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# THE DISTRIBUTION OF TRACE ELEMENTS IN COCKSFOOT *(Dactylis glomerata)* AT FLOWERING

By B. G. DAVEY* and R. L. MITCHELL

Comparison of the contents of 24 trace and major elements in the various parts of cocksfoot at the flowering stage shows marked diversity in the distribution of the elements. The different plant parts contribute very different amounts of different elements. The stem, which accounts for 54% of the dry matter, generally contributes most, although on a dry matter basis there is seldom concentration in the stem. Concentration occurs mainly in the leaves and the spikelets which respectively contribute 9 per cent and 20 per cent of the dry matter. The spikelets contain nearly half of the Ni, Zn and Si. Calcium is relatively most abundant in the leaf: only Ni, Se and P do not show concentration in this organ. The contributions of elements by the sheath seldom differ much from its dry matter contribution of 17 per cent. Considerable differences also occur between the same organs from different nodes: the rachis leaf contains 8 times as much Zn as the lowest leaves. The possible significance to animal nutrition of the distribution in different plant parts is pointed out.

## Introduction

Although the functions of many trace elements in plants have been established, detailed knowledge of their distribution throughout the plant is still rather incomplete. Information is available for some elements, but there have been few comprehensive examinations covering different plant-parts. In the course of a study of the seasonal variation of trace elements in pasture herbage[1] the effect of variations in the distribution within the plant on analytical interpretation became obvious and this investigation was initiated. A grass, *Dactylis glomerata* (cocksfoot or orchard grass), was examined because samples of adequate size were readily obtainable and because preliminary results suggested that it would be a convenient subject for investigation. A suitable, readily identifiable stage of growth occurs when the plant is in full flower, and this stage was chosen for this initial study. Early in July 1956 sufficient plants were collected from a field of four year old mixed herbage on a poorly drained soil derived from a mixed drift (Tarves association, Pitmedden series[2]) on the University of Aberdeen farm eleven miles north of Aberdeen. Publication of the findings, which include comparative results for major elements, has been delayed until results became available for a number of elements not among those initially determined. The aim of the investigation was to study the contents of trace elements from the aspects of soil–plant and plant–animal relationships rather than from the plant-physiological standpoint.

A number of workers have studied the distribution of one or more elements in various plant parts of cereals or herbage plants. Generally, these have been concerned with major sub-division into leaf, stem, seedhead and on occasion root. Few comprehensive studies covering a number of elements in different parts at various heights within the plant have been found in the literature. Miller[3] reports results covering 14 major and trace elements in leaves, stems, grain, cobs and roots of mature corn plants grown in 1920. Fleming[4] presents information for 20 elements in head, leaf and stem of five constituent species of pasture herbage at early maturity (well-formed heads). Williams[5] dealt with Cu, Zn, Mn, Mo and Fe in subterranean clover at different stages of growth, and Bishop[6] determined Mn in maize. Semi-quantitative radio-autographic techniques have been applied by numerous workers, including Evans *et al.*,[7] Romney and Toth[8] and Riceman and Jones.[9]

Herman, Cornil and Ledent[10] have reported results for 14 elements in rib, heart and green and yellowed leaves of healthy and unhealthy endives. No marked differences between healthy and unhealthy plants were found in the distribution of any element, although the absolute levels varied. Wilkinson & Gross[22] have examined the distribution in *Trifolium repens* grown in solution culture, of ten elements in seven different plant parts including leaflets and petioles of three different ages.

## Experimental

Samples of spikelets, leaf blades (leaves), leaf sheaths (sheaths) and stems were obtained by separation of the above-ground portion, cut two inches above soil level, into fractions corresponding to the different nodes, as illustrated in Fig. 1. In all, ten separate plant parts were sampled. The plants selected were as nearly uniform in height as possible and generally had four nodes. The method of separation ensured that the uppermost, young leaves from the rachis node (A) and those from the node immediately below this (B) were always contained in the same samples whatever the number of nodes in the plant. In order to give an adequate sample for analysis, the leaves from the lower third and fourth nodes (C), which were often becoming senescent, were combined, as were the sheath and stem samples from these nodes. Some hundreds of plants with a total dry weight of 1200 g were sub-divided in order to provide adequately large composite samples of the various plant parts. A sample of plants that had not been sub-divided was analysed in order to obtain results for whole plants to compare with those for the summation of the contents of the plant parts.

The contents of 19 major and trace constituents were determined by the spectrochemical methods described by Mitchell.[11,12] For Fe[13], Se[14], P, S and N appropriate chemical methods were employed. Analyses for Se were provided by Johnstown Castle Agricultural College, Wexford, Ireland.

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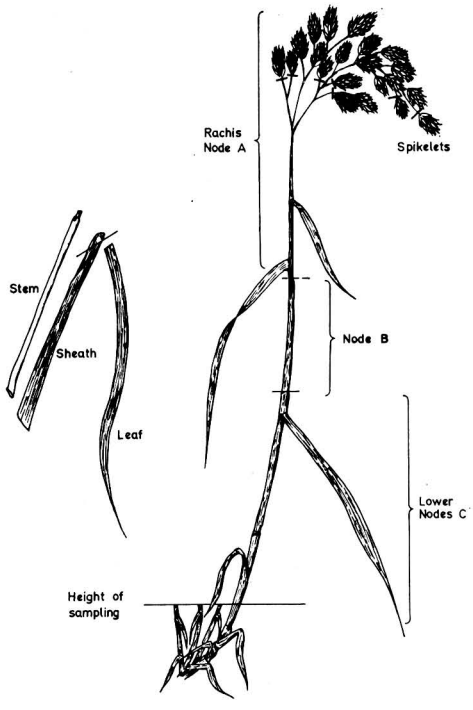



FIG. 1. *Details of sampling and separation procedure*

## Results

### Proportion and contribution of the plant parts

The contributions of the various plant parts to the weight of the whole plant, on an oven-dry basis, and the ash contents of the oven-dry materials were as follows:

| Plant part | % Contribution | % Ash |
|---|---|---|
| Spikelets | 20·2 | 7·1 |
| Leaf rachis node A | 2·5 | 9·1 |
| Leaf node B | 3·2 | 10·4 |
| Leaf lower nodes C | 3·2 | 10·0 |
| Sheath rachis node A | 5·6 | 5·8 |
| Sheath node B | 6·7 | 5·8 |
| Sheath lower nodes C | 4·5 | 7·3 |
| Stem rachis node A | 12·8 | 3·5 |
| Stem node B | 20·6 | 3·7 |
| Stem lower nodes C | 20·6 | 3·7 |
| Whole plant | — | 4·5 |

The plants thus comprised approximately 54% stem, 17% sheath, 9% leaf and 20% spikelet. The small contribution of the leaves to the total dry weight is noteworthy. On the other hand, the leaf had an ash content considerably higher than that of the other parts, the contribution to the total ash content being 36·9% stem, 19·7% sheath, 16·5% leaf and 26·8% spikelet.

To facilitate comparison with the findings of earlier investigators, the percentage contributions of combined leaf, sheath and stem samples have been calculated and are given in Table I. Unfortunately, direct comparison with Fleming's results is difficult because only contents and not relative contributions of the plant parts were given in his report.[4]

From Table I it will be seen that the various major and trace constituents were distributed very differently in the various plant parts. The largest amounts of many of the elements, notably P, S, K, Na, Ba, Co, Mo, Mn, Cu and Se were in the stem, some 40%–50% of their total contents being found there, but even for K for which the stem contribution is greatest there was no significant concentration in the stem on a dry weight basis. The proportion of Ni in the stem was as high as for several of the above elements, but, exceptionally, an even greater proportion was located in the spikelet. A

TABLE I

**Percentage contribution of the different plant parts to the contents of different elements in the whole of the above-ground portion of the plant**

| | Si | P | N | S | K | Na | Mg | Ca |
|---|---|---|---|---|---|---|---|---|
| Spikelets | 49·8 | 33·2 | 37·6 | 33·5 | 12·6 | 11·6 | 21·4 | 17·6 |
| Leaf | 16·5 | 8·9 | 17·0 | 14·3 | 12·5 | 21·3 | 20·5 | 41·5 |
| Sheath | 25·4 | 11·7 | 16·1 | 14·0 | 19·5 | 22·1 | 18·4 | 22·1 |
| Stem | 8·4 | 46·1 | 29·3 | 38·3 | 55·5 | 45·2 | 39·9 | 18·8 |
| | Sr | Ba | Co | Ni | Fe | Al | Zn | Mo |
| Spikelets | 17·8 | 13·2 | 27·2 | 46·6 | 36·0 | 22·5 | 47·1 | 26·6 |
| Leaf | 26·0 | 23·5 | 11·8 | 7·2 | 29·0 | 31·2 | 17·1 | 22·6 |
| Sheath | 23·2 | 19·6 | 11·1 | 6·2 | 20·7 | 20·9 | 11·5 | 11·6 |
| Stem | 33·0 | 43·6 | 49·9 | 40·1 | 14·3 | 25·3 | 24·3 | 39·2 |
| | Cu | Mn | B | Pb | Se | Cr | V | Ti |
| Spikelets | 27·2 | 23·4 | 27·8 | 24·2 | 30·0 | 29·0 | 29·5 | 27·0 |
| Leaf | 13·3 | 14·4 | 21·8 | 35·9 | 6·7 | 34·4 | 27·7 | 39·3 |
| Sheath | 12·2 | 24·0 | 17·7 | 13·6 | 14·5 | 18·3 | 18·6 | 24·2 |
| Stem | 47·4 | 38·2 | 33·0 | 26·2 | 49·0 | 18·4 | 24·6 | 9·4 |

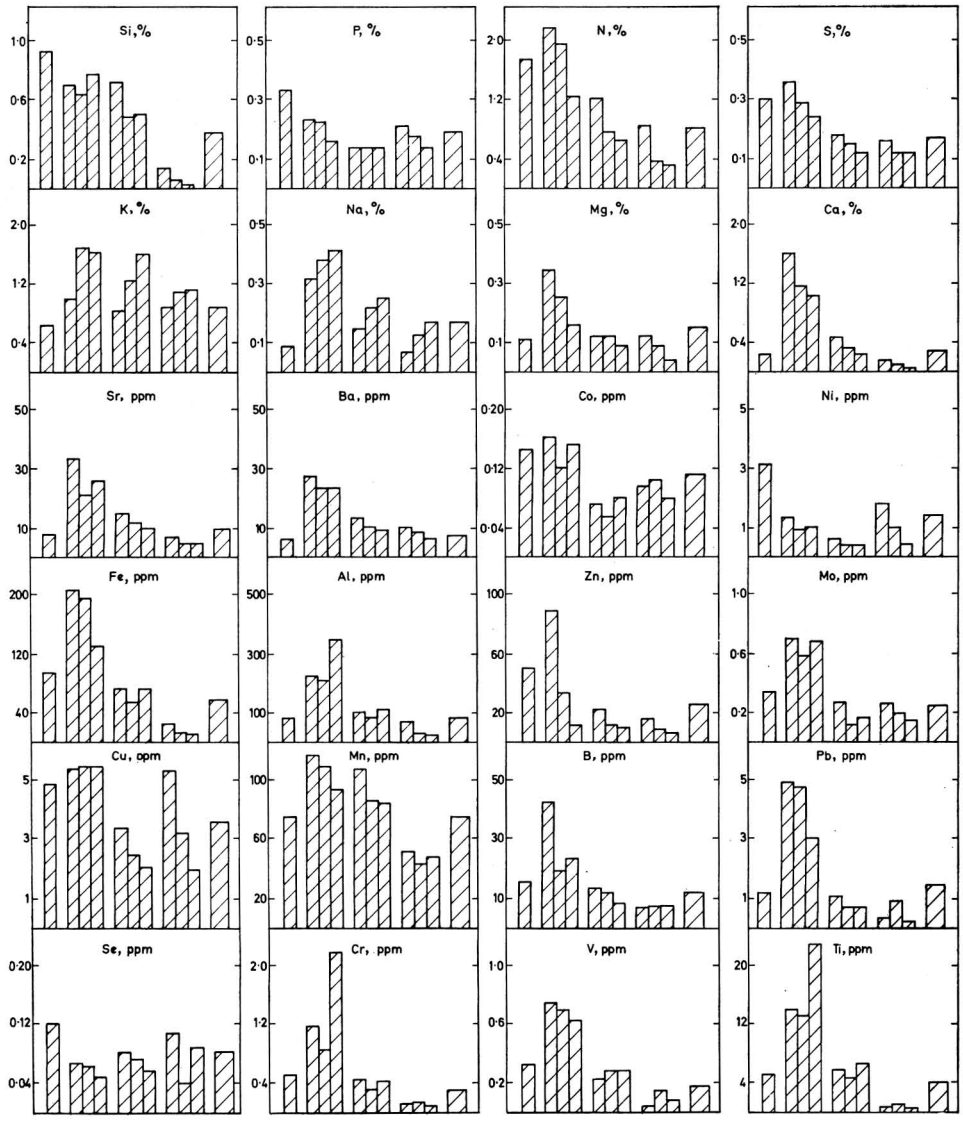



FIG. 2. *Contents of different elements in individual plant parts, expressed as parts per million or percent in dry matter*
Histograms for each element reading from left to right: Spikelet; Leaf A, B, C; Sheath A, B, C; Stem A, B, C; Whole plant

particularly small proportion of Si occurred in the stem, most being found in the spikelets. The stem also contributed relatively small amounts of Ti, Fe, Ca and Cr. Only 8·9% of the dry weight of the plant was contributed by the leaves, so that for all elements except Ni, Se and P there was, on this basis, some concentration in the leaf. The contributions of the sheaths were generally much closer to the dry weight distribution, with Ni again an obvious exception.

**Results for individual plant parts**

The analytical findings for the 24 elements in the individual plant parts are presented graphically in Fig. 2 and Fig. 3. In Fig. 2 the results are expressed in terms of the contents of the individual parts as % or parts per million. In Fig. 3 the percentage contributions of the individual parts to the amount in the whole plant are shown diagrammatically, the plant parts, separated by horizontal marks, being identified by the positions they occupy, as illustrated in the key diagram. The contribution of any part is indicated by the length of the line representing that plant part: the total length of line, corresponding to the total content in the whole plant, i.e. to a contribution of 100%, is shown and is the same in each of the element diagrams.

In general, reasonably good agreement was obtained between the contents found for the whole plant and the summations of those for the individual parts, taking into account their relative proportions. This comparison is presented in Table II, in which it will be seen that only for Mg and B was the difference greater than might be expected in view of the various sources of error which can arise in such comparisons. The reason for the discrepancy with Mg, for which the porous-cup solution-spark analytical technique is quite precise, is not known. The results for B are more suspect, as slight B contamination may have occurred during storage of the samples in paper before the possibility of this contamination was appreciated (see Mitchell[11]). For Ag, for which insufficient information was available to justify presentation in diagrammatic form, the whole plant content was 0·02 ppm.

## Discussion

**Variability of different elements**

The elements in Table II are arranged in order of total content in the above-ground portion of the plant. The 10-fold increase in content between Al and Mg is worthy of notice: in other plant species with high normal Al, Mn and Fe contents such a wide gap may not occur.

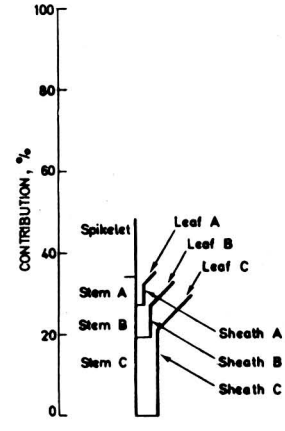
It is apparent from a superficial inspection of Fig. 2 that there are great differences in the pattern of distribution of various elements throughout the plant, but before considering these in more detail it is of interest to examine the magnitude of the variation occurring for each element. This can be assessed either from the ratio of the highest to the lowest concentration found in the ten parts examined, or as the ratio of the absolute amounts by weight in these parts. The latter is of course particularly dependent on the chosen sub-division. Results by both methods of calculation are presented in Table III. On the concentration basis, P, K, Mn, Ca, Cu, Co, S, Se and Ba are least variable throughout the plant, with a spread of less than 5 times, while Si and Ti are 50 times as abundant in some parts as in others, chiefly because of a very low content in the lower part of the stem. When the absolute amounts in each plant part are considered, only Sr, Ca, Al, Ba and Na have ranges of less than 5-fold and only for Ni and Si is the range greater than 30-fold. Much greater ranges might be expected if a more detailed separation on a plant-physiological basis were made.

**Distribution within the plant**

In the first 8 elements in Fig. 2, i.e. those present in the whole plant in amount exceeding 0·1%, namely Si, P, N, S, K, Na, Mg and Ca, the most obvious differences in distribution is in the content of the spikelet, which is high for Si and P, intermediate for N and S, and low, particularly relative to the leaves, for the others. Both K and Na show an increase in content of all organs towards the base of the plant, while the others show the opposite trend. It is perhaps surprising to find the oldest leaves to be highest in K, as Miller[3] states that all meristematic tissues and organs with high metabolic rates are rich in K. The explanation is possibly related to the removal of K from the aerial parts during the period of reduced anabolic activity at flowering, observed by Burd[15] in barley.

It is interesting to compare the distribution of Ca, Sr and Ba. The ratios of leaf to stem contents for Ca are about twice as great as those for Sr and three times those for Ba, indicating a preferential transport of Ca from the stem to the leaf compared with Sr and Ba. Comparison of the Ca/Sr and Ca/Ba ratios in the whole plant and in the various parts substantiates this:

| | Ca/Sr | Ca/Ba |
|---|---|---|
| Whole Plant | 715 | 1420 |
| Spikelet | 660 | 1320 |
| Leaf A | 1000 | 1950 |
| B | 1220 | 1720 |
| C | 880 | 1500 |
| Sheath A | 695 | 1210 |
| B | 595 | 1090 |
| C | 610 | 1000 |
| Stem A | 490 | 540 |
| B | 375 | 370 |
| C | 290 | 360 |




Key to FIG. 3

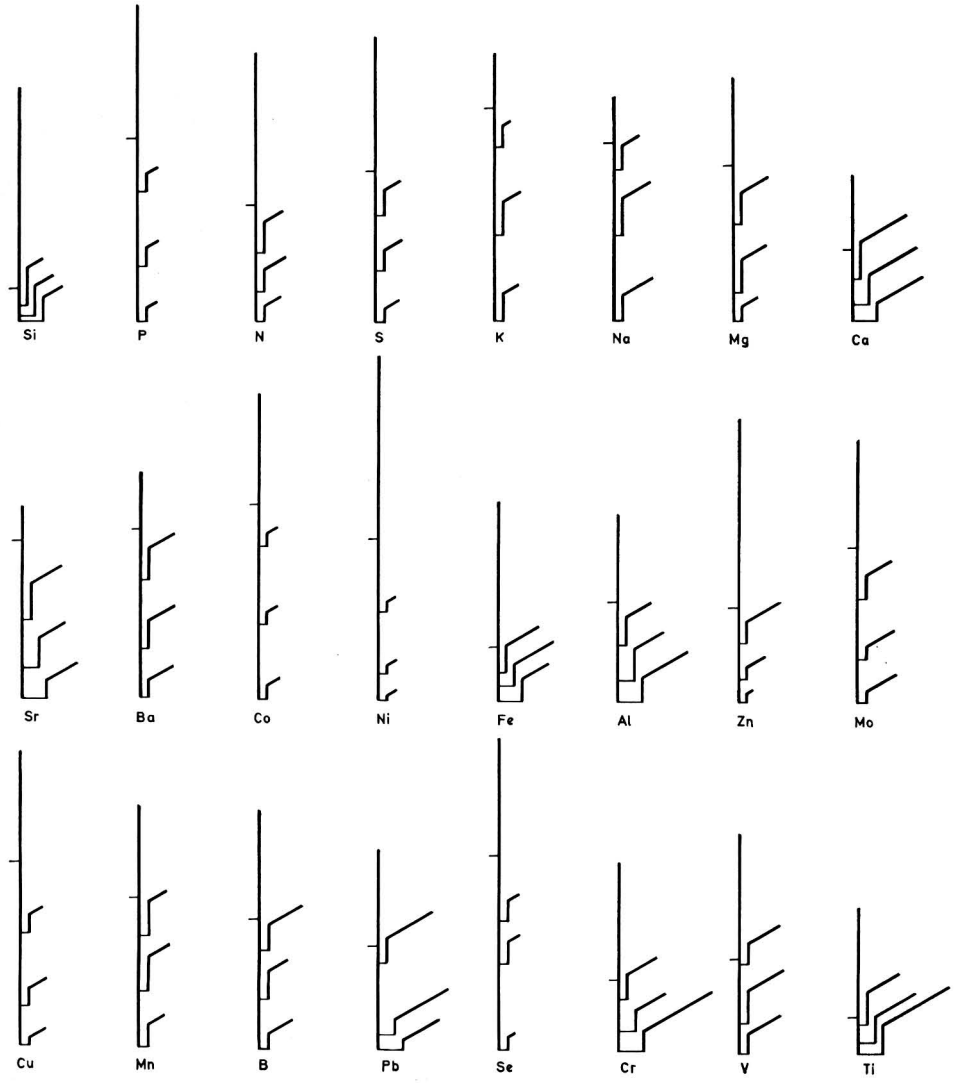



FIG. 3. *Diagrammatic representation of the percentage contribution of individual plant parts to the content of different elements in the whole plant*

% contributions of the ten plant parts together = 100

TABLE II

**Contents in the whole plant as determined and as calculated by summation of the contributions of the individual parts**

(parts per million or per cent in dry matter)

| | | Determined | Calculated | | | Determined | Calculated |
|---|---|---|---|---|---|---|---|
| K | % | 0·91 | 1·05 | Sr | ppm | 8·9 | 9·0 |
| N | % | 0·82 | 0·92 | Ba | ppm | 6·8 | 9·3 |
| Si | % | 0·34 | 0·37 | B | ppm | 6·7 | 11·3 |
| Ca | % | 0·29 | 0·27 | Ti | ppm | 3·9 | 3·8 |
| P | % | 0·19 | 0·20 | Cu | ppm | 3·5 | 3·6 |
| S | % | 0·17 | 0·18 | Pb | ppm | 1·4 | 1·0 |
| Na | % | 0·17 | 0·16 | Ni | ppm | 1·4 | 1·4 |
| Mg | % | 0·15 | 0·10 | Cr | ppm | 0·31 | 0·37 |
| Al | ppm | 80 | 75 | Mo | ppm | 0·24 | 0·26 |
| Mn | ppm | 74 | 65 | V | ppm | 0·18 | 0·22 |
| Fe | ppm | 55 | 53 | Co | ppm | 0·14 | 0·14 |
| Zn | ppm | 24 | 21 | Se | ppm | 0·10 | 0·10 |

TABLE III

**Order of variability of different elements in different plant parts as ratio of maximum to minimum content**

| Absolute Amount Basis | | Concentration Basis | |
|---|---|---|---|
| Si | 45 | Ti | 56 |
| Ni | 34 | Si | 51 |
| Zn | 28 | Fe | 25 |
| Se | 16 | Pb | 25 |
| Ti | 14 | Cr | 22 |
| P | 13 | Al | 16 |
| V | 13 | Zn | 15 |
| Fe | 11 | Mg | 8·5 |
| N | 11 | V | 8·2 |
| S | 11 | Ni | 7·5 |
| Cu | 11 | Sr | 6·6 |
| K | 9·4 | B | 6·5 |
| Mo | 9·4 | N | 6·4 |
| Pb | 8·3 | Na | 5·9 |
| Co | 8·1 | Mo | 5·8 |
| B | 7·8 | Ba | 4·5 |
| Cr | 6·3 | S | 3·0 |
| Mg | 5·5 | Se | 3·0 |
| Mn | 5·2 | Co | 2·9 |
| Na | 4·4 | Cu | 2·8 |
| Ba | 4·0 | Ca | 2·7 |
| Al | 3·8 | Mn | 2·7 |
| Ca | 3·8 | K | 2·6 |
| Sr | 3·6 | P | 2·4 |

Should a similar process operate in other species the effect may be of significance in restricting the transfer of radioactive Sr into the leafy parts of plants.

The distributions of Co and Ni are quite dissimilar. Cobalt is one of the elements that is relatively uniform in content throughout the various parts, while high Ni contents occur in the upper stem and spikelets. Fleming[4] has confirmed the high Ni content of the heads of pasture grasses. This movement towards the seed-head may suggest the site of a possible biological function for Ni. The distribution of Fe, with a high content in the leaf, is different from that of Co or Ni and bears some resemblance to that of Ca, although the spikelets are relatively richer in Fe than in Ca.

The feature of the distribution of Zn is the high content of the rachis node leaf (A), which is eight times that of the lowest leaves. This doubtless reflects the function of Zn in leaf development and auxin activity. One practical implication of the variation in leaf content is that the content of Zn found analytically in a whole plant sample might be appreciably affected by the sampling procedure. If the lower senescent leaves were rejected, the Zn content found would be higher. Comparison with the pot-culture results of Wood and Sibly[16] and Williams and Moore[17] for oats suggests that, with Zn, significant differences occur between varieties, but the state of maturity is undoubtedly important. This is confirmed by the results of Paribok[18] who gives results for Cu, Zn, Mn and B in leaves, stems and seedheads of wheat and flax at different stages of growth. It is however open to question whether pot-culture results for trace element contents are ever directly comparable with those obtained under more natural conditions in the field.

The contents of Mo, Mn and Cu in the different leaves are relatively uniform. The Mo contents of the spikelets, sheaths and stems are considerably lower than those of the leaves. Copper, and particularly Mn, are more uniformly distributed throughout the plant, although the Cu gradient in the stem is interesting. Williams and Moore[17] also report differences in the distribution of Mo in Algerian oats in different soil types, the stem being highest on most of the soils, which were studied in pot-culture. These authors found a marked increase in the Mn content of the inflorescence during seed formation.

The distribution of Pb is remarkably similar to that of Ca and Fe, and may give rise to suspicion regarding the existence of a biological function. The possibility of major surface contamination of leaves by exhaust fumes from motor vehicles can be excluded at this site. Boron also resembles Ca and Fe to some extent: here the biological requirement is well established. The content of Se is high in two of the stem samples and the overall distribution does not resemble that of any of the other elements studied, with the leaves generally having the lowest contents.

It is interesting to consider Cr, V, Ti and Al together. The contents of at least the first 3 are higher than normal in a grass, and it is possible, as has been suggested elsewhere[19] that the increased uptake is related to the occurrence of plant-available organic-matter complexes of these elements in the soil in question. The high contents of Cr, Ti and Al, and of Si, in the lower leaves do not appear to be due primarily to soil contamination: if they were, it is to be expected that the same effect would be shown by V and Fe.[20] It appears possible that these lower leaves are providing a site for the

deposition of elements present in unwanted excess, and the slight suggestion of the same effect for Sr, Co, Mo and B may be significant. The effect is often apparent to a lesser extent in the sheaths.

In this connection it should be pointed out that it must not be assumed that the location of high contents of a trace element in a particular organ always indicates that the trace element performs its essential functions there. It is conceivable that for certain elements some control mechanism will supply only the required amount to the essential location and store or dispose of any excess in other plant parts.

### Contribution of the different plant parts

The foregoing discussion has been concerned with the concentrations or contents of the various trace elements in the different parts of the plant. It is equally enlightening to examine the percentage contribution which each part makes to the content of the whole plant. This could be of importance in animal nutrition, as the trace elements in the various organs may be in different forms and their digestibility may vary.

The results presented in Fig. 3 illustrate the percentage contribution of each plant part. These diagrams show the different distributions of the various elements. They demonstrate, for instance, the relatively large contribution of the leaves towards the Ca content compared with that of other essential major plant nutrients such as P or K. The concentration of Ni in the spikelets and upper stem is again emphasised. In general, these diagrams show the relatively small contribution of the leaves to the total content of most elements in the above-ground portion of cocksfoot at the stage at which it is cut for hay. For instance, as seen also in Table I, only 10–20% of the Co, Cu, Mn, Zn or Se is located in the leaf samples.

## Conclusions

There are marked differences between elements in their distribution throughout the plant: there is an obvious requirement for similar field investigations at different stages of growth, on soil types with different contents of trace elements and under different climatic conditions, before definite conclusions can be drawn. Such studies, now in progress at the Macaulay Institute, should indicate which parts are most susceptible to changes in age and environment. For assessment of soil availability it is obviously desirable to find some plant part in which the content varies with soil status rather than with climatic factors or stage of growth.

From the animal nutrition standpoint the results would appear to merit consideration in view of the possibility that the trace elements in the different plant parts are not equally available, either because of the different digestibilities of the plant materials or of the different availabilities of the forms in which the trace element may be present. In this connection, the finding by Ferguson *et al.*[21] that Mo is less toxic when present in dried hay than in the same material grazed as herbage may be significant.

### Acknowledgments

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# ACTIVATION ANALYSIS OF TRACE ELEMENTS IN FISHMEAL

By G. LUNDE

Fishmeals produced from herring (*Clupea harengus*), mackerel (*Scomber scomber*), capelin (*Mallotus villosus*) and Norway pout (*Gadus esmarki*) were analysed by neutron activation. The elements tested were mercury, bromine, arsenic, selenium, antimony, copper, cobalt, iron, zinc, molybdenum and tungsten. The distribution of these elements in the solid and aqueous phases in boiled fish used as raw material for fishmeal was also studied.

## Introduction

Interest in trace elements and their physiological significance has increased considerably in recent years, for example in relation to animal feedingstuffs. Feed mixtures should contain sufficient amounts of trace elements necessary for the animals. Of these elements the following may be mentioned specially: molybdenum, zinc, fluorine, copper, manganese, cobalt, chromium and selenium. There is also a certain amount of interest in arsenic; although this element has not been localised in any metabolic process, additions of certain arseno-organic compounds have a favourable influence on growth of chickens and pigs.[1]

Relatively few data are available for trace elements in feeds of marine origin.[2,3] The purpose of this work was to analyse the most common types of fishmeal commercially produced in Norway.

Fishmeal is usually produced by boiling the fish at 95–100°. The material is then pressed in a screwpress where the insoluble phase (the press cake) is separated from the liquid phase consisting of oil and nitrogenous liquor. The oil is separated by centrifugation and the nitrogenous liquor is concentrated by evaporation.

In Norway the normal procedure for making fishmeal is to mix the press cake and the solubles and dry the mixture to so-called full meal. The dehydration of the meal is normally carried out at 60–80°.

In connexion with the project it was also of interest to investigate more thoroughly how the various trace elements are distributed in the press cake and the nitrogenous liquor. For this reason some samples of press cake and N liquor were produced from the same raw material.

## Experimental

Neutron activation was used as analytical method. This method is sensitive for several among the typical trace elements. It is also suitable when several elements are to be analysed simultaneously in the same sample. Owing to a high content of Na and P in the fishmeal it is necessary to perform a chemical separation after the irradiation. This separation was carried out according to the directions given by Samsahl.[4–6] A general introduction to the method has been given by Bowen & Gibbons.[7]

The following elements were analysed or looked for: Hg, Br, As, Se, Sb, Cu, Co, Fe, Zn, Mo and W. All of these elements, with the exception of Fe, show high sensitivity by this method. The radioactive isotopes induced in these elements by pile neutrons disintegrate, giving off gamma emissions; they can therefore be recorded by means of a multi-channel gamma spectrometer after a relatively simple chemical separation.

### Sample preparation

The common types of fish used in the production of fishmeal in Norway are herring (*Clupea harengus*), mackerel (*Scomber scomber*), and capelin (*Mallotus villosus*), but smaller landings of trash fish, such as Norway pout (*Gadus esmarki*), are also used. The samples for the analyses were obtained directly from factories or they were produced in the laboratory. The raw material for the laboratory samples was obtained from the commercial fish market and treated as follows: about 2 kg fish were ground in a blender, and boiled in a glass apparatus for about half an hour. Some distilled water was added. The oil was then separated and the residue was filtered and washed once on the filter with distilled water. The insoluble portion, the press cake, was dehydrated at 50–60° and finally homogenised in an agate mortar. The water-soluble portion was concentrated by evaporation at 80–90°. Prior to the neutron irradiation all the samples were dehydrated at 105° to constant weight. The yield of press cake and N liquor from the samples produced in the laboratory is presented in Table I.

### Irradiation and registration

Approximately $\frac{1}{2}$ g fishmeal was used in each irradiation. The samples of the concentrated N liquor were diluted with distilled water (1 : 2) in order to facilitate the transfer of the N liquor after irradiation to the dissolution flasks. All samples were sealed in quartz ampouled and irradiated for 3 days at an approximately constant neutron flux $2{\cdot}4 \times 10^{2}$ neutron $cm^{-2}$ $sec^{-1}$ in the nuclear reactor JEEP I, Kjeller, Norway. Standards (PA reagents from A. Merck, Darmstadt) for the elements to be analysed were irradiated simultaneously.

The chemical separation of the trace elements to be analysed[4–6] was based on a wet dissolution ($H_2SO_4$), distillation and ion exchange technique. By varying the eluting conditions groups of elements were adsorbed in different ion-exchange columns. After the separation the fractions of resin were collected in measuring glasses and the activities were recorded by a 400-channel $\gamma$ spectrometer (Victoreen

Scipp 400) with 2 × 2′ sodium iodide crystal. The standards were subjected to identical treatment; they were dissolved and adsorbed on to ion-exchange resin in the same way as the corresponding elements from the activated samples. A precision of ±10–15% can be expected by this method when the concentrations of the respective elements are not close to the limit of the sensitivity

### Results and Discussion

The analytical results for commercially produced fishmeal samples are given in Table II, and those for samples produced in the laboratory are in Table III. Zn and Fe are adsorbed to the same ion-exchange resin, and owing to the high activity of the 1·11 MeV γ-peak from $^{67}$Zn the activity from 1·29 MeV of $^{59}$Fe was difficult to measure. Only a few such measurements were performed, and low precision was to be expected (±40–50%). The variations in the results of the Fe determinations may also be due to contamination by this element throughout the processing of the meal. The concentrations of Co, Zn and Cu in the fishmeal are in agreement with other analytical results.[2,8] Results concerning Hg, As, Br and Sb content in fish can be found in 'The elementary chemical composition of marine organisms' by Vinogradov.[9] The Br content was only determined in the samples produced in the laboratory (Table III). No previous determinations of Se in marine fish have been found.

Table III indicates how the various trace elements are distributed in the solid and liquid phases (i.e. press cake and concentrated N liquor). It may be noted that among the essential elements, Zn and Mo seem to occur mainly in the press cake while Co and Cu are more concentrated in the N liquor. The way in which the different trace elements are distributed on the two phases may have some effect on the living organism's ability to resorb these elements. The Se, As and Br contents are greater in the liquid phase than in the solid phase. In some preliminary studies using the radioactive isotope, $^{76}$As, it has been shown that the As does not exchange with inorganic arsenite and arsenate (Lunde, G. unpublished results). The As may therefore exist as an arseno-organic compound.

From the analyses there does not appear to be any significant difference in the trace element content of fishmeal produced from different types of fish. The variations found seem to be as much an expression of the difference in basic food intake and habitat of the fish as of species differences.

TABLE I

**Yield of press cake and nitrogenous liquor produced from fish raw material in the laboratory**

| Sample | Raw material, g | Press cake, dehydrated, g | N liquor, conc., g |
|---|---|---|---|
| Herring (Mature | 2140 | 232 | 80 |
| Herring (Immature) | 1520 | 358 | 47 |
| Mackerel | 1460 | 258 | 49 |

TABLE II

**Trace elements ppm in press cake and concentrated N liquor**

| Sample | Fraction | Locality | Se | As | Sb | Br | Co | Cu | Zn | Fe | Hg | Mo | W |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Herring (Mature) | Press cake | Western Norway | 1·9 | 2·3 | 0·061 | 4·7 | 0·17 | 11·2 | 86 | — | 0·26 | 0·26 | <0·005 |
| Herring (Mature) | N liquor | Western Norway | 2·8 | 13·0 | <0·01 | 65 | 0·28 | 17·2 | 7·7 | — | <0·01 | <0·05 | <0·005 |
| Herring (Immature) | Press cake | Western Norway | 1·3 | 3·6 | 0·013 | 2·2 | 0·087 | 3·5 | 100 | 23 | 0·16 | 0·13 | <0·005 |
| Herring (Immature) | N liquor | Western Norway | 5·6 | 24·0 | <0·01 | 63 | 0·11 | 6·2 | 6·7 | 4·0 | <0·02 | <0·05 | <0·005 |
| Mackerel | Press cake | North Sea | 2·7 | 4·5 | 0·017 | 8·3 | 0·10 | 2·0 | 84 | 15·0 | 0·41 | 0·10 | <0·005 |
| Mackerel | N liquor | North Sea | 15·1 | 15·2 | <0·01 | 130 | 0·22 | 10·4 | 8·7 | 4·4 | <0·01 | <0·05 | <0·005 |

— Not measured

TABLE III

**Trace elements ppm in factory-produced fishmeal**

| Sample | Locality | Se | As | Sb | Co | Cu | Zn | Fe | Hg | Mo | W |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herring | North Sea | 2·9 | 4·9 | 0·029 | 0·51 | 3·0 | 88 | — | 0·18 | 0·05 | <0·005 |
| Herring | North Sea | 3·2 | 4·0 | 0·028 | 0·07 | 2·8 | 76 | — | 0·26 | 0·30 | <0·005 |
| Herring | Shetland | 3·7 | 15·0 | 0·040 | 0·25 | 2·0 | 84 | — | 0·36 | 0·03 | <0·005 |
| Herring | Western Norway | 2·7 | 4·0 | 0·015 | 0·19 | 1·6 | 75 | 14 | 0·18 | 0·07 | <0·005 |
| Herring | Western Norway | 2·2 | 2·7 | 0·029 | 0·15 | 2·3 | 85 | 106 | 0·09 | 0·13 | <0·005 |
| Herring | Western Norway | 2·0 | 4·0 | 0·017 | 0·18 | 1·6 | 74 | — | 0·11 | 0·07 | 0·014 |
| Herring | From stock, Oslo | 3·6 | 5·3 | 0·012 | 0·34 | 2·4 | 65 | — | 0·13 | 0·16 | <0·005 |
| Mackerel | North Sea | 5·3 | 3·8 | 0·015 | 0·17 | 1·2 | 88 | — | 0·21 | <0·05 | <0·005 |
| Mackerel | North Sea | 2·2 | 2·7 | 0·047 | 0·23 | 2·1 | 77 | 87 | 0·09 | 0·15 | <0·005 |
| Capelin | Northern Norway | 1·3 | 2·6 | 0·015 | 0·05 | 1·7 | 108 | — | 0·026 | 0·2 | <0·005 |
| Capelin | Northern Norway | 1·7 | 19·1 | 0·018 | 0·13 | 4·1 | 134 | — | 0·13 | 0·06 | 0·008 |
| Norway pout | Western Norway | 1·5 | 3·9 | 0·20 | 0·82 | 2·9 | 180 | — | 0·40 | <0·05 | 0·030 |

— Not measured

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# IDENTIFICATION OF 3α-HYDROXY-5α-ANDROST-16-ENE AS THE MUSK ODOUR COMPONENT OF BOAR SUBMAXILLARY SALIVARY GLAND AND ITS RELATIONSHIP TO THE SEX ODOUR TAINT IN PORK MEAT

By R. L. S. PATTERSON

The salivary glands of boars, hogs and gilts have been examined for the presence of sex odour. The submaxillary salivary glands of all boars examined possessed a musk-smelling compound, which was absent from the glands of the hogs and gilts. The compound, which has been isolated and identified as 3α-hydroxy-5α-androst-16-ene, is closely related to 5α-androst-16-ene-3-one, previously isolated from boar fat.

## Introduction

About thirty years ago, a number of papers[1-4] described the unpleasant odour, referred to as 'sex odour', which was produced when meat from mature boars or incompletely castrated boars was cooked. Because of its adverse effect on the acceptability of the meat various attempts were made to eliminate or suppress the odour, for example, castration before slaughter, the swift removal of the testicles at the time of slaughter, prolonged hanging of the carcass or prolonged pickling or curing.

One of the problems was to find a convenient and reliable means of detecting this sex odour, or taint, and the two most satisfactory methods described[2] consisted of boiling small pieces of diced fat or meat in a small quantity of water and smelling the steam, or warming the material slowly in the dry state on a watch-glass, frying pan etc., and smelling the

odour produced. These methods are still in use today and can yield information very quickly to the experienced nose.

It was believed that the reliability of this method for detecting taint was increased by testing the salivary glands in the same way, because there appeared to be a greater concentration of odour in these glands. Gereke[1] and Lerche[2] referred to the parotid salivary gland as possessing the greatest degree of odour, whereas Keller[4] cited the submaxillary salivary gland.

In the work now reported, a comparison of the salivary glands as sources of sex odour has been made and the submaxillary gland is shown to be the most important when heated under the conditions of the tests described above. The actual odour evolved, however, is not the same as the characteristic sex odour of boar flesh or fat, already identified as 5$\alpha$-androst-16-ene-3-one.[5] The more musk-like odour from the submaxillary gland is now shown to be the corresponding secondary alcohol, 3$\alpha$-hydroxy-5$\alpha$-androst-16-ene, and it has also been identified by olfactory examination of the compounds eluted from the gas chromatographic separation of compounds stripped from boar fat.

## Experimental

The three main salivary glands of the pig are the parotid, submaxillary and sublingual glands.[6] The largest of these is the parotid which is flat and distinctly triangular in shape and overlies the darker-coloured submaxillary gland which has a rounded oval structure. The sublingual gland is composed of two parts and is much smaller than either the parotid or submaxillary glands. The glands were either removed from the heads of the pigs in the laboratory or were received labelled from the slaughterhouse.

### Odour testing

5 g of glandular material, freed from adhering fatty tissue, were boiled in 25 ml distilled water in an Erlenmeyer flask, and the steam was smelled by a panel of 5 judges.

### Extraction of odorous compounds

The pair of submaxillary glands from a 3-yr-old, 650 lb (liveweight) Large White boar were frozen at $-10°$, chopped whilst frozen and minced with a chilled hand mincer. Six Green's 603 Extra Strong extraction thimbles (33 × 145 mm) were filled and separately extracted with 150 ml diethyl ether for 2½ hours, followed by a similar extraction with acetone.

The ether extracts were combined and concentrated in a rotary evaporator to approximately 100 ml. The solution was pale yellow and possessed an unpleasant odour. A glass rod, dipped in the solution, acquired and retained the musk odour for an hour or more after the solvent and other volatile compounds had evaporated.

The acetone extracts were combined and concentrated, and in addition to the unpleasant odour noted in the ether extract, there was also a distinct 'cat urine' smell. This odour is sometimes produced when proteinaceous material is exposed to ketonic solvents, but it disappeared very quickly from a glass rod, along with other volatiles, and left the musk odour. By further rotary evaporation, firstly at room temperature, then at 50°, both extracts were reduced almost to dryness. Dark yellow precipitates appeared in the two solutions during the final stages of concentration.

### Analysis of volatile compounds

The volatile components of the residues were recovered in two fractions by vacuum distillation, firstly at 35° and 0·01 torr for 1½ hours, then at 70° and 0·001 torr for 4 hours.

### Gas–liquid chromatography

A 3 ft × 4 mm i.d. glass column, packed with 5% SE-30 on Chromosorb G (80–100 mesh) and 5% phenyldiethanolamine succinate on Chromosorb G (80–100 mesh) in the ratio of 5 : 2 by length, was used in a Pye Series 104 chromatograph with a flame ionisation detector. Argon carrier gas was used at approximately 60 ml/min with a column temperature of approximately 180°. The compound with the musk odour was purified and isolated by repetitive semi-preparative scale gas–liquid chromatography as previously described,[5] until sufficient material had been obtained for mass spectrum analysis. The purity of the trapped sample was confirmed by the appearance of a single peak on the analytical gas–liquid chromatograph, before recording the mass spectrum on an A.E.I. MS9 instrument, using the direct inlet probe to insert the sample.

## Results

### Odour survey of the salivary glands

The steam issuing from odorous material when boiled in water had a readily detectable musk odour, whereas that from a sample free of sex odour was bland and described as similar to that of boiling potatoes. The results of the panel's estimates of the intensity of sex odour from various samples of salivary gland tissue are given in Table I. The submaxillary gland of boars possessed, in all cases examined, the musk-smelling compound associated with sex odour; this confirms the earlier work of Keller.[4] The parotid gland gave very weakly positive results only in a few samples where the odour of the submaxillary gland was most intense. The sublingual gland did not possess any sex odour except in one case in which the odour of the submaxillary gland was very strong.

The range of samples examined shows that the musk smell was detectable in the submaxillary salivary gland of all boars from the age of 4 months upwards, the youngest age available in this series. The intensity of the smell increased with age and, in the oldest boars, traces of the smell also appeared in the tissues from the other glands; it may be that this resulted from contamination of the latter tissues by saliva originating from the submaxillary gland.

The glands from four gilts showed no trace of odour, other than the bland potato-like smell. Similarly with three of the four samples from the hogs; in the remaining case, a trace of musk odour was detected, indicating the presence of some 'boar' characteristics, probably resulting from incomplete castration.

The quality, as well as the intensity of the odour changed with increase in age and weight of the boars. The odour of the heated submaxillary glands from very young boars was musky, but that from the glands of older boars possessed the perspiration-like characteristics of 5$\alpha$-androst-16-ene-3-one, typical of tainted boar fat,[5] superimposed on the musk odour.

A further examination was made of swabs of saliva obtained from three live boars, two Large Whites, aged 1 year 2 months and 2 years 6 months, and one Landrace, aged 2 years. The swabs were boiled separately in water and the steam was

TABLE I

**Intensity of sex odour detectable in salivary glands (means of assessments by a panel of 5 members)**
Each sample was judged by smelling the steam evolved when 5 g glandular tissue was boiled with water

| Animal | Breed | Age | Liveweight (approx. lb) | Intensity of odour in gland | | |
|---|---|---|---|---|---|---|
| | | | | Parotid | Submaxillary | Sublingual |
| Boar | Large White | 5½ years | 800 | Very weak | Very strong | None |
| ,, | ,, ,, | 3 ,, | 650 | Weak | ,, ,, | Trace |
| ,, | ,, ,, | 2 ,, | 420 | Very weak | Strong | None |
| ,, | ,, ,, | 1 ,, | 344 | None | ,, | ,, |
| ,, | ,, ,, | 4 months | 120 | ,, | Weak | ,, |
| ,, | Landrace | 10¾ ,, | 400 | Very weak | Very strong | ,, |
| ,, | ,, | 10 ,, | 336 | None | Strong | ,, |
| ,, | ,, | 9 ,, | 310 | ,, | ,, | ,, |
| ,, | ,, | 7¾ ,, | 201 | ,, | Medium | ,, |
| ,, | ,, | 7¾ ,, | 173 | ,, | ,, | ,, |
| ,, | ,, | 4 ,, | 146 | ,, | Weak | ,, |
| ,, | Pietrain | 8½ ,, | 265 | ,, | Medium | ,, |
| ,, | ,, | 8 ,, | 240 | ,, | ,, | ,, |
| ,, | ,, | 8 ,, | 218 | ,, | ,, | ,, |
| ,, | ,, | 5 ,, | 192 | ,, | ,, | ,, |
| ,, | ,, | 5 ,, | 163 | ,, | Weak | ,, |
| ,, | ,, | 4¾ ,, | 163 | ,, | Strong | ,, |
| ,, | ,, | 4¾ ,, | 142 | ,, | Medium | ,, |
| ,, | ,, | 4¾ ,, | 141 | ,, | ,, | ,, |
| ,, | ,, | 4¾ ,, | 136 | ,, | Weak | ,, |
| Hog | Wessex | 6 months | 213 | None | None | None |
| ,, | Large White | 6 ,, | 200 | ,, | Trace | ,, |
| ,, | ,, ,, | 6 ,, | 200 | ,, | None | ,, |
| ,, | ,, ,, | 6 ,, | 200 | ,, | ,, | ,, |
| Gilt | Wessex | 6 months | 202 | None | None | None |
| ,, | Large White | 6 ,, | 200 | ,, | ,, | ,, |
| ,, | ,, ,, | 6 ,, | 200 | ,, | ,, | ,, |
| ,, | ,, ,, | 6 ,, | 200 | ,, | ,, | ,, |

smelled. All samples exhibited sex odour but the quality was that of the androstenone rather than the musk compound. The saliva may therefore transfer the odorous compound from the salivary glands to the body fat.

### Identification of the musk odour

Gas–liquid chromatography of the fractions distilled from the ether and acetone extracts at 35° revealed few peaks, none of which possessed the sex odour when smelled at the outlet of the column with the flame of the detector extinguished. The 70° fraction from the ether extract gave two peaks of approximately equal size, the later at the same retention volume as 5α-androst-16-ene-3-one and possessing the perspiration odour characteristic of this compound. The earlier peak possessed the musk odour. The corresponding fraction from the acetone extract yielded a much larger amount of musk-smelling material, chromatography of which gave the same two peaks, but with the musk compound present in about twenty times greater concentration than the ketone.

The mass spectrum of the musk compound is shown in Fig. 1 (a) and of 5α-androst-16-ene-3-one in Fig. 1(b). In Fig. 1(a), the base peak of the spectrum is the molecular ion $(M)^+$ at *m/e* 274, with major peaks at *m/e* 259, 241, 148, and 94. The molecular weight of this compound is two units greater than that of 5α-androst-16-ene-3-one ($C_{19}H_{28}O$, mol wt. 272) but the fragmentation pattern is similar. The peak at *m/e* 259 (Fig. 1a) represents the loss of a methyl

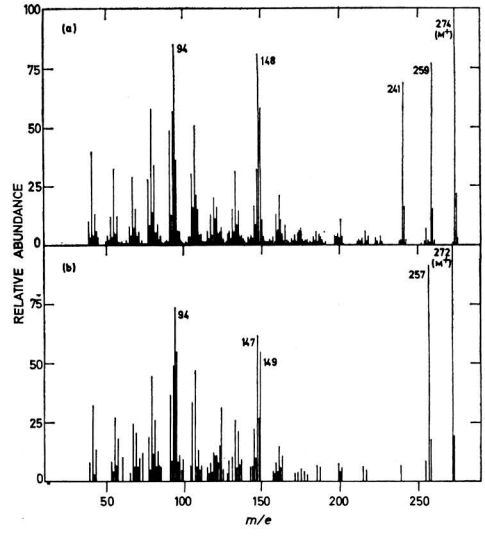


FIG. 1. *Comparison of the mass spectra of (a) the musk compound isolated from boar submaxillary salivary gland with (b) 5α-androst-16-ene-3-one*

group from the parent molecule $(M-15)^+$, and the peak at *m/e* 241 $(M-33)^+$ represents the simultaneous loss of a methyl group and the elements of water. The small peak at *m/e* 256 results from the direct loss of water from the molecule, but since the intensity of this peak is only 10% of the peak for the simultaneous elimination of water and a methyl group, the latter reaction is greatly favoured. There are only very small peaks at *m/e* 254 and 239 indicating similar losses from the ketone.

This evidence strongly indicates the presence of a hydroxyl group in the molecule, and the close similarity to the fragmentation pattern of the ketone indicates that the two compounds are very closely related structurally. The difference of 2 in the molecular weights can therefore be most satisfactorily accounted for by assuming that the hydroxy compound is the corresponding secondary alcohol of the androstene ketone. The alternative addition of a molecule of hydrogen to the double bond at $C^{16}$ of the ketone does not account for the appearance of the intense peak at *m/e* 241, which can arise only from the presence of a hydroxyl group. The structure of the musk compound was therefore identified as either 3α-hydroxy- or 3β-hydroxy-5α-androst-16-ene, $C_{19}H_{30}O$.

Authentic samples of the 3α- and 3β-hydroxy-5α-androst-16-ene steroids were obtained from the M.R.C. Steroid Reference Collection. Both compounds possessed musk odours, but that of the α-epimer was stronger. The mass spectra of the compounds were almost identical (Figs 2a and b) and showed the same major ions as the musk compound isolated from the salivary gland (Fig. 1(a)). The spectra of the epimers differed, however, in the relative abundances of the ion at *m/e* 241. The ratio of the intensities of the peaks *m/e* 241: 274 for the compound with hydroxyl group in the α-orientation was 0·73, whereas the ratio for the β-epimer was 0·39. The ratio for the compound isolated from the salivary gland was 0·68 showing it to be almost exclusively the α-epimer. It was concluded that the compound with the musk odour isolated from the submaxillary salivary gland of the boar was principally 3α-hydroxy-5α-androst-16-ene.

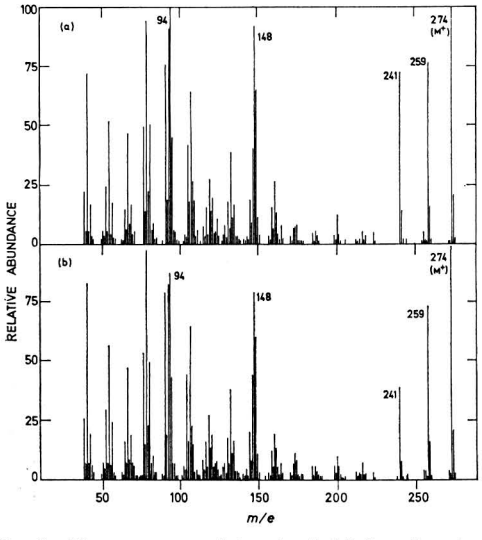


FIG. 2. *The mass spectra of the epimeric 3-hydroxy-5α-androst-16-enes. (a) the α-epimer; (b) the β-epimer*

Confirmatory evidence of the secondary alcohol structure of the musk compound in the salivary gland and its relationship to the 'taint' compound of the fat was obtained from *in vitro* oxidation of the alcohol to the ketone. A small quantity of androstenol, extracted from the salivary gland and purified and isolated by semi-preparative-scale gas chromatography, was oxidised in acetone solution to androstenone by a few drops of 8N chromic acid.[7] The orange colour of the chromic acid disappeared simultaneously with the musk odour, which was replaced by the sharper odour characteristic of the ketone. The reaction proceeded smoothly to completion at room temperature, and no trace of the androstenol was detected by gas chromatography afterwards. A single major peak, that of androstenone, indicated that an efficient oxidation had occurred, free from side reactions producing compounds detectable under these chromatographic conditions. A sample, trapped from the chromatograph, gave a mass spectrum identical to that obtained for the 'taint' compound, 5α-androst-16-ene-3-one, isolated from boar fat.[5]

## Discussion

The chemical structure of the odorous compounds shows that they are related to the hormone system of the boar, and their absence in castrated animals and females indicates that their formation is under testicular control. Prelog & Ruzicka[8] first isolated both the 3α- and 3β-hydroxy-5α-androst-16-enes from pig testes over twenty years ago but, apparently, their studies did not extend to the fat or salivary glands. Since then, the 3α-isomer has been isolated in very small amount from the hydrolysed urine of both men and women,[9] and also from the urine and tumour tissue of women with adrenal cortical tumours, which gave rise to the suggestion that androstenol might be of adrenal origin.[10]

The work of Stylianou *et al.*[11,12] and Dorfman,[13] in which testosterone was transformed into small amounts of androst-4,16-diene-3-one and 3β-hydroxy-5α-androst-16-ene, led to the suggestion that the double bond at $C_{16}$ was formed by elimination of the elements of water between $C_{16}$ and the hydroxyl group of $C_{17}$ in testosterone. Subsequent reduction of the carbonyl group at $C_3$ and the double bond at $C_4$ could then lead to formation of the 3β-hydroxy-5α-androst-16-ene. However, other workers have failed to confirm this, notably Gower & Ahmad[14] who found that no $\triangle^{16}$ compounds were formed *in vitro* when [4-$^{14}$C] testosterone was incubated with minced boar adrenal and testicular tissue and, more recently, they have suggested[15] that pregnenolone or progesterone may give rise to the $\triangle^{16}$-steroids without invoking the dehydration of testosterone as the means of introducing the double bond. The intervening steps have not yet been elucidated.

5α-Androst-16-ene-3-one does not appear to have been reported before as a natural product in other animal species. The presence of the corresponding alcohol in small amount in human urine, tumour tissue, etc., suggests that the reduced form is the end product of the catabolic pathway. The detection of the alcohol by sensory means in the submaxillary glands of young boars before the ketone appeared in either

the gland or fat, and the detection of the ketone, as well as the alcohol, in the glands of older boars, shows that the ketone is formed from the alcohol at a later stage.

The relatively high concentration of androstenol compared to androstenone in the submaxillary salivary gland of mature boars, and the reverse situation in the depot fat, suggests that the salivary gland is either the site of formation of the alcohol or that it acts as a reservoir for the compound, presumably abstracting it from the blood stream during saliva production. Compounds with musk odours are known to be sex attractants and it is possible that 3$\alpha$-hydroxy-5$\alpha$-androst-16-ene acts in this way. However, since the urine-like odour of the ketone is more readily detectable in the saliva and breath of a sexually aroused boar, it may be that the ketone is more important to the sow *in oestrus*. Evidence that an attractant of some description is present in the head of a boar is found by observing the behaviour of boars and sows. One of the actions of a mature boar in the presence of sows in heat is to work his jaws and stimulate saliva production until it runs from his mouth. Some sows will then stand with their snouts directed towards his jaws, as though detecting an odour, whilst assuming the characteristic mating stance. If the musk-smelling alcohol is the attractant, it will be released by champing of the jaws and increased saliva production. Alternatively, if the ketone is the active principle, the oxidation process converting the alcohol to the ketone may be stimulated in the gland by sexual arousal.

Since the saliva was found to possess the odour of 5$\alpha$-androst-16-ene-3-one, ingestion of the ketone in the saliva will result in its incorporation in the body tissues, as a result of the normal processes of digestion. It will be preferentially deposited in the fatty tissue, because of its lipophilic ketonic structure. On the other hand, the presence of the hydrophilic hydroxyl group in the alcohol will tend to facilitate its elimination from the body, either as the free compound, or as the water-soluble ester conjugate of glucuronic or sulphuric acid. This would explain the low concentration of the alcohol, but the relatively high concentration of the ketone, found in the depot fat of mature boars.

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# IRON CONTENT OF TEFF *(Eragrostis abyssinica)*

By SHEKEEB SUFIAN and L. R. PITTWELL

The iron content of locally purchased teff has been determined before treatment (0·05% mean), and after repeated sequential washing with N/10 hydrochloric acid and water (0·0033% mean).

## Introduction

Teff (*Eragrostis abyssinica*) is a cereal grain peculiar to Ethiopia, where it is one of the chief food grains. It has been claimed by Darby,[1] that it is a rich source of dietary iron, whilst Almgård has reported that all except 0·052–0·059% of this iron was present as soil on the outside of the grains.[2] One or two local scientists have even wondered whether even this figure was too high due to ferrugineous soil ground into the outside surface of the grains. Tests showed that hydrochloric acid was a good solvent for the iron in Ethiopian soils, and that whilst concentrated hydrochloric acid was much more rapid in its action, acid as dilute as N/10 was as effective if used in sufficient quantity for a long enough time at room temperature. On the other hand concentrated hydrochloric acid hydrolysed the grain wall whilst N/10 acid had no visible effect on the grain. It was therefore decided to check the iron content of teff once more.

## Experimental

Three 1 kg samples of commercial teff (two white, one red or black) were purchased from different dealers in the Addis Ababa grain market. Each sample was divided into 32 equal fractions by riffling, and each of these three series of fractions was then treated separately as follows: one fraction from each series was set aside as received, the remainder were washed thoroughly in N/10 hydrochloric acid and water, one more fraction chosen at random from each series was set aside, the remaining fractions were given a further acid and water wash and so on until all the fractions had been set aside. All the fractions were then dried at about 35°, and triplicate subsamples from each were weighed for determination of iron by the thiocyanate method, the grain being first completely oxidised by nitric and perchloric acid oxidation; these acids were removed by evaporation before analysis. Triplicate blanks were run by evaporating similar amounts of nitric and perchloric acids in empty beakers and analysing these along with each series of samples. Each large sample of teff was treated separately, all sub-samples being analysed for the first series; with the second series of fractions only one subsample was analysed, between the tenth and thirtieth washing fractions; for the third series it was between the fifth and thirtieth washing fractions.

## Results

The results (on a dry weight basis) are summarised in Table I.

TABLE I

**Effect of dilute acid washing on the iron content of teff**

| | percentage iron | |
|---|---|---|
| | Mean of 3 Series | Range |
| Initial total iron | 0·050 | 0·035–0·058 |
| Residual iron after 1st acid and water washing | 0·016 | 0·010–0·021 |
| ,, ,, after 2nd washing | 0·006 | 0·008–0·004 |
| ,, ,, ,, 3rd ,, | 0·0036 | 0·0038–0·0032 |
| ,, ,, ,, 4th ,, | 0·0035 | 0·0038–0·0031 |
| ,, ,, ,, 30th ,, | 0·0033 | 0·0036–0·0031 |
| ,, ,, ,, 32nd ,, | 0·0033 | 0·0036–0·0031 |

It is concluded from these results that the true iron content of the actual dirt-free teff grain is about 0·0033%. However Melak[3] has obtained higher values than Almgård using teff fresh from the plant, threshed in the laboratory. This may mean either that the iron content is very variable, that Melak's sample was contaminated by wind-blown dust embedded in the grain wall, or that the outer seed wall as distinct from the husk is richer in iron than the central grain. On the other hand, iron actually embedded in the grain walls must be considered to be a dietary source of iron along with the actual true iron content of the grain itself.

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# CHANGES IN THE LIPIDS OF TURKEY MUSCLE DURING STORAGE AT CHILLING AND FREEZING TEMPERATURES

By M. J. FISHWICK

Diced turkey leg and breast muscle stored for 22 days at 0° and −3°, and for up to one year at −10°, −20° and −60° was examined at intervals for lipid composition. Free fatty acids increased at all temperatures except −60°, with a $Q_{10}$ of 3–4 between 0° and −20°; 90% of the fatty acids liberated were unsaturated, matching in composition the unsaturated acids of the muscle glycerophospholipids. Demonstration of linear relationships between increase in free fatty acids and decrease in phosphatidylethanolamine or increase in lysophosphatidylcholine confirmed phospholipase $A^2$ as the enzyme mainly responsible. The composition of the fatty acids liberated during storage at 0° and −3° showed that both lipase and phospholipase were active. A slight decrease in extractability, resulting in an apparent loss of phospholipid-P, was observed after storage at low temperatures.

## Introduction

Lipid hydrolysis is known to occur during the cold storage of fish[1,2] and of peas.[3] The proportion of the phospholipids of cod muscle hydrolysed during storage of temperatures of −7° to −29° is considerable (60–75%), and the rate reaches a maximum at −4° but the degree of hydrolysis varies from species to species: there was no detectable hydrolysis of the phospholipid in shark tissue, for example, during storage at −14° for 16 weeks whilst the degree of hydrolysis in cod under comparable conditions was 60%.[3] Although extensive hydrolysis of both triglyceride and phospholipid occurred in lamb's liver held at 0° or higher temperatures, hydrolysis was hardly detectable at −10° or −20° after 24 weeks, as judged by loss of lipid phosphorus and of total carboxylic ester groups.[4]

In view of these marked species differences in lipid hydrolysis, the present work on turkey muscle was designed to ascertain the nature and extent of hydrolysis over a range of storage temperatures. Such hydrolysis would be likely to affect the properties of lipoprotein membranes and, if the analogy with fish muscle holds, the liberated free fatty acids might be expected to accelerate deteriorative changes associated with protein denaturation during frozen storage.[5–7] A secondary objective of the work was to ascertain the degree of cooling necessary to preserve experimental poultry muscle tissue for subsequent lipid analysis.

Turkey muscle after storage at −25° for several months has been reported to give a lower yield of extractable lipid phosphorus than fresh tissue,[8] the losses being tentatively attributed to the formation of complexes of low solubility. This observation, if confirmed, would affect the validity of following phospholipase action in tissues by measuring changes in the extractable lipid phosphorus content of the sample.

## Experimental

### Materials

Broadbreasted White turkeys (Robert Spillars and Bros., Keystone, Hunts.) were removed from the processing line, after evisceration and plucking but before the freezing stage, and chilled in polythene bags over ice. Breast and thigh muscles were removed from four 6 lb birds by dissection on a 'cold slab' within 1 hour of collection, and each composite sample was diced into 5 mm cubes and mixed. Storage treatments commenced immediately.

### Storage conditions

Representative samples of the diced muscle were packed into small sealed cans, each holding 34 g, and placed in cold rooms maintained at 0°, −3°, −10° and −20°. Further samples were held over solid carbon dioxide at −60° to serve as controls. From portions of the diced muscle pre-treated with 2% of an aqueous solution of 0·05% (w/v) aureomycin and 0·05% (w/v) nystatin (to inhibit microbial growth) further cans were packed and stored at 0° and −3°.

### Methods

*Extraction of lipid from tissues*

Samples of the fresh muscle were extracted immediately after they had been diced and subsequently, at intervals, after storage. The single-stage extraction procedure used for the lipid was essentially that of Winter.[9] The tissue (25 g) was mixed in a high-speed blender for 2 min with chloroform–methanol (1 : 1 v/v, 160 ml) and 1 M magnesium chloride (0·5 ml). Chloroform (80 ml) was added, and blending was continued for a further 2 min. Water, to give a total volume (including that present in the tissue) of 60 ml, was then added, the extract was mixed for a further 1 min and the whole homogenate was centrifuged at 2000 rev/min for 20 min. The cup and contents were then cooled at −20° for 20 min. and re-centrifuged for 20 min to produce two clear liquid layers separated by a tightly packed layer of disintegrated tissue. The aqueous upper layer was discarded and the lower layer was sampled for analysis. Its volume was calculated to be 175 ml.

*Storage of lipid samples*

70 ml portions of the lower extraction phase were taken down to dryness in a rotary evaporator and re-dissolved in 5 ml chloroform–methanol (2 : 1 v/v) containing 5 mg% (w/v) 2,6-di-t-butyl-4-methylphenol (BHT). The samples were

stored at −60° over solid carbon dioxide until required for analysis.

*Phosphorus*

Phosphorus was determined by the routine method of Allen.[10] A micro method[11,12] was used when phosphorus assay was required of a phospholipid fraction from a thin-layer plate.

*Total lipid*

20 ml portions of the lower extraction phase were dried in a vacuum oven at 40° and weighed.

*Free fatty acid*

Free fatty acid was determined colorimetrically by the method of Duncombe,[13] a small silica column being used to remove interfering substances.[14]

*Thin-layer chromatography*

The total lipid was fractionated into phospholipid, free fatty acid and individual neutral lipids by chromatography at 1° on plates of silica gel H (20 × 20 cm) using hexane–diethyl ether–glacial acetic acid (79 : 20 : 1 v/v) as developing solvent. Phospholipids were separated into their individual constituents by chromatography on silica gel H with chloroform–methanol–glacial acetic acid–water (50 : 30 : 8 : 4 v/v).[15] Layer thicknesses of 250 $\mu$m and 500 $\mu$m respectively were used for quantitative analysis by densitometry and for separation of lipid fractions prior to gas chromatography analysis of their constituent fatty acids as methyl esters. Samples were applied as individual spots for quantitative phospholipid analysis, or as narrow bands across the full width of the plate for fatty acid analysis. Separated bands were visualised by spraying the plates with rhodamine B–fluorescein reagent (rhodamine B, 100 mg, and dichlorofluorescein, 32 mg, dissolved in 150 ml ether, 70 ml ethanol and 17 ml water) and viewing them under u.v. light.

*Conversion to methyl esters and analysis by gas liquid chromatography*

Lipid fractions scraped from the t.l.c. plates were refluxed with 5% (v/v) sulphuric acid in anhydrous methanol for 2 h under nitrogen, without prior separation from the silica gel. The method used was substantially that of Bowyer *et al.*[16] except that the methyl esters were prepared for gas chromatography as 1% (v/v) solutions in iso-octane containing 5 mg % BHT. Analysis of the methyl esters was carried out on a Pye argon gas chromatograph, with Peg A column at 190° and argon flow rate of 40 ml/min. Retention times of the methyl esters were calibrated by use of a standard mixture of $C_{14}$ to $C_{24}$ saturated methyl esters, supplied by the National Institutes of Health, Bethesda 14, Maryland.

*Quantitative analysis of phospholipids*

Separated phospholipid fractions were determined directly on thin-layer plates by charring the material with chromic acid–sulphuric acid mixture under carefully controlled conditions and comparing the densities of the charred areas with those of standard pure phospholipids by means of a photodensitometer[17] (Photovolt Corporation of America). Values obtained in this way were checked at intervals by scraping off each individual spot from the plate and determining the amount of phosphorus present.[18]

*Presentation of results*

Analyses for phosphorus, free fatty acids, phospholipids and total lipid are all expressed as mg/100 g wet muscle.

## Results and Discussion

### Bacterial spoilage

Samples of leg and breast muscle sprayed with antibiotic solution and stored at 0° and −3° for up to three weeks showed the same rate of hydrolysis as untreated samples at the same temperatures. Bacterial growth on the meat was therefore not a significant factor at these temperatures.

### Influence of storage temperature on rate of hydrolysis

Samples stored at 0°, −3°, −10° and −20° showed a progressive hydrolysis of lipid during storage, whilst controls at −60° showed no detectable change, even after 12 months (Figs 1 and 2). After about 4 months at −10° or −20° the free fatty acid level ceased to rise. Similar decreases in free fatty acid production have been reported for frozen peas[3] and fish.[1] The highest level of free fatty acid reached corresponded to approximately 8% and 7% respectively of the phospholipid of leg and breast muscle held at −20°, and to 31% and 22% respectively for leg and breast muscle held at −10°. These figures are calculated on the assumption that hydrolysis was due solely to phospholipase $A^2$ activity. This assumption arises from a consideration of the composition of the fatty acids resulting from hydrolysis.

The rate of hydrolysis decreased with the lowering of storage temperature without passing through a maximum at about −4° (Fig. 1) which has been observed for cod.[2] Calculation of temperature coefficients over the range 0° to −20°, based on the linear parts of the hydrolysis curves, gave values between 3·3 and 4·2, which lie near to reported ranges for enzymically catalysed reactions.[2] A marked lag period in hydrolysis of 10 to 13 days was observed in samples stored at 0° and −3°, but there was no obvious delay in the

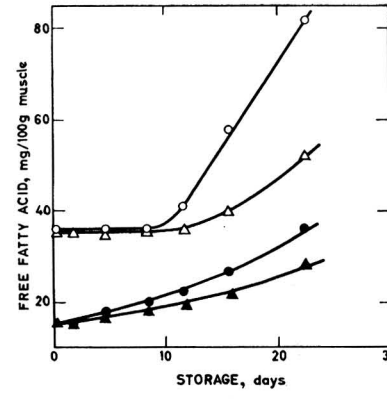


FIG. 1. *Accumulation of free fatty acids in leg and breast muscle during storage at* 0° *and* −3°
○ leg 0° △ leg −3°
● breast 0° ▲ breast −3°

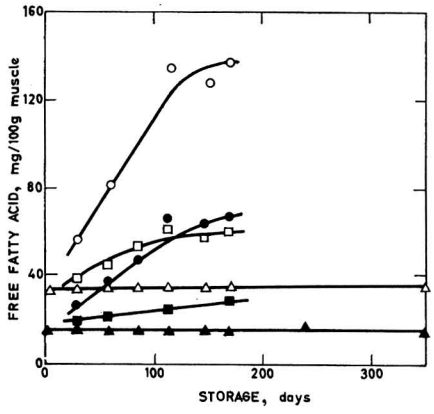


FIG. 2. *Accumulation of free fatty acids in leg and breast muscle during storage at −10°, −20° and −60°*

○ leg −10° ● breast −10° □ leg −20° ■ breast −20° △ leg −60° ▲ breast −60°

onset of hydrolysis at lower storage temperatures. A similar lag period for fish stored at 0° (but not in the frozen fish) has been noted by Lovern & Olley,[3] and by Rhodes & Lea[4] for lambs' liver at 15°. In the latter case the delay was eliminated when the enzyme was activated by pre-freezing the tissue.

**Extraction of total lipid and loss of extractable phospholipid during storage in the frozen state**

The breast muscle used contained approximately 1·0% of total lipid, of which the phospholipid content was 66%: leg muscle contained about 2·4% lipid of which 37% was phospholipid. The amount of extractable phospholipid, as measured by lipid phosphorus determination, decreased with time of storage at −10° or below, up to a maximum loss of the order of 10%, and this decrease appeared to be largely independent of the actual temperature. Total lipid, extracted from tissue stored for various lengths of time at −60° under an atmosphere of carbon dioxide, was analysed for the amount of each individual phospholipid present. These values, when plotted against the total phospholipid content, gave a series of straight lines the slopes of which were proportional to the respective initial concentration, indicating that the apparent losses of phospholipid during low-temperature storage were non-selective.

Autoxidation is unlikely to be involved in loss of phospholipid from tissue stored at −60° in an atmosphere of carbon dioxide and samples of extracted lipid showed only negligible peroxide values. Breast muscle stored for 4 months at −60°, for example, still gave a peroxide value of $< 1$ μmole per g lipid, although the amount of extractable phospholipid had fallen to 89% of the initial amount.

The nature of the process that apparently binds phospholipid more firmly to protein and renders it more resistant to extraction by the mild single-stage procedure used is not clear. It might perhaps be connected with changes in salt concentration caused by the freezing out of water.[19] Losses of phospholipid from cold-stored muscle during the extraction procedure have been reported by Acosta *et al.*[8] for chicken muscle whilst Davidkova & Khan[20] noted an increased yield of triglyceride apparently as a result of increased extractability after storage.

**Phospholipid composition of turkey muscles**

Amounts of individual phospholipids found in breast and leg muscle, as determined by densitometry, are given in Table 1. Leg muscle contained about 40% more total phospholipid than breast muscle, and proportionally slightly more phosphatidylethanolamine and phosphatidylserine, but slightly less phosphatidylcholine and sphingomyelin.

**Fatty acid composition of the lipid fractions**

Total lipid obtained from fresh muscle was separated into phospholipid, triglyceride, free fatty acid and diglyceride fractions by thin-layer chromatography on silica gel H. The various fractions were methylated and their constituent acids resolved as methyl esters by gas–liquid chromatography.

Table II shows that the phospholipids were comparatively rich in polyunsaturated fatty acids, especially 20 : 4 and 22 : 6, whereas these acids were virtually absent from the triglyceride fraction.

**Changes in the composition of the free fatty acid fraction during storage**

Tables III and IV give an amount of each fatty acid

TABLE I

**Composition of the phospholipids of turkey muscle**

| Phospholipid | Breast | | Leg | |
|---|---|---|---|---|
| | mg/100 g (mean + S.E.) | Percentage of total | mg/100 g (mean + S.E.) | Percentage of total |
| Phosphatidylethanolamine | 186 ± 4·5 (8)* | 29·2 | 322 ± 6·1 (6) | 36·0 |
| Phosphatidylserine | 10·7 ± 1·0 (8) | 1·7 | 21 ± 1·5 (6) | 2·4 |
| Phosphatidylcholine + Phosphatidylinositol | 388 ± 5·7 (8) | 60·9 | 493 ± 5·7 (6) | 55·1 |
| Sphingomyelin | 52 ± 0·7 (8) | 8·2 | 59 ± 0·4 (6) | 6·6 |
| Lysophosphatidylcholine | tr | nil | tr | nil |
| Total | 637 | — | 895 | — |

* Number of samples analysed given in parenthesis

liberated by hydrolysis during storage of the muscle, as calculated from the formula:

$$\%\text{ of a particular acid in the free fatty acids liberated by hydrolysis} = \frac{100\,(By - Ax)}{\begin{matrix} n = 22:6< \\ n = 14:0< \end{matrix} (By - Ax_n)}$$

where $A$ (mg/100 g) = amount of total free fatty acids initially present in the muscle; $B$ = the corresponding value after storage at a particular temperature for a particular time; $x$ = percentage of a particular fatty acid in the initial total free fatty acid fraction; and $y$ = the corresponding value after storage.

Examination of Tables III and IV shows that at least 90% of the fatty acids liberated by hydrolysis during storage at $-10°$ or $-20°$ were unsaturated; this indicates that the main enzyme involved has a specificity for the ester linkage at position 2 of a triglyceride or a phospholipid.

Comparison of the percentage composition of the liberated fatty acids with the unsaturated fatty acids of the phospholipid and triglyceride fractions (Table V) shows that the fatty acids liberated by hydrolysis contained too much polyunsaturated acid to be derived in any major degree from triglyceride, but that they approximated in composition to the unsaturated acids of the phospholipid. The hydrolysis which occurs during storage at $-10°$ and $-20°$ must therefore be due, largely if not entirely, to the action of phospholipase A[2], which is known to hydrolyse the ester linkage at the 2-position in a glycerophospholipid, removing the predominantly unsaturated fatty acids bound there. The relatively small proportion (10%) of unsaturated fatty acid found in the liberated fatty acids is not inconsistent with this view, since a small proportion of saturated acid is commonly present also in the 2-position.[20] Neudoerffer & Lea,[22] in fact, found approximately 10% of saturated acids among the predominantly unsaturated acids liberated from turkey

TABLE II

**Fatty acid composition % of the lipids of fresh turkey muscle***

| Carbon No. | Identity | Breast | | | | Leg | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PL | DG | FFA | TG | PL | DG | FFA | TG |
| 14.00 | 14 : 0 | tr | 0·5 | 0·6 | 1·2 | 0·2 | 1·4 | 0·6 | 1·2 |
| 15.54 | 16 : A | 9·9 | 3·3 | — | — | 7·8 | 1·5 | — | — |
| 16.00 | 16 : 0 | 19·0 | 25·9 | 14·6 | 27·6 | 14·5 | 18·8 | 18·4 | 23·7 |
| 16.35 | 16 : 1 | 1·1 | 5·9 | 2·6 | 7·8 | 1·3 | 4·8 | 2·7 | 6·9 |
| 17.02 | 17 : 0 | — | — | — | — | — | — | — | 0·7 |
| 17.54 | 18 : A | 2·1 | 0·5 | 0·8 | — | 2·2 | — | — | — |
| 18.00 | 18 : 0 | 12·9 | 11·0 | 8·7 | 7·3 | 13·9 | 8·1 | 8·4 | 6·4 |
| 18.35 | 18 : 1 | 11·8 | 25·1 | 14·0 | 27·6 | 10·8 | 25·9 | 19·2 | 28·4 |
| 18.90 | 18 : 2 | 22·3 | 17·8 | 30·1 | 27·4 | 24·9 | 28·4 | 29·6 | 28·1 |
| 19.65 | 18 : 3 | — | 2·4 | 0·7 | 1·0 | — | 0·9 | 0·8 | 1·6 |
| 20.00 | 20 : 0 | — | — | — | — | tr | — | — | — |
| 21.25 | 20 : 3 | 0·2 | 2·0 | — | — | 0·7 | 0·9 | 11·7 | — |
| 21.54 | 20 : 4 | 7·8 | 2·0 | 12·4 | — | 9·8 | 4·5 | 5·2 | 0·4 |
| 22.10 | 20 : 5 | 2·9 | 2·7 | 6·0 | tr | 3·9 | 0·5 | — | 0·4. |
| 24.05 | 24 : 0 | — | — | 0·7 | — | — | — | — | — |
| 24.31 | 22 : 5 | 1·9 | tr | 2·0 | — | 1·6 | 1·2 | 0·3 | 0·6 |
| 24.54 | 22 : 6 | 8·2 | 0·9 | 6·7 | tr | 8·3 | 3·0 | 3·1 | 1·6 |

* Phospholipid (PL); diglyceride (DG); free fatty acid (FFA); triglyceride (TG); saturated aldehyde (A)

TABLE III

**Turkey leg muscle—percentage composition of the fatty acids liberated by hydrolysis during storage of leg muscle at 0° to −20°**

| Carbon No. | Identity | −20° | −10° | −10° | −10° | −10° | −3° | 0° | Temperature |
|---|---|---|---|---|---|---|---|---|---|
| | | 6 m | 1 m | 3 m | 5 m | 6 m | 22·5 d | 22·5 d | Storage period |
| 14.00 | 14 : 0 | 0·1 | 0·3 | 0·4 | 0·5 | 0·2 | 1·8 | 1·8 | |
| 14.45 | — | — | — | — | 0·3 | — | — | — | |
| 15.47 | 16 : A | — | — | — | — | — | 2·5 | 1·2 | |
| 16.00 | 16 : 0 | — | — | 6·0 | 5·8 | 5·3 | 18·0 | 24·8 | |
| 16.37 | 16 : 1 | 3·5 | 5·0 | 4·4 | 3·4 | 2·4 | 11·5 | 8·5 | |
| 16.95 | 17 : 0 | 1·0 | — | 0·8 | 0·4 | — | — | — | |
| 17.32 | — | 1·0 | — | — | 0·3 | — | — | — | |
| 18.00 | 18 : 0 | 4·1 | 5·0 | 4·9 | 4·1 | 4·9 | 11·3 | 13·3 | |
| 18.31 | 18 : 1 | 13·0 | 10·9 | 15·2 | 13·4 | 14·0 | 34·2 | 26·6 | |
| 18.90 | 18 : 2 | 40·5 | 45·9 | 41·2 | 39·2 | 36·6 | 13·7 | 18·9 | |
| 19.64 | 18 : 3 | 0·3 | 1·8 | 1·4 | 0·7 | 0·5 | 1·3 | 1·7 | |
| 21.25 | 20 : 3 | 1·5 | — | — | 0·6 | 0·5 | — | — | |
| 21.50 | 20 : 4 | 12·4 | 12·6 | 11·4 | 13·0 | 15·9 | — | — | |
| 22.24 | 20 : 5 | 4·6 | 5·4 | 4·1 | 5·2 | 6·1 | — | — | |
| 23.48 | — | 3·2 | — | — | 0·6 | 0·5 | — | — | |
| 24.22 | 22 : 5 | 3·2 | 4·6 | 2·5 | 2·7 | 2·4 | 3·1 | 0·7 | |
| 24.53 | 22 : 6 | 11·7 | 8·4 | 7·6 | 9·8 | 10·6 | 2·5 | 2·6 | |

muscle phosphatidylcholine or phosphatidylethanolamine by the *in vitro* action of snake venom phospholipase A.

By contrast, 30–40% of the free fatty acids liberated after storage of the tissue at −3° and 0° were saturated (Tables III and IV); this indicates that lipase action was probably making a major contribution at these temperatures. The polyunsaturated free fatty acids, 20 : 4 and 22 : 6, present in the samples of stored muscle (particularly breast), however, could only have come from phospholipid, and phospholipase action must also have been occurring at these temperatures.

### Changes in the phospholipids during storage

The amount of each phospholipid present in the tissue after various periods of storage at −10° was measured by densitometry, after separation by thin-layer chromatography. Phosphatidylethanolamine was the best separated of the major phospholipids and a plot of the loss of this muscle lipid constituent against increase in free fatty acid gave a linear relationship (Fig. 3A). The slopes of the lines calculated on a molar basis gave phosphatidylethanolamine/free fatty acid ratios of 36 : 100 and 38 : 100 respectively for leg and breast muscle, values which are of the same order of magnitude as the respective concentrations (38% and 32%) of phosphatidylethanolamine in the total phospholipid, excluding sphingomyelin.

The decrease in the phosphatidylcholine content of the tissue during storage could not be followed directly because lysophosphatidylethanolamine, produced by enzymic hydrolysis, was not separated from phosphatidylcholine on the thin-layer plates. A linear relationship was, however, established between the increase in lysophosphatidylcholine and increase in free fatty acid, despite some difficulty in measuring accurately the small amounts involved (Fig. 3B). The molar ratio of lysophosphatidylcholine to free fatty acid was found to be 41 : 100 and 59 : 100 for leg and breast muscle respectively, compared with concentrations of phosphatidylcholine in the total phospholipid, excluding sphingomyelin, of 59% and 66%. The latter figures, however, are too high, probably by about 10 units, because of the inclusion of phosphatidylinositol in the phosphatidylcholine fraction from the thin-layer plates.

The relationships between phosphatidylethanolamine and phosphatidylcholine hydrolysis and free fatty acid production, therefore, give additional support to the conclusion already reached from studying the composition of the free fatty acids

TABLE IV

**Turkey breast muscle—percentage composition of the fatty acids liberated by hydrolysis during storage of breast muscle at 0° to −20°**

| Carbon No. | Identity | −20° | −10° | −10° | −10° | −10° | −3° | 0° | Temperature |
|---|---|---|---|---|---|---|---|---|---|
| | | 6 m | 1 m | 3 m | 5 m | 6 m | 22·5 d | 22·5 d | Storage period |
| 14.00 | 14 : 0 | 0·4 | — | 0·3 | 0·1 | — | 1·4 | 0·8 | |
| 15.43 | 16 : A | — | — | — | — | — | — | — | |
| 16.05 | 16 : 0 | 3·7 | 2·1 | 6·8 | 4·8 | 7·8 | 21·2 | 20·7 | |
| 16.35 | 16 : 1 | 1·1 | 0·4 | 2·2 | 1·7 | 5·6 | 3·7 | 4·5 | |
| 17.35 | — | — | — | — | — | — | — | — | |
| 18.00 | 18 : 0 | 1·9 | 1·2 | 3·5 | 2·7 | 3·7 | 15·6 | 11·3 | |
| 18.36 | 18 : 1 | 9·8 | 8·1 | 9·8 | 8·8 | 11·7 | 19·2 | 19·6 | |
| 18.90 | 18 : 2 | 28·6 | 38·0 | 33·7 | 33·4 | 35·4 | 23·8 | 24·1 | |
| 19.30 | — | 0·6 | — | — | — | — | — | — | |
| 19.70 | 18 : 3 | 0·5 | — | — | 1·1 | — | — | 1·0 | |
| 20.40 | — | — | — | — | 0·1 | — | — | — | |
| 20.93 | — | 2·4 | 0·2 | — | 0·3 | — | — | — | |
| 21.10 | — | 4·5 | — | — | 0·8 | — | — | — | |
| 21.25 | 20 : 3 | — | 2·0 | — | 0·7 | — | — | 1·2 | |
| 21.54 | 20 : 4 | 16·2 | 16·4 | 17·4 | 15·1 | 14·1 | 7·4 | 6·4 | |
| 22.31 | 20 : 5 | 7·0 | 7·9 | 6·9 | 7·4 | 6·2 | — | 2·1 | |
| 23.57 | — | — | 1·1 | — | 0·9 | — | — | — | |
| 24.31 | 22 : 5 | 3·2 | 4·8 | 1·7 | 3·6 | 2·8 | 1·7 | 2·2 | |
| 24.62 | 22 : 6 | 20·1 | 17·8 | 17·8 | 18·4 | 12·7 | 6·0 | 6·2 | |

TABLE V

**Compositions of the unsaturated fatty acids present in the triglyceride and phospholipid fractions of fresh turkey muscle**

| Carbon No. | Identity | Breast | | Leg | |
|---|---|---|---|---|---|
| | | Triglyceride | Phospholipid | Triglyceride | Phospholipid |
| 16.35 | 16 : 1 | 12·2 | 2·0 | 10·1 | 2·1 |
| 18.35 | 18 : 1 | 43·2 | 21·0 | 41·7 | 17·6 |
| 18.90 | 18 : 2 | 42·9 | 39·7 | 41·4 | 40·6 |
| 19.65 | 18 : 3 | 1·6 | — | 2·4 | — |
| 21.25 | 20 : 3 | — | 0·4 | — | 1·1 |
| 21.54 | 20 : 4 | — | 13·9 | 0·6 | 16·0 |
| 22.10 | 20 : 5 | tr | 5·2 | 0·6 | 6·4 |
| 24.31 | 22 : 5 | — | 3·4 | 0·9 | 2·6 |
| 24.54 | 22 : 6 | tr | 14·6 | 2·4 | 13·6 |

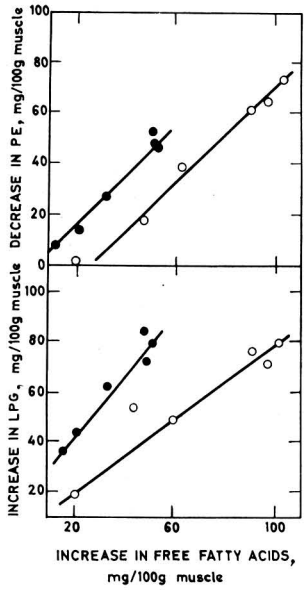


FIG. 3. *Linear relationship between increase of free fatty acids and* (A) *loss of phosphatidylethanolamine or* (B) *increase in lysophosphatidylcholine at* $-10°$

○ leg muscle ● breast muscle

liberated during storage at $-10°$ and $-20°$; together, these indicate that lipid hydrolysis at these temperatures was mainly due to the action of phospholipase $A^2$ action on the glycerophospholipids of the muscle to give corresponding lyso compounds and (mainly unsaturated) fatty acids. It was not possible to ascertain whether phosphatidylserine and phosphatidylinositol, present only in very small quantity, were being attacked in addition to the two major glycerophospholipids. There was no evidence of any loss of sphingomyelin.

The accumulation of lyso compounds during storage of turkey muscle is in contrast to the work of Olley & Lovern[1] who concluded that in *cod* muscle lyso compounds do not accumulate, and that both α- and β -fatty acids are simultaneously attacked and hydrolysed. Since this work was completed, Davidkova & Khan[20] have published data on changes in the lipids of boiler chickens stored for two years at $-10°$. Using composite muscle samples these authors found that phospholipids (lecithins and 'cephalins') decreased, and free fatty acids increased. There was also some increase also in lysophosphatidylcholine. They suggest that about 70% of the total increase in free fatty acid content of the muscle was probably due to phospholipase B activity, and the remaining 30% to breakdown of triglyceride.

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# VARIATION IN THE COMPOSITION OF BARLEY AND ITS EFFECT ON THE PERFORMANCE OF PIGS

By A. S. JONES, A. CADENHEAD and R. M. LIVINGSTONE

The crude protein and basic amino acid contents of 26 samples of barley were determined. The lysine, histidine and arginine contents of barley were positively and significantly related to the crude protein content. The proportion of lysine in barley protein decreased significantly as the crude protein level increased. Differences in the lysine and protein content of barley were shown to affect the growth and carcass quality of bacon pigs.

## Introduction

The protein content and amino acid composition of barley may be affected by manurial treatment and by the climate of the region in which it is grown. The composition also varies from one variety to another.

A high proportion of the total protein in diets of pigs in this country comes from barley, so that, theoretically, large variations in the protein content or amino acid composition of barley are of importance. Variations in the lysine content of barley are likely to have effects on the growth of pigs greater than those caused by variations in other amino acids.

There is considerable variation in the nitrogen content of barleys, and often those with a low nitrogen content are grown for the distilling and malting trade. The late application of nitrogen was studied by Coïc *et al.*,[1] who found it increased the protein content of the grain by up to 40%. There were increases in the percentage of some amino acids in the barley proteins, notably of glutamic acid and proline, but the percentages of others—the sulphur-containing amino acids, lysine, and glycine—decreased even though their content expressed as a % of the dry matter of the grain increased.

Recently, McGeown & Maguire[2] found that the application of nitrogen at the time of sowing significantly increased the crude protein content of barley by 2% without appreciably altering the amino acid composition of the grain.

In this laboratory, the variations in the crude protein and the basic amino acid contents of barleys purchased in a 6 month period were examined together with a number of samples of known variety, manurial treatment and origin. Based on the results obtained, an experiment was carried out to determine the effect of extreme variations in lysine and crude protein in barley on the growth of pigs.

## Variation in barley composition

### Methods

The basic amino acids were separated on 15 cm Amberlite column using 0·35 M sodium citrate buffer, pH 5·28, and the concentrations of each amino acid in the sample were determined according to the method of Moore *et al.*[3] Nitrogen was determined by the Kjeldahl method.

### Results

The protein contents of the 26 samples of barley analysed ranged from 8·2 to 14·0% on a dry matter basis. The lysine contents of the same barleys ranged from 0·30 to 0·47%. The values obtained for the various samples are given in Table I. As can be seen from Fig. 1 the lysine content of the barley increased as the crude protein content increased ($r = 0·77$, $P < 0·001$). In a similar manner the contents of histidine and arginine were related to the crude protein content), the correlation coefficients being $r = 0·67$ ($P < 0·01$) and $r = 0·78$ ($P < 0·001$) respectively. The concentration of lysine in the protein however significantly decreased as the protein concentration increased ($r = 0·66$, $P < 0·001$) while the concentrations of histidine and arginine in the protein did not change significantly with increasing protein concentration.

## Performance of pigs

### Animals

Twelve sets of 3 littermates were used, 6 trios consisting of males only and 6 trios of females only. Within each trio pigs were allocated at random to 3 experimental treatments, A, B and C. The pigs were housed with 2 trios in a pen. The pigs were weaned at 6 weeks of age. Males had been castrated at 3 weeks of age. The experimental diets were given over the period from 20 to 90 kg liveweight.

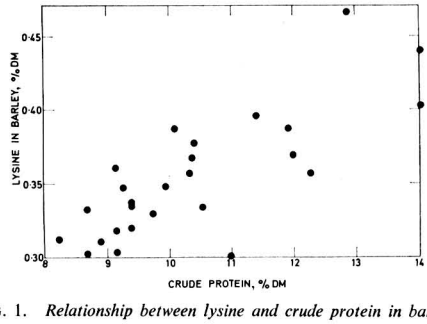


FIG. 1. *Relationship between lysine and crude protein in barley*

TABLE I

**The composition of the barleys examined**

| Variety | Year | Manurial Treatment | % in dry matter | | | |
|---|---|---|---|---|---|---|
| | | | Crude Protein | Lysine | Histidine | Arginine |
| Pallas | 1963 | 0 units N | 9·4 | 0·336 | 0·126 | 0·344 |
| Pallas | 1963 | 40 units N | 9·1 | 0·317 | 0·124 | 0·268 |
| Pallas | 1963 | 80 units N | 10·3 | 0·376 | 0·224 | 0·571 |
| Pallas | 1965 | | 9·2 | 0·347 | 0·148 | 0·385 |
| Ymer | 1963 | 0 units N | 9·4 | 0·334 | 0·089 | 0·277 |
| Ymer | 1963 | 40 units N | 8·9 | 0·310 | 0·209 | 0·506 |
| Ymer | 1963 | 80 units N | 10·1 | 0·386 | 0·170 | 0·415 |
| Ymer | 1965 | Unknown | 8·7 | 0·302 | 0·118 | 0·307 |
| A. Deba | 1965 | ,, | 8·2 | 0·312 | 0·129 | 0·359 |
| Delisa | 1965 | ,, | 9·4 | 0·319 | 0·127 | 0·357 |
| Ingrid | 1965 | ,, | 8·7 | 0·332 | 0·125 | 0·264 |
| Zephyr | 1965 | ,, | 9·7 | 0·329 | 0·187 | 0·369 |
| Unknown | ,, | ,, | 10·3 | 0·356 | 0·196 | 0·491 |
| ,, | ,, | ,, | 14·0 | 0·439 | 0·251 | 0·642 |
| ,, | ,, | ,, | 14·0 | 0·401 | 0·205 | 0·569 |
| ,, | ,, | ,, | 10·5 | 0·333 | 0·160 | 0·418 |
| ,, | ,, | ,, | 11·0 | 0·300 | 0·198 | 0·449 |
| ,, | ,, | ,, | 12·0 | 0·368 | 0·193 | 0·500 |
| ,, | ,, | ,, | 12·2 | 0·356 | 0·230 | 0·548 |
| ,, | ,, | ,, | 9·1 | 0·360 | 0·210 | 0·434 |
| ,, | ,, | ,, | 12·9 | 0·465 | — | — |
| ,, | ,, | ,, | 9·1 | 0·303 | — | — |
| ,, | ,, | ,, | 11·4 | 0·396 | — | — |
| ,, | ,, | ,, | 11·9 | 0·388 | — | — |
| ,, | ,, | ,, | 11·3 | 0·367 | — | — |
| ,, | ,, | ,, | 9·9 | 0·347 | — | — |

## Diets

The pigs were fed individually according to a feeding scale based on liveweight.[4] The diets given as Treatments A, B and C contained the same amounts of each ingredient. The compositions of the barleys used are shown in Table II and were such that Treatments A and B provided the same concentrations of protein, but different concentrations of lysine, and Treatments B and C the same concentrations of lysine, but different concentrations of protein.

When the pigs reached 54 kg liveweight, the composition of the diets was changed so that the concentration of crude protein was reduced by 2 percentage units in all treatments. The composition of the diets given is shown in Table III.

In diets providing the greatest concentration of lysine (Treatments B and C) the lysine concentration was less than the estimated lysine requirement of pigs of the appropriate weight.[4] This lower concentration was given to ensure that treatment effects were not masked by intakes of lysine higher than the amounts required by the pigs.

TABLE II

**The lysine and protein content of the barleys used**

| | Treatment | | |
|---|---|---|---|
| | A | B | C |
| % Crude Protein in barley | 9·5 | 9·5 | 12·0 |
| % Lysine in protein | 3·26 | 4·43 | 3·28 |

## Results

The mean growth rates and feed conversions of the pigs are shown in Table IV. There were no sex × treatment interactions and the values shown in Table IV are combined means. There were no significant differences in growth rate between treatments over the whole experiment or up to 54 kg liveweight. In the period when the pigs were between 54 and 90 kg liveweight, the extra lysine (cf. Treatments A and B) tended to increase growth rate, but the increase was not statistically significant. The supply of extra protein in addition to the lysine significantly increased growth rate (cf. Treatments A and C). Differences in feed conversion followed a similar pattern.

TABLE III

**The composition of the diets**

| Treatments | A | | B | | C | |
|---|---|---|---|---|---|---|
| Type of barley used | Low Protein/ Low Lysine | | Low Protein/ High Lysine | | High Protein/ Low Lysine | |
| Diet* | 1 | 2 | 1 | 2 | 1 | 2 |
| Barley | 72·5 | 75·5 | 72·5 | 75·5 | 72·5 | 75·5 |
| Weatings | 20·0 | 20·0 | 20·0 | 20·0 | 20·0 | 20·0 |
| Fishmeal | 7·4 | 4·5 | 7·5 | 4·5 | 7·5 | 4·5 |
| Crude Protein (%) | 14·9 | 12·9 | 14·9 | 12·9 | 16·8 | 14·9 |
| Total Lysine (%) | 0·65 | 0·53 | 0·73 | 0·61 | 0·72 | 0·60 |

Diet 1 given up to 54 kg liveweight and diet 2 from 54–90 kg
* A mineral and vitamin supplement was added to each

TABLE IV

**Daily liveweight gain and feed conversion**

| | Treatment means | | | Approx. S.E. of means |
|---|---|---|---|---|
| | A | B | C | |
| Liveweight gain (g/day) | | | | |
| Start–54 kg | 566 | 574 | 563 | ± 6·7 |
| 54–90 kg | 688 | 724 | 746 | ±16·3* |
| Start–90 kg | 628 | 638 | 648 | ±13·5 |
| Feed conversion (kg feed/kg liveweight gain) | | | | |
| Start–54 kg | 2·91 | 2·89 | 2·95 | ±0·035 |
| 54–90 kg | 4·04 | 3·89 | 3·76 | ±0·094* |
| Start–90 kg | 3·52 | 3·42 | 3·39 | ±0·053 |

* Significant at 5% level

TABLE V

**Carcass measurements**

| | Treatment means | | | Approx. S.E. of means |
|---|---|---|---|---|
| | A | C | C | |
| Fat depth 'C' mm | 18·2 | 16·2 | 18·8 | ±0·95 |
| Fat depth 'K', mm | 23·6 | 21·3 | 23·2 | ±0·94 |
| Mid-back fat, mm | 18·8 | 18·4 | 19·2 | ±1·02 |
| Average rump fat, mm | 28·8 | 27·0 | 28·2 | ±0·85 |
| Average back fat, mm | 32·1 | 31·0 | 31·6 | ±0·90 |
| Length, mm | 813 | 814 | 812 | ±4·3 |
| Specific gravity | 1·0523 | 1·0564 | 1·0555 | ±0·00099** |
| Eye muscle area, $cm^2$ | 26·9 | 29·8 | 27·7 | ±0·90* |
| Killing-out percentage | 76·3 | 76·8 | 76·0 | ±0·41 |

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

Treatment means for the various measures of carcass quality are given in Table V. These results show that the extra lysine supplied in the diets produced significantly leaner carcasses as indicated by the various measures of carcass quality such as area of eye muscle, specific gravity, and linear measurements.

## Discussion

It is pointed out that although there were differences in the crude protein content of the barleys, not all of these differences necessarily apply to the true protein content.

The increase in the concentration of lysine with a decrease in the protein content of the barley means that if lysine were the most limiting amino acid in the diet the quality of the dietary protein would improve as the crude protein content of the barley decreased. The proportion of lysine in the protein of the barley increased by about 20% for a reduction of 33% in protein concentration so that the effect of these changes on performance in the non-ruminant may not be as great as might be expected from an examination of crude protein content alone. It is known that dietary protein concentration may be reduced without adversely affecting growth rate provided that the concentrations of the limiting amino acid are increased.[5,6]

The other amino acids examined are usually found in British diets in amounts in excess of those required by the pig and it seems unlikely that the changes in histidine and arginine shown in Table I would affect performance.

The effect of changes in the lysine and protein composition of barley on the growth of pigs will depend on how near to the pigs requirements of these nutrients are the daily amounts supplied. The carcass quality of the pigs on Treatment C was poorer than that of pigs on Treatment B even though the diets contained the same concentration of lysine. This would suggest that the balance of amino acids in Treatment B was more favourable for lean tissue development than in Treatment C. Pigs on Treatment C, however, actually consumed less total lysine by virtue of their better feed conversion. It is not possible from this experiment to say whether the lysine effect observed was exerted before or after the pigs reached 54 kg liveweight. Until the pigs weighed 54 kg the amounts of lysine and protein supplied by the poorest diet (Treatment A) were 0·65% and 14·9% respectively which are lower than the R.A.C. estimates of requirement.[4] Similar comparisons with the A.R.C. estimates of requirement suggest that in the period from 54 to 90 kg liveweight protein was limiting, and the response to protein confirm this.

It may be noted that the difference in lysine content between the diets given in Treatments A and B was about 0·07%, which would be equivalent to the addition of approximately 1·5% of white fishmeal to the diets. Using the barleys examined, the greatest difference that could have been produced in the lysine contents of the diets would have been 0·16%, but since this difference would also be associated with changes in the quality of the protein of the barley, it would be unreasonable to equate this with an amount of white fishmeal.

## Acknowledgments

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# EFFECT OF BAKING AND AMINO ACID SUPPLEMENTATION ON THE PROTEIN QUALITY OF ARABIC BREAD

By M. MALEKI* and A. DJAZAYERI†

The effect of supplementation of flour with lysine, lysine–threonine, lysine–methionine, and lysine–threonine–methionine on the protein quality of Arabic bread was studied. In addition the effect of baking on the protein quality of non-supplemented and supplemented flour and bread was investigated. For the preparation of bread samples the dough was made in the laboratory and baked in a local bakery. Rats were used for evaluation of protein by protein efficiency ratio, weight gain and feed consumption.

The results showed that the addition of 0·3% L-lysine to flour improved the protein quality of flour and bread to a significant degree. Supplementation of lysine-fortified flour with 0·62% DL-threonine caused further improvement in protein quality, whereas methionine had no effect. Baking did not change the protein quality of lysine or lysine–threonine supplemented bread.

## Introduction

In Lebanon it has been reported that cereals provide over 55% of the daily protein and calorie intake of an average person.[1] Wheat, in the form of Arabic bread, is by far the most common cereal product consumed in the Arab world. This bread is flat and circular (1 cm thick, 10–30 cm diameter) it is baked at 400–500° for about one minute.

Wheat protein is known to be of poor quality because of its low content of certain essential amino acids, particularly lysine.[2–4] Lysine is the first limiting amino acid in wheat flour and bread[5,6] and Ericson[7] found threonine to be the second. Bender[2] confirmed Ericson's result and reported methionine to be the third limiting amino acid. Contrary to these reports, King *et al.*[6] claim that the order of limiting amino acids in wheat protein is: lysine, tryptophan, methionine, isoleucine and threonine.

Gates & Kennedy[5] and Rao *et al.*[8] reported that baking caused partial unavailability of lysine, threonine and methionine in European bread whereas Clegg & Davis[9] observed very little loss of available lysine in bread. Thus, there are conflicting reports with regard to second and third limiting amino acids in bread and the effect of baking on the protein quality of European bread.[7,10,11]

Since there is no published work on the protein quality of Arabic bread, the present work was initiated to study the effect of baking on the protein quality of Arabic bread with and without supplementation with lysine, threonine and methionine.

## Experimental

### Preparation of bread

The ingredients used for the preparation of experimental bread (wheat flour (65% extraction), 1000 g; water, 500 ml; salt, 12 g; and compressed yeast, 5 g) were mixed in a Crypto mixer (Model EB 12, Crypto Ltd., London) for 12–15 minutes and the resulting dough was allowed to ferment at room temperature for 1·0–1·5 hours. After fermentation, the dough was rounded, flattened and baked in a local bakery as described by Pelshenke.[1] For incorporation into animal diets, the bread samples were dried at 70° and ground to 100 mesh.

### Lysine analysis

Total lysine was determined microbiologically.[12] For the measurement of available lysine, the 2,4-dinitrofluorobenzene (DNFB) procedure of Bruno & Carpenter[13] was followed.

### Animal experiments

For the assessment of protein quality in rat diets containing supplemented and unsupplemented flour and bread, protein efficiency ratio (PER) was determined according to the method described by Campbell.[14] Male weanling rats of the Sprague–Dawley strain were used throughout the experiments. The animals were individually housed in mesh-bottomed cages in an air conditioned room held at 22 ± 1° and the percentage composition of their basal diet was: corn starch, 80; USP salt mixture, 4; non-nutritive cellulose (Alphacel), 5; vitamin mixture (Nutritional Biochem. Crop.), 1; vegetable oil, 10. Test materials (flour, bread or casein) were added at the expense of corn starch to a 10% protein level. The rats were allocated to the diet according to a randomised design. During the experiment, feed intake and weight gain were recorded weekly for each animal.

## Results and Discussion

*Results obtained from the experiments on the effect of baking on the protein quality of supplemented bread are summarised in Tables I and II.*

*According to Table I the PER of non-supplemented flour was 0·57, which was decreased to 0·40 by baking. Any food with a PER below 1 is considered to have little nutritional value. The addition of 0·30% L-lysine increased the PER*

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TABLE I

**Effect of lysine supplementation and baking on PER, total and available lysine and feed consumption and weight gain of rats fed such diets**

| Diets | Lysine added (% of flour) | PER* Mean ± SE | Feed Consumption (g/4 Wks) | Weight gain (g/4 Wks) | Total Lysine (mg/gN) | | | Available Lysine | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | calculated | determined | % recovery | mg/gN | % recovery† |
| Flour | 0·0 | 0·57 ± 0·05 | 168 | 8·4 | — | 118 | — | 98 | — |
| Flour | 0·30 | 1·40 ± 0·11 | 214 | 26·8 | 268 | 224 | 71 | 160 | 41 |
| Flour | 0·50 | 1·38 ± 0·19 | 211 | 26·5 | 368 | 284 | 66 | 202 | 42 |
| Flour | 0·70 | 1·38 ± 0·15 | 192 | 23·9 | 468 | 351 | 66 | 240 | 41 |
| Bread | 0·0 | 0·40 ± 0·06 | 159 | 6·1 | — | 118 | — | 100 | — |
| Bread | 0·30 | 1·50 ± 0·14 | 214 | 27·5 | 268 | 202 | 56 | 150 | 33 |
| Bread | 0·50 | 1·45 ± 0·13 | 184 | 23·5 | 368 | 283 | 66 | 198 | 39 |
| Bread | 0·70 | 1·58 ± 0·29 | 186 | 25·0 | 468 | 350 | 66 | 231 | 37 |

* PER of 10% casein diet was 2·41. The data are corrected to casein PER of 2·5

† Percentage recovery $= \frac{\text{Lysine determined (mg/gN)} - \text{Original lysine content (mg/gN)}}{\text{Lysine added (mg/gN)}} \times 100$

TABLE II

**Effect of threonine and methionine supplementation and baking on PER of lysine-supplemented flour and bread and on feed consumption and weight gain of rats fed such diets**

| Diet | Amino acids added (% of flour) | PER Mean ± SE | Feed Consumption (g/4 Wks) | Weight Gain (g/4 Wks) |
|---|---|---|---|---|
| Flour | 0·30L* | 1·05 ± 0·11 | 221·7 | 22·5 |
| Flour | 0·30L + 0·60T | 2·16 ± 0·10 | 310·9 | 62·3 |
| Flour | 0·30L + 0·28M | 1·11 ± 0·23 | 204·2 | 17·7 |
| Flour | 0·30L + 0·28M + 0·60T | 2·44 ± 0·07 | 332·1 | 76·2 |
| Bread | 0·30L | 1·151 ± 0·12 | 224·6 | 29·7 |
| Bread | 0·30L + 0·60T | 2·30 ± 0·12 | 285·3 | 60·1 |
| Bread | 0·30L + 0·28M | 1·33 ± 0·09 | 236·1 | 29·4 |
| Bread | 0·30L + 0·28M + 0·60T | 2·06 ± 0·08 | 287·9 | 57·5 |

*L = L-lysine, T = DL-threonine, M = L-methionine

of flour from 0·57 to 1·40 and of bread from 0·40 to 1·50. However, the addition of higher levels of this amino acid had no further improving effect. These results are in agreement with those of Hutchinson *et al.*[3] who found that the level of lysine supplementation for optimum growth of rats fed flour diets was 0·20–0·30%. There was also an increase in feed consumption and weight gain due to the supplementation of flour and bread with 0·30% of L-lysine. Rerat & Jacquot[4] also reported an increase in weight gain due to lysine supplementation. Levels higher than 0·30% lysine caused a statistically non-significant decrease in food consumption and weight gain.

As indicated in Table I the recovery of available lysine was about 40% in flour and 33 to 39% in the case of bread; the difference was not significant. Clegg & Davis[9] also observed no significant reduction in the available lysine content of flour due to baking.

In another experiment, diets containing the flour and bread supplemented with 0·30% lysine had PER values of 1·05 and 1·51, respectively (Table II). The addition of 0·60% DL-threonine to the lysine-supplemented flour before baking increased these values to 2·16 and 2·30, while the addition of 0·28% methionine to the lysine-supplemented flour did not change the PER value of either flour or bread to any significant degree. These results indicate that, in the flour and bread, of the three studied threonine was the second limiting amino acid; similar results have been reported by Bender[2] for European bread. When 0·28% of L-methionine was added to flour already fortified with lysine and threonine, there was no statistically significant increase in the PER of the flour diet, whereas, the value for the bread diet was in fact significantly lower than its corresponding flour.

Similar results were observed in feed consumption and weight gain. There was no significant change in feed consumption and weight gain of rats due to addition of methionine to lysine-supplemented flour, but the addition of threonine caused increases in these measurements (Table II). Addition of 0·60% threonine and 0·28% methionine to 0·30% lysine-enriched flour caused a further increase in the rats' food consumption and weight gain, whereas when the flour was baked into bread and fed to rats there was a drop in feed consumption and weight gain.

From these findings one can conclude that: baking has no damaging effect on the protein quality of bread except when the flour is made from flour supplemented with its first limiting amino acid (lysine) or its first two limiting amino acids (lysine plus threonine). Methionine does not appear to be the third limiting amino acid.

According to this study, Arabic bread, as reported for other kinds of breads, seems deficient in lysine and threonine. However, some workers[15,16] have shown that, in mixed diets, especially those containing animal protein, the amino acid which becomes limiting is methionine rather than lysine or threonine; since bread is seldom used as a sole diet and since most meals contain some sort of animal protein, the addition of methionine to Arabic bread would improve the general nutrition of people using this kind of bread in their daily meals.

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# PESTICIDE RESIDUES IN FOODSTUFFS IN GREAT BRITAIN

## VII.*—Demeton-methyl and dimethoate residues in Brussels sprouts, lettuce, green peas and French beans, potatoes and strawberries

By D. F. LEE

Samples of Brussels sprouts, lettuce, green peas and French beans, potatoes and strawberries from commercial crops that had been treated with demeton-methyl, oxydemeton-methyl, dimethoate and/or formothion were analysed at the time of harvest. 168 of 191 samples (88%) contained residues of these pesticides not exceeding 0·2 ppm; the residues in the remainder did not exceed 0·8 ppm.

### Introduction

In Great Britain aphids are controlled on many crops by the use of organophosphorus pesticides. Demeton-methyl, oxydemeton-methyl, dimethoate and formothion are, or have been, used on Brussels sprouts, lettuce, green peas, French beans, potatoes and strawberries to control cabbage aphid (*Brevicoryne brassicae*), glasshouse-potato aphid (*Aulacorthum solani*), peach-potato aphid (*Myzus persicae*), potato aphid (*Macrosiphum euphorbiae*), shallot aphid (*Myzus ascalonicus*), pea aphid (*Acyrthosiphon pisum*), black bean aphid (*Aphis fabae*) and buckthorn-potato aphid (*Aphis nasturtii*). Red spider mite (*Tetranychus telarius*) may be controlled by these chemicals.

The Pesticides Safety Precautions Scheme[1] has recommended that a minimum interval of three weeks should elapse between the last application and harvest of crops sprayed with demeton-methyl and oxydemeton-methyl and one week for dimethoate and formothion.

In 1963 the Panel on Residues of Pesticides in Foodstuffs[2] recommended that studies of residues of organophosphorus insecticides in the crops listed above should be undertaken. When this work started demeton-methyl and dimethoate were the organophosphorus insecticides most commonly

* Part VI: *J. Sci. Fd Agric.*, 1968, **19,** 315

used on these crops but later oxydemeton-methyl replaced demeton-methyl and formothion was introduced in competition with dimethoate. In plants demeton-methyl is rapidly converted to oxydemeton-methyl, and formothion to dimethoate.

## Experimental

Samples were taken just before harvest from commercial crops known to have been treated only with the named organophosphorus pesticides. Details of the rates, dates and method of application of the pesticides to the crops were also obtained where possible. Except where later indicated the intervals between the last treatment and harvest were greater than those recommended. Samples consisted of a minimum of 1 kg of Brussels sprouts, two heads of lettuce, 1 kg of peas in the pod, 500 g of bean pods, twelve potato tubers (average total weight about 1·5 kg) and 500 g of strawberries.

Samples of untreated crops for blank value determinations were usually specially grown but untreated commercial crops were also examined.

### Preparation and storage of samples before analysis

The sprouts and lettuce were prepared as for human consumption by removal of dirty and blemished leaves and the main stalk. The leafy parts remaining were finely chopped and thoroughly mixed. The peas were shelled and the pods were discarded. After the ends had been cut off and discarded, bean pods were finely chopped and thoroughly mixed. The potatoes were scrubbed free of soil under running water and, after air-drying, were macerated to a thick slurry. Strawberries were also macerated after removal of stalks and calyces. The prepared samples were stored in polythene bags or screw-capped bottles at −20° until required for analysis.

### Methods of analysis

All samples were analysed at least in duplicate. Samples from the 1963 and 1964 crops were examined by methods based upon that described by the Joint Demeton-methyl Residues Panel.[3] In 1965 and 1966 sub-samples from the prepared field samples were initially screened by two-dimensional Kieselgel G thin-layer or paper chromatography methods, based upon the extraction and clean-up procedures described by Bates.[4] The limit of positive identification was 0·2 ppm of oxydemeton-methyl or dimethoate, using 4-(*p*-nitrobenzyl)-pyridine and 2,6-dibromo-*p*-benzoquinone-4-chlorimine as the chromogenic reagents. Samples containing residues in excess of 0·2 ppm were re-examined, the cleaned-up extracts being oxidised with nitric and perchloric acids and the phosphate being estimated as above. Recoveries of demeton-methyl sulphoxide and dimethoate added at the initial solvent extraction stage at the 0·1 ppm level were better than 80% with the exception of dimethoate from peas and beans, where the recovery was occasionally as low as 60%.

TABLE I

**Residues of oxydemeton-methyl and dimethoate in vegetables**
(parts per million in excess of blank value)

| Crop | Year | No. of samples | No. of samples with residues less then 0·2 ppm | Residues above 0·2 ppm (Total phosphorus method) | 'Apparent residues' in samples from untreated crops (Total phosphorus method) | Interval between last treatment and harvest, weeks |
|---|---|---|---|---|---|---|
| Brussels sprouts | 1964 | 19 | 15 | 0·4[a], 0·5[a], 0·7[b], 0·7[c] | 0—0·02 | 3–18<br>a 8; b 14; c 16 |
| | 1965 | 15 | 8 | 0·3[a], 0·3[d], 0·4[c], 0·5[a], 0·5[b], 0·7[f], 0·8[c] | | 18–28<br>a 18; b 19; c 20; d 21; e 23; f 26 |
| | 1966 | 34 | 34 | | | 1–28 |
| Lettuce | 1965 | 27 | 20 | 0·4[a], 0·6[a], 0·6[c], 0·8[b]; 0·2[d], 0·4[e], 0·6[f] | 0–0·04 | 1–3 for dimethoate; 3–5 for demeton-methyl<br>dimethoate a 5; b 9; c 24;<br>demeton-methyl d 4; e 28; f 35 |
| | 1966 | 15 | 15 | | | 2–5 for dimethoate; 3–12 for demeton-methyl |
| Green peas | 1965 | 20 | 18 | 0·23[a], 0·26[b] | 0–0·08 | 1–2 for dimethoate; 3–6 for demeton-methyl<br>dimethoate a1; b 2 |
| | 1966 | 18 | 15 | 0·3[b], 0·4[a], 0·4[a] | | 1·5–6 for dimethoate; 3–6 for demeton-methyl<br>dimethoate a 1·5; b 2 |
| French beans | 1965 | 3 | 3 | | Paper chromatography only | 4–9 |
| | 1966 | 10 | 10 | | | 8–10 for dimethoate; 5–11 for demeton-methyl |
| Potatoes | 1964 | 5 | 5 | | 0–0·07 | greater than 8 |
| Strawberries | 1963 | 4 | 4 | | 0·7–0·9 | 4–5 |
| | 1964 | 21 | 21 | | | 4–9 |

## Results

The results are summarised in Table I. All residue levels quoted are uncorrected for recovery and are in excess of the 'apparent residue' levels in equivalent untreated crops as determined by the total phosphorus method.

### Brussels sprouts

Usually not more than two treatments are needed to control *Brevicoryne brassicae* but in 1964, a dry late summer and autumn made control difficult and up to four treatments were applied. The 4 samples from the 1964 crop that contained residues in excess of 0·2 ppm had been treated 3, and in one case 4 times, but a sample from another crop that had been treated 4 times contained 0·1 ppm. None of the 1965 samples was from crops that had been treated more than twice with demeton-methyl, but one sample with a residue of 0·7 ppm oxydemeton-methyl had been treated twice with demeton-methyl and once with disulfoton.

### Lettuce

Two of the 1965 crops that had been treated with dimethoate and sampled after 5 days contained residues at 0·4 and 0·6 ppm but two other crops sampled after 9 and 24 days contained 0·8 and 0·6 ppm respectively. One demeton-methyl treated crop sampled after 14 days contained 0·2 ppm, one sampled after 28 days 0·4 ppm and one after 35 days 0·6 ppm. Only 2 samples from 15 examined from the 1966 crop gave an indicative reaction in the screening test; they both contained 0·1 ppm demeton-methyl sulphoxide.

### Peas

Two samples from the 1965 crop contained residues of dimethoate in excess of 0·2 ppm (0·23 and 0·25 ppm). The method of the pesticide to the first of these crops was not known but the second had received a low-volume aerial application. The other samples for which treatment dates were available had been treated by high-volume ground application. The three 1966 samples which contained residues in excess of 0·2 ppm had all received low-volume aerial applications of dimethoate.

## Discussion

Of a total of 191 samples taken at the time of harvest from various crops that had been treated with demeton-methyl, oxydemeton-methyl, dimethoate and/or formothion, 168 (88%) did not contain residues exceeding 0·2 ppm and in many of these the 'apparent residue' was indistinguishable from that in untreated crops. In none of the remaining 23 (12%) samples did the residues exceed 0·8 ppm. The indications are, therefore, that the use of these particular pesticides on the crops investigated, according to the instructions issued by the manufacturer and approved by the Pesticides Safety Precautions Scheme, should not present a hazard to the consumer.

## Acknowledgments

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# DETERMINATION OF ELEMENTAL SULPHUR IN SOILS

By N. J. BARROW

Elemental sulphur was extracted from soil with chloroform and the sulphur was reduced to hydrogen sulphide by iron powder in the presence of hydrochloric acid, acetone and chloroform. The hydrogen sulphide was transferred in a stream of nitrogen to sodium hydroxide solution and determined by titration with mercuric chloride using dithizone as an indicator. The reduction technique was not specific to elemental sulphur but soils appeared to contain few compounds which were soluble in chloroform and which were also reduced. Wet soils could be extracted without preliminary drying. The analysis is rapid and suitable to routine application.

## Introduction

In southern Australia, sulphate fertilisers are often rapidly leached from soil,[1,2] and sulphur deficiency may ensue within a few months of application. Under such conditions more slowly available forms of sulphur—such as elemental sulphur—may have advantages. Research into optimum amounts, and frequencies of application would be aided by a method of determining residual elemental sulphur. If such a method were sufficiently accurate it could also be used to study rates of oxidation of elemental sulphur under field conditions.

Of the previously published methods for the determination of elemental sulphur, that of Hart[3] relies on turbidimetric measurement of the sulphur precipitated when an acetone solution of sulphur is mixed with water. It suffers from interference from organic matter[4] and is only suitable if the sulphur content of the soil is greater than 1 mg/g. The method of Chopra[4] is more sensitive. In this method elemental sulphur is extracted from the soil with benzene and reduced with Raney nickel under alkaline conditions. Hydrogen sulphide is then released from the nickel sulphide with acid and determined as methylene blue. According to Chopra[4] it is necessary to remove organic matter before the soil is extracted. He used a preliminary treatment with hydrogen peroxide but this seems undesirable because of possible oxidation of elemental sulphur. Alternative procedures were therefore sought. Elemental sulphur can be reduced to sulphide by stannous chloride, by iron and hydrochloric acid,[5] or by tin and hydrochloric acid.[6] These reactions were investigated as the basis of the present method.

## Experimental

### Reagents

*Chloroform.* Analytical grade reagent or re-distilled reagent grade.

*Iron powder.* Merck analytical grade reagent.

*Hydrochloric acid.* Equal volumes of concentrated hydrochloric acid and water were mixed.

*Sodium hydroxide solution.* An approximately 1 N solution.

*Acetone–dithizone solution.* Approximately 0·01 g dithizone was dissolved in a litre of glass-distilled acetone. This had to be prepared fresh each day.

*Mercuric chloride solution.* A 0·001 M solution.

*Standard sulphur solution.* 0·100 g sulphur were dissolved in chloroform and adjusted to 100 ml. This solution was diluted with chloroform to give appropriate standard solutions.

### Apparatus

The reduction and distillation apparatus is shown in Fig. 1. Nitrogen gas obtained from Commonwealth Industrial Gases was found to be free from sulphur compounds and therefore was not purified before use. If sulphur compounds are present in the nitrogen they may be removed as described by Johnson & Nishita.[7]

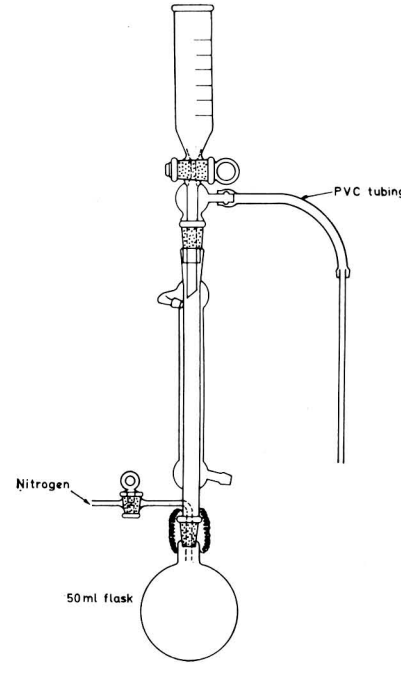


FIG. 1. *Apparatus used for the reduction of sulphur*

**Procedure**

The experimental procedure adopted can be used as a set of recommended instructions as follows:

To a sub-sample of at least 50 g soil in a glass bottle add 100 ml chloroform. Seal the bottle with a rubber bung protected by a small piece of thin polyethylene and shake it intermittently for about 30 minutes. Pipette an aliquot of the supernatant containing 10–400 μg S into the 50 ml round-bottomed flask (Fig. 1) and evaporate the extract to dryness on a rotary film evaporator. Unused chloroform may be recovered from the soil by distillation.

Add 2 ml acetone and ~0·2 ml chloroform to the dried sample and place ~0·2 g iron powder in the flask. Attach the flask to the apparatus and sweep it with nitrogen for a few minutes to remove air. Place 10 ml N sodium hydroxide solution in a 1 in. dia. test tube and attach it to the side arm so that the glass tube dips below the NaOH surface. Run 10 ml HCl into the apparatus. Apply a low flame from a microburner and allow 10–15 minutes for transfer of sulphide. Detach the glass tube from the apparatus and leave it in the test tube; add 10 ml acetone-dithizone solution and wash the inside of the glass tube with it. Titrate the solution against the mercuric chloride to a pink end-point. Stir the solution during titration by bubbling nitrogen through it. Standardise the mercuric chloride solution against solutions of sulphur reduced in the same way.

**Results**

Powdered iron was found to be effective in reducing sulphur to sulphide in the presence of hydrochloric acid. It was used in the development of the method mainly because supplies were more readily available than powdered tin. Powdered tin was also effective. It had the advantage that little reaction occurred in the cold so that acid could be added directly to the flask immediately before it was attached to the apparatus and the separating funnel was not needed. Stannous chloride solution also reduced sulphur, but variable recovery of hydrogen sulphide was obtained.

Iron did not give complete reduction of the sulphur added. The titre was about 7% lower than that obtained when sulphate was reduced to hydrogen sulphide with hydriodic acid and hypophosphorus acid as described by Steinbergs *et al.*[8] Granatelli[9] similarly found that reduction of sulphur with Raney nickel gave titrations about 5% lower than were expected. In agreement with results of Granatelli,[9] attempts to find the cause of this loss were unsuccessful. However, a consistent calibration line was obtained, and the method was therefore placed on an empirical basis.

Provided that analytical grade iron was used, so that the blank is low, the amount of iron used could range from 0·05 to at least 0·5 g with little adverse effect (Table I). Hence iron could be dispensed by volume measure. The method was also insensitive to the volume of acid used (Table II) so that acid could be dispensed with sufficient accuracy by calibration marks on the vessel at the top of the apparatus.

TABLE I

**Effect of amount of iron on reduction of sulphur**

| Sulphur taken, μg | Sulphur recovered, μg* for different amounts of iron added, g | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0·05 | 0·10 | 0·2 | 0·3 | 0·5 | 0·8 | 1·0 |
| 100 | 95 | 100 | 103 | 103 | | | 100 |
| 200 | 199 | 202 | 203 | 200 | 198 | 201 | 200 |
| 300 | 300 | 302 | 300 | 297 | 303 | 290 | 282 |

* Values read from standard calibration line

The reduction step was not specific to elemental sulphur. Sulphate was not reduced, but inorganic salts in which the sulphur was in a lower valency state were partly reduced (Table III). Sulphur in sulphamic acid, or in two sulphonic acids was not reduced, nor was sulphur in a thioether as in methionine or a ring as in methylene blue (Table III). The sulphydryl group of cysteine and the thio groups of dithiocarbamate and of dithizone were partly reduced. However, in the soils tested there were few sulphur compounds which were soluble in chloroform and which were also reduced by iron and hydrochloric acid. Soils with no history of application of elemental sulphur gave the following values for 'apparent' content of elemental sulphur: alpine humus, 0·2 ppm; reddish chocolate soil 0·2 ppm; chocolate soil 0·2 ppm; lateritic red earth, 0·5 ppm; meadow podzolic, 0·1 ppm.

Chloroform was found to dissolve commercial samples of sulphur rapidly. However, μ-sulphur (i.e. sulphur which is insoluble in carbon disulphide) has a low solubility in chloroform; when μ-sulphur was prepared by pouring molten sulphur into water and extracting the soluble sulphur with

TABLE II

**Effect of volume of acid on reduction of sulphur**

| Volume of acid ml | Sulphur recovered, μg* for different amounts of sulphur taken, μg | | | | |
|---|---|---|---|---|---|
| | 100 | 150 | 200 | 250 | 300 |
| 5 | 99 | 145 | 197 | 251 | 304 |
| 10 | 98 | 152 | 199 | 251 | 299 |
| 15 | 100 | 152 | 204 | 247 | 301 |

* Values read from standard calibration line

TABLE III

**Reduction of sulphur in a range of organic and inorganic sulphur compounds**

| Compound | Nature of sulphur group | S reduced (100 μg of S taken) |
|---|---|---|
| Elemental sulphur | S | 100 |
| Sodium thiosulphate | $S_2O_3^=$ | 92 |
| Sodium dithionite | $S_2O_4^=$ | 45 |
| Sodium metabisulphite | $S_2O_5^=$ | 61 |
| Sodium sulphite | $SO_3^=$ | 41 |
| Potassium pyrosulphate | $S_2O_7^=$ | 3 |
| Ammonium persulphate | $S_2O_8^=$ | 3 |
| Potassium sulphate | $SO_4^=$ | 1 |
| Cysteine | −C−S−H | 79 |
| Ammonium pyrrolidine dithiocarbamate | S ‖ −C−S⁻ | 90 |
| Dithizone | \| −C=S | 53 |
| Methionine | C−S−C | 1 |
| Methylene blue | C−S−C | 2 |
| Sulphamic acid | N−$SO_3$H | 2 |
| Sulphanilic acid | C−$SO_3$H | 2 |
| 1 : 2 naphthaquinone-4 sulphonic acid | C−$SO_3$H | 2 |

carbon disulphide in a Soxhlet apparatus, the solubility of the residue in chloroform was found to be 7 μg-ml. This is unlikely to be a disadvantage in practice because the results of Weir[10] suggest that there is little insoluble sulphur in sulphur-fortified superphosphate. However, sulphur condensed from a vapour, may contain a large proportion of insoluble sulphur and should be avoided in preparing standards.

Because of the low solubility of water in chloroform, wet soils can be extracted without any preliminary drying. This is an advantage over acetone, which was used by Hart.[3] Furthermore, chloroform is less toxic than benzene, which was used by Chopra.[4] Soils usually settled rapidly in chloroform so that centrifugation was seldom necessary.

Chloroform extracts must be concentrated because, if large quantities of chloroform are present in the reduction step, low results are obtained. Presumably the chloroform occludes some of the sulphur in droplets. Furthermore, if large quantities of chloroform are present it distils into the absorption vessel and produces a turbidity which makes titration difficult. However, unless a solvent for sulphur is present during the reduction step, erratic results are obtained. For small amounts of sulphur, adding acetone to the flask before the reduction overcame the difficulty, but for large amounts of sulphur low recoveries were sometimes obtained even when acetone was added (Table IV). This was most marked when small volumes of concentrated solutions were evaporated so that the sulphur was presumably localised in a small part of the flask. This suggested that the sulphur was not redissolving rapidly. The difficulty was overcome by adding 0·2 ml of chloroform to the flask (Table IV).

Sulphur added to both dry and wet soil in the laboratory was completely recovered (Table V). However, the method is intended to measure elemental sulphur in samples collected from the field. This involves collecting separate samples, bulking them to give a composite sample, and sub-sampling this composite sample to give a sample for analysis. The standard deviation associated with each step was measured and found to be: field sampling, 30·7 lb/ac; sub-sampling, 4·4 lb/ac; analysis, 1·0 lb/ac (mean value 39 lb/ac). These values can be used as a guide in estimating optimum sampling procedure. Even though in the above data, the sub-samples were of 80 g, the precision of sub-sampling was lower than is usually obtained with soil analysis. This reflects the difficulty of mixing *particles* of sulphur through a large bulk of soil. This difficulty may vary with the soil used. It would be expected that the variance of sub-sampling would be inversely proportional to the size of the sub-sample. Hence it is important that sub-samples should be as large as practicable—certainly larger than the 2 g suggested by Chopra.[4] It is because of this need for large sub-samples that the less sensitive titration finish has been preferred to the sensitive methylene blue finish used by Chopra.[4]

TABLE IV

**Effect of the presence of acetone and of chloroform on the recovery of sulphur using iron plus hydrochloric acid for the reduction**

| Acetone ml | Chloroform ml | Sulphur recovered,* for different amounts of sulphur added, μg | | | |
|---|---|---|---|---|---|
| | | 100 | 200 | 250 | 300 |
| 0 | 0 | 70 | 156 | 140 | 127 |
| 2 | 0 | 92 | 185 | 230 | 265 |
| 0 | 0·2 | 101 | 203 | 216 | 290 |
| 2 | 0·2 | 100 | 202 | 251 | 298 |

* Values read for standard calibration line

TABLE V

**Recovery of sulphur added to 50 g of wet or dry soil**

| Soil | Sulphur added mg | Sulphur recovered mg |
|---|---|---|
| Meadow podzolic dry | 4·5 | 4·5 |
| | 10·8 | 11·0 |
| | 16·1 | 16·1 |
| Meadow podzolic wet* | 6·1 | 6·3 |
| | 11·1 | 11·2 |
| | 18·8 | 18·8 |
| Lateritic red earth dry | 4·2 | 4·5 |
| | 9·6 | 9·3 |
| | 14·6 | 14·3 |
| Lateritic red earth wet* | 6·6 | 6·8 |
| | 11·0 | 11·1 |
| | 18·8 | 19·1 |

* Soil moistened to field capacity before sulphur added

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# LEAF ANALYSIS AS A GUIDE TO THE NUTRITION OF FRUIT CROPS

## VII*.—Sand culture N, P, K, Mg experiments with red raspberry *(Rubus idaeus)*

By C. BOULD

The main effects and interactions of N, P, K and Mg on cane growth, leaf nutrient composition and crop yield of red raspberry, grown in sand culture, were investigated. Results indicated that leaf-lamina samples taken from non-fruiting canes from late June to early August may be used as an index of the nutritional status of the crop. Leaf nutrient concentrations at this growth stage were related to crop yield in the following season. Optimum leaf-lamina concentrations in late July were thought to be: K, 1·5%; Mg, 0·3%; N, > 2·3%; P, > 0·2% in dry weight.

### Introduction

Previous papers in this series have dealt with the general principles of leaf analysis, sampling errors and techniques, sample storage conditions and analytical methods[1–3] and with their application to strawberry nutrition.[4] The current paper deals with the use of leaf analysis, in conjunction with cane growth and yield data from plants grown in sand culture, for studying the nutritional requirements of the red raspberry. The first two experiments have been partly described in a previous publication[5] but in order to give a complete picture, and because the previous publication is not readily available, some of the results from the first two experiments have also been included here.

Ljones[6] has reviewed the general position with regard to bush fruit nutrition, including raspberry, from which it appears that little experimental work has been done on leaf analysis in relation to the nutrition of this crop.

Ramig & Vandecaveye[7] grew red raspberry in water culture and studied the effect of treatments on yield, leaf and petiole composition and their relationships. Using samples comprising the first six physiologically active leaves from each cane, beginning with the 3rd or 4th leaf from the growing tip, they found that leaf petioles reflected P, K and Ca status better than did leaf laminae, but laminae were preferable for N and Mg. They suggested critical nutrient levels, in samples from non-bearing canes, as follows: N in leaf laminae, 2·9%; P in laminae or petioles, 0·3%; K in laminae, 1·0%; K in petioles, 0·7% dry matter. They noted that bearing canes grown under field conditions contain less N, P and K and more Ca than leaves of non-bearing canes.

Wood[8] studied the seasonal variation in the leaves of fruiting canes, using the 1st mature leaf behind the growing tip. Potassium content was greatly influenced by potassic fertilisers and by stage of growth. The lowest leaf-K level was found in the post-harvest sampling. Wood concluded that critical K levels must be associated with stage of growth and suggested that a more sensitive leaf, possibly the oldest leaf or a leaf adjacent to the fruiting tip, might be used for earlier sampling dates. He found that leaf scorch deficiency symptoms appeared when the leaf-K concentration, prior to the fruiting stage, was less than 1% in dry matter.

Clark & Powers[9] used leaf analysis as an indicator of the potassium requirements of red raspberry. Using samples consisting of the 1st leaf below the growing tip at harvest time, they found that normal plants contained 1·2% K in leaf dry matter.

Naumann[10], using plants grown in gravel culture, suggested critical nutrient levels, in leaves from the central part of annual shoots just before bloom, as follows: N, 1·8–2·0%; P, 0·28–0·30%; K, 1·8–2·0%; Ca, 0·8–1·0% in dry matter. Deficiency of N and P resulted in a considerable reduction in cane growth whereas deficiency of Ca and K caused only a minor reduction in growth.

From a survey of the nutrient ranges and crop yields of fruit trees and small fruits, Ljones[11] found that the leaf-N content of raspberry, cv. Malling Promise, ranged from 2·34 to 3·29%. No yield increase was observed when leaf-N values exceeded 2·4% in dry matter, for samples collected between 30 August and 20 September. He concluded that the critical point, between the optimum range and the excess range for leaf-N in relation to crop yield, was approximately 2·9% N in dry matter. He gives the deficiency range for leaf potassium as being below 1·2%; optimum range, 1·2–1·6%, and the excess as being greater than 1·6% K in dry matter. For leaf-P, no yield increase was obtained when values exceeded 0·22% P in dry matter. The deficiency level for calcium is below 0·8% Ca in dry matter. No critical value is given for leaf-Mg.

In a sampling study of red raspberry grown under different soil fertility regimes in the field, Bould, Bradfield & Clarke[1] found that the chemical composition of the lamina and

* Part VI: *J. Sci. Fd Agric.*, 1965, **16**, 33

petiole is considerably affected both by position and by soil treatment. For samples taken at the fruit ripening stage, from non-fruiting canes, the lamina has a higher nitrogen concentration, and is more sensitive to nitrogen supply than the petiole, and of all positions the lower third best reflects soil nitrogen treatment. Lamina and petiole from any position on the cane reflect soil potassium treatment. The petiole is more sensitive than the lamina to changes in potassium supply, but the values are more variable and a greater difference is required for the same level of significance. The phosphorus concentration in the lamina is significantly greater than in the petiole for all positions. They suggested that, bearing in mind the sensitivity to nutrient supply, the variability between samples, and the introduction of errors in sub-sampling, the lamina from the middle to lower third region of non-fruiting canes best reflects N and K supply and may be the most suitable organ for relating nutrient supply and crop yield. For these reasons, leaf laminae from the mid-third region of non-fruiting canes were used in the current study.

## Experimental

### Pot experiments, materials

A non-calcareous coarse pit sand was used in all experiments. It was not purified, by acid treatment, but was washed thoroughly before use. Rainwater, collected from a large experimental glasshouse, was used for making up solutions and for watering the plants. Purification of salts and materials was deemed to be unnecessary because absolute deficiency was not required in this series of experiments with major nutrients.

*Pots*

Unglazed 12 in. clay pots were used, after being given two coats of bituminous paint. Drainage was provided by covering the basal holes with a circle of coarse-mesh Tygan.

*Nutrient solutions*

The nutrient compositions were based on the standard Long Ashton solution (as described by Hewitt)[12] except for iron which was supplied initially as Fe-EDTA and subsequently as Fe-EDDHA. Magnesium and potassium, when reduced, were substituted by sodium. Nitrate and phosphate were replaced by equivalent amounts of sulphate.

*Plants*

Single canes, cv. Lloyd George (virus-tested), were planted in 12 in. pots in early winter. In the following April the canes were pruned to 9 in. from the sand surface. All plants were given a complete nutrient solution throughout the first growing season. In the following April, they were transferred to the experimental cages; canes were pruned again to 9 in. from sand level and differential nutrient treatments were started. When the young shoots were about 9 in. high they were reduced in number to 8 per pot.

### Treatments and layout

*Experiment 1.* *N × P factorial* (K = 4; Mg = 3 mequiv./l)
Nitrogen, 5, 10, 15 and 20 mequiv. $NO_3$/l.
Phosphorus, $\frac{1}{2}$, 1, 2 and 4 mequiv. $PO_4$/l.
Three replications of 16 treatments, 2 pots per plot, giving a total number of 96 pots. Layout, three randomised blocks.

*Experiment 2.* *K × Mg factorial* (N = 15; P = 4 mequiv./l.)
Potassium, $\frac{1}{2}$, 1, 2, 4 mequiv. K/l.
Magnesium, $\frac{1}{2}$, 1, 2, 4 mequiv. Mg/l.
Three replications of 16 treatments, 2 pots per plot, giving a total of 96 pots. Layout, three randomised blocks.

*Experiment 3.* *N × K factorial* (P = 4; Mg = 4 mequiv./l.)
Nitrogen, 10, 15 and 20 mequiv. $NO_3$/l.
Potassium, $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 4 mequiv. K/l.
Three replications of 18 treatments, 2 pots per plot, giving a total number of 108 pots. Layout, three randomised blocks.

*Experiment 4.* *N × Mg factorial* (P = 4; K = 4 mequiv./l.)
Nitrogen, 10, 15, 20 mequiv. $NO_3$/l.
Magnesium, $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 4 mequiv. Mg/l.
Three replications of 18 treatments, 2 pots per plot, giving a total number of 108 pots. Layout, three randomised blocks.

*Management*

Nutrient solutions were applied weekly at the beginning of the season, then twice a week, and finally every other day; water being given as required.

*Leaf sampling*

Leaf samples (excluding the main petiole[2]) were taken from the middle position of non-fruiting canes, one leaf per cane, at the flowering and fruit ripening stages in Experiments 1 and 2 and in early August and early September in Experiments 3 and 4. Chemical analysis of samples was by methods previously described by Bould *et al.*[1]

*Cane measurement*

The variety Lloyd George has a tendency to produce some fruit on the tips of the current season's canes. These fruiting tips died during the winter and were removed. Measurements were made on the canes after the removal of the dead tips. A covariance correction was made on plots with one or more missing canes.

## Results and Discussion

The experimental results for cane length, crop yield and leaf nutrient status are given in Tables I–IX, and the relationships between leaf nutrient status and crop yield are illustrated in Figs 1–5.

### Nitrogen

*Cane length*

Increasing concentrations of nutrient nitrogen, over the range 5 to 20 mequiv. $NO_3$/litre, resulted in a curvilinear increase in cane length (Fig. 1a). From the shape of the curves it is clear that the optimum level of nitrogen supply had not been reached. There was no significant interaction (at the 5% level) between N and P, or between N and K, on cane length although the response to N was greatest at the higher levels of nutrient P (Table I) and K.

*Crop Yield*

The response to nitrogen varied with the supply levels of phosphate (Table II) and of potassium (Table VI) but not of magnesium (over the range 0·5 to 4 mequiv. Mg/l.). Highest yields were given by treatments $N_{20}$ $P_4$ (Experiment 1) and $N_{20}$ $K_3$ (Experiment 3).

*Leaf analysis*

In Experiment 1, mean leaf-lamina-N concentrations (Table III) from non-fruiting canes varied from 1·94% to 2·87% at the first sampling (22 June), and from 1·61% to 2·26% in dry matter at the second sampling (22 July). In Experiment 3, leaf lamina-N (Table VII) varied from 2·14% to 2·51% at the first sampling (1 August), and from 1·74 to 2·10% in dry matter at the second sampling (3 September). There was no significant interaction between N and P, or between N and K, on leaf-N.

*Leaf-N, growth, crop yield relationships*

The relationship between leaf-N and cane growth in Experiments 1 and 3 (Fig. 1b) is curvilinear over the range 1·5 to 3·0% N in dry matter. For leaf samples taken on 22 June, the highest mean value (2·87%N) was still too low for maximum cane growth. Fig. 2a shows that crop response to nitrogen was affected by the supply of phosphate. With adequate supplies of nutrient-P, crop response to leaf-N was almost linear over the range 1·6 to 2·3% N in dry matter for leaf laminae sampled on 22 July. Crop response to leaf-N was also affected by the supply of nutrient-K (Fig. 4b), and was linear over the range 2·0 to 2·5% N in dry matter of leaf lamina samples taken from non-fruiting canes on 1 August. Fig. 4b shows that the optimum leaf-N level for maximum crop yield had not been reached.

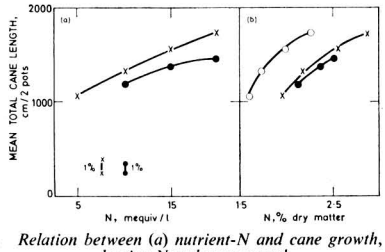


FIG. 1. *Relation between (a) nutrient-N and cane growth, (b) leaf lamina-N and cane growth*

× = Experiment 1, sampled June 22
○ = Experiment 1, sampled July 22
● = Experiment 3, sampled August 1
Significant difference is indicated

**Phosphorus**

*Cane length*

Nutrient-P had a significant effect on cane length up to concentrations of 1 mequiv. P/l. (Table I) above which there was no further significant response. The NP interaction was not significant at the 5% level.

*Crop yield*

The response to nutrient-P varied with the supply of nutrient-N (Table II), highest yield being given by treatment $P_4$ $N_{20}$. The yield response to increased levels of nutrient-P varied from 113 g/2 pots at the lowest level of nutrient-N to 1,417 g/2 pots at the highest level of nutrient-N.

*Leaf analysis*

In Experiment 1 leaf lamina-P (Table III) varied with treatments from 0·123% to 0·262% in dry matter on June 22, and from 0·099% to 0·230% in dry matter on July 22. Nitrogen supply had a significant effect on leaf-P; in general increased nutrient-N depressed leaf-P at both sampling dates.

TABLE I

**Pot Experiment 1. (N × P). Mean total cane length in cm/2 pots**
(Raspberry, cv. Lloyd George)

| Treatments mequiv./l | $P_0$ | $P_1$ | $P_2$ | $P_4$ | N-means | Sig. effects. "F" | Sig. diff. 1% | means 0·1% |
|---|---|---|---|---|---|---|---|---|
| $N_5$ | 1024 | 1081 | 1039 | 1110 | 1064 | N*** | 117 | — |
| $N_{10}$ | 1132 | 1373 | 1436 | 1370 | 1328 | P** | 117 | 155 |
| $N_{15}$ | 1507 | 1603 | 1537 | 1573 | 1555 | NP n.s. | — | — |
| $N_{20}$ | 1643 | 1661 | 1697 | 1863 | 1716 | | | |
| P-means | 1327 | 1430 | 1427 | 1479 | | | | |

In this and the following Tables, significant effects are *5%; **1%; ***0·1%

TABLE II

**Pot Experiment 1. (N × P). Mean crop yield in g/2 pots**
(Raspberry, cv. Lloyd George)

| Treatments mequiv./l | $P_0$ | $P_1$ | $P_2$ | $P_4$ | N-means | Sig. effects. "F" | Sig. diff. 1% | means 0·1% |
|---|---|---|---|---|---|---|---|---|
| $N_5$ | 935 | 681 | 847 | 1048 | 878 | N*** | 308 | 408 |
| $N_{10}$ | 1472 | 1555 | 1703 | 1653 | 1596 | P*** | 308 | 408 |
| $N_{15}$ | 1667 | 2085 | 2278 | 2248 | 2070 | NP** | 816 | — |
| $N_{20}$ | 1776 | 2026 | 2647 | 3193 | 2410 | | | |
| P-means | 1463 | 1587 | 1869 | 2036 | | | | |

*Leaf-P, growth, crop yield relationships*

Leaf-P values ($P_1$) greater than 0·163% on June 22, or greater than 0·143% in dry matter on July 22, had no significant effect on cane growth of plants with leaf-N concentrations ranging from 1·94 to 2·87 and 1·61 to 2·26% respectively (Tables I and III). With enhanced nitrogen levels the leaf-P values for optimum growth would probably have been higher. The relationship between leaf-P and crop yield shown in Fig. 2b indicates that nitrogen supplies below 20 mequiv. $NO_3$/l. severely limited response to leaf-P, and that at the highest level of nutrient-N the optimum level of leaf-P for maximum crop yield had not been reached.

**Potassium**

*Cane length*

Potassium supply over the range ½ to 4 mequiv. K/l. had no significant effect on growth of one-year-old canes. The interaction between K and Mg, and between N and K (Table VI), on cane length was not significant. The regular supply of low levels of nutrient-K was apparently adequate for growth but inadequate to prevent leaf scorch developing in older leaves, or to prevent adverse effects on crop yield.

*Crop yield*

The effect of potassium on yield was highly significant, the response to K depending on the level of Mg (Table IV) and of N (Table VI). Highest yields were given by treatments $K_4$ $Mg_4$ (Experiment 2) and $N_{20}$ $K_3$ (Experiment 3).

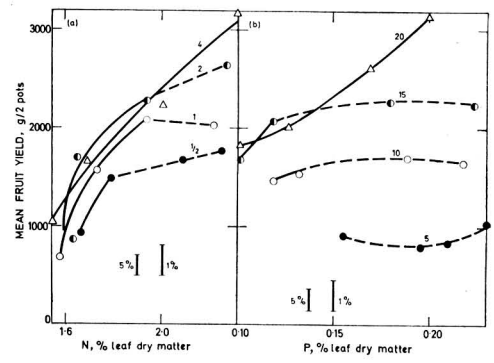


FIG. 2. *Relation between leaf lamina nutrient composition and fruit yield in the following season; (a) effect of nutrient-P on response to leaf-N, (b) effect of nutrient-N on response to leaf-P. Nutrient N and P levels in mequiv./l. Samples taken from non-fruiting canes on July 22*

(In this and subsequent figs a broken line indicates that one nutrient is limiting that response to the other nutrient)

Significant difference is indicated

TABLE III

**Pot Experiment 1. (N × P). Effect of treatments on leaf nutrient status of non-fruiting canes**
(Raspberry, cv. Lloyd George)
(Mean values as % lamina dry matter)

| Sampling date | Treatments mequiv./l | $P_{\frac{1}{2}}$ | $P_1$ | $P_2$ | $P_4$ | N-means | Sig. effects "F" | Sig. diff. means 1% | 0·1% | N-means | | 'F' Sig. diff. 1% | 0·1% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *% Phosphorus* | | | | | | | *% Nitrogen* | | |
| June 22 | $N_5$ | 0·173 | 0·204 | 0·225 | 0·260 | 0·215 | N*** | 0·013 | 0·017 | 1·94 | N*** | 0·12 | 0·16 |
| | $N_{10}$ | 0·128 | 0·148 | 0·189 | 0·236 | 0·175 | P*** | 0·013 | 0·017 | 2·17 | NP n.s. | — | — |
| | $N_{15}$ | 0·123 | 0·148 | 0·213 | 0·262 | 0·186 | NP** | 0·026 | — | 2·57 | | | |
| | $N_{20}$ | 0·135 | 0·152 | 0·185 | 0·231 | 0·176 | | | | 2·87 | | | |
| | P-means | 0·140 | 0·163 | 0·203 | 0·247 | | | | | | | | |
| July 22 | $N_5$ | 0·157 | 0·197 | 0·209 | 0·230 | 0·198 | N*** | 0·016 | 0·021 | 1·61 | N*** | 0·087 | 0·12 |
| | $N_{10}$ | 0·119 | 0·132 | 0·188 | 0·218 | 0·164 | P*** | 0·016 | 0·021 | 1·72 | NP n.s. | — | — |
| | $N_{15}$ | 0·099 | 0·118 | 0·180 | 0·224 | 0·155 | NP* | 0·032 | — | 1·99 | | | |
| | $N_{20}$ | 0·100 | 0·126 | 0·169 | 0·199 | 0·149 | | | | 2·26 | | | |
| | P-means | 0·119 | 0·143 | 0·187 | 0·218 | | | | | | | | |

TABLE IV

**Pot Experiment 2. (K × Mg). Mean crop yield in g/2 pots**
(Raspberry, cv. Lloyd George)

| Treatments mequiv./l | $K_{\frac{1}{2}}$ | $K_1$ | $K_2$ | $K_4$ | Mg-means | Sig. effects "F" | Sig. diff. means 1% | 0·1% |
|---|---|---|---|---|---|---|---|---|
| Mg ½ | 841 | 1627 | 1584 | 1575 | 1407 | K*** | 276 | 367 |
| Mg 1 | 1254 | 1721 | 1736 | 2005 | 1679 | Mg*** | 276 | 367 |
| Mg 2 | 1086 | 2001 | 2145 | 2012 | 1811 | K Mg*** | 559 | 743 |
| Mg 4 | 766 | 1759 | 2145 | 2927 | 1899 | | | |
| K-means | 987 | 1777 | 1903 | 2130 | | | | |

*Leaf analysis*

In Experiment 2, leaf lamina-K values (Table V) ranged from 0·41% to 1·73% in dry matter on 22 June, and from 0·23% to 1·53% in dry matter on 22 July. In Experiment 3 leaf lamina-K values ranged from 0·32% to 1·72% in dry matter on 1 August and from 0·24% to 1·56% on 3 September. Increasing supplies of nutrient-Mg (Table V) and of nutrient-N (Table VII) depressed leaf-K concentration.

*Leaf-K, crop yield relationships*

The relationship between leaf-K and crop yield varied with the supply of Mg (Fig. 3a) and of N (Fig. 4a). When both these nutrients were adequate ($Mg_4$ and $N_{20}$) the relationship between leaf-K and crop yield in the following season was curvilinear over the range 0·3 to 1·5% K in leaf-lamina dry matter for samples taken on 22 July. Above 1·0% K there is a tendency for the curve to flatten, suggesting that the optimum, or 'critical', concentration may be approximately 1·5% K in the dry matter.

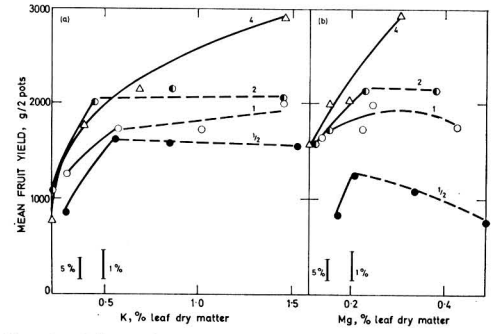


FIG. 3. *Relation between leaf-lamina nutrient composition and fruit yield in the following season; (a) effect of nutrient-Mg on response to leaf-K, (b) effect of nutrient-K on response to leaf-Mg. Nutrient K and Mg levels in mequiv./l. Samples taken from non-fruiting canes on July 22*
Significant difference is indicated

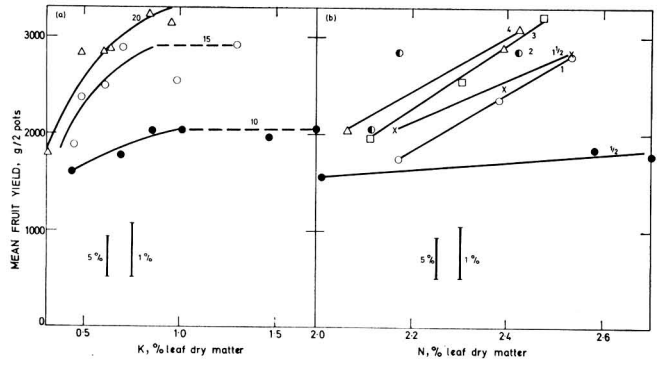


FIG. 4. *Relation between leaf-lamina nutrient composition and fruit yield in the following season; (a) effect of nutrient-N on response to leaf-K, (b) effect of nutrient-K on response to leaf-N. Nutrient N and K in mequiv./l. Samples taken from non-fruiting canes on August 1*
Significant difference is indicated

TABLE V

**Pot Experiment 2. (K × Mg). Effect of treatments on leaf nutrient status of non-fruiting canes**
(Raspberry, cv Lloyd George)
(Mean values as % lamina dry matter)

| Sampling date | Treatments mequiv./l | $K_{\frac{1}{2}}$ | $K_1$ | $K_2$ | $K_4$ | Mg-means | Sig. effects "F" | Sig. diff. 1% | Sig. diff. 0·1% | $K_{\frac{1}{2}}$ | $K_1$ | $K_2$ | $K_4$ | Mg-means | Sig. effects "F" | Sig. diff. 1% | Sig. diff. 0·1% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % Potassium | | | | | | | | % Magnesium | | | | | | | |
| June 22 | Mg ½ | 0·503 | 0·707 | 1·017 | 1·727 | 0·988 | K*** | 0·076 | 0·100 | 0·233 | 0·202 | 0·193 | 0·151 | 0·195 | K*** | 0·031 | 0·042 |
| | Mg 1 | 0·493 | 0·730 | 1·277 | 1·666 | 1·040 | Mg*** | 0·076 | 0·100 | 0·300 | 0·256 | 0·213 | 0·192 | 0·240 | Mg*** | 0·031 | 0·042 |
| | Mg 2 | 0·483 | 0·647 | 1·163 | 1·600 | 0·973 | KMg*** | 0·151 | 0·201 | 0·441 | 0·377 | 0·274 | 0·217 | 0·327 | KMg*** | 0·063 | 0·083 |
| | Mg 4 | 0·410 | 0·560 | 0.727 | 1·470 | 0·792 | | | | 0·576 | 0·521 | 0·444 | 0·363 | 0·476 | | | |
| | K-means | 0·473 | 0·661 | 1·046 | 1·614 | | | | | 0·387 | 0·339 | 0·281 | 0·231 | | | | |
| July 22 | Mg ½ | 0·300 | 0·567 | 0·857 | 1·533 | 0·814 | K*** | 0·064 | 0·085 | 0·177 | 0·139 | 0·124 | 0·107 | 0·137 | K*** | 0·027 | 0·036 |
| | Mg 1 | 0·317 | 0·573 | 1·033 | 1·467 | 0·848 | Mg*** | 0·064 | 0·085 | 0·209 | 0·226 | 0·154 | 0·155 | 0·186 | Mg*** | 0·027 | 0·036 |
| | Mg 2 | 0·233 | 0·460 | 0·867 | 1·467 | 0·757 | KMg** | 0·128 | — | 0·238 | 0·244 | 0·229 | 0·194 | 0·251 | KMg** | 0·055 | — |
| | Mg 4 | 0·233 | 0·407 | 0·680 | 1·460 | 0·695 | | | | 0·491 | 0·426 | 0·379 | 0·305 | 0·400 | | | |
| | K-means | 0·271 | 0·502 | 0·859 | 1·482 | | | | | 0·304 | 0·259 | 0·221 | 0·190 | | | | |

TABLE VI

**Pot Experiment 3. (N × K). Mean total cane length in cm/2 pots and mean crop yield in g/2 pots**
(Raspberry cv. Lloyd George)

| | Treatments mequiv./l | $N_{10}$ | $N_{15}$ | $N_{20}$ | K-means | Sig. effects "F" | Sig. diff. means 1% | Sig. diff. means 0·1% |
|---|---|---|---|---|---|---|---|---|
| Cane length, cm | | 1185 | 1368 | 1449 | | N*** | 96 | 127 |
| | | | | | | K n.s. | — | — |
| | | | | | | NK n.s. | — | — |
| Crop yield, g | K ½ | 1592 | 1871 | 1780 | 1748 | N*** | 226 | 298 |
| | K 1 | 1757 | 2352 | 2822 | 2311 | K*** | 319 | 422 |
| | K 1½ | 2019 | 2485 | 2854 | 2453 | NK* | 553 | — |
| | K 2 | 2027 | 2874 | 2868 | 2590 | | | |
| | K 3 | 1948 | 2544 | 3236 | 2576 | | | |
| | K 4 | 2044 | 2900 | 3120 | 2688 | | | |
| | N-means | 1898 | 2505 | 2780 | | | | |

TABLE VII

**Pot Experiment 3. (N × K). Effect of treatments on leaf nutrient status of non-fruiting canes**
(Raspberry, cv. Lloyd George)
(Mean values as % lamina dry matter)

| Sampling date | Treatments mequiv./l | $N_{10}$ | $N_{15}$ | $N_{20}$ | K-means | Sig. effects "F" | Sig. diff. means 1% | Sig. diff. means 0·1% |
|---|---|---|---|---|---|---|---|---|
| | | | | % *Potassium* | | | | |
| August 1 | K ½ | 0·43 | 0·45 | 0·32 | 0·40 | N*** | | |
| | K 1 | 0·69 | 0·49 | 0·49 | 0·56 | K*** | | |
| | K 1½ | 0·86 | 0·62 | 0·60 | 0·69 | NK*** | 0·22 | 0·29 |
| | K 2 | 1·02 | 0·71 | 0·64 | 0·79 | | | |
| | K 3 | 1·45 | 0·98 | 0·84 | 1·09 | | | |
| | K 4 | 1·72 | 1·31 | 0·95 | 1·33 | | | |
| | N-means | 1·03 | 0·76 | 0·64 | | | | |
| | | | | % *Potassium* | | | | |
| September 3 | K ½ | 0·42 | 0·29 | 0·24 | 0·32 | N*** | | |
| | K 1 | 0·55 | 0·32 | 0·24 | 0·37 | K*** | | |
| | K 1½ | 0·63 | 0·47 | 0·38 | 0·49 | NK*** | 0·25 | 0·33 |
| | K 2 | 0·89 | 0·55 | 0·47 | 0·64 | | | |
| | K 3 | 1·33 | 0·63 | 0·61 | 0·85 | | | |
| | K 4 | 1·56 | 1·10 | 0·78 | 1·15 | | | |
| | N-means | 0·89 | 0·56 | 0·45 | | | | |
| | | | | % *Nitrogen* | | | | |
| August 1 | | 2·14 | 2·37 | 2·51 | | N*** | 0·16 | 0·21 |
| September 3 | | 1·74 | 1·92 | 2·10 | | N*** | 0·14 | 0·19 |

TABLE VIII

**Pot Experiment 4. (N × Mg). Mean total cane length in cm/2 pots and mean crop yield in g/2 pots**
(Raspberry, cv. Lloyd George)

| | Treatments mequiv./l | $N_{10}$ | $N_{15}$ | $N_{20}$ | Sig. effects "F" | Sig. diff. means 1% | Sig. diff. means 0·1% |
|---|---|---|---|---|---|---|---|
| Cane length, cm | | 1091 | 1317 | 1427 | N*** | 92 | 122 |
| | | | | | Mg n.s. | | |
| | | | | | NMg n.s. | | |
| Crop yield, g | | 1939 | 2560 | 3023 | N*** | 272 | 359 |
| | | | | | Mg n.s. | | |
| | | | | | NMg n.s. | | |

TABLE IX

**Pot Experiment 4. (N × Mg). Effect of treatments on leaf nutrient status of non-fruiting canes**
(Raspberry cv. Lloyd Goerge)
(Mean values as % lamina dry matter)

| Sampling date | Treatments mequiv./l | % Magnesium | Sig. effects "F" | Sig. diff. means 1% | Sig. diff. means 0·1% | Treatments mequiv./l | % Nitrogen | Sig. effects "F" | Sig. diff. means 1% | Sig. diff. means 0·1% |
|---|---|---|---|---|---|---|---|---|---|---|
| August 1 | Mg ½ | 0·255 | Mg*** | 0·40 | 0·053 | N 10 | 1·96 | N*** | 0·13 | 0·17 |
| | Mg 1 | 0·296 | N n.s. | | | N 15 | 2·10 | Mg n.s. | | |
| | Mg 1½ | 0·316 | NMg n.s. | | | N 20 | 2·29 | NMg n.s. | | |
| | Mg 2 | 0·349 | | | | | | | | |
| | Mg 3 | 0·358 | | | | | | | | |
| | Mg 4 | 0·407 | | | | | | | | |
| September 3 | Mg ½ | 0·286 | Mg*** | 0·53 | 0·070 | N 10 | 1·84 | N*** | 0·12 | 0·16 |
| | Mg 1 | 0·331 | N n.s. | | | N 15 | 1·93 | Mg n.s. | | |
| | Mg 1½ | 0·345 | NMg n.s. | | | N 20 | 2·08 | NMg n.s. | | |
| | Mg 2 | 0·365 | | | | | | | | |
| | Mg 3 | 0·405 | | | | | | | | |
| | Mg 4 | 0·464 | | | | | | | | |

## Magnesium

*Cane length*

Magnesium had a significant effect on cane length in Experiment 2 but not in Experiment 4 (Table VIII). Leaf-Mg differed in these two experiments.

*Crop yield*

Magnesium had a significant effect on crop yield in Experiment 2 only, the KMg interaction being highly significant (Table IV). Increasing supplies of nutrient-Mg, at low levels of K, first enhanced but later depressed crop yield whereas at high levels of K the response to nutrient-Mg progressively increased.

*Leaf analysis*

In Experiment 2 (Table V) leaf lamina-Mg ranged from 0·15% to 0·58% (22 June) and from 0·11% to 0·49% in dry matter (22 July). In general, increasing the supply of nutrient-K depressed leaf lamina-Mg. In Experiment 4 (Table IX) the leaf lamina-Mg range was narrower than in Experiment 2, i.e. from 0·26% to 0·41% on 1 August and from 0·29% to 0·46% in dry matter on 3 September. Nutrient-N levels had no significant effect on leaf lamina-Mg concentrations.

*Leaf-Mg, growth, crop yield relationships*

The relationship between leaf lamina-Mg and cane growth in Experiment 2 was slightly curvilinear over the range 0·195% to 0·476% Mg in dry matter for leaves sampled on 22 June. Growth response over this range was small but significant There was no significant cane response to Mg in Experiment 4. The relationship between leaf lamina-Mg and crop yield is shown in Fig. 3b and Fig. 5. Crop response to increasing concentrations of leaf lamina-Mg depends on leaf lamina-K (Fig. 3b). When potassium is not limiting (at 4 mequiv. K/l.) then the relationship is almost linear over the range 0·1% to 0·3% Mg in dry matter. At low levels of K, concentrations of leaf lamina-Mg above 0·2% in dry matter do not increase, and may even depress, crop yield (Fig. 3b). Fig. 5 shows the combined results from Experiments 2 and 4. It indicates that the optimum, or 'critical' level for leaf lamina-Mg in samples from non-fruiting canes sampled in late July–early August is about 0·3% Mg in dry matter (when other major nutrients are non-limiting).

## Conclusions

These experiments show that leaf lamina samples taken from the mid-third region of non-fruiting canes from late June to early August may be used as an index of the nutritional status of the crop, and that leaf nutrient concentrations at this growth stage are related to crop yield in the following season. The data show quite clearly that the response to one nutrient varies with the supply of other essential nutrient elements, and that in attempting to establish the optimum leaf nutrient concentration for any one nutrient precautions should be taken to see that no other essential nutrient is limiting crop performance. This can best be done by a series of factorial or response surface experiments. Optimum levels of leaf-N and -P were not attained in these experiments, but the results suggest that for samples taken from non-fruiting canes in late July they are greater than 2·3% N and 0·2% P (Figs 2a and 2b) in dry matter. The optimum level for leaf lamina-K is probably about 1·5% K (Figs 3a and 4a), and for leaf lamina-Mg about 0·3% Mg in dry matter (Figs 3b and 5).

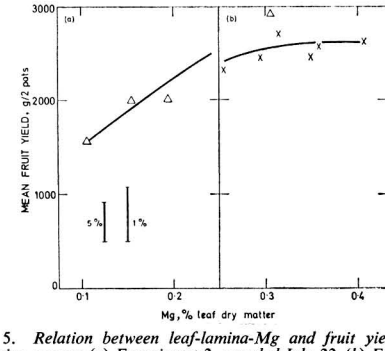


FIG. 5. *Relation between leaf-lamina-Mg and fruit yield in the following season: (a) Experiment 2, sampled July 22, (b) Experiment 4, sampled August 1*
Significant difference is indicated

**Acknowledgments**

The author is indebted to Mr. G. M. Clarke (Statistics Section) for the experimental designs and for the statistical analysis of the results; to Mr. R. Parfitt and Mr. W. A. Taylor for the pot experiments and to Mr. E. G. Bradfield, Miss J. V. O'Neill and others for the chemical analyses.

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# EFFECT OF ASCORBIC ACID ON WHEAT GLUTEN

By H. ZENTNER

The effect of ascorbic acid on the consistency of hydrated wheat gluten was investigated. The addition of ascorbic acid to gluten in the Farinograph resulted in a drop in the maximum consistency of the gluten. Chemical tests failed to show either reduction of disulphide groups of the gluten or any blocking of sulphydryl groups by ascorbic acid. A hypothesis has been put forward, ascribing the observed changes in the consistency of the gluten to interference of the ascorbic acid with the water structure in the gluten.

## Introduction

The addition of ascorbic acid imparts certain characteristics to dough which give it an advantage over other bread improvers such as potassium bromate and iodate, particularly in important variants of mechanical dough development and in 'no-time' dough development with conventional mixing,[1-5] which have become well established in the baking industry.

The dough characteristics produced under these conditions by ascorbic acid are very distinctive but they can, in the absence of a description in chemical and physical terms, be described as organoleptic. The dough is softer, but feels drier to the touch than doughs containing other improvers. Theories of the improver effect of ascorbic acid[6-11] do not account for these dough properties and therefore an attempt was made to find an explanation for this phenomenon by investigating the effect of ascorbic acid on wheat gluten, the component considered to govern largely, though by no means wholly, the rheological properties of doughs.

It was decided to search in ascorbic acid-treated gluten for evidence of chemical reactions known to induce softening in bulk gluten as well as in dough. As it was essential that the tests should be carried out on uniform gluten samples at accurately known water absorptions, several batches of high quality commercial dry gluten were used in preference to freshly washed gluten in view of the large amount of gluten required for each test. However, freshly washed gluten was used in the amperometric titrations.

## Experimental

The maximum consistencies of gluten at various levels of water absorptions were determined, using the technique for gluten farinograms devised by Bushuk[12] in which the 50 g bowl of the Farinograph is used with the Durum setting of the machine.

The point of maximum consistency of the glutens was determined in the Farinograph at various water absorptions and with varying amounts of ascorbic acid.

The effects of sulphydryl blocking agents, of reducing agents, of denaturing agents and of pH on the consistency of wheat gluten were investigated by adding in solution 2-mercapto ethanol, *N*-ethylmaleimide (NEMI), mercuric chloride, silver nitrate, sodium chloride, urea alone, and urea followed by solid ascorbic acid, and 0·07 N acetic acid.

To observe possible reduction of disulphide bonds by

ascorbic acid, amperometric titrations of sulphydryl groups were carried out in the presence, and in the absence, of urea. 6 g freshly washed wet gluten were suspended in 50 ml Tris-buffer (0·2 mM, pH 8·9) and the suspensions were centrifuged. Titrations were carried out with 2 mM methyl mercuric iodide in 25% dimethyl formamide[13] using a dropping mercury electrode at −0·70 V vs. S.C.E. at 40° with a Radiometer Polarograph Type PO3.

To test for a possible reaction between ascorbic acid and sulphydryl groups, 6 mM solutions of ascorbic acid in acetate buffer of pH 5·6 were titrated with 6 mM solutions of cysteine, sodium nitroprusside being used as external indicator.

## Results

The addition of ascorbic acid to wheat gluten resulted in a drop of consistency as measured in the Brabender Farinograph; sulphydryl blocking agents, reducing agents as well as denaturants produced a similar effect (Fig. 1).

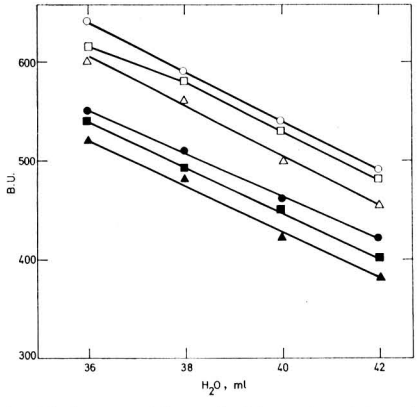


FIG. 1. *Maximum consistency of gluten–water mixtures in the Brabender Farinograph containing different levels of* L-*ascorbic acid (AA) at different water absorptions*

○ Blank (no ascorbic acid)
□ 40 mg ascorbic acid (AA)/50 gm gluten
△ 80 mg ascorbic acid (AA)/50 gm gluten
● 158 mg ascorbic acid (AA)/50 gm gluten
■ 316 mg ascorbic acid (AA)/50 gm gluten
▲ 632 mg ascorbic acid (AA)/50 gm gluten

In this work, the maximum consistency of the gluten (i.e. the height of the Farinograph curve) was regarded as the most significant parameter. No attempt was made to interpret other differences in the character of the curves, such as curve width or slope of curve (i.e. rate of break-down of gluten).

Amperometric titration failed to show the presence of −SH in gluten dispersions, even in the presence of 8 M urea, although the addition of bisulphite produced titrable −SH, indicating the presence of measurable amounts of disulphide groups in the gluten dispersion. No free sulphydryl could be detected even after prolonged standing of the protein dispersions after 1–18 mg of solid ascorbic acid had been dissolved in them.

Addition of mercuric chloride or silver nitrate, which would be expected to react predominantly with the −SH groups accelerated the breakdown of the gluten in the Farinograph (Fig. 2, I). If, after about 13 minutes, solid ascorbic acid was added, a sharp drop in the consistency indicated that ascorbic acid does not exert its softening effect by reacting with the −SH groups which at this stage should be blocked. In a control experiment, even twice the amount of solid sodium chloride had no effect on the consistency of the gluten; this showed that the effects observed with silver nitrate and mercuric chloride were not salt effects (Fig. 2, F).

## Discussion

Although the effect of ascorbic acid on gluten in the Farinograph resembles the effect of reducing agents and sulphydryl blocking agents it has been shown that neither reduction of disulphide bonds nor blocking of −SH groups by ascorbic acid takes place. Ascorbic acid does not normally reduce disulphide bonds in proteins but such a reduction seems possible. Therefore high levels of ascorbic acid were used in these experiments in an attempt to shift the thiol–disulphide equilibrium towards the reduced form.

However, amperometric titrations showed that even high levels of ascorbic acid do not produce titrable −SH, and therefore it may be assumed that the softening effect which ascorbic acid exerts on gluten is not brought about by reduction of disulphide bonds.

Acetic acid (0·07 N) which has a pH similar to that of the ascorbic acid solution (pH 2·95) does not produce a drop in the consistency of the gluten; it is therefore not a pH effect.

It was found on titration of cysteine with ascorbic acid that although there is an addition reaction taking place between ascorbic acid and sulphydryl the extent of this reaction is too small to be important in the present context. It may therefore be assumed that no blocking of sulphydryl groups takes place when ascorbic acid is added to wheat gluten, despite the similarity between the ascorbic acid curve and the NEMI curve of gluten (Fig. 2, H).

If mechanical scission[14] and increased reactivity of disulphide bonds under strain[15] are excluded as being unlikely under the conditions of the experiment, the possibility that ascorbic acid addition to dough may affect hydrogen bonding has to be considered.

The addition of urea to gluten in the Farinograph produces a similar pattern to the ascorbic acid/gluten curve (Fig. 2, E). If gluten is treated first with urea, and solid ascorbic acid is added later, a further drop in the consistency occurs; this suggests that the effect of ascorbic acid is different from that of urea which is known to be a hydrogen bond-breaker, although there is also the possibility that the effect of ascorbic acid may be additive, in that the ascorbic acid may affect a different site of the protein from that affected by the urea (Fig. 2, K).

Just as the softening effect of ascorbic acid on gluten can be simulated by the addition of an appropriate amount of water (Fig. 2, C) so, the consistency of an ascorbic acid-containing gluten can be restored to that of the ascorbic acid-free gluten by an appropriate reduction in the water content (Fig. 2, G). Thus, it appears that the addition of ascorbic acid to dough affects hydrogen bonding, L-ascorbic acid taking up some of the sites previously occupied by water. This it might be able to do because of its particular molecular structure. This is inferred from the fact that analogues of L-ascorbic acid (which is L-xyloascorbic acid, vitamin C) do not have the same effectiveness as improvers. Analogues tried as improvers by other authors[16] were D-arabo-ascorbic acid and D-gluco-

ascorbic acid. After oxidation to the corresponding dehydro-ascorbic acids, these compounds showed somewhat increased effectiveness but they were still not as effective as L-dehydroxyloascorbic acid.

The weak improver effect of the unoxidised analogues of L-xyloascorbic acid may be due in part to the limited ability of the flour ascorbic acid oxidase to oxidise these compounds to the corresponding dehydro-acids, but the limited effectiveness of even the oxidised compounds makes it likely that other mechanisms are involved in the characteristic and distinctive improver effect of the L-xyloascorbic acid, i.e. factors related to the molecular configuration of this compound. It may be that L-xyloascorbic acid has such outstanding characteristics as an improver because it just fits into particular sites of the gluten complex. There it may influence hydrogen bonding, and the Farinograph results could be explained by assuming that L-ascorbic acid displaces 'bound' water, making it available to other dough constituents and available as a lubricant, thereby causing the distinctive softness of the dough, particularly under intensive mixing. The flattening out of the maximum consistency curve at high concentrations of ascorbic acid (Fig. 3) may be an indication that the sites from which ascorbic acid can displace water in gluten are limited in number and that saturation with ascorbic acid has taken place (Fig. 3). The 'stoicheiometry' of such a reaction could be ill-defined at this stage because attachment of a small amount of ascorbic acid at a particular site would result in the displacement of a large cluster of water aggregates.

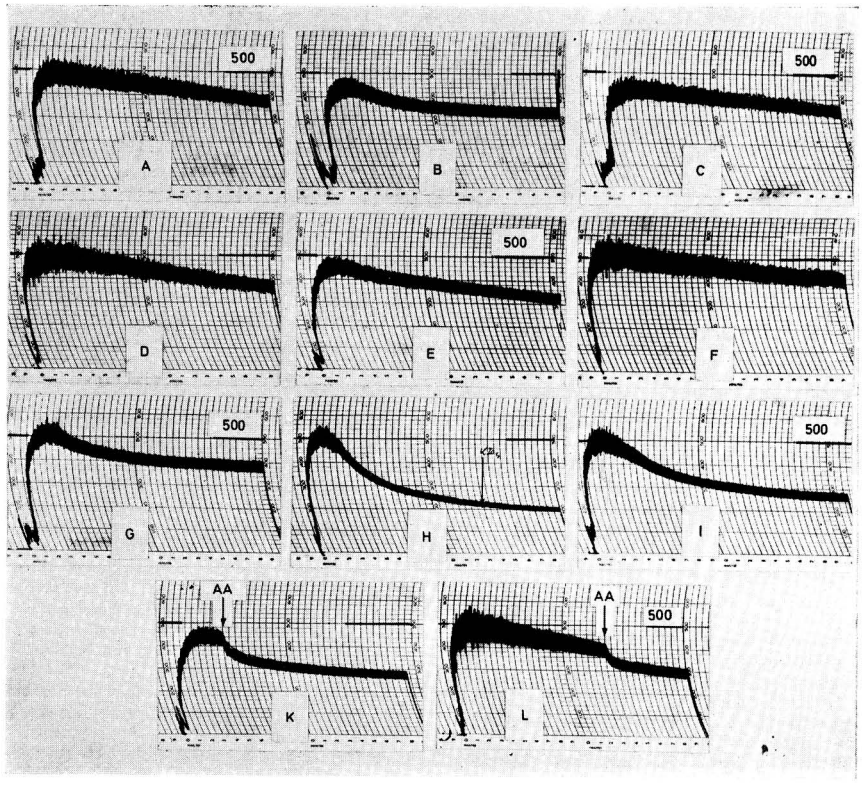


FIG. 2. *Farinograms of gluten.* 50 *g commercial dry gluten*

A: Gluten Blank, 36·5 ml water
B: gluten, 36·5 ml water, AA (316 mg)
C: gluten, 39·2 ml water
D: gluten, 36·5 ml 0·07 N acetic acid, pH 2·95
E: gluten, 36·5 ml water, urea 2·7 g
F: gluten, 36·5 ml water, NaCl (32 mg)
G: gluten, 33·8 ml water, 316 mg AA
H: gluten, NEMI (167 mg)
I: gluten, 32 mg $HgCl_2$
K: gluten, urea, after 5 min AA (632 mg) added
L: gluten, 32 mg $HgCl_2$, after 13 min AA (316 mg) added

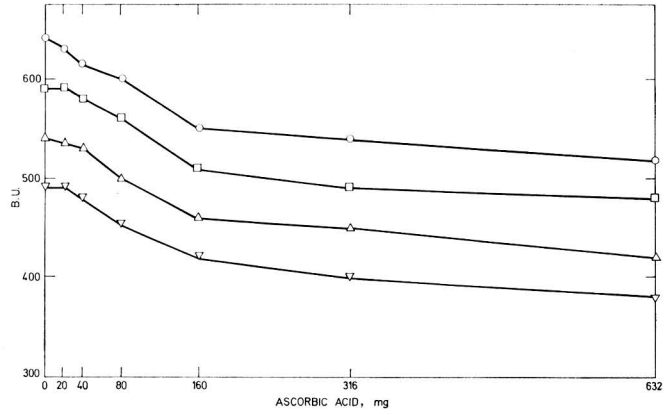


FIG. 3. *Maximum consistency of gluten–water mixtures in the Brabender Farinograph at different water absorptions, plotted against different levels of* L-*ascorbic acid (AA)*

○ 36 ml water/50 g gluten
□ 38 ml water/50 g gluten
△ 40 ml water/50 g gluten
▽ 42 ml water/50 g gluten

## Conclusions

An attempt has been made to explain the softening effect of ascorbic acid on wheat gluten. The possibility that the observed effect is exerted by splitting of gluten disulphide bonds, by blocking of —SH groups or by a pH effect has been ruled out by these experiments. An alternative hypothesis, involving the interference of ascorbic acid with the water structure of wheat gluten is suggested, but its tentative nature at this stage cannot be overemphasised.

It is hoped that the idea might serve as a working hypothesis for further studies.

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# FRUIT RIPENING DISORDERS IN RELATION TO THE CHEMICAL COMPOSITION OF TOMATO FRUIT

By J. N. DAVIES and G. W. WINSOR

The incidence of ripening disorders in relation to the chemical composition of tomato fruit from a $3^2$NK × $2^3$PMgCa factorial nutritional trial has been investigated for three seasons. Highly significant negative correlations were obtained between $\log_{10}$ wt.-% of unevenly ripened fruit and K content, specific conductivity, acidity (titratable, total and combined) and refractive index of the expressed fruit juices. The importance of K in these relationships is emphasised. Differences between the regressions relating fruit grading to the specific conductivity or to the K content of the expressed juices of the varieties studied are described and discussed.

The use of specific conductivity together with the refractive index for the rapid assessment of tomato fruit quality is advocated.

## Introduction

Some effects of nutrition on the composition of fruit from a $3^2$NK × $2^3$PMgCa factorial nutritional trial with maincrop tomatoes[1] were reported in a previous paper.[2] Throughout this experiment detailed records were taken of the grading of the fruit for uniformity of colour and associated defects, a summary of which has also been published.[3] The present paper is concerned with the relationships between the incidence of ripening disorders and various aspects of fruit composition; an attempt has been made to assess the usefulness of the compositional parameters as indices of tomato fruit quality.

## Experimental

The experiment comprised 72 plots including three levels each of N and K in factorial combination with two levels of phosphate, magnesium and calcium (lime). Full details of the design and layout of this trial have been given elsewhere.[1] Details of the methods used in the analysis of the fruit juices have also been published.[2] The grading procedure has been described previously,[3] but for convenience it is repeated here.

Fruit showing uneven ripening in any form, later referred to as NUC (non-uniformly coloured), were recorded by weight and further sub-divided into mild (grade C1) and severe (grade C2) forms of disorder. In addition the number of fruits in each of the following sub-categories was recorded:

NUC/A Greenback, in both green and yellow forms
NUC/B Yellow blotch.
NUC/C Green blotch
NUC/D Waxy patch
NUC/Z Any fruit showing uneven colour but not included in the preceding grades.

The data used in the present investigation were obtained with variety Potentate (1960 and 1961) and varieties J168 and Moneymaker grown on a split-plot basis in 1962. The soil was steamed except for the 1960 crop.

## Results

The relationships between the grading data (expressed as a percentage of total yield) and the K content, titratable, total and combined acidities, specific conductivity and refractive index of the expressed fruit juices were investigated. A preliminary examination of the results showed a negative linear relation between all these aspects of fruit composition and $\log_{10}$ of the grading data (% NUC and % grade C2). Further statistical examination showed, however, that in no instance could the results from the different varieties and seasons be combined. Thus the residual variations for variety Potentate in 1960 and 1961 invariably differed significantly, as also did the regression coefficients in many instances. In the relations between fruit grading and specific conductivity, K content and combined acidity, the regression coefficients for varieties J168 and Moneymaker were similar in each case, but the regression lines for the varieties were separated ($P < 0{\cdot}001$). The various sets of results have therefore been treated individually.

Correlation coefficients relating the composition of the expressed juices and the $\log_{10}$ % NUC and $\log_{10}$ % grade C2 are given in Tables I and II, respectively. All the values, each obtained from 72 pairs of observations, were negative and highly significant ($P < 0{\cdot}001$). Apart from the results for Potentate in 1961, higher correlations were generally obtained for the total non-uniformly coloured fruit (Table I) than for the severe category alone (Table II). Generally speaking the closest relationships were obtained when the specific conductivities and K contents of the fruit juices were correlated with the grading figures. Although the correlations between fruit grading and titratable acidity or refractive index were also highly significant, considerably more variation was apparent.

In the relation between fruit grading ($\log_{10}$ % NUC) and the specific conductivity of the expressed fruit juices, the regression coefficients for the two seasons of variety Potentate did not differ significantly, neither did those for varieties J168 and Moneymaker in 1962. However, the regression coefficients differed significantly ($P < 0{\cdot}001$) from those of the other two varieties. Curves relating the actual % NUC fruit to the specific conductivity of the juices were derived from these results, and examples for Potentate (1961) and J168 are shown in Fig. 1. The values plotted for each variety represent the 18 NPK treatment combinations, each being the mean of 4 individual observations. Low specific conductivity of the fruit juices was associated with a high incidence of ripening disorders; maximum values of 93, 94, 71 and 76% NUC by weight were encountered in Potentate (1960), Potentate (1961), J168 and Moneymaker respectively.

TABLE I

**Correlation coefficients relating the composition of the expressed juices of tomato fruit and fruit grading ($\log_{10}$ % NUC fruit)**

| | Variety and year | | | |
|---|---|---|---|---|
| | Potentate (1960) | Potentate (1961) | J168 (1962) | Moneymaker (1962) |
| Potassium content | −0·911 | −0·856 | −0·847 | −0·796 |
| Titratable acidity | −0·760 | −0·797 | −0·684 | −0·739 |
| Total acidity | −0·864 | −0·862 | −0·834 | −0·813 |
| Combined acidity | −0·900 | −0·834 | −0·861 | −0·813 |
| Specific conductivity | −0·913 | −0·864 | −0·853 | −0·741 |
| Refractive index | −0·743 | −0·624 | −0·760 | −0·734 |

Minimum value of 'r' required for significance at $P < 0{\cdot}001 = 0{\cdot}380$

TABLE II

**Correlation coefficients relating the composition of the expressed juices of tomato fruit and the incidence of severe forms of ripening disorder ($\log_{10}$ % grade C2)**

| | Variety and year | | | |
|---|---|---|---|---|
| | Potentate (1960 | Potentate (1961) | J168 (1962) | Moneymaker (1962) |
| Potassium content | −0·797 | −0·879 | −0·798 | −0·761 |
| Titratable acidity | −0·621 | −0·789 | −0·738 | −0·740 |
| Total acidity | −0·734 | −0·872 | −0·825 | −0·796 |
| Combined acidity | −0·793 | −0·859 | −0·795 | −0·689 |
| Specific conductivity | −0·805 | −0·873 | −0·821 | −0·703 |
| Refractive index | −0·639 | −0·634 | −0·723 | −0·726 |

Minimum value of 'r' required for significance at $P < 0{\cdot}001 = 0{\cdot}380$

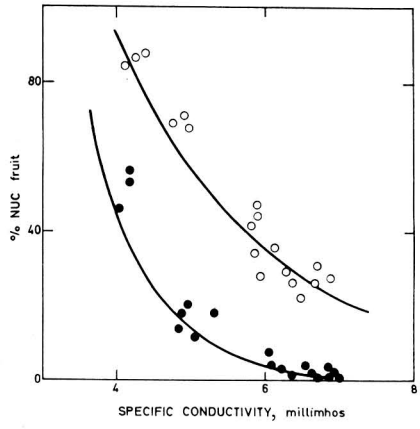


FIG. 1. *Relation between fruit grading (% NUC fruit) and specific conductivity of the expressed juices of tomato varieties Potentate and J168*

○ Potentate, 1961 ● J168, 1962

Where the conductivity was high, however, the incidence of ripening disorders at a given conductivity was much lower for varieties J168 and Moneymaker than for variety Potentate.

Results for the regression of $\log_{10}$ % NUC fruit on the K content of the fruit are shown in Fig. 2. Variety Potentate differed markedly from the other two varieties in the slope of the regression lines (see discussion).

As might be expected from the foregoing, the specific conductivity and K content of the expressed juices were extremely closely correlated, coefficients greater than + 0·97 being obtained for each set of samples. Comparison of the data for 1960 (unsteamed soil) with those for 1961–62, however, showed significant differences between the regression coefficients and separation of the regression lines ($P < 0{\cdot}001$). The regressions of specific conductivity on K content for 1960 and for 1961–62 (combined) are shown in Fig. 3, together with data for the 18 NPK treatment combinations; each value shown is the mean of 4 combinations of magnesium and lime. The corresponding regression equations were: $y = 0{\cdot}69x + 1{\cdot}72$ and $y = 0{\cdot}64x + 1{\cdot}48$ for 1960 and 1961–62 respectively, where $y$ = specific conductivity and $x$ = K content.

The relationships between the compositional parameters and the various forms of ripening disorders are illustrated by results for variety Potentate in 1961 (Table III). Yellow blotch (NUC/B), green blotch (NUC/C) and waxy patch (NUC/D) were all fairly closely correlated ($P < 0{\cdot}001$) with the various aspects of fruit composition. The greenback category (NUC/A) showed a lower, but still highly significant

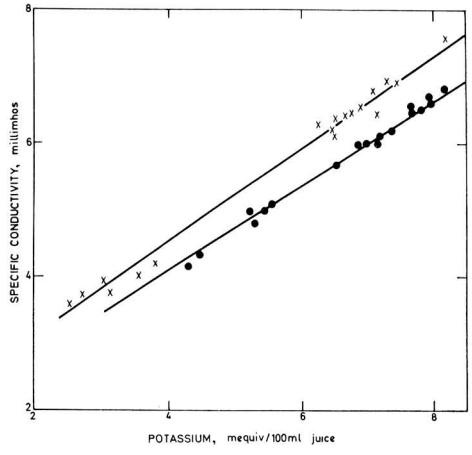


FIG. 3. *Relation between the K content and the specific conductivity of the expressed juices of tomato fruit* (1960–1962)
× 1960 ● 1961–62 combined

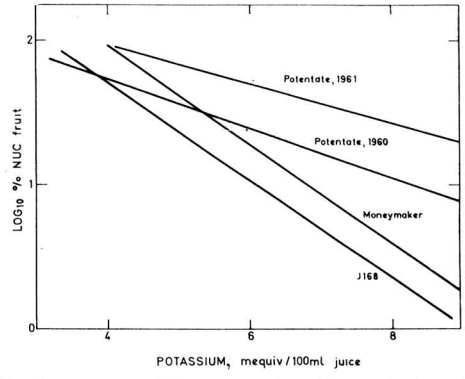


FIG. 2. *Regression of fruit grading ($log_{10}$ % NUC fruit) on the K content of the expressed juices of varieties Potentate* (1960 *and* 1961), *J*168 *and Moneymaker*

TABLE III

**Correlation coefficients relating the composition of the expressed juices of tomato fruit and various forms of ripening disorder (variety Potentate, 1961)**

| | Ripening disorder | | | | |
|---|---|---|---|---|---|
| | NUC/A | NUC/B | NUC/C | NUC/D | NUC/Z |
| Potassium content | −0·501 | −0·764 | −0·843 | −0·665 | −0·105 |
| Titratable acidity | −0·354 | −0·844 | −0·743 | −0·756 | −0·152 |
| Total acidity | −0·475 | −0·811 | −0·832 | −0·704 | −0·142 |
| Combined acidity | −0·531 | −0·706 | −0·828 | −0·593 | −0·120 |
| Specific conductivity | −0·511 | −0·784 | −0·838 | −0·676 | −0·154 |
| Refractive index | −0·414 | −0·586 | −0·604 | −0·583 | −0·061 |

Minimum value of 'r' required for significance at $P < 0{\cdot}001 = 0{\cdot}380$

degree of correlation. The unclassified forms of ripening disorder (NUC/Z) were not significantly correlated with fruit composition.

## Discussion

Although tomato fruit affected by ripening disorders differ in composition from normal fruit,[4–6] they are usually removed during the commercial grading procedure. Fruit reaching the market are thus evenly pigmented, but uniformity of colour is no criterion of uniformity of composition. Results from the present glasshouse trial, published previously,[2] show the wide variation in the composition of evenly pigmented fruit that can occur in response to different levels of the major nutrients. The visual grading of the fruit was also markedly affected by nutrition.[3] The results presented here show how closely the composition of the fruit reflected the overall incidence of ripening disorders.

It seems likely that the high degree of correlation observed between the grading data and the compositional parameters can be attributed to the influence of K. Thus the K content of the fruit was itself closely correlated with the grading results (Tables I and II). K is also closely correlated with fruit acidity.[2,7,8] On calculating the partial correlations between fruit grading and K content with acidity (titratable, total or combined) held constant, the relation was significant ($P < 0{\cdot}01$) for 8 out of the 12 sets of results. In contrast, where K was held constant, the correlation between acidity and fruit grading was significant in only two instances, both with variety Moneymaker.

The K content and specific conductivity of the fruit juices were very closely correlated (Fig. 3). The regression of the percentage of unevenly ripened fruit on either specific conductivity (Fig. 1) or K content (Fig. 2) showed clear separation between the varieties, however. The susceptibility of Potentate to greenback, and the virtual absence of this disorder in the other varieties tested, clearly contributes to such differences between varieties. The K status of the soil has less influence on the incidence of greenback (NUC/A)

than that of other forms of disorder (NUC/C, NUC/D, NUC/Z); greenback is far more closely associated with environmental factors such as light intensity and temperature. Even after excluding the greenback category, however, the regression of fruit grading on specific conductivity or K content still differed between varieties. Whilst allowance must also be made for the variations inherent in any subjective grading procedure, some varieties, however well grown, are more susceptible than others to ripening disorders. The results show, nevertheless, that specific conductivities of the fruit juices in excess of about 6 mmho are associated with a relatively low proportion of poor quality fruit for the variety concerned. Below this level, declining specific conductivities are accompanied by a rapidly increasing incidence of ripening disorders.

More than thirty years ago two Japanese workers[9] suggested the use of conductivity determinations as an indicator of the degree of fruit ripeness, but since then such measurements do not appear to have been employed as an index of fruit quality. On the other hand, by virtue of its close relation with soluble solids and sugar content, the determination of refractive index has been widely used as a rapid quality assessment of fruit and fruit products.[10–13] In the present study the visual grading of the fruit was less well correlated with refractive index than with acidity (total or combined), K content or specific conductivity. This is not, perhaps, surprising in the light of the following considerations. Firstly, the concentration of sugar in the fruit was little influenced by the nutrient treatments whereas the effects on acidity were pronounced.[2] Secondly, unpublished work at this Institute has shown that the relation between the refractive index and acidity of fruit juices is subject to considerable variation. As a result, significant effects of nutrition on the refractive index were comparatively few and differences between treatments were small.[2] This in no way detracts from the value of refractometric measurements under conditions in which the sugar content of the fruit varies considerably.

From the present results it is apparent that conductivity measurements would be a useful adjunct to determinations of refractive index. These two simple determinations, which with the aid of modern portable equipment could be carried out in the field, provide a rapid assessment of the main taste components of the tomato. Moreover, with suitable preliminary calibration to take account of varietal effects, conductivity measurements provide a means of assessing the overall visual quality of the crop.

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# DEHYDRATION OF MEMBRANE-COATED FOODS BY OSMOSIS

By W. M. CAMIRAND, R. R. FORREY, K. POPPER, F. P. BOYLE and W. L. STANLEY

Preliminary studies have shown that pieces of fruit, meat, fish, and whole shellfish can be dehydrated osmotically. Fifty percent invert sucrose was used as a solute; edible semipermeable membranes maintained osmotic differential and reduced solute contamination of the product. The greatest weight loss was observed with component items of high molecular weight such as meats and fish. Some of the products when re-hydrated show a marked similarity to the fresh food. Calcium pectate membranes gave the best results.

## Introduction

Dehydration of foods by transfer of the water across a semipermeable membrane into a hypertonic solution has been carried out with clear liquids[1] and with turbid liquids such as orange juice.[2] A process that uses successive treatments with syrups or salt solutions of increasing concentration has been described.[3] More recent work[4] has shown the feasibility of direct osmotic concentration of food items, fruit in particular, against sucrose. In this system the extent of dehydration is determined by the equilibrium between the osmotic pressure exerted by the solutes of the food portion on one side of the natural vegetative membrane and that of saturated sucrose solution on the other side. The extent of dehydration is also determined by the extent to which the natural vegetative membrane rejects sucrose. The solubility of sucrose increases with increasing temperature; therefore, the osmotic pressure of saturated sucrose solutions will be higher at higher temperatures, resulting in greater dehydration of the food. Process temperature, however, is limited to no more than 30° because of thermal degradation. At this temperature the normality of a saturated sucrose solution is about 2·7 and when fruit is dried against this solution, the resulting product at equilibrium will still contain substantial free water which must be removed by some other means.

Drying of osmosis against solutions of sugars or salts results in the passage of the solute into the dehydrated item. This is not objectionable in some products. In other products, however, it is a serious deterrent. For these products, membranes with a higher degree of rejection than that exhibited by the natural membranes of the food item are necessary. Membranes of greater solute rejection lead to drier products at equilibrium.

These factors led to the concept of a membrane cast around the item to be dehydrated. The membrane selected should exhibit sufficient selectivity and be edible. It was thought in the light of available information[5–9] that pectate and pectinate membranes should be suitable. They are easily cast and they selectively retard the diffusion of sugars into the food and of large molecules out of it. It was also felt that the membrane should stay intact up to and during re-hydration and thus furnish a product that would lose but a modicum of ingredients by diffusion during re-hydration.

## Experimental and Results

Pitted 'Jumbo' size olives were weighed, sliced in halves and placed in a 2% solution of low-methoxy pectin for 1 min. Upon removal from the solution they were dipped in a saturated solution of calcium nitrate for half a minute. This brought about the formation of calcium pectate as a layer on the item causing a 25% increase in the weight of the sample. The coated items were then washed free of excess nitrate. The coated, washed pieces were then placed in a beaker containing a 75° Brix syrup on half invert sugar and half sucrose and kept from floating by a perforated porcelain plate. The olives were removed from the solution after 118 h, rinsed, and allowed to drain. Weight loss for the coated olives was 37·6% (calculated from the original non-coated weight). A non-coated control batch showed a weight gain of 13·6%. The sugar and solids content of the coated and non-coated samples are given in Table I.

When the above procedure was tested with pieces of pineapple ($1\frac{1}{2} \times 1\frac{1}{2} \times 1\frac{1}{4}$ in.), weight loss for the coated pineapple was 36·3% and that for the non-coated pineapple was 35·9%. The results obtained for apple, banana, and papaya are given in Table II.

Coated and non-coated fresh shelled prawns were osmotically dehydrated. The prawns were removed from the 50% inverted sucrose syrup after 72 h, rinsed, and drained (Fig. 1). Weight loss for the coated prawns was

TABLE I

**Total sugar and total solids content of coated and non-coated prawns and olives dehydrated in a 75° Brix 50% invert sugar–sucrose solution at 25°C**

| Food | Total solids % | Total sugar % | Time of osmotic dehydration, h |
|---|---|---|---|
| *Prawns* | | | |
| Coated | 88·1 | 21·7 | 72 |
| Non-coated | 80·0 | 45·1 | 72 |
| *Olives* | | | |
| Coated | 80·2 | 54·2 | 118 |
| Non-coated | 85·5 | 70·0 | 118 |

TABLE II

**Comparative weight loss of various coated and non-coated food items after dehydration in a 75° Brix 50% invert sugar–sucrose solution at 25°C**

| Food | Weight loss | | Time of osmotic dehydration, h |
|---|---|---|---|
| | Coated % | Non-coated % | |
| *Fruit* | | | |
| Pineapple | 36·3 | 35·9 | 98 |
| Papaya | 60·7 | 60·5 | 72 |
| McIntosh apples | 72·5 | 73·6 | 144 |
| Bananas | 22·0 | 18·4 | 99 |
| Olives | 37·6 | −13·6 | 118 |
| *Meat* | | | |
| Meat balls | 42·0 | 13·0 | 72 |
| Beef | 54·0 | 21·0 | 120 |
| *Fish* | | | |
| Swordfish | 36·0 | 32·0 | 93 |
| Oysters | 51·0 | 46·0 | 96 |
| Prawns | 55·0 | 41·0 | 72 |
| Salmon | 38·0 | 2·0 | 143 |

55%, while for the non-coated prawns it was 41% (Table I).

The coated prawns and controls were re-hydrated by immersion in water at 9° for 24 h. Fig. 2 shows the fresh coated dehydrated prawns and coated re-hydrated prawns. The re-hydrated coated and non-coated prawns were fried. The uncoated controls were excessively sweet; the coated prawns had good flavour and texture. The sugar and solids content of the coated and non-coated dehydrated samples are given in Table I.

The calcium pectate membrane was cast around other foods including swordfish cut into $1\frac{1}{2}$ in. cubes, beef rump steak sliced into strips $2\frac{1}{2} \times 1 \times \frac{3}{8}$ in., salmon strips $4 \times 1\frac{1}{2} \times \frac{1}{4}$ in., ground chuck-steak meat balls 1 in. in diameter and whole shucked Pacific oysters. The oysters were of special interest as they had been considered difficult to treat. Fig. 3 shows a coated dehydrated oyster compared with one that has been re-hydrated. The re-hydrated product was virtually identical with fresh oysters.

Since fish and meat do not contain dissolved components of relatively low molecular weight to the same degree that fruits do, the most noteworthy dehydration was with these high-molecular-weight component items.

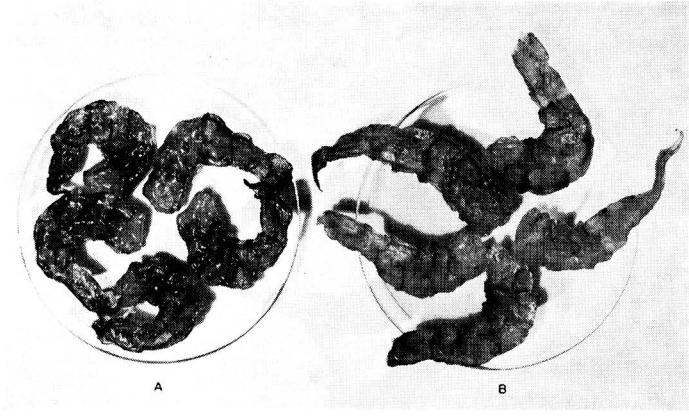


FIG. 1 A. *Coated osmotically dehydrated prawns.* B. *Non-coated osmotically dehydrated prawns*

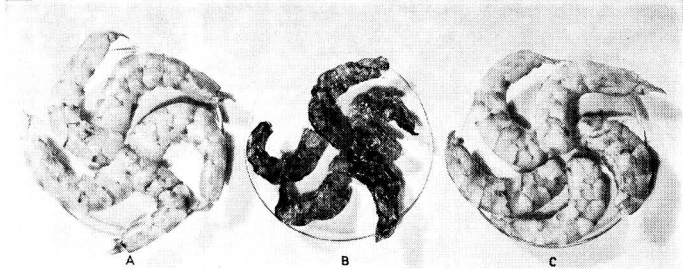


FIG. 2 A. *Fresh prawns.* B. *Coated osmotically dehydrated prawns.* C. *Product B re-hydrated*

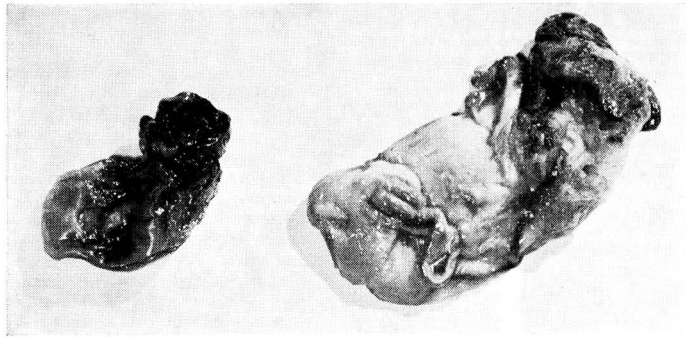

FIG. 3 A. *Coated dehydrated oyster.* B. *Product A re-hydrated*

TABLE III

**Comparative weight loss of prawns with different membrane coatings after 73 h in a 75° Brix 50% invert sugar–sucrose solution**

| Membrane coating | | Weight loss, % |
|---|---|---|
| 1st dip | 2nd dip | |
| 2% Low-methoxy pectin | Saturated calcium nitrate | 57·8 |
| 2% Low-methoxy pectin | Saturated aluminium chloride | 50·0 |
| 2% Carboxymethylcellulose | Saturated aluminium chloride | 28·5 |

Cast-membrane dehydration could become an important preservation process once appropriate membranes are found. This work was limited to calcium and aluminium pectates and aluminium carboxymethylcellulose. Table III shows the results obtained with these various membranes on prawns.

## Discussion

The value of a food dehydration process can best be determined by the degree to which it meets the following requirements. There must be sufficient moisture reduction to retard chemical deterioration, microbial growth, and enzyme activity; and no irreversible reaction should take place. Only the reversibly bound water[10] should be removed. Thus, the re-hydrated product should be identical with, or very similar to, the starting material. If this condition cannot be met, then the re-hydrated product after cooking or other normal processing methods, should be identical with the cooked or processed fresh product; re-hydration time must be commensurate with expected type of use. Osmotic membrane dehydration results in minimum texture degradation. Since heating is not necessary, undue thermal changes are avoided. Since freezing is not necessary, ice crystal formation and concomitant physical disruptions are avoided.

Many other membranes besides those tested may be contemplated: Calcium alginate, methyl or ethyl cellulose, starch and starch derivatives such as carboxymethyl starch, carboxymethyl amylopectin, modified gums or proteins such as partly denatured gelatin, casein, zein, gluten and any other edible film-forming polymer. A good dehydration membrane must reject sodium chloride or other low-molecular-weight compounds and allow a high water flux.

Development of such membranes will lead to rapid dehydration and very dry final products.[11] A salt-rejecting membrane could be used for inexpensively drying fish against sea water or brine immediately after catch. As has been pointed out previously,[4] direct use of solar energy for brine concentration could become more important.

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# ORGANOPHOSPHORUS COMPOUNDS. VI.*—Structure and activity of certain 4-(diethoxyphosphinothioylthiomethyl)-4*H*-1,3,4-oxadiazoline-5-thiones, of certain 4-diethoxyphosphinothioylthiomethyl-thiazoles, and related compounds

By M. PIANKA

Contact activity of 4-diethoxyphosphinothioylthiomethyl-4*H*-1,3,4-oxadiazoline-5-thiones is affected by the nature of the 2-substituent. Alkyl substitution gives high activity against *Aphis fabae*, *Tetranychus telarius* and *Plutella maculipennis* (Curtis), but not against *Musca domestica; p*-nitrophenyl substitution gives high activity, and *p*-chlorophenyl substitution lower activity against all of these organisms. Replacement of oxygen of the heterocycle by sulphur reduces acaricidal activity, and replacement by *N*-methyl increases toxicity to insects and mammals. The compounds lack systemic activity. 4-Diethoxyphosphinothioylthiomethyl-2-phenylthiazole, whether substituted or not in the heterocycle or on the phenyl ring with a nitro-group, lacks activity. 3-Diethoxyphosphinothioylthiomethyl-5-methyloxazolid-2,4-dione has good activity. The structures of the compounds were tentatively assigned on the basis of infra-red and p.m.r. spectra.

## Introduction

In B.P. 978,854[1] were reported the insecticidal properties of condensation products of chloromethyl-1,3,4-oxa- and thiadiazoles with potassium dialkyl phosphorothiolothionates to which structure (**I**; R,R′=alkyl; Z=O or S) was given.

Condensations of potassium diethyl phosphorothiolothionate (**II**) and the chloromethyl compounds (**III**, Q=O or NMe, Y=Cl; **V**, Y=Cl) were carried out, in order to study the activity of the resulting products (**IV**, Q=O or NMe; **VI**), with special reference to the effects of the substituent R (alkyl, thioalkyl or aryl) and of the heterocycle (oxadiazole, thiadiazole or triazole) on the insecticidal and acaricidal activities.

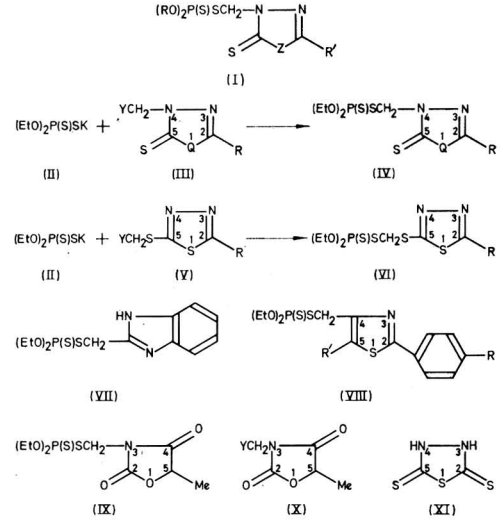
Since substitution with a nitro-group on the benzene nucleus rendered 2-(diethoxyphosphinothioylthiomethyl) benzimidazole (**VII**) and related compounds insecticidally active[2] it became of interest to examine also the effect of substitution by a nitro- group on the activity of 4-(diethoxyphosphinothioylthiomethyl)-2-phenylthiazole (**VIII**). The activity of compound (**IX**) in which the diethoxyphosphinothioylthiomethyl group is attached to the nitrogen atom of a heterocycle containing two electron-attracting carbonyl groups was also examined.

## Experimental

The hydroxymethyl and chloromethyl derivatives (**III**, **V**; Y=$CH_2OH$ or $CH_2Cl$) being the precursors of phosphates (**IV**, **VI**) and by analogy with these phosphates, were tentatively assigned thiono-(**III**) or thio-(**V**) structures.

**Preparation of hydroxymethyl derivatives** (Table I)

A mixture of the heterocyclic compound (0·1 mole), methanol (10 ml), anhydrous potassium carbonate (0·25 g) and an aqueous solution of formaldehyde (38% w/v; 10·5 ml) was kept at 50–60° for 30 min. Acetone (200 ml) and a little charcoal were then added and the hot mixture was filtered, the acetone was removed from the filtrate and the residue was crystallised where appropriate.



* Part V: *J. Sci. Fd Agric.*, 1968, **19**, 403

**Preparation of chloromethyl derivatives** (Table II)

To the hydroxymethyl compound (0·1 mole) thionyl chloride (0·3 mole) was cautiously added, the mixture being shaken. A vigorous reaction ensued. The solution was warmed (steam bath) until evolution of gases ceased, diluted with cold water, then extracted with ether. The ether extract was washed with a saturated solution of sodium chloride, then with water, and dried (sodium sulphate). The ether was removed under reduced pressure.

**Preparation of phosphates (IV, VI, VIII, IX;** Table III)

The method was essentially as described previously.[8] Decimolar amounts of potassium diethyl phosphorothiolothionate and of the chloromethyl compound, in acetone (~100 ml), were heated under reflux or kept at room temperature or both until no more potassium chloride was precipitated. The reaction mixture was then filtered and the volatile components were removed from the filtrate under reduced pressure below 50° (bath temperature). The residual oil was diluted with ether (~200 ml) and washed with a 1% aqueous solution of sodium carbonate or potassium carbonate saturated with sodium chloride (2×100 ml), with a saturated solution of sodium chloride (50 ml), then with water, and dried (sodium sulphate). The volatile components were then removed under reduced pressure. The residue was crystallised where appropriate.

**Infra-red measurements**

The infra-red absorption bands of Compounds 15–24 are summarised in Table IV. The measurements were conducted as described previously.[11]

**Proton magnetic resonance (p.m.r.) measurements**

These were conducted as described previously.[12] P.m.r. spectra in deuteriochloroform of Compounds 15–24 showed triplets (J=7 c/sec) centred at τ 8·63–8·69 and double quadruplets (J=7 & 15 c/sec) centred at τ 5·72–5·88 for the protons of the ethyl group of P–OEt. They also showed a doublet (J=16 c/sec) centred at τ 4·35–5·73 for the protons of the S–$CH_2$– group.

Compound 15 gave a triplet at τ 8·68 and quadruplet at 7·27 (J=7 c/sec) for the protons of the 2-ethyl- and Compound 16 gave peaks at τ 7·32, 8·22 and 8·97 for the protons of the 2-propyl-substituent on the 1,3,4-oxadiazole ring. Compound 19 showed peaks centred at τ 6·14 and 8·54 for the protons of the 5-thioisopropyl substituent of the 1,3,4-thiadiazole ring and Compound 20 gave peaks at τ 7·64 for the *N*-methyl and at τ 6·48 for the 2-methyl substituents of the 1,3,4-triazole ring. Compound 24 showed a quadruplet (J=7 c/sec) at τ 5·08 for the proton and a doublet at τ 8·40 for the methyl group in the 5-position of the oxazolid-2,4-dione ring.

Compounds 17, 18, 21–23 showed also two doublets (J=9 c/sec) centred at τ 1·60–2·13 and 1·90–2·7 for the 3-

TABLE I

**Hydroxymethyl compounds** (III, V, X; Y=OH)

| No. of compound | Name of hydroxymethyl compound | | Q | R | Obtained from heterocycle | Physical state | Solvent of crystallisation | Yield, % |
|---|---|---|---|---|---|---|---|---|
| 1 | *2-Ethyl-4-hydroxymethyl-4*H-*1,3,4-oxadiazoline-5-thione** | (III) | O | Et | 2-Ethyl-4*H*-1,3,4-oxadiazoline-5-thione[3] | White crystals, m.p. 63–66° | Ethanol (−50°) | 95 |
| 2 | *4-Hydroxymethyl-2-propyl-4*H-*1,3,4-oxadiazoline-5-thione** | (III) | O | $Pr^n$ | 2-Propyl-4*H*-1,3,4-oxadiazoline-5-thione[3] | Off white mobile oil | | 100 |
| 3 | *2-(p-Chlorophenyl)-4-hydroxymethyl-4*H-*1,3,4-oxadiazoline-5-thione** | (III) | O | *p*-ClPh | 2-*p*-Chlorophenyl-4*H*-1,3,4-oxadiazoline-5-thione[4] | White crystals, m.p. 133–134° | Acetone | 92 |
| 4 | *4-Hydroxymethyl-2-p-nitrophenyl-4*H-*1,3,4-oxadiazoline-5-thione** | (III) | O | *p*-$NO_2$Ph | 2-*p*-Nitrophenyl-4*H*-1,3,4-oxadiazoline-5-thione[5] | Yellow crystals, m.p. 105–108° | Acetone | 99 |
| 5 | *5-Hydroxymethylthio-2-isopropylthio-1,3,4-thiadiazole** | (V) | | $SPr^i$ | 2-Isopropylthio-4*H*-1,3,4-thiadiazoline-5-thione‡‡ | Yellow oil | | 100 |
| 6 | *1,2-Dimethyl-4-hydroxymethyl-4*H-*1,3,4-triazoline-5-thione** | (III) | NMe | Me | 4,5-Dimethyl-1,2,4-triazole-3-thiol[6] | White crystals, m.p. 129–131° | Ethanol | 83 |
| 7 | *3-Hydroxymethyl-5-methyl-oxazolid-2,4-dione*†* | | (X; Y=OH) | | 5-Methyl-1,3-oxazolid-2,4-dione[7] | Yellow oil | | 100 |

* The compound was not analysed and was used directly for the next step
† The mixture of the dione, formalin, methanol and potassium carbonate was kept for 24 h at room temperature. The water present in the reaction mixture was removed on distilling with chloroform. The volatile components were then removed under reduced pressure leaving an oil
‡ Prepared from 3*H*,4*H*-1,3,4-thiadiazolidine-2,5-dithione and isopropyl iodide

TABLE II

**Chloromethyl compounds** (III, V; X; Y=Cl)

| No. of compound | Name of compound | | Q | R | From Compound no. | Physical state | Yield, % |
|---|---|---|---|---|---|---|---|
| 8 | *4-Chloromethyl-2-ethyl-4*H-*1,3,4-oxadiazoline-5-thione** | (III) | O | Et | 1 | $n_D^{20}$ 1·5573 | 93 |
| 9 | *4-Chloromethyl-2-propyl-4*H-*1,3,4-oxadiazoline-5-thione** | (III) | O | $Pr^n$ | 2 | $n_D^{20}$ 1·5471 | 96 |
| 10 | *4-Chloromethyl-2-p-chlorophenyl-4*H-*1,3,4-oxadiazoline-5-thione** | (III) | O | *p*-ClPh | 3 | M.p. 96–98° | 75 |
| 11 | *4-Chloromethyl-2-p-nitrophenyl-4*H-*1,3,4-oxadiazoline-5-thione** | (III) | O | *p*-$NO_2$Ph | 4 | Yellow crystals | 63 |
| 12 | *5-Chloromethyl-2-isopropylthio-1,3,4-thiadiazole** | (V) | | $SPr^i$ | 5 | Red oil | 67 |
| 13 | *4-Chloromethyl-1,2-dimethyl-4*H-*1,3,4-triazoline-5-thione** | (III) | NMe | Me | 6 | M.p. 135–137° | 52 |
| 14 | *3-Chloromethyl-5-methyl-oxazolid-2,4-dione*†* | | (X; Y=Cl) | | 7 | Oil | 100 |

* The compound was not analysed and was used directly for the condensation with potassium diethyl phosphorothiolothionate
† The mixture was heated for 2·5 h and the volatile components were removed from it under reduced pressure

TABLE III

**Diethoxyphosphinothioylthiomethyl compounds (IV, VI, VIII, IX)**

| No. of compound | Name of compound | Preparative details | Q | R | R′ | From (II) and Compound no. | Period of reaction h: At reflux | Period of reaction h: At room temp. |
|---|---|---|---|---|---|---|---|---|
| 15 | 4-Diethoxyphosphinothioylthiomethyl-2-ethyl-4*H*-1,3,4-oxadiazoline-5-thione | (IV) | O | Et | | 8 | 1 | 16 |
| 16 | 4-Diethoxyphosphinothioylthiomethyl-2-propyl-4*H*-1,3,4-oxadiazoline-5-thione | (IV) | O | $Pr^n$ | | 9 | | 3 days |
| 17 | *4-Diethoxyphosphinothioylthiomethyl-2-*p*-chlorophenyl-4*H*-1,3,4-oxadiazoline-5-thione* | (IV) | O | *p*-ClPh | | 10[c] | 2 | |
| 18 | *4-Diethoxyphosphinothioylthiomethyl-2-*p*-nitrophenyl-4*H*-1,3,4-oxadiazoline-5-thione* | (IV) | O | *p*-$NO_2$Ph | | 11[c] | 2 | |
| 19 | *5-Diethoxyphosphinothioylthiomethylthio-2-thioisopropyl-1,3,4-thiadiazole* | (VI) | | $SPr^i$ | | 12[c] | 2 | |
| 20 | *4-Diethoxyphosphinothioylthiomethyl-1,2-dimethyl-4*H*-1,3,4-triazoline-5-thione* | (IV) | NMe | Me | | 13[c] | 2 | |
| 21 | *4-Diethoxyphosphinothioylthiomethyl-2-phenylthiazole* | (VIII) | | H | H | 25[e,f] | 5 | 16 |
| 22 | *4-Diethoxyphosphinothioylthiomethyl-5-nitro-2-phenylthiazole* | (VIII) | | H | $NO_2$ | 26[g,f,h] | 0·5 | |
| 23 | *4-Diethoxyphosphinothioylthiomethyl-2-*(p-*nitrophenyl)thiazole* | (VIII) | | *p*-$NO_2$Ph | H | 27[j,h] | 1 | 16 |
| 24 | *3-Diethoxyphosphinothioylthiomethyl-5-methyloxazolid-2,4-dione* | (IX) | | | | 14 | | 2 days |

**Physical data and analyses**

| No. of compound | M.p., °C | Appearance of solid or appearance and $n_D^{20}$ of oil | Yield, % | Formula | Found, % N | Found, % P | Found, % S | Required, % N | Required, % P | Required, % S |
|---|---|---|---|---|---|---|---|---|---|---|
| 15 | | Yellow oil, 1·5622[a] | 82 | $C_9H_{17}N_2O_3PS_3$ | 8·2 | 8·8 | | 8·5 | 9·4 | |
| 16 | | Orange oil, 1·5565[b] | 90 | $C_{10}H_{19}N_2O_3PS_3$ | 7·7 | 8·7 | | 8·2 | 9·1 | |
| 17 | 91–93[d] | Light brown crystals | 95 | $C_{13}H_{16}ClN_2O_3PS_3$ | 6·8 | 7·8 | | 6·8 | 7·5 | |
| 18 | 95–96[d] | Light yellow crystals | 60 | $C_{13}H_{16}N_3O_5PS_3$ | 10·4 | 7·5 | 22·4 | 10·0 | 7·4 | 22·8 |
| 19 | | Red oil, $n_D^{22}$ 1·6201 | 91 | $C_{10}H_{19}N_2O_2PS_5$ | 7·1 | 7·6 | 40·1 | 7·2 | 8·0 | 41·0 |
| 20 | 68–69[d] | White crystals | 82 | $C_9H_{18}N_3O_2PS_2$ | 12·8 | 9·5 | | 12·8 | 9·5 | |
| 21 | | Pale yellow oil, 1·6160 | 88 | $C_{14}H_{18}NO_2PS_3$ | | 8·6 | | | 8·5 | |
| 22 | 91–92[i] | Yellow needles | 94 | $C_{14}H_{17}N_2O_4PS_3$ | | 7·8 | | | 7·7 | |
| 23 | 77–78·5[i] | Yellow prisms | 81 | $C_{14}H_{17}N_2O_4PS_3$ | | 7·8 | | | 7·7 | |
| 24 | | Brown oil, 1·5250 | 78 | $C_9H_{16}NO_5PS_2$ | | 9·9 | | | 9·7 | |

[a]Ref. 1 reported b.p. 125°/0·02 mm and no analyses. [b]Ref. 1 reported b.p. 130°/0·02 mm and no analyses. [c]Centimolar amounts of the reagents were used and 100 ml of acetone. [d]The residue that remained after the removal of the acetone was crystallised from ethanol. [e]4-Chloromethyl-2-phenylthiazole (Compound no. 25) was prepared by the method of Silberg *et al.*[9] [f]200 ml of acetone were used. [g]4-Chloromethyl-5-nitro-2-phenylthiazole (Compound no. 26) was prepared by the method of Simiti and Farkas.[10] [h]Benzene was used for the extraction. [i]From propan-2-ol. [j]4-Chloromethyl-2-*p*-nitrophenylthiazole (Compound no. 27) was prepared by the method of Simiti and Farkas[10]

and 2-protons of the benzene ring. Compound 21 gave a peak at $\tau$ 2·77 and compound 23 a peak at $\tau$ 2·58 for the 5-proton of the thiazole ring.

### Infra-red absorption and p.m.r. spectra of 3H,4H-1,3,4-thiadiazolidine-2,5-dithione

The product of the condensation of hydrazine and carbon disulphide described as 2,5-dithiolo-1,3,4-thiadiazole[13] showed as a nujol mull absorption bands at 3200 $cm^{-1}$ (NH) and at 1250 $cm^{-1}$ (probably C=S), and in bromoform solution it showed bands at 3260 $cm^{-1}$ (NH) and at 1250 $cm^{-1}$ (probably C=S). It showed a p.m.r. peak at $\tau$ −0·70 indicative of NH. The spectra suggest therefore structure (**XI**) for this compound.

### Formulation

The compounds were formulated as 10% w/v (nos. 15, 16, 17, 19, 20, 24), 5% w/v (nos. 21, 22, 23) and 1% w/v (no. 18) emulsifiable concentrates in acetone and Lissapol NX (a polyethylene glycol ether). Their activities were tested by various methods.

TABLE IV

**Infra-red absorption bands ($cm^{-1}$) for $CS_2$ solutions**

| No. of compound | Infra-red bands: P–O–C | P–O–Et | P=S | C=S? | Phenyl | $NO_2$ |
|---|---|---|---|---|---|---|
| 15 | 1015 | 1165 | 648 | 1235 | | |
| 16 | 1018 | 1165 | 650 | 1240 | | |
| 17 | 1018 | 1166 | 652 | 1240 | 836† | |
| 18 | 1018 | 1164 | 652 | 1240 | 850† | 1345 |
| 19 | 1015 | 1160 | 654 | | | |
| 20 | 1012 | 1162 | 648 | 1240 | | |
| 21§ | 1015 | 1158 | 654 | | 685; 760 | |
| 22 | 1014 | 1158 | 654 | | 682; 754 | 1335 |
| 23 | 1012 | 1156 | 654 | | 848† | 1345 |
| 24§‡ | 1010 | | 652 | | | |

§ Weak absorption at 3680 $cm^{-1}$ in $CHBr_3$ suggests water

‡ Bands at 1825 and 1752 $cm^{-1}$ associated with an oxazolid-2,4-dione ring

† Associated with *p*-disubstituted aromatic ring

**Insecticidal and acaricidal tests**

Contact acaricidal activity, contact activity against *Musca domestica*, contact and stomach activity against *Plutella maculipennis* (Curtis), systemic aphicidal activity on broad-bean plants by root-uptake from culture solutions and systemic aphicidal activity on broad-bean plants by soil application were assessed as described previously.[2] Contact aphicidal activity and systemic aphicidal activity by spraying of broad-bean plants were assessed as described previously.[14] Two standards were used in the systemic tests: demeton-methyl which is the common name for a mixture of 2-(ethylthio) ethyl dimethyl phosphorothionate and S-[2-(ethylthio) ethyl] dimethyl phosphorothiolate, and dimefox- the common name for *N,N,N′,N′*-tetramethylphosphorodiamidic fluoride.

**Mammalian toxicity**

The acute oral toxicity to mice and rats was determined as follows.

The finely ground compounds were suspended in 25% propylene glycol and dispersed with the addition of 0·5% Tween 80 (Compounds 15, 16, 19) or suspended in 0·5% Tween 80 (Compound 20). The concentrations ranged from 1 to 30 mg/ml. Fifty (with Compound 20 only 14) male Imperial Chemical Industries Ltd. mice (bodyweight range 19–21 g), and 24 male (Compounds 15, 16) or female (Compounds 19, 20; only 14 rats were used with Compound 20). Wistar rats (bodyweight 130–330 g) were used per determination. The doses were administered by stomach tube. Mortalities were observed after 7 days.

## Results

The results of the insecticidal and acaricidal tests are presented in Tables V–VIII. Median lethal doses to mice and rats are presented in Table IX.

TABLE V

**Contact activities against *Aphis fabae*, *Tetranychus telarius*, *Musca domestica* and *Plutella maculipennis* of phosphates (IV, VI, VIII, IX)**

| No. of compound | Q | R | *Aphis fabae* | | | | | | *Tetranychus telarius** | | | | *Musca domestica* Kill (%) at ppm | | | *Plutella maculipennis* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 300 | 100 | 30 | 10 | 3 | 1 | 300 | 100 | 30 | 10 | 300 | 100 | 30 | 300 | 100 | 30 | 10 | 3 |
| | | A. *4-Diethoxyphosphinothioylthiomethyl-2-R-4H-1,3,4-oxadiazoline-5-thiones and related compounds* | | | | | | | | | | | | | | | | | | |
| 15 | (IV) | O Et | –† | 100 | 100 | 99 | 82 | 59 | 100 | 98 | 95 | 92 | 35 | – | – | 100 | 85 | 35 | – | – |
| 16 | (IV) | O $Pr^n$ | 100 | 99 | 98 | 86 | 42 | 27 | 100 | 92 | 86 | 75 | – | – | – | 100 | 95 | 100 | 50 | 20 |
| 17 | (IV) | O $C_6H_4Cl$-*p* | 90 | 82 | 55 | – | – | – | 83 | 81 | 60 | – | 60 | – | – | 85 | – | – | – | – |
| 18 | (IV) | O $C_6H_4NO_2$-*p* | 95 | 90 | 59 | – | – | – | 99 | 92 | 67 | – | 95 | 20 | 4 | 100 | 90 | 35 | – | – |
| 20 | (IV) | NMe Me | – | 99+ | 99+ | 98 | 92 | 62 | – | 95 | 96 | 90 | 100 | 84 | 32 | 100 | 100 | 90 | – | – |
| 19 | (VI) | $SPr^i$ | – | 100 | 100 | 89 | 90 | – | – | 81 | 37 | 6 | 88 | – | – | 100 | 45 | – | – | – |
| | | B. *4-Diethoxyphosphinothioylthiomethyl-2-phenylthiazoles* (VIII) | | | | | | | | | | | | | | | | | | |
| | R | R′ | | | | | | | | | | | | | | | | | | |
| 21 | H | H | 20 | 17 | – | – | – | – | – | 26 (49 | 30 16 | 27 –) | – | – | – | 0 | 0 | – | – | – |
| 22 | H | $NO_2$ | 11 | 11 | 8 | 7 | – | – | 41 (48 | 30 30 | 35 27 | – 20) | – | – | – | 0 | – | – | – | – |
| 23 | $NO_2$ | H | 67 | 51 | – | – | – | – | 67 (18 | 23 16 | – 21 | – –) | – | – | – | 18 | – | – | – | – |
| 24 | | C. *3-Diethoxyphosphinothioylthiomethyl-5-methyloxazolid-2,4-dione* (IX) | – | 99 | 72 | 9 | – | – | – | 98 | – | – | 87 | – | – | – | 50 | – | – | – |

**T. telarius* mites not resistant to organophosphorus compounds; in brackets is given activity against *T. telarius* mites resistant to organophosphorus compounds

†Not tested at this concentration

TABLE VI

**Systemic aphicidal activity by spraying of broad-bean plants**

| No. of compound | | Q | R | I Test | | II Test | | III Test | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Kill (%) of *Aphis fabae* at ppm | | | | | |
| | | | | 300 | 100 | 300 | 100 | 300 | 100 |
| 15 | (IV) | O | Et | 0 | 0 | | | | |
| 16 | (IV) | O | $Pr^n$ | 0 | 0 | | | | |
| 18 | (IV) | O | $C_6H_4NO_2$-*p* | 1 | – | | | | |
| 20 | (IV) | NMe | Me | | | | | 56 | 5 |
| 19 | (VI) | | $SPr^i$ | | | 15 | 19 | | |
| Demeton-methyl | | | | 100 | 100 | 100 | 75 | 99 | 98 |

TABLE VII

**Systemic aphicidal activity on broad-bean plants by root-uptake from culture solutions**

| No. of compound | | Q | R | Kill (%) of *Aphis fabae* at ppm | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 30 | | 10 | | 3 | |
| | | | | A* | B† | A* | B† | A* | B† |
| 15 | (IV) | O | Et | 0 | 0 | – | – | – | – |
| 16 | (IV) | O | $Pr^n$ | 4 | Plants died | – | – | – | – |
| 19 | (VI) | | $SPr^i$ | 0 | 0 | 0 | 0 | 0 | 0 |
| Demeton-methyl | | | | 100 | 100 | 99 | 100 | 99 | 94 |

* After 2 days † After 6 days

TABLE VIII

**Systemic aphicidal activity on broad-bean plants by soil application**

| No. of compound | | R | Kill (%) of *Aphis fabae* at ppm | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1000 | | | 500 | | |
| | | | C* | D† | E‡ | C* | D† | E‡ |
| 19 | (VI) | $SPr^i$ | 3 | 1 | 0 | – | – | – |
| Dimefox | | | – | – | – | 92 | 96 | 95 |

* After 2 days † After 5 days ‡ After 7 days

TABLE IX

**Mammalian toxicity of certain phosphates**

| No. of compound | | Q | R | Acute oral $LD_{50}$ mg/kg Mice | Rats |
|---|---|---|---|---|---|
| 15 | (IV) | O | Et | 104 | 16 |
| 16 | (IV) | O | $Pr^n$ | 86 | 50 |
| 20 | (IV) | NMe | Me | <5 | 5 |
| 19 | (VI) | | $SPr^i$ | 640 | 56 |

## Discussion

### Structure of phosphates

The infra-red spectra of oxadiazoline phosphates (Compounds 15–18) show bands at 1240–1235 $cm^{-1}$; this band might be associated with C=S and, if this is so, it would support structure (**IV**). The thiadiazole phosphate (Compound 19) does not show such a band. The nomenclature of the phosphates is based on the tentative assumption that Compounds 15–18 have the thionocarbonyl structure (**IV**) and Compound 19 has structure (**VI**). The evidence is, however, not conclusive.

### Structure–activity relationships

The oxathiazoline, thiadiazole and triazoline derivatives had moderate to excellent contact activity against *Aphis*, *Tetranychus* and *Plutella* (Table VA). Only the highly toxic triazoline derivative (no. 20) had high activity against *Musca*. Increase in the 2-alkyl chain of the oxadiazoline compounds (nos. 15, 16) had the effect of reducing aphicidal and acaricidal activity [as it did with diethyl *S*-(*N*-alkoxycarbonylcarbamoylmethyl) phosphorothiolothionates],[8] and of enhancing activity against *Plutella*. Substitution with a *p*-chlorophenyl (no. 17) brought about reduction in activity, but the *p*-nitrophenyl compound (no. 18) was highly active in conformity with the greater electron-withdrawing effect of the nitro-group.[2] The 5-thioisopropyl thiadiazole derivative (no. 19) had excellent aphicidal activity, but lower acaricidal activity. Reduction in acaricidal activity was also observed on replacing oxygen by sulphur in the carbonate chain of 2-alkyl-4,6-dinitrophenols.[15,16] As already observed the triazoline derivative (no. 20) was highly toxic to insects and to mammals (Table IX). None of these compounds had systemic activity when tested on broad-bean plants by leaf- or root-uptake (Tables VI–VIII). Demeton-methyl was highly active by leaf- or root-uptake and dimefox by root-uptake from soil.

In Part V[2] it has been shown that 2-(diethoxyphosphinothioylthiomethyl) benzimidazole requires for high activity an electron-withdrawing group such as a nitro-group. A similar effect was observed with the oxadiazoline derivatives (nos. 17, 18; Table VA). It was therefore surprising to find that 4-diethoxyphosphinothioylthiomethyl-2-phenylthiazoles substituted with a nitro-group (nos. 22, 23) had very low activity; substitution in 5-position (no. 22) had no effect on activity and substitution in the phenyl ring enhanced activity only slightly.

The oxazolid-2,4-dione derivative (no. 24) with its two electron-attracting carbonyl groups proved more active than *N*-(diethyl phosphorothionothiolomethyl) acetamide,[8] where the electromeric effect of the imino-group would be expected to reduce substantially the electrophilic character of the phosphorus atom and the formation of the enzyme-inhibitor complex.

As with the benzimidazole derivatives examined[2] the oxadiazoline derivatives (nos. 15, 16) and the thiadiazole derivative (no. 19) proved less toxic to mice than to rats, perhaps because of reduced absorption. The triazoline derivative (no. 20) was more toxic to mice than to rats.

### Acknowledgments

The author thanks Mr H. T. Foley and Miss C. M. Creighton for assistance in the preparation of the compounds, Mr C. B. F. Smith and the staff of the Biology Department for the contact and systemic tests, Mr T. R. Middleton for the acute oral $LD_{50}$ determinations in mice and rats and Dr J. E. Page for the i.r. and p.m.r. spectra.

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## ERRATA

In the paper by Massey & Winsor in the June issue (*J. Sci. Fd Agric.*, 1968, **19,** 332) the running headline should read 'Soil Salinity Studies. II' not 'Soil Salinity Studies. IV"

The authors' address should read: Glasshouse Crops Research Institute,
Worthing Road, Rustington,
Littlehampton, Sussex

In the paper by Currah & Meigh (*J. Sci. Fd Agric.*, 1968, **19,** 409) the authors' address should read:
Agricultural Research Council,
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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

# ABSTRACTS

AUGUST, 1968

## 1.—AGRICULTURE AND HORTICULTURE

### General: Soils and Fertilisers

**Selenium in horizons of soil profiles.** N. Wells (*N. Z. Jl Sci.*, 1967, **10**, 142–179).—Reports and discusses extensive determinations of total Se in N. Zealand topsoils and parent rocks. Concn. of Se (mean ~ 0·6 ppm) during soil formation arises from its high retention by clay particles. Concn. are very high (mean ~ 1·4 ppm) in well-developed $B_2$ horizons, being max. in concretions of ironstone soils and in Fe-pan of podzols. High concn. of Se occur in topsoils of some yellow-brown earths, of rendzinas, yellow-brown loams and brown granular clays from andesites, loams from basalts, and recent soils from basaltic or andesitic ash. Concn. are min. in AC profiles, especially those derived from granite and rhyolitic pumice or those which have undergone podzolisation. The gley process has little effect on Se concn., but hydrothermal activity in the volcanic zone can raise the concn. as can bird burrowing on the smaller islands. W. J. BAKER.

**Relationship between water potential of soil and of leaves of *Theobroma cacao* L.** R. Iserentant (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1167–1170).—Water potential of the leaves of plants in a gradually drying soil, remained constant at ~ 8 atm. During this period the soil-moisture was sufficient for adequate maintenance; beyond this point, at soil moisture ~9%, the leaf potential graph commenced to rise, keeping 2–4 atm. ahead of the soil curve, until the point of withering was reached. Results obtained by other workers are confirmed. P. S. ARUP.

**Present-day concepts of soil organic matter.** B. R. Nagar and N. P. Datta (*J. scient. ind. Res.*, 1967, **2**, 131–134).—A general review of soil fertility with special reference to humic and non-humic soil org. matter, plant nutrients and plant diseases. Plant growth in relation to the absorption of org. compounds is also considered. Physical, chemical and physico-chemical properties of humic substances are discussed. (130 references.) J. LAMBORN.

**Effect of competition on *Beijerinckia* spp. in soil supplemented with calcium carbonate.** H. W. Strijdom (*S. Afr. J. agric. Sci.*, 1967, **9**, 849–855).—An increase in pH (~ 5·9–7·5) in sterilised soil caused a moderate reduction in the growth of these bacteria (*Azotobacter indicum*), but the same change in unsterilised soil caused their elimination due to competition by other soil micro-organisms. The addition of 0·25% of sucrose to the soil markedly reduced the effect of competition in the acid or alkaline soils. (16 references.) P. S. ARUP.

**Estimation of capacity of soils for phosphorus nutrition of plants. Analyses of soils and experimental results.** L. Gachon (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1966, **52**, 1313–1318).—The fertility indices $I_L$ and $I_E$ for 16 Auvergne soils were calculated as functions of P assimilated by ryegrass as previously described (cf. *Idem, ibid.*, 1966, **52**, 1108). The results were generally valid for values of $I_L$ and $I_E$ of < 80, and < 35, respectively. P. S. ARUP.

**Phosphatic fertilisation of legume improves soil physical condition.** T. D. Biswas, K. S. Pharande and M. H. Ali (*Fertil. News*, 1967, **12**, No. 7, 31–33).—Phosphate application in the form of super-phosphate resulted in significant improvement in org. C and soil structure; moisture retention was increased and bulk density, air space and permeability showed improvement. The application of superphosphate to a legume (*Trifolium alexandrinum* in this investigation) is not only effective in increasing the yield of the legume, and of the succeeding crop, but is also conducive to improvement of soil structure and other related soil properties. (11 references.) I. DICKINSON.

**Phosphate manuring in depth. II.** E.-M. Batisse (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1152–1157).—Previously reported percolation experiments with $Ca(H_2PO_2)_2$ (**I**) and $Na_2HPO_3$ (**II**) (*ibid.*, 1967, **53**, 1079) were repeated with a French soil taken from three horizons, and three African laterite soils; 120 days after treatment with the solutions, appreciable amounts of the added P were found to have been fixed as $P_2O_5$, the amounts fixed from **II** being much greater than those from **I**. In the experiments with French soil, P fixed as $P_2O_5$ was approx. proportional to clay content. In the African soils, which contained appreciable amounts of $Al_2O_3$ and $Fe_2O_3$, the relationship was masked by other factors. P. S. ARUP.

**Mineral elements in temperate crop and pasture plants. I. Zinc.** J. S. Gladstones and J. F. Loneragan (*Aust. J. agric. Res.*, 1967, **18**, 427–446).—Twenty-five annual crop and pasture plant varieties were grown on a lateritic gravelly sand plus a trace element mixture containing Cu, Zn, Mn, Mo, Co, Se and iodine (as NaI). Zn concn. in the tops fell with increasing age in all species and trace element treatments, but total Zn in the tops rose throughout the growing season except in lupins. Based on overall treatments and harvests, legumes and herbs had higher concn. of Zn than did cereals and grasses and results suggest that a high Zn uptake could be one of the mechanisms which enable certain species to grow better than others on sandy soils of low fertility. E. G. BRICKELL.

**Sampling bulk fertilisers.** C. W. Gehrke, W. L. Baker, G. F. Krause and C. H. Russell (*J. Ass. off. analyt. Chem.*, 1967, **50**, 382–392).—A stream sampling cup is passed through the entire flow of material at equally time-spaced intervals during the loading of a truck. A.O.A.C. triers did not secure accurate samples. The use of two compartmented triers and the stream sampler is recommended. A. A. ELDRIDGE.

**Mechanisms of sampler bias.** W. L. Baker, C. W. Gehrke and G. F. Krause (*J. Ass. off. analyt. Chem.*, 1967, **50**, 407–413).—The compositions of samples of mixed fertiliser taken horizontally and vertically were determined. Vertical cores, or cores drawn at 60–75° from the horizontal were the more representative. The bias in horizontal cores is attributed to downward drifting of small particles. A. A. ELDRIDGE.

**Composition of fertiliser granules and residues [thereof] recovered from soil.** E. L. Pocharkoff, S. Kuczynski and S. Macchia (*J. agric. Fd Chem.*, 1967, **15**, 123–126).—Chemical analyses and sp. gr. determinations of residual NPK granules (resembling the original granules) revealed that, after one season, all the N and K, and 70% of the P originally present had migrated into the soil. P. S. ARUP.

**Thermal decomposition of compound concentrated fertilisers.** M. Man (*Revta Chim.*, 1967, **18**, 145–152).—Mixed conc. fertilisers were prepared, containing NPK mixtures of: $NH_4Cl$, $NH_4NO_3$, $(NH_4)_2SO_4$, $NaNO_3$, $Ca(H_2PO_4)_2 \cdot H_2O$, $CaHPO_4$, KCl, $K_2SO_4$, $KNO_3$; these were studied by several methods, (mainly by thermo-gravimetry). Presence of only 0·05% $Cl^-$ was sufficient, but essential, for initiation of $NH_4NO_3$ decomposition; its rôle was catalytic, max. effect being obtained with 0·5% $Cl^-$. The free acidity of the fertilisers ($HNO_3$, $H_3PO_4$) also favoured N losses. The joint presence of $NH_4^+$ and $NO_3^-$ was necessary for decomposition by reactions leading finally to $N_2$ and $N_2O$; $SO_4^{2-}$ as $K_2SO_4$ or $(NH_4)_2SO_4$, (1 : 1 with respect to $NH_4NO_3$) had a stabilising effect. In ternary fertilisers based on $NH_4$ or mono-Ca phosphates, urea was hydrolysed, especially during storage, and hence cooling beforehand is essential. With $NH_4$ phosphates, the losses occur as $NH_4Cl$ and $NH_3$. Locally initiated decomposition was propagated at room temp. in the entire mass of fertiliser at the rate of 5–6 cm/h in mixtures based on $NH_4$ phosphates, or 60–150 cm/h in those based on superphosphate. Self-ignition and propagation appeared at > 15% $NH_4NO_3$, presence of $Cl^-$ being essential. M. L.

**Comparison of slow-release nitrogen and water-insoluble nitrogen [analytical] methods for fertilisers.** K. E. Holt (*J. Ass. off. analyt.*

*Chem.*, 1967, **50**, 414–418).—Collaborative results obtained by the slow-release method (*ibid.*, 1965, **48**, 1105; 1966, **49**, 206) and by the A.O.A.C. method 2.057 have achieved higher precision, so that the methods will give significantly different results for some fertilisers. A. A. ELDRIDGE.

**Agricultural value of anhydrous ammonia on arable land.** P. F. J. Van Burg, G. D. Van Brakel and J. H. Schepers (*Neth. Nitrogen Tech. Bull.*, 1967, No. 3, 39 pp).—Experiments with potatoes, sugar-beet, oats and spring wheat (1963–1966) are described with particular emphasis on (1) the date of injection for spring-sown crops, (2) injection into a winter-cereal crop and (3) the spacing between injection tines for different crops. Results indicate that an average injection could be started 6 to 7 weeks before sowing or planting though 4 to 5 weeks is safer; injection into a winter cereal crop gave poor results compared with a standard fertiliser; a tine spacing of 60 cm can be used for potatoes but a lesser spacing (⩾ 30 cm) is imperative for cereals. E. G. BRICKELL.

**Comparative efficiency of superphosphate and bonemeal.** S. K. Choudhury (*Fertil. News*, 1967, **12**, No. 6, 32–34).—The effect of different doses of $P_2O_5$ in the form of superphosphate (**I**) (16% $P_2O_5$) and bonemeal (**II**) (20% $P_2O_5$) on the yield of paddy was studied over a period of three years. Applications of 22·5 kg and 45 kg of $P_2O_5$/ha as **II** and as **I** were made. **I** was always superior. The residual effect was studied, the higher doses in both cases were equally effective. The lower dose of **II** gave a significantly lower yield. Maximum profits were obtained from the application of 22·5 kg of $P_2O_5$/ha applied as **I**.
I. DICKINSON.

**Importance of water solubility of phosphorus fertilisers.** O. P. Engelstad and G. L. Terman (*Fertil. News*, 1967, **12**, No. 7, 9–12).—Results are tabulated for three categories of phosphate fertilisers. (1) High (40% or more of the total P) is sol. in water; (2) Low (5 to 40%) sol. in water and (3) Very low (less than 5%) sol. in water). The results relate to soils deficient in P where crops respond readily to added phosphate; water solubility is less important when soil P levels are relatively high. As the water solubility decreases, more restriction must be placed on fertiliser use. P fertilisers of high solubility can be used under all conditions; those of low solubility should be used for long season crops and where soils are acid. Rock phosphates can be effective when residual value is more important than immediate effectiveness; they should be broadcast in finely ground form and applied only to acid soils. I. DICKINSON.

**Ion-exchange determination of trace components in phosphorus fertilisers.** I. K. Tsitovich and N. G. Gaidukova (*Zh. prikl. Khim.*, 1967, **40**, 1708–1712).—Samples of superphosphates containing trace elements Cu, Zn, Mo, Co and Mn were prepared in the laboratory by mixing in the metals (as sulphates). Trace elements were calculated as kg per 100 kg of $P_2O_5$: Cu 0·16–0·30, Mn 0·80, Mo 0·10–0·20, Co 0·10–0·15, Zn 0·60–0·80. Experiments show that it is possible to separate trace elements (obtained by citrate extraction of superphosphate) by percolation of the extracts through a cation exchanger KU–1 ($NH_4^+$ form). The following pairs of elements were separated: Cu–Co, Cu–Mo, Cu–Mn, Zn–Co, Zn–Mn, Zn–Mo. A. L. B.

**Differences in potassium values [for fertilisers] determined by official A.O.A.C. extraction procedures.** P. R. Rexroad and C. W. Gehrke (*J. Ass. off. analyt. Chem.*, 1967, **50**, 714–717).—The A.O.A.C. carbonate procedure (2·079*a*) gave lower results than the oxalate procedure (2·070*a*) when applied to mixed fertilisers. Residues from the former procedure contained much more P and Fe than did those from the latter procedure. A. A. ELDRIDGE.

**Determination of secondary and minor plant nutrients in fertilisers by atomic absorption spectrophotometry.** C. H. McBride (*J. Ass. off. analyt. Chem.*, 1967, **50**, 401–407).—In a further collaborative study (cf. *Idem, ibid*, 1965, **48**, 406, 1100) Ca, Na, K, Cu, Fe, Mg, Mn and Zn were determined with a between-laboratories precision of 4–7% in the range employed. Chemical results were well within the range of atomic absorption results.
A. A. ELDRIDGE.

**Crop response to zinc oxide applied in liquid and granular fertilisers.** J. J. Mortvedt and P. M. Giordano (*J. agric. Fd Chem.*, 1967, **15**, 118–122).—Better responses with lucerne were obtained with liquid than with granular fertilisers as carriers containing 2% of Zn. Mixed placements gave better responses than did band or spot placements. Among various liquid formulations tested, $NH_4NO_3$, urea–$NH_4NO_3$ and $NH_4$ pyro- and poly-phosphates were almost equally effective. Solution and clay suspension fertilisers were equally effective. Spot-placed anhyd. $NH_3$ was ineffective.
P. S. ARUP.

**Influence of applications of copper to soil on copper content of certain crops.** C. P. de L. Beyers (*S. Afr. J. agric. Sci.*, 1967, **9**, 907–910).—Single applications of 20 lb of $CuSO_4$ per morgen (2·12 acres) to an acid, Cu-deficient, sandy soil on which various cereals and legumes were grown in rotation, sufficed to increase yields of wheat, millet, and peas. Applications of 40 and of 60 lb had no further effects and did not cause abnormal Cu-uptake.
P. S. ARUP.

**Determination of copper in trace-element superphosphate by a.c. polarography.** G. Curthoys and J. R. Simpson (*Analyst, Lond.*, 1967, **92**, 565–566).—From 0·01 to 5% of Cu are determined in ~1 h by polarography of the prepared solution in M-HCl, the sharp peak being at −0·26 V vs. the Hg-pool anode. There is no interference from $PO_4^{3-}$, $SO_4^{2-}$, Fe or Al, and the results agree with those obtained by the sulphide separation procedure.
W. J. BAKER.

**Determination of iron in fertilisers by dichromate titration.** W. J. Ingram (*J. Ass. off. analyt. Chem.*, 1967, **50**, 397–400).—Satisfactory collaborative results are reported. In the use of $HClO_4$ for oxidation a ppt. which contains much Fe may be formed; it must be redissolved before the titration is carried out. High $P_2O_5$, $K_2O$ and $NH_4NO_3$ contents do not interfere.
A. A. ELDRIDGE.

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**Production of fertiliser product from molten ammonium nitrate and powdered solid calcium carbonate.** Chemical Construction Corp. (B.P. 1,063,419, 1.9.65. U.S., 4.9.64).—To prevent formation of deliquescent $Ca(NO_3)_2$, 0·1–1 wt.-% of a silicofluoride (Na, K, or $NH_4$ silicofluoride) is added to the molten $NH_4NO_3$, before or after incorporation of the powdered limestone. The resulting mixture is then cooled (to < 170°) F. R. BASFORD.

**Fertiliser compositions.** Shell Internationale Research Mij. N. V. (B.P. 1,067,635, 21.2.66. Neth., 23.2.65).—Compositions containing a nitrate fertiliser and a mineral oil product as binder have improved storage properties when the pH of a 10% aq. dispersion of the fertiliser is at least 4 (preferably at least 4·8). This can be achieved by addition of ammonia during manufacture. Storage tests on fertiliser grains treated with a paraffin wax-containing distillate lubricating oil showed that air concn. of acrolein (formed by oxidative decomposition of the mineral oil) after 16 h storage of fertiliser at 70° in half-filled bottles was only 5 ppm for fertiliser of pH 4·8 but 170 ppm for fertiliser of pH 3·8.
S. D. HUGGINS.

**Composts or plant growth media.** L. Bulmer (B.P. 1,068,753, 27.4.65).—A soil-free compost or plant growth medium consists of dry fly-ash, furnace bottom ash or pptd. ash and dry sphagnum moss peat, with the optional addition of suitable fertiliser or plant nutrient, in intimate mixture. Thus, a seed compost consists of ash mixed with an equal wt. of sphagnum moss peat, together with 1·5 oz. superphosphate, 0·75 oz $CaCO_3$ and 0·25 oz $KNO_3$ per bushel of ash–peat mixture. S. D. HUGGINS.

**Tablet-type plant fertiliser.** Leslie-Angriform Corp. (B.P. 1,068,960, 4.11.64. U.S., 19.11.63).—The tablet comprises at least a major portion of slow-acting fertiliser ingredients, e.g., urea–$CH_2O$, Ca phosphate, and fritted potash; a binder to render the tablet rapidly disintegratable by water, e.g., expanded vermiculite, alginic acid, or a salt thereof (e.g., Na alginate, 0·5–5); and optionally a lubricant (e.g., talc, 4–10 wt.-%) and a water-sol. fertiliser, e.g., $Fe(NH_4)_2(SO_4)_2$. F. R. BASFORD.

**Fertiliser compositions.** L. Bulmer (B.P. 1,070,357, 8.4.65).—An improved composition comprises a mixture of fly-ash and sewage sludge, optionally together with clay, sand or other additives.
F. R. BASFORD.

**Production of multi-component fertilisers.** VEB Stickstoffwerk Piesteritz (Inventors: G. Naumann, H. Stephan, O. Jeitner, M. Dinter, L. Zipfel and O. Konig) (B.P. 1.077,184, 30.9.64).—$NH_3$ is passed into a slurry of crude phosphate digested with $HNO_3$ and $H_2SO_4$, $H_3PO_4$, $(NH_4)_2SO_4$ or alkali metal sulphates containing Mg salts, in 3 stages at 110–160° and pH ranges of 3·3–3·8, 4·3–4·9 and 5·2–6·0 respectively, while the water content is simultaneously reduced to 3–8% by wt. in the third stage. S. D. HUGGINS.

**Mono-alkali metal phosphates.** Israel Mining Industries—Institute for Research and Development (B.P. 1,080,661, 1.2.65. Israel, 30.3.64).—Aq. $H_3PO_4$ (65%), alkali chloride (KCl) or

$NH_4Cl$ and an inert, org. solvent (**I**) are distilled at $\geq 180°$. The b.p. of **I** must be greater than that of the azeotropic mixture of HCl and water at the reaction pressure; suitable **I** are kerosene, n-octane, $Bu_2O$. **I** is recycled, and aq. HCl distillate discarded, until the distillate is composed wholly of **I** and the residue consists of the double compound $KH_2PO_4.H_3PO_4$ (**II**) and ~5% of KCl. After cooling, $H_3PO_4$ is extracted with BuOH, KCl removed, and $KH_2PO_4$ recovered. Alternatively, **II** can be reacted with $NH_3$ for use as fertiliser. J. A. SUGDEN.

**Fertiliser manufacture.** Fisons Fertilisers Ltd., (Inventors: J. D. C. Hemsley and S. M. Janikowski (B.P. 1,081,296, 26.3 and 17.6.64).—Solid ammonium phosphates (**I**) are produced by reacting $H_3PO_4$ containing < 60% $P_2O_5$ with $NH_3$ under pressure to give a fluid **I** solution at its b.p. containing 4–15% by wt. of water, and subsequently expelling the resulting solution into a zone at ambient pressure (e.g. a prilling tower) to reduce the water content and give a solid product. E. ENOS JONES.

## Plant Physiology, Nutrition and Biochemistry

**Effect of DDT on photosynthesis in varieties of barley.** P. D. Lawler and L. J. Rogers (*Nature, Lond.*, 1967, **215**, 1515–1516).—Reports and discusses (*i*) gas exchange during successive periods of light and darkness after treatment of 8-day-old barley plants (resistant and susceptible varieties) with DDT, (*ii*) changes in Hill activity of chloroplasts from untreated and treated plants as measured by rate of photoreduction of 2,6-dichlorophenolindophenol at pH 6·5. Chloroplasts from DDT-treated susceptible barley showed a large decrease in Hill activity, whilst those from treated resistant barley had Hill activity approx. equiv. to that of unsprayed plants. Chloroplasts from unsprayed plants (susceptible variety) showed decreased Hill activity when suspended for 45–60 min. in aq. suspension of DDT, whereas those from the resistant variety were unaffected. Results indicate that DDT affects the light reaction during photosynthesis in susceptible ('Rika') varieties of barley. W. J. BAKER.

**Photosynthesis in algae. III. Distribution of soluble carbohydrates and dimethyl-β-propiothetin in marine unicellular Chlorophyceae and Prasinophyceae.** J. S. Craigie, J. McLachlan, R. G. Ackman and C. S. Tocher (*Can. J. Bot.*, 1967, **45**, 1327–1334).—Carbohydrates accumulated by 10 species of Chlorophyceae and 13 of Prasinophyceae after photosynthesis in $NaH^{14}CO_3$ were separated and identified by paper chromatography. Major products in the Chlorophyceae were sucrose, glycerol and mannitol but mannitol and sucrose were never found together, and glycerol and sucrose occurred together in only two cases. $H_2S$ and dimethyl-β-propiothetin (DMPT) each occurred in only one species. All the 13 Prasinophyceae produced mannitol and all with one exception, contained DMPT. $H_2S$ was observed in one strain but sucrose and glycerol were not found in this class. (18 references.) J. L. WALPOLE.

**Phenol synthesis and photomorphogenesis.** G. Engelsma (*Philips tech. Tijdschr.*, 1967, **28**, 105–114; *Landbouwdocumentatie*, 1967, **23**, 548).—The light-induced stimulation of the synthesis of the phenols *p*-coumaric acid and ferulic acid in the hypocotyl of cucumber seedlings is caused by the increased rate of production from amino-acids of the enzymes phenylalanine diaminase and cinnamic acid dehydroxylase. A possible connection between this stimulation and the inhibition of stem-elongation is considered in view of the fact that phenols are regarded as regulators of the production of indolylacetic acid. P. S. ARUP.

**Effects of herbicides on carbon dioxide uptake by pine seedlings.** S. Sasaki and T. T. Kozlowski (*Can. J. Bot.*, 1967, **45**, 961–971).—Experiments with eight herbicides showed them to have varied effects on the $CO_2$ uptake of 3-year old *Pinus resinosa* Ait. seedlings In soil applications, monuron (*M*) caused the greatest inhibition, $CO_2$ absorption virtually ceasing after 10 days; atrazine (*A*) and 2,4-D depressed absorption at a steady rate as did EPTC (ethyl *N*,*N*-di-n-propylthiolcarbamate) after an initial delay, while DCPA (dimethyl 2,3,5,6-tetrachloroterephthalamate), CDAA (2-chloro-*N*,*N*-diallylacetamide), CDEC (2-chloro-allyl diethyldithiocarbamate) and NPA (*N*-1-naphthylphthalamic acid, Na salt) had no significant effect. *M* applied as a spray depressed the $CO_2$ uptake at an even faster rate and very rapid inhibition was also observed with spray applications of 2,4-D and EPTC while *A* again depressed absorption at a steady rate and DCPA as before had no effect. The wide difference in response to individual herbicides is discussed; it suggests that *M* affects the photosynthetic mechanism more directly than others such as *A*, 2,4-D and EPTC. (33 references.) J. L. WALPOLE.

**Phosphate uptake along attached and excised wheat roots measured by an automatic scanning method.** G. D. Bowen and A. D. Rovira (*Aust. J. biol. Sci.*, 1967, **20**, 369–378).—The method which records accurately and with good resolution sites of uptake and accumulation of radioactively labelled nutrients has been developed and applied to phosphate uptake in wheat roots. Attached roots absorbed 40–60% more phosphate in 2 and 15 min. than did roots excised immediately prior to uptake. These differences could not be ascribed to transpiration nor to translocation in whole plants and hence rapid physiological changes upon excision are suggested. The lower uptake of excised roots occurred in both the apical and the mid-root portions. S. A. BROOKS.

**Exchange capacity of roots; cationic equilibrium of plants [as influenced by nitrogenous fertilisation].** D. Blanc (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1220–1225).—Increase in N-fertilisation increased the ratio of (Ca + Mg)K which was absorbed by the roots, and found in the shoots. This experimentation was carried out on tomatoes in the field, and on carnations in the glasshouse. P. S. ARUP.

**Incorporation of nitrogen-15 into the constituents of the wheat kernel.** W. B. McConnell and A. J. Finlayson (*Cereal Chem.*, 1967, **44**, 353–359).—Metabolism of $^{15}N$ was studied by injection as $NH_4Cl$ solution into the top internode of maturing wheat plants and subsequent analysis of lower and upper stem, chaff, rachis, kernel and kernel fractions of the mature plants. $^{15}N$ was mainly incorporated into the kernel (92%). Specific activity was higher in the gluten fraction than in the salt-sol. proteins, and higher in glutamic acid than in the other amino-acids. (11 references.) E. C. APLING.

**Metabolism of aromatic compounds in healthy and rust-infected primary leaves of wheat. I. Studies with $^{14}CO_2$, quinate-U-$^{14}C$ and shikimate-U-$^{14}C$ as precursors.** R. Rohringer, A. Fuchs, J. Lunderstadt and D. J. Samborski (*Can. J. Bot.*, 1967, **45**, 863–889).—Studies employing $^{14}CO_2$, quinate-U-$^{14}C$ and shikimate-U-$^{14}C$ showed that the two acids were interconvertible but differed in their efficiency as precursors of phenylalanine and tyrosine, suggesting that phenylalanine can be obtained from quinate (*Q*) in wheat leaves but not necessarily via shikimate (*S*). Rust infection led to an increase of the carbon flow from $CO_2$ to *S* and to higher levels of both *Q* and *S*, the trend being more marked in susceptible leaves. Utilisation of *Q* and *S* was increased in infected susceptible leaves but not in infected resistant ones. Considerable differences were observed in the amount of activity accumulated in sol. and insol. esters resulting from the metabolism of *Q* or *S* according to whether the leaves were healthy or infected, resistant or susceptible. (51 references.) J. L. WALPOLE.

**How plants fix nitrogen.** W. D. P. Stewart (*Span*, 1967, **10**, 110–113).—A review of recent studies on biological N-fixing enzyme systems discusses the inter-relations between the enzymes, hydrogenase and nitrogenase, and ferredoxins, pyruvic acid and ATP. J. L. WALPOLE.

**Temperature coefficient of invertase from leaves of cold-hardened and cold-susceptible wheat plants.** D. W. A. Roberts (*Can. J. Bot.*, 1967, **45**, 1347–1357).—The energy of activation of invertase (**I**) obtained from the leaves of winter wheat, Kharkov 22MC, grown at 6°, is lower than that from leaves grown at 20°, whether or not the extracted juice is purified by $(NH_4)_2SO_4$ pptn. With Rescue wheat leaves (a frost-sensitive variety) the energy of activation of the extracted **I** is substantially the same for plants grown at 6° or at 20° while Thatcher wheat, which is moderately cold-hardy, is intermediate between the two in its behaviour. Various possible explanations for the changes in temp. coeff. of **I** are discussed. (63 references.) J. L. WALPOLE.

**Oxidation–reduction enzymes of wheat. II. Quantitative investigation of the dehydrogenases.** G. R. Honold, G. L. Farkas and M. A. Stahmann (*Cereal Chem.*, 1967, **44**, 373–382).—Quant. assays of dehydrogenases (**I**) of glucose-6-phosphate, 6-phosphogluconate, malate, isocitrate, succinate, glutamate, lactate and ethanol are reported for two hard red winter and two hard red spring wheats and the milling fractions of each. Assays were made by densitometric tracing of reduced nitro-blue tetrazolium on polyacrylamide gel (cf. Part I, J.S.F.A. abstr., 1967, ii-62) and by conventional spectrophotometric methods. Malate **I** activity was 100 to 1,000 tmes greater than the activity of the other **I** studied, and its activity was similar in spring and winter wheats. Only low levels of activity of **I** of lactate, succinate and glutamate

were detected but the remainder were found in significant amounts; levels were generally higher in spring than in winter wheat. Substantial activities were found in flour, particularly in flour from spring wheat. (12 references.) E. C. APLING.

**A soluble ATP-ase from bean roots stimulated by monovalent cations.** J. Neumann and N. Gruener (*Israel J. Chem.*, 1967, **5**, 107–116).—The roots of 7-day old bean seedlings (var. Brittle wax) grown in vermiculite in the dark were homogenised and the homogenate filtered. Mitochondria were removed from the filtrate by centrifuging at 20,000 × g and microsomes at 120,000 × g; the various fractions were dialysed for 24 h against $3 \times 10^{-3}$M-Tris–$5 \times 10^{-4}$M-EDTA of pH 7·7. ATP-ase activity was determined by measuring the $^{32}P$ (inorg.) liberated from γ-labelled ATP (synthesised enzymically); 90% of the activity resided in the final supernatant; the mitochondria and microsomes contained only about 10%. The activity increased in presence of NaCl and considerably more so in presence of $Mg^{2+}$ and $Ca^{2+}$. While ATP, ADP and ITP were readily hydrolysed, AMP, pyrophosphate and *p*-nitrophenyl phosphate were not attacked. Activity was not affected by NaF, dinitrophenol, *p*-hydroxymercuribenzoate or ouabain (which is a potent inhibitor of animal transport ATP-ase). (15 references.) J. I. M. JONES.

**Phenoloxidases and content of *o*-diphenols in olives.** E. Ragazzi and G. Veronese (*Annali Chim.*, 1967, **57**, 1476–1492).—The enzymic activity of various types of olive pulp has been examined in relation to the darkening of olive pulp on exposure to air; this is probably associated with the action of phenol oxidases on phenols present and formation of catechol melanic pigments. The catecholasic activity was very high and the cresolasic activity low and similar to that of other vegetable tissues. The laccasic and peroxidasic activity was very low while the ascorbic acid oxidasic activity was absent. The content of *o*-diphenols (0·5 to 3·5% calc. as caffeic acid) was much higher than in most other vegetables studied. (25 references.) L. A. O'NEILL.

**Nodulation and growth of *Trifolium subterraneum* L. cv. Mount Barker in agar culture.** J. R. Cannon, N. H. Corbett, J. Brockwell, A. H. Gibson and G. A. McIntyre (*Aust. J. biol. Sci.*, 1967, **20**, 285–295).—The effect of root exudates on nodulation of *Trifolium subterraneum* L. cv. Mount Barker in agar culture was investigated by means of preplanting technique. When the use of this technique for the bioassay of substances influencing nodule formation was examined it was found that preplanting removed unidentified N compounds from the agar medium; some of the data were subject to observer effects. Environmental conditions and type of agar had large and significant effects on the number of nodules formed but preplanting did not. Plant yield was significantly affected by environmental conditions. S. A. BROOKS.

**Rapid method for determination of iron in plant material with application of automatic analysis to the colorimetric procedure.** C. Quarmby and H. M. Grimshaw (*Analyst, Lond.*, 1967, **92**, 305–310).—The sample solution, in 1% $H_2SO_4$ is mixed with reagent [sulphonated bathophenanthroline–$NH_2OH$,HCl–Na acetate (3 : 1 : 4)], and the extinction measured at 536 nm. Beer's law holds up to 0·4 mg per 50 ml; for ~250-mg samples of oak-leaf litter containing 0·044% Fe the coeff. of variation was 0·001% (10 determinations). Conditions for ensuring complete recovery of Fe during digestion of sample with $HClO_4$–$HNO_3$–$H_2SO_4$ are discussed. Method is applicable directly to the Auto Analyser, the flow diagram being shown. $PO_4^{3-}$ rarely interferes; 4 ppm Fe are fully recovered in presence of a max. of 50 ppm $PO_4^{3-}$. W. J. BAKER.

**Identification of plant material by its phenolic content.** M. B. Duggan (*J. Ass. off. analyt. Chem.*, 1967, **50**, 727–734).—Flavonol glycosides of apple and pear were extracted with ethanol and transferred to ethyl acetate; pptn. with Pb acetate can be used to separate flavonoids from sugars. The extracts are chromatographed on microcryst. cellulose TLC plates and developed with butanol–acetic acid–water (6 : 1 : 2) for differentation. The results were confirmed by ultra-violet spectrophotometry. A. A. ELDRIDGE.

**Occurrence of 5-hydroxy-7,4′-dimethoxyflavone in the leaves of *Rosmarinus officinalis* L.** C. H. Brieskorn and H. J. Dömling (*Arch. Pharm., Berl.*, 1967, **300**, 1042–1044).—The presence of very small amounts of a flavone is reported in the petroleum ether extract of powdered *Rosmarinus officinalis* L. after the extraction of carnosol. The flavone which has u.v. absorption max. at 328 nm and 269 nm is identified as 5-hydroxy-7,4′-dimethoxyflavone, m.p. 178–179°. G. R. WHALLEY.

**Formation of perylenequinones in etiolated cucumber seedlings infected with *Cladosporium cucumerinum*.** J. C. Overeem, A. K. Sijpesteijn and A. Fuchs (*Meded. Lab. Phytopath.*, 1967, **228**, 99–105).—Five wine-red pigments, extracted from the infected seedlings and separated by TLC, were characterised as deriv. of 4,9-dihydroxyperylene-3,10-quinone. A tentative structural formula is proposed for the main pigment, cladochrome A. (10 references.) P. S. ARUP.

**Alteration of chloroplast pigments by chromatography with siliceous adsorbents.** H. H. Strain, J. Sherma and M. Grandolfo (*Analyt. Chem.*, 1967, **39**, 926–932).—The conflicting results obtained from the chromatography of leaf pigments on various $SiO_2$-gels, siliceous earths and synthetic silicates are discussed with respect to factors which promote the isomerism of neoxanthin (**I**) and violaxanthin (**II**) on these adsorbents. **I** yields neochrome (one furanoid group) and **II** yields first luteoxanthin and then auroxanthin (two furanoid groups). This isomerisation of epoxy carotenoids is accelerated by (1) non-polar org. wash liquids that permit very strong pigment adsorption, (2) exposure of adsorbent plus pigment and wash liquid to air after separation of pigments by thin-layer or column chromatography, (3) evaporation of org. wash liquid from adsorbent plus pigment *in vacuo* or in moist or dry $N_2$. Isomerisation is retarded by $NH_3$ vapour and is sometimes accompanied by alteration of the chlorophylls. Siliceous adsorbents cannot be relied on as inert chromatographic media. (35 references.) W. J. BAKER.

**Detection, isolation and characterisation of chlorophylls and related pigments during ripening of fruits and vegetables.** D. Yen-ching Lynn Co (*Diss. Abstr.*, B, 1967, **28**, 227).—The pigments were separated by TLC using silica gel G absorbent and several solvent systems, on pigment extracts from the peels of progressively ripening bananas, peppers and cucumbers. Two compounds (**I**) and (**II**) were the last to disappear and are thought to be degradation products somewhat more stable than chlorophylls *a* and *b*. In addition, two green pigments appeared in acetone extracts of all three fruits, with visible absorption peaks at 418 and 444 nm. It is suggested that **I** and **II** could be deriv. of chlorophylls or of chlorophyll precursors, or could themselves be precursors of chlorophylls. F. C. SUTTON.

**Changes in the aqueous ammonia-soluble proteins of rapeseed (*Brassica napus* L.) during the maturing period.** A. J. Finlayson (*Can. J. Biochem.*, 1967, **45**, 1225–1231).—Changes in the amino-acid composition of the two aq. ammonia sol. proteins from rapeseed (*Brassica napus* L.) have been studied from the time that the seed contains a small amount of protein N until it is mature. Amino-acid analyses and N-terminal amino-acid analyses indicated that protein synthesis proceeds from the N-terminus of the protein towards the C-terminus. Although results of the analyses cannot be interpreted unequivocally, they ruled out the possibility that the storage protein is synthesised by the condensation of similar polypeptide subunits. S. A. BROOKS.

**Nucleic acids as related to juvenility in *Pyrus* species.** N. Ali and M. N. Westwood (*Proc. Am. Soc. hort. Sci.*, 1966, **89**, 123–131).—In general leaves from adult shoots of *Pyrus* species contained more RNA and had a greater RNA/DNA ratio than did leaves from juvenile shoots. It is suggested that the inability of juvenile plants to initiate flower buds is related to the failure of DNA to mediate synthesis of the specific m-RNA which is responsible for synthesis of specific 'floral protein'. A. H. CORNFIELD.

**Inhibition of germination of non-dormant apple seeds (*Pirus malus* L.). Possible rôle of integumentary phenols.** D. Come (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1359–1362).—The possible role of seed-coat phenols is considered with respect to known facts concerning dormancy. P. S. ARUP.

**Inhibition of Brussels sprout leaf senescence by kinins.** D. T. Dennis, M. Stubbs and T. P. Coultate (*Can. J. Bot.*, 1967, **45**, 1019–1024).—Kinins (kinetin, kinetin riboside and benzyladenine) inhibit the senescence of leaf discs cut from mature Brussels sprout leaves but accelerate the process of senescence in young expanding leaves. A number of plant growth regulators and antibiotics were without effect. Electron microscope studies showed that in mature leaves the chloroplast senesces first and the breakdown is retarded by *N*-6-benzyladenine (**I**). In young leaves, however, **I** appears to over-stimulate the chloroplast which swells and leads to excessive membrane synthesis. (21 references.) J. L. WALPOLE.

**Growth retardants in relation to the germination of seeds. I. A paradoxical concentration effect of *N,N*-dimethylaminosuccinamic**

**acid on the germination of kale seeds affected with coumarin.** J. S. Knypl (*Can. J. Bot.*, 1967, **45**, 903–913).—Increasing concn. of *N,N*-dimethylaminosuccinamic acid (**I**) initially reduce and then increase the germination of kale seeds inhibited by treatment with coumarin (**II**) at 100 mg/l, the response curve having a min. at $1–5 \times 10^{-3}$M. Kinetin (**III**) and gibberellic acid at $10^{-4}$M reduce and reverse the inhibition of germination caused by **II** and **I** while **I** at $5 \times 10^{-3}$M reduces the toxic effect of **III** used at the high rate of $5 \times 10^{-4}$M. Indole-3-acetic acid, on the other hand, increases the inhibitory effect of **II** and **I**. The mechanisms of these interactions are discussed. (39 references.)

J. L. WALPOLE.

**Plant growth regulators. I. Isolation of indole-3-acetamide, 2-phenylacetamide and indole-3-carboxaldehyde from etiolated seedlings of *Phaseolus* [*mungo*].** Y. Isogai, T. Okamoto and T. Koizumi (*Chem. Pharm. Bull., Tokyo*, 1967, **15**, 151–158).—The isolation of these growth promoting substances and their identification by paper chromatography is described. Dehusked oat seeds (*Avena sativa* var. Victory) were used in the bioassay.

C.V.

**Effect of benzyl adenine on isolated apple-shoots.** O. P. Jones (*Nature, Lond.*, 1967, **215**, 1514–1515).—The effect of benzyl adenine (**I**) (1 mg per l) during 5–10 weeks on the shoots in sterile culture medium at pH 5·8 was reflected in increased leaf production (poorly-developed small leaves) and internode extension, delayed senescence of shoots, but no rooting. There was hardly any growth after 5 weeks, but cultures receiving **I** were still green after 10 weeks. Effects, which partly simulate those of roots, were max. on cultures initiated from 1·5 cm shoots. At 0·1 mg per l **I** merely increased callus production in basal regions, but at 10 and 50 mg per l, **I** inhibited shoot extension and promoted profuse callus growth. The results, in conjunction with evidence for cytokinins (**II**) in ascending sap, indicate that **II** from the roots are essential for continued shoot-growth in apple. Amino-compounds and gibberellin-like compounds in the roots may also influence normal shoot-growth. W. J. BAKER.

**Genetic traits in tetraploid and diploid lucerne.** D. K. Barnes and C. H. Hanson (*Tech. Bull., U.S. Dep. Agric.*, 1967, No. 1370, 39 pp).—An illustrated summary of published and unpublished information on genetic markers studied in the *Medicago sativa* L. species, is presented. Information on each trait consists principally of a description and a brief account of its mode of inheritance. (56 references.) E. G. BRICKELL.

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**Derivatives of *s*-triazine and their use as plant-growth regulating agents.** Deutsche Gold-u. Silber-Scheideanstalt (B.P. 1,068,036, 19.11.63. Ger., 2.7.63).—The regulating agents are 2-$NR^{II}R^{III}$-4-$XR^{I}$-6-$NR^{IV}$·Y·$NR^{V}R^{VI}$-*s*-triazines wherein X is O or S; Y is CO or $SO_2$; $R^{I}$-$R^{V}$ are low-mol. alkyl, alkenyl, or alkoxyalkyl or $R^{II}$-$R^{III}$ are H or halogeno or nitroaryl—e.g., 2-isopropyl-amino-6-carbamylisopropylamino-4-methoxy-*s*-triazine m.p. 215–216°. F. R. BASFORD.

**2,4-Pentadienoic acid derivatives.** Shell Internationale Research Mij N. V. (Inventor: J. W. Cornforth) (B.P. 1,073,882, 14.4.65.)—Used as plant growth regulants, the 3-methyl-5-(1-hydroxy-4-oxo-2,6,6-trimethyl-2-cyclohexen-1-yl)-*cis*, *trans* or -*trans*, *trans* 2,4-pentadienoic acids (**I**) are obtained from the corresponding *cis*- and *trans*-dehydro-β-ionylidene acetic acids treated with activated $O_2$ to give the epidioxides; these are rearranged, under alkaline conditions, to give **I**. Thus, *cis*-dehydro-β-ionylidene acetic acid, mixed with EtOH, benzene and eosin (as photosensitiser) is irradiated while passing in $O_2$ for 1·5 h; evaporation *in vacuo* gives 3-methyl-5(1,4-epidioxy-2,6,6-trimethyl-2-cyclohexen-1-yl)-*cis*, *trans*-2,4-pentadienoic acid, m.p. 158–160° (decomp.). This is dissolved in 0·1N NaOH, heated in a water bath for 7·5 min., cooled, acidified and the solution extracted with $Et_2O$ and worked up to give 3-methyl-5-(1-hydroxy-4-oxo-2,6,6-trimethyl-2-cyclohexen-1-yl)-*cis*, *trans*-2,4-pentadienoic acid, m.p. 188–190°.

S. D. HUGGINS.

## Crops and Cropping

**Effect of seeding rates upon survival of genotypes in oat populations.** R. Reyes and K. J. Frey (*Iowa St. J. Sci.*, 1967, **41**, 433–445).—$F_3$ oat seeds derived from 250 crosses were sown at three rates (1, 3 and 5 bushels/acre) and observations made of the number of panicles (*P*) per plot, number of spikelets per *P*, grain yield, heading date and plant height for five generations ($F_3$–$F_7$). The number of *P* per plot decreased in each line of descent, the number of spikelets per *P* and the plant height increased while the other two features showed no consistent change. The reduction in *P* number was less at the 1 bushel/acre seeding rate but in general, varying the sowing rate had little effect in modifying the genotypic structure of oat populations. (23 references.) J. L. WALPOLE.

**Effect of temperature on the growth of wheat. I. 'Marquillo', 'Kenya Farmer' and two dwarf progeny.** D. T. Canvin and Yun-te Yao (*Can. J. Bot.*, 1967, **45**, 757–772).—Two wheat varieties and two dwarf crosses were grown under continuous illumination and in all combinations of temp. of 16°, 21°, 26° and 31° for 16 and 8 h periods in a 24 h cycle. Wheat dwarf No. 1 elongated and produced seed only when the air and soil temp. was kept at 26° or higher and if exposed to 21° or below for 8 h or more, only a 'grass-clump' type of growth was observed. Wheat dwarf No. 2 required a temp. of at least 21° to induce elongation but fertile tillers were produced at lower temp. and only at 16° was the 'grass-clump' habit produced. In contrast, 'Marquillo' and 'Kenya Farmer' varieties produced the tallest plants at the lowest temp. and max. shoot and seed yields were obtained under a heat regime corresponding to a temp. of only 17°–20°.

J. L. WALPOLE.

**Fertilising wheat in an intensive cereal rotation. II.** S. L. Chowdhury and S. S. Bains (*Fertil. News*, 1967, **12**, No. 6, 22–27).—An application of 100 kg per ha of N to irrigated wheat following a fodder crop of sorghum, gives the highest wheat yields. The level of fertilisation depends on capital available; lower levels of fertilisation, for example 30 kg per ha to cover large areas, can increase the yield appreciably. (18 references.) I. DICKINSON.

**Fertilising wheat in an intensive cereal rotation.** S. L. Chowdhury and S. S. Bains (*Fertil. News*, 1967, **12**, No. 4, 11–16).—The effects of treatment on plant characteristics, weed growth and lodging of crops is briefly discussed. (12 references.) I. DICKINSON.

**Competition between wheat and adventitious grasses; rôle of cultural rotations.** M. Sebillotte (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1374–1388).—Weed growth amongst wheat following colza was much greater than amongst wheat following maize; in the former case a negative linear relationship was found between grain-yield and the dry wt. of weed production; in the latter case no such relationship was found. Weeds in wheat following two years under grass were reduced to a very low level. The presence of ryegrass (*Lolium rigidum*) decreases the sucker- and ear-production of wheat in proportion to the dry wt. of the grass. The production of total dry matter (including weeds) in a plot at a given level of fertilisation was constant. P. S. ARUP.

**When and how to apply nitrogen for maize.** K. C. Sharma, P. C. Gupta and Virendra Singh (*Fertil. News*, 1967, **12**, No. 6, 29–31).—A review. Maize is mainly grown during the rainy season. To minimise leaching losses of N, and to maintain an adequate supply of it in the soil, it is desirable to split the dose of N for its proper utilisation. Various methods of application are discussed. The results are inconclusive; most workers report non-significant differences in grain yield between for instance, $NH_4Cl$ and $(NH_4)_2SO_4$. (24 references.) I. DICKINSON.

**Development of frost-resistant potatoes: I.** J. L. Blanco and J. L. Ubeda (*An. Inst. nac. Invest. agron.*, 1967, **15**, 571–618).—Preliminary studies of the genetics of frost-resistance and of appropriate test procedures are reported in detail. $F_1$ and back-crosses (1st to 3rd) of wild species (*Solanum acaule*, *S. demissum*, *S. toralapanum*, *S. simplicifolium* and *S. andigenum*) with *S. tuberosum* as recurrent parent were intercrossed and back-crossed to *S. tuberosum*. Populations were subjected to temp. between −1° and −5° for periods of 1–8 h. The results suggest the feasibility of developing potato varieties of normal productivity but resistant to −5° and a scheme, based on continuous back-crossing from hybrids (*S. acaule* × *S. tuberosum*) to *S. tuberosum* with selection in each generation for resistance to −5° for 4 h is proposed. (16 references.) E. C. APLING.

**Frost injury systemic in sweet potatoes.** B. D. Ezell and M. S. Wilcox (*J. agric. Fd Chem.*, 1967, **15**, 729–735).—Experimental injury by frosting with solid $CO_2$, flaming, or spraying with herbicides, restricted to the aerial tissues of the plants appeared to cause systemic injury to the plants as a whole. This was evidenced by decreased carotenoid synthesis in the roots during subsequent storage. (24 references.) P. S. ARUP.

**Growing table beets.** V. R. Boswell (*Leafl., U.S. Dep. Agric.*, 1967, No. 360, 4 pp).—Soils and fertilisers, varieties, planting and culture are discussed. E. G. BRICKELL.

**Varieties of sugar-beet.** Anon (*Fmrs' Leafl. natn. Inst. agric. Bot.*, 1967, No. 5, 7 pp).—Recommended varieties, with descriptive notes, are listed. E. G. BRICKELL.

**Pasture growth in relation to defoliation.** L. R. Humphreys (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 40).—Growth and development of green panic and of buffel grass in relation to variations in frequency, intensity, and stage of defoliation, are reported. Intensity of defoliation had effects on leaf area/leaf wt. ratio, leaf wt. ratio, shoot and leaf differentiation, max. size of leaves after defoliation, and leaf senescence. Growth was more closely associated with residual leaf area than with plant carbohydrate status.
E. G. BRICKELL.

**Nitrogen-manuring and utilisation of grassland.** D. Oostendorp and T. Boxem (*Landbouwvoorlichting*, 1967, **24**, 204–206, 211).—In trials over 4 years, the utilisation by grass of applied N was 51–62% on clay soil, 34–51% on sandy soil, and 31–45% on peat soil. For optimum utilisation, N should be applied at ≤ 60 kg/ha during spring, but in decreasing amounts as the season progresses. The manuring improved the botanical quality of the crop, even under constant mowing. P. S. ARUP.

**Growth of an annual pasture on virgin land in S. W. Australia including effects of stocking rate and nitrogen fertiliser.** E. A. N. Greenwood, H. L. Davies and E. R. Watson (*Aust. J. agric. Res.*, 1967, **18**, 447–459).—A pasture of *Bromus mollis* L. and *Trifolium subterraneum* L. sown on virgin land, was grazed by sheep for 4 years. Three rates of $(NH_4)_2SO_4$ were factorially combined with two stocking rates. Stocking rates of eight and twelve sheep/ha had similar effects on growth rate of the sward and influenced botanical composition only in the 2nd and 3rd year of grazing. $(NH_4)_2SO_4$ increased the proportion of *B. mollis* and volunteer grasses, and reduced *T. subterraneum* and dicotyledonous volunteers. N fertiliser can increase the rate of production of dry matter of the sward provided that pasture re-establishment is good.
E. G. BRICKELL.

**Changes in the lipids of lucerne prior to and after dehydration.** J. van der Veen and H. S. Olcott (*J. agric. Fd Chem.*, 1967, **15**, 682–684).—Lucerne lost 75% of its β-carotene in the first 24 h after harvest when kept at room temp. The remainder, in the freeze dried product could be protected during storage at 37° for 46 days by the addition of 0·0125 %of Ethoxyquin (**I**), 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline. Max. protection was obtained when **I** was applied and the lucerne dehydrated immediately after harvesting. Changes occurring in the amount and composition of the $CHCl_3$ -extractable lipids during storage at 46°, were not affected by the addition of **I**. (14 references.)
P. S. ARUP.

**Varietal resistance to clover rot in white clover.** D. T. A. Aldrich and D. A. Doling (*Nature, Lond.*, 1967, **214**, 946–947).—A brief survey of field trials and crops reveals differences in resistance to clover rot of white clover varieties, e.g. 'Aberystwyth S. 100' and 'Grasslands Huia' were seriously affected whilst 'Pajbjerg Milka' was highly resistant. Distribution of the disease, which causes loss of vigour and even plant mortality, requires further investigation. W. J. BAKER.

**Response of jute to nitrogen as ammonium sulphate in cultivators' fields.** K. L. Gurnani and S. Pathak (*Fertil. News*, 1967, **12**, No. 5, 27–30).—Application of N as $(NH_4)_2SO_4$ at 22·4 kg/ha for *C. olitorius* and 44·8 kg/ha on *C. capsularis* gave an average increased yield of 17·1% and 23% respectively over the control. The economics of this increase are discussed. I. DICKINSON.

**Nutrition of glasshouse and other horticultural crops.** G. W. Winsor (*Fertil. Soc.*, 1968, 32 pp).—A review with 86 references.
P.P.R.

**Young apple orchards.** Anon (*Fmrs' Bull., U.S. Dep. Agric.*, 1967, No. 1897, 22 pp).—Selection of sites, planning and planting, soil management, pruning, and spraying are discussed.
E. G. BRICKELL.

**Virus-induced wood pitting in the root systems of apple seedlings and its effects on tree vigour.** M. F. Welsh and J. May (*Can. J. Pl. Sci.*, 1967, **47**, 51–59).—Wood pitting (*WP*) symptoms induced in open-pollinated Hyslop and Columbia crabapple seedlings, by inoculation with stem pitting virus, were as severe in the root systems as in the trunks and branches. Severe *WP* also occurred in open-pollinated Delicious seedlings used as rootstocks for Virginia crab trees—usually on both the rootstock and the stem, but in seven out of 113 tests the rootstock only was affected; in nine other cases the rootstock symptoms were more severe than those on the trunk. *WP* of the root systems was as effective in reducing tree vigour as pitting of the trunks and branches.
J. L. WALPOLE.

**Effects of excessive moisture on peach orchards.** M. Deffontaines (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 957–961).—Observations on the Tournon plateaus confirmed the prevalence of trees of stunted growth and low vitality on soils that became water-logged due to heavy rainfall and close soil structure. Mortality rates were higher for 4 to 5-year-old trees than for older trees. P. S. ARUP.

**Nitrogen nutrition of the peach tree: I. Seasonal changes in nitrogenous constituents in mature trees. II. Storage and mobilisation of nitrogen in young trees. III. Metabolism and translocation of *l*-[*guanido*-$^{14}$C) arginine hydrochloride and *l*-(U-$^{14}$C) asparagine in young dormant trees.** B. K. Taylor (and L. H. May, II, III) (*Aust. J. Biol. Sci.*, 1967, **20**, 379–387; 389–411; 413–418).—I. Peak concn. of all the nitrogenous constituents in tissues of 25-year old peach trees were found before growth commenced, and after growth ceased. This pattern of change was not influenced by the amount of N supplied, but the concn. of constituents was usually proportional to N supply.

II. Young peach trees accumulated N in proportion to supply during the first year. Tree growth in early spring was significantly correlated with the level of storage N but after November it was markedly dependent upon external N supply. (20 references.)

III. Storage N in dormant trees consisted mainly of sol. org. N of which free arginine was the principal constituent. Results from application of *l*-(*guanido*-$^{14}$C) arginine hydrochloride and *l*-(U-$^{14}$C) asparagine to dormant 2-year old trees indicate that both compounds are metabolised by the trees. Small amounts of the applied $^{14}$C were translocated both upwards and downwards from the point of application. S. A. BROOKS.

**Spraying of orange trees with nutrients during flowering.** A. Bar Akiva and M. Kaplan (*Fruits*, 1967, **22**, 153–154).—No harmful effects were observed as the result of spraying with urea, ZnO, or $KNO_3$. P. S. ARUP.

**Influence of density and disposition of planting on the production from vineyards.** L. Hidalgo and M. R. Candela (*Inst. nac. Invest. agron.*, 1966, 68 pp).—Results of a study of the effects of planting density (*PD*) and distance between lines, on production from an experimental plantation of the variety 'Tempranillo', are reported in detail. Total production of grapes from a given area progressively increased, but production per vine, vegetative potential, and vigour of the vinestock, decreased with *PD*. At a given *PD*, grape production and vigour retention were best with equal spacing of the vines, but wider spacing of lines is necessary for economy in the use of labour and of mechanisation. Most economical results resulted from a vinestock density of 2,500 per ha, with a separation between lines of from 2·5 to 3·0 m. (41 references.) E. C. APLING.

**Factors limiting crop production. VII. Hops.** R. A. Neve (*Span*, 1967, **10**, 92–95).—In addition to a good supply of moisture, hops require a high level of nutrition which can be met by simple inorg. fertilisers; apart from Zn lack, deficiency diseases are not common. The major pests, aphids (*Phorodon humuli*) and the red spider mite (*Tetranychus urticae*), are normally controllable with organophosphorus insecticides; downy mildew and powdery mildew are controllable with standard fungicides but *Verticillium* wilt and root rot can be adequately dealt with only by breeding resistant varieties of hops. The major criterion in hop breeding for quality is the α-acid content of the hop resins. The best varieties should combine this with wilt-resistance. The economic factors involved in hop growing and production are discussed.
J. L. WALPOLE.

**Effect of artificial illumination on growth of melon plant.** C. Costes and Y. Milhet (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1303–1314).—Growth rates increased with intensity of illumination and length of photo-period. Max. rates were attained with continuous illumination at 10,000 lux. Continuous illumination had no effect on the reproductive capacity of the mature plants.
P. S. ARUP.

**Effect on tomato yields of spacing rows and spacing plants within the rows.** M. Vergniaud (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1158–1166).—In experiments with the same no. of plants per ha, greater yields were obtained by spacing the rows 1·2 m apart than by spacing them 1·5 or 1·8 m apart. Probable explanations for these results are considered. P. S. ARUP.

**Use of protein-based foams to protect plants against frost.** D. Siminovitch, W. L. Ball, R. Desjardins and D. S. Gamble (*Can. J. Pl. Sci.*, 1967, **47**, 11–17).—Tomato and coleus plants can be protected from frost damage by spraying with a foam prepared from a commercial hydrolsyed protein concentrate containing a stabiliser and 1% gelatin. In small scale trials protection was obtained against temp. of 20°F; the foam was non-phytotoxic and easily washed off the plants. Further research is needed to increase the stability so that the foam will last for at least 12 h.
J. L. WALPOLE.

**Foliar diagnosis of crop value and chemical composition of pea seed.** N. P. Sherstov and N. K. Boldyrev (*Dokl. Akad. Nauk SSSR.*, 1967, **174**, 489–491).—The authors report their investigation of fertilising conditions in relation to size of crop and quality of peas. By means of empirical analysis of the leaves, critical level of feeding during 'flat formation' of the pea was 4·34% N, 0·29% P and 2·17% K. Optimal ratio between elements in the leaves is expressed as N% = 15P% = 2K%. If % content of N in the leaf during this phase achieves 'critical level' and ratio of N content to P content is more than 15, the pea plant does not need N but in experiments P was required. If this ratio is less than 15 the plant has a surplus of P and requires N. With normal growth and achievement of critical level of feeding and optimal ratio between elements in the leaves, crops were obtained with 26–30 centner of peas per hectare. Assessment of correct method of 'feeding' the plants resulted in accurate prediction of crop yield and quality 20–25 days before harvest with 87–95% accuracy. A.L.B.

**Morphological properties of fruit and quality of seeds in some varieties of Paprika (*Capsicum annuum* L. *syn. C. mexicanum* Hazenb.) from aspect of seed production.** M. Čirkova-Dordievska (*Bull. scient. Cons. Acads RSF Yougosl.*, A, 1967, **12**, 84–85).—Botanical observations of four varieties of paprika have led to a proposal for an improved system for the grouping of the fruits and seeds. Details are given of deviations from normal types as a guide to systematic selective breeding. (In English.)
P. S. ARUP.

**Three-year experiments in fertilisation of sweet pepper.** V. Morani, G. Gattorta and G. de Philippis (*Annali Staz. chim.-agr. sper., Roma*, 1965, **III**, No. 233, 18 pp).—Results reported show the need for three distributions of N during the 2 months subsequent to transplanting, to replace leaching losses; additional advantages are obtained from org. manuring and/or application of superphosphate to a soil level of 150 ppm of $P_2O_5$. No advantage resulted from additions of nicotinamide to foliar manurial treatments, and in hydroponic cultivation the optimal concn. of N is close to 100 ppm. (17 references.) E. C. APLING.

**Better yields of garlic through potassic fertilisers.** J. R. Singh and Janardan Tiwari (*Fertil. News*, 1967, **12**, No. 7, 13–15).—Field trials with five levels of potash (0, 50, 75, 100 and 125 kg/ha) were carried out; the growth characteristics of garlic at 52, 104 and 156 days after planting were observed. Significant improvement in height, dry wt. of stem and bulb, fresh wt. of bulb and yield of bulb per ha were obtained with 100 kg $K_2O$ and for dry wt. of stem 125 kg dose proved best. I. DICKINSON.

**Field drainage of sugarcane.** Anon (*Bull. Exp. Stn S. Afr. Sug. Ass.*, 1968, No. 18, 19 pp).—Discusses farm management and various types of drainage. P.P.R.

**Study of some chemical quality indices of flue-cured tobacco with reference to grades and leaf position on stalk.** B. V. Ramakrishnayya, N. C. Gopalachari and K. S. N. Murty (*Indian J. appl. Chem.*, 1966, **29**, 170–180).—Chemical features, such as total N, protein-N, nicotine, sugars (total and reducing), polyphenols, starch, petroleum ether extracts, total org. acids, water sol. acids, ash and pH were studied. A comparative study of chemical data for Indian, American and Canadian leaf is presented and the need for improvement of nicotine content in Indian leaf is pointed out. (24 references.) I. DICKINSON.

**Semi-wild oil palm and its industry in Africa.** A. C. Zeven (*Versl. landbouwk. Onderz. Ned.*, 1967, No. 689, 178 pp).—A survey comprising botanical information, habitats, distributions and classes of palm groves, methods of exploitation and yields, diseases and pests, deterioration and regression of palm groves, efforts at improvement, and economic aspects. (267 references.)
P. S. ARUP.

**Fuel-breaks for forest fire control.** L. R. Green and J. R. Bentley (*Span*, 1967, **10**, 96–101).—Fuel-breaks, strips of low vegetation generally 200 ft wide, serve to break up large expanses of inflammable forest and reduce the risk of fires spreading over very large areas. The siting, construction and maintenance of fuel breaks are described; although they are expensive they offer many other advantages including the provision of forage for livestock. J. L. WALPOLE.

**Physiological variation of Scotch pine seedlings in relation to provenance and nitrogen.** G. F. Dykstra and G. E. Gatherum (*Iowa St. J. Sci.*, 1967, **41**, 487–502).—Photosynthesis, respiration, growth and distribution of assimilated N of 9 months old Scotch pine seedlings were found to show marked variation in relation to four provenances ranging from 42° to 60° north latitude in eastern Europe and to five sand culture N levels from 25 to 500 ppm. The variations depended not only on the source of the seed but also on its latitude and indicate that differences exist in the physiological processes underlying the factors of vigour and quality in Scotch pine. (24 references.) J. L. WALPOLE.

## Pest Control

**Integrated control in protected cultivation.** N. W. Hussey (*Chemy Ind.*, 1968, 498–502).—Integrated control is based chiefly on hygienic and biological techniques, sometimes supplemented by limited chemical measures. The theory of and practical requirements for successful biological control (mainly by introducing predators and/or parasites and hyperparasites) are explained fully by reference to the pest-complex on cucumbers and on mushrooms grown in protected environments. Trials for control of spider mite by *Phytoseiulus* and of phorids and sciarids by parasitic eelworms, as well as attempts to control *Aphis gossypii* by integrated biological-chemical methods are reported. The economics of an integrated control programme are briefly considered.
W. J. BAKER.

**Structures and pesticidal activities of derivatives of dinitrophenols. IV. Preparation of certain 2-(α-branched alkyl)-4,6-dinitro- and 4-(α-branched alkyl)-2,6-dinitro-phenols. V. Reactions of certain dinitro-aryl thiocarbamates with potassium hydroxide in methanol and with various nucleophiles.** M. Pianka and J. D. Edwards (*J. chem. Soc.*, C, 1967, 2281–2290; 2290–2295).—IV. Some 180 compounds are reviewed; twenty-one 2-(α-branched alkyl) phenols and thirty-one 4-(α-branched alkyl) phenols were synthesised by condensation in ether of a 2- or 4-acyl-phenol or the more sol. -anisole with alkyl-MgBr, dehydration to the alkenyl deriv., hydrogenation and where required, demethylation of the anisole. These 2- and 4-alkyl phenols were converted to title compounds for testing against the causal agents of powdery mildew of apple *Podosphaera leucotricha*, of barley *Erysiphe graminis* Mérat, and of cucumber *E. cichoracearum* Mérat. (30 references.)

V. Describes colour reactions of certain thiocarbamates and iminothiocarbonates. None of these compounds had significant pesticidal activity. (27 references.) J. I. M. JONES.

**Thiadiazoles. II. Preparation of certain azo dyes as potential pesticides.** S. Giri and H. Singh (*Indian J. appl. Chem.*, 1967, **30**, 14–16).—Several 2-amino-5-aryl-1,3,4-thiadiazoles have been diazotised and coupled with the requisite phenols to yield the corresponding azo dyes, with a view to studying their pesticidal properties. The m.p., yields, colours, formulae and % N of the compounds are presented; their pesticidal properties will be published at a later date. I. DICKINSON.

**Insecticidal derivatives of 3-nitro-4-methyl-benzenesulphonyl chloride and chloraceto-*p*-toluidine.** K. C. Srivastava (*Chem. Age India*, 1967, **18**, 561–562).—3-Nitro-4-methylbenzenesulphonyl chloride (**I**) was condensed with *p*-nitrophenol (**II**), 2-hydroxyquinoline (**III**) and *p*-nitraniline to produce sulphonates and sulphones, and with diethyl sodium phosphite to yield a disulphone. Chloraceto-*p*-toluidine was condensed with $Et_3$- and $Pr^i_3$- phosphites. Condensation of **I** with **II** or **III** was carried out to obtain compounds analogous to Ovotran, a known miticide.
K. GRAUPNER.

**Cyclic organophosphorus compounds. VI. Hydrolysis of some 1,3,2-dioxaphospholans and 1,3,2-dioxaphosphorinans in aqueous alkali–dioxan solution.** R. S. Edmundson and A. J. Lambie (*J. chem. Soc.*, B, 1967, 577–581).—The rate of hydrolysis of 1,3,2-dioxaphosphorinans substituted on the C atoms of the ring is measured at 25° in 50% (vol./vol.) aq. NaOH–dioxan by conductimetric methods and for *p*-nitrophenyl esters by measurement of the extinction at 407 nm. Replacement of H by substituents at 4 and 5 in the 2-thiono-1,3,2,-dioxaphospholan ring stabilises it to hydrolysis by a factor of 2 in the rate. Some preparative hydrolyses are carried out to identify and confirm the products. The work is part of an attempt to explain variations in anticholinesterase activity. (21 references.) E. J. H. BIRCH.

**Enol phosphates.** M. Schuler (*Chimia*, 1967, **21**, 342–349).—After a review of the chemistry of enol phosphate insecticides and their prep. by the Perkow reaction, the production of new enol phosphates by addition of trialkyl phosphites to α-trichloroamides in which N is joined to COO-, CO- or $SO_2$- or forms part of a herero-ring is reported. Their properties, alkaline and acid hydrolysis and their contact and systemic insecticidal activities are tabulated and structure–activity relations are discussed. A correlation between insecticidal effectiveness and hydrolysis rate has been found. (14 references.) M. SULZBACHER.

**Possibilities of organotin compounds as pesticides with wide spectrum of activity.** S. Byrdy *et al.* (*Meded. Rijk-Fac. Landbouw. Wet.*, **30**, 876–888; *Landbouwdocumentatie*, 1967, **23**, 553).—Some triphenyltin fungicides of the fentin type have an anti-feeding effect on the larvae of the Colorado beetle; this effect reduces egg-production by the mature insects. P. S. ARUP.

**Trifluoromethyl-benzimidazoles—a new family of acaricides.** D. T. Saggers and M. L. Clark (*Nature, Lond.*, 1967, **215**, 275–276).—Promising acaricidal properties of 5,6-dichloro-1-phenoxy-carbonyl-2-trifluoromethyl benzimidazole (**I**), m.p. 103°, are reported; herbicidal action and mammalian toxicity have been decreased by suitable N-substitution in the parent benzimidazole. Partition ratio of **I** between cyclohexane and water is 320 : 1; it has moderate acute oral and low dermal toxicity to domestic animals, and is active against eggs and adults of red spider and larvae of the cabbage white butterfly. It is inactive against bean aphid, flour beetle, housefly, brown ear-tick, *Aedes aegypti*, German cockroach and mealworm. One application of 0·03% **I** to French bean foliage effectively controls adult *Tetranychus telarius* and their eggs for > 24 days; and **I** is also active against dimethoate-, phenkapton- and chlorobenzilate-resistant strains of *T. urticae*. Field trials confirm these results; hazard to worker bees is low. **I** is inactive against mildews. W. J. BAKER.

**Antifungal activity of isothiocyanates and related compounds. I. Naturally occurring isothiocyanates and their analogues. II. Mono-nuclear aromatic isothiocyanates.** L. Drobnica, M. Zemanova, P. Nemec, P. Kristián, A. Antos, (**I.** A. Štullevova, V. Knoppova, P. Nemec jun.) and (**II.** A. Hulka) (*Appl. Microbiol.*, 1967, **15**, 701–709; 710–717).—I. The antifungal activity of 11 natural isothiocyanates (**I**) and 27 synthesised analogues on *Aspergillus niger*, *Penicillium cyclopium* and *Rhizopus oryzae* as well as on additional 13 saprophitic and parasitic fungi is studied, that of β-phenyl ethyl-**I** being specially marked; these compounds have not previously been described. In the benzyl-**I** compounds, a correlation is noted which is inversely proportional between the $ED_{100}$ values for *A. niger* and *R. oryzae* and the corresponding molar solubilities in water. There was no relationship between antifungal and chemical reactivity. (26 references.)

II. A large number of further subsitituted deriv. of phenyl-**I** was studied; most displayed an equal activity against the three fungi, unlike analogues of natural benzyl- and β-phenylethyl-**I** with their characteristic low activity agaisnt *R. oryzae*. Differences occurred in the type of activity when the -NCS group was bound to the aromatic moiety as compared with those compounds in which this group is bound to the aliphatic radical or indirectly to the aromatic moiety. The results confirm the negative influence of ionised substituents on the aromatic moiety as well as of substituents which result in extreme reactivity of the -NCS group. (13 references.) C.V.

**Cyclic diquaternary salts of 1,10-phenanthroline as one-electron transfer agents related to bipyridylium herbicides.** L. A. Summers (*Nature, Lond.*, 1967, **215**, 1410–1411).—The two cryst. salts prepared and examined are 5,6-dihydropyrazino (1,2,3,4-*l,m,n,*)-1,10-phenanthrolinium dibromide (**I**) and 5*H*-6,7-dihydro (1,4)-diazepino (1,2,3,4-*l,m,n*)-1,10-phenanthrolinium dibromide (**II**); both are reduced to radical cations more easily than diquat (*D*) and paraquat (*P*), the one-electron transfer being largely reversed by atm. oxidation. Both **I** and **II**, applied at 4–8 lb per acre, have low herbicidal activity but are much inferior to *D*. Toxic action of *D* and *P* type is thus not confined to diquaternary salts of bipyridyls, the herbicidal action of which seems to be associated with reversible one-electron transfer (Black and Myers, *Weeds*, 1966, **14**, 331). W. J. BAKER.

**Insecticide mode of action. Effect of dieldrin on ion movement in nervous system of *Periplaneta americana* and *Blatella germanica* cockroaches.** M. Hayashi and F. Matsumura (*J. agric. Fd Chem.*, 1967, **15**, 622–627).—The first effect of dieldrin on the isolated nervous tissues of the insects was to stimulate the uptake of $Na^+$ from saline solutions. After this stage, the $Na^+$ accumulated in the tissues whilst the uptake of K was inhibited. Inhibition of Ca transport was also indicated, but this was not considered to be the main cause of dieldrin poisoning. (11 references.) P. S. ARUP.

**Effect of rearing and breeding density on maturation rate of brown locust, *Locustana pardalina* (Walker).** L. G. Venter and R. J. Mansfield (*S. Afr. J. agric. Sci.*, 1967, **9**, 857–862).—Increases in hopper rearing and adult breeding population densities both retarded and synchronised the incidence of sexual maturation. P. S. ARUP.

**Rearing of [the bollworm] *Heliothis armigera* Hübn and [the leaf cutter] *Prodenia Litura* F. on an artificial diet.** J. Bot (*S. Afr. J. agric. Sci.*, 1966, **9**, 535–538).—The composition is given of an aseptic and very satisfactory diet. The ingredients, mixed with water, comprise wheat germ, yeast, casein, agar, ascorbic acid, cholesterol, choline chloride, inositol, cysteine hydrochloride, and Nipagin M (*p*-hydroxybenzoate). The laboratory-reared pests are suitable for pesticide studies. (11 references.) P. S. ARUP.

**Systematic approach to preparation and identification of glucuronic conjugates.** J. B. Knaak, J. M. Eldridge and L. J. Sullivan (*J. agric. Fd Chem.*, 1967, **15**, 605–609).—The enzymic synthesis of the compounds (**I**) from components, $^{14}C$-labelled in the aglycone or the glucuronic moiety was accomplished by the method of Knaak *et al.* (cf. *ibid.*, 1965, **13**, 537). After clean-up on a column of diethylamino-ethylcellulose and conversion to the acid form on a cation-exchange resin, the acids were quant. acetylated with $Ac_2O$ and methane sulphonic acid and methylated with diazomethane for GLC with $^{14}C$ detection. The separation of **I** is of significance in investigations on the metabolism of e.g. carbaryl. P. S. ARUP.

**Wood tannins. Isolation and significance in host resistance to *Verticillium* wilt disease.** T. C. Somers and A. F. Harrison (*Aust. J. biol. Sci.*, 1967, **20**, 475–479).—The phenolic substances extracted with MeOH and 50% MeOH from diseased apricot wood (purified by pptn. with Pb acetate, followed by liberation with methanolic HCl) were separated in aq. $COMe_2$ solution on a column of Sephadex G-25 (fine). A main tannin fraction of mol. wt. > 2000 inhibited the spore-germination and hyphal growth of *Verticillium alboatrum* to a greater extent than did an intermediate fraction of lower average mol. wt. A monomeric fraction inhibited germination very slightly. P. S. ARUP.

**Metabolism of 2-chloro-4,5-dimethylphenyl *N*-methylcarbamate in bean plants.** A. R. Friedman and A. J. Lemin (*J. agric. Fd Chem.*, 1967, **15**, 642–647).—The synthesis of this insecticide, Banol (**I**), with 4-methyl-$^{14}C$ is described. **I** was readily absorbed from culture solutions and almost entirely metabolised into a water-sol. compound with the carbamate side-chain intact and the ring modified by introduction of an OH group functioning as a link with glucose. The phenolic character of the O introduced into the ring was surmised from mass-spectral evidence. P. S. ARUP.

**Plant-parasite relationship between root-knot nematode *Meloidogyne javanica* and some resistant and susceptible plants.** H. Koen (*S. Afr. J. agric. Sci.*, 1967, **9**, 981–989).—No difference was found between the degree of attraction of the larvae to the roots of susceptible plants (beans and tomatoes) and those of resistant plants (*Eragrostis curvula*, asparagus, *Crotalaria spectabilis*, and *Tagetes* spp.). Root extracts (in water drained from the roots) had no effect on the eggs or the larvae. Extracts from the macerated roots inactivated the larvae during 96 h, the effect of extracts from resistant plants being greater than those from susceptible plants; an inhibitory substance is probably formed by decomposition in all the extracts, but more readily in the former than in the latter. (22 references.) P. S. ARUP.

**Significance of atrazine dealkylation in root and shoot of pea plants.** R. H. Shimabukuro (*J. agric. Fd Chem.*, 1967, **15**, 557–562).—Atrazine (**I**) is shown to be de-ethylated to the less toxic 2-chloro-4-amino-6-isopropylamino-*s*-triazine in both the shoots and roots of pea plants. This probably accounts for the ability of pea plants to tolerate **I** in low concn. (19 references.) P. S. ARUP.

**Responses of processing peas to applications of 4-(2-methyl-4-chlorophenoxy)butyric acid.** H. J. Vostral (*Diss. Abstr.*, B, 1967, **28**, 417–418).—The shelled pea yields of five processing pea varieties differed when treated with 0·5, 1·0 and 2·0 lb/A of the herbicide 4-(MCPB) (Na salt) as a foliar application during a 3-year study. The test material was treated at different growth stages. Early applications caused no yield reductions. Low shelled pea yields resulted when the plants were treated during a

period commencing two nodes prior to initial bloom and continuing through the early bloom stage. Yield decreases also occurred following treatments made during pod swelling. The yield reductions were associated with high tenderometer readings, reduced pod numbers and reduced numbers of peas per plant.
F. C. SUTTON.

**Uptake, distribution, and metabolism of monuron and diuron by several plants.** J. W. Smith and T. J. Sheets (*J. agric. Fd Chem.*, 1967, **15**, 577–581).—No difference was observed between soya-bean (*SB*) and cotton plants (*CP*) as regards the uptake and translocation of $^{14}$C-diuron (**I**), supplied in culture solutions. The susceptibility of *SB* is probably due to its conversion of **I** into 1-(3,4-dichlorophenyl)-3-methylurea (also phytotoxic), and the tolerance of *CP* to the formation of 1-(3,4-dichlorophenyl)urea (non-phytotoxic). Residual **I** was identified in *SB* leaves but not in *CP* leaves. In similar experiments with monuron, 1-(*p*-chlorophenyl)-3-methyl urea occurred in *SB* leaves and 1-(*p*-chlorophenyl)urea and *p*-chloroaniline in *CP*. In comparative experiments with oats, soya-bean and maize, the differences in sensitivity were probably caused mostly by differential uptake. (15 references.)
P. S. ARUP.

**Review of metabolism and decomposition of diquat and paraquat.** H. H. Funderburk, jun. and G. A. Bozarth (*J. agric. Fd Chem.* 1967, **15**, 563–567).—A review. (46 references.) P. S. ARUP.

**Influence of physicochemical properties on biodegradability of phenylcarbamate herbicides.** P. C. Kearney (*J. agric. Fd Chem.*, 1967, **15**, 568–571).—The relative rates of hydrolysis of 12 of these herbicides by an enzyme obtained from *Pseudomonas striata* are determined by the method previously described by Kearney (*ibid.*, 1965, **13**, 561). Inductive effects caused by meta substitution of electron-withdrawing groups and steric effects caused by altering the size of the alcohol group resulted in significant changes in reaction rate; a positive correlation between relative acidity and hydrolysis rate was found. P. S. ARUP.

**Metabolism of 2-methyl-2-(methylthio)-propionaldehyde-O-(methylcarbamoyl)oxime in plant and insect.** R. L. Metcalf, T. R. Fokuto, C. Collins, K. Borck, J. Burk, H. T. Reynolds and M. F. Osman (*J. agric. Fd Chem.*, 1966, **14**, 579–584).—The insecticide (Temik) was shown by means of radiotracer and chromatographic techniques to be metabolised along similar lines both in the cotton plant and the housefly. Temik is at first readily and completely oxidised to the sulphoxide which shows greater cholinesterase activity than does Temik itself, and is responsible for the persistent systemic activity achieved by use of the latter. Oxidation to the sulphone is very slow; the sulphoxide oxime is the main degradation product in the plant. P. S. ARUP.

**Food-chain toxicity of systemic acaricides to predaceous mites.** R. J. McClanahan (*Nature, Lond.*, 1967, **215**, 1001).—The differential effect of root drenches of acaricides (35–225 μg per plant) on the two-spotted spider mite and the predator *Phytoseiulus persimilis* during 24 h was compared with the 48 h toxicity of similarly treated cucumber-leaves to *Tetranychus urticae* alone. Results reveal a food-chain toxicity when predators fed on prey mites which were feeding on toxic plant-juices. The order of decreasing toxicity was dimethoate, thionazin, phorate, and Temik, the last-named (not an organo-P compound) consistently favouring *P. persimilis*. Because of this established absence of complete ecological selectivity, materials must be evaluated to find those that do not show food-chain toxicity to the predators.
W. J. BAKER.

**Metabolism of 2-methyl-2-(methylthio)-propionaldehyde-*O*-(methylcarbamoyl)oxime (Temik, UC-21149) in insects.** D. L. Bull, D. A. Lindquist and J. R. Coppedge (*J. agric. Fd Chem.*, 1967, **15**, 610–616).—With the use of the $^{14}$C- and $^{35}$S- labelled compounds Temik was shown to be absorbed rapidly by adult boll-weevils *Anthonomus grandis* Boheman, but very slowly by third instar tobacco budworms *Heliothis virescens* F. The main changes in both insects were oxidation to the sulphoxide deriv. followed by partial further oxidation to the sulphone. Other very limited changes were indicated. P. S. ARUP.

**Enzymic degradation of 2-chloro-4,5-dimethylphenyl-*N*-methylcarbamate by fat bodies of *Blaberus giganteus*.** E. G. Gemrich (*J. agric. Fd Chem.*, 1967, **15**, 617–621).—Enzymic prep of the fat bodies acting on this insecticide produced $CHCl_3$-sol. degradation products of which at least three could be separated by TLC and one identified as 2-chloro-4,5-dimethylphenyl-*N*-hydroxymethyl carbamate. (21 references.) P. S. ARUP.

**Dursban insecticide.** [A] **Uptake and translocation of $^{36}$Cl-labelled *O,O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate and of the $^{14}$C-ring-labelled compound, by beans and maize.** [B] **Metabolism of $^{36}$Cl-labelled *O,O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate in rats.** G. N. Smith, B. S. Watson and F. S. Fischer (*J. agric. Fd Chem.*, 1967, **15**, 127–131, 132–138).—[A] Transmission of radioactivity into the plant from the soil was 1–2% only, mostly as breakdown products, e.g., 3,5,6-trichloro-2-pyridinol. In foliar applications most of the radioactivity was lost by volatilisation.

[B] Single doses were excreted largely in the urine (~ 90%) and the faeces (~ 10%), mostly as 3,5,6-trichloro-2-pyridyl phosphate (75–80%), trichloropyridinol (15–20%), with traces of Dursban. Small amounts of Dursban accumulated in the fat from which it was slowly liberated. P. S. ARUP.

**Dechlorination of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane by *Aerobacter aerogenes*. I. Metabolic products.** G. Wedemeyer (*Appl. Microbiol.*, 1967, **15**, 569–574).—Whole cells or cell free extracts catalyse the degradation of DDT *in vitro* to at least seven metabolites. These are studied and the use of metabolic inhibitors are discussed. (17 references.) C.V.

[A] **Fate of trifluralin [herbicide] in soils and plants.** [B] **Metabolism of carbon-14 trifluralin in carrots.** R. J. Herberg, S. J. Parka, T. Golab, J. B. Tepe and [A] C. van der Schans, G. W. Probst and F. J. Holzer (*J. agric. Fd Chem.*, 1967, **15**, 592–599; 638–641).—[A] The concn. of $^{14}$C-labelled trifluralin (**I**) in a field soil decreased very rapidly by ~ 80–90% during the first 40 days, then very slowly for over a year. Aerobic degradation commenced with dealkylation, followed by progressive reduction; in anaerobic degradation the dealkylation was preceded by a reduction step. The main pathway of ultimate degradation appears to be through a mixture of unidentified polar compounds. Tolerant crops did not readily absorb **I**, but a metabolite of **I**, not found in soil, occurred in carrots.

[B] Unaltered **I** was the major source of radioactivity in carrots grown in soil that had been treated with 0·75 lb/acre of **I**. The chief metabolite found (cf. [A]) was α,α,α-trifluoro-2,6-dinitro-*N*-(n-propyl)-*p*-toluidine. Most of the **I** and its metabolite occurred in the carrot peel. (14 references.) P. S. ARUP.

**Degradation of carbamate herbicides in soil.** D. D. Kaufman (*J. agric. Fd Chem.*, 1967, **15**, 582–591).—A review. (92 references.)
P. S. ARUP.

**Aldrin absorption by soils and clays.** B. Yaron, A. R. Swcboda and G. W. Thomas (*J. agric. Fd Chem.*, 1967, **15**, 671–675).—The absorption of $^{14}$C-labelled aldrin (**I**) dissolved (in concn. $\geq$ 0·2 μg/l) in aq. electrolyte solutions to simulate field conditions was studied by means of batch and column experiments. No difference was found between the absorptive capacities of kaolinite and montmorillonite clays. Absorption was promoted by presence of org. matter, and was dependent on the mechanical soil structure. Most of **I** deposited on a clay soil was too firmly held to be removed by aq. leaching; **I** could, however, be entirely removed with $COMe_2$. (13 references.) P. S. ARUP.

**Degradation of some persistent chlorinated hydrocarbon insecticides applied to different soil types.** I. H. Wiese and N. C. J. Basson (*S. Afr. J. agric. Sci.*, 1967, **9**, 945–969).—In micro-plot experiments with three soil types the rapidity of degradation of the insecticides was considerably greater than has been generally reported. Relative persistency ratings were in the (descending) order DDT, dieldrin, chlordanes, aldrin, and heptachlor. Considerable adsorption occurred, especially in clay soil, immediately after incorporation. Persistence was greatest in sandy soil and loam. Max. downward movement by leaching occurred in the light soils, but dieldrin and aldrin showed considerable downward movement even in clay soil. Disproportionately high retention of heavy dosages was observed, but no definite conclusions were drawn. (54 references.) P. S. ARUP.

**Chemistry and biochemistry of pyrethrins.** J. B. Moore (*Pyrethrum Post*, 1966, **8**, No. 4, 27–31).—A comprehensive review of recent advances in the study of the constituents of pyrethrum. The relative toxicities of pyrethrins I and II and cinerins I and II alone, or with six different ratios of synergists, are discussed. Some of the theories proposed for the toxic action of pyrethrum are given but a very large number of factors is evidently involved. (35 references.) J. L. WALPOLE.

**Factors influencing the stability of pyrethrins in powders.** D. R. Maciver (*Pyrethrum Post*, 1966, **8**, No. 4, 23–26).—High grade talcs and silica are the most satisfactory bases for impregnating with pyrethrins (**I**); china clays, fuller's earth, soapstones (impure talcs), limestone, diatomite, gypsum, MgO and $CaCO_3$ cause

rapid degradation of **I**. Attempts to stabilise **I** with urea, triethanolamine, aerosol OT and Octaphen [benzyldiethyl-2-*p*-(1,1,3,3-tetramethylbutyl)phenoxyethyl ammonium chloride] were unsuccessful but even poor fillers become suitable if first mixed with an equal wt. of fine pyrethrum marc. The stabilising factor of marc may be a naturally occurring antioxidant such as a phenolic cpd. (17 references.) J. L. WALPOLE.

**Synergism of pyrethrum. I. Piperonaldehyde acetals and mercaptals. II. Comparison of certain *endo*- and *exo*-*N*-alkyl- and *N*-alkoxy-5-norbornene-2,3-dicarboximides.** L. O. Hopkins and D. R. Maciver and N. K. Sylvester (II) (*Pyrethrum Post*, 1966, **8**, No. 4, 10–12; 13–17).—I. Alkoxyacetals containing the methylenedioxyphenyl radical are shown to be potential pyrethrum synergists; this confirms earlier work. In addition, alkoxymercaptals are also potential synergists. Acetals and mercaptals having O-interrupted side chains synergise both knockdown and kill, the max. effect being given by n-butoxyethoxy-ethyl chains. None of the attempts to explain the mechanism involved is wholly satisfactory. (24 references.)

II. Comparison of pyrethrum synergism by the *exo*- and *endo*-isomers of *N*-(2-ethylhexyl)-5-norbornene-2,3-dicarboximide showed the *exo*-isomer to be slightly more potent whereas the *endo*-isomers were the more potent in the case of *N*-(3-ethoxyethoxypropyl)- and *N*-(3-ethoxyethoxyethoxypropyl)- deriv. Both *N*-alkoxy imides showed higher overall synergism for knockdown and kill than the *N*-alkyl compound. (13 references.)
J. L. WALPOLE.

**Analytical methods for pesticide formulations.** R. L. Caswell (*J. Ass. off. analyt. Chem.*, 1967, **50**, 565–566).—Investigations in progress under the auspices of the A.O.A.C. are listed.
A. A. ELDRIDGE.

**Programmed temperature gas chromatography of pesticides, using electron capture and thermionic detectors.** D. C. Bostwick and L. Giuffrida (*J. Ass. off. analyt. Chem.*, 1967, **50**, 577–581).—Operating parameters for temp. programming with a single column system are given. A constant rate of gas flow from column to detectors was achieved by means of a differential flow controller placed close to the column gas inlet. Programmed temp. columns may cause decomposition of certain pesticides; moreover re-equilibration is slow. The use of tritium sources with ion strengths higher than usual is recommended. A. A. ELDRIDGE.

**Gas chromatographic determination of residues of organophosphorus pesticides with a modified flame ionisation detector.** J. H. Ford and M. Beroza (*J. Ass. off. analyt. Chem.*, 1967, **50**, 601–604).—The advantage of the use of a wire coated with KCl (Giuffrida, Ives and Bostwick, *ibid.*, 1966, **49**, 12) is confirmed. The use of He as carrier gas instead of $N_2$ further enhances the response to P.
A. A. ELDRIDGE.

**Additional clean-up of samples on GLC columns for TLC determination of pesticide residues.** K. T. Hartman (*J. Ass. off. analyt. Chem.*, 1967, **50**, 615–623).—Siliconised glass wool is used as a trap in the application of GLC to the clean-up of samples instead of the use of acid-Celite or saponification procedures. TLC can then be used for the detection of lower levels of pesticide residues than is possible with other clean-up procedures.
A. A. ELDRIDGE.

**New extraction system for [pesticide] residue analyses.** R. R. Schnorbus and W. F. Philips (*J. agric. Fd Chem.*, 1967, **15**, 661–666).—Examples are described in which propylene carbonate (4-methyl-2-dioxolone) is shown to be an efficient extractant for a variety of polar and non-polar pesticidal compounds from animal and plant products; also from soil. Different techniques of extraction, clean-up, and determination are summarised. (22 references.) P. S. ARUP.

**Determination of DDT, BHC and methoxychlor in their mixtures by thin layer chromatography.** A. Kotarska and K. Mosinska (*Chemia analit.*, 1967, **12**, 329–338).—The mixture of DDT, BHC and methoxychlor (**I**) is separated by TLC on silica gel–gypsum (9 : 1) with cyclohexane–$CHCl_3$ (9 : 1) as solvent. The spots are extracted with ethanol, and the pesticide contents determined polarographically vs. the S.C.E. (−0·4 to −1·4V for DDT and **I**, and −0·7 to −1·7V for BHC). For 60 to 600 μg of BHC the error is ⩾ −4%, for 200 to 2000 μg of DDT and **I** the error is ⩾ −5% and ⩾ ±1% respectively (4 determinations each).
B. KAMINSKI.

**Determination of Dursban [insecticide] and its oxygen analogue in maize and grass by gas chromatography with flame-photometric detection.** M. C. Bowman and M. Beroza (*J. agric. Fd Chem.*, 1967, **15**, 651–653).—The compounds were extracted with $C_6H_6$ in the presence of $Na_2SO_4$, and separated on a column of silica-gel and $Na_2SO_4$, Dursban (**I**) being eluted with $C_6H_6$ and the oxygen (phosphate) analogue (**II**) with $COMe_2$. Determinations were carried out on a specially prepared and conditioned column. Recoveries of **I** and **II** were 96–99% and 85–90%, respectively, in the 0·1–5·0 ppm range. The sensitivity of the method was 0·002–0·01 ppm with slight interference occurring only at levels ⩾ 0·01 ppm. P. S. ARUP.

**[Analysis of] chlorinated insecticides and miticides.** J. A. Burke (*J. Ass. off. analyt. Chem.*, 1967, **50**, 575–577).—Brief reference is made to investigations in progress under the auspices of the A.O.A.C. A. A. ELDRIDGE.

**Field weathering of Toxaphene and chlordane.** A. K. Klein and J. D. Link (*J. Ass. off. analyt. Chem.*, 1967, **50**, 586–591).—After weathering on crops for 28 days only traces of Toxaphene (**I**) and chlordane remain. The colorimetric method of determination (Graupner and Dunn, *J. agric. Fd Chem.*, 1960, **8**, 286) using Na *p*-diphenylamine sulphonate instead of *p*-diphenylamine, is satisfactory at levels of **I** ⩽ 5 ppm. Chlordane and DDT interfere; large amounts of DDT interfere with gas chromatomatic determination of **I**. A. A. ELDRIDGE.

**Separation, identification and measurement of DDT and its metabolites.** M. Siewierski and K. Helrich (*J. Ass. off. analyt. Chem.*, 1967, **50**, 627–633).—GLC and TLC have been combined with i.r. spectrophotometry for the separation and identification of DDT and 12 of its possible reaction products. A. A. ELDRIDGE.

**Distribution of heptachlor residues in pond ecosystems in South Western Virginia.** W. M. Weatherholz, G. W. Cornwell, R. W. Young and R. E. Webb (*J. agric. Fd Chem.*, 1967, **15**, 667–670).—After the discontinuation of the use of heptachlor against the lucerne weevil, in 1964, the water, fauna, flora, and mud from 35 farm ponds were examined for heptachlor content. Residues ⩾0·3 ppm (⩾300 parts per U.S. billion) were found in all watersheds up to 25 months after the last application. The only other chlorinated hydrocarbon found was DDT. (11 references.)
P. S. ARUP.

**Quantitative determination of pyrethrins by gas-liquid chromatography. I. Detection by electron capture.** S. W. Head (*Pyrethrum Post*, 1966, **8**, No. 4, 3–7).—The six active insecticidal constituents of pyrethrum (**I**) extract can be detected, and estimated, by GLC using electron capture. Separation is carried out at 160° on a glass column packed with 1% neopentylglycol succinate on acid-washed 60–80 mesh Chromosorb W. The results of analysing five **I** extracts are given and compared with those given by the official A.O.A.C. method. (13 references.) J. L. WALPOLE.

**Insecticidal constituents in *Chrysanthemum cinerariaefolium*. (I). Development in the flower head. (II) Distribution in the plant.** S. W. Head (*Pyrethrum Post*, 1966, **8**, No. 4, 32–37).—Distribution of the six active constituents present in all parts of the pyrethrum plant, including the leaves, stem and roots as well as the heads, was examined. GLC using electron capture was employed for detection of the constituents and the results, when expressed in terms of total 'pyrethrins', were seen to be in good agreement with earlier work. J. L. WALPOLE.

**Effects of humidity in the laboratory on thin layer chromatography of insecticides.** W. I. Reichel (*J. Chromat.*, 1967, **26**, 304–306).—After coating with aluminium oxide G, plates were spotted and exposed to a given humidity for 10 min. They were then developed and spots were located as usual. Generally $R_F$ values increased with increasing humidity, but some compounds were more affected than others. This offers the possibility of improving separation in some cases by varying the humidity. G. RUSSELL.

**Complexometric determination of mercury in Fungitox OR.** H. Romanowski (*Chemia analit.*, 1966, **11**, 1027–1028).—The pesticide (1 g) is wet-oxidised with $HNO_3$; the resulting solution is boiled until colourless and made up to 100 ml. An aliquot (1 to 15 ml) is treated with 25 ml of 0·001 N E.D.T.A., the pH is adjusted to 10 with ammonia buffer, and after adding Eriochrome Black indicator, excess EDTA is back-titrated with 0·001N $ZnSO_4$. Average error for 10 to 150 mg of Hg is ±3·0%. (In Polish.)
P. BRYCH.

**Determination of Terraclor [fungicide] in crops and soil by electron-capture gas chromatography.** T. P. Methratta, R. W. Montagna and W. P. Griffith (*J. agric. Fd Chem.*, 1967, **15**, 648–650).—Extraction of Terrachlor [pentachloronitrobenzene, (**I**)] was carried out with hexane and clean-up by passing the extract through a column of silicic acid containing 10% of moisture, topped with

anhyd. $Na_2SO_4$. **I** was determined by GLC in the hexane extracts in the range 0·01–0·27 ppm. Recoveries ranged from 74% from lettuce leaves to 119% from groundnut hay. Other crops examined included celery, flax seed, peanuts, potatoes, radishes and strawberries. P. S. ARUP.

**[(Terraclor)] pentachloronitrobenzene residues in potatoes.** S. Gorbach and U. Wagner (*J. agric. Fd Chem.*, 1967, **15**, 654–656).—The residues were determined in potatoes grown in soil treated with Terraclor (**I**). Extraction was carried out with $C_6H_6$– 2-propanol (2 : 1) in presence of anhyd. $Na_2SO_4$. The extract was washed with 2% aq. NaCl, dried with $Na_2SO_4$, and concentrated for GLC with a halogen-specific coulometric detector. Most of the residues occurred in the peel together with a metabolite identified as pentachloroaniline (**II**), and another unidentified metabolite. **II** was also found to be produced by fermentation of **I** in potato homogenates. (10 references.) P. S. ARUP.

**Iodometric determination of insoluble dialkyldithiocarbamates.** M. M. Lorenzo and J. G. M. Luengo (*Boln. Inst. nac. Invest. agron. Madr.*, 1967, **26**, 199–216).—Active material is extracted into $CHCl_3$ (reflux and filtration) and the solution is shaken with 0·25 M EDTA-$Na_2$ and successive portions of 0·1 N $I_2$ until an excess of $I_2$ is observed in the $CHCl_3$ layer. The excess of $I_2$ is back-titrated with 0·1 N $Na_2S_2O_3$. Oxidation is quant.; the method gives precise results for both Zn and Ag dimethyl- and diethyldithiocarbamates, but with the Fe compounds, results are variable. (40 references.) E. C. APLING.

**Bensulide residues in turf grass soil following annual treatments for crabgrass control.** S. W. Bingham and R. E. Schmidt (*Agron. J.*, 1967, **59**, 327–329).—Annual treatments with bensulide [*N*-(2-mercaptoethyl)benzenesulphonamide *S*-(*O*,*O*-diisopropyl phosphorodithiolate)] at 15 lb per acre controlled crabgrass in bent-grass turf, and increased turf grass vigour. Bensulide residues were present in large amounts 11 months after the fourth annual application of a granular formulation. The toxicity of bensulide residues to milo was inversely related to org. matter and nutrient content of the soils. A. H. CORNFIELD.

**Infra-red absorption spectra and polymeric structures of three *s*-triazine herbicides and their metabolites.** J.-Y. T. Chen (*J. Ass. off. analyt. Chem.*, 1967, **50**, 595–600).—The i.r. spectra of the 2-chloro-4,6-bis(alkylamino)-*s*-triazine herbicides atrazine, propazine and simazine and the corresponding hydroxy-compounds have been charted and their characteristic absorption frequencies listed. A. A. ELDRIDGE.

**Electron capture determination of Cotoran, Tenoran, and Tupersan herbicides following hydrolysis and bromination.** W. H. Gutenmann and D. J. Lisk (*J. Gas Chromat.*, 1966, **4**, 424–425).—Cotoran [*N'*-(3-trifluoromethyl)-phenyl-*N'*,*N*-dimethylurea], Tenoran [*N*-4-(4'-chlorobiphenylyl)oxy-*N'*,*N'*-dimethylurea], and Tupersan [*N*-(2-methylcyclohexyl)-*N'*-phenylurea] were acid hydrolysed to their respective substituted anilines and the latter were converted into brominated deriv. which were analysed by GLC. As little as 0·2 ng of each herbicide could be detected. D. I. REES.

**Determination of the herbicide Tordon (4-amino-3,5,6-trichloropicolinic acid) in soil by electron capture gas chromatography.** J. G. Saha and L. A. Gadallah (*J. Ass. off. analyt. Chem.*, 1967, **50**, 637–641).—The Tordon is extracted with acetone in presence of $H_3PO_4$ and is then treated with diazomethane to afford the Me ester, which is then determined. Recoveries were 80·0 to 96·0%. A. A. ELDRIDGE.

**Titrimetric assay of amitrole formulations.** J. R. Bishop (*J. Ass. off. analyt. Chem.*, 1967, **50**, 568–572).—Excess of standard acid solution is added to a solution of the 3-amino-1,2,4-triazole and the excess is titrated potentiometrically with standard alkali to measure the amount consumed between the first and second inflexion points. The method yields results of 98–100% for pure amitrole. A. A. ELDRIDGE.

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**Carbamoyl oxime derivatives [pesticides].** Union Carbide Corp. Assee of L. K. Payne, jun. and M. H. J. Weiden (B.P. 1,046,407, 24.9.63. U.S., 25.9.62) (22 pp, 42 claims).—The title compounds have insecticidal, miticidal, and nematocidal activity and the structure X·C·C:N.O.C(O)N wherein X is O, S, SO, or $SO_2$ and free valencies are satisfied by H or optionally substituted hydrocarbon radicals. In an example, MeSH is added to a solution of Na in EtOH, followed by a solution of dimerised Cl.$CMe_2$.$CH_2$NO in hot EtOH. After 5 h at the boil the mixture is worked up, to give 2-methyl-2-methylthiopropionaldoxime, b.p. 78–81°/5 mm. Treatment of this in acetone with MeNCO in presence of 2 drops of $SnBu_2(OAc)_2$ gives after 4 h at the boil, *O*-methylcarbamyl-2-methylthio-2-methylpropionaldehyde oxime [1-(methylcarbamyloximino)-2-methylthio-2-methylpropane], m.p. 95° (from $Pr^i_2O$). F. R. BASFORD.

**Heterocyclic esters of phosphorus acids.** Shell Internationale Research Mij N. V. (Inventors: J. T. Hackmann and J. Wood) (B.P. 1,055,093, 10.12.63).—Compounds $R^IR^{II}P(X)YR^{III}$, (**I**) useful as pesticides against e.g., housefly, mosquito larvae, aphids, red spider mite, are claimed. $R^I$ and $R^{II}$ are alkyl or alkoxy; X and Y are O or S; $R^{III}$ is an isoindol-1,3-dione or 1,2-benzisothiazol-3-on-1,1-dioxide radical, optionally substituted on the N-atom by (substituted) hydrocarbon, e.g., Me, and in the benzene ring by halogen, $NO_2$ or alkyl. **I** are made by reacting a compound $R^IR^{II}P(X)Z$ (Z is halogen) with a suitable hydroxy compound, or $R^IR^{II}P(S)SM$ (M is alkali metal) with a diazonium fluoborate. Thus, 5-diethoxyphosphinyloxy-isoindol-1,3-dione is prepared by heating 5-hydroxy-isoindol-1,3-dione and $(EtO)_2P(O)Cl$ in $MeBu^tCO$ at 100–105° for 24 h, in presence of $K_2CO_3$. More than 50 products are tabulated. E. ENOS JONES.

**[Insecticidal] phosphorus-containing esters.** Shell Internationale Research Mij N. V. (Inventors: G. O. Osborne and R. H. Davis) (B.P. 1,055,094, 23.2.65).—The esters (**I**) have a general formula $R^IR^{II}P(S)OR^{III}$; $R^I$ = phenyl, optionally bearlng one or more halogen atoms, $R^{II}$ is alkoxy and $R^{III}$ is an isoindole-1,3-dione group, with an aryl or aralkyl optionally bearing one or more halogen atoms, nitro groups, alkyl or alkoxy groups (1–4 C atoms) on the N-atom. Thus 5-(phenylethoxyphosphinothioyloxy)-2-benzylisoindole-1,3-dione is obtained from 5-hydroxy-2-benzylisoindole-1,3-dione + EtPhP(S)Cl. **I** are particularly insecticidally active against flies, mosquito larvae, beetles, moth larvae, aphids and mites. They can be applied in an inert diluent or admixed with a carrier and/or surface-active agent. E. ENOS JONES.

**Bridged-ring isonitriles.** Farbenfabriken Bayer A.-G. (Inventors: H. Fetzer, I. Hgi, H. Knupfer, J. A. Renner and F. Grewe) [B.P. 1,064,835, 22.6.65. Ger., 9.7.64).—Possessing acaricidal, fungicidal and insecticidal properties, the title compounds have the formula B.A.N:C, where A represents a bivalent, optionally substituted, saturated or unsaturated aliphatic or cycloaliphatic radical which may contain O, S and/or N or for an optionally substituted aromatic radical or for a direct bond between B and the N of the ·NC function; B represents an optionally substituted, saturated or unsaturated bridged-ring radical, which may contain 1 or 2 fused aliphatic or aromatic rings and also O, S and/or N. A formamide B.A.NH.CHO is reacted with an acid halide in presence of a base at −20 to 60°. Thus, 3-(β-aminoethyl)-bicyclo-[3-2,2]-3-aza-nonane is reacted with H.CO.OMe at 120°, giving 3-(β-formylaminoethyl)-bicyclo-[3,2,2]-3-aza-nonane, m.p. 69–71°, which is then dissolved in $Et_3N$ and $CH_2Cl_2$. $COCl_2$ is introduced at 0–10° and the mixture saturated with $NH_3$, filtered, concentrated and the residue distilled *in vacuo* to give 3-(β-isocyanoethyl)-bicyclo-[3,2,2]-3-aza-nonane, b.p. 104–106°/0·1 mm Hg. S. D. HUGGINS.

**Sulphoxides and their use as pesticides.** Chemagro Corp. (Inventors: P. C. Aichenegg and C. D. Emerson) (B.P. 1,065,336, 15.2.66).—Compounds, of formula $SO(CHX.CHX_2)_2$ (X is Cl or Br) are useful as fungicides and nematocides without vesicant hazards. In an example, $SCl_2$ is added at 30–35° during 2·75 h to a suspension of $FeCl_3$ in *cis*-$C_2H_2Cl_2$ then after keeping overnight $FeCl_3$ is filtered off. The filtrate is washed with dil. aq. HCl and distilled, to give a 22·2% yield of di-(1,2,2-trichloroethyl) sulphide, b.p. 102–108°/0·03 mm. This is diluted with AcOH, then 30% aq. $H_2O_2$ is added gradually. After 42 h at room temp. and 2 h at 60–70° the mixture is diluted with water, and pptd. oil is dissolved in $CHCl_3$. The extract is worked up to give di-(1,2,2-trichloroethyl) sulphoxide, b.p. 102–105°; m.p. 88° (light petroleum). F. R. BASFORD.

**Esters of *N*-alkyl-*N*-phenoxyalkanoyl carbamic acids.** Boots Pure Drug Co. Ltd. (B.P. 1,068,056, 2.9.64. Norway, 9.10.63. Addition to B.P. 982,235, 1.5.63).—The compounds have the formula $R{\cdot}O{\cdot}CO{\cdot}N(R^{II})COR^I$ where $R^{II}$ is a lower alkyl radical; R is a substituted or unsubstituted phenyl, naphthyl or oxa-, thia- or aza- 5- or 6- membered ring heterocyclic radical and $R^ICO$ is an acyl residue of a phenoxyalkanoic acid; 'lower' alkyl, cycloalkyl or alkoxy radicals are defined as those containing ⩾ 7C atoms. E.g., 2-isopropoxyphenyl *N*-methylcarbamate is reacted with phenoxyacetyl chloride in xylene and dimethylaniline to give 2-isopropoxyphenyl *N*-methyl-*N*-phenoxyacetyl carbamate, b.p. 218–220°/1·5 mm; m.p. 73–75°. The claimed compounds possess activity as insecticides, acaricides, molluscicides, nematocides and fungicides. E. ENOS JONES.

**Tetrachlorothiophene.** R. N. Haszeldine, R. E. Banks and J. M. Birchall (B.P. 1,069,943, 24.5.65).—The claimed pesticide is obtained by reacting S with tetra- or tri-chloroethylene or hexachloroethane at 200–400° and 1–50 atm. pressure. Thus, tetrachloroethylene and S are sealed *in vacuo* into a steel autoclave and heated at 250° for 90 h, the max. observed pressure being 18 atm. The reaction products are refluxed with excess water to remove S halides, and then steam distilled to recover (40%) tetrachloroethylene. The water is decanted from the residues of the steam distillation and the brown tarry mixture heated under vacuum to give a pale yellow liquid that is purified by sublimation and identified as a 15% yield of tetrachlorothiophene.

S. D. HUGGINS.

**Dithiophosphonic esters.** Esso Research and Engng Co. (B.P. 1,071,033, 31.8.64; U.S. 30.9.63).—Esters, useful as active ingredients in pesticidal compositions (claimed) are prepared by reacting a substituted dithiophosphoric acid $RO(R^1O)P(S)SH$ (where R and $R^1$ are $C_1$–$C_{30}$ hydrocarbon radicals, preferably Me or Et) with 2–5 moles of a conjugated or cumulative diene (butadiene, allene) at −80 to +100° under free radical conditions. The compounds, e.g. *O,O*-$Me_2$- and *O,O*-$Et_2$-***S*-allyldithiophosphate** are active as insecticides, miticides, nematocides.

E. ENOS JONES.

**Organotin complex salts.** Boehringer Ingelheim G.m.b.H. (B.P. 1,073,650, 18.12.64. Ger., 18.12.63).—Salts claimed have good microbial activity, especially bactericidal, fungicidal, and molluscidal activity (e.g., against *Phytophthora infestans* or *Cercospora beticola*) and are produced by reacting $PR^IR^{II}R^{III}R^{IV}X$ with $SnPh_3Y$ (where $R^I$–$R^{IV}$ are H, straight- or branched, saturated or unsaturated aliphatic hydrocarbon group of 1–12 C, alicyclic, araliphatic, or aromatic radical—at least one of them not being H; X and Y are anions). E.g., a solution of $SnPh_3Cl$ in MeOH is added to MeOH containing dissolved $PhCH_2{\cdot}PEt_3Br$, then solvent is evaporated off, and residue is recrystallised from $Pr^iOH$, to give (benzyltriethylphosphonium)(triphenylbromochlorostannate) m.p. 129° in 71% yield. It may be admixed (20) with kaolin (71·5), Ca ligninsulphonate (5), wetting agent (2), and sticking agent (methylcellulose) (1·5%) to provide a wettable powder.

F. R. BASFORD.

**Xanthic acid esters.** VEB Farbenfabrik Wolfen (Inventors: W. Wildgrube and M. Born) (B.P. 1,077,227, 13.8.65).—The claimed esters of formula $R^1S{\cdot}CS{\cdot}O{\cdot}C_2H_4SR^2$, wherein $R^1$ is alkyl of 1–4C, alkylmercaptoalkyl or aralkyl radical and $R^2$ is an alkyl, are obtained by reacting compounds of formula $R^2S{\cdot}C_2H_4OH$ with $CS_2$ and NaOH or KOH in water or inert org. solvent to give the corresponding xanthate which is then alkylated with an alkyl halide or dialkyl sulphate. The esters are active against housefly, granary weevils, aphids etc.

S. D. HUGGINS.

[A] **Chlorobenzimidazoles [fungicides], [B] preparation of 4,5,6,7-tetrachlorobenzimidazole.** Fisons Pest Control Ltd. (Inventors: D. W. J. Lane and G. T. Newbold) (B.P. 1,063,472–3, 23.11.62).—[A] There is claimed a fungicidal composition containing 4,5,6-trichloro- or 4,5,6,7-tetrachlorobenzimidazole (**I**) (or a salt thereof) admixed with a surface-active agent and/or a solid diluent (or, in the case of a salt, water and optionally an alcohol). The effect on *Phytophthora palmivora*, *Alternaria solani*, *Botrytis fabae*, *Fusarium oxysporum*, *Fomes annosus*, and *Verticillium alboatrum* is described. [B] A mixture of 1,2,3,4,5,6-$(NH_2)_2C_6Cl_4$ and 98% $HCO_2H$ is boiled for 2 h, then made alkaline with NaOH, and diluted with water. The filtered solution is adjusted to pH 6 with aq. HCl, with pptn. of **I**, m.p. 327–328°.

F. R. BASFORD.

**Fungicidal compositions.** Fabriek Van Chemische Producten Vondelingenplatt N. V. (Inventor: K. v. D. Boogaart) (B.P. 1,063,794, 31.12.63).—The claimed compositions contain one or more asymmetrical thiuram monosulphides (**I**) of formula $R^1R^2NCS(S)CSNR^3R^4$ (where $R^1$–$R^4$ are hydrocarbon radicals), in which the groups $R^1R^2N$- and $R^3R^4N$- are dissimilar. Thus, a powder for use against powdery mildew, consists of 25% of **I**, 2–5% wetting agent (alkylaryl sulphonate), 2–5% dispersing agent (lignin sulphonate) and a carrier (clay or silicate) up to 100% and is diluted with water to give spraying liquids which are used on gherkin plants with just-developed cotyledons; dinitro-(1-methylheptyl)phenyl crotonate (**II**) is used as a comparative fungicide. The dried plants are infected with mildew spores; after 18 days, mildew-coverage of the plants is: untreated, 100%; **II**-treated, 5%; **I**-treated, 0% at 25% concn. (1·8% at 12·5% concn. and 9·6% at 6·25% concn.).

S. D. HUGGINS.

**3-(Benzthiazol-2-yl)-1-ethylureas and their application as fungicides.** Chevron Research Co. (B.P. 1,065,589, 8.11.65. U.S., 9.11.64).—1-Ethyl-(**I**) and 1,1-diethyl-**3-(benzthiazol-2-yl)urea** are claimed and are active against, e.g., *Phytophthora infestans*, *Uromyces phaseoli*, *Erysiphe cichoracearum*, *E. polygoni*, and *Sphaerotheca pannosa*. In an example, EtNCO is added during 20 min. to a solution of 2-aminobenzthiazole in benzene at 75°, temp. rising to the b.p. After a further 2 h the mixture is cooled to 25°, with pptn. of **I**, m.p. 198°.

F. R. BASFORD.

**[Fungicidal] 1-substituted carbostyrils.** Upjohn Co. (B.P. 1,065,737, 13.8.64. U.S., 16.9.63).—The 1-$C_{3-8}$-alk-2-enoxy-2-oxo-1,2-dihydroquinolines (1-alkenoxycarbostyrils) are produced by reacting a 2-$C_{1-3}$-alkoxyquinoline-1-oxide with RX (X is Cl, Br, or I; R is alkenyl) and are useful in the treatment of topical fungal infections in mammals (humans, cattle, horses, etc.) caused by, e.g., *Microsporum canis*, *Trichophyton rubrum*, plant infections caused by, e.g., *Alternaria solani*, *Fusarium oxysporum* var. *cubense*, and *Sclerotina fructicola*, as mothproofing agents (in the form of salts with $H_2SiF_6$), etc. In an example, 1-allyloxycarbostyril, m.p. 43–44° (hexane), b.p. 135°/0·13 mm, is prepared by boiling a mixture of 2-ethoxyquinoline-1-oxide and allyl bromide during 1·5 h.

F. R. BASFORD.

[A] **1-Hydroxy-2,2,6,6-tetrachlorocyclohexane-carbonamide-1.** [B] **2,6-Dichlorobenzonitrile.** N. V. Philips' Gloeilampenfabrieken (B.P. 1,066,811–2, 30.4.64. Neth., 2.5.63).—[A] The title compound, having fungicidal activity is obtained by saponifying 2,2,5,6-tetrachlorocyclohexanone cyanhydrin (**I**) at 40–70° with $H_2SO_4$. Thus, **I** in conc. $H_2SO_4$ is heated, at 55–60° for 1 h and then poured on to ice to give a 98% yield of 1-hydroxy-2,2,6,6-tetrachlorocyclohexane-carbonamide-1 (**II**), m.p. 166°, which can be recrystallised from benzene to melt at 204°.

[B] 2,6-Dichlorobenzonitrile (**III**), a good herbicide, is economically made by removing water and HCl from **II** and then converting the resulting 2,6-dichlorobenzamide into **III**. Thus, **II** in 1,2,4-trichlorobenzene and anhyd. $ZnCl_2$ are heated at 120–125° for 15 min. and at 130–135° for 30 min., when 95% of the theoretical HCl has escaped. After cooling, the 2,6-dichlorobenzamide contaminated with $ZnCl_2$ is treated with $PCl_5$, $P_2O_5$ or $POCl_3$. The non-purified oil-like reaction product is then heated at 105° for 1 h and **III** is obtained by steam distillation of the mixture.

S. D. HUGGINS.

[A] **Bicyclic sulphur-containing organic compounds.** [B] **Bicyclic sulphur-containing olefines.** Hooker Chemical Corp. (B.P. 1,061,472–3, [A] 6.2.64. [B] 7.2.64. U.S., 25.2.63).—[A] Compounds claimed are 9-thiabicyclo[4,2,1 (or 3,3,1)]nonanes and *S*-oxides thereof substituted in $\beta$- and/or $\beta^1$-position (with respect to S) by halogen, ZR (Z is O or S; R is H, hydrocarbon radical, or acyl), hydrocarbon radical, NCS, $CO_2H$ (which may be in ester, amide, or salt form), CN, or $NR^IR^{II}R^{III}{}_nYn'{}_m$ ($R^I$ and $R^{II}$ are as R or $R^I$ is 9-thiabicyclo [4,2,1 (or 3,3,1)]-nonyl or $NR^IR^{II}$ is heterocyclyl; $R^{'II}$ is H or hydrocarbon radical, *n* is 0 or 1, *n* being 0 when $R^I$ or $R^{II}$ is acyl; *n'* is 0 when N is tertiary and 1 when N is quaternary, and Y is anion of valency *m*), and optionally elsewhere by up to 12 Cl. They are herbicides and pesticides. Thus, 1,5-cyclo-octadiene and $SCl_2$ are mixed in hexane at 10–20°, then 1 h later solid is filtered off, to give 2,6-dichloro-9-thiabicyclo [3,3,1]nonane (**I**), m.p. 102–103° (benzene–heptene). A further 67 compounds are described, also their activity against, e.g., early blight, *Pythium*, nematodes, *Staphylococcus aureus*, *Escherichia coli*. [B] Compounds claimed comprise 9-thiabicyclo[4,2,1 (or 3,3,1)]non-2-enes substituted in the 6- or 5-positions respectively by similar groups as in [A]. They are similarly active. Thus, 6-chloro-9-thiabicyclo[3,3,1]non-2-ene, b.p. 64–69°/0·3 mm is prepared by heating **I** at 166–184° during 20 h, then distilling.

F. R. BASFORD.

**Herbicidal compositions.** Mirvale Chemical Co. Ltd. (Inventor: S. Everest-Todd) (B.P. 1,063,234, 4.3.65).—The herbicidal action of **α-naphthyl**-phthalamic acid (**I**) and -phthalimide is synergised by a phenoxy-$C_{1-3}$-alkane carboxylic acid (or an ester, metal salt or amine salt thereof) in a 1 : 6 to 6 : 1 ratio. E.g. 3 pints of an aq. solution containing 10% each of the diethanolamine salts of **I** and of 2,4-dichlorophenoxyacetic acid were further diluted with water to make 20 gal, which was applied to 1 acre of barley at the 5-leaf stage, without damage, yet with good control of both *Stelaria media* and of *Sonchus oleracus*.

F. R. BASFORD.

**Anilide derivatives.** Takeda Chemical Industries Ltd. (Inventors: Y. Okada and M. Ochiai) (B.P. 1,063,528, 3.4.64).—Used as weed killers, title compounds have formula $X{\cdot}(Y){\cdot}C_6H_3NRCOR^1$ (X and Y are H, halogen or straight-chain alkyl radical with 1–5C but X and Y are not both H; R is H or alkyl radical with 1–5 C and $R^1$ is an aliphatic hydrocarbon radical with 1–5 C) and

are prepared from the acid or salt $R^1 \cdot COOM$ (M is H or alkali metal) and a carbamoyl chloride deriv. $X(Y) \cdot C_6H_3 \cdot NR \cdot CO \cdot Cl$. Thus, 3,4-dichlorophenylcarbamoyl chloride in $o\text{-}Cl_2 \cdot C_6H_4$ is added to propionic acid in $o\text{-}Cl_2 \cdot C_6H_4$ at 160–165°. The mixture is heated for 1 h at this temp. to yield 3,4-dichloropropionanilide, m.p. 91–92°. S. D. HUGGINS.

**2-Mercapto-4,6-diamino-1,3,5-triazine derivatives.** Nippon Kayaku K. K. (B.P. 1,064,570, 14.12.64. Jap., 13.12.63).—The herbicidally active title compounds are obtained by reacting cyanuric chloride (**I**) at < 0° with an amine $R^1R^2NH$ or $R^3R^4NH$ ($R^1$,$R^3$ and $R^4$ are independently H or lower aliphatic hydrocarbon radicals, optionally containing O or S and $R^2$ is a lower aliphatic radical, optionally containing O or S) in presence of an acid binding agent (or an excess of the amine), and then reacting the intermediate 2,4-dichloro-6-amino-*s*-triazine with an alkenyl- or alkyl-mercaptan in presence of, e.g., NaOH, followed by reaction of the product with a further amine, $R^3R^4NH$ or $R^1R^2NH$. Thus, water and $Me_2CO$ are added to **I**, followed by addition, at $< -5°$, of 28% aq. $NH_3$ and 4N-NaOH. MeSH is then added at 5°, followed by 4N-NaOH until the solution is neutral (35–40°). 70% aq. $EtNH_2$ is added and the mixture refluxed at 70° for 3 h to give an 89% yield of 2-methylmercapto-4-amino-6-ethylamino-1,3,5-triazine, m.p. 119–120°. S. D. HUGGINS.

**Diamino-mono-azido-1,3,5-triazines.** Deutsche Gold- und Silber-Scheideanstalt (B.P. 1,065,402, 3.4.64. Ger., 9.5.63 and 30.1.64).—Used as pesticides (for affecting plant growth) the title compounds are prepared by treating the corresponding 6-halo-2,4-diamino-1,3,5-triazines (in which the amino groups can be substituted with O, S, N, P and halogen-containing groups) with $NaN_3$ or $NH_4N_3$ in e.g., $Me_2$-formamide, from 20° to the b.p. of the solvent. Thus, 2-ethylamino-4-t-butylamino-6-chloro-1,3,5-triazine is introduced to a stirred suspension of $NaN_3$ in $Me_2$-formamide and the mixture stirred for 3 h on a boiling water bath and then poured into water, to give 2-ethylamino-4-t-butylamino-6-azido-1,3,5-triazine, m.p. 101–102°. S. D. HUGGINS.

**Cycloalkyl carbonamides and herbicidal compositions containing them.** Chemical Investors, S.A. (B.P. 1,065,533, 18.3.64. U.S., 28.3 and 8.5.63).—The compounds have the formula $R \cdot CONR^1R^{II}$ (wherein R is cyclopropyl or cyclobutyl substituted in the 1-position by alkyl, alkoxy, or halogen; $R^1$ and $R^{II}$ are H, alkyl, alkenyl, alkynyl, cycloalkyl, alkaryl, halogeno-aryl, halogeno-alkaryl, aralkyl, or $NR^1R^{II}$ is heterocyclyl- at least one of them being other than H). Their effect on lucerne, brome-grass, flax, oats, radish, sugar beet, maize, coxcomb, cotton, crabgrass, millet, and soyabean is described, and they can be prepared by known methods (see also B.P. 1.065,532). E.g., a mixture of Me 1-methylcyclopropane-1-carboxylate l, $m\text{-}NH_2 \cdot C_6H_4Cl$, NaOMe and benzene is boiled during 12 h, with azeotropic removal of water. The cooled residue is mixed with water and conc. aq. HCl, then the org. phase is washed with dil. aq. HCl, clarified with C, and evaporated to give 1-methylcyclopropane-1-carb-*m*-chloroanilide, m.p. 118–120° (light petroleum). F. R. BASFORD.

**Dinitroaniline derivatives.** Eli Lilly and Co. (Inventor: Q. F. Soper) (B.P. 1,071,478, 23.2.65).—Herbicidal action is claimed for the title *N*-alkyl-2,6-dinitro-4-trihalogenomethylanilines, obtained by heating a primary amine with the corresponding-4-halogeno-benzene. Thus, 4-chloro-3,5-dinitrobenzofluoride, s-$BuNH_2$ and $C_6H_6$ are refluxed for approx. 2 h, when $Et_2O$ is added and the org. layer washed with water, followed by 10% aq. HCl and then by water. The org. layer is separated, dried and $Et_2O$ removed *in vacuo* to leave a residue that is crystallised from $C_6H_{14}$ as *N*-(s-Bu)-2,6-dinitro-4-trifluoromethylaniline, m.p. 69·5–71·5°. S. D. HUGGINS.

**Weed control.** Farbenfabriken Bayer A.-G. (Inventors: H. Hack, L. Eue and W. Schafer) (B.P. 1,079,848, 6.7.66. Ger., 20.7.65).—Compositions contain 0·1–95% by wt. of 1-Me-3-(2-benzothiazolyl)-urea and $NHPhCO_2Pr^i$ in the ratio by wt. 1 : 1–9 : 1, together with a solid or liquid diluent. Examples of weeds destroyed by the combination are those occurring among beet and carrots; they include dicotyledons such as common chickweed, fumitory, broad-leafed dock, French mercury and knot-grass and monocotyledons such as slender foxtail and green panic grass. S. D. HUGGINS.

## Animal Husbandry

**Study of rapeseed meal. XII. Non-enzymic browning reaction of extracted rapeseed meal during production.** A. Rutkowski and H. Kozlowska (*Oléagineux*, 1967, **22**, 173–176).—The presence of glucosides, which liberate S compounds and glucose by hydrolysis, creating conditions which favour the non-enzymic browning of rapeseed meal is discussed. The technical conditions affect its biological value and are in direct relation to the quantity of melanoidins formed. Changes in the α-amino-N content, in lysine amino groups, in free amino-acids, in free sugars (particularly free reducing sugars) and the solubility of nitrogenous substances, suggest that a non-enzymic browning reaction (of the Maillard type) is present. Studies on the treatment of rapeseed meal at different temp., in wet and dry environments show that the Maillard reaction is slower in a dry than in a wet environment. (14 references.) M. DUDLEY.

**Importance of cellular constituents to cottonseed meal protein quality.** W. H. Martinez, L. C. Berardi, V. L. Frampton, H. L. Wilke, D. E. Greene and R. Teichman (*J. agric. Fd Chem.*, 1967, **15**, 427–432).—Further to earlier work, by Martinez *et al.* (cf. *ibid.*, 1961, **9**, 64) meals were prepared from glandless cottonseed to investigate the importance of the phospholipins, carbohydrates, and gossypol as regards nutritive value. The nutritive value of the meals was equal to that of the soybean control, but autoclaving decreased the protein efficiency ratio; the decrease was less in the meals from which the phospholipins and the carbohydrates had been removed. The binding effects of gossypol on protein quality depended on its physical state and on the other cellular constituents of the meal. Under comparable conditions, the protein efficiency ratios were correlated with the ε-free lysine content. (11 references.) P. S. ARUP.

**Yeast supplement to food.** J. N. Tuli (*Chem. Age India*, 1967, **18**, 568).—The production of yeast primarily for use in animal feeds is briefly discussed. K. GRAUPNER.

**Composition and nutritive value of meals from alewife, sheepshead, maria and tullibee.** B. E. March, J. Biely, E. G. Bligh and A. W. Lantz (*J. Fish. Res. Bd Can.*, 1967, **24**, 1291–1298).—The composition and nutritive value of meals manufactured from four species of freshwater fish, alewife (*Alosa pseudoharengus*), sheepshead (*Aplodinotus grunniens*), maria (*Lota lota*) and tullibee (*Coregonus artedii*), were determined and compared with meals of marine origin (herring and white fish). The meals from freshwater fish contained 62–68% protein, 7–13% fat and 14–20% ash. Na, K and Mg values were lower than in the marine fish meal but Fe and Cu were higher. Riboflavin, pantothenic acid, niacin and cobalamin levels were lower than in herring meal but similar or higher than in white fish meal. No consistent differences in amino-acid composition were noted. Protein value, pepsin digestibility and metabolisable energy values compared satisfactorily. (11 references.) S. A. BROOKS.

**Varieties of green fodder crops.** Anon (*Fmrs' Leafl. natn. Inst. agric. Bot.*, 1967, No. 2, 15 pp).—Summaries are given of the trial data obtained for varieties of the following crops: kales—marrow stem and thousand head; fodder cabbage; forage rape; fodder radish; maize—silage, green, grain; sorghum; catch crops. E. G. BRICKELL.

**Conservation and feed value of low-moisture orchardgrass stored in gas-tight and bunker silos.** C. H. Gordon, J. C. Derbyshire and J. R. Menear (*J. Dairy Sci.*, 1967, **50**, 1109–1115).—Trials with gas-tight and plastic-sealed bunkers for storage of orchardgrass showed that the latter bunkers are not ideally suited to obtaining a consistently low moisture content in forage stored during inclement weather. (17 references.) M. O'LEARY.

**Accelerating effect of magnesium on increase of phosphorus and calcium in animals.** C. L. Kervran (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1368–1373).—Increases in total Ca and P in animals (mice) receiving supplements of 100 mg/kg daily during 5 days were ~33% greater than corresponding increases in control animals. P. S. ARUP.

**Extent of cellulose and hemicellulose digestion in various forages by pure cultures of rumen bacteria.** B. A. Dehority and H. W. Scott (*J. Dairy Sci.*, 1967, **50**, 1136–1141).—The ability of nine pure strains of rumen bacteria to digest the cellulose (**I**) and hemicellulose (**II**) portions of intact forages at various stages of maturity was investigated. Two strains of *Bacteroides succinogenes* were capable of digesting significantly greater amounts of **I** from forages than were the other species. Two of four strains of *Ruminococcus flavefaciens* had a markedly reduced ability to digest lucerne **I**. Only three, all rumincococci, of the seven cellulolytic strains were capable of digesting forage **II**. With all organisms, the extent of **I** and **II** digestion decreased with increase in forage maturity. A synergistic effect resulting in increased **I** digestion was observed

when the non-cellulolytic *Bacteroides rumincola* was combined with any of four cellulolytic strains. (16 references.)
M. O'LEARY.

**Effect of moist heat treatment of cereal grains on growth and feed utilisation by cattle.** W. H. Hale (*Feedstuffs*, 1967, **39**, 29, 32; *Landbouwdocumentatie*, 1967, **23**, 748).—The effects on growth and feed utilisation of maize and barley were shown in feeding experiments to be greatly improved by steaming the grain at 20lb pressure for 1·5 min. Increase in pressure to 60lb did not improve the results. The increase in growth rate of the cattle was 11%. P. S. ARUP.

**Effect of stage of maturity at harvest on nutritive value of combine-type grain sorghum silage.** C. B. Browning and J. W. Lusk (*J. Dairy Sci.*, 1967, **50**, 81–85).—Relative feeding value of RS 610 combine-type grain sorghum cut at the following three stages of maturity was determined: **(I)** milk to early dough; **(II)** soft to hard dough; **(III)** hard seed. Average silage dry matter % obtained were: **I**, 25·3; **II**, 27·4; **III**, 34·6. Crude fibre decreased and N-free extract (*NFE*) increased with maturity. Daily silage dry matter intake of lactating cows over a 2 year period was 1·64, 1·80, and 1·96 kg per 100 kg body wt. for stages **I**, **II**, and **III** respectively. No significant difference in average daily *FCM* production or in milk fat % was detected. The digestion coeff. for crude protein decreased significantly as maturity increased. (16 references.) M. O'LEARY.

**Comparative feeding values of oat-pea forages ensiled at two stages of maturity.** A. L. Brundage and W. J. Sweetman (*J. Dairy Sci.*, 1967, **50**, 696–699).—The feeding values of oat-pea forages ensiled at early-head and late-milk stages of maturity were compared in feeding trials with lactating dairy cows. *Ad lib.* silage intake was not significantly different for the two silages on a dry matter basis. Daily *FCM* differences did not exceed 0·3 kg/cow/day. No consistent differences in live weight changes were observed. (19 references.) M. O'LEARY.

**Beef cattle breeds.** Anon (*Fmrs' Bull., U.S. Dep. Agric.*, 1968, 2228, 28 pp).—Describes the selection and development of beef cattle breeds, and the characteristics of ten European and six U.S. breeds, also of two dual-purpose breeds of cattle. P. P. R.

**European breeds of cattle.** M. H. French, I. Johannson, N. R. Joshi and E. A. McLauchlin (*F.A.O. Agric. Stud.*, 1966, **I**, No. 67, 389 pp., 1966, **II**, No. 67, 424 pp).—I. This volume deals with breeds that have evolved where the climate is temperate and where feed supplies are generally adequate for the support of high levels of production. The most highly specialised dairy and beef breeds have been developed. Many dual- and triple-purpose breeds were evolved by settlers in the Americas and Australia and N.Z. Regarding the world cattle population as a whole, factual information and its analysis are presented, especially the physiological characteristics of the cattle, their potential genetic capacities, their abilities to withstand various environmental stresses and their possible production levels. (100 references, 170 illustrations).

II. Improvement through breeding is frequently in advance of practical potentials. Body conformation, physiological characteristics and production ability have segregated in the different breeds in conformity with the environmental circumstances with which they have been equilibrated. Breeds and strains have been selected for polygenic, quant. characters with distinguishing external and production traits. Improvements in feeding, housing and management have permitted certain breeds to develop in areas where this would have been impossible 50 years ago. Management in various countries is reviewed. (41 references.) E.M.J.

**Factors limiting animal production: I. Beef cattle.** E. J. Warwick (*Span*, 1967, **10**, 102–105).—The main factors limiting beef cattle production are high temp., parasites (especially in tropical and sub-tropical areas) and shortage of suitable feedstocks. Selective breeding of satisfactory beef-producing cattle is still inadequate, particularly for the hotter regions of the world. Variations in the labour and investment costs obtaining in the U.S. are discussed.
J. L. WALPOLE.

**Performance of a dairy herd under beef herd management.** T. G. Martin and M. W. Alderfer (*J. Dairy Sci.*, 1967, **50**, 1178–1180).—Results of an experiment in which 33 dairy cows were transferred to beef management showed that the correlation (*C*) between previous lactation level under dairy management and 36 h milk production under beef management was 0·27. The *C* between 180-day calf wt. and milk consumption by the calf in 36 h was 0·26; the *C* between previous level of lactation and 180-day calf wt. was 0·16. None of these *C* values were statistically significant. The incidence of mastitis and calf diarrhoea was very low.
M. O'LEARY.

**Nutritive value of dehydrated sweet potato trimmings fed to beef steers.** J. Bond and P. A. Putnam (*J. agric. Fd Chem.*, 1967, **15**, 726–728).—Growth and carcass ratings and ruminal data for steers receiving rations containing 51% of the ground trimmings (instead of maize) were fairly satisfactory, but not equal to results obtained with maize. (22 references.) P. S. ARUP.

**Increased feed intake by steers receiving salts of volatile fatty acids in high-energy rations.** P. J. S. Pieterse and J. A. De Waal (*S. Afr. J. agric. Sci.*, 1967, **9**, 1019–1028).—Supplementation of a maize-meal mixture with the Na salts of these acids greatly improved the performance of the fattening steers. P. S. ARUP.

**Nitrate poisoning of cattle through turnips grown on stubble.** H. A. te Velde (*Landbouwvoorlichting*, 1967, **58**, 250–252).—The incidence of cases of poisoning during Nov. 1966 is attributed mainly to excessive N-manuring of the turnips; an $NO_3$ content in the dry matter of the turnips approaching 3% is considered dangerous. Factors contributing to a high N content are sowing later than mid-July and growth under unfavourable climatic or nutritional conditions. The $NO_3$ content is greatly reduced by ensiling or clamping the turnips. P. S. ARUP.

**Effect of level of urea on utilisation of poor quality roughage by steers.** S. F. Lesch and P. J. S. Pieterse (*S. Afr. J. agric. Sci.*, 1967, **9**, 881–887).—Daily supplementation of the hay with 24 g of urea-N sufficed to secure a positive N-balance and increased the voluntary intake of dry solids. Urea supplied in excess of this amount tended to retard reticulo-rumen functioning and was eventually excreted in the urine. (15 references.) P. S. ARUP.

**Corn and sorghum silage for lactating dairy cows.** P. T. Nordquist and M. G. A. Rumery (*J. Dairy Sci.*, 1967, **50**, 1255–1261).—Comparison of maize with three forage sorghums showed that highest total dry matter production was obtained from the hybrid sorghum FS-22 and that maize produced higher grain yields. Maize was lowest in crude fibre content and highest in crude protein. No significant differences were detected in crude fat or ash content. Feeding trials with lactating dairy cows indicated that excellent milk production may be obtained with maize or forage sorghum. It is suggested that feeding of the latter should be considered in areas where heat or drought stress may occur. (10 references.) M. O'LEARY.

**Influence of stage of maturity at harvest and level of grain feeding on intake of wheat silage [fed to milking cows].** M. E. McCullough and L. R. Sisk (*J. Dairy Sci.*, 1967, **50**, 705–708).—Trials with dairy cattle showed that intake of wheat silage, cut at the early heading stage, was superior to that of later cut silages. When fed as the only feed, or when up to 50% of the silage dry matter was replaced with a grain mixture, this superiority was maintained. M. O'LEARY.

**Effect of dietary fibre level on lactating dairy cows in the humid tropics.** Y. C. Tsai, L. S. Castillo, W. A. Hardison and W. J. A. Payne (*J. Dairy Sci.*, 1967, **50**, 1126–1129).—Feeding trials with dairy cows in the Philippines, showed that feeding of a ration containing 12·5% crude fibre resulted in significant increases in the production of 4% fat-corrected milk and in ruminal propionic acid content and in significant decreases in milk fat content, rectal temp., pulse rate, respiration rate, and ruminal AcOH content as compared with a ration containing 18·5% crude fibre. (14 references.) M. O'LEARY.

**Effect of concentrate level on milk production of cattle grazing high-quality pasture.** J. D. Donker, G. C. Marten and W. F. Wedin (*J. Dairy Sci.*, 1968, **51**, 67–73).—Over a six-year period lactating cows grazed on pasture were given various levels of concentrates to determine effect on milk production. The results indicated that most Holstein cows producing close to 23 kg milk daily could be supported by good pasture alone. Higher levels of production appeared to need supplementation with concentrates. (13 references.) M. O'LEARY.

**Effect of grinding, pelleting, and frequency of feeding of forage on fat percentage of milk and milk production of dairy cows.** G. D. O'Dell, W. A. King and W. C. Cook (*J. Dairy Sci.*, 1968, **51**, 50–55).—Feeding of pelletted lucerne was shown to result in a significant ($P < 0·01$) decline in milk fat % compared with baled lucerne. Feeding of pelletted Coastal Bermuda-grass gave similar results. The results of a further experiment, in which dehydrated Coastal Bermuda-grass was fed as 0·64 cm ground, 0·64 cm ground and pelletted, 0·95 cm ground and pelletted, and 0·16 cm ground and pelletted indicated that the critical grind size in relation to milk fat test depression is about 0·64 cm. (24 references.) M. O'LEARY.

**Occurrence of methyl sulphide in milk from cows fed fresh, dried, or stored alfalfa.** J. R. Dunham, G. Ward, R. Bassette and M. C. Reddy (*J. Dairy Sci.*, 1968, **51**, 44–46).—$Me_2S$ (**I**) concn. in milk and **I** peak heights in blood plasma and rumen fluid from cows fed various lucerne test meals were determined. **I** levels in milk, rumen fluid and blood plasma reached max. ~ 3 h after feeding. Neither oven drying or sun curing reduced **I** production significantly from that obtained with green lucerne forage. Feeding immature lucerne forage resulted in significantly more milk **I** than did feeding bloom-stage lucerne. Feeding lucerne hay stored three months resulted in very low levels of **I** in the milk. M. O'LEARY.

**Comparison of milo and barley for lactating cows. II. Effects of roughage intake and season.** W. H. Brown, A. O. Jareed and J. W. Stull (*J. Dairy Sci.*, 1967, **50**, 700–704).—Results of feeding trials with lactating dairy cows indicates that milk production was not affected by increasing the ratio of milo to barley in the ration from 1 : 3 to 3 : 1. Feed conversion and body wt. gain were greater in animals fed the high barley ration. On both rations, milk fat % was lower in summer than in winter. Reduction of roughage content of the feed from 60 to 40% (by wt.) increased propionate and decreased acetate levels in the rumen during summer. (30 references.) M. O'LEARY.

**Comparison of low moisture and unwilted Coastal Bermuda-grass silages for lactating dairy cows.** W. J. Miller, C. M. Clifton, P. R. Fowler and R. P. Gentry (*J. Dairy Sci.*, 1967, **50**, 1262–1272).—Unwilted Coastal Bermuda-grass silage, containing from 32·7 to 40·2% dry matter, was compared with low-moisture silage, having 58·4 to 76·4% dry matter, in a two year feeding trial with lactating cows. In the first year cows fed wilted silage consumed 1·2 kg/day more forage dry matter but produced 1·2 kg/day less fat-corrected milk ($P = 0·05$) than those fed unwilted silage plus 5% ground barley. The latter also produced milk with 0·12% more solids-not-fat ($P = 0·01$). No significant differences were detected in milk or solids-corrected milk production, weight gains, or level of fat or protein in milk. In the second year no significant differences were detected in any of the above factors. Invisible or gaseous dry matter losses were slightly lower for low moisture silage but there was a great incidence of mouldy material. Low moisture silage had a higher pH but less ammoniacal -N than unwilted silage. (26 references.) M. O'LEARY.

**Varying levels of urea for dairy cows fed corn silage as the only forage.** J. T. Huber, R. A. Sandy, C. E. Polan, H. T. Bryant and R. E. Blaser (*J. Dairy Sci.*, 1967, **50**, 1241–1247).—Trials with Holstein cows indicated that significant decreases in milk yields occurred when urea (**I**) formed 21% or more of ration N. No effect was detected at the 11% level. In general, **I** had no adverse effect on voluntary intake of corn [maize] silage. Rumen valerate and isovalerate contents tended to be depressed when **I** was fed, but acetate and propionate, were not affected. Small, but significant, depressions occurred in dry matter, crude fibre, and N-free extract digestibilities when **I** formed 11% or more of the dietary N. At the 22% level, N recovered in milk, was significantly lower and that in the urine significantly higher than with the 11% level, or the control. (22 references.) M. O'LEARY.

**Effect of high-urea supplementation on feed intake and milk production of dairy cows.** H. H. Van Horn, C. F. Foreman and J. E. Rodriguez (*J. Dairy Sci.*, 1967, **50**, 709–714).—Trials with lactating dairy cows indicated that the addition of 2·2 and 2·7% urea (**I**) and 15·9 and 19·0% maize cobs (**II**) to a concentrate ration significantly depressed feed intake but there was no interaction between **I** and **II**. Milk production was directly related to concentrate intake. Addition of 4·7% molasses did not prevent the reduction in feed intake attributed to the inclusion of **I** in the ration. Further trials indicated that 1% **I** in the concentrate is acceptable to dairy cows. (29 references.) M. O'LEARY.

**Effect of molasses in normal- and high-grain rations on utilisation of nutrients for lactation.** F. G. Owen, D. W. Kellogg and W. T. Howard (*J. Dairy Sci.*, 1967, **50**, 1120–1125).—Experiments with dairy cows showed that addition of 10% molasses (**I**) to the grain in a ration containing grain and wilted silage (66% moisture), in a ratio of 40 : 60 (dry basis) depressed milk and fat-corrected milk (*FCM*) yields and milk fat content. Raising of the grain level to 60% increased milk fat content and *FCM* yield. **I** did not affect dry matter intake or efficiency of estimated net energy utilisation. When wilted silage was replaced by haylage (47% moisture) the added **I** had no detrimental effect. Similar results were obtained when 6% sucrose was added to rations of lucerne hay and 40 or 60% grain indicating that the effect of **I** is *via* its sucrose content. (22 references.) M. O'LEARY.

**Effect of abrupt ration change on rumen micro-organisms and the niacin and vitamin $B_6$ content of rumen fluid and milk.** K. M. Nilson, F. G. Owen and C. E. Georgi (*J. Dairy Sci.*, 1967, **50**, 1172–1176).—Feeding trials with three Holstein cows indicated that the niacin and vitamin $B_6$ content of rumen fluid and milk are altered by abrupt ration changes and that the response is related to the type of ration fed. M. O'LEARY.

**Effect of environmental factors on lactone potential in bovine milk fat.** P. S. Dimick and J. L. Harner (*J. Dairy Sci.*, 1968, **51**, 22–27).—The lactone (**I**) content of butteroil samples (from 276 animals) collected weekly over a 52 week period was found to average 96·0 ppm and 67·2 ppm while the cows were on barn feed and pasture feed, respectively. Analysis of weekly milk samples from an individual Holstein during a 310 day lactation showed that **I** concn. increased from 25–30 ppm following parturition to 170–180 ppm at about 150 days and subsequently decreased. Throughout lactation **I** concn. showed a negative correlation ($P < 1\%$) with fat yield and fat % and a positive correlation ($P < 1\%$) with short-chain fatty acid composition. Analyses of milk from various breeds, on identical feeding regimens, revealed a slightly higher **I** potential in Holstein fat. Ketotic animals were characterised by a marked depression in steam volatile components in the butteroil steam distillate. The data seem to indicate that **I** precursors are biological in origin and that they may be involved in fatty acid synthesis. (19 references.) M. O'LEARY.

**Transfer of fall-out manganese from feed to milk.** D. W. Wilson and G. M. Ward (*J. Dairy Sci.*, 1967, **50**, 592–593).—Transfer coeff. from a lucerne hay diet to milk of $^{54}Mn$ was shown to be $7 \times 10^{-6}$ per l. $^{54}Mn$ was not detected in muscle tissue of dairy cattle but was found in cow liver at levels of 30 to 341 pCi per kg of wet tissue. Observations on $^{137}Cs$ are included. M. O'LEARY.

**Transfer coefficients of fallout caesium-137 to milk of dairy cattle fed pasture, green-cut [alfalfa] or stored feed.** G. M. Ward, J. E. Johnson and L. B. Sasser (*J. Dairy Sci.*, 1967, **50**, 1092–1096).—The transfer coeff. (% intake per litre of milk) of fallout $^{137}Cs$ was found to average 0·35 under pasture feeding in Colorado during the summers of 1962, 1963, and 1964. A mean of 0·41 was found during the winter of 1963 when the cows received lucerne hay, maize silage and a grain mix. When green-cut lucerne was fed in the summer of 1965 a mean value of 0·25 was obtained. The level of crude fibre intake was not related to transfer coeff. (14 references.) M. O'LEARY.

**Effect of ethanol infusion on milk fat content and composition and on volatile fatty acids in the rumen liquor.** E. R. Orskov, R. W. Hemken and L. A. Moore (*J. Dairy Sci.*, 1967, **50**, 692–695).—Infusion of ~ 875 g ethanol per day into the rumen of lactating dairy cows, on a pelleted ration of 20% lucerne hay and 80% concentrate, resulted in increases in milk-fat content and in increased proportion of acetic, isovaleric, and valeric acids in the rumen liquor. Up to 7 molar % caproic acid appeared and the proportion of propionic acid was depressed. Blood ethanol increased from a trace to 5 mequiv/l. (10 references.) M. O'LEARY.

**Effect of milking throughout pregnancy on milk secretion in the succeeding lactation.** A. Smith, J. V. Wheelock and F. H. Dodd (*J. Dairy Res.*, 1967, **34**, 145–150).—Experiments with five Friesian cows showed that quarters regularly milked throughout pregnancy produced less milk in the next lactation than quarters allowed a normal dry period. Milking during pregnancy did not affect milk composition. (12 references.) M. O'LEARY.

**Effect of a temporary suspension of milking in mid-lactation on milk secretion after the resumption of milking and in the following lactation.** J. V. Wheelock, A. Smith and F. H. Dodd (*J. Dairy Res.*, 1967, **34**, 151–161).—Suspension of milking of two quarters of each of four Friesian cows for 2 weeks caused a reduction in milk yield when milking was resumed. However, in the following lactation, the yield from the quarters, rested in the first lactation, was greater than that from the quarters milked continuously. Suspension of milking caused an increase in Na and Cl contents of the subsequent milk and a decrease in the lactose and K content for an 8 week period; after this milk composition returned to normal. M. O'LEARY.

**Liver vitamin $B_{12}$ status of the lactating dairy cow.** K. A. Wilson, J. M. Elliot and M. M. Mathias (*J. Dairy Sci.*, 1967, **50**, 1280–1282).—Vitamin $B_{12}$ values of 41 samples of liver homogenates from 31 dairy cows were found to range from 2·8 to 6·6 $\mu$g per g of protein. A significant part ($P < 0·05$) of the variation was accounted for by the multiple regression of liver vitamin $B_{12}$ on

days fresh milk production to date during lactation, daily milk yield, and the interactions of these variables. Five of seven cows sampled during early lactation, and again at variable intervals, but within the first 180 days of lactation showed a higher liver vitamin $B_{12}$ level at the second sampling. Liver and blood vitamin $B_{12}$ levels were found to be poorly correlated. M. O'LEARY.

**Fish flour as a protein source in calf milk replacers.** J. T. Huber and L. M. Slade (*J. Dairy Sci.*, 1967, **50**, 1296–1300).—The results of trials with Holstein calves showed that average daily gains and feed efficiencies were not significantly depressed when fish flour furnished up to 40% of dietary protein. At the 60 to 67% level significant ($P < 0{\cdot}01$) decreases were detected and at the 100% level death occurred. (21 references.) M. O'LEARY.

**Influence of supplements of protein and non-protein nitrogen to winter veld grass on wool growth.** C. G. Coetzee and P. J. S. Pieterse (*S. Afr. J. agric. Sci.*, 1967, **9**, 889–898).—As regards feed utilisation, growth, and wool production, supplements of fish meal proved more efficient than N-equiv. supplements of urea plus maize meal. (20 references.) P. S. ARUP.

**Permissible restriction of feed intake in digestion trials with roughages.** J. B. J. Van Ryssen and W. J. Stielau (*S. Afr. J. agric. Sci.*, 1967, **9**, 1005–1010).—Reduction of the feeding level of wethers below voluntary intake did not affect the outcome of digestibility trials. The N-digestibility was, however, greater at high than at low feeding levels. (14 references.) P. S. ARUP.

**Some effects of feeding sheep on low-sodium hay with and without sodium supplement.** D. I. H. Jones, D. G. Miles and K. B. Sinclair (*Br. J. Nutr.*, 1967, **21**, 391–397).—After a preliminary period on pelletted perennial ryegrass hay (0·28% Na on dry matter) groups of sheep were fed on timothy hay (**I**) (0·04% Na) with and without added NaCl or $NaHCO_3$. Feeding **I** lowered urinary and faecal Na and balances became negative; Na content of saliva fell markedly and K content increased. Supplementary Na prevented these changes but low levels of dietary Na appeared to have no effect on the well-being of the sheep. (12 references.) C.V.

**Effects of feeding frequency on energy and nitrogen balance in sheep given a ground and pelletted forage.** N. McC. Graham (*Aust. J. agric. Res.*, 1967, **18**, 467–483).—Wether sheep were fed at intervals of 3 h, 24 h, and 4 days on a diet consisting of ground and pelletted lucerne containing 19% crude protein and 28% crude fibre. Results demonstrated appreciable improvements in both digestive and metabolic efficiency at the 3 h feed rate. There was evidence that the lucerne underwent a rapid initial fermentation, causing an intense phase of lipogenesis immediately after feeding. E. G. BRICKELL.

**Nitrogen balance studies with the milk-fed lamb. IV. Effect of different nitrogen and sulphur intakes on live-weight gain and wool growth and on nitrogen and sulphur balances. V. Effect of frequency of feeding. VI. Effect of starvation and realimentation.** D. M. Walker, L. J. Cook (IV and V) and K. T. Jagusch (V) (*Br. J. Nutr.*, 1967, **21**, 237–256; 275–287; 289–308).—IV. Sixteen male cross-bred lambs were given four diets, the protein content on dry matter bases being 6·1, 11·9, 17·5 and 22·9%. The mean digestibilities of energy, N, ether extractives and dry matter significantly increased as protein content increased; live-wt. gain also increased and the N and S balances increased with increasing intake of apparently digested N and S. All differences between the means of individual diets were highly significant. There was a significant correlation between N balance and live-wt. gain for each diet but when compared with the same rate of gain, N balance increased as protein content increased. Wool growth increased with increase of protein content but there was no significant difference in the N and S content of the wool, the mean value being 15·7% N and 2·87% S; % of fat in carcass decreased and % of protein increased with increased protein content of diet but moisture and ash were not affected. The % of moisture in liver, pancreas and muscle decreased while % of protein in the liver and muscle increased with increasing dietary protein. (25 references.)

V. Thirty-three cross-bred lambs were given reconstituted dried whole cow's milk. Feeding frequency had no effect on live-wt. gain, N retention or total body composition but lambs given two feeds daily had significantly heavier abomasums than pair-fed lambs given six feeds daily. Wt. of fat, protein ($N \times 6{\cdot}25$) and water in the body were closely related to empty body wt. but body composition (% of empty body) was not significantly affected by level of milk intake or growth rate. (41 references.)

VI. Five male cross-bred 3-week old lambs were starved for four days. The findings are discussed but give reasonable agreement with those found for the calf, except for a marked ketone acidosis and considerable increase in excretion of purine N. Losses during starvation differed from those with the adult sheep in showing a relatively greater loss of body protein. (34 references.) C.V.

**Effects of level of intake and other factors on digestibility of Climax timothy hay.** M. R. Hogan, B. W. Henderson, E. R. Berousek, R. C. Wakefield and R. W. Gilbert (*J. Dairy Sci.*, 1967, **50**, 86–89).—Results of total-collection digestion trials with sheep showed that *ad lib.* feeding plus 10% refusal of Climax timothy hay resulted in a significant depression in energy digestibility compared to two limited feeding rates. Voluntary consumption of early harvested forage was significantly higher than that of late harvested forage. Energy digestibility decreased significantly with increase in maturity of the hay at harvesting. Forage composition was not significantly affected by level of N fertilisation. M. O'LEARY.

**The effect of equal and unequal intervals in twice-daily milking on the milk yield of ewes.** R. Morag and S. Fox (*J. Dairy Res.*, 1967, **34**, 163–167).—Experiments with milch ewes indicated that variation of intervals between milking from 12 h to 8 and 16 h had no effect on milk yield or fat content. (20 references.) M. O'LEARY.

**Regulation of food intake in ruminants. V. Effect of amphetamine administered intraruminally and intravenously to sheep and goats.** B. R. Baumgordt (*J. Dairy Sci.*, 1967, **50**, 1176–1178).—Intraruminal administration of up to 2·1 mg *d*-amphetamine sulphate per kg body wt. had no effect on feed intake of either sheep or goats. Intravenous injection of 2·5 mg *dl*-amphetamine phosphate significantly reduced feed intake by both species. M. O'LEARY.

**Nutritional effects of autoxidised fats in animal diets.** J. L. L'Estrange, K. J. Carpenter, C. H. Lea and L. J. Parr (*Br. J. Nutr.*, 1967, **21**, 377–390).—In two experiments 100 pigs were fed from age 4–5 weeks to 16–19 weeks on practical-type diets containing 10% meat meal (15–17% lipid) of either low (3–17) or high (114–150 $\mu$moles/g lipid) peroxide value. The cereal basis of the diet in the first experiment was wheat and maize; in the second barley. Other variants were the inclusion of a stabilised vitamin E supplement, the presence of 100 or 250 ppm Cu and the storage of the diet for 6–21 weeks before commencing the experiment. Apart from the occurrence of an unusually high incidence of stomach ulcers (equally distributed between the control and oxidised fat groups) which did not appear to affect health or nutrition the pigs grew well with no significant difference in wt. gain, food conversion ratio, liver wt., vitamin A storage in liver, etc. Other observations are recorded. (19 references.) C.V.

**Some factors affecting the biological availability of phosphorus in wheat by-products.** J. D. Summers, S. J. Slinger and G. Cisneros (*Cereal Chem.*, 1967, **44**, 318–323).—The results of *in vitro* and *in vivo* experiments are reported. Autoclaving of wheat bran, shorts, middlings and germ is shown to result in a marked decrease in their content of phytin P (**I**). The P requirement of chicks for max. growth, efficiency of feed utilisation and bone ash formation were lower when either the complete diet or the wheat bran portion of the diet, was steam-pelletted. The results suggest that a significant proportion of **I** of wheat bran can be made biologically available by steam-pelletting. (11 references.) E. C. APLING.

**Effect of lactose on egg production and shell quality.** S. Hurwitz, A. Bar and S. Bornstein (*Poult. Sci.*, 1967, **46**, 1024–1025).—Addition of 5% lactose (in the form of skimmed milk powder) to the diet of hens receiving either normal (3%) or low (2%) Ca diets had no effect on egg production, egg wt. or shell wt. per unit area. Egg production and shell wt. per unit area were lower with 2% than with 3% dietary Ca. A. H. CORNFIELD.

**Effects of forest sprayings with insecticides.** [A] **1952–63: Fish and aquatic invertebrates in New Brunswick streams: introduction and summary.** [B] **Fish losses, 1952–62, as shown by caged specimens and other observations.** C. J. Kerswill and H. E. Edwards [B] (*J. Fish Res. Bd Can.*, 1967, **24**, 701–708; 709–729).—[A] The spray programme developed to counteract an outbreak of the spruce budworm (*Choristoneura fumiferana*) in New Brunswick is described. Details of a research programme for the affected fisheries, mainly for Atlantic salmon (*Salmo salar*), are given. The main effects of insecticide spraying on caged young salmon and trout (*Salvelinus fontinalis*), on aquatic insect production, on feeding habits of native young salmon and on population levels of young salmon are described. (20 references.)

[B] The survival of young Atlantic salmon and eastern brook trout, held in cages and free-living, was observed in New Brunswick

streams inside and outside forested areas sprayed operationally and experimentally from aircraft with DDT and other insecticides for budworm control. DDT-in-oil at 0·5 lb/acre caused heavy loss of underyearling salmon and parr within 3 weeks. DDT-in-oil at 0·25 lb/acre, DDD at 0·5 and 0·25 lb/acre and malathion at 0·125 lb/acre had no apparent short term effects on salmon parr but killed many underyearlings. Experimental spraying of phosphamidon-in-water at 1 lb/acre had no apparent harmful effects. Wild young salmon were found dead in streams when autumn water temp. approached freezing after June sprayings of water sheds with DDT. S. A. BROOKS.

**Pyrimidines. V. Some higher 5-substituted 2,4,6-trichloropyrimidines.** H. Gershon, R. Parmegiani and R. D'Ascoli (*J. medn1. Chem.*, 1967, **10,** 113; *Contr. Boyce Thompson Inst. Pl. Res.*, 1966, 2068, 3 pp).—The compounds were screened, but proved inactive, against four animal tumours, lymphoid leukemia L.1210, lymphosarcoma P.1789 or sarcoma 180, Dunning ascites leukemia, adenocarcinoma 755 or Lewis lung carcinoma, and Walker 256 carcinosarcoma. Cytotoxicity in the KB cell culture system reached a max. when the side chain was propyl and diminished with increasing chain length. Branching of the side chains yielded less toxic products than the corresponding, straight-chain, parent compounds. E. G. BRICKELL.

**New broad-spectrum anthelmintic, methyl 5(6)-butyl-2-benzimidazolecarbamate.** P. Actor, E. L. Anderson *et al.* (*Nature, Lond.*, 1967, **215,** 321–322).—The compound (**I**) (m.p. 224°–227°, decomp.) is prepared by reaction of 4-butyl-*o*-phenylenediamine with carbomethoxycyanamide in boiling 2-propanol. Data are reported for the excellent therapeutic activity of **I** (10–50 mg per kg per day) in mice infected with *Syphacia, Nematospiroides* or *Ascaris* and in sheep and cattle infected with *Haemonchus, Ostertagia, Trichostrongylus, Strongyloides, Cooperia, Nematodirus, Oesophagostomum* and *Chabertia*. In nearly all instances there was > 90% removal of the infection by one or more doses. **I** was just as effective in swine infected with mature *Ascaris suum, Strongyloides ransomi* and *Trichuris suis*, and in chickens infected with *Ascaridia* and *Heterakis*. With sheep and pigs **I** and its metabolites were almost entirely excreted, the tissue residues containing < 0·1 ppm 16–21 days after oral administration of 45–50 mg per kg. W. J. BAKER.

**Effects of oxytocin administered during the dry period on the succeeding lactation.** G. M. Gorman and E. W. Swanson (*J. Dairy Sci.*, 1968, **51,** 60–66).—Intravenous injection of 5 IU oxytocin twice daily during a 60 day dry period resulted in a significant ($P < 0·01$) reduction in the milk yield of 7 cows compared with that of 7 control animals. (16 references.) M. O'LEARY.

**Passage of pentobarbital and phenobarbital into bovine and caprine milk after systematic administration.** G. E. Miller, R. D. Peters, R. V. Engebretsen and C. M. Stowe (*J. Dairy Sci.*, 1967, **50,** 769–772).—Results of experiments with lactating cows and goats indicated that, under equilibrium conditions, pentobarbital passes into the milk of these animals in accord with pH partition concepts. It appeared that phenobarbital also enters the milk of the goat via passive diffusion. (13 references.) M. O'LEARY.

**Estimation by gas chromatography of 5β-pregnane-3α, 20α-diol in the urine of normal lactating and ketosed cows.** R. J. Heitzman (*J. Dairy Res.*, 1967, **34,** 21–25).—Mean pregnane diol (**I**) level in the urine of normal lactating cows was found by gas chromatography to be 30·7 μg/l. Experimentally induced ketosed cows were found to have a mean level of 22·3 μg/l urine and primary spontaneously ketosed cows 31·4 μg/l. The difference in urine **I** levels of the three groups was not significant. (16 references.) M. O'LEARY.

**Performance and iodine metabolism of dairy cattle with iodine irradiation injury.** J. K. Miller and E. W. Swanson (*J. Dairy Sci.*, 1967, **50,** 90–95).—Administration of a single oral dose of 160–120 μc $^{131}$I per kg body wt. to growing heifers caused blood plasma $^{125}$I concn. to rise three-fold within 10 days. Urinary and faecal excretion of $^{125}$I also increased. Plasma $^{125}$I concn. returned to control values within a further 10 days. After 6 months a marked reduction in thyroid activity was indicated by a decrease of 64% in plasma protein-bound iodine, 80% in thyroid $^{131}$I uptake, and 56% in thyroid secretion rate, Three cows which had received 100 mc of $^{131}$I calved 15, 123, and 136 days later. The first calf was a cretin without detectable thyroid activity, the second calf died on birth, and the third appeared to be normal. (13 references.) M. O'LEARY.

**Purification of bovine plasma arylesterase.** S. S. Choi and T. L. Forster (*J. Dairy Sci.*, 1967, **50,** 1088–1091).—A description is given of a procedure for preparing bovine plasma arylesterase in pure form. (16 references.) M. O'LEARY.

**Structure, biochemical properties and origin of the aflatoxins $B_{2a}$ and $G_{2a}$.** M. F. Dutton and J. G. Heathcote (*Chemy Ind.*, 1968, 418–421).—Gives a more detailed account of the work reported previously (*Idem, Biochem. J.*, 1966, **101,** 21P), with emphasis on methods used to distinguish aflatoxins $B_{2a}$ (**I**) and $G_{2a}$ (**II**) from aflatoxins $M_1$ and $M_2$. These methods included spectrometric analyses of **I, II** and their deriv. (acetyl, $Me_3$ silyl ether, etc.) and also proton magnetic resonance absorption. Mol. wt., m.p., extinction max. and p.m.r. spectra are listed. The evidence confirms that **I** and **II** are hydroxyl deriv. of aflatoxins $B_2$ and $G_2$, respectively; since **I** and **II** are produced by treatment of the more toxic aflatoxins ($B_1$, $G_1$) with cold, dil. aq. mineral acid, this would appear to be a method of reducing toxicity in aflatoxin-contaminated feedstuffs. W. J. BAKER.

**Isolation of salmonellae from animal feeding stuffs.** R. W. S. Harvey and T. H. Price (*J. Hyg., Camb.*, 1967, **65,** 237–244).—A technique is described for routine isolation of salmonellae from meat and bone meal samples; this more than doubles the number of positives obtained by an orthodox method. Primary and secondary enrichment is employed and the number of isolations are compared. (19 references.) C.V.

**Chemical composition of the cell-wall constituent and acid detergent fibre fractions of forages.** M. W. Colburn and J. L. Evans (*J. Dairy Sci.*, 1967, **50,** 1130–1135).—Cellulose plus lignin was shown to constitute from 51 to 69% of the total cell-wall constituent of 14 grasses, four lucernes and three lucerne–grass mixtures. The acid detergent fibre (*ADF*) fractions of the forages consisted of 79% cellulose. Lignin content of the grasses *ADF* (10·6%) was significantly lower than that of the lucernes and lucerne–grass mixtures (16·3%). (22 references.) M. O'LEARY.

**Difference in composition between crude fibre and acid-detergent fibre.** J. T. Kim, J. T. Gillingham and C. B. Loadholt (*J. Ass. off. analyt. Chem.*, 1967, **50,** 340–343).—Fibre separated by the A.O.A.C. method (*Official Methods of Analysis*, 10th ed., 1965, 22.038–22.042) retained the original cellulose but lost 60–84% of the original lignin and 80–86% of the pentosans. Fibre separated by the acid-detergent method retained all the lignin and cellulose but lost 82–84% of the original pentosans. The procedure of Van Soest and Marcus (*J. Dairy Sci.*, 1964, **47,** 704) is preferred. A. A. ELDRIDGE.

**Determination of xanthophyll [in hay].** G. O. Kohler, R. E. Knowles and A. L. Livingston (*J. Ass. off. analyt. Chem.*, 1967, **50,** 707–711).—For the precise determination of carotene and xanthophyll in hay or lucerne rigid adherence to the recommended procedure is required. The chromatographic column is composed of MgO and diatomaceous earth (1 : 1) in a Pyrex glass tube 30 cm × 12·5 mm. Fresh material is blended with acetone, dried material is soaked in hexane–acetone (7 : 3). Carotene passes through the column; xanthophylls are eluted with hexane–acetone –methanol (80:10:10). Chlorophyll can be eliminated by saponification with methanolic KOH in the cold before chromatographing. A. A. ELDRIDGE.

**Assay for neomycin in feed.** A. R. Barbiers and A. W. Neff (*J. Ass. off. analyt. Chem.*, 1967, **50,** 462–467).—The use of tris (hydroxymethyl) aminomethane at pH 8 as buffer instead of phosphate buffer is recommended. Yields are improved by saturation with 20% aq. NaCl. The addition to the buffer of $CaCl_2$ or $MgCl_2$ enhances neomycin activity. The A.O.A.C. method (*Official Methods of Analysis*, 10th ed., 1965, 33.112–33.117) was used. Recoveries ranged from 26 to 113%. A. A. ELDRIDGE.

**Microbiological assay for low chlortetracycline concentrations in final feeds.** A. Abbey (*J. Ass. off. analyt. Chem.*, 1967, **50,** 446–449).—Collaborative results (cf. Abbey and Hewel, *ibid.*, 1965, **48,** 271) were subject to a coeff. of variation of 9·6% for a single assay in a single laboratory. A. A. ELDRIDGE.

**[Determination of] oxytetracycline in feed.** D. C. Billman, jun. and H. Clark (*J. Ass. off. analyt. Chem.*, 1967, **50,** 454–457).—Minor modifications in the A.O.A.C. method (*Official Methods of Analysis*, 10th ed., 1965, 33.152–33.154) led to recoveries of 100 to 121%. At the levels used there was no significant difference in recoverability for mineral and non-mineral feeds. The absorption column method of preparing a compensating curve is not recommended. A. A. ELDRIDGE.

**Microbiological assay of lincomycin in animal feed.** A. W. Neff, A. R. Barbiers and J. I. Northam (*J. Ass. off. analyt. Chem.*, 1967, **50**, 442–446).—Collaborative results, obtained by the A.O.A.C. method at levels of ~4 g per ton, were satisfactory, recoveries being from 95·3 to 113·3%. A. A. ELDRIDGE.

**Microbiological assay of procaine penicillin in feeds.** J. J. Mayernik (*J. Ass. off. analyt. Chem.*, 1967, **50**, 450–454).—By incorporating a phosphate buffer into the aq. acetone used for extraction, the activity of the penicillin can be stabilised. Collaborative results gave recoveries of 91·2 to 101·7% (cf. Mayernik and Fiori, *ibid.*, 1965, **48**, 268). A. A. ELDRIDGE.

**Determination of sulphamethazine in mixed feeds containing procaine penicillin.** V. B. Hill and E. E. Martin (*J. Ass. off. analyt. Chem.*, 1967, **50**, 42–45).—To eliminate interference by procaine, protein is pptd. from the methanol extract and the azo-dyes are produced by the Bratton-Marshall reaction. The mixed dye solution is shaken with $CHCl_3$, made alkaline, and reshaken; the aq. phase containing the sulphamethazine dye is acidified and the extinction is measured at 540 nm. Very good recoveries are reported. A. A. ELDRIDGE.

**Determination of piperazine in feeds.** M. F. Loucks and L. Nauer, jun. (*J. Ass. off. analyt. Chem.*, 1967, **50**, 268–273).—An aq. extract of the sample is treated at 80° with ethanolic *p*-benzoquinone acidified with AcOH (Cavett and Heotis, *ibid.*, 1955, **41**, 323) and, after cooling, the extinction at 490 nm is measured. Average recoveries of 94 to 100 % are reported. A. A. ELDRIDGE.

**Determination of residual plant preservatives: Procedure for concentration and determination of 9-hydroxyfluorene carboxylic acid 9-n-butyl ester in straw.** K. Fickentscher and E. Jackwerth (*Z. analyt. Chem.*, 1967, **227**, 23–30).—The material is separated from straw by $CHCl_3$ extraction and concentrated by preparative TLC. After saponification and oxidation the fluorenone formed is condensed with *p*-nitrophenylhydrazine to give the *p*-nitrophenylhydrazone; this after purification, is reacted with methanolic KOH to give a stable blue compound, the absorption of which is measured at 597 nm. (10 references.) P. N. R. NICHOLS.

**Ultra-violet spectrophotometric and gas chromatographic methods for [determining] Ronnel in feeds.** A. J. Gehrt (*J. Ass. off. analyt. Chem.*, 1967, **50**, 257–261).—The Ronnel is (*a*) extracted with $CHCl_3$ for u.v. spectrophotometry or (*b*) extracted with acetone for electron capture gas chromatography, the extract in either case being cleaned up on a Florisil column. In collaborative work average recoveries were (*a*) 98, 105%, (*b*) 92, 95%. A. A. ELDRIDGE.

**Colorimetric method for [determining] Nitrodan in feeds.** D. V. Bloom (*J. Ass. off. analyt. Chem.*, 1967, **50**, 261–263).—The sample is ground with a mixture of dimethylformamide and ethanol (1 : 1), the extract is made slightly alkaline with NaOH, and the extinction is measured at 575 mμ. Recoveries ranged from 90·7 to 106·3%. A. A. ELDRIDGE.

**Determination of Buquinolate in chicken feeds by thin layer chromatography and fluorometry.** H. Borfitz, J. Para, J. V. Stickles, G. B. Ginther and B. C. Southworth (*J. Ass. off. analyt. Chem.*, 1967, **50**, 264–268).—Buquinolate is extracted into $CHCl_3$, the conc. extract is subjected to TLC on $SiO_2$ gel, and an 80% aq. ethanolic extract of the spot is irradiated at 265 nm, the emission at 375 nm being measured. Recoveries of 93·2 to 108·1% are reported. A. A. ELDRIDGE.

**Specific determination of nitrofurans in feeds containing interfering pigments.** G. F. Bories (*J. agric. Fd Chem.*, 1967, **15**, 217–220).—Extraction of nitrofurans is carried out with a mixture of dimethylformamide (**I**) and water (2 : 1 by vol.). The furans are converted into 5-nitrofurfuraldehyde phenylhydrazones (**II**) which are extracted with toluene and purified by passing through $SiO_2$, then through $Al_2O_3$, and eluting with toluene followed by **I**. The eluate containing **II** (red band) is treated with KOH to form a blue coloration which is measured at 630 mμ (accuracy ±3%). The nitrofurans can be separated into two groups, one in which the semicarbazide function is destroyed by treatment with $I_2$ in warm solution at pH 8, and a second group which continues to form phenylhydrazone after the above treatment. In this way furazolidone and nitrofurazone can be determined separately (accuracy ±6%). P. S. ARUP.

**[Determination of] minerals in feeds by atomic absorption spectrophotometry.** M. Heckman (*J. Ass. off. analyt. Chem.*, 1967, **50**, 45–50).—Satisfactory results for Ca and Mg were obtained, but for Zn, Mn, Fe and Cu the coeff. of variation were unsatisfactory. A. A. ELDRIDGE.

**Colorimetric determination of urea in feeds.** T. J. Potts (*J. Ass. off. analyt. Chem.*, 1967, **50**, 56–58).—The *p*-dimethylaminobenzaldehyde colorimetric method has been modified by taking the spectrophotometer reading at 420 instead of 440 mμ and using a reference standard with each set of samples. A. A. ELDRIDGE.

**Cobalt loss by dry ashing [of feeds] in porcelain crucibles.** D. L. Mays, W. V. Kessler, J. A. Chilko and E. D. Schall (*J. Ass. off. analyt. Chem.*, 1967, **50**, 735–737).—Radiochemical measurements using $^{60}Co$ showed that ashing of feed samples is accompanied by a much smaller loss of Co than is ignition of $CoCl_2$ derived from its aq. solution. This is attributed to the Co being retained in the ash whereby contact with the porcelain surface is prevented. A. A. ELDRIDGE.

**Fate of plant regulator 2,3,5-tri-iodobenzoic acid (TIBA) in the bovine.** W. H. Gutenmann, C. A. Bache and D. J. Lisk (*J. agric. Fd Chem.*, 1967, **15**, 600–604).—After a cow had been dosed daily with 5 ppm of TIBA in the feed, TIBA and 2,5-di-iodobenzoic acid (**I**) (free and combined) were found in the urine, but not in the milk or in the faeces. TIBA was rapidly decomposed into **I** and other products when exposed to sunlight but remained stable when incubated with rumen fluid, beef liver or beef thyroid. P. S. ARUP.

**Secretion of DDT by lactating cows fed thyroprotein.** J. W. Stull, W. H. Brown, F. M. Whiting, L. M. Sullivan, M. Millbrath and J. M. Witt (*J. Dairy Sci.*, 1968, **51**, 56–59).—Six lactating cows were intravenously administered DDT (rate ≡4 ppm dietary intake) for 14 consecutive days. After a ten-day decline period three of the animals were given a thyroactive casein at the rate of 1 g/11 kg body wt. for 28 consecutive days. Body wt. decreased and milk and milk fat yields increased in the animals receiving the thyroprotein. The yield figures declined below the values in the control group after thyroprotein feeding ceased. After reaching 30 ppm during administration, DDT content of the milk declined to about 7 ppm for both groups prior to thyroprotein feeding. DDT content of the milk of the control animals declined steadily to 2 ppm over 12 weeks whereas the content in the experimental group only reached 4 ppm. (11 references.) M. O'LEARY.

**Danger of chlorinated hydrocarbon insecticides in birds' eggs.** J. H. Koeman, R. C. H. M. Oudejans and E. A. Huisman (*Nature, Lond.*, 1967, **215**, 1094–1096).—Reports and discusses (*i*) concn. of dieldrin (**I**) in blood of embryos and in chicks hatching from eggs injected with 240 μg of **I** before incubation, (*ii*) **I** residues in blood, yolk and body of 14-day embryos and 0–6-h chicks developing from eggs dosed with 40 μg of **I**, and (*iii*) concn. of **I** in blood of starved chicks hatched from **I**-dosed eggs. Results (cf. Dunachie and Fletcher, *ibid.*, 1966, **212**, 1062), show that absorption of the yolk after hatching can cause poisoning of young birds by insecticides at a concn. of the residue which does not affect the rate of hatching. This toxicity is especially hazardous when recently hatched chicks are deprived of food for long periods and then rapidly absorb the remaining yolk. W. J. BAKER.

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**Iyomycin B and its manufacture.** Kitasato Institute. (B.P. 1,073,531, 7.7.64. Japan, 19.7.63).—Iyomycin B, a new tumour-inhibiting antibiotic, is produced by aerobic fermentation of an aq. nutrient medium, (pH 7) in presence of a strain of *Streptomyces phaeoverticillatus* at 25–35° during 2–5 days. The antibiotic, extracted from the broth by pptn. or by extraction with e.g., MeOH, can be separated into five fractions by chromatography on acidified $Al_2O_3$. F. R. BASFORD.

**Alkenyloxypyridones.** Upjohn Co. (B.P. 1,065,657, 13.8.64. U.S., 16.9.63).—2-Oxo-1,2-dihydropyridines (and acid-addition salts thereof) substituted in the 1-position by $C_{3-8}$-alk-2-enyloxy and optionally elsewhere by Me are produced by interaction of a 2-$C_{1-3}$-alkoxypyridine-*N*-oxide with an alk-2-enyl halide at 25–125° during 1–10 h. They are useful in the treatment of topical fungal infections in mammals (humans, cattle, etc.) caused by *Trichophyton rubrum*, inhibit growth of *Pseudomonas fluorescens*, and also have sedative and muscle relaxant properties. In an example, a mixture of 2-ethoxypyridine-1-oxide and allyl bromide is boiled during 3·5 h, then distilled, to give 1-allyloxy-2-oxo-1,2-dihydropyridine, b.p. 89–92°/0·1 mm, $n_D^{20}$ 1.5442. F. R. BASFORD.

**Complexes of benzimidazoles with thiobisphenols.** Merck & Co. Inc. (B.P. 1,056,022, 2.4.64. U.S., 8.1.64).—The claimed, anthelmintics are obtained by reacting a substituted benzimidazole (**I**) with a thiobis(halophenol) (optionally containing $NO_2$). **I** are substituted (optionally) in the benzene ring (5- and 6-positions) with $C_{1-5}$ alkyl, alkoxy, $CF_3$; in the imidazole ring (1-position)

by $C_{1-5}$ alkyl or $C_{2-5}$ alkenyl (optionally) and in 2-position by (iso)thiazolyl, thiadiazolyl, pyrryl, furyl, thienyl or Ph. E.g., a mixture of 2-(4′-thiazolyl)benzimidazole and 2,2′-thiobis(4,6-dichlorophenol) in MeOH is refluxed for 5 min. Upon cooling the adduct crystallises and is dried at 50°. The products are active against tapeworm, roundworm and fluke in sheep, cattle, horses, etc. C.V.

**Thiocarbanilide.** CIBA Ltd. (Inventor: H. Martin) (B.P. 1,069,850, 21.12.65).—The claimed 3,5-bis(trifluoromethyl)-4′-nitrothiocarbanilide (**I**), with valuable anthelmintic properties, is obtained from **3,5-bis(trifluoromethyl)phenyl-isothiocyanate (II)** or -amine and *p*-nitroaniline (**III**) or *p*-nitrophenylisothiocyanate, respectively. Thus, **II** and **III** in $Me_2$-formamide are heated for 4 h at 80°, cooled to room temp. and water added to ppt. **I**, of m.p. 175–177° (aq. $Me_2$-formamide). It can be used in conjunction with starch, colloidal $H_2SiO_3$, gelatin, arrowroot, Mg stearate and talcum to make grooved tablets for pharmaceutical and veterinary application. S. D. HUGGINS.

**Pesticide compositions for destroying internal worm parasites in animals.** Stecker International S.p.A. (Inventor: [B] E. Lienert) (B.P. 1,079,177–8, [A] 2.12.63 [B] 27.7.66. U.S., [A] 11.6.63).—Compositions for use in the control of e.g., lung and liver fluke in animals contain as active agent compounds of formula 2,3,4,5,6-OH·$C_6$ $R^{III}$ $R^{IV}$ $R^{V}$ $R^{VI}$ CO·NH·$C_6$ $X^{II}$ $X^{III}$ $X^{IV}$ $X^{V}$ $X^{VI}$-1,2,3,4,5,6, wherein [A] $R^{III}$-$R^{VI}$ are H, Cl, Br, or I, or $R^{IV}$- is $CF_3$ or $NO_2$ or $R^{V}$ and $R^{VI}$ are F; and $X^{II}$-$X^{V}$ are H or $CF_3$, or $X^{II}$ and $X^{VI}$ are $OCF_3$, or $X^{VI}$ is H, or $X^{II}$ and $X^{IV}$ are Cl, Br, or I, or $X^{II}$ is $SO_2Et$, there being at least one directly-connected halogen and ⩾ 2 substituents on the salicyl nucleus and ⩾ 3 substituents in the anilide nucleus—e.g., 3,5,4′-tribromosalicylanilide; [B] $R^{IV}$, $R^{VI}$, $X^{II}$, $X^{III}$, $X^{V}$, and $X^{VI}$ are H, and $R^{III}$, $R^{V}$, and $X^{IV}$ are I or one of them is Cl or Br, e.g., 3,4,5-tri-iodosalicylanilide. F. R. BASFORD.

**4-Hydroxy-6,7-disubstituted-3-carbalkoxyquinolines.** Norwich Pharmacal Co. (B.P. 1,065,567, 8.12.65. U.S., 24.2.65).—The title compounds, of use in the control of coccidiosis in poultry (the 6- and 7-substituents being alkoxy of 3–4 C), are prepared in good yield by acid-hydrolysis of corresponding 4-chloro analogues (new substances). Thus, a mixture of $Me_2$ 3,4-diisopropoxyanilinomethylenemalonate and $POCl_3$ is heated at 95° during 2 h, then excess of $POCl_3$ is distilled off. The residue is diluted with MeOH, the solution poured into water and neutralised with 30% aq. NaOH to give a ppt. of Me 4-chloro-6,7-diisopropoxyquinoline-3-carboxylate, m.p. 109–110° (MeOH). This is boiled with MeOH containing 5 drops of conc. aq. HCl during 460 min., then the cooled solution is poured into water, with pptn. of crude Me 4-hydroxy-6,7-diisopropoxyquinoline-3-carboxylate in 81% yield. F. R. BASFORD.

**Preparations containing 3-(5-nitro-2-furfurylideneamino)-2-oxazolidone.** C. F. Boehringer and Soehne G.m.b.H. (B.P. 1,065,708, 25.3.66. Ger., 27.3.65).—The title compound (for use in the prophylaxis of fowl pest, etc.) in admixed with a powdered wetting agent, e.g., $C_{12}H_{25}C_6H_4SO_3Na$ (Maranil) 0·1–10 and a powdered dispersing agent, e.g., Na lignin sulphonate (Darvan 2) 0·1–10 wt.-%, to provide a composition which forms a stable dispersion in water. F. R. BASFORD.

**Derivatives of nitrofuran.** Norwich Pharmacal Co. (B.P. 1,067,992, 16.12.63. U.S., 27.12.62).—Compounds claimed comprise 1-R-3-(5-nitrofur-2-yl)-5-$R^{I}$-1,5-dihydro-1,2,4-triazolines wherein R is H, Me, Ac, $[CH_2]_2OH$, or $PS(OEt)_2$, and $R^{I}$ is NH, oxo, or NAc. They are prepared by reacting an alkyl 5-nitrofurimidate with $NH_2NR^{II}CZNH_2$ ($R^{II}$ is H, Me, or $[CH_2]_2OH$; Z is O or NH), then acylating where desired. E.g., a suspension of 5-nitrofuronitrile in EtOH is gassed at 10° with HCl for 1·5 h until almost clear, then introduction of HCl is continued during a further 2–3 h at a reduced rate. Pptd. product, viz., Et 5-nitro-2-furimidate hydrochloride, m.p. 158–160°, is heated with semicarbazide, and EtOH at 50–55° for 30 min. The cooled solution is diluted with water, with pptn. of *N*-ureido-5-nitro-2-furamidine, m.p. 274–275° in 63% yield, then this is boiled in $PhNO_2$ for 15 min. The cooled mixture is diluted with ether to give a ppt. of 3-(5-nitrofur-2-yl)-5-oxo-1,5-dihydro-1,2,4-triazole, m.p. 277–279° (from water). The products are bactericidal and coccidicidal agents. F. R. BASFORD.

**[Preparation of] carbanilides.** Merck Co., Inc. (B.P. 1,068,887, 1.7.64. U.S., 17.7.63).—Compounds of the formula $XC_6R_4$-O·$C_6R_4$·NHCONH·$C_6R_4X$ in which each X is Cl, Br or $NO_2$ and each R is H, Cl, Br, $NO_2$ or alkyl $C_{1-5}$ are prepared by reaction of a substituted diphenyl ether $XC_6R_4$·O·$C_6R_4Y$ with a substituted phenyl compound $XC_6R_4Y$ in both of which one Y is an amino radical and the other is an isocyanate radical. The products e.g., 4-(4-chlorophenoxy)-4′-nitrocarbanilide (m.p. 234–237°), prepared by reacting 4-chlorophenoxy-aniline and 4-nitrophenyl-isocyanate in benzene, are used as anti-coccidial agents, mixed with poultry feeding stuffs at a concn. of 0·006–0·025 wt.-%. J. M. JACOBS.

# 2.—FOODS

## Carbohydrate Materials

### Cereals, flours, starches, baking

**Effect of γ-irradiation of wheat flour on the microflora and vitamin $B_1$ content.** J. Poisson *et al.* (*Fd Irrad.*, 1967, **8**, Nos 1 & 2, 2–11).—Flours of same extraction rate with moisture 1·5, 9·9, 15·0, 17·9 and 22%, both sterilised and unsterilised were studied. The micrɔ-organisms are more sensitive to γ-irradiation than is vitamin $B_1$, a dose of 150 krad being sufficient to render flour 'hygenically clean' although 500 krad is insufficient to eliminate all the organisms. Storage of flours at 5° after irradiation invariably results in a lower micro-organism count in irradiated flours as compared with the controls. (21 references.) C.V.

**Determination of uric acid in flour.** N. P. Sen (*J. Ass. off. analyt. Chem.*, 1967, **50**, 776–781).—In determinations by the method of Sen and Smith (*ibid.* 1966, **49**, 899) collaborative studies gave a mean recovery of 103%. A. A. ELDRIDGE.

**Micro-determination of nitrogen [in flour].** R. Rougieux and G. Cesaire (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 736–738).—In the apparatus described for the determination (within 3 min.) of volatile acids, EtOH, or $NH_3$ in 5-ml samples, steam generated in a large flask passes through the sample contained in a small vessel, partly submerged in the boiling water, and thence through a vertical reflux condenser to the receiver. The two main parts of the apparatus are connected by a ground-glass joint. Results obtained by this method agreed well with those obtained by the usual method. P. S. ARUP.

**Determination of starch and soluble carbohydrates. I. Grains, stock feeds, cereal foods, fruits and vegetables.** T. E. Friedemann, N. F. Witt and B. W. Neighbors (*J. Ass. off. analyt. Chem.*, 1967, **50**, 944–958).—An isopropanol extract of the sample is diluted with water, NaCl and Celite are added, and the residue on filtration is incubated at 50° with an acetate buffer and Rhozyme-S. After clarification with $Zn(OH)_2$ the reducing sugars are determined with $K_3Fe(CN)_6$ titrimetrically or spectrophotometrically. Procedures for the determination of sol. starch oligosaccharides, maltose and other sugars are given. A. A. ELDRIDGE.

**Determination of starch and soluble carbohydrates. II. Starch determination in cereal grains and cereal products.** T. E. Friedemann and N. F. Witt (*J. Ass. off. analyt. Chem.*, 1967, **50**, 958–963).—Collaborative results are reported. The authors' method requires experience for satisfactory results. A. A. ELDRIDGE.

**Milk proteins, protein–protein interactions, and their effects on dough properties and bread quality.** W. B. Sanderson (*Diss. Abstr.*, B, 1967, **28**, 399–400).—Nonfat dry milk, when incorporated into continuous mix bread, causes loaf depression and poor texture. Increasing the forewarming heat treatment of the milk to 165°F for 30 min. and heating the concentrate to 175°F for 10 min. improved baking quality slightly. Acid and rennet wheys adversely affected loaf vol.; this effect was eliminated by heating the wheys at 185°F for 30 min. Raw acid casein had an even greater effect on bread quality; heating the casein solution only slightly improved baking properties. Salt whey, blood serum albumin, α-lactalbumin, β-lactoglobulin and the heat-induced interactions of κ-casein-β-lactoglobulin and α-lactalbumin-2-lactoglobulin had no significant effect on bread properties. F. C. SUTTON.

**Technology of production of high quality, long-life bread from flour derived from high-yielding wheats, by using a combined additive, 'Panvit'.** Lj. Milaković (*Kemija Ind.*, 1968, **17**, 119–136).—The new product 'Panvit' (**I**) which contains leguminose-endosperm, powdered emulsifiers, acidified Ca acetate salts, sucrose and *l*-ascorbic acid, was tested as a flour additive. Bread made with **I** (0·4% for white flour and 0·5% for dark flour) had greater vol., finer texture, longer shelf-life and was more aromatic than that produced in its absence. Flour fortified with **I** had increased water absorption (by 2–4%), resulting in improved glutinisation; flour made from soft types of wheat can be used satisfactorily if **I** is added. P.P.R.

**Proteinaceous materials and farinaceous compositions.** Welfare Foods (Stockport) Ltd. (Inventors: H. B. Hawley and D. J. Heath) (B.P. 1,074,087, 30.7.64).—Used to prepare baked products having a high protein content, the title material for addition to flour consists of one or more non-proteinate proteins and 8–50% (dry wt.) of one or more ingestible alkaline earth- or alkali metal-proteinate. The farinaceous composition is formed into a dough at 29–30° and then leavened and baked at 232–260°. Thus, strong Canadian flour, NaCl, corn oil, dry vital wheat gluten, dextrose, low-heat skimmed milk powder, Ca caseinate, lactalbumin and the Ca salt of isolated soy protein are dry mixed for ∻2 min. and any fat, oil etc. is optionally added. The baker's yeast, water and any optional minerals, trace elements etc. are mixed and added to the mixture and blended for 5 min. at high speed. A final dough temp. of 29–30° is achieved and the dough is further processed for baking.
S. D. HUGGINS.

**Extraction of the germ from kernels of maize.** Ocrim, Società per Azioni (Inventor: L. Grassi) (B.P. 1,078,985, 14.4.65).—A mass of maize kernels is subjected to impact breaking to produce a mixture of grains of germ (*G*), endosperm (*E*) fragments, meal (*M*), bran (*B*) and unbroken kernels (*K*). A certain amount of *M* and *B* is separated, by means of perforated steel plates, from the mass during treatment in the impact breaker. The bulk of the mass is subsequently conveyed to a sifting machine in which the remaining *B* and *M*, together with unbroken *K*, are separated. The *G* grains and *E* fragments are also selected, in order to render them of uniform size, and are thereafter conveyed to a gravity separator with a vibrating deck; the to-and-fro mechanical movement of a screen, combined with the action of an air stream, cause the separation of *G* grains and *E* fragments of equal size from each other. The maize germ is used for extraction of edible oil.
J. M. JACOBS.

**Continuous dough making.** Wallace and Tiernan Ltd. (Inventor: W. Dixon) (B.P. 1,073,892, 19.2.65).—A quantity of yeasted bread dough, in which the characteristics of one portion differ from those of another portion, is obtained by adjusting the pressure of the pre-mixed dough in transit between a dough pump and a developer. The apparatus consists of a pre-mixer for the dough ingredients, a dough pump for receiving the undeveloped dough and feeding it under pressure along a circuit to a closed developer, and an adjustable throttling member in the conduit. The developer has two co-operating impellers turning on parallel shafts to give a low pressure on one side, the inlet being on the low pressure side. Use of a fairly high pressure gives a bread having a fine texture, while a low pressure results in a more open-textured product.
S. D. HUGGINS.

**Whey product.** National Dairy Products Corp. (B.P. 1,080,946. 1.7.66. U.S., 19.7.65).—A yeast fermented dough constituent comprises whey solids [the serum solids in the whey obtained from cheese-making, with additional amounts of lactalbumin sufficient to provide a level of lactalbumin in the whey product of 1–4 (2–3) wt.-%]. Buffering and fortifying salts, minerals comprising harmless edible Ca and P compounds, and, as a hydrophilic agent, 5–20 wt.-% of maize flour (particle size < 50 mesh, U.S. standard sieve) are added; the pH of the product is 7–8. J. M. JACOBS.

**Decorating and filling confectionery.** J. MacManus (B.P. 1,073,889–90, 10.9.65. U.S., 15.9.64).—The method and apparatus for depositing thick, flowable material, e.g. whipped cream, on to cakes, pastries and sweetmeats etc. consists of (I) pumping a continuing flow of the material to a collapsible forcing bag, with an open discharge tip, and manually moving the tip to deposit the material, the bag being partially collapsed, and then releasing the compression so as to reduce the flow of the material while the tip is sent to the next unit; (II) a pump for pumping the material along a flexible hose formed by a flexible tube enclosed within a helix of flexible wire to an unobstructed flexible discharge device at the lower end of the hose which is moved from unit to unit for deposition.
S. D. HUGGINS.

### Sugars and confectionery

**Practical aspects of aeration [in confectionery].** J. W. Mansvelt (*Mfg Confect.*, 1967, **47**, No. 9; 43, 46, 48, 50–52; No. 10; 21–26).—A review and discussion. Some 29 tables and formulations are presented.
C.V.

**Icing and preserving fruit.** G. Bandot (B.P. 1,074,040, 6.6.66. Fr., 29.6.65).—Chestnuts and candied fruit (cherries, plums) are impregnated with sugar, coated with a syrup consisting of sugars and at least one gelatinous product such as alginates and carraghenates and sprayed or dipped with aq. $CaCl_2$ solution to fix and coagulate the coating. Fruit from both treatments may be preserved and sterilised in a syrup bath and after storage and removal from the syrup bath, is still satisfactorily iced.
S. D. HUGGINS.

**D-Ribose-5-phosphate and D-ribose.** Kyowa Hakko Kogyo Co. Ltd. (B.P. 1,067,840, 9.7.64. Jap., 29.7. and 8.8.63).—The title compounds are prepared by culturing a micro-organism of the genus *Micrococcus*, *Brevibacterium*, *Aerobacter*, *Corynebacterium* or *Bacillus*, aerobically, at 20–40° in a saccharide-containing aq. medium containing 0·20–2·0% by wt. $PO_4^{3-}$, 0·01–0·2% by wt. Mg salt and 0·1–1·5% by wt. K salt and recovering D-ribose (**I**) if the culture contains 0·0001–1·0% wt./vol. of a salt of Mn, Fe or Zn and I-5-phosphate if the culture contains none of the Mn, Fe or Zn salts. Thus, *Brevibacterium ammoniagenes* is cultured for 120 h in an aq. medium containing glucose, urea, $KH_2PO_4$, $K_2HPO_4$, $MgSO_4$, $CaCl_2$, $MnSO_4$ and biotin, at pH 8. The broth containing **I** is filtered and the filtrate adjusted to pH 5 with HCl, then passed through a cationic resin, eluted with water and passed through an -OH type resin. The fractions containing **I** are adjusted to pH 6·5, concentrated under vac. at 50°, decolorised with C, mixed with EtOH and crystallised. S. D. HUGGINS.

## Fermentation and Alcoholic Beverages

**Fermentation of grape juice.** I. Holzberg (*Diss. Abstr.*, B, 1967, **28**, 656–657).—The two phases of grape juice fermentation: alcoholic fermentation and the cultivation of the yeast, were studied separately. Formulae are given for the exponential growth phase and also for the stationary phase. The use of a continuous cultivator of yeast as a research tool for finding growth limiting factors, is described.
F. C. SUTTON.

**Fermenter yeast cropping and washing device.** John Labatt Ltd. (B.P. 1,075,370, 14.6.66. U.S., 29.6.65).—The claimed device consists of a header pipe extending horizontally in the upper portion of the fermenter vessel so that at least one end of the pipe extends outside the vessel. The header pipe is provided with a series of orifices opening into the vessel and rotates about a horizontal axis. The apparatus is for use in the brewing industry. (4 drawings.)
S. D. HUGGINS.

**Concentrating wort.** Jos. Schlitz Brewing Co. (B.P. 1,069,867. 15.9.65. U.S., 18.9.64).—A continuous process for concentrating brewer's wort consists of continuously preheating wort free of suspended solids to 185–230°F, evaporating the preheated wort by passing it through a series of evaporative, heat exchange systems to concentrate the wort to a solids content > 78% by wt. and then immediately cooling the conc. wort to < 105°F, the whole process being performed in < 10 min.
S. D. HUGGINS.

**Increased alcohol yields from cereal worts.** Associated British Maltsters Ltd. (Inventor: K. C. Stowell) (B.P. 1,070,796, 7.5.64).—Crushed cereal malt is mashed to form a fermentable wort and cereal malt, in finely divided form, containing limit dextrinase together with yeast is added to the wort; fermentation is carried out at 80–85°F. It is found that the addition of 12·5%, 37·5% and 50% malt flour prior to fermentation gives an increased yield of EtOH of 1%, 1% and 25% respectively, taking no malt flour addition as control.
S. D. HUGGINS.

**Concentration of fermented beverages.** A. Guinness Son & Co. (Dublin) Ltd. (B.P. 1,072,758, 21.9.64. Ireland, 23.12.63).—Fermented beverage is concentrated (for storage or transport purposes) without prejudicing the flavour after reconstitution by passing through it a first stream of stripping gas so as to remove most of the volatile components other than water; then passing through the residue a further stream of stripping gas so as to remove most of the water; and combining with the second residue the volatile components from the first stage (at a temp. which is 18–60° lower than that of the first stage).
F. R. BASFORD.

**Mashing of grains.** Associated British Maltsters Ltd. (Inventor: K. C. Stowell) (B.P. 1,080,093, 9.7.65).—An improved procedure for mashing grain for making wort comprises introducing grains in admixture with a starch-containing flour and a starch-degrading enzyme (e.g., a bacterial or fungal amylase prep.) into the mash.
F. R. BASFORD.

**Brewers wort production.** Chas. Pfizer and Co., Inc. (B.P. 1,083,485, 21.9.65. U.S., 7.4.65).—Used in beer, ale and lager

production, the wort is obtained by adding proteolytic enzyme to a malt mash, increasing the temp. to 60–70°, maintaining at 60–70° for 1·5 h, adding liquefied cereal grain to the mash (45–70% by wt. cereal to total wt.), increasing the temp. to 65–75° and keeping at this temp. for 30 min. to 1 h. The use of lower malt to cereal ratios than normal gives a good quality beer at lower cost.
S. D. HUGGINS.

**A method of artificially maturing alcoholic beverages.** A.-G. für Brauerei-Industrie (Inventor: P. Flesch) (B.P. 1,083,066, 8.8.66).—The drink is irradiated with $^{60}Co$ ($1 \times 10^5$ to $5 \times 10^5$ r/h during 10–30 or 10–20 min. respectively for beverages rich or poor in extract). Prior to irradiation, the beverages may be saturated with air, $O_2$, $N_2$ or $CO_2$.
F. R. BASFORD.

**Improving alcoholic liquors.** M. Morita (B.P. 1,083,835, 22.6.65. Jap., 10.7.64).—Improved taste is obtained by subjecting the liquor (whiskey, saké) to cavitation resulting from agitation at a high rotational speed at $\geq 42°$ for $\geq 20$ min. Rapid mellowing, with no deterioration of flavour, is achieved. Preferably the temp. of treatment is 0–25°, maintained by adding a refrigerant (e.g. liquid $N_2$) to the liquor.
S. D. HUGGINS.

**Alcoholic beverages.** Kyowa Hakko Kogyo Co. Ltd. (B.P. 1,066,084, 6.5.64. Japan, 8.5. and 1.6.63).—0·1–1·0% by wt. of ornithine–aspartic acid-salt or -peptide is added to alcoholic beverages (e.g. beer) to improve their quality and to increase their flavour.
E. ENOS JONES.

**Preparation of hop extract emulsions.** Chas. Pfizer & Co. Inc. (B.P. 1,071,867, 8.2.66. U.S., 29.9.65).—A hop extract is intimately admixed with gum arabic, water, a non-toxic emulsifying agent, e.g., dioctyl Na sulphosuccinate, and optionally sorbitol, to provide a stable emulsion suitable for use in flavouring beer and ale.
F. R. BASFORD.

**Hop extracts.** A. Guinness, Son & Co. (Dublin) Ltd. (Inventor: R. O. V. Lloyd) (B.P. 1,074,644, 23.6.65).—Improved hop extracts or concentrates yielding beers of better flavour are obtained by extracting hops with an org. solvent (lower alkanol, aliphatic ketone, aliphatic or aromatic hydrocarbon, or halogenated solvents) and a mild reducing agent (**I**) ($SO_2$) that is sol. in the solvent; **I** is removed from the extract by evaporation, neutralisation, or salting out. When desired the extract may thereafter be isomerised by treatment with hot aq. alkali. Conventionally prepared hop extracts may also be improved by treating them with **I** (in solution in a non-polar solvent such as benzene or light petroleum).
H. L. WHITEHEAD.

**Flavouring ingredients from hops.** Wolton Hop Extracts Ltd. (Inventors: D. A. Wolton, D. Attwood and J. E. F. Marshall) (B.P. 1,082,725, 13.6. and 29.10.63).—Hops are subjected to solvent extraction using an org. solvent substantially insol. in water, e.g. pentane, and partitioning the org. extract with an aq. alkaline solution, e.g. of $Na_2CO_3$ or $NaHCO_3$, followed by separation of the phases. Hop oils are obtained from the org. phase by distillation and steam distillation of the residue, while the aq. phase is acidified to precipitate hop resins, preferably in presence of a physiologically inert carrier (e.g. Me cellulose) so as to form a flavouring ingredient for beer, etc.
S. D. HUGGINS.

**Clarification and stabilisation of beer.** Schwarz Laboratories, Inc. (B.P. 1,069,404, 11.5.64. U.S., 10.5.63).—The clarity and flavour of beer is retained during packaged storage by adding a non-swelling, adsorbing agent ($\leq 70\%$ by wt. $CaSiO_3$, $MgSiO_3$ or $ZnSiO_3$) prior to the final filtration, removing the adsorbing agent and then packaging the beer. Thus, 500 ppm of non-swelling, particulated synthetic hydrated $CaSiO_3$ ($>70\%$ $CaO + SiO_2$) is added in transfer from the fermentor to a primary storage vessel, when a large portion of the $CaSiO_3$ settles out before filtration. A portion of the same beer is used as a control and the turbidity of each is analysed after final filtration and packaging. After (*i*) 6 days at 75°F, (*ii*) 6 days at this temp. and 2 days at 32°F, and (*iii*) 6 days at 75°F, then 5 days at 122°F and 2 days at 32°F, the formazin turbidity readings for the control beer are, respectively, 29, 86 and 380 against 18, 25 and 170, respectively, for the $CaSiO_3$-treated beer.
S. D. HUGGINS.

**Reduction of haze in beer.** A.-G. für Brauerei-Industrie Glarus (B.P. 1,073,924, 4.8.64. Ger., 2.8.63).—Haze is reduced by adding to the beer a layered mineral with a swellable lattice, together with a coarse- or medium-pored $SiO_2$ gel in the state of a xerogel, which is ground so that $\leq 75\%$ by wt. has a grain size of $\geq 44\ \mu$. The preferred mineral is a montmorillonite (optionally acid-activated); its use reduces the amount of $SiO_2$ gel required to remove haze-forming compounds. Several examples are given of cold turbidity tests carried out on the treated beers.
S. D. HUGGINS.

## Fruits, Vegetables, etc.

**Thermal processes for canned cherries.** K. D. Dastur (*Diss. Abstr.*, B, 1967, **28,** 730–731).—Heat processing procedures based on F and z values of organisms *Saccharomyces fragilis, Pichia membranaefaciens,* YB-826 *Saccharomyces* sp. and the enzyme peroxidase were developed. Thermal death times of the three organisms in the stationary phase were determined at five temp. in cherry: water and cherry: syrup media. Tests on inoculated packs indicated that the suggested procedures were sufficient to inactivate the organisms and peroxidase, while contributing substantially toward improved product quality.
F. C. SUTTON.

**Pectinous material from cranberry fruit: isolation, purification and characterisation.** N. D. Pintauro (*Diss. Abstr.*, B, 1967, **28,** 733–734).—It is concluded that the pectinous material from cranberries is a true pectic substance and the uronide components are admixed with polygalacturonic acid in the same way in which these materials co-exist in citrus and other fruit pectins. The problem of acid sensitivity in high solid jellies is probably due to higher ester content; it was necessary to increase the pH to 3·7–3·9 at 65% sol. solids to give a satisfactory jelly.
F. C. SUTTON.

**Determination of biphenyl in citrus fruits by thin layer chromatography and ultra-violet spectrophotometry.** P. E. Corneliussen (*J. Ass. off. analyt. Chem.*, 1967, **50,** 934–938).—A mixture of the sample and boiling water is extracted into n-heptane; the extract is cleaned up by TLC on plates coated with $SiO_2$ gel containing phosphors to facilitate identification of biphenyl spots in u.v. light. The spots are removed and an ethanol extract is examined spectrophotometrically at 248 nm. Average recoveries of 92 to 102% are reported.
A. A. ELDRIDGE.

**Determination of bitterness of olives.** S. Cohen, A. Lifshitz and Z. Samish (*J. Ass. off. analyt. Chem.*, 1967, **50,** 1194–1195).—The bitter principle is extracted into acetone, the solution is decanted after being shaken with gelatin and then with $Al_2O_3$, and its extinction is measured at 345 nm. Results are correlated with those of organoleptic tests.
A. A. ELDRIDGE.

**Texture of peppers used in stuffing of olives. Effect of acidity and time of conservation.** M. J. Fernández Díez, J. Fernández Villasante and F. González Pellisso (*Grasas aceit.*, 1968, **19,** 16–18).—The texture of red peppers used for stuffing olives is measured with an Allo-Kramer Shear Press. The texture changes with time of conservation in brine, but org. acids (citric or lactic) reduce this change.
L. A. O'NEILL.

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**Banana storage.** Whirlpool Corp. (B.P. 1,067,018, 3.2.65. U.S., 20.4.64).—In the process claimed, the storage atm. contains less $O_2$ and more $CO_2$ than is found in normal air and the unsaturated hydrocarbon gases, such as $C_2H_4$ liberated from the bananas are removed so that harvesting can take place at a more mature stage. The $O_2$ content of the storage atm. is 1·5–3·5% by vol., the $CO_2$ content 5% and the remainder inert gases, the R.H. being 85–95%.
S. D. HUGGINS.

**Citrus fruit derivatives.** Aktieselskabet Grindstedvaerket (Inventor: K. J. S. Villadsen) (B.P. 1,072,733, 26.8.63).—There is claimed an agent for imparting stable cloudiness and improved colour to citrus fruit juices and other juices comprising a stable aq. suspension of natural citrus fruit cloudifyer and citrus fruit colouring matter in finely divided form and derived from peel and/or 'rags' of such fruit by boiling them with water. The viscosity of the extract is reduced by treatment with a pectolytic enzyme.
F. R. BASFORD.

**Sweetened fruit.** Kellogg Co. (B.P. 1,074,950, 3.6.65. U.S., 18.8.64).—Freeze-dried fruit (moisture $\geq 2\%$) can be rapidly artificially sweetened at 100° and then redried with heated air to a moisture content of $\geq 2\%$ for stable transportation. Sweetening agents include Na cyclamate, Ca cyclamate, saccharin Na and cyclohexylsulphamic acid. Thus, a typical composition consists of 100 g freeze-dried peach slices, 1·114 g Ca cyclamate, 0·0832 g saccharin Na and 4·45 ml water which gives a 40% relative sucrose equiv.
S. D. HUGGINS.

**Cleaning of fruit and vegetables.** W. E. Zimmie A.-G. (B.P. 1,073,946, 9.9.64. U.S., 8. and 19.11.63).—Soil, dirt or mud can be cleaned from the surfaces of fruit and vegetables by the application of an aq. solution of a water-sol. org. ethylenic polymer (**I**) or one of its salts, which has an average mol. wt. of 800,000–25,000,000 and contains the grouping C:C·R. R is ·CN, ·$CONH_2$, substituted amide or ·COOM (where M is H, $NH_4$ or alkyl of 1–4 C) or

the polymer is an aminoalkyl acrylate, *N*-alkyl substituted aminoalkyl acrylate or methacrylate, hydrogenation product of nitriles, polymer containing lactone or lactum groups, reaction product of polyvinyl alcohol with dibasic acids or polymer containing $\cdot SO_3H$ groups. The fruit or vegetables are washed in an aq. bath containing 0·001–50 ppm I at >90°F; the bath may also contain >2% alkaline detergent and/or solvent. S. D. HUGGINS.

**Treatment of fruit and vegetables.** J. Daudin and R. Hayot (B.P. 1,083,245, 21.1.66. Fr., 18.2.65).—Fruits and vegetables with inedible skins, particularly citrus fruits and bananas are treated with a fungicide in association with a physiologically active complex, consisting of (*i*) at least one paraffinic liquid hydrocarbon of 23–26 C, (*ii*) a non-ionic emulsifying agent sol. in hydrocarbons and water, and (*iii*) a salt of at least one of the following org. acids: 2,4-dichlorophenoxyacetic, α-naphthylacetic or 2,4,5-trichlorophenoxyacetic acids or gibberellic acid with an amine or with an alkali metal. S. D. HUGGINS.

**Treatment of vegetables.** Nestlè's Products Ltd. (B.P. 1,067,705, 31.1.66. Switz., 12.2.65).—Vegetables are preserved by treatment with an aq. antioxidant composition containing 0·01% by wt. of extract of an aromatic plant (*Labiatae* spp) and 0·1–1·0% of a molecularly dehydrated phosphate containing at least one P·O·P linkage in its mol. and having the empirical formula $(M_2O)_m \cdot (X_2O)_n \cdot P_2O_5$ where M is alkali metal, X is H or alkali metal, $m$ is <1 and >2, $n$ is <0 and >1, $m + n$ is >2. Thus, carrots are peeled, washed and machine-cut into 8 mm cubes and dipped for 3 min. into a bath of aq. Na pyrophosphate and alcoholic rosemary extract at 50°. After draining, the cubes are blanched for 5 min. in steam at 98°, cooled by a water spray for 30 sec. and freeze-dried to ~1% final moisture content. After storage for 4 months at 37° in air, the product has normal colour, odour and flavour. S. D. HUGGINS.

**Preserving new potatoes.** Unilever Ltd. (Inventor: R. Lester and T. P. Williams) (B.P. 1,083,817, 15.4.65).—The potatoes are pre-heated to 40–65° and then partially blanched, to a centre temp. >60°, followed by freezing. Colour, flavour and waxy texture are maintained. S. D. HUGGINS.

### Tea, coffee, cocoa

**Determination of caffeine in non-alcoholic beverages.** A. R. Johnson (*J. Ass. off. analyt. Chem.*, 1967, **50**, 857–858).—A spectrochromatographic procedure, which is a modification of Levine's method (Borker and Sloman, *ibid.* 1965, **48**, 705) gave recoveries of 93 to 102% of caffeine from a simulated beverage prep. A. A. ELDRIDGE.

**Determination of loss on drying in roasted ground coffees.** E. A. McCarron (*J. Ass. off. analyt. Chem.*, 1967, **50**, 835–837).—Drying of finely ground roasted coffee for 6 h in air at 105° or in a vac. oven at 100° gives concordant results. Pre-grinding of coarsely ground samples to 20–30 mesh is recommended. A. A. ELDRIDGE.

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**Improvements to C.T.C. machines for processing tea.** T. C. Martin (B.P. 1,072,433, 25.3.63).—The claimed machine has a framework of two vertical side frames adapted to hold the ends of two rollers mounted in housings clamped to the frames. Each frame consists of a bottom extended pedestal and a sliding arm frame, which, fitted together, form a slotted portion for receiving the housings and each sliding arm is fitted by a dovetail joint at the rear and top of the pedestal, so that it can slide towards the feeding end of the pedestal. The bearings have a long life and also facilitate an even cut on the tea leaves being processed. (4 drawings.) S. D. HUGGINS.

**Instant coffee production.** W. J. Mach and C. Mach (B.P. 1,073,738, 26.10.64. Ger., 26.10.63 and 8.5.64).—The coffee containing the hepatotropic agents present in *Carbo Coffeae* is obtained by recovering the melanoidines (sol. in dil. NaOH of pH 8–8·5 and in acidified org. water-sol. solvent of pH 1–3) from the coffee grounds of an aq. extract of the roasted coffee and adding the extracted melanoidines to aq. or dried coffee. Thus, ground roasted coffee is extracted at 120° with water giving a 28% solution. The moist grounds remaining are extracted with 0·1 N aq. NaOH; the filtrate is then passed over a cation exchanger in H form so that the pH becomes 6·2. This weakly acid solution is mixed with hot water used for extracting the ground roasted coffee so that a total 34% extraction is achieved, the product obtained possessing improved physiological compatibility and having the qualities of Turkish coffee. S. D. HUGGINS.

## Milk, Dairy Products, Eggs

**Milk flavour. I. As it comes from the cow. II. Changes during storage and processing. III. Flavour quality control.** W. L. Dunkley (*Dairy Inds.*, 1968, **33**, 19–22; 91–94; 162–164).—A general survey. C.V.

**Flavour stability studies on vacuum dried whole milk.** M. G. Cooper (*Diss. Abstr.*, B, 1967, **28**, 730).—Studies included such factors as moisture content, preheat treatments, addition of pancreatin extract, folic acid addition, variations in homogenisation procedures, and immersion of magnesium ribbon in milk prior to drying. Promising possibilities for improving dried whole milk flavour stability by changes in the preheat treatments and homogenisation sequence presently employed are indicated. F. C. SUTTON.

**Studies on physical and chemical changes in concentrated milk in frozen storage.** R. S. Kadan (*Diss. Abstr.*, B, 1967, **28**, 732).—Frozen, conc. milk was studied for the effects of forewarming temp., storage temp., extent of concn., and for the mechanism of action of certain additives on its storage stability. A forewarming temp. of 63° for 30 min. gave the highest storage stability value. There was a linear relationship between protein pptn. and lactose crystallisation. The stability of the product was also a direct function of concn. and storage temp. It is proposed that polyhydroxy compounds (e.g. sucrose) improve the stability of milk by increasing viscosity, while salts such as NaCl, KCl, citrates and polyphosphates, do so by lowering colloidal Ca and P. F. C. SUTTON.

**Fortification of milk for children.** J. G. Davis (*Dairy Inds*,1968, **33**, 27–29).—The possible reasons are considered with special reference to the use of vitamin D and fluoride. The advantages of preferentially using milk as a vehicle are examined. (28 references.) C.V.

**Quality grading of raw milk by electronic cell counting.** H. Klein and H. J. Thomas (*Milchwissenschaft*, 1968, **23**, 153–156).—A total of 9699 raw milk samples taken from seven dairies in general showed the applicability of this form of control. C.V.

**Investigations on the modified resazurin test.** V. M. Thom (*Dairy Inds.*, 1968, **33**, 238–242).—Some 544 winter and 319 summer milks were examined by this test (*MR*) and by one in which a further modification had been introduced. Statistical analysis showed that cell count had a significant influence on *MR* results in winter but not in summer. Correlation between bacterial count and *MR* test was poor in winter but rather better in summer. Using the further modification (*FMR*) the results appeared to be unaffected by cell count at either season and they were more closely correlated with bacterial count than were the *MR* results. In general the *FMR* not only fulfilled the requirements of the Scottish Milk Marketing Board but it gave a better indication of hygiene in production than the *MR* test. (16 references.) C.V.

**Use of the Milkotester as a turbidity meter in the determination of the degree of dispersion of milk fat emulsion.** M. E. Schulz, D. Thiele and E. Höft (*Milchwissenschaft*, 1968, **23**, 14–19).—The instrument is illustrated and is described. (21 references.) C.V.

**Estimation of sulphydryls in milk with n-ethyl maleimide.** A. S. Narang, J. Singh, R. V. Rao and V. R. Bhalerao (*Milchwissenschaft*, 1967, **22**, 682–685).—Using a spectrophotometer in the u.v. range, the required optical conditions are discussed. The free-SH groups (mmole/litre) in pasteurised cow- (0·193) and buffalo-milk (0·098) are recorded, the average being shown. (28 references.) C.V.

**Investigation of factors affecting perception and preference of flavour levels in sour cream.** W. L. Hempenius (*Diss. Abstr.*, B, 1967, **28**, 731).—Two types of sour cream appear to be suitable for the retail market. These are (*i*) a bland, acidified cream containing less than 2 mmoles AcOH/kg plus flavour concentrate and (*ii*) a highly flavoured sour cream containing approximately 8 ml volatile acidity (*VA*) plus associated flavour compounds, where *VA* is measured as the no. of ml of 0·1 N NaOH required to neutralise the steam distillate from a 50 g sample. F. C. SUTTON.

**Changes in content of volatile substances during the ripening of yoghurt.** F. Görner, V. Palo and M. Bertan (*Milchwissenschaft*, 1968, **23**, 94–100).—Gas chromatographic studies confirm the view that acetaldehyde is one of the flavour substances in yoghurt; in fact it is the only volatile that is produced in significant amounts during ripening. Concn. is 23·1–41·0 ppm in the ripe state. The pasteurised bulk milk contained acetone, butanone-2, and ethanol. (19 references.) C.V.

**Fatty acid composition of German winter and summer butter, Turkish and Italian butter and German margarine.** A. Janoschek and M. Metin (*Milchwissenschaft*, 1968, **23,** 29–33).—The ratios of the different fatty acids show a quite marked difference. A table of values for $C_6 : C_8$, $C_{12} : C_{10}$ and $C_{14} : C_{12}$ acid ratios is given. C.V.

**Continuous milk coagulation in the Multilub cheesemaking machine.** J. T. Lomasow and E. Schmanke (*Milchwissenschaft*, 1968, **23,** 83–93).—To calculate the time for cold renneting, an empirical formula similar to that of Berridge is used: $t_1 = t_2 \times 1{\cdot}96^n$ where $t_1$ is time required for cold renneting, $t_2$ the time of normal coagulation at 35° and $n$ is $\frac{1}{10}$ of the temp. difference between the two temp. values. $CaCl_2$ reduces coagulation time of cold renneted milk but the time remains constant whether $CaCl_2$ is added before commencing the renneting or before warming the milk afterwards. Other aspects are discussed. (10 references.) C.V.

**Manufacture of Blue cheese by direct acidification methods.** A. El-Sayed Shehata (*Diss. Abstr.*, B, 1967, **28,** 401).—Direct acidification was more economical than traditional processes (manufacturing time and amount of rennet reduced by 50% and amount of starter by 75%). Use of $CaCl_2$ in the direct acidification process reduced levels of proteolysis and lipolysis during ripening. Addition of C- or K-lipase to milk (28·4 g/453·6 kg) containing 0·02% $CaCl_2$ produced cheese with typical Blue cheese flavour and satisfactory mould growth. Use of KL-lipase with 0·02% $CaCl_2$ produced cheese which did not develop Blue cheese flavour and possessed off-flavours during ripening. F. C. SUTTON.

**Factors affecting the B-vitamin content of cheese.** K. M. Nilson (*Diss. Abstr.*, B, 1967, **28,** 398).—Niacin, vitamin $B_6$, biotin, folic acid, pantothenic acid, and vitamin $B_{12}$ were assayed in the milk, whey, and cheese curd. The temp. (4·4, 10·0, and 15·6°) and length of ripening markedly affected the vitamin content of the cheese. In general, cheese ripened at higher temp. contained more vitamins than at lower temp., except for pantothenic acid. The microflora of Cheddar cheese were studied throughout a 12-month ripening period. Total counts increased to 5·8 billion after one week of ripening and then decreased to 0·3 million after the 12th month. Streptococci predominated for the first six months, but were overtaken by lactobacilli and micrococci during the last six months. Niacin was synthesised to some degree by all the cheese isolates. Vitamins $B_6$ and $B_{12}$ were synthesised only by the composite flora of all the isolates and by one of the miscellaneous groups. Vitamin contents of Swiss and Blue cheeses were also studied. F. C. SUTTON.

**Market quality of eggs in Australia.** F. S. Shenstone (*Fd Preserv. Q.*, 1967, **27,** 86–94).—Control of quality is discussed. (52 references.) P.P.R.

**Updating analytical constants for [determining] egg content of food.** L. J. Stauffer (*J. Ass. off. analyt. Chem.*, 1967, **50,** 851).—New values for the sterol content of dried whole eggs, dried egg yolk, frozen egg yolk, flour and semolina are tabulated. A. A. ELDRIDGE.

**[Determination of] sodium lauryl sulphate in egg whites.** J. Wiskerchen (*J. Ass. off. analyt. Chem.*, 1967, **50,** 847–849).—The method of Swift & Co. (*Instrumental methods for the analysis of food additives*, New York, 1961) is modified by standardising the benzethonium chloride by titration with $HClO_4$ in glacial AcOH in presence of Hg acetate. Recoveries of 94·1 to 100·2% are reported. A. A. ELDRIDGE.

**A rapid method of identification of salmonellae in spray-dried whole egg solids.** L. D. Rasplicka (*Diss. Abstr.*, B, 1967, **28,** 734).—The method consists of pre-enrichment in Hv broth for 16 to 18 h, followed by incubation in a selective enrichment broth of tetrathionate and cystine-selenite-F for 10 to 12 h. After incubation in selective broth, small aliquots were removed for a standard tube agglutination test, used to determine the presence or absence of salmonellae. Time for test was 30 h. F. C. SUTTON.

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**Brief heating of liquids particularly milk.** Sulzer Bros. Ltd. (B.P.1,070,444, 26.8.64. Switz., 4.9.63).—There is claimed a plant for the brief heating of liquids (especially sterilising of milk) by treating the liquid successively to preheating; heating with direct steam; expansion and cooling by heat exchange. Apparatus is described. (3 diagrams.) F. R. BASFORD.

**Milk products.** Taisho Pharmaceutical Co. Ltd. (B.P. 1,083,661, 11.1.65. Jap., 30.1.64).—The products, used to protect infants from suffering intestinal disorders arising from various pathogenic organisms, are obtained by promoting the growth of *Lactobacillus bifidus* in animal milk treated with the enzyme muramidase (0·05–0·1 mg/ml milk) from egg white or albumen. Additives such as β-lactose, glucose, linoleic acid and vitamins may be included in the animal milk (cow, goat, sheep) and treatment is carried out for 0·3–3·0 h at 30–50°, after which the enzyme is inactivated and the milk product sterilised. S. D. HUGGINS.

**Preparation of an improved milk product.** Ajinomoto Co. Inc. (Inventors: T. Hino, K. Hayashi and N. Lizuka) (B.P. 1,070,400, 30.9.65).—The improvement comprises adding to milk leucine, isoleucine, valine, threonine, lysine, methionine, phenylalanine, and/or tryptophan (10–100 mg per g of N content of the milk) (to improve nutritive value), mono-Na *l*-glutamate, and flavouring agent selected from glycine, alanine, serine, and threonine (5–30 wt.-% on amino-acid additive). The product may be in condensed or dried form. F. R. BASFORD.

**Sour cream powder.** Beatrice Foods Co. (Inventor: P. P. Noznick) (B.P. 1,072,308, 10.12.65).—Cream, whole milk or skim milk is cooled to a temp. between the f.p. of the cream etc., and 40°F, acidified to a pH of 4–4·7 with nontoxic acid and the acidified product spray dried to a powder. Thus, 18% cream (18% butterfat content) is pasteurised at 155°F for 30 min. and homogenised at 150°F and 1500 p.s.i. After cooling to 38°F, lactic acid is added to give a pH of 4·6 and 0·15% starter distillate flavour is added before the cold mixture is spray dried at a spray drier outlet temp. of 175–180°F to give a sour cream powder of good flavour and consistency. S. D. HUGGINS.

**Yoghurt manufacture.** G. S. Deeb (Inventor: G. S. Deeb) (B.P. 1,081,365, 13.11.64).—Packaged yoghurt is obtained by placing inoculated yoghurt mix in containers, stacking these containers and passing them through a processing tunnel possessing an incubating zone in which heated gas is circulated to raise the yoghurt to incubation temp., an intermediate zone where the yoghurt cools while incubation continues and a cooling zone in which a gas or vapour circulates to cool the yoghurt. The zones of the processing tunnel are separated by partitions. The apparatus is described. (3 diagrams.) S. D. HUGGINS.

**Continuously manufactured cheese.** B. E. Budahn (B.P. 1,062,148, 9.11.65).—Slabs of cheese curd are continuously milled by a power driven device and conveyed to the receiving end of a continuously rotatable open-ended tumbling drum with a generally horizontal axis, a predetermined quantity of NaCl being added as the curd is transferred to the drum and steam being injected into the interior of the salted curd as it is tumbled. The tumbled and salted curd is then discharged into containers for pressing into cheese. S. D. HUGGINS.

**Cheese-making.** Cow & Gate Ltd., G. M. Robertson and G. K. Charles (B.P. 1,069,081, 1.8.62).—In a continuous process, cheese curd is fed to a mill, then the crumbled material is discharged and intimately mixed with NaCl in a rotary drum forming part of conveyor means along which the curd is conveyed to a station where it is filled into moulds. Two diagrams of apparatus. F. R. BASFORD.

**Method and apparatus for combating seaminess in Cheddar, and like cheese.** Commonwealth Scientific & Industrial Research Organisation (B.P. 1,069,829, 4.5.66. Austral., 5.5.65).—In the manufacture of the Cheddar in which particles of milled curd are carried along in a stream to the location at which NaCl is added, seaminess is controlled by washing the particles (while moving) with water at 90°F to 145°F (at the moment of contact). An apparatus for the washing process is claimed. F. R. BASFORD.

**Apparatus for handling cheese curd.** National Dairy Products Corp. (B.P. 1,072,390, 26.9.65. Austral., 6.10.64).—An apparatus is described and illustrated whereby a block of cheese curd can be divided into strips or ribbons which can then be continuously and automatically fed for further processing. S. D. HUGGINS.

**Apparatus for separating cheese curd and whey.** National Dairy Products Corp. (B.P. 1,074,298, 9.8.65. Austral., 14.8.64). —Apparatus is illustrated and claimed. F. R. BASFORD.

## Edible Oils and Fats

**Rapid method for determination of the egg content of mayonnaise and salad cream.** R. E. Fresenius (*Z. analyt. Chem.*, 1967, **229,** 353–355).—The egg content is related to the concn. of lecithin phosphoric acid. The latter is converted to phosphate, which is determined spectrophotometrically by the molybdenum blue method. A. TOWNSHEND.

**Correlation of fatty acid structure with preferential order of urea complex formation.** J. L. Iverson and R. W. Weik (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1111–1118).—A standard urea fractionation procedure was applied to the methyl esters of a complex mixture of fatty acids. The preferential order so disclosed was correlated with GLC retention times and principles governing the order of formation of such compounds are enunciated.
A. A. ELDRIDGE.

**Programmed temperature gas chromatographic technique for detecting trace amounts of fatty acids.** J. L. Iverson (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1118–1123).—The urea fractions of the methyl esters of fatty acids present in cocoa butter, palm kernel oil, butter fat and fish oils have been subjected to programmed temp. gas chromatography. By changing the rate of change of temp., and sometimes by overloading the chromatograph, fatty acids (present as a few ppm) can be detected. Although a generally applicable procedure cannot be specified, general principles are stated.
A. A. ELDRIDGE.

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**Treatment of vegetable materials [oil extraction].** J. C. Cavanagh and R. A. Couche (B.P. 1,081,640, 3.12.65. Australia, 3.12.64).—Oil is separated from vegetable materials by subjecting the latter to a multi-stage counter-current solvent extraction with one or more hydrophilic org. solvents, e.g. $Me_2CO$ or EtOH, EtOAc and $(Pr^i)_2O$ or EtOH, EtOAc and $Me_2CO$, the quantity of solvent and water being adjusted to a low water content as the extraction progresses so that no oil separates as a separate phase in the first stage or in the last. The oil which separates out in the remaining stages is separated from the extraction liquor before this liquor is cycled to later stages of the process. This treatment is specially suitable for rice bran, soyabean, groundnuts, maize, coconut meat, coffee grounds, etc.
S. D. HUGGINS.

**Refining process comprising deacidification and deodorisation of glyceride oils and fats, and equipment for this purpose.** G. B. Martinenghi (B.P. 1,080,057, 15.10.64).—In the improved method for deacidifying and deodorising these fats and oils by steam stripping under vacuum, the stripping steam, having similar low pressure to that of the charge (0·1–0·8 mm) is introduced into the hot charge to be stripped (which is preferably at 170–230°) at temp. much below the charge temp. (preferably 10–35°), and after stripping, the steam is condensed by refrigeration (at −40 to −20°), and the source of vacuum (a rotary pump) is applied to the system after the refrigeration stage.
H. L. WHITEHEAD.

**Selective hydrogenation of fatty oils.** Engelhard Industries Inc. (Inventor: M. Zajcew) (B.P. 1,062,121, 29.4.64. Addition to B.P. 886,477).—A hard butter-like composition is produced by hydrogenating cottonseed oil in the presence of a supported catalyst of Pd or a Pd-compound modified by a mixture of a Bi compound with Ag (or one of its compounds) until I-value is reduced to 40–60; the product is then dissolved in a solvent (acetone), cooled to 15–30° and the hydrogenated product is recovered from the solution. This product is a satisfactory substitute for cocoa butter.
E. ENOS JONES.

**Selective hydrogenation of esters of poly-unsaturated fatty acids.** Unilever Ltd. (Inventor: R. F. A. Schulz) (B.P. 1,080,891, 19.11.63).—To effect selective hydrogenation of these esters in such manner that the reduction of the poly-unsaturated acid groups to mono-unsaturated acid groups is favoured at the expense of the reduction of the latter to saturated acid groups, the esters (e.g. soyabean oil or desulphurised marine oil) are hydrogenated in presence of conventional hydrogenation (Ni) catalysts (at 60–180° under $H_2$ pressure of 1–8 atm.) in the additional presence of 0·01–4·5% of an org. compound (**I**) that is non-acid and contains ≤1 alcoholic OH group, e.g. a 3–8 C alkanol, methoxy- or butoxy-ethanol, glycols, polyhydric alcohols or their partial esters with fatty acids. The products have lower softening points than those formed in absence of **I**, and the method yields almost completely liquid products of better keeping properties than the initial ester mixture. **I** may be formed *in situ* by reduction of an aldehyde.
H. L. WHITEHEAD.

**Salad oil from zero-erucic acid rapeseed oil.** Canada Packers Ltd. (B.P. 1,073,598, 5.11.65; Canada 13.2.65).—Zero-erucic acid rapeseed oil is hydrogenated to 0·1–1·5% (0·5%) linolenic acid content and is then winterised at ~45°F to provide good yields (94–96%) of a salad oil having good flavour stability. A 'salad oil' is defined as an edible oil that will remain liquid at temp. as low as 50°F. To obtain one that will remain liquid at lower temp., lower winterising temp. should be used.
E. ENOS JONES.

**Dessert composition mix.** General Foods Corp. (Inventors: M. E. Thoma and E. A. Pence) (B.P. 1,072,768, 10.11.65).—An aq. slurry of fat, emulsifier therefor [e.g., edible partial ester of a glycol and a $C_{12-22}$-fatty acid (**I**), or a mixture of partial esters of glycerol, lactic acid, and **I**, or a mixture of propylene glycol monopalmitate and glycerol monostearate—optionally employed in conjunction with lecithin], and proteinaceous fat encapsulating material e.g., non-milk solids is spray-dried (after or before addition of gelatin), to give a dessert powder (in which fat and emulsifier together at <30° have a solids content of ≤19·6 and a liquid content of ≥80·4%, and at >40° have a solids content of ≥12 and a liquid content of ≤88%). The powder is suitable for dispersion in aq. medium.
F. R. BASFORD.

**Edible whipping composition.** General Foods Corp. (Inventor: A. J. Perkins) (B.P. 1,077,338, 11.3.66).—The powdered product, which can be mixed with water and whipped to give a substitute for whipped cream, consists of a spray-dried mixture of (*a*) an edible fat, a sweetener, a water-sol. protein and a coating agent (water-sol. gum, or milk solids) and (*b*) a spray-dried mixture of a water-sol. gum and an acidic stiffening agent. Further ingredients include an emulsifier, a moisture-reducing agent, a stabiliser, further sweetening agents, flavouring etc. Thus, spray-dried product (*a*) can contain 53·1% hydrogenated cottonseed oil (**I**) (plus 10% propylene glycol monostearate and 2·2% monoglyceride esters of **I** as emulsifiers), 30·8% of sugar, 10·65% of Na caseinate and 5·31% of gum acacia, while product (*b*) can contain 83·4% of gum acacia and 16·6% of cream of tartar. Product (*a*) (66·15%) and (*b*) (2·5%) are mixed with sugar (29·4%) and rice flour (1·94%) to give the final composition, which when mixed (5 oz.) with water (6–7 oz), can be whipped in 4 min. to a stiff foam, stable for ≤24 h.
S. D. HUGGINS.

**Cream-like products based on fat and sugar.** Henkel & Cie., G.m.b.H. (B.P. 1,080,998, 13.7.66. Ger., 16.7.65).—Anhyd. cream-like products are prepared by blending a fat mixture (containing 10–15% solids at 20° and 5–8% solids at 30°) 50–70 (55–65), sugar 20–30 and taste-refining and/or taste-giving constituents (milk powder, egg powder, chocolate powder, etc.) 0–30%. An inert gas (solid $CO_2$ added to the heated mixture) is worked into the mixture in amounts of 20–150 (40–100) ml (measured at 20°) per 100 g of the mixture. The fat mixture preferably consists of natural vegetable oils and fats, such as palm oil and cottonseed oil (wt. ratio 70–50 : 30–50) or palm oil and soyabean oil 60 : 40. Coconut and palm kernel oils cannot be used as they melt in a very narrow temp. range. The products, after conditioning at 30° for 24–48 h, do not change their consistency upon storage, being superior in this respect to prep. based on butter or margarine.
J. M. JACOBS.

**All-purpose shortening composition.** Procter & Gamble Co. (B.P. 1,069,669, 29.12.65. U.S., 29.12.64).—The shortening consists of (*a*) monoester of glycerol and fatty acid (**I**) e.g. derived from partly hydrogenated vegetable oil (I-value 70–80) containing $C_{16-18}$-acids; (*b*) monoester of polyoxyethylene-sorbitan containing ~20 oxyethylene units per mol. of acid, e.g., polyoxyethylene-sorbitan monostearate; (*c*) decaglycerol ester of fatty acid containing 2–5 fatty acid groups of 16–18 C and preferably derived from partly hydrogenated vegetable oil of I-value ≥45; (*d*) half-ester of a $C_{3-6}$-dicarboxylic acid with a monoester of **I** and a straight-chain glycol of 3–6 C. The product is useful for cake-making.
F. R. BASFORD.

**Pumpable shortening.** Procter & Gamble Ltd. (Inventor: C. F. Bruce) (B.P. 1,074,121, 17.5.63. Addition to B.P. 1,029,237, 5.10.62).—Shortening is prepared in a stable fluid state capable of being pumped at 70–80°F. As an example, 75 pt/wt. of a hydrogenated mixture of equal pt. whale oil and herring oil (I-value 76), 12 pt. of an unhydrogenated soyabean oil and 12 pt. hydrogenated palm oil (I-value 3) were melted at 120°F, and chilled in <1 min. to 92°F. The chilled blend was then further cooled (with mild agitation) at a rate of 4°F per h to 75°F. The freshly prepared product had a viscosity of 1800 cP at this temp. and after standing 7 days without agitation showed a viscosity of 2800 cP.
E. ENOS JONES.

## Meat and Poultry

**Catty odours in food: their production in meat stores from mesityl oxide in paint solvents.** R. L. S. Patterson (*Chemy Ind.*, 1968, 548–549).—The thinner, accelerator and polyurethane base in the paint responsible for the catty odour reported previously (*ibid.*, 1967, 2003) were analysed. The thinner was a mixture of xylene

and pentoxone (**I**), the latter being closely related to 4-mercapto-4-methylpentan-2-one (formed by addition of $H_2S$ to mesityl oxide and having a strong catty odour at high dilution). About 0·4% of mesityl oxide was present in **I** and was mainly responsible for the catty odour in the chill-room by combining with $H_2S$ in the meat. This was confirmed from results of reaction of $H_2S$ with **I** samples, and of meat with paint constituents, as well as from examination of contaminated meat. Solvent systems containing mesityl oxide by formulation or as impurity should be rigorously excluded from paints likely to be in contact with biological matter in a confined space or for long periods. Even 0·009% mesityl oxide in **I** produced a catty odour in meat. W. J. BAKER.

**Salmonella in meat imported from South American countries.** M. van Schothorst and E. H. Kampelmacher (*J. Hyg. Camb.*, 1967, **65**, 321–325).—This organism was isolated from 278 out of 800 (34·7%) samples of frozen carcass or boneless horsemeat from South America and from 101 out of 751 (13·5%) samples of frozen boneless beef. Contamination was mostly external, pointing to slaughterhouse contamination and boneless meat was contaminated to a higher degree than carcass meat. Thus slaughterhouse practice plays a most important rôle in establishing contamination. C.V.

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**Meat-curing compositions.** Griffith Laboratories, Ltd. (Inventor: L. Sair) (B.P. 1,077,112, 16.2.65).—The composition consists of NaCl, an ene-diol (e.g., water-sol. salt of an ascorbic acid isomer such as Na erythorbate), and (as curing salt) crystals of alkali metal nitrite with or without nitrate (or a fusion thereof). The ene-diol and the nitrite are prevented from reacting, without addition of an alkaline stabiliser, by ensuring that at least one of them is present as a minor component in aggregated particles of NaCl. The other compound can be present in free form (as crystals) or can also form a minor component of other aggregated NaCl particles. An alkaline agent can be included if desired. F. R. BASFORD.

### Fish

**Origins and development of the typical flavour and aroma of Thai fish sauce.** P. Saisithi (*Diss. Abstr.*, B, 1967, **28**, 734–735).—Studies of the bacteria isolated from fish sauce samples showed that the typical fish sauce aroma was produced by the homofermentative lactic acid bacterium, *Pediococcus halophilus*. The possible mechanisms by which the bacterium produced volatile acids (*VA*) from amino-acid catabolism were discussed. Principal amino-acids present were lysine, aspartic acid, glutamic acid, glycine, alanine, histidine, threonine, valine, leucine or isoleucine and phenylalanine; *VA* identified were formic, acetic, propionic and isobutyric.

**Determination of 2,4-D and its butoxyethanol ester in oysters by gas chromatography.** J. R. Duffy and P. Shelfoon (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1098–1102).—An acetonitrile extract is heated with ethanolic KOH; after acidification an ethyl ether–light petroleum extract is treated with $Na_2CO_3$ and re-extracted after acidification. After evaporation of the solvents the residue is methylated with diazomethane, cleaned up on a Florisil column and the 2,4-D methyl ester is determined by electron capture gas chromatography followed by TLC. Recoveries were 68 to 110%. A. A. ELDRIDGE.

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**Recovering fish oil.** Alfa-Laval Aktiebolag (B.P. 1,070,128, 29.10.65. Swed., 30.10.64).—The flushing water (centrifuged so as to separate out coarse solids) and the fish oil are separated by centrifuging so that three components are obtained: fine solids, oil-free water and an oil–water emulsion. Separation is thus speeded up and putrefaction processes in the sludge avoided. S. D. HUGGINS.

**Preservation of shellfish.** AB Skaldjur (B.P. 1,072,430, 24.5.66. Nor., 24.5.65).—All the cavities of the unshelled fish, particularly shrimps, are filled with preserving liquid, e.g. brine, excess liquid forming a layer around the fish which are contained in an airtight resilient bags of synthetic resin. The air in the bag is then evacuated so that the resinous material collapses; the shrimps are left in their natural shape and delicacy is maintained for approx. 4 weeks at a cooling temp. of 4–6°. This provides a cheaper reserve supply to the markets, without the use of canning or deep freezing. S. D. HUGGINS.

## Spices, Flavours, etc.

**Determination of geographical origin of spices. I. Cassia and cinnamon by thin layer chromatography.** W. A. Voelker, J. N. Skarzynski and W. H. Stahl (*J. Ass. off. analyt. Chem.*, 1967, **50**, 852–856).—By means of TLC using glass plates coated with silica gel G and cold extracts of the sample in EtOAc, Saigon cassia, Batavia cassia, Korintji cassia, Ceylon cinnamon and Seychelles cinnamon can be differentiated. About 20% of one sample mixed with another can be estimated. A. A. ELDRIDGE.

**[Determination of] vanillin and ethylvanillin in flavouring material.** J. Fitelson (*J. Ass. off. analyt. Chem.*, 1967, **50**, 859–860).—The two A.O.A.C. u.v. absorption methods and the A.O.A.C. paper chromatographic method, when studied collaboratively, gave satisfactory results. A. A. ELDRIDGE.

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**Production of uridylic acid by fermentation.** Kyowa Hakko Kogyo Co. Ltd. (B.P. 1,065,380, 27.11.64. Japan, 27.11.63).—The acid (flavour-improver in foodstuffs) is economically produced by culturing *Brevibacterium ammoniagenes* under aerobic conditions at 20–40°, pH 5·5–9 in aq. nutrient medium containing uracil (0·1–10 g per l). F. R. BASFORD.

**Improving the keeping properties of flavouring powders.** Kogei Co. Ltd. (B.P. 1,065,655, 2.7.64. Jap., 4.7.63).—The powder, which comprises flavouring agent (an essential oil) dispersed in a solid matrix (a sugar composition), is washed with a solvent selected from alcohols and/or ethers to remove the flavouring agent from the surface zones of the individual powder particles. The solvent also contains straight-chain polyhydric alcohols, monosaccharides and/or sorbitol fatty acid esters, surface-active agents, solid fats, resins or mineral waxes which, on evaporation of solvent from the powder, form a coating on the individual particles. The products retain their flavour for 70 days compared with untreated compositions (⩾28 days). J. M. JACOBS.

**Production of cured vanilla beans from green or partly cured vanilla beans.** R. J. Kaul (B.P. 1,067,967, 27.4.65. U.S., 27.4.64).—Curing is effected at 95°F to 175°F in an atm. of ⩽70% humidity until the enzymatic action and associated curing reactions have been substantially completed. The beans are then dried. F. R. BASFORD.

**Food products.** Unilever Ltd. (Inventors: J. C. M. Schogt, P. H. Begemann, K. de Jong and J. Rademaker-Koster) (B.P. 1,068,712, 24.7.62).—Hept-4-enal is claimed for addition to foodstuffs, imparting to them a cream- or butter-flavour, together with, if required, additional flavouring agents such as a lactone of a γ- or δ-hydroxy straight chain, aliphatic $C_8$-$C_{14}$ monocarboxylic acid. Thus, a flavouring agent is made by dissolving the following substances in refined groundnut oil: δ-hydroxy-n-dodecanoic acid lactone, γ-hydroxy-n-butyric acid lactone, γ-hydroxy-n-octanoic acid lactone, ε-hydroxy-n-dodecanoic acid lactone, butyric acid, caproic acid, caprylic acid, capric acid, diacetyl, acetylmethylcarbinol and *cis*-hept-4-enal. A portion of this mixture is incorporated in a milk margarine during manufacture. S. D. HUGGINS.

**Flavouring composition.** International Flavours and Fragrances Inc. (B.P. 1,069,104, 14.3.66. U.S., 22.3. and 21.10.65).—The claimed composition consists of the reaction product obtained by heating a mixture of a S-containing polypeptide, or a mixture of amino-acids, at least one of which contains S, and thiamine (optionally, in presence of an alkanone or hydroxyalkanone with 4–8 non-quaternary C atoms, and an alkyl aldehyde, having 5–8 C atoms), at 200–420°F for 10 min.–3 h. Thus, a mixture is prepared of fat, NaCl, glutamic acid, *l*-cysteine hydrochloride, β-alanine, glycine, thiamine hydrochloride, $Na_2$-inosinate and $Na_2$-guanylate, and is fed, continuously, to a scraped-wall heat exchanger. After heating at 325°F for 0·5 min., it is immediately cooled to 100°F in a second, similar exchanger when diacetyl and hexanal are added. The resulting mixture has an excellent chicken flavour suitable for gravies, sauces, soups etc. S. D. HUGGINS.

**Manufacture of seasoning material by the decomposition of whole yeast cells.** Kikkoman Shoyu K. K. (B.P. 1,071,027, 25.11.63. Japan, 26.11.62).—The seasoning (of delicious taste and not unpleasant odour) is produced in high yield by culturing *Streptomyces satsumaensis* nov. sp. in aq. medium containing inorg. phosphate material; then adding the resulting solution of enzymes (preferably after separation of any solids and heating at 50–60° to inactivate 5′-nucleotidase) to a suspension of yeast cells in aq. medium containing antiseptic (toluene, EtOAc or nitrofurazone); and allowing the enzymes to digest the yeast cells. The digest, now containing the seasoning bodies, may be adjusted to pH 5, extracted with EtOAc, then further adjusted to pH 6·5, distilled/$<$1 atm. with removal of 20% of distillate, adjusted to pH 5–6 and spray-dried. F. R. BASFORD.

**Production of flavourful protein hydrolysate.** Griffith Laboratories Ltd. (B.P. 1,071,248, 20.12.65. U.S., 26.2.65).—Protein (yeast, casein, wheat, rice, soya) is hydrolysed in aq. acid to a hydrolysate of 35–58% α-amino N content (on total N content), then acid is neutralised to pH 4·5–7, and the solution is dried to give a food flavouring. Aq. HCl is the preferred dilute acid used; if the hydrolysate is then neutralised with a Na alkali, the resulting NaCl byproduct is allowed to remain in the flavouring material.
F. R. BASFORD.

**Process for intensifying and supplementing the flavours of dairy products.** Takeda Chemical Industries Ltd. (Inventors: S. Hori and H. Shimazono) (B.P. 1,075,149, 25.8.64).—An improved dairy product is prepared by treating milk or a milk product with a flavouring enzyme produced by *Trametes*, e.g., *T. sanguinea*.
F. R. BASFORD.

**Improvement in the taste of seasoning, food and drinks.** Kyowa Hakko Kogyo Co. Ltd. (B.P. 1,080,659, 22.7.66. Jap., 23.7.65).—The foodstuff contains 0·01–1·0% by wt. of a mixture of *l*-glutamine and 5′-nucleotide in the ratio 100 : 1–1 : 1 by wt., the nucleotide being selected from Na 5′-inosinate, Na 5′-guanylate, Na 5′-adenylate and Na 5′-adenosine triphosphate.
S. D. HUGGINS.

**Flavouring composition.** Chas. Pfizer and Co. Inc. (B.P. 1,082,504, 6.10.65. U.S., 9.10.64).—A beef-flavoured substance is obtained by heating a hexose or pentose monosaccharide with cysteine in the presence of water until a beef-flavoured mixture is obtained and then adding for each pt by wt. of the mixture, 5–15 pt of vegetable protein hydrolysate and also 0·5–1·5 pt of a 5′-ribonucleotide and heating for approx. 2 h at ≮70°.
S. D. HUGGINS.

**Improved quality of seasonings, foods and beverages.** Kyowa Hakko Kogyo Co. Ltd. (B.P. 1,083,189, 26.10.64. Jap., 31.10.63).—The above are treated with a copolypeptide derived from one or two kinds of monoaminodicarboxylic acid e.g. glutamic or aspartic acid and one or two kinds of diaminocarboxylic acid e.g. ornithine or lysine. Additional flavouring agents (a 5′-nucleotide and Na glutamate) may also be incorporated. The copolypeptides are heat-stable and help to retain the moisture content of foods containing them, due to their hygroscopic properties.
S. D. HUGGINS.

### Colouring matters

**Colour coating compositions for edible solids.** H. Kohnstamm & Co. Inc. (B.P. 1,073,366, 4.3.66. U.S., 10.3.65).—A non-toxic colour composition for coating edible solid (food, drugs, tablets, pills, pellets, candy, condiments, and chewing gum) comprises an aq. suspension of an *in situ* formed non-toxic colour lake containing a non-toxic water-sol. dyestuff, adsorbed on aluminium hydrate gel, $CaSO_4$ etc. An edible preservative, a viscosity-reducing non-toxic org. acid, and $TiO_2$ powder may also be included.
F. R. BASFORD.

### Preservatives

**Effects of meat curing ingredients on heat resistance of putrefactive anaerobic spores.** C. L. Duncan (*Diss. Abstr.*, B, 1967, **28**, 457).—The mechanism by which the curing ingredients NaCl, $NaNO_3$ and $NaNO_2$ supplement the action of heat in effecting preservation of canned cured meat products was studied. Depending on concn. and pH, $NaNO_2$ was shown to inhibit emergence of vegetative cells from spores, or cell division of newly emerged and elongated vegetative cells. Data obtained on heating spores in presence of nitrite supports the assumption that nitrite stimulates the germination of spores, since spores so heated had a much lower D value than did those heated without nitrite.
F. C. SUTTON.

**Preservatives: a review of methods of analysis.** P. L. Schuller and E. Veen (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1127–1145).—A review of methods for determining $SO_2$, sorbic acid, nitrite, nitrate and benzoic acid in food is presented with 246 references. Methods officially specified or used in various countries are indicated.
A. A. ELDRIDGE.

**Rapid determination of sorbic acid in orange juice.** K. M. Floyd (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1123–1127).—After a screening test involving spectrophotometry at 400 to 220 nm of a filtered mixture of the sample and ethanol (1 : 19) an acidified sample is extracted into a mixture of ether and light petroleum (1 : 1) and a dil. ethanolic NaOH extract of this solution is then examined spectrophotometrically at 255 nm. Sorbic acid (**I**) is distinguished from benzoic acid by paper chromatography followed by spraying with aq. $K_2Cr_2O_7$ and treatment with 2-thiobarbituric acid which with **I** produces a pink spot.
A. A. ELDRIDGE.

**Gas chromatographic determination of butylated hydroxyanisole and butylated hydroxytoluene in breakfast cereals.** D. M. Takahashi (*J. Ass. off. analyt. Chem.*, 1967, **50**, 880–883).—The procedure previously used (Takahashi, *ibid.*, 1966, **49**, 704) has been modified by eluting the antioxidants with $CS_2$. The eluate, containing 3,5,-di-t-butyl-4-hydroxyanisole as internal standard, is concentrated under $N_2$ and analysed by GLC using a $H_2$ flame detector. Recoveries ranging from 96 to 106% are reported.
A. A. ELDRIDGE.

**[Determination of] benzoates in foods.** M. H. Lewis and G. Schwartzman (*J. Ass. off. analyt. Chem.*, 1967, **50**, 985–988).—The aq. sample is distilled with steam after addition of $MgSO_4$ and $H_3PO_4$; the distillate is collected in aq. NaOH which is extracted with $CHCl_3$ and ether (2 : 1) after acidification. The benzoic acid is determined by TLC, using $SiO_2$ gel GF254 and kieselguhr G, followed by spectrophotometry. Recoveries were 48·0 to 116·8%.
A. A. ELDRIDGE.

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**Preservation of foods.** Chas. Pfizer & Co. Inc. (B.P. 1,077,690, 8.2.66. U.S., 29.9.65).—Foodstuffs especially processed foods, e.g., dog food, cheese, bakery goods (cookies, cakes), also fresh fruit and vegetables are preserved against spoilage by microorganisms by treatment with 0·01–1 wt.-% of isomaltol (**I**) (3-hydroxy-2-acetylfuran). **I** can also be added to wrapping films for preventing microbial spoilage of e.g. raspberries inoculated with *Botrytis cinerea*.
F. R. BASFORD.

## Pesticides in Food

**Determination of nicotine residues in foods.** R. J. Martin (*J. Ass. off. analyt. Chem.*, 1967, **50**, 939–940).—In a modification of the A.O.A.C. method (*Official Methods of Analysis*, 10th Ed., 1965, 24.162–7) a glass column 6 ft × 4 mm packed with 10% DC-200 on Gas Chrom Q was used, with $N_2$ as carrier gas, and a thermionic detector coated with KCl. At 1–3 ppm recoveries were 95 to 97%.
A. A. ELDRIDGE.

**Determination of ethylene dithiocarbamate residues in plants, fruits and vegetables.** C. F. Gordon, R. J. Schuckert and W. E. Bornak (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1102–1108).—In a modification of the method of Pease (*ibid.*, 1957, **40**, 1113) a sample of the frozen crop is blended in ice-cold water and an aliquot is decomposed with hot 50% $H_2SO_4$, the $CS_2$ evolved being removed in a current of air. For its colorimetric determination, Viles' procedure (*J. ind. Hyg. Toxicol.*, 1940, **22**, 188) is used for 1–200 μg of ethylene dithiocarbamate and Cullen's procedure (*Analyt. Chem.*, 1964, **36**, 221) for 200–1000 μg.
A. A. ELDRIDGE.

**Extraction of a polar insecticide (Imidan) from milk.** M. C. Bowman and M. Beroza (*J. Ass. off. analyt. Chem.*, 1967, **50**, 940–941).—Recovery of Imidan (*O,O*-dimethyl 5-phthalimidomethyl phosphorodithioate) from goat's milk is more complete by the low fat extraction procedure than by the high fat procedure, whether the insecticide is fed to the goat or added to the milk. A high dosage is required to cause even a small amount to appear in the milk.
A. A. ELDRIDGE.

**Screening method for the detection of chlorinated hydrocarbon pesticide residues in the fat of milk, cheese and butter.** R. P. Moubry, G. R. Myrdal and H. P. Jensen (*J. Ass. off. analyt. Chem.*, 1967, **50**, 885–888).—Fat is separated from milk with a detergent at 85–100° or from cheese by extraction with ether. A mixture of fat and partially deactivated Florisil is added to a Florisil column which is then eluted with hexane and diethyl ether and the chlorinated hydrocarbon content is determined by GLC using electron capture detection. Recoveries ranging from 77·8 to 102·3% are reported.
A. A. ELDRIDGE.

**Modifications of the Mills procedure for [determining] chlorinated pesticides in dairy products.** W. B. Furman and N. V. Fehringer (*J. Ass. off. analyt. Chem.*, 1967, **50**, 903–908).—Theoretical and experimental results for the extraction of lindane, heptachlor, aldrin, heptachlor-epoxide, dieldrin, endrin and *p,p′*-DDT by the procedure of Mills (*ibid.*, 1961, **44**, 171) have led to several proposals for minor changes in the method of extraction, resulting in improved recoveries.
A. A. ELDRIDGE.

## Food Processing, Refrigeration

**Preservation of bread by exposing to γ-irradiation.** G. Stehlik (*Atompraxis*, 1968, **14**, 195–200).—Prolongation of storage-life of sliced bread was studied using γ-irradiation alone; in certain

experiments, heating and chemical pretreatment were also carried out. Organoleptic observations were also made. No difference could be noted immediately after irradiation even at $5 \times 10^5$ rad, as compared with the controls. After storing 2 months at room temp. a degree of deterioration was noted. The findings are tabulated. (14 references.) C.V.

**Effects of freezing on dairy product quality.** P. Swartling (*Dairy Inds*, 1968, **33**, 30–36).—Despite the fact that many adverse reactions occur which are detrimental to quality, refrigeration is the best available method of preservation. Effects of ice formation, protein destabilisation, fatty acid oxidation, etc. are studied, tables and graphs being given. (35 references.) C.V.

## Packaging

**Plastic crates.** L. W. McNair (*Dairy Inds.*, 1968, **33**, 235–237).—C.V.

**Odour control by chromatography.** S. Sacharow (*Am. Ink Mkr*, 1967, **45**, No. 8; 39, 64–65).—Many food taint problems arise from printing ink used on packaging. Examples of the use of gas chromatography to examine residual odours in inks, e.g., from retained solvent, are described. L. A. O'NEILL.

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**Laminating compositions.** Shell Internationale Research Mij., N.V. (B.P. 1,081,312, 15.7.65. U.S., 17.7.64).—A composition with good adhesion to paper cartons, etc., is a dispersion of 2–10 wt.-% of a polyethylene (mol. wt. < 50,000) in a microcryst. wax, the initial crystallisation temp. of which is below the complete crystallisation temp. of the polyethylene. 5–10 wt.-% of a paraffinic wax (with ASTM m.p. 57·2–62·8° and containing >30% of non-normal paraffins) and 2–10 wt.-% of a sealing strength additive (polyterpene resin, or hydrogenated rosin esterified with glycerol or pentaerythritol) may also be present. J. A. SUGDEN.

## Miscellaneous

### Nutrition, proteins, amino-acids, vitamins

**Nutrition and public health.** (*Proc. Nutr. Soc.*, 1968, **27**, 1–43).—A joint symposium; some eight papers were submitted. C.V.

**Milk and lactose intolerance in healthy Orientals.** Shi-Shung Huang and T. M. Bayliss (*Science, N.Y.*, 1968, **160**, 83–84).—Nineteen out of 20 healthy oriental adults living in the U.S.A. developed abdominal cramps and diarrhoea after ingesting an amount of lactose equivalent to one-quart of milk; 14 showed similar symptoms after one or two glasses of milk. All had consumed milk in infancy without ill effect. Two out of 20 Caucasians were intolerant to milk and lactose. It would appear that many Orientals may have a lactase deficiency (*LD*) which would lead to milk intolerance. *LD* is also common amongst Negroes, which suggests that a large % of the world's adult population is thus genetically constituted. (13 references.) C.V.

**Sensory evaluation of soya flour.** H. A. Moser, C. D. Evans, R. E. Campbell, A. K. Smith and J. C. Cowan (*Cereal Sci. Today*, 1967, **12**, 296, 298–299, 314).—A description is given of the satisfactory performance of a taste panel in the detection of soyabean flavours in flours resulting from different methods of processing. P. S. ARUP.

***l*-Ascorbic and *d*-isoascorbic acids: quantitative separation and assay.** C. E. Weeks and M. J. Deutsch (*J. Ass. off. analyt. Chem.*, 1967, **50**, 793–798).—*l*-Ascorbic and *d*-isoascorbic acids, which are not differentiated by chemical assay, can be separated by ascending chromatography on a glass fibre paper previously treated with $HPO_3$ and glycerol after impregnation with silicic acid. Extracts of the chromatogram are assayed by the microfluorometric method (Deutsch and Weeks, *ibid.*, 1965, **48**, 1248). Average recoveries were: *l*-ascorbic acid (alone) 99%, (mixture) 102%; *d*-isoascorbic acid 104%. A. A. ELDRIDGE.

**Assay for vitamin C.** M. J. Deutsch (*J. Ass. off. analyt. Chem.*, 1967, **50**, 798–806).—Comparison with the methods of assay using 2,6-dichloroindophenol and 2,4-dinitrophenylhydrazine shows that the microfluorometric method (preceding abstr.) using *o*-phenylenediamine gives satisfactory results and is the more specific for the measurement of antiscorbutic activity. A. A. ELDRIDGE.

**Determination of ascorbic acid in enriched evaporated milk.** O. Pelletier (*J. Ass. off. analyt. Chem.*, 1967, **50**, 817–820).—Proteins and their thermal decomposition products are removed by pptn. with acetic and metaphosphoric acids before determination of the ascorbic acid (I) by titration with 2,6-dichloroindophenol. A correction for interfering substances is made by blank titration after condensing I with $CH_2O$. Recoveries of 96·0 to 100·6% are reported. A. A. ELDRIDGE.

**Polarographic determination of vitamin E. Determination of total tocopherols.** K. Wisser, W. Heimann and Ch. Fritsche (*Z. analyt. Chem.*, 1967, **230**, 189–199).—Total tocopherols are determined by polarography of the quinones after oxidation. The method is applicable to plant and animal materials as well as to pure prep., and six to eight determinations per day are possible. R. WASPE.

**Gas–liquid chromatographic method for determination of fat-soluble vitamins. IV. Vitamin $K_1$.** D. A. Libby, A. R. Prosser and A. J. Sheppard (*J. Ass. off. analyt. Chem.*, 1967, **50**, 806–809).—By the use of SE-30 on silanised Gas Chrom P the vitamin $K_1$ peak and the tocopherol peak were resolved. A $^{226}Ra/Ar$ ionisation detector and $H_2$ flame ionisation detector permitted the determination, respectively, of 0·5 and 2 μg of vitamin $K_1$. A. A. ELDRIDGE.

### Unclassified

**Removal of pigments from chloroform extracts of aflatoxin cultures with copper carbonate.** H. G. Wiseman, W. C. Jacobson and W. C. Harmeyer (*J. Ass. off. analyt. Chem.*, 1967, **50**, 982–983).—The $CHCl_3$ extract of aflatoxins produced by *Aspergillus flavus* is treated with 1% of ethanol and shaken with basic Cu carbonate on which the pigments are adsorbed and from which they may be recovered. A. A. ELDRIDGE.

**Water-based aflatoxin standard.** R. Peterson and A. Ciegler (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1201–1202).—Errors due to concn. of $CHCl_3$ solutions of aflatoxins by exposure to air are minimised by the use of aq. standard solutions. A. A. ELDRIDGE.

**Photochemical changes of aflatoxin $B_1$.** P. J. Andrellos, A. C. Beckwith and R. M. Eppley (*J. Ass. off. analyt. Chem.*, 1967, **50**, 346–350).—Irradiation of aflatoxin $B_1$ and $G_1$ at 365 nm gives fluorescent products having lower $R_F$ values when chromatographed on $SiO_2$ gel thin layer plates, thus affecting fluorescence comparison assays. Moreover the photo-product from aflatoxin $B_1$ is less toxic than the parent aflatoxin. Appropriate precautions in the assay procedure are listed. A. A. ELDRIDGE.

**Identification of aflatoxin $B_1$ by derivative formation.** L. Stoloff (*J. Ass. off. analyt. Chem.*, 1967, **50**, 354–360).—The procedure of Andrellos and Reid (*ibid.*, 1964, **47**, 801) was improved by a modification of the $SiO_2$ gel column clean-up. In collaborative work good results were obtained with trifluoroacetic acid and formic acid/thionyl chloride reagents; some difficulty was experienced with the acetic acid/thionyl chloride reagent. A. A. ELDRIDGE.

**Detection and estimation of aflatoxin in fungal fermentation products.** L. J. Denault and L. A. Underkofler (*Cereal Chem.*, 1967, **44**, 1–7).—The method of W. V. Lee (*Analyst, Lond.*, 1965, **90**, 305) offers a simple, rapid procedure for the examination of fungal enzymes (e.g. produced by *Aspergillus flavus* and used in the food industry). TLC of the $CHCl_3$ extracts of seven commercial prep. on silica gel showed in all cases a fluorescing spot having an $R_F$ value > that of aflatoxin (I) $B_1$, due to a component with completely different u.v. and i.r. spectra which does not produce a fluorescent deriv. with formic acid in presence of $SOCl_2$. Recovery of added I by $CHCl_3$ extraction of the enzyme prep. was complete and no I was found in any of the samples examined. The detection limit was 0·005 μg of I-$B_1$ (≡ 0·05 ppm). E. C. APLING.

**[Determination of] extractables in rubber materials.** D. E. Korte (*J. Ass. off. analyt. Chem.*, 1967, **50**, 840–844).—The wt. of material extracted by water or hexane from samples of rubber articles that repeatedly come into contact with food has been determined with reference to the appropriate U.S. Food Additives Regulation. A. A. ELDRIDGE.

**Flame photometric determination of calcium in food, using a reversed oxy-acetylene flame, a radiation buffer and an acid mixture.** J. Lehmann and E. G. Zook (*J. Ass. off. analyt. Chem.*, 1967, **50**, 814–817).—Interferences from Na, K, P, Mg, Fe, S and Al are controlled by use of a radiation buffer (aq. NaCl, KCl and $MgCl_2$) or aq. HCl + $H_3PO_4$ when a reversed oxy-acetylene flame is employed in the spectrophotometric determination. Recoveries of 96·0 to 101·6% of added Ca are reported. A. A. ELDRIDGE.

**Polarographic determination of inorganic constituents (natural or derived) in foodstuffs.** P. Souchay (*Chim. analyt.*, 1967, **49**, 496–511).—Reviews extensively the methods, especially the problems associated therewith, for determining $SO_3^{2-}$, $NO_2^-$, $SO_4^{2-}$, $PO_4^{3-}$, $O_2$, peroxides, and traces of metals. Subjects discussed are: preliminary treatment or concn. of sample; selection of basal electrolyte; adjustment of pH; separate determination of the metals (Cu, Pb, Cd, Ni, Zn, Sn, Sb) or other species either by use of an appropriate basal electrolyte or by rapid and simple separation before polarography; indirect polarography of non-electroactive species by use of a reducible or non-reducible reagent to form a reducible compound in solution or as a ppt. Ways of improving sensitivity and/or selectivity are also examined, e.g. by large-area electrodes, derivative curves, and oscillographic, sinusoidal-voltage or square-wave polarography. (70 references.)
W. J. BAKER.

**Chemical indexes of decomposition and filth in foods.** H. Salwin (*J. Ass. off. analyt. Chem.*, 1967, **50**, 774–776).—A brief review of advances in methods for the determination of acids and volatile compounds. A. A. ELDRIDGE.

**Bacteriological quality of soft-serve frozen desserts.** J. H. Martin, R. E. Roberts and J. J. Sheuring (*J. Milk Fd Technol.*, 1968, **31**, 31–33).—In a 3-year survey it was found that 76% of all samples analysed contained < 50,000 bacteria/ml while 72% contained < 10 coliform organisms/ml. Setting these figures as a max. limit would pose a problem since in the retail sample group, only 42% and 51% would achieve this standard. Spore counts are of little consequence in serve mixes or frozen products and a max. allowable standard of 300/ml would be a reasonable figure that both the processor and retailer could meet. C.V.

**Heat resistance of spores of *Clostridium welchii*.** M. Nakamura and J. D. Converse (*J. Hyg., Camb.*, 1967, **65**, 359–365).—Eight strains of this organism were studied. Spores emanating from food poisoning cases possessed greater heat resistance than those isolated from the soil or from faeces. D-values and trend values are calculated from the thermal death curves. (23 references.)
C.V.

**Heat and radiation resistance and activation of spores of *Clostridium welchii*.** T. A. Roberts (*J. appl. Bact.*, 1968, **31**, 133–144).—Spores of the 'classical' strains (*CS*) are more sensitive to heat ($D_{90}$° 3–5 min.) than those of 'food poisoning' (*FPS*) strains ($D_{90}$° 15–145 min.). The *z* values for *CS* (*z* = 11–14°F) were lower than for *FPS* (*z* = 16–29°F); 30–50% spores of *CS* grew without heating and no heat activation was noted. In the *FPS*, only 0·13–3·6% grew without heating and heat activation was detected at 75–80°C and in one case at 75–100°C. Spores of *FPS* were more resistant to γ-radiation (D = 0·26–0·34 Mrad) than spores of *CS* (D = 0·12–0·21 Mrad). (25 references.) C.V.

## 3.—SANITATION, WATER, etc.

**Specifications for pesticides used in public health: Insecticides, rodenticides, molluscicides, repellents, methods.** (*World Health Organisation*, Geneva, 1967, 3rd Ed., 300 pp.). C.V.

**Fly identification by the morphology of the head and head appendages.** N. A. Carson and E. F. Martinez (*J. Ass. off. analyt. Chem.*, 1967, **50**, 1146–1193).—A description of the characteristics useful in identifying 10 fly species is accompanied by many illustrations.
A. A. ELDRIDGE.

**Housefly age as a factor in response to certain carbamates.** L. R. Green and H. W. Dorough (*J. econ. Ent.*, 1968, **61**, 88–90).—Baygon, Banol and carbaryl were applied topically to adult flies. Susceptibility decreased from 1–5 days and then increased so that at 15 days it was similar to that on the 1st day. This pattern could not be explained by total cholinesterase (**I**) activity or its sensitivity to the insecticides, neither did rates of penetration or excretion of carbaryl-$^{14}$C show any relation to the mortality data. However, *in vivo* inhibition of **I** did correlate with observed toxicity of the carbamates to adult flies. C. M. HARDWICK.

**Integrated housefly control: populations of fly larvae and predaceous mites *Macrocheles muscaedomesticae*, in poultry manure after larvicide treatment.** R. C. Axtell (*J. econ. Ent.*, 1968, **61**, 245–249).—Manure under caged laying hens was treated with various insecticides. Counts of mites and fly larvae showed that any treatment that did not destroy the mite population gave very little control of fly larvae. Compounds that controlled fly larvae were deleterious to the mites for a far longer period, allowing rapid fly resurgence. (24 references.) C. M. HARDWICK.



**Biochemical genetics of housefly resistance to carbamate insecticide chemicals.** M. Tsukamoto, S. P. Shrivastava and J. E. Casida (*J. econ. Ent.*, 1968, **61**, 50–55).—The use of susceptible multi-chromosomal markers is described. Resistance to Baygon and Matacil was dominant; the 5th chromosome was the most important but 3rd and 2nd were also involved. Simultaneous topical application of piperonyl butoxide decreased the effect of the 5th chromosome. C. M. HARDWICK.

**Petroleum oils as mosquito larvicides and pupicides. I. Correlation of physiochemical properties with biological activity.** D. W. Hagstrum and M. S. Mulla (*J. econ. Ent.*, 1968, **61**, 220–225).—The mortality of *Culex pipiens quinquefasciatus* was evaluated by log-dosage response lines and probit mortality. Larvicidal and pupicidal activity of the oils varied with aromatic and paraffin content and with boiling range, viscosity being a function of boiling range. These factors affected the rate at which the oil film was reduced by evaporation. (31 references.)
C. M. HARDWICK.

**The control of rats in coconuts, using rat blocks.** R. W. Smith (*Oleagineux*, 1967, **22**, 159–160).—A technique for the chemical control of rats was developed by which an anticoagulant is mixed with maize flour and coated with paraffin wax, to protect it against moisture. For a shock treatment, half-pound blocks are placed on the ground along a line of trees, one for every five or six trees; however, three or four treatments a year at the rate of 2 lb to the acre are usually sufficient. A simple method to assess the extent of rat damage and effectiveness of control is given.
M. DUDLEY.

**Enzymes of thermophilic aerobic, sporeforming bacteria.** A. Baillie and P. D. Walker (*J. appl. Bact.*, 1968, **31**, 114–119).—Esterase patterns of 217 strains were examined by starch gel electrophoresis; classification by this method corresponded closely with results obtained by biochemical methods. Protein patterns were also examined by acrylamide gel electrophoresis. In order to study heat resistance and other properties, an attempt was made at purification, using high voltage carrier-free electrophoresis.
C.V.

**Amino-acid flux in an estuary.** J. E. Hobbie, C. C. Crawford and K. L. Webb (*Science, N.Y.*, 1968, **159**, 1463–1464).—Dissolved org. matter in York river estuary included 38 μg free amino-acids per l. The highest concn. were of glycine, serine and ornithine. Of the 14 amino-acids studied for uptake by planktonic bacteria, glycine, methionine and serine had the greatest flux rates, the total amino-acid flux representing 1–10% of the daily photosynthetic C-fixation. (11 references.) C.V.

**Anaërobic lagoon treatment of milking-parlour wastes.** R. C. Loehr and J. A. Ruf (*J. Wat. Pollut. Control Fed.*, 1968, **40**, 83–94).—A study of the milking parlour waste (flow 2870 l per day, $BOD_5$ 1030 mg per l) from an 80-cow dairy, which is allowed to flow into two lagoons operated in series, shows that anaërobic lagoons can treat such wastes adequately. The average $BOD_5$ reductions produced by the first cell loaded at ~9 lb $BOD_5$/day/1000 cu. ft (144 g BOD/day/cu. m), were 85% in summer (liquid temp. 29°) and 20% for the winter (liquid temp. 2°). The average $BOD_5$ of the effluent from the first cell during the summer was ~150 mg per l. During the summer the $BOD_5$ reduction in the second cell, loaded at 0·6 lb $BOD_5$/day/1000 cu. ft (9·6 g BOD/day/cu. m) was ~67%, or 95% for the system. Removal of the solids after sedimentation in winter without leaving adequate seed had an adverse effect on the performance of the lagoons. The total and faecal coliform reduction of the supernatant liquid for the first cell during the summer was ~99%, while the reduction for the system with the second cell was 99·8%. Key factors involved in re-establishment of equilibrium conditions, were (*a*) increase of temp. in spring, (*b*) increase in microbial no. stimulated by high food-to-organism ratio and (*c*) control of acidity due to volatile acids. (14 references.) J. M. JACOBS.

## 4.—APPARATUS AND UNCLASSIFIED

**Rubbers in agriculture.** P. S. Byrne (*Rubb. J.*, 1967, **149**, (12), 46–55).—New agricultural uses for synthetic rubber polymers and blends include the manufacture of milking liners, cow mats, dairy floor coverings and membranes for reservoir and pit linings, silos and feed hoppers. A specification and various formulations suitable for agricultural applications are provided. (11 references.)
J. L. WALPOLE.

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# ABSTRACTS

AUGUST, 1968

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

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