave 98–100% control of histomoniasis, without affecting growth. A. H. Cornfield.

Bacillus flora of the colon of large domestic animals, antibioresistance reserves. P. Pohl and J. Thomas (*Revue Ferment. Ind. aliment.*, 1969, **24** (6), 196–198. Fr., 3 ref.).—Studies on calves and pigs show that *Escherichia coli* may have acquired a resistance to antibiotics in these animals by continued contact with the normal intestinal flora. M. T. Rawnsley.

Possible relationship between mercury retention and resistance to lymphoid leukosis. V. L. Miller, G. E. Bearse, C. F. McClary and E. Csonka (*Poult. Sci.*, 1969, **48** (4), 1199–1202. 11 ref.).—Chicks resistant to lymphoid leukosis retained larger amounts of Hg from $HgCl_2$ or Ph-Hg acetate injections than did susceptible chicks. No difference was noted in Hg retention with respect to resistance to Marek's disease. A. H. Cornfield.

Kidney alkaline phosphatase in mercuric chloride-injected chicks resistant and susceptible to leukosis. V. L. Miller, J. A. McIntyre and G. E. Bearse (*Poult. Sci.*, 1969, **48** (4), 1487–1490. 19 ref.).—AP-activity was greater in the kidneys of chicks from a susceptible strain, previously shown to retain small amounts of Hg, than in the kidneys of chicks from a resistant strain, which retained large amounts of Hg. Injection of $HgCl_2$ depressed the *in vivo* kidney AP in the resistant chicks more than in the susceptible chicks, but *in vitro* AP was affected similarly by Hg in both strains.
A. H. Cornfield.

Ovicidal (pesticidal) compositions. G. E. F. A. Rohner (Br. Pat. 1,178,757, 20.6.67. Ger., 1.7.66).—CH_2Cl_2 and MeOH are claimed as the active constituents of compn. for killing coccidia in oocyst form and worm eggs, e.g., in poultry houses. The compn. are also effective against tubercle bacteria. S. S. Chissick.

Animal feed composition. Chas. Pfizer & Co. Inc. (Br. Pat. 1,180,143, 10.5.67. U.S., 18.10.66).—The compn., which are esp. useful in improving the condition of poultry infected with chronic respiratory disease (e.g., caused by *Mycoplasma gallisepticum*) and in affecting the rate of growth and feed efficiency in animals, contain 7,8,9,10-tetrahydro-6*H*-cyclohepta[*b*]quinoxaline-5,11-dioxide and/or 2,3-dihydro-1*H*-cyclopenta[*b*]quinoxaline-4,9-dioxide.
F. R. Basford.

2-Substituted pyridines as anthelmintic agents. C. Pfizer & Co., Inc. (Br. Pat. 1,188,959, 4.8.67. U.S., 18.8.66).—The agents, of value in the veterinary control of helminthiasis, comprise 1-(thien-2-yl)-2-(pyrid-2-yl)ethylene, 1-(thien-2-yl)-2-(pyrid-2-yl) ethane (I) (and acid addn. salts thereof) and deriv. containing Me in the 3-position of the heterocyclic rings. The I (hydrochloride, m.p. 80–81°) is prepd. by adding 2-methylpyridine to a soln. of KNH_2 in liquid NH_3, followed 15 min later by 2-(chloromethyl)-thiophen. F. R. Basford.

Sanitation, Hygiene and Safety

General Sanitation, Pollution

Agriculture and public health. W. R. Thrower (*Br. med. J.*, 1970, **ii** (5701), 69–74. 12 ref.).—The more common of the 140 listed zoonoses are summarized and are discussed. Compd. used in agriculture, the use of antibiotics, 'factory farming', slurry disposal, etc. are also reviewed. C. V.

L-Amino acid oxidase as bactericidal system. R. C. Skarnes (*Nature, Lond.*, 1970, **225** (5237), 1072–1073. 15 ref.).—The bactericidal activity (estimated LD_{50}) of this system vs. 8 pathogens ranged from 0·7 μg for *M. aureus* to 50 μg for *E. coli*, for enzyme concn. of 0·2–50 μg in 1 ml phosphate-buffered saline (pH 6·9) contg. mM L-leucine. The enzyme is heat-labile, insensitive to mN-KCN, and inhibited by excess L-leucine; 3 μg will kill up to 10^6 staphylococci. Catalase effectively inhibits the antibacterial activity, probably by destruction of the lethal H_2O_2 produced during aerobic oxidn. Site and mechanism of action of this H_2O_2 is discussed. W. J. Baker.

Action of amine ovicides on *Aedes aegypti* mosquitos. D. P. Wilton and R. W. Fay (*Mosquito News*, 1969, **29** (3), 361–365).—When eggs were dipped sequentially in the separate components of the amine ovicides, a 4-h interval between dips had no marked influences on effectiveness if the lipo-sol. amine was applied first. If water-sol. ethanolamine (I) came first, ½-h between dips caused substantially increased hatches. This, with other data, suggests that aliphatic amines produce a quant. change in the water impermeability of the egg shell. C. V.

Laboratory testing of promising mosquito larvicides. B. M. Glancey, K. F. Baldwin and C. S. Lofgren (*Mosquito News*, 1969, (1), 41–43).—Toxicity of 14 larvicides to larvae of fourth-instar *Anopheles quadrimaculatus* was studied; the chem. compn. is given in each case. In a further screening test, Bay 48792, 48772, 52553 and 51294, Staufer N-4548 and Velsicol FCS-303 were compared, the last three showing 100% mortality with 0·025 ppm concn. C. V.

Laboratory evaluation of some organophosphorus compounds against the larvae of *Aedes* (*o.*) *detritus* (Haliday), *Aedes* (*o.*) *caspius* (Pallas) and *Culex pipiens pipiens* L. G. Gras and J. A. Rioux (*Mosquito News*, 1969, **29** (2), 202–209. 15 ref.).— C. V.

New insecticides evaluated as non-thermal aerosols against *Aedes taeniorhynchus* (Wiedemann). G. A. Mount, N. W. Pierce and C. S. Lofgren (*Mosquito News*, 1969, **29** (1), 53–54).—Geigy GS-13005, Bay 77488 and 78182, Montecatini L561 and CIBA C9643 were tested against the standard bromophos, fenthion, malathion and naled. The findings are tabulated. C. V.

A laboratory thermal aerosol generator for testing insecticidal aerosols. C. B. Rathburn, jun. (*Mosquito News*, 1969, **29** (1), 1–6). C. V.

Field effectiveness of five organophosphorus compounds as thermal fogs. R. T. Taylor and H. F. Schoof (*Mosquito News*, 1969, **29** (1), 7–8).—Malathion, Gardona (= tetrachlorvinphos), bromophos, Abate (*O,O*-dimethyl phosphorothioate *O,O*-diester with 4,4′-thiodiphenol), Bay 77488 [(*o*-chlorphenyl) glyoxylonitrile oxime *O,O* diethyl phosphoroate)] and Bay 78182 (phenyl glyoxylonitrile oxime *O,O*-diethyl phosphorothioate) were tested and compared. C. V.

Absorption of sodium monofluoroacetate ('1080') solution by carrot baits. E. L. J. Staples (*N.Z. Jl agric. Res.*, 1969, **12** (4), 783–788. 3 ref.).—Poison was absorbed better by skinned carrots, but an immersion technique and a cutting machine to produce uniform size and uniform distribution must be devised to obtain full efficiency. It is pointed out that uneaten bait must be removed or thoroughly washed to ensure safety of domestic stock.
M. T. Rawnsley.

Food Hygiene and Safety

Chemistry of aflatoxins. K. K. Maggon, L. Viswanathan and T. A. Venkitasubramanian (*J. scient. ind. Res.*, 1970, **29** (1), 8–17. 137 ref.).—Methods for their separation, purification and estimation are described. Their phys. and chem. properties, as well as recent reports on their stereochemistry and synthesis, are collated. Research covering the past decade is discussed and also the biological effects of aflatoxins in relation to their structures. Current ideas on their biogenesis are presented and attention is focussed on the scope for future work. F. C. Sutton.

Problems of transport and storage of groundnut seeds and meal. R. D'Agata (*Riv. ital. Sostanze Grasse*, 1970, **47** (2), 67–72. It.).—The problems are discussed with particular reference to infection with *Aspergillus flavus* and prevention of aflatoxin formation.
L. A. O'Neill.

Bacteriology of foodstuffs. R. Lambion and A. Veulemans (*Revue Ferment. Ind. aliment.*, 1969, **24** (5), 168–176. Fr., 20 ref.).—Figures given since 1965 for Belgian cases of typhoid, paratyphoid and other *Salmonella* infections are analysed, and show that the most dangerous foods are meats, esp. pork. Other organisms

besides *Salmonella* have been shown to cause toxic infections, e.g., *Shigella, Clostridium botulinum, Cl. perfringens, Proteus, Escherichia coli, Klebsiella, Mycobacterium bovis, Bacillus cereus, Streptococcus* sp., *Pasteurella* sp., *Brucella* sp., *Listeria* sp., and *Staphylococcus* spp. The symptoms of food poisoning caused by these organisms are discussed. Legal requirements for testing and specification are also considered in detail. M. T. Rawnsley.

Incidence of various groups of bacteria in dehydrated onions and garlic. R. H. VAUGHN (*Fd Technol., Champaign*, 1970, **24** (2), 189–191. 12 ref.).—Samples were diluted as required from the stock soln. of 20 g sample/180 ml of sterile distilled H_2O. Tests were made for detection of thermophilic spores, enumeration of coliform and lactic acid bacteria, and detection of pectinolytic and psychrophilic bacteria. No one group of the bacteria served as an index for microbial quality of the dehydrated onions and garlic. However the total viable count was useful for microbial quality control purposes. M. J. Rawlins.

Micro-organism content of baby and infant foods, tonics and deep frozen vegetables—a comparative study. G. FROMM and U. STRAHTMANN (*Dt. LebensmittRdsch.*, 1970, **66** (1), 1–6. Ger., 20 ref.).—Milk based foods, fruit juices and purées have been examined by standard methods from the food regulations. 0–500 organisms/ml were present in milk products whilst other prepn. had < 100/ml and half were germ-free. All were well below legal limits. 70% of frozen vegetables were above the limit of 10^4/g. Tests for coli were positive. It is recommended that frozen foods should only be used with extreme caution for infants. J. B. Woof.

Deep-frozen, ready to eat dishes having a meat base. Methods and results of bacteriological and organoleptic tests. R. LEVETZOW, H.-J. BAUMGARTEN and D. GROSSKLAUS (*Fleischwirtschaft*, 1969, **49** (8), 1025–1032. Ger., 32 ref.).—Eighty-eight ready to eat menus, which had been stored and thawed under different conditions, were examined for total count, Enterobacteriaceae, enterococci, micrococci/staphylococci, aerobic spore formers, moulds and clostridia. No salmonellae could be isolated, and the products were considered to be hygienically and organoleptically satisfactory. P. P. R.

4′-(3,3-Dimethyl-1-triazeno) acetanilide to protect packaged cereals against stored products insects. S. R. LOSCHIAVO (*Fd Technol., Champaign*, 1970, **24** (4), 485–489. 9 ref.).—The compd. was used as a surface coating on a variety of cereal packages. Poorly constructed bags, whether coated or not, were infested within 2 months. Plastic or plastic-lined bags were successful for 6 months, and treated bags, of closely-woven unbleached cotton, were not infested during 6 months. M. J. Rawlins.

Contamination by Pesticides, Pesticide Toxicity

Pesticides in water. K. H. DEUBERT and I. E. DEMORANVILLE (*Pestic. Monitoring J.*, 1970, **4** (1), 11–13. 7 ref.).—Rate at which Cu disappeared from H_2O of Cranberry bogs treated with $CuSO_4$ was measured. H_2O samples taken 1, 3, 6, 8, 10 and 28 days after treatment were analysed. After 6 days, 91% of Cu had disappeared leaving 0·07 ppm in the floodwater. There was a difference between surface and subsurface H_2O. M. J. Rawlins.

Distribution of arsenic from poultry litter in broiler chickens, soil and crops. J. L. MORRISON (*J. agric. Fd Chem.*, 1969, **17** (6), 1288–1290, 27 ref.).—The As content in litter from poultry houses in which arsenicals had been used, did not affect the As content of poultry tissue or feathers. As content of soils or crops grown on soils treated with litter was not affected. I. Dickinson.

Extraction and colorimetric determination of picloram in soil. H. H. CHENG (*J. agric. Fd Chem.*, 1969, **17** (6), 1174–1177. 17 ref.).—Picloram (4-amino-3,5,6-trichloropicolinic acid) is diazotised with nitrite in H_2SO_4 soln. The colour developed is stable in the absence of u.v. radiation without a coupling agent; the reaction is specific and not subject to interference from substances commonly present in soil. Picloram can be quant. extracted from most soils by 1 N-NH_4OAc, 2 N-KCl, or a KCl–KOH soln. The method is rapid and suitable for routine analyses for a picloram content of > 0·5 ppm. I. Dickinson.

Fate of diazinon in submerged soil. Accumulation of hydrolysis product. N. SETHUNATHAN and T. YOSHIDA (*J. agric. Fd Chem.*, 1969, **17** (6), 1192–1195. 11 ref.).—In a study of the persistence of diazinon (^{14}C-labelled at the 4-position on the pyrimidine ring) in submerged soils, soil microflora appeared to assist in its degradation into a less toxic hydrolysis product (2-isopropyl-6-methyl-4-hydroxy pyrimidine). This hydrolysis product was resistant to further degradation under submerged conditions. I. Dickinson.

Removal of [the organophosphate insecticides] Gardona and Azodrin from vegetable crops by commercial preparative methods. J. E. FAHEY, G. E. GOULD and P. E. NELSON (*J. agric. Fd Chem.*, 1969, **17** (6), 1204–1206. 23 ref.).—Sweetcorn, green beans and tomatoes were treated with Gardona at rates of 1 and 2 lb/acre. Two spray applications were made 10 days and 24 h resp. before harvesting. Most of the insecticide residue on sweetcorn was removed in husking. No residue was found in canned corn, and ≤ 0·01 ppm in canned beans. Azodrin was more resistant to removal by processing than Gardona; canned green beans contained 0·01 to 0·28 ppm of Azodrin. Juice from washed tomatoes contained 0·03 to 0·09 ppm of Gardona; tomatoes canned after blanching and peeling contained 0·01 to 0·03 ppm. I. Dickinson.

Extraction and clean-up methods to determine malathion and its hydrolytic products in stored grains by gas–liquid chromatography. A. M. KADOUM (*J. agric. Fd Chem.*, 1969, **17** (6), 1178–1180. 10 ref.).—Malathion (*O,O*-dimethyl dithiophosphate of diethyl-mercaptosuccinate) (**I**) is used to protect grains from insects. The acids are quant. converted to their Me esters by reaction with BF_3–MeOH. *O*-demethyl deriv. of **I** apparently cannot be esterified by BF_3–MeOH, and incomplete esterification was obtained by the use of diazomethane. This method is suitable for detn. of **I**, half-ester, and dicarboxylic acid deriv. of **I** in the presence of waxes and pigments of the grain sorghum, corn and wheat. I. Dickinson.

Previously unreported homologue of malathion found as residue on crops. A. M. GARDNER, J. N. DAMICO, E. A. HANSEN *et al.* (*J. agric. Fd Chem.*, 1969, **17** (6), 1181–1185. 10 ref.).—Quant. data from g.l.c. indicated that residues of an unidentified compd. were more persistent than those of malathion (**I**). The compd. was isolated and collected from malathion, 50% emulsifiable concentrate, by g.l.c. fractionation. By the use of n.m.r. and mass spectrometry, it was identified as the Et Bu mercaptosuccinate, *S*-ester with *O,O*-dimethyl phosphorodithioate, a homologue of **I**. The analogue of this compd. was five times as inhibitory as the O analogue of **I** towards bovine erythrocyte cholinesterase. I. Dickinson.

Extraction, clean-up, and gas-liquid chromatographic method for the analysis of captan, folpet and difolatan in crops. I. H. POMERANTZ, L. J. MILLER and G. KAVA (*J. Ass. off. analyt. Chem.*, 1970, **53** (1), 154–157. 12 ref.).—Carrots, cabbage or soyabeans (≡ from 0·1 to 2 ppm of each fungicide) were extracted with CH_3CN. The fungicides were partitioned into CH_2Cl_2–light petroleum, cleaned up on a Florisil column and analysed by g.l.c. with use of QF-1 or XF-60 as column packing and electron capture detection. Recoveries of from 80 to 110% were obtained. D. I. Rees.

Decontamination of malathion from Ladino clover seed screenings. T. E. ARCHER (*Poult. Sci.*, 1969, **48** (6), 2075–2080. 17 ref.).—Several extraction methods tested removed 82% or more of malathion (*M*) residues from clover seed screenings, but had no significant effects on gross chem. compn. except for a decrease in fat % with some methods. 'Vapour' washing with C_6H_6, water, or 15% aq. NH_3, which removed 90% or more of *M*, is the preferred method. A. H. Cornfield.

Chemical studies on tobacco smoke. IX. Quantitative analysis of chlorinated hydrocarbon insecticides. D. HOFFMANN, G. RATHKAMP and E. L. WYNDER (*Beitr. Tabakforsch.*, 1969, **5** (3), 140–148. Engl., 21 ref.).—The insecticides are isolated from the smoke by liquid–liquid extractions, chromatog. on deactivated Al_2O_3 and g.c. The effluent from the g.c. column is used for mass spectrometric analysis; for quant. analysis ^{14}C-labelled DDT is used as internal standard and the amt. of insecticides detd. by g.c. with electron capture detection. Some pyrolysis expt. were carried out on DDD at 880; the products identified include 9-methylene fluorene and 1-chloro-2,2-(*p*-chlorophenyl) ethane. P. P. R.

Pesticide residues as health hazard: United Kingdom viewpoint. R. GOULDING (*Wld Rev. Pest Control*, 1969, **8** (4), 171–177. 7 ref.).— W. J. G.

DDT-induced inhibition of avian shell gland anhydrase: a mechanism for thin eggshells. J. ZITMAN, H. C. CECIL and G. F. FRIES (*Science N.Y.*, 1970, **168** (3931), 544–546. 16 ref.).—It was found that feeding with *p,p′*-DDT or *p,p′*-DDE resulted in a 16–19% lower carbonic anhydrase activity than those kept on pesticide-free diet. C. V.

Teratogenic evaluation of 2,4,5-T. K. D. COURTNEY, D. W. GAYLOR, M. D. HOGAN, H. L. FALK *et al.* (*Science, N.Y.*, 1970, **168** (3933), 864–866).—This herbicide was also found to be foeto-

cidal, using two strains of mice, when administered orally or subcutaneously. The incidence of cystic kidney was also measured in rats. Other evidence is presented and is discussed. C. V.

Acute and subchronic toxicity to Japanese quail of the carbamate insecticides, carbofuran and SD 8530. M. SHERMAN and E. ROSS (*Poult. Sci.*, 1969, **48** (6), 2013–2018. 13 ref.).—The LD_{50} of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) to the male and female quail was 0·0019 and 0·0017 g per kg body wt., resp. For SD 8530 (3,4,5-trimethylphenyl methylcarbamate), the values were 0·0173 and the 0·0164 g per kg body wt. Addn. of SD 8530 (> 400 ppm) to the feed depressed wt. gains, feed efficiency and egg production. Carbofuran was toxic at levels > 100 ppm in the feed. A. H. Cornfield.

Fate of a single dose of carbaryl (1-naphthyl *N*-methylcarbamate) in the chicken. G. D. PAULSON and V. J. FEIL (*Poult. Sci.*, 1969, **48** (5), 1593–1597. 17 ref.).—Tests with ^{14}C carbonyl- or ring-labelled carbaryl (used for control of ectoparasites in poultry litter) showed that urine was the primary source of excretion when a single dose (0·01 g per kg body wt.) was given. ^{14}C remaining in the carcass 48 h after dosing accounted for 1·4–7·1% of the dose. Eggs laid 12 days after dosing contained 0·33% of the ^{14}C administered. A. H. Cornfield.

Effect of various levels of dietary malathion on performance of chicks. B. M. REHFELD, D. E. PRATT and M. L. SUNDE (*Poult. Sci.*, 1969, **48** (5), 1718–1723. 6 ref.).—Chicks receiving 1000 ppm malathion (*M*) to 6 weeks of age showed no adverse effects, whilst 5000 ppm *M* was lethal within 19 days. Except for decreased wt. gains, 1- and 25-day-old chicks tolerated 5000 ppm *M* for up to 1 week, and then showed normal wt. gains when returned to a *M*-free diet. When given free choice, chicks showed a strong preference for normal over *M*-contg. diets. A. H. Cornfield.

4.—MISCELLANEOUS

Biological air filtration for animal quarters. J. E. GEHRKE-MANNING (*Filtration Separation*, 1969, **6** (1), 25–26, 29).—The laminar aseptic airflow barrier system at the Rikev laboratories is illustrated and the system is discussed. The possible extension to hospitals is also examined. C. V.

Bonn radiocarbon measurements. I. H. W. SHARP-ENSEEL, F. PIETIG and M. A. TAMERS (*Radiocarbon*, 1968, **10** (1), 8–28. 32 ref.).—Ground water samples, soils, winter wheat and rye, grass, sugar and fodder beet leaves and wines (1958–66) are examined. C. V.

Determination of mineral elements in biological material by atomic absorption spectrophotometry. G. K. JUDEL and S. HEILENZ (*Z. PflErnähr. Bodenk.*, 1969, **124** (1), 43–51. Ger., 6 ref.).—Lower limits of concn. are given for Ca, Co, Cu, Fe, Mg, Mn, Pb and Zn, for both the propane and acetylene burner. Optimum working conditions for these two burners are suggested and their advantages or otherwise are discussed. Interferences from accompanying elements were best eliminated by addn. of the interfering element in excess. M. Long.

The Harvey lectures. 1969, No. 63, 323 pp. (New York, London: Academic Press).—The following are amongst the nine communications listed: **Microbial persistence.** W. MCDERMOTT (27 ref.). This state is defined as the susceptibility of a drug *in vitro* as compared to the capacity to survive long-term exposure *in vivo*. **Polypeptide conformation.** H. A. SCHERGA (79 ref.). **Synthesis and utilisation of glutamine.** A. MEISTER (98 ref.). **Structure and function of haemoglobin.** M. F. MUNTZ (57 ref.). C. V.

Plants used against cancer: a survey. J. L. HARTWELL (*Lloydia*, 1969, **32** (2), 153–205).—A continuation of earlier work (*ibid.*, **30**, 379–436; **31**, 71–170; **32**, 79–107), with more than 900 ref. C. V.

'Review of microbiology'. C. E. CLIFTON, S. RAFFEL and M. P. STARR (Eds.) (*A. Rev. Microbiol.*, 1969, **23**, 622 pp.).—20 contributions are presented; specially mentioned are: **Regulation of enzyme function.** D. E. ATKINSON (169 ref.). ***Bacillus thuringiensis*: microbiological considerations.** M. H. ROGOFF and A. A. YOUSTEN (105 ref.). **Chemotherapy of drug-resistant malarias.** L. H. SCHMIDT (176 ref.). **Carbohydrate metabolism in microorganisms.** R. L. ANDERSON and W. A. WOOD (211 ref.). C. V.

5.—RECENT BOOKS AND JOURNALS

Agricultural potentialities of world climates. J. PAPADAKIS. (Buenos Aires: Imprenta coni S.A.C.I.F.I.). P. P. R.

Climates of the world—their classification, similitudes, differences and geographic distribution. J. PAPADAKIS (Buenos Aires: Imprenta coni S.A.C.I.F.I.).— P. P. R.

Fundamentals of agronomy (Compendium of crop ecology). J. PAPADAKIS (Buenos Aires: Imprenta coni S.A.C.I.F.I.).— P. P. R.

Pest control in rice (PANS Manual No. 3), 1970, 270 pp. Price: 12/6 (London: Ministry of Overseas Development).—The distribution, symptoms, life-cycles and methods of control for the major diseases and pests of rice are discussed under the following headings: weeds; diseases; nematodes; molluscs; crustacea; insects; birds; rodents; storage. The book is well illustrated with clear line drawings of the pests mentioned, together with many plates including 15 in colour. A useful check-list of diseases is also given. C. J. R.

Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilisers, anticaking agents, and certain other substances. XIIIth Report FAO/WHO Committee on Food Additives. (*Wld Hlth Orgn tech. Rep. Ser.*, 1970, (445), 36 pp. Price 6/-. Fr., Russ. and Sp. edn in prepn.).—Principles governing the toxicological evaluation of food colours, modified starches and certain natural spices, gums, etc. are reviewed. The next section contains details of acceptance or rejection of certain members of the following classes of compd.: Food colours from natural sources; synthetic food colours; (modified) starches; natural gums; modified fats and fatty acids; other emulsifiers and stabilisers, e.g., hydroxypropyl cellulose, fruit pectin; anticaking agents (ferrocyanates, phosphates, silicates, fatty acid salts); miscellaneous additives (e.g., glutamates, ascorbyl stearate, $CaSO_4$). Certain recommendations to FAO and WHO are set forth. P. P. R.

***CRC Critical Reviews in Food Technology*, 1970, 1** (1), quarterly. (Cleveland, Ohio: The Chemical Rubber Co.).— W. J. G.

***CRC Critical Reviews in Environmental Control*, 1970, 1** (1), quarterly. (Cleveland, Ohio: The Chemical Rubber Co.).— W. J. G.

***La Recherche*, 1970, 1** (1), Fr., monthly. (Paris: Société d'édition scientifiques).— W. J. G.

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