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THE PLACE OF FERTILISERS IN FORESTRY*

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Apart from their regular use in forest nurseries and on certain nutrient-deficient sites at the time of planting or to rejuvenate plantations that have checked, fertilisers are at present very little used in forestry, mainly because of lack of knowledge of the magnitude and persistence of responses that may be expected under given conditions. The literature records many instances of responses to different fertilisers, applied mainly at the time of planting, phosphate being the most frequently effective nutrient, though in certain circumstances N, K and Ca have also produced worthwhile responses. Classical work on liming in older conifer forests in Germany is mentioned, and recent work on nitrogen is discussed, with particular reference to the need for discovering a slow-acting form which will produce lasting effects.

Much research is needed to determine conditions for economic growth responses to fertiliser applications, and it is stressed that the physical and biological factors affecting tree nutrition must be studied in addition to the purely chemical factors.

Introduction

COMPARED with agriculture, where in little over a century the ever-increasing use of fertilisers has resulted in enormously increased yields from the soil, forestry is, relatively speaking, almost at the beginning. The amounts of fertiliser used, except in a few special cases, such as in nurseries and on particular sites, are negligible. Scarcely ever does one find fertilisers being used to improve the yield from normally growing forest crops.

There are various reasons for this. Foresters have been brought up in a school founded on the management of natural forests. It was almost axiomatic that healthy and well-managed forest was in equilibrium with the soil on which it grew and with the other ecological factors of the site, and the forester sought to improve his yields by regulating the composition of the forest stand and so influence the soil. The alternative method of deliberately employing technical measures to interfere with and regulate nature, as has been done in agriculture so successfully, was scarcely considered—at least until things began to go wrong.

The late Sir John Stirling-Maxwell, a pioneer of tree planting on peat and the first to use phosphate fertilisers for peat planting in this country, wrote :[1] ' Most foresters are, or were, brought up to distrust the use of manures in the forest. They do not disdain their help—indirectly at least—in the nursery where seedlings are made to grow at least three times as fast as those under natural conditions. But they feel, not without reason, that it is futile to offer a tablespoonful of manure to a plant which is intended to live 150 years, grow 100 feet high and weigh more than a ton.' Such ideas are by no means unknown today and the fact that tree crops take so long to mature, some 60–90 years for conifers and 120–200 years for broadleaved trees, encourages the feeling that the use of fertilisers in them is rarely likely to be economic.

This attitude, plausible as it may seem, depends on the tacit assumption that the magnitude and persistence of growth responses to fertilisers will be negligible and the most cogent reason for their very limited use in forest crops is the lack of knowledge of what responses to expect under given conditions. A great deal of experimentation has been done over the last 70 years or so, a large part of which has produced negative results and very few general principles to guide the forester have as yet emerged, though in a number of particular cases, local techniques of using fertilisers have been worked out.

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That fertilisers will become more widely used in the future is probable for two reasons, (1) that much afforestation is being done on infertile soils, too poor for agriculture, and, without the artificial addition of nutrients, too poor sometimes for economic forestry; and (2) because a forest crop extracts from the soil a considerable quantity of mineral nutrients. Through the removal of trees in thinnings and final fellings, this constitutes a drain on the mineral reserves in the soil which may have to be replenished by artificial means.

This point has been examined by Rennie[2] who collected such figures as were available of the nutrient content of the wood, bark, twigs and leaves of trees, calculated the equivalent quantities of calcium, potassium and phosphorus per acre and showed that the danger of soil degradation from loss of mineral nutrients was a real one especially in respect of calcium on our poorer acid heathland soils. Forests must be managed so as to maintain and if possible improve their productivity. It is an important part of the programme of the soils section of the Forestry Commission Research Branch to collect data on this very point so as to be able to follow the changes in the nutrient balance sheet of forests of different species and on different soils. In this way it is hoped to detect any tendencies towards the depletion of mineral reserves and to arrive at a means of diagnosing conditions under which economic responses to additions of particular fertilisers are likely.

The use of fertilisers in forestry can be conveniently divided into four phases according to the stage and state of growth of the crop, namely (1) in forest nurseries; (2) at the time of planting in the forest; (3) for the amelioration of crops that have gone into check; and (4) in crops that have closed canopy.

Fertilisers in nurseries

This subject is discussed by Benzian[2a] and it is only necessary to add that it is a phase of forestry operations that is more akin to horticulture than the others and the regular use of both mineral fertilisers and bulky organic manures is general practice.

Application at time of planting and to checked crops

The use of fertilisers at the time of planting is closely linked with the use for the rejuvenation of checked or unhealthy young crops. In fact nearly all the prescriptions for manuring at the time of planting have been developed from experience in treating plantations that have grown unsatisfactorily or even stopped growing altogether. A great deal of experimentation has been done in almost every country that has an extensive plantation programme. Much of it has, unavoidably, been of an *ad hoc* nature, testing out likely substances in conditions where trees either fail or grow very slowly, and in very few cases have data from soil or foliar analyses been found useful as a guide to the treatments most likely to produce beneficial results.

On the deep peats and peat bogs, even after the first essential of good drainage has been provided, trees frequently fail to grow without manuring. In contrast with some heathland soils, nitrogen supplies appear always to be adequate, but frequently phosphorus and sometimes potash may be lacking in sufficient amounts.

One of the earliest experiments on peat was started in Sweden in 1928 (Alund, see[3]), in which applications of wood ashes to stagnant Scots pine and Norway spruce resulted in greatly improved growth which has persisted for 30 years and is attributed mainly to the phosphate content of the wood ash. In other Swedish investigations on peat bogs[4] phosphorus and potassium were both found to be limiting growth and responses were obtained to fertilisers containing these elements. In some cases it was found that phosphate alone had no effect. Experience in Finland[5] has been similar, good results being obtained from fertilisers containing both P and K. Lime, even in large quantities, was found to have no effect.

In Germany also[6] clear responses were obtained to both phosphate and potash in experiments in planting Norway spruce on blanket peat in Upper Bavaria. After 10 years the potash effect appeared to be diminishing while the phosphate effect was apparently increasing. The effect of lime was again negligible.

In this country, some 30 years of research comprising a very large number of experiments on the problems of establishing plantations on peat has been summarised by Zehetmayr.[7] The results show that on the better-quality peats, usually containing a small admixture of

mineral soil and characterised by a vegetation of *Molinia* grass, applications of phosphate and other fertilisers are unnecessary and have no appreciable effect. The extensive grass peat areas in the border country are of this type. On the poorer peats dominated by short heather or deer grass vegetation, addition of phosphate is found to be highly beneficial and in many cases essential for the survival of the trees. Ground mineral phosphate is the form generally preferred and is applied as a routine measure on such sites at the rate of 1½–2 oz. per planted tree which is equivalent to approximately 1½–2 cwt. per acre with trees spaced 5 ft. apart. Superphosphate and basic slag have also been used successfully but the latter should be a high-grade Bessemer slag as some inferior slags have been found to be toxic. The method of application is by spreading the powdered fertiliser by hand on the surface of the ground around the stem of the planted tree. This has been found as effective as placing it in the planting notch or in holes a few inches away from the roots. Triple superphosphate has recently been tried out[8] and found equally satisfactory and has the advantage that the weight of material that has to be carried up the mountain side is greatly reduced.

Trials with other nutrients—potassium, nitrogen, complete NPK fertilisers, calcium, magnesium, boron, copper, iron and even iodine—were also carried out. No positive responses were obtained, phosphorus being the only nutrient that has been effective, and its use has often made all the difference between success and failure.

These results are broadly in line with those on the Continent with the notable exception that on the peats in this country we have so far never obtained any responses to initial applications of potash, whereas on peats in Sweden, Finland and Germany responses to potash are usual and sometimes it seems to be the more essential element.

The interesting phase on the deep peats will be reached when the tree crops come into canopy and start making much bigger demands on the soil. This problem is being studied by Wright and others at the Macaulay Institute, Aberdeen.[9] It has been found, for instance, that in an experimental plot of Lodgepole pine planted in 1928, part of which was dressed with ground mineral phosphate in 1939, the amounts of all major nutrients, particularly potassium and inorganic phosphorus, are lower under the trees with fertiliser which are growing vigorously than under the unfertilised control. It is evident that as tree crops on these deep peats develop, new nutritional problems are likely to arise.

Use on heathland

A far greater range of nutrient deficiencies is found on heathlands and on other mineral soils, although, here again, phosphate is the commonest nutrient in deficit. The levels of nutrients in some of the mineral soils used for forestry are exceedingly low, well beyond the normal experience of the agriculturist. In Western Australia,[10] for instance, phosphate levels are commonly under 200 p.p.m. of total P_2O_5, and in some cases down to only 2 p.p.m. In the pine plantations there it was found that, provided other causes such as trace-element deficiencies were not limiting growth, applications of phosphate fertilisers at the rate of 1½–2 cwt. per acre in most cases restored the plantations to healthy growth. As a rough guide a total P_2O_5 content of over 400 p.p.m. was required for the satisfactory growth of *Pinus radiata* and of 150 p.p.m. for the more tolerant *Pinus pinaster*. Below these levels application of phosphate was necessary. Growth responses appeared in the third year after application, and increased or repeated applications did not result in a further stimulus to growth. In South Australia,[11] in contrast, it was found that on lateritic soils with a P_2O_5 content of 130 p.p.m., proportionately greater growth responses in *Pinus radiata* were obtained with increasing applications up to 8 cwt. of basic slag per acre, and worthwhile additional growth resulted from second similar dressings at 15 years old.

In Europe, most of the fertiliser trials at the time of planting have been done on heathland of one kind or another. Contrary to experience in Britain, liming on acid heaths with, or sometimes without, the use of phosphate has on occasions given good results. In Belgium,[12] for instance, a fairly heavy liming of 2 tons per acre before planting Norway spruce in 1909 resulted in larger increases of growth than a heavy dressing of basic slag at 12 cwt. per acre, and the effects still persist after 44 years. Sometimes basic slag alone gives large responses. Jaeger[13] reports a case where, after strip ploughing and harrowing, an area was sown with mixed conifer seed in 1940. Plots in it were given 3·2 and 1·6 cwt. of basic slag per acre while a third plot was

left untreated as a control. Sixteen years later the total stem volumes standing in the plots was in the ratio of $7\frac{1}{2} : 5\frac{1}{2} : 1$, Japanese larch accounting for most of the growth.

In another heathland experiment, in Holland,[14] checked chlorotic Norway spruce 15 years old and only about $4\frac{1}{2}$ ft. high was subjected to some 24 different combinations of fertilisers in the early spring of 1955. Foliar analysis showed deficiencies of nitrogen and phosphorus in the needles. Height growth was markedly increased by the addition of superphosphate but only slightly increased by nitrogen fertilisers. Heavy applications of nitrogen caused a reduction of growth due to a dense invasion of grass.

By way of contrast Berg,[15] summarising experiments on wet moorlands and poor sandy heaths in Norway, states that while phosphate, in addition to nitrogen, is necessary on the wet peat areas, nitrogen alone, in the form of calcium nitrate, is all that is needed on the sandy heaths.

It is clear that conditions differ widely in different localities. Duchaufour[16] recognises this in his summary of fertiliser experiments on acid heaths with a deep layer of raw humus in France. He makes a clear distinction between those with very acid humus (pH 3·5–4) poor in bases occurring on infertile gravels and sands on the one hand, and those that are less acid (pH 4·5–5) in which the humus is richer in bases occurring on brown podzols on schists and crystalline rocks. For the former, liming plus a complete fertiliser is recommended. For the latter, liming may make them unsuitable for conifer growth, the recommended treatment being 16 cwt. of basic slag together with $1\frac{1}{2}$ cwt. of calcium cyanamide per acre.

In Britain, a great deal of experimental work with fertilisers has been done on heathland, a comprehensive summary of which is under compilation. Conditions vary greatly in different localities and fertiliser practice has to be adjusted accordingly. On the better heaths, characterised by grassy vegetation, fertilisers often have no beneficial effect. On the poorer, more acid heaths, usually dominated by a heather vegetation, an initial dressing of phosphate at the rate of about 2 oz. per plant is generally necessary, and gives a lasting stimulus to growth. Many tree species, in particular the spruces, are very susceptible to heather competition, and deep ploughing to smother the heather is necessary to give the trees a start before the heather grows again. If, in a spruce plantation, growth has not been sufficiently rapid to prevent re-invasion by heather, the spruce goes into complete check, the needles turn yellow and all the symptoms of acute nitrogen starvation set in. The application of nitrogen fertilisers, however, has little or no effect and only occasionally does phosphate alone bring the plants out of check. The salvage of such areas is difficult and expensive, and it is better to take precautions at the start, by ensuring that heather is so well suppressed in the ploughing operation that its return will be long delayed or by growing susceptible species like spruce in mixture with pines, Japanese larch or broom which are less susceptible and will suppress the heather sufficiently to enable the spruce to root in heather-free ground beneath them. The effects of broom are particularly dramatic and may be partly due to the improved nitrogen status of the soil.

Response to phosphate application

Exceptional cases are found where 2 oz. of phosphate fertiliser at the time of planting is not enough to produce a lasting response. An instance of this was found at Wilsey Down in Cornwall,[17] where Sitka spruce planted in 1934 with the usual 2 oz. of basic slag per plant very soon went into check. Twenty years later, the plants, which were only 2–4 ft. high, were treated experimentally with graduated doses varying from 2 to 12 cwt. per acre of a compound phosphate and potash fertiliser broadcast over the area. The response was dramatic and increased with the rate of application, the growth by the third year after application being greater for the heavier doses than in the previous 20 years. Factorial experiments laid down in 1955 on the same area proved that the response was entirely due to phosphate and that the potash did not contribute anything towards it.

In order to obtain more information about the conditions under which phosphate fertilisers produce worthwhile responses, an extensive trial on new plantings was started in 1950. Simple comparisons of plants with and without fertiliser were laid down in 184 localities and covered nine species, the manure being 2 oz. per plant of a 5 : 10 : 5 NPK fertiliser, except that on grassy sites a 16 : 16 PK fertiliser was used. In 1955 an assessment[18] was made of 45 plantations in which adequate numbers of the original seedlings survived. Height growth was measured

as well as the total phosphate content of the subsoil in the control plots. Only some of the experiments showed growth responses and when the growth increases were plotted against the total subsoil phosphate levels there was an indication that, for Sitka spruce, there is a threshold value of about 1300 p.p.m. of P_2O_5 below which responses to phosphate fertilisers may be expected, and that for Japanese larch the figure is probably lower.

In connexion with phosphate levels in the soil, a recent inspection[19] of some very poor Douglas fir plantations on the Hastings beds in Kent has revealed surprisingly low phosphate levels for this country, ranging from 190 to 670 p.p.m. of total P_2O_5, the average being in the region of 250 p.p.m.

As regards nutrients other than nitrogen, phosphorus, potassium and calcium, much less is known. Clear cases of deficiencies are relatively seldom encountered, although Stone[20] describes a magnesium deficiency in *Pinus resinosa* that was corrected by applications of magnesium sulphate. Trace elements also do not often feature in forest nutritional problems, although there is the famous zinc deficiency in Western Australia[10] in *Pinus pinaster* and *P. radiata* plantations which is corrected by spraying with minute quantities of zinc sulphate solution, or even by knocking a small galvanised nail into a tree. Numerous trials have been made in this country with applications of mixed trace elements, but, with the exception of a copper deficiency in Wareham nursery in Dorset, no trace-element deficiencies have so far been detected.

A selection of cases has been given above where applications of various fertilisers at the time of planting have resulted in improved growth, but there was a far greater number of fertiliser trials that have given negative results. No very clear picture emerges except that the variation in conditions on mineral soils is large, requiring correspondingly varied treatment. In most cases, and particularly in Britain, phosphate is the limiting factor. In others it may be lime or nitrogen or comparatively rarely potassium. There are suggestions that it may be possible to get an indication of whether phosphate will produce a worthwhile response by determining the level of total phosphate in the subsoil. No clear criteria for other nutrients yet exist, although it is hoped that as more is learnt about how to interpret foliar analyses, some guidance may be obtained from them.

Use of fertilisers in forest crops

The use of fertilisers in forest crops after they have reached the pole stage and have closed canopy, has to be discussed firstly in relation to counteracting any drain of mineral nutrients from the soil that may be taking place and secondly in relation to the possibility of increasing yields from the forest. Our knowledge is very limited indeed and, apart from some remedial measures mentioned later, there is no regular use of fertilisers in these older crops.

In the absence of reliable information on the size and nature of the growth responses that might result from the use of fertilisers, it is clearly impossible to make any useful economic forecasts. It would, from an economic point of view, be more profitable to improve our better-quality forests than to produce an equal increase in production in slower-growing crops. It is expected, however, that any responses to fertiliser applications are likely to be larger in plantations on poorer sites.

A large proportion of the continental work on fertilisation of forest crops has been carried out in connexion with the deterioration of sites under pure conifer stands associated with the accumulation of a deep layer of raw humus on the forest floor. In many instances this has resulted in reduced growth and difficulties in regeneration. Experiments have been in progress for over 20 years to find a means of rectifying these conditions, Prof. Wiedemann[21] being one of the pioneers in this work. The general conclusion is that heavy liming is the best remedial measure. The ecologically minded forester would probably say that these troubles are due to growing pure crops on sites where mixtures are needed, and that the admixture of a deep-rooted hardwood with the shallow-rooted conifer such as Norway spruce would result in more calcium being brought up from deeper soil layers and the litter layer enriched through the calcium in the broadleaved leaf-fall. This may be true, but as Wittich[22] points out, it would be useless as a remedial measure since it has been calculated that a broadleaved mixture of this kind would

at the best only draw up from the lower layers of the soil about 13 lb. of calcium per acre per annum, which is about one three-hundredth part of what experiments have shown to be necessary to secure the breakdown of the litter layer. A single application of lime of about 40 cwt. per acre (costing about £7 5*s.* 0*d.*) would supply this requirement. Increases in growth of 20–30% are frequently obtained, and in the oldest experimental areas additional increment of 45–55 cu. ft. per acre per annum resulted and has been maintained for 18 years.

There are many other reports of the beneficial effects of liming on old spruce sites. Lohwasser[23] describing experiments dating from 1927, showed that liming transformed the densely compacted raw humus into a black amorphous mass. He calculated that the CaO requirement for proper decomposition is about 4–6½ cwt. per acre for every cm. depth of humus. The total amounts applied in the trials ranged from 16 to 80 cwt. per acre. The increases in increment in the first 3 or 4 years were sufficient to cover the cost of the liming, and the effects still persist after 20 years. He concluded that, with liming, it should be possible to grow successive crops of pure conifers without fear of soil degradation.

There appear to be two possible effects from liming in forests where a thick layer of acid raw humus has been formed, namely the breakdown of the humus with consequent release of nitrogen and other nutrients, and the replenishment of calcium reserves in the soil.

Nitrogen fertilisers

Apart from lime, the other nutrient that has received most attention in established forest crops is nitrogen. Hesselman,[24] who did much pioneer work on forest manuring in Sweden, asserts that nitrogen is the element in greatest deficiency in Swedish forests. He showed that by treating a 200-year-old spruce stand for 11 years with small annual dressings of only 32 lb. of nitrogen in the form of ammonium nitrate per acre, a considerable increase in height and girth increment was obtained, even though the amount of nitrogen given as fertiliser was less than 5% of the total amount of nitrogen locked up in the raw humus layer.

Many records of responses to dressings of various forms of nitrogen fertilisers are to be found in the literature, both from Europe and America, but nowhere is it made clear whether the stimulus to increased growth persists. Since so many of the accounts refer to repeated dressings, one can only surmise that a continuation of the response after stopping applications was not anticipated. If this is in fact the case, nitrogen fertilisers become less attractive economically. More information on this point is required, and in particular on the possibility of finding a nitrogen fertiliser that is so slow acting that its effect will last for several years after application.

Much publicity has recently been given to the use of nitrogen fertilisers in the forest. Mayer-Krapoll[25] gives a comprehensive account of experiments with nitrogen fertilisers in all stages of forest operations. He states that in old forest crops, the breakdown of raw humus by the use of lime is very slow and that much more rapid breakdown in a matter of days or weeks can be achieved by injecting ammonia gas into the litter by means of a special roller with hollow spikes. It is claimed that the ammonia gas is immediately absorbed by the litter and that practically none of it escapes into the open air. Being rather sceptical of this result, we carried out trials with hand injection of ammonia into the litter layer in a pine pole crop in Hampshire. The results appear to confirm the German experience. Although no measurable growth responses have so far appeared, there is a change in the condition of the litter that is obvious to the eye.

A difficulty with most nitrogen fertilisers is that they are rapidly leached out of the soil before the tree roots are able to absorb them. In some preliminary lysimeter experiments, Hinson & Reynolds[26] have found that the leachability of both ammonium and potassium ions from the soil is much greater when they are added as salts of strong acids (e.g., as sulphates or nitrates) than when they are in the form of salts of weak acids or hydroxides. It is suggested that cation exchange with the soil humus constituents takes place more readily in the latter case so that the NH_4 or K ions are retained in the soil. This work has led to practical trials of alternative forms of nitrogen fertilisers including ammonium carbonate, ammonia and urea. The discovery of a slow-acting form of nitrogen fertiliser is a matter of prime importance both in the nursery and in the forest. Materials such as urea–formaldehyde are of some interest in this connexion but are too costly for general forest use.

Phosphate and potassium

Fertilising with other elements, notably phosphorus and potash, has received much less attention although there are some records of growth improvements achieved with them. It appears that, in general, phosphate is less effective in this later stage of crop development than when applied when crops are first established. Potash appears to be more often important, one paper[27] describing the correction of potash deficiencies in conifer stands growing on degraded agricultural land in the Eastern United States, by aerial dressings of granular KCl at the rate of 200 lb. per acre.

Methods of application

In nurseries and on flat land cleared for planting, ordinary agricultural techniques can be employed. It was even found possible to apply fertiliser with a tractor-mounted spinner when the young tree crop was 2–4 ft. high amidst dense heather and dwarf gorse vegetation. As soon as crops grow larger, the problem becomes more difficult. Attempts have been made in Germany[28] to apply lime by blowing it from a mechanical blower moving along the forest rides but satisfactory distribution is not obtained unless the particle size is very uniform. Aerial application is widely used in the United States, the costs usually being under 10 dollars per acre for application alone, fertiliser costs being extra. In this country, quotations for aerial application have been received at under £1 per acre. This method, however, is limited to fertilisers that are not heavy or bulky. Aerial liming on the scale of the German practice would, for instance, be quite impracticable.

Conclusion

The picture delineated above of the place of fertilisers in forestry is rather distorted. Much has been said about cases where responses have been obtained to fertiliser applications but little about the far more frequent occasions when the results are negative.

In practice the use of fertilisers is restricted to nursery work, to some cases of plantings on peat and heathlands and to the improvement of checked crops where the cause is a nutrient deficiency. The use of fertiliser additions to enhance the growth of established forest crops has not yet been sufficiently explored in this country, although experiments have now been laid down to examine the possibilities of obtaining worthwhile increases in growth. Even on the Continent, fertiliser treatments in older crops are almost entirely confined to remedial measures where site fertility has declined, and no instances have been encountered of the use of fertilisers to raise the production of normally vigorous forest stands.

Much more work is necessary, especially experiments designed to analyse the nutritional factors and determine the reasons for any results achieved. We now have more powerful tools at our disposal than ever before. The fuller knowledge of how to interpret foliar analyses gives us a better idea of what is happening, and the collection of data on a wide range of factors that may affect the nutrition of a forest crop need no longer daunt us now that we have electronic computors that can carry out multivariate analyses and determine partial correlations that hitherto have been too laborious to contemplate. As our knowledge of cause and effect grows it is safe to say that the intelligent use of fertilisers in forestry will increase, but it is important to remember that there may be little progress by studying the purely chemical factors in isolation. It will be necessary to learn the significance of the physical and biological factors affecting the breakdown of litter, the stimulation of root activity and the absorption of nutrients by the tree roots, as well as other factors influencing the physiological processes of the tree. The more complete our knowledge of the way in which all these factors act and interact, the more likely we are to be able to determine what factors are limiting growth and to take steps to remedy them.

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INACTIVITY OF THE CAROTENE-OXIDISING SYSTEM IN IRIS LEAF

By V. H. BOOTH*

When green leaves were severely damaged by grinding to pulp, a quarter of the carotene was lost by enzymic activity in about 13 min. at room temperature. The enzyme was found in 20 species of leaves; also in pods, green fruits, stems and other chlorophyll-containing parts of plants. (Some of the species treated have nutritional value.) The enzyme was not found in most of the chlorophyll-free materials that were tested.

Carotene was stable in macerated leaf of iris. The iris was the only exception found to the rule that chlorophyll in higher plants is accompanied by the carotene-destroying enzymic system. Other respects in which iris differed from other species are discussed.

Introduction

Chlorophyll is never found in higher plants without carotene and luteol—a fact so well established that it can be considered a law of Nature. The converse is not true: carotenoids occur without chlorophyll in roots, fruits, seeds and flowers of many plants.

In green leaves there is also a system, presumed enzymic, under whose influence carotene is oxidised when the leaves are damaged (Hauge & Aitkenhead.[1] For other references see Walsh & Hauge[2]). The leaf system that oxidises carotenoids has been likened to the lipoxidase system in soya-beans in which an enzymically-produced peroxide of a fatty acid oxidises carotene by a secondary or coupled reaction; but the identity of the green leaf system with the soya-bean system has not been unequivocally established (Wijesinha;[3] Friend & Nakayama[4]).

The enzyme system, although absent from normal red carrot roots, has been found to develop simultaneously with chlorophyll near the surface of carrots exposed to sunlight (Booth[5a]). This type of observation has been extended, the distribution of the system has been studied, and in this paper it is shown that the enzyme system is found in all parts of plants examined that contain chlorophyll, except in the leaf of the iris.

Part of this work has been reported briefly in an oral communication (Booth[5b]).

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Methods

Determination of carotene

Pigments were quickly extracted by grinding small samples (0·25–0·75 g.) of plant materials with quartz under cold acetone and quinol. Light petroleum* was added, and the solution was decanted into a layer of light petroleum over aqueous ammonium sulphate in a separating funnel. The extraction was repeated until all the pigment was removed. Acetone was washed from the combined solution with water in an automatic apparatus. The light petroleum solution of pigments was then chromatographed on a 1 : 1 mixture of aluminium oxide and Na_2SO_4, from which adsorbent the carotene was eluted with 2% acetone in light petroleum. The carotene in the eluate was measured in a spectrophotometer. Details of the method have been described elsewhere (Booth[5c, d]). All carotene values are based on fresh weight.

Precision.—For small leaves, such as cress, grass, carrot and lucerne, the coefficient of variation of a single determination of carotene is about 3–6%.[5c, 6] For large leaves the coefficient is a little greater. For other tissues, it might be much greater, especially where the concentration gradient of pigment is steep enough to be visible, as with fruits or green-skinned potatoes.

Test for the presence of the enzyme system

Exploratory tests for the enzyme were made as follows. Two similarly prepared samples were weighed in extraction beakers. One sample was extracted with solvent immediately. The other was ground to pulp or paste with the aid of quartz powder and a few drops of water or acetate buffer pH 4·6, and left for ½ hour or other period, screened from bright light, before it was extracted with solvent. The carotene values of the two extracts were determined, and the difference between them, namely the carotene lost in the pulp, was expressed as a percentage of the initial value.

In some experiments four samples of leaves were weighed. Two were treated as above. The other two were immersed in boiling water, cooled, and ground to paste after decanting the water. Carotene was determined immediately in one, and after ½ hour in the other.

Assessment of chlorophyll concentration

Small samples were extracted with 80% acetone (80 ml. of acetone + 20 ml. of water) by a technique similar to that used for carotene. The chlorophyll was removed in about five extractions. Extinctions of the pooled acetone extract, diluted if necessary, were read at 663 mμ with an optical path length of 1 cm. Concentrations of chlorophyll *a* were calculated by multiplying by 9. This is approximate only and ignores chlorophyll *b* but is satisfactory for present purposes which were comparative only.

Results

Green leaves

The results in Table I show that carotene was lost from green leaves of diverse types when they were severely damaged. Evidently the carotene-destroying system was active in all these autolysing leaves. Each of the several percentage values in the last column of the table was obtained in a separate test, using a different sample of leaves, and each value is the average of duplicate sets.

The values for the carotene contents in the second column are the averages of the initial values found in the experiments.

The method of testing for the presence of the enzyme was only qualitative, as may be inferred from the considerable variation within groups in the last column of Table I, the pooled coefficient of variation of the percentage loss in a single test within a species being 42%. Only a small part of the variation is due to experimental error in the determination of carotene. Preliminary experiments indicated that enzyme activity was dependent upon the degree of grinding, the amount of water present or added, the temperature, the pH, and other conditions. Since these conditions were not standardised, no attempt is made to rank the types of leaf in order of enzyme activity, although it can be said that cress, clover and nasturtium were rich sources.

* Boiling range 40–60° or higher

Table I

Loss of carotene from damaged green leaves

Leaf	Initial carotene, p.p.m.	Time in pulp, h.	Loss, as % of initial carotene*
Achillea millefolium (Yarrow)	103	2	18, 37
Betula alba (Silver birch)	127	½	13, 5, 4
Crocus flavus (Croeus)	64	½	7
Daucus carota L. (Carrot)	90	½	20, 9, 22
Grass (various pasture)	106	1½	21, 17, 33
Ligustrum vulgare (Privet)	86	½	19
Lepidium sativum (Cress)	52	½	36, 41, 31, 28
Medicago sativa (Lucerne)	158	½	7, 17, 18
Narcissus (cultivars of)	53	½	13, 25, 22
Phaseolus coccineus (Runner bean)	128	½	26, 22, 39
Physalis alkekengi (Chinese lantern)	88	½	12
Populus alba (Poplar)	103	½	26, 15, 30, 13
Rubus idacus (Raspberry)	130	½	12, 6
Sambucus nigra (Elder)	102	½	7
Seseli bocconei (Sicilian parsley)	86	½	29
Solanum lycopersicum (Tomato)	80	½	10
Solanum tuberosum (Potato)	103	½	13, 34, 19
Taraxacum officinale (Dandelion)	97	½	20, 12
Trifolium repens (Clover)	119	½	29, 35, 28, 24, 38
Tropaeolum majus (Nasturtium)	108	1	52, 30, 23, 15

* Each value is the mean for duplicated pairs

The effect of boiling

Leaves of clover, cress, grass and poplar were boiled as described under 'Methods'. Negligible amounts of carotene were lost during ½ hour in the pulp. When the leaves were boiled for several minutes, the 'initial' carotene value was slightly less than the 'initial' value for unboiled leaves, as though carotene were destroyed or isomerised during the boiling. The boiling time for maximum yield of carotene was about 1 min. The prevention by boiling of loss of carotene from pulp was taken as evidence, as is customary, that the loss was enzymic in these species so tested. It is supposed that boiling would have the same effect in other species.

Other green tissues

Chlorophyll and carotene occur in many other parts of plants besides leaves. Some of these were examined and the results are shown in Table II. Here, also, the variation in apparent activity was considerable. The green skin only was used from potatoes that had been exposed to sunlight: tubers grown underground normally have neither carotene nor chlorophyll. Carotene was present and the enzyme was active in this damaged green skin.

Table II

Enzyme in green parts other than leaves of plants

Plant	Part	Initial carotene, p.p.m.	Time in pulp, h.	Loss % of initial carotene
Amaryllis belladonna (Belladonna)	Berry	11	½	15
Cucumis sativus (Cucumber)	Skin	17	½	37, 40
Medicago sativa (Lucerne)	Stem	14	½	24, 27
Papaver somniferum (Poppy)	Bud sheath	37	1	2, 9, 14, 12
Phaseolus coccineus (Runner bean)	Pod	3·5	1	46, 48, 3, 31
Physalis alkekengi L. (Chinese lantern)	Green lantern	23	½	30, 66
Pimenta dioica (Pimiento)	Green fruit	2	½	37
Pisum sativum (Green pea)	Pod	7	1	29
Prunus domestica sp. *italica* (Greengage)	Fruit	4	1	1, 11, 34
Solanum lycopersicum (Tomato)	Green fruit	2	1	16, 28, 41
Solanum tuberosum (Potato)	Tuber*	3	1	25, 16
Vicia faba (Broad bean)	Pod	6	½	29, 26

* Green, after exposure to sunlight

Plant tissues without chlorophyll

It has already been reported[5a] that the enzyme is not found in the root of the carrot. When the red fruit of mountain ash was ground to pulp and left for one hour, there was no loss of carotene, indicating the absence of the enzyme ; also it was not found in the fruit of *Pyracantha* or the corona from the flower of *Narcissus* (Table III).

Table III

Plant tissues containing carotene but not showing the enzyme activity

Plant	Part	Carotene, p.p.m.
Daucus carota	Root	120
Narcissus	Corona	740
Pyracantha	Berry	34
Sorbus aucuparia	Berry	67

Other carotene-containing tissues in which no chlorophyll was obvious, although small amounts may have been present, included ripe tomato fruit. Some of the carotene disappeared from the pulped fruits, but the percentage loss was much less than in green fruits.

A test for the presence of the enzyme in white roots of a variety of fodder carrots was done as follows. Three similar samples of red carrot were weighed into extraction beakers. From one sample the carotene was extracted and determined immediately. In another the carrot was ground to pulp and set aside for one hour before extraction of the carotene. A portion of white carrot root was added to the third and ground with it, the combined pulp being also extracted after one hour. The carotene contents of the three samples were the same. An extract of the white carrot contained a negligible amount of carotene. The test was repeated and the result confirmed. Evidently the enzyme system is not active in white carrot roots.

Although the green skins of potato tubers that had been exposed to sunlight contained chlorophyll together with carotene and the enzyme system, tubers protected from sunlight by normal methods of cultivation were devoid of both carotene and chlorophyll. Ordinary uncoloured potato and red carrot were thoroughly ground together and left for one hour as in the test with white carrots. No evidence was found for the presence of the carotene-oxidising system in white potatoes.

Time course of the loss

Several other specimens of the leaf materials listed in Table I were left in pulped condition at room temperature for longer or shorter periods than indicated. The 59 observations were pooled and grouped according to test intervals, and the results are shown in Fig. 1, which is plotted on a logarithmic time scale. The diagram is composite and the accuracy may be low, but it serves to show the general course of the loss of carotene. On average a quarter of the carotene was lost in about 13 min. at 18°. The curve is nearly straight. The falling off in activity with time is interpreted to mean that the enzyme was progressively inactivated by autolytic processes, or that an intermediary substrate was being used up.

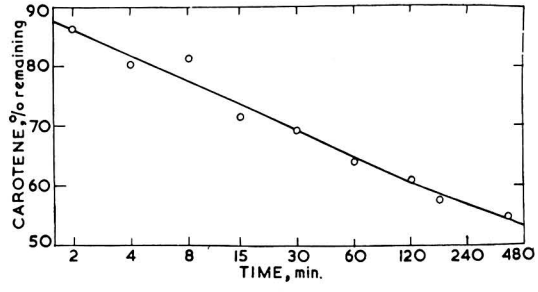

FIG. 1.—*The time course of the loss of carotene from pulped green leaves of several species. Pooled results from 59 experiments at room temperature (approx. 18°)*

Chloroplasts

The ratio of the contents of carotene and of chlorophyll *a* in leaves of runner bean was 0·077. Chloroplasts were prepared from similar leaves by the usual method of moderate maceration in a blendor followed by fractional centrifugation. Pigments were extracted, and the carotene/chlorophyll ratio was found to be 0·054. The experiment was repeated on leaves of bean and other species (Table IV). The ratio was always lower for the chloroplasts than for leaves, with one exception to be discussed later.

Table IV

Comparison of the ratios of carotene to chlorophyll a *content of extracts from leaves, and in similar extracts from chloroplast preparations*

Source	Carotene/chlorophyll ratio		Lf/Ch*
	Leaf	Chloroplast	
Phaseolus coccineus (Runner bean)	0·077	0·054	1·4
,, ,, ,, ,,	0·076	0·048	1·6
,, ,, ,, ,,	0·067	0·049	1·4
Hedera helix (Ivy)	0·071	0·045	1·6
Sambucus nigra (Elder)	0·087	0·080	1·1
Taxus baccata (Yew)	0·088	0·067	1·3
Urtica dioica (Nettle)	0·081	0·064	1·3

* The carotene/chlorophyll ratio for leaf divided by the ratio for chloroplasts from the same samples of leaves

Chloroplasts from bean leaves were divided into several portions. Some were ground with quartz to a paste, and after ½ hour carotene and chlorophyll were determined. Both had decreased only by about 5%.

Iris, an exception

When a sample of leaves of the purple iris was pulped, no loss of carotene was observed in ½ hour. The experiment was repeated with other samples of *Iridaceae* of several varieties at different seasons (during and after flowering), and left for an hour or longer. Sometimes a small loss and occasionally a smaller gain was observed. The average for the 30 tests was 2·2% loss, and it was significantly greater than 0 ($P < 0{\cdot}05$). This average is close both to the practical limits of experimental error and to the non-enzymic loss (the thermal or dark reaction).

The optimum pH for the oxidation of carotene in leaf macerate is between 4 and 5 (Walsh & Hauge[2]). As the liquid obtained by grinding and pressing iris leaves had pH 5·6, acetate buffer at pH 4·6 was added to iris leaves before they were ground. Other buffers were also tried. There was no significant enhancement of the oxidation of carotene.

The ratio of carotene to chlorophyll for a sample of iris leaves was 0·080, as was that for an extract made with chloroplasts separated from the same leaves. The experiment was repeated twice more, with comparable results. The mean value obtained by dividing the chloroplast ratio into the leaf ratio for the three experiments, as for the last column in Table IV, was 0·98, i.e., practically unity.

After pigments had been extracted from leaves of most species the residue was usually pale greenish buff in colour. The colour varied with species, but the residue from the paste that had been left to autolyse before extracting the pigments was darker in colour than that obtained by immediate extraction. The iris leaf was exceptional here in showing no darkening of the residue during autolysis.

Discussion

Chlorophyll is accompanied by the carotene-oxidising system

The carotene-destroying enzyme system was found in macerated leaves and other green parts of plants. The enzyme was not normally found in potato, carrot and other chlorophyll-free parts of plants, yet it was found when chlorophyll was formed in the surface parts of potatoes and carrots exposed to sunlight. Indeed the data accumulated so far suggest that, to the rule

that chlorophyll in higher plants is always accompanied by carotenoids, may be added another, namely that chlorophyll is also accompanied by the enzymic system that destroys carotene. The simplest hypothesis would be that the enzyme is situated inside the chlorophyll-containing chloroplasts, and this has already been postulated by Friend & Nakayama.[8]

Chloroplasts

Since both the carotene and the chlorophyll of leaves are situated inside the chloroplasts the ratio between these pigments should be the same for whole leaves as for separated but unchanged chloroplasts. Observation showed that the latter was lower (Table IV). The fall in the ratio for separated chloroplasts was interpreted (*a*) to mean that carotene was oxidised during the preparation of the chloroplasts; (*b*) to confirm the view that the enzyme is in the chloroplasts; and (*c*) to confirm that the system became active when the cells were damaged. It is possible of course that chlorophyll was also lost, but obviously not to the same extent as carotene. When separated chloroplasts were ground and the paste set aside there was little further loss of carotene. From this it was concluded that most of an intermediary substrate had already been used up in the oxidation that had occurred during the separation of the chloroplasts.

Leaf of the iris

The carotene-oxidising activity in damaged leaves of the iris is too low to be distinguished from the non-enzymic thermal oxidation. This lack of activity is in keeping with the lack of change in the carotene/chlorophyll ratio during the separation of chloroplasts from iris leaves, and with the failure of the macerate to darken on keeping.

The leaf of the iris thus appears to offer an exception to the rule that chlorophyll is always accompanied by the carotene-oxidising system. There is a possibility, however, that the enzyme is not absent but only inhibited, according to the following argument. Sodium ascorbate added to leaves of clover prevented the loss of carotene from the pulp produced by grinding them. Iris leaves were analysed and found to be exceptionally rich in ascorbic acid (Booth & Constable[7]): they had 4·1 mg./g. based on fresh weight. Possibly this indigenous ascorbic acid protects the carotene in macerated iris leaves. Leaves of nasturtium (*Tropaeolum majus*) are also very rich in ascorbic acid, the content varying from 2·6 to 6 mg./g. fresh weight in samples taken at various times. These leaves were therefore tested for the carotene-destroying system which was found to be very active. However, leaves of nasturtium are also rich in ascorbic oxidase: after leaves were ground to a fine paste the ascorbic acid had almost disappeared in 10 min.[7] Therefore even though ascorbic acid inhibits the carotene-destroying system, it could not do so for long in macerated nasturtium leaves. Ascorbic oxidase in iris leaves on the other hand is too feebly active to remove an appreciable part of the ascorbic acid during a carotene test.

Perhaps in autolysing iris leaf, carotene is protected, and polyphenolases are inhibited, by ascorbic acid. Alternatively the enzymes, including ascorbic oxidase, are inhibited by another antioxidant.

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THE FREE AMINO-ACIDS OF CERTAIN BRITISH FRUITS*

By L. F. BURROUGHS

The total and alcohol-soluble nitrogen contents have been determined and the free amino-acids examined in strawberry, gooseberry (green and ripe), blackcurrant, redcurrant, loganberry, raspberry, blackberry and tomato. Most of the common amino-acids were present, alanine and glutamine being the two most generally prominent.

Free amino-acids have been examined in 20 non-edible species of apple and pear, in medlar (unripe and ripe) and in three species of *Vaccinium*.

1-Aminocyclopropane-1-carboxylic acid was not found in any fruit except ripe cowberries.

Introduction

During recent years the application of paper chromatographic methods has greatly increased our knowledge of the amino-acids present in fruits. Attention has naturally been directed mainly to those fruits of greatest commercial importance, such as the apple,[1–3] citrus fruits,[4–8] and the grape.[9–13] A review of present knowledge of the amino-acids (and organic acids) of fruit juices in general will shortly be published.[14]

Another feature of paper chromatographic studies of amino-acids in biological materials has been the discovery of new compounds not previously known in natural products. One such amino-acid, 1-aminocyclopropane-1-carboxylic acid, was found in perry pears[15] and was later reported in the fruits of the cowberry.[16] The present survey of amino-acids in various soft fruits was undertaken mainly to look for this substance in other species. It was in fact not found, but the results are nevertheless of interest from the point of view of the amino-acid content of the fruits themselves.

Experimental

The fruits were analysed for total and alcohol-soluble nitrogen content and the free amino-acids in the alcoholic extracts were examined by paper chromatography.

Sampling

Only the edible portion of the fruit (approx. 200 g.) was used, i.e. the stem and calyx, where possible, were removed. Each sample of fruit was divided into four equal parts; two parts were macerated with alcohol and the others were used for total N determination. To provide more uniform samples, the strawberries were cut in halves and the tomatoes in quarters.

Nitrogen determinations

Known weights of fruit (30–50 g.) were digested in 300-ml. Kjeldahl flasks using 5 g. of the catalyst described by Chibnall *et al.*[17] with the addition of 10% of HgO. Alcoholic extracts (5 ml. in micro-Kjeldahl flasks) were acidified with a trace of H_2SO_4 and evaporated to dryness before adding 0·5 g. of catalyst and more H_2SO_4. The amount of H_2SO_4 required for digestion was calculated according to Middleton & Stuckey[18] at the rate of 4 ml. of conc. H_2SO_4 per g. of dry matter plus 2 ml. per g. of catalyst. Digestions were continued for one hour after clearing. A suitable aliquot of each digest was pipetted into a Markham apparatus, made alkaline with 10N-NaOH containing 5% $Na_2S_2O_3,5H_2O$ and steam-distilled into 3 ml. of 0·5% boric acid.[19] The distillate (15 ml.) was titrated with 0·0071N-H_2SO_4.

From the total and alcohol-soluble N contents of a sample the alcohol-insoluble N content was calculated by difference. All nitrogen contents are reported as mg. of N/100 g. of fruit.

Alcoholic extracts

A known weight of fruit was macerated in a Waring Blendor with a measured volume of absolute alcohol to give approximately 70% ethanol by volume in the extract (most fruits contain about 80% moisture). The extract was either centrifuged or filtered with precautions to prevent loss of alcohol. The exact alcohol content of the extract was then determined, by the usual distillation method, so that the volume of extract equivalent to a given weight of fruit could be calculated.

* Read at meeting of Food Group, 5 March, 1958

Separation of amino-acids

A suitable volume of alcoholic extract, e.g., 10 or 20 ml., was percolated slowly through a column of Zeokarb-225 (0·5 g. of air-dried resin ; 80–100 mesh*) in the H^+ form. The column was washed with about 5 ml. of water and the N content of the percolate and washings was determined.

After a more thorough wash with water the column was eluted with 5 ml. N-aq. NH_3 and the eluate evaporated to dryness *in vacuo* over $CaCl_2$ and H_2SO_4. The residue was dissolved in a suitable volume of water to give a concentrate containing about 5 mg. of N/ml., based on the amount of N adsorbed by the resin. This technique has been shown[3] to give substantially complete recovery of amino-acids (except glutamine), although traces of other nitrogenous substances may also be present.

Paper chromatography of amino-acids

Suitable volumes of the amino-acid concentrates, representing 125 μg. of N adsorbed by Zeokarb-225, were chromatographed on Whatman No. 1 paper ($22\frac{1}{2} \times 18\frac{1}{4}$ in.) first in n-butanol : acetic acid : water (65 : 10 : 25) and then in the second dimension in phenol : water (80 : 20) in the presence of NH_3 and KCN.

After being dried in air the chromatograms were heated at 80° for 10 min. to remove traces of phenol and NH_3. They were then dipped in 0·2% ninhydrin in acetone and heated at 80° for 5 min. to develop the colour.

The relative amounts of the individual amino-acids on the chromatogram were assessed visually on an arbitrary scale (0–10). Proline gave a yellow colour and was scored A (small), B, C, D (strong). The results obtained by this method, which has previously been used with apple and pear juices,[3] are not claimed to be quantitative but are an approximate indication of the relative proportions of amino-acids in a known volume of extract. Since each chromatogram represented the same weight (125 μg.) of N adsorbed by Zeokarb-225, the results for the different fruits are comparable on a basis of N content, although they represented different weights of fruit.

The above procedure was used for the fruits described in Table I. Smaller chromatograms (9 in. square, with a loading of approx. 80 μg. of N) were sometimes used for other samples.

Results

The nitrogen and amino-acids present in tomatoes and in seven commonly grown species of soft fruits are shown in Table I.

The total N contents of these fruits, ranging from 150 to 300 mg./100 g., were much higher than that normally found in the apple (40–80 mg. of N/100 g.). The alcohol-soluble N contents (except that of redcurrants) were also higher than in apple, which usually contains 5–20 mg. of N/100 g. The alcohol-soluble N content of gooseberries was extremely high. The alcohol-insoluble N of these fruits can be regarded as protein N and includes the N present in the seeds. The amounts of N not adsorbed from the alcoholic extracts by Zeokarb-225 ranged from 3·6 (strawberry) to 14·2 mg. of N/100 g. of fruit (blackcurrant). This suggests that these fruits contain much more non-basic N than is present in apples, which give Zeokarb percolates containing about 1 mg. of N/100 g. of fruit.

The outstanding features of these results were :

(1) the amino-acid patterns of all the fruits, except tomato, were broadly similar and no unusual amino-acids were detected. The tomato was remarkable for the predominance of glutamic acid and γ-aminobutyric acid.

Only single samples of each fruit were examined and it is possible that the amino-acid patterns may be somewhat influenced by varietal and environmental factors and by the degree of ripeness of the fruit. With apples, however, it has been found that these factors do not greatly alter the general pattern of amino-acids except in so far as they affect the soluble N content of the juice ; very high and very low N contents influence chiefly the content of asparagine and, to a lesser extent, of aspartic and glutamic acids.

(2) Alanine and glutamine were the two most generally prominent amino-acids : in this

* One-g. columns of the commercially available 100–200 mesh microbeads (Permutit Co. Ltd., London) are equally suitable ; the resin as supplied contains about 50% moisture.

Table I

Amino-acids of soft fruits

	Straw-berry	Goose-berry (green)	Black-currant	Red-currant	Logan-berry	Rasp-berry	Black-berry	Tomato
	(mg. of N/100 g. of fruit)							
Total N	148	256	290	183	278	177	181	211
Alcohol-sol. N	44	165	53	15	94	44	40	98
Alcohol-insol. N	104	91	237	168	184	133	141	113
Amino-acids (visual assessments on arbitrary scale 0–10)								
Aspartic acid	3	2	2	3	2	2	3	3
Asparagine	7*	3	3	1	6*	3	6*	1
Glutamic acid	5	5	3	3	2	3	6*	10*
Glutamine	8*	10*	5*	5*	2	3	5	4
Serine	2	5	4	3	3	5*	5	2
Glycine	tr	1	1	1	tr	0	0	0
Threonine	1	2	2	1	1	1	1	1
α-alanine	3	8*	7*	8*	6*	8*	5	1
β-alanine	tr	tr	2	1	0	tr	0	tr
γ-aminobutyric acid	1	3	3	3	1	3	4	8*
Valine	tr	2	2	1	2	2	2	tr
Leucine(s)	tr	2	2	1	1	1	1	1
Proline	0	A	A	A	tr	tr	tr	A
Arginine	0	tr	1	tr	1	tr	tr	tr
Lysine	0	tr	tr	tr	0	tr	0	tr
Tyrosine	0	tr	0	0	tr	tr	tr	0

* dominant amino-acid tr = trace A = small amount

respect these soft fruits differ markedly from apples, where asparagine and aspartic acid are the main constituents.[3]

(3) Methyl-hydroxyproline,[20, 21] which is almost invariably present in apples and pears, was not detected in any of these fruits. It was found in trace amount in medlars (see below) and traces have been reported in Australian apricots[22] but it was not detected in a sample of imported (Spanish) apricots examined by the writer. It has also been reported tentatively in cherries.[23] This cyclic imino-acid may be characteristic of the apple and pear; if this is so, its presence in other fruit products might be useful analytically as evidence of adulteration.

Changes in nitrogen content of gooseberries during ripening

The gooseberries in Table I were unripe and in this way differed from the other fruits examined; they differed also in the ratio of alcohol-soluble to alcohol-insoluble N and in their exceptionally high content of glutamine. A further sample of fruit was therefore examined (Table II) to see whether these characteristics of N content changed during ripening. Both green and ripe samples were picked from the same bush (variety Careless) at an interval of 33 days.

The change in the ratio of alcohol-soluble to alcohol-insoluble N content (per 100 g.) with increasing maturity suggests that the green stage of this sample was in fact more mature than that of Table I. The data for N content per 100 g. of fruit show that the *concentration* of protein N remained constant during growth and ripening, while the concentration of soluble N decreased. Calculated on a '*per fruit*' basis, the amount of total N in each berry increased, but practically all this went into protein N while the amount of soluble N per fruit remained the same.

The chromatograms of green and ripe gooseberries represented the same amounts of N adsorbed by Zeokarb and were therefore comparable on a 'per fruit' basis. The main differences due to ripening were increases in the amounts of alanine, glutamic acid and γ-aminobutyric acid and a decrease in glutamine.

Examination of other fruits for the presence of 1-*aminocyclopropane*-1-*carboxylic acid*

This survey of the free amino-acids of fruits was undertaken primarily to look for the presence of 1-aminocyclopropane-1-carboxylic acid (hereafter abbreviated to A.C.P.C.) previously found in certain perry pears and cider apples.[15]

Apple and pear species.—All the commonly grown edible varieties of apple and pear belong

Table II

Changes in nitrogen content of gooseberries during ripening

Average weight per fruit, g.	*Green* 6·9		*Ripe* 15·4	
	per 100 g.	per fruit	per 100 g.	per fruit
Total N, mg.	157	10·8	117	18·0
Alcohol-soluble N, mg.	77	5·3	36	5·5
Alcohol-insoluble N, mg.	80	5·5	81	12·5
Amino-acids (visual assessment)				
Aspartic acid	3		3	
Asparagine	6		6	
Glutamic acid	5		8	
Glutamine	10		6	
Serine	3		3	
Threonine	2		2	
Alanine	4		10	
γ-aminobutyric acid	tr		4	
Valine	1		2	
Leucine(s)	1		2	
Proline	0		A	
Lysine	0		tr	
Tyrosine	0		tr	

to the species *Malus pumila* and *Pyrus communis* respectively. Fruits of 20 different species of apple and pear were available at Long Ashton and their amino-acids were examined, but A.C.P.C. was not detected in any sample. Methyl-hydroxyproline was present in about half the species examined. The amino-acid patterns varied considerably among the different species but the majority showed the preponderance of asparagine and aspartic acid typical of the edible species. No unusual amino-acids were detected.

Medlar (Mespilus germanica).—The occurrence of A.C.P.C. in perry pears was found to be related in some way to the process of ripening, since the amounts present increased considerably during storage of the fruit after harvesting. The medlar is botanically a close relative of the apple and pear and particularly resembles the pear in its ripening process. The amino-acids of medlars in the green and brown states were therefore examined. The change from the unripe green state to the soft brown condition was associated with a five-fold decrease in alcohol-soluble N content, from 9·8–1·9 mg. of N/100 ml. of extract. The relatively high N content of the extract from green medlars was due largely to asparagine, which disappeared almost completely in the brown fruit. A.C.P.C. was not detected in either green or brown fruits but both contained traces of methyl-hydroxyproline.

Vaccinium species

Shortly after the publication reporting the presence of A.C.P.C. in perry pears, Vähätalo & Virtanen[16] reported its occurrence in cowberries (*Vaccinium vitis-idaea*). Accordingly, other species of *Vaccinium* were examined at Long Ashton. The common bilberry (*V. myrtilis*) was found to contain chiefly glutamic acid and valine together with smaller amounts of α-alanine, serine, γ-aminobutyric acid and several others; the unknown 'yellow spot' reported by Vähätalo & Virtanen[24] in *V. vitis-idaea* was also present.

Six cultivated varieties of blueberry (*V. corymbosum*) were also examined. The amino-acid patterns of these samples were most unusual in that arginine was much the most prominent amino-acid, particularly in varieties of high nitrogen content. Other amino-acids present, in decreasing order of prominence, were γ-aminobutyric acid, α-alanine, serine, glutamic acid, glutamine, valine, leucine(s) and aspartic acid; the yellow spot was absent.

Finally, a sample of *V. vitis-idaea* was obtained from Scotland. A large number of amino-acids was present; the most prominent were aspartic and glutamic acids, asparagine, serine and γ-aminobutyric acid with small amounts of 12 others, including A.C.P.C. and the yellow spot reported by Vähätalo & Virtanen. Although A.C.P.C. was present in the ripe fruit, it could not be detected in unripe berries from the same sample, confirming the observation of Vähätalo & Virtanen. The metabolic rôle of this amino-acid and its association with the changes occurring during ripening of pears and cowberries, are at present unknown.

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DETERMINATION OF MOISTURE CONTENT IN CEREALS. I.—Interaction of Type of Cereal and Oven Method

By T. A. OXLEY, S. W. PIXTON and R. W. HOWE

This paper gives the moisture content values obtained by five different oven-drying methods, in common use, for a number of non-oily cereal grains at different moisture levels.

It is shown that the values obtained for the moisture content of a grain sample given by various methods are different and that the amount of difference between the methods is influenced by the type of grain being investigated.

The difference is less for high-protein hard wheats, parboiled rice and flint maize than it is for the soft wheats, milled rice, dent maize, oats or barley.

Hence it is concluded that, when more than one type of grain is to be dealt with, no two methods of the oven type will give the same results for all grains and, it is not valid, therefore, to have alternative standard methods, or a standard method and sub-standard, except for one precisely specified kind of grain.

Introduction

Whenever cereals are to be stored or processed, a knowledge of their moisture content is most important. A large number of methods of determining moisture are in common use, all giving slightly different results. This investigation was designed to compare the values given by several standard methods and to determine how consistent were the differences between them.

It was quickly found, however, that the differences between the results obtained by various methods are influenced to a large extent by the type of grain used. This investigation was,

therefore, directed to a study of the interaction of method of moisture determination and type of grain on the moisture results, the choice being restricted to oven-drying methods and non-oily grains.

It is well known that different combinations of time and temperature can be chosen which will give identical results with a particular grain. It has now been found that methods which are equivalent for one grain do not give identical results for other grains.

Experimental

Materials and methods

In Experiment I three different types of wheat and four other varieties of grain, namely, oats, barley, yellow flint maize and milled rice, were used. This was followed by experiments comparing first (Expt. II) six different types of wheat, then (Expt. III) parboiled,* converted* and untreated rice, and then (Expt. IV) powdered wheat gluten and pure starch derived from wheat, maize and rice. In addition the moisture content of an extra sample of maize was determined by several methods.

All grains were ground using a Regent Maskin burr type grinder, set to give a fairly coarse grind. Results of sieving tests on ground grain, expressed as the average of three tests, sieved by hand to clear, are given in Table I.

Table I

Sieving tests on throughs of grinder

Sieves	Aperture width, mm.	Soft English wheat (18·5% moisture), %	Canadian wheat (12·0% moisture), %
Overtails 16 wire	1·24	56·2	59·4
,, 30 ,,	0·94	10·1	14·2
,, 30 ,,	0·62	14·8	11·3
Throughs 30 ,,		17·1	14·1

The ovens used in this investigation were: (1) Gallenkamp, fitted with an internal fan; (2) Laboratory Thermal Equipment (LTE), still air oven; (3) Brabender Semi-Automatic Moisture Tester.

Except with the Brabender apparatus, which has its own tins, the tins used were 4·9 cm. diameter, 1·2 cm. deep, with tight-fitting lids. The amount of ground material used per tin was sufficient to give 0·2–0·3 g. per sq. cm. Moisture determination, on all samples, was done in duplicate and the average of the two results recorded.

The methods of determination were:

A. 105°, 16 h. LTE still air oven.
B. 113°, 4 h. Gallenkamp ventilated oven.
C. 120°, 4 h. LTE still oven.
D. 120°, 4 h. Gallenkamp ventilated oven.
E. 130°, 1½ h. Brabender Semi-Automatic Moisture Tester.

It was expected, from previous experience, that results would be lowest from method A and would rise to the highest from method E.

All five methods were used only in Expt. I. With only one Gallenkamp oven and one LTE oven available, all the methods could not be used simultaneously in this experiment, and the temperature settings of the ovens had to be readjusted between determinations. Therefore, in later experiments, one of the methods (B) using the Gallenkamp oven and one (C) using the LTE oven were excluded, these methods having given, as expected, values intermediate between A and D in Expt. I. In each of these experiments all the moisture determinations by the methods A, D and E on the prepared samples were done on the same day. There was no replication, and consequently no means of determining how the results given by any method differ from day to day. A final experiment was, therefore, conducted in which the moisture content

* ' Converted ' rice is rice which has been subjected to a form of ' parboiling ', i.e., soaking, and gelatinisation of the starch by heat before the removal of the husk.

of three types of wheat, at two moisture levels, was determined by the three methods, on each of four days spread over one week.

Conditioning of samples

Before each experiment, moisture determinations were made on all samples of the cereals to be used in the experiment, by all the methods to be tested, and the mean of the results found. Each of these samples was then sub-divided into 300-g. sub-samples whose moisture content was then adjusted, approximately, to selected levels.

Where the moisture content had to be raised, distilled water, calculated to give a level about 1·0% above the final moisture content required, was added, and the samples, after being well mixed, were kept in airtight containers at room temperature for 24 h. All samples were then dried back, at 28–30° on a laboratory tray dryer, with frequent weighing, until the desired weight was reached.

This method of conditioning grain, by dampening to a slightly excessive extent and then drying to the desired level, is frequently adopted in this laboratory for the following reasons:

(1) On a laboratory scale it is more convenient to adjust moisture content precisely by measuring weight loss during drying than by adding precisely calculated quantities of water.

(2) There is evidence that grain to which water has very recently been added has an excess of water in the outer layers, and in an unbound condition. Slight drying can be expected to remove this water preferentially, and thus leave the grain in as nearly 'normal' condition as possible.

(3) Most commercial grain (whether artificially dried or not) may be considered to have been subject to drying, i.e., is on a desorption isotherm, since the process of ripening in the field is always accompanied by drying, and this process is seldom reversed by uptake of moisture during storage.

When the moisture content of the samples had to be reduced, the tray dryer was again used and the samples, after drying, stored in the same way as the moistened samples.

To increase the moisture content of the starches and gluten, the samples were exposed to a humid atmosphere. The starches picked up moisture quite easily, but the gluten reacted more slowly. To bring the gluten to the highest moisture level, 17%, it was mixed with English wheat at 20% moisture, kept in an airtight container for 10 days and then sieved out.

To reduce the moisture content of the starches they were slowly dried in a warm oven at a temperature not exceeding 60°. It was not necessary to dry the gluten because the moisture content, as received, was already at a low level, 9·37%.

After damping or drying, all samples were placed in airtight containers and left in a cool place to equilibrate, being turned over at intervals.

Results and discussion

All the results from Expt. I are tabulated in Table II. For each of the other experiments the results for selected materials are plotted individually in Figs. 1–4, using for this purpose the deviations of each method from the mean for the material of all the methods at each level of moisture content.

The results of all experiments were examined by analysis of variance as is illustrated by Table III for Expt. I. The 'block' sum of squares is calculated from the mean moisture content of the 21 samples (seven varieties, three levels of moisture). It represents the main moisture content and variety effects and their interaction, but these are irrelevant to the present study. The main moisture content differences were deliberately arranged, and the variety differences depend, mainly, on the varying success with which the selected moisture levels were achieved in the artificial conditioning of the different grains.

Experiment I

Three varieties of wheat, and one variety each of barley, oats, rice and maize were used.

The analysis shows that the differences between the methods of Expt. I are highly significant; indirectly, by the interactions, it allows an evaluation of the effects of variety and of

Table II

Results obtained by different methods at different moisture levels on various grains

(figures are % moisture)

Moisture level	Method	Canadian wheat	Atle wheat	Bersee wheat	Oats	Barley	Yellow flint maize	Milled rice	Totals	
									Method × moisture level	Moisture level
Low	A	11·50	12·40	12·41	12·04	12·81	12·60	11·93	85·69	
	B	11·30	12·60	12·67	12·33	12·95	12·59	12·05	86·49	
	C	11·24	12·32	12·51	12·18	13·05	12·77	11·95	86·02	
	D	11·60	12·77	12·76	12·40	13·26	12·90	12·20	87·89	
	E	11·30	13·10	13·10	12·80	13·40	12·80	12·40	88·90	
	Mean	11·39	12·64	12·69	12·35	13·10	12·73	12·11		
Moisture × variety	Total	56·94	63·19	63·45	61·75	65·47	63·66	60·53		434·99
Medium	A	14·53	14·51	15·40	14·51	14·56	15·17	13·83	102·51	
	B	14·48	14·48	15·54	15·11	14·81	15·06	13·95	103·43	
	C	14·34	14·52	15·60	15·09	14·75	15·04	13·69	103·03	
	D	14·66	14·86	15·78	14·94	14·96	15·21	14·28	104·69	
	E	14·60	14·80	15·90	14·90	15·10	15·20	14·30	104·80	
	Mean	14·52	14·63	15·64	14·91	14·84	15·14	14·03		
Moisture × variety	Total	72·61	73·17	78·22	74·55	74·18	75·68	70·05		518·46
High	A	18·45	17·91	17·69	17·98	18·33	18·17	18·31	126·84	
	B	18·55	18·20	18·01	17·97	18·54	18·05	18·30	127·62	
	C	18·64	18·23	17·96	18·26	18·75	18·33	18·13	128·30	
	D	18·71	18·33	18·23	18·45	18·74	18·13	18·57	129·16	
	E	18·70	18·50	18·30	18·10	18·80	18·30	18·80	129·50	
	Mean	18·61	18·23	18·04	18·15	18·63	18·20	18·42		
Moisture × variety	Total	93·05	91·17	90·19	90·76	93·16	90·98	92·11		641·42
Variety × method	Total								Method	Grand total
	A	44·48	44·82	45·50	44·53	45·70	45·94	44·07	315·04	
	B	44·33	45·28	46·22	45·41	46·30	45·70	44·30	317·54	
	C	44·22	45·07	46·07	45·53	46·55	46·14	43·77	317·35	
	D	44·97	45·96	46·77	45·79	46·96	46·24	45·05	321·74	
	E	44·60	46·40	47·30	45·80	47·30	46·30	45·50	323·20	
Variety	Total	222·60	227·53	231·86	227·06	232·81	230·32	222·69		1594·87

Table III

Experiment I. Analysis of variance

Source	Sums of squares	Degrees of freedom	Mean square	'F'	'F' at 5%	'F' at 1%
Blocks	634·8307	20				
Methods	2·1752	4	0·54380	40·25	2·57	3·75
Method × moisture level	0·1446	8	0·01807	1·33	2·14	2·58
Method × variety	0·7720	24	0·03217	2·38	1·75	2·22
Error (by difference)	0·6486	48	0·01351			
Total	638·5711	104				

moisture content on the differences between the methods. The triple interaction serves as error (Table IV).

It will be seen, from Table II, that the individual deviations from the mean for flint maize and hard Manitoba wheat are less than for the five softer grains. This difference is responsible for the significant method and variety interaction indicated by the analysis of variance.

The similarity between flint maize and Manitoba wheat was presumed to be due to their common hardness, but, in case it should be a characteristic common to all varieties of maize, an additional test was performed using a soft white ' horse-tooth ' dent maize. For this purpose the moisture content of the soft maize was determined, for comparison, at three levels of moisture

content, by methods A, D and E. The results were not examined statistically but are shown, together with those for the flint maize, in Fig. 1, where it can be seen that the softer maize gave a somewhat wider range of variations than the hard variety.

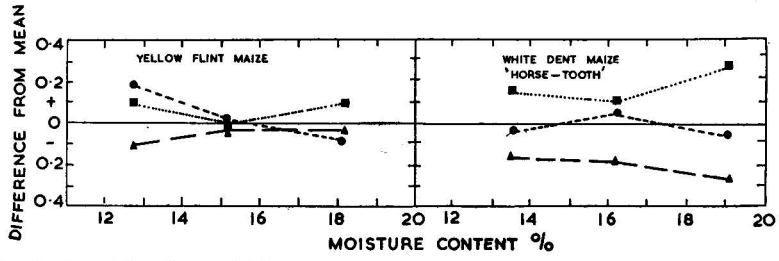

FIG. 1.—*Results from* (a) *a flint and* (b) *a dent maize, showing the deviations of the methods from the mean, plotted against moisture content*

Legend

Mean
105° 16 h. no fan
120° 4 h. fan
130° 1·5 h. Brabender

Experiment II

As Expt. I had shown less variation with a hard than with a soft wheat, six wheats, three hard with high protein and three soft with low protein were prepared to three moisture levels and tested by three methods. Table V gives the mean of the moisture contents, and the range obtained by all three methods at each moisture level for each variety, and the protein content of each variety calculated on a 13·5% moisture content. Three of these wheats were used again in the final 'replication' experiment.

Table IV

Replication test. Analysis of variance of moisture content values obtained by three methods on three varieties of wheat at two moisture levels on four occasions spread over one week

Source	Mean square	'F'	'F' at 5%
Methods	0·3133	19·70	
Method × variety	0·0359	2·26	2·54
Method × moisture level	0·0107	0·67	
Variety × method × moisture level	0·0267	1·68	
Replication	0·0159		

Table V

Experiment II. Mean and range of moisture contents obtained by three methods at three moisture levels for six varieties of wheat

(Protein content of each variety is also shown)

Group	Variety	Protein, %	Moisture level					
			Low		Medium		High	
			Mean	Range	Mean	Range	Mean	Range
Hard	Up River Plate	12·9	11·61	0·49	16·09	0·48	19·27	0·28
	No. 1 Dark Hard Winter	12·4	12·43	0·32	16·17	0·34	20·05	0·34
	No. 2 Atlantic Manitoba	12·4	11·04	0·29	16·61	0·27	19·27	0·34
Soft	No. 2 Red Winter	10·3	11·75	0·45	16·51	0·46	19·62	0·43
	Swedish	9·9	11·64	0·59	16·21	0·30	19·45	0·36
	No. 2 Soft White Winter	9·3	12·05	0·47	16·10	0·42	19·46	0·57

Results are figured for Dark Hard Winter and Soft White Winter wheats in Fig. 2. This figure and Table V show that there was a smaller range of results with hard, high-protein wheat than with soft, low-protein wheat. The analysis, however shows (Table VI), that this varietal effect just fails to reach significance at a 5% level (F = 2·36). However, as pointed out earlier, if the hard and soft wheats are grouped together, the differences between the groups are significant at the 5% level.

In this experiment variations due to the interaction of method and moisture level were highly

Table VI

F ratio and degree of significance for all experiments

Experiment No.	Source							
	Method		Method × moisture level		Method × variety (all grains)		Method × variety (hard versus soft)	
I	40·25	**	1·33	N.S.	2·38	**		
II	296·00	**	5·36	**	2·06	N.S.	5·44	*
III	55·00	**	2·90	N.S.	3·85	*		
IV	160·00	**	0·59	N.S.	4·55	**		
V	19·70	**	0·67	N.S.	2·26	N.S.	3·53	*

** Significant at 1% level * Significant at 5% level
N.S. not significant

significant (Table VI). Inspection of the original data showed that this was mainly due to results from method D which increased in relation to the others with increasing moisture content. This can also be seen in Fig. 2.

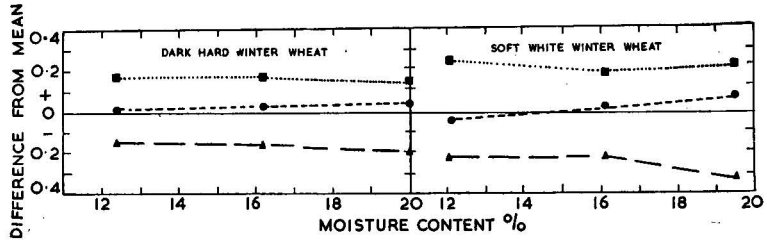

FIG. 2.—*Results from* (a) *Dark Hard Winter and* (b) *Soft White Winter wheat, showing the deviations of the methods from the mean plotted against moisture content*

Legend as Fig. 1

Experiment III

This experiment was designed as a further test to determine whether the difference found between vitreous and non-vitreous grains was due to protein content or to the physical nature of the grains. For this, five samples of different varieties of rice, a grain notoriously low in protein content, were used. Three of these were normal milled rices, the other two were relatively vitreous as the result of having been parboiled before being milled. The samples were prepared to three moisture levels.

Table VII gives the mean results and range for each variety at each level of moisture content. Results for the Siam rices, untreated and parboiled, are shown in Fig. 3. The figure and table show that gelatinised rices give a narrower range of variation than the untreated rices. This difference is confirmed by the analysis of variance (Table VI).

Experiment IV

The results of Expts. I and II suggested that wheat protein tended to reduce the range of moisture content given by different methods. Expt. IV was, therefore, designed to compare wheat gluten with pure starch from wheat, maize and rice. The materials were conditioned to four levels of moisture content.

Table VII

Experiment III. Mean and range of moisture contents obtained by three methods at three moisture levels for five varieties of rice

Group	Variety		Moisture level					
			Low		Medium		High	
			Mean	Range	Mean	Range	Mean	Range
Vitreous	Meedone	Converted	12·57	0·20	16·12	0·11	18·99	0·44
	Siam	Parboiled	13·05	0·14	16·21	0·16	18·91	0·45
Non-vitreous	Siam		13·55	0·66	16·40	0·40	18·91	0·63
	Italian		14·05	0·30	17·16	0·47	18·81	0·44
	Meedone		13·58	0·52	15·57	0·49	18·58	0·59

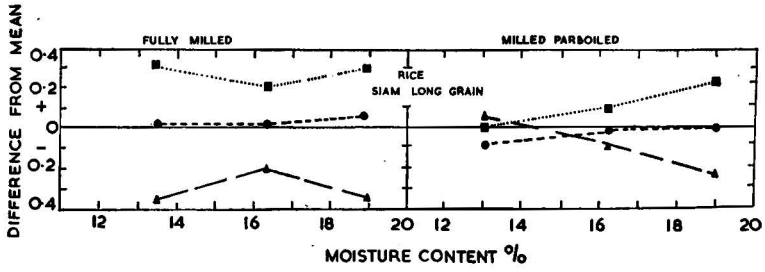

FIG. 3.—*Results from* (a) *a fully milled untreated, and* (b) *a parboiled rice, showing deviations of the methods from the mean plotted against moisture content*

Legend as Fig. 1

Table VIII gives the mean results and range for each material at each level of moisture content. Results for gluten and wheat starch are shown in Fig. 4. The Figure and Table show that the range of results given by the starches is wide, whereas the range given by the gluten is narrow. This is confirmed by the analysis of variance (Table V).

Table VIII

Experiment IV. Mean and range of moisture contents obtained by three methods at four moisture levels for wheat gluten and three starches

Material	Moisture level							
	Low		Low medium		High medium		High	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Gluten	9·37	0·25	12·99	0·35	14·90	0·26	17·23	0·20
Wheat starch	12·69	0·48	14·25	0·41	16·84	0·36	17·53	0·51
Maize starch	12·75	0·52	14·39	0·36	16·41	0·62	17·32	0·57
Rice starch	13·08	0·61	13·77	0·61	15·80	0·48	17·40	0·38

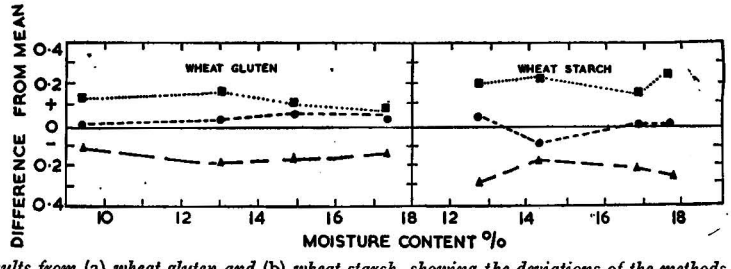

FIG. 4.—*Results from* (a) *wheat gluten and* (b) *wheat starch, showing the deviations of the methods from the mean plotted against moisture content*

Legend as Fig. 1

Experiment V (Replication experiment)

This final experiment was a repetition of part of Expt. II, using only three of the varieties of wheat, but repeated on each of four days spread over one week. Once again the two hard wheats exhibited a narrower range of variation in the results than was given by the softer wheat. This is not apparent from Table V, in which the value for the 'F' ratio falls just short of the level required for statistical significance, but if the values for the two hard wheats are grouped together, then the 'F' value for the hard versus soft wheats is significant.

This experiment revealed, rather surprisingly, a marked systematic occasion variation. The replication error of Table V includes a main occasion effect and a series of interactions, the former being much the greater. The subject of day-to-day variations will be considered in a later paper, but it is worth noting here that it is clear from the detailed results of this experiment that daily variation is much the biggest with the Brabender method (E).

In all experiments the values obtained from the various methods differ significantly (Table VI) and are in the order expected. The main purpose of the experiments, however, was to determine whether or not the differences between methods vary from one variety to another,

and from one moisture level to another. This may be examined by reference to Table VI in which the values of the ' F ' ratio obtained by analysis of variance and the degree of significance of the values are given for all experiments.

It is clear that variety has a marked effect on the method, for the ' F ' ratio for the method and variety interaction exceeds the 5% level in three experiments, and is only slightly below it in the other two. It may be noted in passing that both of these two barely significant ' F ' ratios relate to the same varieties of wheat, and that even with these, if the hard and soft wheats are grouped together, differences between them are significant at the 5% level. The effect of moisture level is less important, for the 5% value for the ' F ' ratio for the moisture level and method interaction is reached only once.

Conclusions

The data presented give evidence that the harder, more vitreous materials give a narrower range of differences between methods and, conversely, that soft, non-vitreous materials give a wider range. It is the nature of the material that mainly affects the differences between the methods but, in one instance at least, the level of moisture content affected the relative results of the methods.

These results preclude the possibility of alternative standard methods for moisture determination being specified if more than one kind of grain is to be tested. This in itself is not important, since it is well known that different methods give different results. It will be of importance, however, if the claim is made that two different methods give identical results, as indeed they may do for one type of grain, and the inference is made that they will do so for all other grains.

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STUDIES ON PROTEIN CONCENTRATES FOR ANIMAL FEEDING

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The protein quality of a wide range of common protein foods has been investigated by means of chemical, biological and microbiological tests. B vitamin contents have also been determined in some materials. Results for a large range of meat meals, whalemeat meals, fish meals and miscellaneous samples are presented and discussed in separate sections, and attention drawn to the existence of certain corelations.

Introduction

As is well known, protein concentrates used as supplementary sources of protein in the rations of farm livestock differ widely in nutritive quality. The term ' protein concentrate ' embraces not only materials of animal origin, such as fish meal, meat meal and whale meal, but also the oil cakes and extracted meals as well as the leguminous grains or pulses. All

these materials have their place in animal feed formulation as sources of dietary nitrogen, and indeed their commercial value is assessed very largely on their total nitrogen content as measured by the conventional Kjeldahl method.

To facilitate the proper selection of protein concentrates for particular purposes numerous tables of analytical data are available which present typical figures of total nitrogen content, but qualitative as well as merely quantitative considerations must be borne in mind in the calculation of rations. In assessing the suitability of a given protein to meet the physiological needs of a particular animal species, the protein is obviously of value only in so far as it is digestible by the animal and in so far as it contributes, along with the other constituents of the ration, to the essential amino-acid requirement of the species concerned. Not only are there great differences in the nutritive value of different types of protein of the same, or similar, total nitrogen content, but there are also wide variations in the nutritive value of different samples of the same feedingstuff. Such variations may arise in a number of ways—from basic variations in raw material sources, from strain or varietal differences, from changes induced by processing, etc., but inevitably they place a severe limitation on the value of any single figure purporting to be representative of a given material.

What criterion of quality could usefully augment—or even replace—a total nitrogen determination? Such a criterion cannot easily be defined, for it must obviously depend not only on the animal species for which the feed is intended but also on the remainder of the ration of which a single protein concentrate is but one part. Furthermore, for a method of evaluation of protein quality to have practical value it is necessary that it should be fairly simple to perform and fairly rapid to produce a result. Numerous tests of protein quality have been described and whilst many of these are too well known to merit detailed description, it may not be inappropriate to outline very briefly some of the diverse approaches that have been made by various authors to this problem.

Animal tests

A great variety of ruminant and non-ruminant animals have been used for evaluating protein quality. Results have sometimes been assessed on the basis of body-weight gains and the Protein Efficiency Ratio (P.E.R.) (Osborne, Mendel & Ferry),[1] but Mitchell[2] has criticised the use of P.E.R. because it varies with food intake. Growth results have also been assessed by comparison with the performance of the test animal fed a standard protein source as in the Gross Protein Value technique for chicks, as modified by Carpenter, Ellinger & Shrimpton[3] which measures the value of a protein source as a supplement for cereals.

Overall N metabolism has been studied more directly by determining changes in total carcass N (McCollum & Simmonds[4] and Bender & Miller[5]) or N balance as in the Biological Value technique of Mitchell *et al.*[6] and the N Balance Index method of Allison *et al.*[7] Other workers have paid special attention to the effects of dietary protein on a single organ such as the liver (Campbell & Kosterlitz).[8]

In all these tests, the level of protein in the diet is a factor of great importance (see Forbes *et al.*[9]), as is the question of whether the test protein supplies all or only part of the dietary N. Tests involving bacteria and protozoa are described below.

Microbiological tests

Several attempts have been made to devise tests in which micro-organisms serve as indicators of protein quality. The most successful and widely used of these are probably the methods for direct determination of the individual amino-acids in hydrolysates using bacteria (usually lactic acid bacteria). Data so obtained have undoubtedly contributed much to our knowledge of the distribution of the amino-acids in natural products but, in spite of the apparent simplicity of such methods, they have many pitfalls. Choice of suitable hydrolysis procedures—whether enzymic or acid hydrolysis should be used—in order to give maximum liberation and minimum destruction of the amino-acid under test, is at least as important as the choice of organism and assay medium in selection of a method. Amongst numerous reviews of the subject, those by Barton-Wright,[10] Schweigert & Snell[11] and Horn *et al.*[12] might be mentioned.

Alternatively, attempts have been made to use enzymic hydrolysates of protein feedstuffs

as a sole source of essential amino-acid nitrogen for micro-organisms, as in the methods of Halevy & Grossowicz[13] and of Horn *et al.*[14] Such methods, however, are largely empirical and do not appear to have come into widespread use in this country at least.

Finally, there is the method described by Rosen & Fernell[15] and by others in which feedstuffs without prior digestion are compared directly as protein sources for the protozoan *Tetrahymena pyriformis*. The similarity of the nutritional requirements of this organism to those of higher animals and its ability to digest intact protein make it an attractive micro-organism for such studies.

Chemical and physical methods

In addition to microbiological assay, chemical methods and the newer chromatographic techniques are still used extensively for amino-acid determination. Most of these are intended to measure ' total ' amino-acid liberated after a suitable hydrolytic procedure and, as with the microbiological technique, therefore, selection and standardisation of suitable hydrolytic methods are of fundamental importance. Recognising that nutritional damage to animal products during processing is not always reflected in amino-acid assays of acid hydrolysates, Carpenter & Ellinger[16] have attempted to measure only the *available* lysine after reacting the protein with 2, 4-dinitrofluorobenzene (D.N.F.B.). Alternatively, dye adsorption tests for the estimation of total acidic or basic amino-acids by the use of Safranin O or Orange G,[17, 18] respectively, have been proposed.

The nutritional value of a protein must obviously depend, first and foremost, on its digestibility. Numerous *in vitro* enzyme methods have been described by Gehrt *et al.*[19] and Bondi & Birk[20] amongst others. Many of them, however, give incomplete splitting of the proteins and are difficult to relate to digestibility coefficients determined on animals—a difficulty that Mertz and his colleagues[21] have endeavoured to overcome by supplementing the action of pepsin with that of a proteolytic bacterium. Sheffner *et al.*[22] have proposed the use of an integrated index—the Pepsin Digest Residue—in which the essential amino-acid pattern as measured microbiologically after pepsin digestion *in vitro* is combined with the amino-acid pattern of the remainder of the protein, and close correlation with net protein utilisation is claimed for a variety of proteins.

Solubility methods have also been extensively studied. One of the best known is the Protein Quality Index (P.Q.I.) of Almquist and co-workers[23] in which the total nitrogen of an animal protein is divided into four fractions based on copper precipitability, pepsin digestibility, hot water solubility and phosphotungstic acid precipitability, all of which come into the final expression for calculation of P.Q.I. A somewhat similar method involving fractionation of the sample successively with distilled water, 5% KCl, 70% alcohol and 0·2% KOH has been proposed by Lund & Sandstrom.[24]

Although these methods have been claimed to give good correlation with animal tests for certain well-defined groups of feedstuffs, they are time-consuming to carry out and of limited applicability and, as pointed out by Evans & St. John,[25] there is no relationship between P.Q.I. ratings of diverse feedingstuffs. These tests are probably most useful when differences between a number of samples of similar material—differing perhaps as a result of processing—are under investigation. Indeed, it may yet prove impracticable to devise a single test that will be universally applicable to different types of proteins, some of which possess properties peculiar to themselves. It is necessary to take account of the presence of gossypol in unprocessed cottonseed meal, for instance, in consideration of the growth-promoting properties of the sample. On the other hand, the possibility of using a test for residual urease, as a method of detection of underheating, is unique to soya-bean meal.

In the present investigation selected chemical, biological and microbiological methods of protein quality assessment were applied to a range of feedingstuffs that had been deliberately selected as representative of a wide range of sources and types. The meat meal and whale meal samples were also tested for presence of gelatin and for digestibility *in vitro*. Chemical tests for crude and true protein, oil, ash, calcium and phosphorus were also applied and, in addition, it was thought to be of interest to determine the B vitamin content of the meat meal and whale meal samples.

Methods

Sampling

Routinely, a representative sample was withdrawn from the main supply of each protein food and thoroughly mixed and ground before being used for testing.

Chemical tests

Moisture.—Samples were dried to constant weight at 105°.

Crude protein (N × 6·25 unless otherwise stated).—A modification of the Kjeldahl method was used, in which digestion was assisted by mercuric oxide and potassium sulphate. Ammonia was distilled into 2% boric acid and titrated with 0·1N-H_2SO_4, using a mixed indicator. Aliquots of the final digest were taken so as to give a titre of less than 15 ml. of 0·1N-H_2SO_4. With this precaution, the method gave results in good agreement with the A.O.A.C. method.[26]

True protein (*albuminoid nitrogen*).—The A.O.A.C. method[27] was used.

Oil.—Determination was by Soxhlet extraction, using equal proportions of ether and light petroleum (b.p. 40–60°).

Calcium.—After dry ashing, calcium was determined by an oxalate precipitation procedure, followed by titration with potassium permanganate.

Phosphorus.—This was determined by the colorimetric technique of Kitson & Mellow.[28]

Orange-G adsorption.—This was determined by the method of Udy[18, 29] as modified by Bunyan.[30]

Presence of gelatin.—A qualitative test was used.[31]

In vitro *digestion.*—The method of Bondi & Birk[20] was used, in which overnight digestion with pepsin at 37° was followed by overnight digestion with pancreatin at the same temperature. Total nitrogen determination were carried out on the filtered enzyme digests as well as on the original materials.

Biological methods for assessment of protein quality

Net Protein Utilisation (*N.P.U.*).—This was determined by the method of Bender & Miller[5] and Miller & Bender,[32] using Norwegian hooded rats and is reported as N.P.U. Before conventional N analysis, the rat carcasses were pretreated either by drying at 105° or by dissolving them in concentrated H_2SO_4 (as suggested by Rix[33]).

True digestibility coefficient.—This was measured as:

$$\frac{\text{Protein N intake} - (\text{total faecal N} - \text{metabolic faecal N})}{\text{Protein N intake}}$$

(Protein N intake = Total intake − non-protein N intake.) Faeces were collected for the final 8 days of each test, after allowing 2 days for the rats to become accustomed to the experimental diets. The faecal-N output of the protein-depleted rats was found and the metabolic faecal-N ratio calculated, viz., g. of faecal N per kg. of food eaten. This ratio was then used to correct the faecal output of the protein-fed rats by subtraction of the metabolic component.

Biological value was estimated by dividing N.P.U. by the Digestibility Coefficient.

Net protein ratio (*N.P.R.*) by the method of Bender & Doell.[34]

Microbiological methods for assessment of protein quality

Tetrahymena pyriformis *method* (Rosen & Fernell)[15].—In our hands replication of results by this method was very poor and we do not yet feel confident of its reliability. For this reason, the technique was applied only to the meat meals—the first samples to be examined.

Evaluation of enzyme digests with Streptococcus faecalis.—This method is based on that of Halevy & Grossowicz,[13] but differs in certain important particulars. Firstly, a different digestion procedure was used, as outlined above. Graded doses of these digests were then added to 6 in. × $\frac{3}{4}$ in. test-tubes containing the culture medium of Henderson & Snell[35] from which the essential, but not the stimulatory, amino-acids had been omitted. The digests, therefore, served as the sole source of essential amino-acids for the micro-organisms.

Cultures of *Streptococcus faecalis* were carried on liver tryptone agar slopes; 24-h. cultures

in liver tryptone broth were resuspended in saline without washing or dilution and one drop per tube used as inoculum. The assay tubes were incubated for 3 days at 37° and the bacterial growth assessed by titration with 0·1N-NaOH to pH 7·0 estimated electrometrically. Results were plotted against mg. of N in the filtered digests over the range 0 to 0·6 mg. of N. Quantitative assessment of the results was complicated by the fact that the curves showed considerable variation in curvature from sample to sample, hence attempts to make growth comparisons at fixed protein levels were not independent of the level chosen. An attempt has been made, therefore, to overcome this difficulty by calculating the areas bounded by the curves for the various samples and comparing these integrated responses with a similar figure calculated for a standard sample (e.g., casein) under identical conditions.

Amino-acid analyses

Total lysine (microbiological).—Using *Leuconostoc mesenteroides P.* 60 as test organism, and the medium described by Barton-Wright.[10]

'Available' lysine.—Method (1). The dinitrofluorobenzene method of Carpenter & Ellinger;[16] *Method* (2). The methyl chloroformate modification of Method (1).[36]

Methionine.—(1) (*chemical*). Determined by the sodium nitroprusside method of Csonka & Denton;[37] (2) (*microbiological*). Using *Leuconostoc mesenteroides P.* 60 as test organism, and the medium described by Barton-Wright & Curtis.[38]

Vitamin analyses

Vitamins were determined microbiologically, riboflavin and nicotinic acid by the method recommended by the Analytical Methods Committee of the Society of Public Analysts[39] and pantothenic acid by the method of Barton-Wright.[10] For extraction of pantothenic acid, however, a method based on that of Harrison[40] was used, in which a 1-g. sample was steamed for 30 min. at pH 6·8 and then, after being cooled, treated with 5 ml. of 10N-NaOH for exactly 5 min. After addition of 5·1 ml. of 10N-H_2SO_4, the pH was adjusted to 4·5 and the mixture digested under toluene for 3 days at 37° with 0·5 g. each of papain and takadiastase. The volume was then made up to 100 ml. and filtered, and a 50-ml. aliquot adjusted to pH 6·8 and diluted as required for assay.

Protein quality and vitamin contents of meat meals

Materials

Of all the protein concentrates, meat and meat and bone meals are probably unique in the diversity of the raw materials from which they are made and in the variety of techniques used in their production. Results of the present investigations, in which 26 samples of meat meal were studied, are shown in Tables I and II.

Chemical and microbiological test results

The very wide range of 40–90% crude protein was encountered. True protein usually accounted for 80–98% of the crude protein, but meals MM3, MM5 and 220 had anomalously low levels and were also observed to 'gelatinise' in warm water. Wide ranges of oil content (1–17·4%) and of ash content (3–42%) were found, the latter being roughly proportional to the inverse of the protein content. The Ca/P ratio itself ranged from 0·5 to 4·3.

Available lysine results obtained by Method (1) were mainly between 3·0 and 4·6 g./16 g. N, with one high value of 4·9 and two low values of 2·6 and 1·9 g./16 g. N. Method (2) generally gave results within ±10% of these, except for meals 223 and MM20, where the difference was much greater. Comparison of these figures with the microbiological determinations done on 10 samples showed an availability of about 50–75%, which is lower than the availability of lysine measured biologically in a variety of foods tested by Schweigert & Guthneck.[41] It should be pointed out, however, that the total lysine results which, except for that on MM20, are in agreement with the values of 5·4 to 7·8 g./16 g. N given by Block & Weiss[42] for meat meals and meat scraps may be high because of the sensitivity of *Leuconostoc mesenteroides* to hydroxylysine, which may be present in structural proteins.[43, 44]

Results of the chemical assay for methionine were well confirmed by the microbiological

Table I

Meat meals: results of protein quality tests

Number	Protein: Crude % (N×6·25)	Protein: True×100 / Crude	Orange-G-binding, acid equivalents dye per 10^4 g. protein	Lysine, g./16 g. N: 'Available' Method (1)	Lysine, g./16 g. N: 'Available' Method (2)	Lysine, g./16 g. N: Total	Methionine, g./16 g. N: C	Methionine, g./16 g. N: M	Net Protein Utilisation	Digestibility	Biological Value	Net Protein Ratio	*Str. faecalis* growth†	Relative Nutritive Value
MM1	57·9	89	6·4	3·7	3·9	7·1	1·3	1·35	25 (2)	0·79 (2)	32	0·8	~50	78
MM2	55·1	91	6·7	4·0	4·1		1·3		18 (2)	0·81 (2)	22	0·8	~50	80
MM3*	84·4	37	2·8	3·9	3·6	5·2	0·6		9 (2)	0·93 (2)	10	0·4	36	33
MM4	56·3	89	7·0	3·8	3·8		1·0	0·9	21 (2)	0·85 (2)	25	0·6	~50	61
MM5*	83·2	35	3·4	3·8	3·7	5·2	0·6		11 (2)	0·91 (2)	13	0·6	36	33
MM6	48·7	86	6·1	4·1	4·4		1·0		22 (2)	0·86 (2)	27	1·2		51
MM7	49·1	89	6·1	3·7	3·4		1·1	1·2	18 (2)	0·77 (2)	23	0·6	~50	63
MM8	41·4	98	6·0	3·2	3·4	6·8	0·7		21 (2)	0·69 (2)	30	0·9	~50	47
MM9	50·2	89	5·8	3·2	3·6		0·9		21 (2)	0·77 (2)	27	1·3	~50	51
MM10	45·4	84	4·7	4·1	3·7		0·9		14 (3)	0·73 (3)	19	0·8	40	32
MM11	47·5	92	6·7	4·4	4·0	7·1	1·1		28 (2)	0·80 (2)	35	1·5	42	33
MM12	53·0	88	6·1	3·4	3·1	6·0	0·9		30 (1)	0·76 (1)	40	2·2		
MM13	46·0	90	6·2	4·3	4·3		0·9		31 (2)	0·77 (2)	40	1·3	~50	76
MM14	53·7	84	6·1	4·3	4·1				27 (2)	0·85 (2)	32	1·1	~50	66
MM15	50·4	89	6·6	4·3	4·1				29 (2)	0·70 (2)	41	1·3	40	41
MM16	61·0	92	7·1	2·6	2·6	5·2	0·9	0·9	26 (2)	0·78 (2)	34	1·5	36	51
MM17	50·0	97	8·1	3·1	3·2				29 (2)	0·77 (2)	37	1·2	43	47
MM18	55·7	91	8·5	4·6	4·9	7·8	1·6	1·7	31 (2)	0·79 (2)	39	1·8	64	103
MM20	79·4	91	4·5	1·9	1·2	2·1	0·2		32 (2)	0·70 (2)	46	1·1	36	
MM23	57·6	86	8·1	4·3	4·5	7·5			34 (1)	0·90 (1)	38	1·8		
MM25	53·8	81	7·0	3·5	4·0				28 (1)	0·88 (1)	32	1·5		
219	88·8	98	4·9		2·2		0·4	0·7	35 (3)	0·83 (3)	42	1·3		
220*	89·3	45	2·4	3·9	3·7		0·4	1·0	38 (1)	0·87 (1)	44			31
221	65·5	97							21 (1)	0·71 (1)	34			
223	42·2	89		4·9	4·1				21 (1)	0·67 (1)	31	0·3		4
224	40·9	96							23 (1)	0·67 (1)	34	1·1		

* Gelatin present C = Chemical method M = Microbiological method † Relative to casein = 100
Numbers of replicates shown in brackets

Table II

Meat meals: miscellaneous chemical data

Number	Moisture, %	Oil, %	Ash, %	Calcium, %	Phosphorus, %	Riboflavin, μg./g.	Nicotinic acid, μg./g.	Pantothenic acid, μg./g.
MM1	8·8	3·5	22	6·1	3·2	6·5	55·8	12·4
MM2	9·2	4·8	19					
MM3	10·3	1·2	3	0·26	0·49	15·7	83·5	49·4
MM4	8·5	4·8	24					
MM5	9·8	1·0	4	0·44	0·48	15·2	82·3	40·6
MM6	7·4	12·0	30					
MM7	8·1	2·2	35					
MM8	8·0	3·3	42	15·1	6·9	3·5	40·4	8·4
MM9	8·2	2·0	34					
MM10	6·8	4·5	35					
MM11	9·8	4·9	30					
MM12	9·6	9·5	26					
MM13	7·4	16·3	28					
MM14	6·9	11·6	28					
MM15	8·2	10·6	27					
MM16	7·6	8·2	20	6·9	2·6	3·5	56·0	8·5
MM17	7·7	10·1	27					
MM18	10·6	17·4	15	5·2	2·5	7·8	45·9	13·9
MM20	7·9	3·0	7	3·0	0·7	4·2	77·0	10·1
MM23	7·3	4·8	13	3·8	2·5	6·9	54·8	9·6
MM25	10·2	10·3	27					

assay. Twelve samples were in the range 0·9–1·6 g./16 g. N. Meals MM3, MM5 and MM8 were below this range and MM20 was quite extraordinary in having only 0·2 g./16 g. N.

The Orange-G-binding figures, with the exception of Nos. 219, 220 and 223 (recent additions), have been considered elsewhere[30] with regard to the relationship between crude protein % (Y) and mg. of dye bound per g. of sample (X). When the anomalous results for MM3, MM5 and MM20 were omitted, the following regressions were found:

$$Y = 0{\cdot}278X + 30 \qquad (P = 0{\cdot}001)$$

and

$$Y_1 = 0{\cdot}239X + 27 \qquad (P = 0{\cdot}001)$$

where Y_1 = True protein %.

In Table I these results are expressed as acid equivalents of dye bound per 10^4 g. of protein (Y) and they show no relation to crude protein content, but they are correlated with true protein results (X):

$$Y = 0{\cdot}129X + 0{\cdot}67 \qquad (P = 0{\cdot}01).$$

In addition to this, there is a relationship between N.P.U. (Y) and acid equivalents dye per 10^4 g. of protein (X):

$$Y = 3{\cdot}86X - 0{\cdot}43 \qquad (P = 0{\cdot}01)$$

Meals MM3 and MM5 do actually lie on this line, too.

If the abnormal samples are ignored, the dye adsorption expressed as mg. of dye/g. of sample is closely related to true and crude protein content; but when expressed as acid equivalents per 10^4 g. of protein, it may be noted that higher values go with higher N.P.U. and higher true protein. These effects may be partly due to the fact that protein is not the only constituent adsorbing dye and partly to a limitation of N.P.U. by a deficiency in the basic amino-acids.

The *Streptococcus faecalis* method, applied over the range 0–0·6 mg. N, did not distinguish between most of the meat meals—the growth-promoting activity of which ranged from about $\frac{1}{3}$ to $\frac{2}{3}$ that of casein.

At other levels of N, however, other differences became apparent, some of the hydrolysates, e.g., MM20, showing inhibition at higher levels; maximal growth could not be obtained with this meal no matter how much was added. *Tetrahymena* showed a wide range of relative nutritive values in spite of the erratic results which the method has given in our hands. No significant relation was found between R.N.V. and N.P.U., but R.N.V. (Y) was significantly related to available lysine (g./16 g. N) (X):

$$Y = 19{\cdot}4X - 20{\cdot}3 \qquad (P = 0{\cdot}05)$$

Biological test results

The range of N.P.U. results (9–38) and N.P.R. (0·4–2·2) must be considered alongside the fact that an N.P.U. value below about 30 (i.e., an N.P.R. of about 1·6) means a loss of body-weight of the rat during the test period. In fact only four meals produced weight gains when tested and 12 meals led to severe weight losses. Of the 26 Digestibility Coefficients determined, three were below and three above the general range of 0·7–0·9.

Carpenter *et al.*[45] found that when fish meals were fed to chicks as supplements to cereal diets, there was a good correlation between the Gross Protein Value and the available lysine of the supplement. A similar relationship was looked for between Biological Value and either methionine or available lysine in this series of meat meals. In each case there was no significant regression, as determined by the method of least squares.

General comments

It is interesting to note that all the meals with 79% or more crude protein, have proved anomalous on further examination. Three of them, MM3, MM5 and 220, show very little true protein or methionine and also contain gelatin. Nos. MM3 and MM5 also contain noticeably more of the B vitamins than the other samples studied. The other high-protein meals have a normal proportion of N as True Protein N but are low in methionine and available lysine.

In these four characteristics they closely resemble the feather meals studied below (Table VI) and the comparison is further substantiated by their low Orange-G adsorptions. (Other low dye-adsorption results may have been due to high soluble-protein contents.) Biological Value results (i.e., N.P.U. + digestibility) were generally low, indicating partial deficiencies of one or more essential amino-acids, and covered a fairly narrow range. They did not then offer much scope for differentiation between the meals not already found anomalous in other tests.

In spite of the general uniformity found among meat meal samples of approximately 40–60% protein content, it is possible to indicate a few that are ranked high by most of the tests applied; MM18, and possibly MM1 and MM13, are the best examples of this.

Comparison with other published data shows that the results presented here extend over a much wider range. This is particularly true of the N, oil and ash values. The range of total lysine values (2·1–7·8 g./16 g. N) agrees roughly with the data given by Block & Weiss[42] (5·4–7·8 g./16 g. N) and by Lyman *et al.*[46] (3·8–5·5 g./16 g. N), but lies well below the figures given by Pritchard & Smith[47] (8·3–9·9 g./16 g. N). Methionine levels stated here are lower than the results quoted by Block & Weiss and Pritchard & Smith, viz., 0·2–1·6 compared with 1·4–2·8 and 1·6–2·1 g./16 g. N, respectively. Lyman *et al.* found 0·8–1·4 g./16 g. N.

Samples MM3 and MM5, which were atypical by several criteria, gave unusually high figures for all three vitamins tested. The remaining samples gave figures for riboflavin and for nicotinic

acid similar to those reported elsewhere for meat meal and meat and bonemeal; we did not, however, examine many samples with as little as 45% protein, for which Pritchard & Smith[47] found only 16 μg. of nicotinic acid per g. Special mention should be made of the pantothenic acid figures which are about two to three times as high as those reported by other workers. This is due mainly to the extraction method we have used in which papain and takadiastase digestion is preceded by alkali treatment—a procedure which we find, as did Harrison,[40] results in the release of much additional pantothenic acid from certain materials. For maximum release of pantothenic acid from coenzyme A, Novelli, Kaplan & Lipmann[48] have recommended treatment of the sample with intestinal phosphatase plus chicken liver extract, and it is clearly essential that if pantothenic acid figures are to be regarded as comparable, the method of extraction must be stated.

Protein quality and vitamin contents of whalemeat meals

Materials

Sixteen whale meals selected from various commercial sources to cover a wide range of protein contents were submitted to various chemical, biological and microbiological tests as detailed above. Results are shown in Tables III and IV.

Table III

Whale meals: results of protein quality tests

Number	Protein		Orange-G-binding, acid equivalents dye per 10^4 g. protein	Lysine, g./16 g. N			Methionine g./16 g. N		Net Protein Utilisation	Digestibility	Biological Value	Net Protein Ratio	*Str. faecalis* growth
	Crude % (N×6·25)	True×100 / Crude		'Available' Method (1)	'Available' Method (2)	Total	C	M					
152	77·2	93			7·5				40(2)	0·88(2)	46	2·5	
153	87·2	95							43(2)	0·86(2)	49	2·2	
154	76·1	94							44(2)	0·87(2)	51	2·4	
155	25·1	56											
WM1	57·3	77	7·4	4·5	4·5	8·8	1·6	2·2	25(2)	0·73(2)	34	1·0	86
WM2	68·6	84	9·7	5·7	6·3				36(2)	0·75(2)	51	2·0	101
WM3	88·3	86	10·9	6·3	7·0		2·0	2·6	44(3)	0·81(3)	54	2·5	105
WM4	77·3	90	10·7	6·2	8·1	9·7	1·8	2·8	57(1)	0·87(1)	66	3·2	113
WM5	69·0	86	10·5	6·3	7·1		1·7		37(3)	0·85(3)	44	2·0	102
WM6	79·0	89	10·5	6·3	6·4				52(1)	0·92(1)	57	2·9	116
WM7	81·8	88	4·9	4·0	3·3	8·1	1·6	2·1	17(3)	0·54(3)	32	0·6	58
WM9	86·0	94	10·2	6·6	7·0				61(1)	0·88(1)	69	3·5	127
WM10	78·3	88	9·6	5·9	5·4		1·7	2·4	35(1)	0·75(1)	47	1·7	95
WM11	83·9	90	9·6	6·0	6·6		1·7	2·6	41(1)	0·89(1)	46	2·8	106
WM12	58·5	87	9·5	5·1	5·1	8·9	2·0	2·6	31(2)	0·77(2)	41	1·6	94
WM13	83·4	90	11·9	7·3	7·3	9·6	2·0	2·6	62(2)	0·92(2)	66	3·3	127

C = Chemical method M = Microbiological method Numbers of replicates shown in brackets

Table IV

Whale meals: miscellaneous chemical data

Number	Moisture, %	Oil, %	Ash, %	Riboflavin, μg./g.	Nicotinic acid, μg./g.	Pantothenic acid, μg./g.
152	7·2		11			
153	7·1		2			
154	7·1		12			
155*		14·0	53			
WM1	7·6	9·7	23	3·1	75·9	6·5
WM2	6·5	3·2	20			
WM3	7·3	2·8	3			
WM4	7·9	3·5	7	3·7	89·6	7·0
WM5	6·6	2·2	20			
WM6	7·2	2·9	9			
WM7	7·1	4·5	6	3·6	85·1	4·5
WM9	7·9	4·4	2	5·1	96·0	12·5
WM10	8·1	3·9	7			
WM11	7·6	4·0	4			
WM12	5·5	2·3	29	1·6	64·6	5·7
WM13	8·8	4·2	3	6·7	137·7	16·2

* Gelatin present

Chemical and microbiological test results

Six whale meals in the 80–90% crude protein range were studied and five of 70–80%. With one exception, the rest had from 50 to 70% protein. The exception was meal 155 with 25% of crude protein (14% true protein), 14% of fat and 53% of ash. In all the other samples, true protein N ' accounted for 77–95% of the total. As expected, the meals highest in protein were the lowest in ash.

Available lysine determined by Method (1) showed a range of 5·1–7·3 g./16 g. N with two lower values (4·0 and 4·5). Method (2) often gave higher results, perhaps because the shorter hydrolysis time resulted in less destruction of amino-acid. The usual range by Method (2) was 3·3–8·1 g./16 g. N.

Microbiological determination of total lysine where applied showed an availability of 41–84%, when compared with the chemical Method (2). This may in fact be an overestimate of the total lysine present and an underestimate of the availability because of the sensitivity, mentioned previously, of the microbiological test to hydroxylysine.

Chemical and microbiological determinations of methionine did not agree as well as in the meat meal series, but the latter method gave results comparable with the figure of 2·6 g./16 g. N by Block & Weiss.[42]

The Orange-G-binding data expressed as mg. of dye/g. of sample (X) have been shown elsewhere[30] to correlate with crude protein % (Y) (WM7 omitted):

$$Y = 0{\cdot}261X + 30 \quad (P = 0{\cdot}001)$$

and with the true protein % as Y_1:

$$Y_1 = 0{\cdot}287X + 16{\cdot}7 \quad (P = 0{\cdot}001)$$

The same data are expressed here as acid equivalents of dye per 10^4 g. of protein (X) and it is found that the higher values are related to higher crude protein contents (Y):

$$Y = 6{\cdot}6X + 9{\cdot}03 \quad (P = 0{\cdot}05)$$

It is also found that the meals with higher dye adsorptions have higher N.P.U. (Y_1):

$$Y_1 = 6{\cdot}37X - 19{\cdot}8 \quad (P = 0{\cdot}001)$$

These two regressions imply a correlation between N.P.U. (Y_1) and crude protein % (Y) and this is found to be:

$$Y_1 = 1{\cdot}22Y - 48{\cdot}1 \quad (P = 0{\cdot}001)$$

In order to assess the results of the *Str. faecalis* tests, areas bounded by the growth curves were calculated over the range 0–0·3 mg. N/ml. of digest. No casein control samples were included in these series so that, although the results on the whale meal samples are comparable amongst themselves, they cannot be compared directly with those for the meat meals.

The *Str. faecalis* growth test, it will be observed, could detect considerable differences between the samples. In general, samples giving good responses in the rat growth test gave high response with *Str. faecalis* and vice versa. Furthermore, the results (Y) correlate well with the N.P.U. estimation (X_1) and with the available lysine results (X_2):

$$Y = 1{\cdot}25X_1 + 50{\cdot}5 \quad (P = 0{\cdot}001)$$

and

$$Y = 12{\cdot}1X_2 + 28 \quad (P = 0{\cdot}001)$$

Biological test results

Out of a complete N.P.U. range of 17–62, only two results were below 30. Eleven meals produced weight gains in the test rats and three others led to considerable weight losses. Digestibility coefficients were found to lie between 0·73 and 0·92 for all meals tested except WM7, which gave 0·54 and also had the lowest available lysine value of 3·3 g./16 g. N, as well as the lowest Orange-G-binding and *Str. faecalis* figures.

For the general range of whale meals, Biological Values (Y) were closely related to the available lysine results (X):

$$Y = 7{\cdot}52X + 4{\cdot}0 \quad (P = 0{\cdot}001)$$

General comments

These whale meal samples showed a considerable diversity of appearance, ranging in colour from light to chocolate brown. The colour did not, however, appear to be related to any of the criteria of protein quality. In contrast to the meat meal series, no suspicion attaches to a sample of high protein content. In fact the only anomalies found were meal 155 (25% protein) and WM7. The latter showed very poor biological digestibility and its low lysine-availability suggests severe heat damage. The larger Biological Value range found here offers more hope of

grading the meals than in the case of the meat meals. Meals such as WM13, WM9 and WM4 are clearly valuable as supplements to cereals because of their high content of lysine and also as sole sources of dietary N, because of their good Biological Value. Many of the remainder have moderate Biological Value fed alone but would be useful supplements because they contain more available lysine than the requirement of the chick (4·5 g./16 g. N) and the young pig (5·5 g./16 g. N).[42]

The contents of riboflavin, nicotinic acid and pantothenic acid are in general similar to those found by Pritchard & Cawthorne.[49] De Brochard *et al.*[50] quote a rather higher figure for riboflavin (8·4 μg./g.) and a lower one for pantothenic acid (2·6 μg./g.).

Protein quality of fish meals

Materials

Thirteen fish meals were submitted to various chemical and biological tests, with the results shown in Table V.

Table V

Fish meals: results of protein quality tests

Number	Protein: Crude % (N × 6·25)	Protein: True × 100 / Crude	Moisture, %	Orange-G-binding, acid equivalents dye per 10^4 g. protein	'Available' lysine, g./16 g. N: Method 1	'Available' lysine, g./16 g. N: Method 2	Methionine, g./16 g. N: C	Net Protein Utilisation	Digestibility	Biological Value	Net Protein Ratio
FM6	73·0	93	10·1	9·9		6·4	2·0	59(2)	0·90(2)	66	3·6
73	72·3	88				6·9					
FM2	71·9	90	12·0	8·6			1·7	60(3)	0·79(3)	76	3·4
FM8	70·6	91	7·7	9·7			2·1	66(2)	0·89(2)	74	4·1
77	69·4	87				4·8					
72	68·9	94		7·3	3·9			66(1)	0·82(1)	80	
76	66·2	88		8·2		6·0					
FM7	65·4	91	8·7	8·4			2·0	57(3)	0·80(3)	71	3·5
75	61·6	93						45(1)	0·92(1)	49	1·1
71	60·0	70		10·0	6·5	6·2	2·5	56(2)	0·91(2)	62	3·4
74	58·7	91				5·8					
FM4	40·8	86	11·5	6·6		3·4	1·3	24(3)	0·74(3)	32	1·5
FM17	39·7	91	8·2	6·1		4·3	1·5	22(3)	0·80(3)	27	1·4

C = Chemical method Numbers of replicates shown in brackets

Chemical test results

Eleven of the meals come into the normal 60–73% crude protein range, with high proportions of true protein, but the other two were exceptional in having low protein content. One of the latter, on inspection, was clearly a shellfish meal.

The dye-binding data expressed as mg. dye per g. of sample (X) have been shown elsewhere[30] to be linearly related to crude protein % (Y):

$$Y = 0{\cdot}325X + 24 \qquad (P = 0{\cdot}001)$$

When expressed as acid equivalents dye per 10^4 g. protein, the data seem to lie above similar data for the meat meals and below those for whale meals. The dye adsorption data, so expressed, fall sharply into two groups, the normal and the low, corresponding exactly to the normal protein content samples and to those of low protein content and low N.P.U. Available lysine contents are high except in the low, protein meals and can be compared with a figure of 8·8 g./16 g. N given by Block & Weiss[42] for total lysine. The same authors give figures of 2·8–3·0 g./16 g. N for the methionine content, which is higher than that found here.

Biological test results

The two low-protein meals showed up poorly compared with the others, which gave Biological Values between 49 and 80. Three meals from the normal protein range had digestibility coefficients around 0·8, while four other results were approximately 0·9. Relationships between Orange-G binding and nutritive value and between available lysine and gross protein value have been found by Thurston[51] and by Carpenter *et al.*[45] The present results for fish meals were too few to attempt to deduce similar relationships between biological and chemical data.

General comments

Samples within the accepted protein % range for this type of product were uniformly good

as judged by the chemical and biological tests applied. The evidence obtained here suggests that fish meals of low protein content are of poor quality.

Protein quality of miscellaneous samples

Materials

This section deals with the testing of a variety of different protein concentrates. Both the casein and the laboratory-prepared dried egg have been used extensively as reference standards for evaluation of protein quality in this and in other laboratories. Soya-bean and groundnut meals were examined since they are widely used in animal feeding. It was also thought useful to study samples of dried milk and yeast which are used to supply both protein and vitamins in animal feeds. Blood meal was tested chemically, but it was not tested biologically because of its known deficiency of isoleucine. Two feather meals were included in this survey because of the recent interest in their use as protein supplements.[52] Two dried 'Whale meal solubles' and one of fish solubles were also tested (results are shown in Table VI).

Table VI

Miscellaneous samples: results of protein quality tests

Material	Protein: Crude % (N × 6·25)	Protein: True × 100 / Crude	Orange-G-binding acid equivalents dye per 10^4 g. protein	Lysine, g./16 g. N: 'Available' Method 1	Lysine, g./16 g. N: 'Available' Method 2	Lysine, g./16 g. N: Total	Methionine, g./16 g. N: C	Methionine, g./16 g. N: M	Net Protein Utilisation	Digestibility	Biological Value	Net Protein Ratio
Whole dried defatted egg (several batches)	71·2–74·6	98	9·0	5·9 5·7	6·7	7·0	2·6	3·0	80(±1)	0·95(12)	84	4·6
Genatosan casein	94·7*	98	8·0	7·4	6·8		2·3		53(4)	0·97(4)	54	2·9
Roller dried skim milk	34·5*	91	7·2	6·4			2·0		60(2)	0·84(2)	71	3·6
Feather Meal No. 6	82	99	3·9	1·9	1·2		0·4		28(2)	0·81(2)	35	1·4
Feather Meal No. 7	84·2	95	4·5	1·8	1·2	1·7	0·4	0·4	30(2)	0·83(2)	35	0·9
Dried brewers' yeast	45·5	83	6·6	5·1					32(1)	0·86(1)	37	1·8
Torula yeast	40	86	7·1	5·4					40(1)	0·78(1)	53	2·0
Blood meal	87·3	95	10·6	6·7	7·8	8·6	1·1					
Groundnut meals (6 samples)	50·5(±0·8)†	94	9·17(±0·27)†		2·35(±0·14)†	2·9(2)	0·6(1)	0·7(1)	40(2)	0·8(2)	52	2·6
Soya-bean meals (8 samples)	47·0(±0·7)†	95(7)	8·45(±0·11)†		4·4(±0·2)†	6·2(1)	1·1		52(2)	0·76(2)	70	2·6
Whalemeat solubles WS1	85·5	29	2·2		3·4				9(1)			1·0
Whalemeat solubles WS2	78·1	44	4·3		4·3				14(1)			1·2
Fish solubles	71·6	32		5·5	3·6		1·1		16(1)	0·92(1)	17	0·6

Numbers of replicates shown in brackets

* N × 6·38 † Standard error M = Microbiological method C = Chemical method

Results and general comments

Among the chemical results obtained, it is interesting to note the low dye-binding, N.P.U., lysine and methionine values of the feather meals. The similarity of some of the meat meals to these feather meals has already been pointed out in a preceding section.

Comparison of available lysine data with corresponding total lysine figures shows the following percentage availabilities: dried egg 96%; feather meal 71%; blood meal 88%; groundnut meal 72%; soya-bean meal 66%. These figures are comparable with the biological results of Schweigert & Guthneck.[41]

Results for groundnut meals and soya-bean meals are grouped together because they each covered such a narrow range. The low lysine and methionine values for groundnut meals are well shown up.

Dried egg N.P.U. results showed very good agreement over 12 successive determinations, having a mean of 80·3 with standard error ±1·0. This material must obviously come near to satisfying the amino-acid requirements of the young rat. Its total lysine value of 7·0 g./16 g. N agrees well with the figure 6·9 given by Block & Weiss[42] and the available lysine figure of 6·7 g./16 g. N exceeds the rat's requirement for 5 g./16 g. N quoted by the same authors. The methionine content also meets the rat's requirement.

An investigation of possible relationships between the biological and chemical data shows

that there is no significant regression of Biological Value on either methionine or available lysine. However, there is a correlation between N.P.U. (Y) and acid equivalents Orange G bound per 10^4 g. of protein (X):

$$Y = 7{\cdot}13X - 6{\cdot}2 \qquad (P = 0{\cdot}01)$$

These results have been presented elsewhere[30] and it can be shown that after omitting the feather meal and whale solubles results, crude protein % (Y) is related to Orange G mg./g. of sample (X) thus:

$$Y = 0{\cdot}41X + 11{\cdot}6 \qquad (P = 0{\cdot}001)$$

A similar regression is found with X = acid equivalents dye/10^4 g. protein:

$$Y = 12{\cdot}4X - 45{\cdot}9 \qquad (P \simeq 0{\cdot}05)$$

Net Protein Ratio results are closely correlated with N.P.U. results and it may be appropriate to mention here that for these miscellaneous samples and for all the other data presented above, we find the following regression:

$$\text{N.P.U.} = 14{\cdot}6(\text{N.P.R.}) + 7{\cdot}33 \qquad (P = 0{\cdot}001)$$

The error involved in predicting N.P.U. from N.P.R. would be about $\pm 5{\cdot}5$ N.P.U. units. This regression is very similar to that found by Bender & Doell[34] when they presented N.P.R. as a short-cut method:

$$\text{N.P.U.} = 15{\cdot}5(\text{N.P.R.}) + 3{\cdot}3 \qquad (P = \ll 0{\cdot}01)$$

Discussion and conclusions

In attempting to assess the feeding quality of proteins, there are a number of factors that must be borne in mind before a method can be recommended as being of value to the practical compounder of rations. Firstly, one must consider for which particular animal species the feedingstuff is required. Ultimately, the feedstuff will be required for cattle, pigs or poultry; the laboratory (as distinct from the field) test must, of necessity, be done on small animals. The present investigation used the laboratory rat whose amino-acid requirements have been shown to be both qualitatively and quantitatively similar to those of the young pig.[42] The requirements of the chick differ quantitatively from these in regard to the amino-acids, arginine and leucine, and absolutely in that glycine is also essential. For lysine, however, the requirements of all three species are closely similar (pig 5·5, rat 5·0, chick 4·5 g./16 g. N).

Secondly, it must be remembered that the biological data (N.P.U., N.P.R., *in vivo* digestibility and the microbiological methods) are all concerned with single protein sources, whereas in the practical feeding of livestock, these materials are normally fed in mixtures as compound feeds.

Thirdly, differences both between and within types of protein must be considered. A given method, applied to samples of a given material, may reveal either a broad spectrum of values, indicating a diversity of quality within the group or a narrow range of values which merely help to characterise the material in comparison with others. N.P.U. measurements on the whale meals, for example, show quite a wide range of values as also do the available lysine figures. Either of these criteria, therefore, would appear to have value as a grading method within the group, whereas methionine determinations show but little sample-to-sample variation on this material.

One purpose of this investigation was to try and find relatively simple laboratory tests that would correlate with biological (animal) tests with a view to their replacement. Whilst it is clear that no single test can be recommended as universally applicable to the wide variety of materials studied, the various correlations to which attention has been drawn above are, it is considered, a start in this direction. In particular, the correlation between Orange-G adsorption and N.P.U. is of interest, as it applied not only to whale meals and meat meals among themselves but also to the miscellaneous substances in Table VI. As far as the whale meals are concerned, N.P.U. was correlated also with growth of *Str. faecalis* and (via Biological Value) with available lysine. The available lysine content is of particular significance inasmuch as it is this amino-acid that is most commonly deficient in rations based on maize, wheat, etc.

It may well be, in fact, that further study will lead to the selection of particular tests as the methods of choice for particular types of material. Much more data on many more samples are required before these questions can be resolved and work on these lines is continuing.

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SELECTIVE MEDIA FOR YEASTS AND BACTERIA IN APPLE JUICE AND CIDER*

By F. W. BEECH and J. G. CARR

From a large number of antibiotics and other inhibitory compounds surveyed in detail, several have been chosen for the selective isolation of yeasts and bacteria from the mixed microfloras of apple juices and ciders. Bacteria were isolated on a basal medium of apple juice plus yeast extract, in the presence of actidione and oxine, while a mixture of actinomycin and Aureomycin allowed the sole growth of yeasts. Diphenyl was also added to the medium for the suppression of moulds in samples heavily infected with these organisms.

Introduction

The investigation of the microfloras of apple juices and ciders is complicated by the wide variety of organisms naturally present. These vary from strongly aerobic moulds, acetic acid bacteria and film yeasts to fermenting yeasts and anaerobic bacteria such as the lactic acid bacteria and a *Zymomonas* sp. Further, during the different stages of cider-making the number of each type of organism is constantly changing. Thus, the fruit as it hangs on the tree has aerobic or non-sporing yeasts on the surface and in the core, while badly stored fruit has a very heavy population of moulds, bacteria and fermenting yeasts.[1a–e] Infection with fermenting yeasts also occurs from poorly cleaned plant during the processing of the fruit to juice. The types of organisms present during fermentation vary with the composition of the original juice; a dry cider made from a juice of pH 4·0–4·5 contains many more bacteria than yeasts.

It is rarely possible to count all groups of organisms on one plate, since their relative numbers vary widely and their growth rates and requirements are different. Therefore, it is necessary to use selective media that allow the growth of only one group of organism per plate. A preliminary survey[2] in which 46 antibiotics and other inhibitory chemicals were tested against 23 species of yeasts and 20 species of bacteria, showed that only a few of these compounds were satisfactory for this purpose. A description is given of media that have been found suitable for the routine isolation and counting of yeasts and bacteria.

Choice of media

Freshly pressed apple juice contains bacteria other than those already described, but these do not develop during fermentation.[3] However, a wrongly chosen medium, particularly a synthetic one whose selective action is based on a particular combination of nutrients, might allow the growth of these extraneous organisms and give a false impression of their importance in the juice microflora. Hence, apple juice, which allows the growth only of acid-tolerant bacteria, has been chosen as the basic raw material for an isolation medium. To obtain a satisfactory agar gel, while retaining selectivity due to acidity, it is necessary to adjust the natural pH of apple juices (3·0–4·6) to 4·8.[1a, e] The growth of all organisms, and of lactic acid bacteria in particular, is accelerated by the addition of 1% Difco yeast extract before pH adjustment.

Selective isolation and counting of bacteria

The survey[2] had shown that the following compounds were suitable for inhibiting yeasts: A228,† actidione, dehydroacetic acid, ferric dimethyldithiocarbamate, gliotoxin, methyl and ethyl *p*-hydroxybenzoates, *p*-nitrophenol, oxine and thiolutin. No single substance was completely satisfactory for this purpose, including actidione, which is widely used for the detection of bacteria in yeast samples from the brewing, baking and distilling industries.[4] There are several yeast species occurring in apple juices and ciders, e.g., *Kloeckera apiculata* and *Rhodotorula glutinis*, that resist more than 500 p.p.m. of actidione.[2] Mixtures of two compounds were more effective—for instance, actidione and gliotoxin have a complementary inhibitory action. Oxine was used in place of gliotoxin since it was more readily available and, therefore,

* Read at joint meeting of Microbiology Group and Society for Applied Bacteriology, 14 January 1959.
† A228 is an antibiotic supplied by Glaxo Laboratories, Ltd., Stoke Poges, Bucks.

more convenient for routine isolation and counting. Tests showed that the inclusion of 15 p.p.m. of actidione and 25 p.p.m. of oxine inhibited all cider yeasts. The plates were incubated for 7 days anaerobically then 3 days aerobically at 25°, to allow the growth of lactic and acetic acid bacteria.[5] During routine sampling it was possible to detect one bacterium per 60,000 yeast cells; since the medium causes the total suppression of yeasts it is possible to count relative concentrations of bacteria even lower than this.

It is comparatively easy to count the total bacterial population, but the ease with which the separate components of this flora may be counted depends on their sensitivities to other inhibitory compounds. All the cider lactic acid bacteria are very sensitive to actinomycin, hence the acetic acid bacteria can be isolated specifically by adding 2 p.p.m. of this antibiotic to the medium containing the yeast inhibitors.[3, 5]

The lactic acid bacteria are more difficult to isolate since they are usually more sensitive than Gram-negative bacteria to inhibitory compounds; they can be isolated on a medium containing 2000 p.p.m. of sorbic acid and 25 p.p.m. of actidione incubated anaerobically at 25°—this medium prevents yeast growth and largely restricts acetic acid bacteria. It is better to use potassium sorbate rather than the acid or its sodium salt, both of which have low solubilities. For routine counting purposes the number of lactic acid bacteria is obtained by difference between the total bacterial count and that of the acetic acid bacteria.

The Gram-negative *Zymomonas anaerobia*[6a, b] occurs very infrequently in apple juices and low-acid sweet ciders: when it does appear, it is present in such large numbers as to be easily identified. Its reaction to antibiotics is very similar to that of the acetic acid bacteria and it is readily identified by the rapid fermentation it induces when colonies are picked off and inoculated into apple juice. Millis[6b] inhibited yeasts and lactic acid bacteria by incorporating 250 p.p.m. of Malachite Green in the basal medium and incubating the plates in a partial atmosphere of hydrogen. Under these conditions the acetic acid bacteria grew very poorly.

Selective isolation and counting of yeasts

Cider bacteria are inhibited by Aureomycin, chloramphenicol, pentachlorophenol, or mixtures of actinomycin, penicillin or Terramycin with Aureomycin.[2] A combination of 2 p.p.m. of actinomycin and 25 p.p.m. of Aureomycin with aerobic incubation was found most effective for isolating and counting yeasts.[5] This medium gives a total yeast count; a lysine medium can be used for counting yeasts other than *Saccharomyces* spp.[7, 8] Although three yeasts of this genus, namely, *S. fragilis*, *S. marxianus* and *S. fermentati*, have been shown to grow on this medium,[9] the first two of these yeasts differ sufficiently from other members of the genus *Saccharomyces* to be put in a new genus, *Dekkeromyces*.[10a]

When non-*Saccharomyces* spp. form the major part of the yeast flora, the yeast count on the lysine medium is higher than on the apple juice–yeast extract medium which contains only traces of this amino-acid.[11] Hence, some of the yeasts included in the lysine count would not normally develop to any extent during cider-making.

Other media to separate the several components of the yeast flora of apple juices are needed. A medium containing nitrate as sole source of nitrogen might appear suitable for isolating yeasts capable of utilising nitrate: unfortunately, the nitrite produced by these yeasts can diffuse through the agar and stimulate the growth of some *Debaryomyces* spp. that utilise this source of nitrogen.[10b] There is also an urgent need for a medium that would allow the specific isolation of *Saccharomyces* spp.; this would be particularly valuable in the examination of the yeast flora of apples, in which such yeasts form only a minute proportion of the total flora.

Suppression of moulds

It is difficult to count yeasts and bacteria when samples are contaminated with moulds. This does not occur very often on media containing actidione[12] and oxine[13] since both of these compounds suppress many moulds. Diphenyl[14] added at a concentration of 100 p.p.m. prevents the germination of many mould spores. This and other inhibitors are often used in ethanolic solution with the result that the total concentration of ethanol depresses the growth of the desired organisms. This can be overcome by placing the ethanolic solution of diphenyl in the lid of the

Petri dish and allowing the ethanol to evaporate before inoculation. It is desirable to keep such dishes in a closed container to prevent the escape of the volatile diphenyl.[15] Sodium propionate[16] may be incorporated in the medium for the same purpose but is less effective than diphenyl since much greater concentrations are required.

Conclusions

Using the media described it has been possible to count and isolate different components of the microflora at all stages of cider-making. There is still a need, however, for other media, that allow the specific isolation of the genus *Saccharomyces*, and of the lactic acid bacteria. While selective media are indispensable for examining mixed microfloras, there is always the possibility that they may suppress organisms of importance in cider-making.

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RÔLE OF POLYSACCHARIDES IN SOIL AGGREGATION

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Natural aggregates of a Swiss Braunerde and synthetic soil aggregates produced with several polysaccharides were subjected to various chemical treatments designed to destroy polysaccharides or other soil constituents. The water stability of the aggregates was determined before and after the treatment to evaluate the rôle of the constituent destroyed.

The properties of the agent responsible for the aggregation of soil are discussed. The contrasting behaviour of natural and synthetic aggregates provides evidence against polysaccharides being the agents responsible for the natural aggregation and it appears that more than one agent may be involved in this.

Introduction

Many theories have been proposed pertaining to the agents responsible for soil aggregation.[1] Besides clays, amorphous silica, iron and aluminium oxides and lime, various organic constituents such as humic acids, waxes and resins, proteins, microbiol filaments, roots, etc., have been held partly or solely responsible for aggregation of soils. The theory which has gained the most acceptance in recent years, however, proposes that long-chain polysaccharides, predominantly of microbial origin, are responsible for soil aggregation.

The ' polysaccharide theory ' is based on the following evidence : (1) many plant and microbial polysaccharides when added to clay or soil produce water-stable aggregates ;[2-7] (2) soils are inhabited by organisms which are capable of producing aggregating polysaccharides; [2] (3) polysaccharides are present in the soil, and when they are extracted, and added to clay or soil, they cause aggregation ;[8-10] (4) there is a statistical correlation between the estimated polysaccharide content of soils and their degree of aggregation.[8, 11]

This evidence, however, is indirect and does not exclude other active agents such as humic acids or proteins.[1] As has been pointed out,[4] most of the experimenters established only the capacity of polysaccharides to promote aggregation, and it is not permissible to conclude from this fact alone that polysaccharides are responsible for the natural aggregation. Supporting evidence for the polysaccharide theory was that polyuronides were supposed to constitute a substantial part of total soil organic matter,[12] and it seemed logical, therefore, that they should be the predominant aggregating agents. It has been shown recently, however, that earlier estimates for the uronic acid content of soil were erroneous and that polyuronides are present in the soils only in small amounts.[13]

The interpretation of the statistical correlation between the polysaccharide content and soil aggregation[8, 11] can also be questioned on various grounds. First, unpublished work of our group shows that repeated extraction of the soil is necessary for complete extraction of the polysaccharides. The procedure employed using only one alkali extraction[11] might give low and non-comparable results for different soils. Since the easily extractable fraction is probably the one least adsorbed on clays, it would also be the one least important for aggregation. Second, it has been reported in the literature,[9] and further elucidated by our unpublished work, that the so-called ' polysaccharide ' contains a very large percentage of inorganic and organic impurities such as mineral salts, amino-acid-containing substances and other non-carbohydrates of hitherto unidentified nature. The interpretation of a statistical correlation between soil aggregation and an incompletely extracted and insufficiently characterised fraction of soil polysaccharide is doubtful.

On the other hand, if a correlation really does exist between the actual polysaccharide content of soils and soil aggregation, it still cannot be unequivocally concluded that polysaccharide is the cause and aggregation the effect. It has been shown that when a soil is aggregated by synthetic soil-conditioners its biological activity is increased.[14] Moreover, treatment with soil conditioners is reported to increase the population of polysaccharide-producing bacteria of soils.[15] Thus, it can be argued with equal validity that a non-polysaccharide aggregating agent of the soil increases its polysaccharide content by providing a favourable environment for the polysaccharide-producing bacteria.

For the solution of this dilemma additional evidence seems necessary and it may be sought in a direct chemical approach. The investigations reported in this paper are based on the premise that any chemical treatment which destroys polysaccharides should destroy the soil aggregates if polysaccharides are responsible for the aggregation. The efficacy of the treatments used was tested on synthetic aggregates prepared by the addition of polysaccharide to the soil. A preliminary note on the work has been published.[16]

Experimental

Materials

Soil.—A top soil (0–4 in.) of a Braunerde from Dietikon, Zürich, Switzerland, occurring under natural vegetation (*Querco-carpinetum aretosum*), was used. It was well aggregated (moderately strong fine subangular blocky structure), had an organic C content of 3·2%, a clay content of 30%, pH 6·8, and contained traces of carbonate. The soil was dried at 40° for 24 h. For the study of natural aggregation only aggregates between 2 and 4 mm. were used. The soil sample used for the preparation of synthetic aggregates and for the other experiments was ground to pass a 0·2-mm. sieve.

Polysaccharides.—The soil polysaccharide was extracted from the Braunerde by three extractions with 0·1M-sodium pyrophosphate (pH 7), at 35° for 36 h. each. The humic acid was precipitated at pH 2·5 below 5° and the solution containing the fulvic acid decanted. The

polysaccharide was precipitated from the solution with a mixture of acetic acid and ethanol, reprecipitated and dialysed.[9] Chitosan was prepared as described in the literature.[17] The other polysaccharides used (see Table I) were commercial products.

Methods

Preparation of synthetic aggregates.—Ten g. of ground Braunerde soil (<0·2 mm.) were mixed with 100 mg. of the polysaccharide in a Petri dish. Distilled water (5–10 ml.) was added portion-wise to the soil, the mixture being stirred with a spatula and then formed into approximately 4-mm. aggregates. The dish was covered, left at room temperature for 8 h., then opened and heated in the oven at 50° for 16 h. The dry aggregates were used in the later experiments. Tests with guar gum and Krilium aggregates showed that the error of the method of preparation together with the error of measurement of their stability was between 5 and 10%. It was found that 0·3% of guar gum was the minimum necessary to produce completely stable aggregates. The amount of polysaccharide generally used was, however, 1% unless otherwise stated.

Decarbonatation.—The carbonates were removed by treatment of the aggregates with 0·1N-HCl at 0° for 7 days, the acid being changed every day.

Aggregate stability test.—The stability of the aggregates was determined by the wet sieving method.[18] The weight of the aggregates retained on the top sieve (2 mm.) after 1 h. as a percentage of the initial weight was taken as a measure of stability. The aggregates were considered completely stable if the ratio of final weight to initial weight exceeded 90%.

Measurement of periodate consumption.—One hundred ml. of 0·01M-$NaIO_4$ was mixed with 10 g. of sieved soil or aggregates in an Erlenmeyer flask which was then stoppered and placed in the dark for the required length of time. The suspension was then filtered through a sintered glass crucible (G-4) and the excess periodate in the filtrate determined by titration.[19] A different sample was taken for each time interval, and the periodate consumed was calculated from the difference between periodate added and recovered. The results agreed within ±2%.

Periodate treatment.—Ten g. of aggregates were placed in a Petri dish. $NaIO_4$ solution (100 ml.) was pipetted in without disturbing the aggregates, the dish covered and placed in the dark for the required length of time. At the end of the reaction period the solution was sucked out with a pipette and 100 ml. of borate buffer (pH 10) were added slowly. After 2 h. the buffer was sucked out, and the Petri dish placed in the oven at 50° for 16 h. The aggregates were never handled when wet. Distilled water was used instead of periodate solution for the blanks.

Acid treatment.—The aggregates were evacuated, wetted with 1N-HCl under vacuum, and heated under reflux in a boiling water-bath for 8 h.

Chlorine dioxide treatment.—A sintered glass crucible was filled with Celite to about 4 cm. height. An aggregate was imbedded in the powder so that half of it was below the surface and half of it above it. The crucible was then placed in a Petri dish containing enough ClO_2 solution (5·12 g./l.) so that the level of the liquid was above the base of the crucible but below the surface of Celite. The Petri dish was completely covered with aluminium foil (except for the opening for the crucible) to prevent direct evaporation. The assembly was then placed in a desiccator containing $CaCl_2$ to hasten evaporation from the aggregate. After the end of treatment the aggregate was dried at 50° for 16 h., and its water stability determined.

Extraction of polysaccharide after periodate treatment.—Sieved soil (120 g.) was oxidised with 0·05M-$NaIO_4$ for 280 h. and then extracted repeatedly with water at 85°, treated with HF at pH 2·5 (to destroy the clay and facilitate polysaccharide extraction), and successively extracted with hot water, 1N-Na_2CO_3 at room temperature, 0·1N-, 0·5N-NaOH at room temperature and finally with 0·1M-sodium pyrophosphate at 85°. This procedure combined the methods used so far for extraction and should have given the maximum yield of polysaccharide with a minimum of degradation. The extracts were combined, concentrated under vacuum below 30° and dialysed. The polysaccharide was precipitated from the non-dialysable fraction with ethanol.[20] The precipitate was filtered, dissolved in water and freeze dried.

Hydrolysis and paper chromatography.—Ten g. of soil were boiled under reflux with 20 ml. of 1N-H_2SO_4 for 4 h. in a boiling water-bath. The suspension was filtered hot, neutralised to pH 6 with NaOH and the resulting precipitate filtered off. The filtrate was then passed through

a washed charcoal column (' Ultra-sorb.' S.C. 120/240, British Carbo Norit Union Ltd.) for desalting.[21] After the salts had been washed out with water (until $BaCl_2$ gave negative test for sulphate), the sugars were eluted with 80 ml. of 20% ethanol, the eluate concentrated at 30° under vacuum and freeze dried. The sugars were identified by paper chromatography using Whatman paper No. 1 with ethyl acetate–acetic acid–water (3 : 1 : 3) as solvent and aniline phthalate as spray.[22]

Results and discussion

Periodate oxidation

As dilute solutions of periodic acid and of its salts oxidise polysaccharides at room temperature[23] and the oxidised polysaccharides are unstable under alkaline conditions,[24] treatment with $NaIO_4$ followed by treatment with a buffer at pH 10 should destroy soil aggregates, if polysaccharides are responsible for the aggregation.

The conditions necessary for periodate treatment of soils were first investigated. The rate of consumption of periodate by ground soil (<0·2 mm.) and by natural Braunerde aggregates (2–4 mm.) is shown in Fig. 1. The consumption by ground soil is very rapid at first and after the first hour it continues at a slower but a uniform rate. In the case of pure polysaccharides the initial rapid consumption is supposed to be due to the characteristic oxidation (1 mol./1 mol.) and the subsequent slow consumption is attributed to the further oxidation of the reaction products (' over-oxidation ').[23] The method is, however, not accurate enough as a quantitative method for soil carbohydrates.

In contrast to the ground soil, the aggregates consume periodate at a considerably slower rate, particularly at first. This can be attributed to the slow diffusion of the periodate into the aggregates. At the end of 72 h. the total periodate consumption of the two samples was almost identical. As about 6 h. are required for the periodate consumption of the aggregates to equal the characteristic oxidation value obtained with the ground soil, the aggregates were treated with periodate for this time.

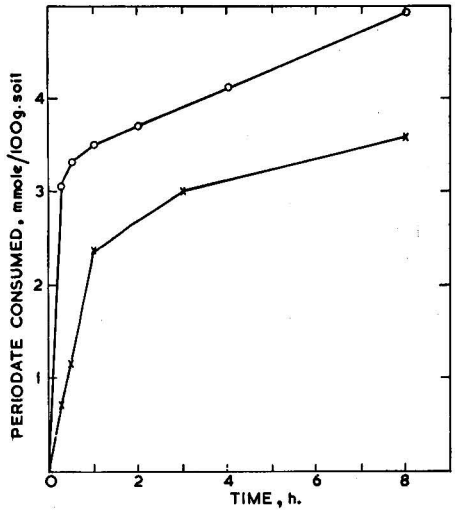

FIG. 1.—*The periodate consumption of ground soil and natural aggregates*

○ Ground soil × Natural aggregates

Table I shows the effect of periodate treatment on synthetic and natural aggregates. The synthetic aggregates were prepared by adding 1% of the various polysaccharides to the ground soil—an amount far greater than the probable polysaccharide content of soils. The stability of the aggregates after the treatment was determined by the wet sieving method.

Wetting and drying a powdered soil which was previously well aggregated does not produce stable aggregates. Whatever is the aggregating substance, it can only be effective once and new agent is necessary to form stable aggregates again.

The alkaline buffer alone has little or no effect on aggregate stability. On the other hand, buffer treatment following periodate oxidation reduces to a powder all the synthetic aggregates except those prepared with guar and locust bean gum.

As guar gum is not easily oxidised with dilute periodate solutions,[25] an experiment was conducted with 0·05M-periodate. In this experiment the locust bean and guar gum aggregates were destroyed in 24 and 120 h. respectively, but the natural aggregates were not affected by even a 280-h. treatment. Various other Swiss soils including two Rendzinas also proved stable against a similar periodate treatment.

The results indicate that periodate-labile polysaccharides cannot be responsible for the

Table I

Effect of a 6-h. treatment with 0·01M-$NaIO_4$ on natural and synthetic Braunerde aggregates
(g. of stable aggregates from 7 g. of initial material)

	Untreated	Buffer* treated	Periodate and buffer* treated
Synthetic aggregates prepared with			
Water alone	0·2	0·0	0·0
Soil polysaccharide	5·9	5·7	0·0
Tamarind gum	6·9	6·7	0·0
Carboxymethylcellulose	7·0	6·8	0·0
Guar gum	6·8	6·8	6·8
Locust bean gum	7·0	6·8	6·1
Pectin	7·0	6·9	0·0
Chitosan	7·0	6·7	0·0
Natural Braunerde aggregates	6·8	6·8	6·8

* borate buffer pH 10

aggregation. This eliminates dextrans, levans, polyuronides, polyhexosamines and a great number of other polysaccharides as agents responsible for aggregation at least in this particular Braunerde soil. If a polysaccharide is still the aggregating agent it must be resistant against periodate oxidation. Such polysaccharides, except for the branched ones, are rare in nature.[23]

To gain an idea as to the amount and composition of the possible periodate-resistant polysaccharides, the Braunerde was oxidised with 0·05M-periodate for 280 h., and its residual polysaccharide exhaustively extracted. The residual soil was then hydrolysed and the sugar in the hydrolysate determined. Only traces of polysaccharides could be extracted from the oxidised soil (<1 mg./100 g.) and the hydrolysate of the residual soil contained less than 5 mg. of sugars per 100 g. of soil. These were identified as glucose, galactose, xylose, arabinose and rhamnose by paper chromatography. This experiment shows the effectiveness of periodate oxidation; it is unlikely that such small amounts of residual sugars are responsible for the aggregation.

Acid hydrolysis

Polysaccharides are depolymerised when heated at 100° with strong acids. In general, amino-polysaccharides require high acid concentrations (6N-HCl) and long periods of time (6–8 h.); polyuronides are intermediate; and most hexosans, pentosans and methyl pentosans are hydrolysed by less concentrated acids (1–2N) in less than 4 h. These conditions are, however, for complete depolymerisation and milder treatment would suffice for a partial degradation. It thus seemed possible to degrade soil polysaccharides by heating at 100° with 1N-HCl for a short time and so to destroy the stability of the aggregates. The destruction of aggregates by such a treatment would, of course, not be conclusive evidence that polysaccharides are the aggregating agent, since other soil organic and inorganic constituents may also be attacked by hot HCl. On the other hand, if the aggregates are not destroyed, polysaccharides may be eliminated as the aggregating agents.

Preliminary experiments showed that the aggregates were labile under the pressure of CO_2 rapidly evolved from the carbonates by the acid, and also under the expansion of entrapped air at the high temperature used. The natural aggregates were, therefore, decarbonatated and the synthetic aggregates were prepared from acid-washed soil. The aggregates were evacuated and wetted with 1N-HCl under vacuum to displace the air.

Synthetic aggregates were prepared from acid-washed Braunerde with 1% of guar gum, pectin and chitosan and heated under reflux in a boiling water-bath with 1N-HCl. In less than 30 min. the aggregates were reduced to a fine powder. This shows the lability of major classes of polysaccharides in the hydrolysis treatment. When the natural Braunerde aggregates were subjected to an identical treatment after low-temperature decarbonatation, they remained stable even after 8 h.

The stability of the natural aggregates as against the lability of the synthetic aggregates provides further evidence of the unimportance of polysaccharides for the aggregation of this soil.

Chlorine dioxide oxidation

Chlorine dioxide is used extensively as a mild agent which oxidises lignin and humic compounds with practically no degradative influence on polysaccharides such as cellulose. It has also been used to oxidise soil humic acids.[26] It therefore seems a suitable agent for testing the rôle of humic acids in soil aggregation.

The main difficulty experienced with ClO_2 treatment was that the evolution of gas during the treatment destroyed the aggregates rapidly. This difficulty was to a great extent overcome by wetting the aggregates from below by capillarity and leaving the top exposed for easy gas exchange and evaporation.

Treatment of the natural aggregates with ClO_2 destroyed them completely in less than 24 h., whereas synthetic aggregates (0·3% guar gum or 0·1% Krilium) remained stable for over a week. This again shows that the usual polysaccharides are not responsible for natural aggregation. However, it cannot be unequivocally concluded from this experiment that humic acids are the responsible agent. It is possible that there was still excessive gas evolution and that natural aggregates were more labile under such slow destructive influence than the synthetic aggregates. The greater stability of sodium-saturated synthetic aggregates compared with that of natural aggregates, as reported later, indicates that this could be the case.

Miscellaneous treatments

The stability of natural and synthetic Braunerde aggregates was tested with various other chemical treatments in order to characterise the aggregating agent. While no definite conclusions can be drawn from the individual experiments, some of the observations seem to be of sufficient interest to be recorded here.

Soaking the natural and synthetic aggregates (0·3% guar gum) in 0·1M-sodium pyrophosphate (pH 7), 0·1M-sodium salt of EDTA (pH 7) and 0·1N-NaOH for 7 days destroyed their stability. However, in addition to the extraction of organic matter, the saturation of the soil with sodium followed by the subsequent swelling in water may have been responsible at least in part for the instability.

To check this possibility the aggregates were soaked in 1N-NaCl for 7 days. This treatment reduced the stability of natural aggregates by 72%, whereas that of the synthetic aggregates (0·3% guar gum) was reduced by only 19%. Krilium-treated aggregates (0·1%) were not affected by the treatment. Since there is practically no extraction with NaCl, only sodium saturation is involved. It appears that compared with synthetic aggregates prepared with long-chain polymers, the natural aggregates are more labile under swelling conditions.

Alcoholic KOH (1N) or aqueous $Ba(OH)_2$ (0·3N) treatment was without effect. Even heating with $Ba(OH)_2$ at 85° for 8 h. (after prior evacuation) did not reduce the stability of the aggregates. This shows that alkalinity *per se* has no influence.

It has been reported[27] that hot water destroys aggregates, from which it was concluded that substances extracted by such a treatment, namely, polysaccharides or proteins, were responsible for soil aggregation. We also found that heating the aggregates in water at 85° destroyed the aggregates in a few minutes, but this was entirely a result of the expansion of the air in the aggregates, since if the aggregates (natural and synthetic) were evacuated prior to wetting they remained completely stable for 24 h.

Treatment with 1N-HCl at room temperature also destroys natural and synthetic aggregates, presumably due to evolution of CO_2 from carbonates. When the aggregates were decarbonatated at low temperature a subsequent treatment with 1N-HCl at room temperature had no effect.

Treatment of the decarbonatated natural aggregates with 1N-HF at room temperature destroyed the aggregates in 48 h. Since 1N-HCl is ineffective it can be concluded that the specific effect of HF is due to the partial destruction of silicates. This provides direct evidence that surfaces of silicates, such as clay minerals, play a rôle in the aggregation of this soil.

Contrary to what has been reported in the literature[28] regarding the stability of earthworm casts when boiled with acidified H_2O_2, the Braunerde aggregates were rapidly destroyed even at room temperature because of rapid gas evolution. Treatment with acidified $KMnO_4$ was inconclusive owing to the formation of a precipitate around the aggregates which effectively prevented the completion of the reaction.

Conclusion

The present results may be qualitatively summarised as in Table II.

Table II

Stability of natural and synthetic Braunerde aggregates against various treatments

Treatment	Aggregates	
	Natural	Synthetic (guar gum)
0·3N-$Ba(OH)_2$, 85°	stable	stable
1·0N-alcoholic KOH	,,	,,
H_2O, 85°	,,	,,
0·1N-NaOH	unstable	unstable
0·1M-sodium pyrophosphate, pH 7	,,	,,
0·1M-EDTA-disodium salt, pH 7	,,	,,
0·1N-HF	,,	,,
3·0% H_2O_2	,,	,,
0·05M-$NaIO_4$	stable	,,
1·0N-HCl, 100°	,,	,,
1·0N-NaCl	unstable	stable
ClO_2	,,	,,

The difference in the behaviour of the natural and synthetic aggregates during treatment with $NaIO_4$, HCl at 100°, NaCl and ClO_2 shows that normal polysaccharides cannot be solely responsible for the natural aggregation of soil. If a polysaccharide is responsible for the aggregation of this soil it would have to be non-extractable, periodate-resistant and non-hydrolysable —a rather unlikely combination of properties.

Proteins, which are partially depolymerised by heating with acid, may also be ruled out as agents primarily responsible for the aggregation.

The nature of the aggregating agent of this soil still remains unknown. The extreme stability of the aggregates under chemical treatments may indicate that more than one agent is involved. There is as yet no experimental evidence that the aggregation of a soil can be traced to a single agent. In fact, the many reported observations and correlations between aggregation and various soil constituents, such as clays, organic carbon, polysaccharides, iron oxides, etc., point to the contrary. The greater lability of natural aggregates to mechanical forces as compared with that of synthetic aggregates produced with long-chain polymers is an indication of the possible rôle of smaller and more isometric organic molecules, such as humic substances, in soil aggregation.

Acknowledgments

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CHEMICAL STUDIES ON THE HERRING (*CLUPEA HARENGUS*). III.*—The Lower Fatty Acids

By R. B. HUGHES

The application of gas chromatography and paper chromatography to a study of the lower volatile fatty acids of herring is reported. Fresh herring flesh contained acetic acid with smaller quantities of formic acid and propionic acid, and occasionally a trace of n-butyric acid. The quantities of all these acids increased in the spoiling fish, while iso-valeric acid became apparent at the later stages. The presence of antibiotics did not affect the rate of production of the acids in fish held in ice. Heat processing at 115·5° in sealed cans did not alter the content of individual acids in the fish.

Introduction

Several studies have been made of the steam-volatile acid fraction of fatty fish, but the information obtained has been concerned mainly with the fraction as a whole, rather than the individual acids, of whose nature and behaviour much less is known. Sigurdsson[1] showed that the volatile acid fraction increased rapidly in American Atlantic Coast herring held at various temperatures, and considered the method equal to the measurement of trimethylamine as an objective index of quality. He also showed that no significant change occurred in the volatile acid fraction during heat processing in cans. Similar observations had previously been made by Hillig & Clark[2] who used them to develop a method for assessing the quality of canned salmon and tuna. According to these authors, the freshest fish contained some formic acid and acetic acid, which increased during spoilage, while traces of higher acids, namely propionic acid and n- and iso-butyric acid, appeared at later stages. Hillig applied the method to a study of quality assessment of canned herring roe[3] and canned sardines,[4] and considered it to be satisfactory in these cases also. Clague[5] studied the behaviour of the volatile acid fraction in sardines before and during canning. Charnley,[6] working with herring, concluded that the volatile acid fraction and the acid value of the oil were equally suitable for assessing quality in herring both before and after canning. Japanese workers[7] used paper chromatography to study the development of acids in fish cake prepared from dog-fish flesh. They detected acetic acid in the fresh cake, and propionic, butyric and valeric acids in older material. Formic, acetic, propionic and butyric acids were detected in cured whale meat.[8]

* Part II: *J. Sci. Fd Agric.*, 1959, **10**, 558

The present paper forms part of a programme of work designed to study factors affecting the quality of canned British herring, and it was considered desirable to investigate the nature of the volatile acids present in herring caught in British fisheries and changes occurring in the individual acids during post-mortem spoilage and heat processing. The development of gas chromatography offers a new analytical approach in this field and the method of James & Martin[9] for separating volatile acids has been used for detecting and estimating the individual acids present. Paper chromatography provided confirmatory evidence of their identity.

Experimental

Preparation of sample for analysis by gas chromatography

The acids were extracted from the fish flesh in the following manner: minced flesh (20 g.) was macerated in a 'Nelco' homogeniser with four successive 50-ml. portions of 80% alcohol (ethanol 4 vol.: water 1 vol.). The bulked extracts were made up to a final volume of 250 ml. with 80% alcohol, filtered and stored at −30° until used.

Before analysis, the acids must be concentrated and transferred to a non-aqueous medium. The most convenient method for separating the acids from the other extractives is by steam distillation, and this technique has been used in all the methods developed for estimating the volatile acid fraction. The volatility of the fatty acids in steam increases with increasing molecular weight. For the lower members, especially formic acid and acetic acid, steam distillation has to be continued for a considerable time and a large volume of distillate collected before a quantitative recovery of these acids is achieved. The addition of substances such as magnesium sulphate to the distillation mixture raises its boiling point and increases the rate of distillation of the acids. Increasing the concentration of the acids in the initial solution by keeping its volume as low as possible also has a very marked effect on their rate of distillation. Because of this difficulty of obtaining quantitative distillation of the acids, the methods used in practice for estimating volatile fatty acids have aimed at estimating only a constant fraction of the total acids, by careful adjustment of the distillation conditions.[10, 11] Clague[5] compared two methods for estimating the volatile acid fraction in fish flesh and found that one gave results approximately 50% higher than the other.

In the present work, the following method was used to prepare the acid fraction: A suitable aliquot (usually 50 ml.) of the aqueous alcohol extract was passed through a short column (height 2 cm., internal diameter 1 cm.) of a strong base ion-exchange resin, Dowex-2 (100–200 mesh, hydroxide form), and the column washed with a small volume of distilled water. The acids, which were retained on the column, were quantitatively eluted with 15 ml. of N-H_2SO_4. This small volume was transferred to a Markham micro-Kjeldahl distillation apparatus.[12] Because of the small initial volume of the solution, all the acids were quantitatively recovered in the first 120 ml. of steam distillate. This was neutralised to thymolphthalein with dilute aqueous NaOH and concentrated by distillation under reduced pressure to a volume of approx. 5 ml. This volume was transferred to a 10-ml. freeze-drying ampoule and evaporated to dryness in a centrifugal freeze-dryer.

Control experiments using standard solutions of fatty acids showed that recoveries were nearly quantitative by the above procedure, the formic acid giving a recovery of at least 95%. If the neutralisation of the fraction after steam distillation is carried out carefully by titration with standard 0·01N-NaOH (indicator, phenolphthalein), an approximate measure is obtained of the total fatty acids present. This value however will be high if lactic and pyruvic acids are present, as these are appreciably volatile in steam. Herring flesh contains large quantities of actic acid (unpublished observation) and therefore a high figure would be obtained in this case.

Application of the sample to the column

The residue of salts after freeze-drying was dissolved in two drops of distilled water and the solution acidified with 1 drop of 60% phosphoric acid solution. [Previous treatment of the internal surface of the ampoule with a water-repelling compound (Repelcote, Hopkin & Williams Ltd.) caused the water to form one large globule which could be manœuvred around the inside surface, so facilitating complete solution of the sodium salts in a small volume.] The acids were quantitatively transferred into ethereal solution in the manner described by James &

Martin.[9] A short column (4 mm. internal diameter) was prepared containing Celite (4 cm.) overlying anhydrous sodium sulphate (1 cm.). The Celite was dried at 300° before use. The acidified solution of acids was carefully transferred to the top of this column and the ampoule rinsed with four successive 0·5-ml. portions of ether, each being then transferred to the column and forced through slowly with compressed air. The ether solution, which was collected from the column in a 2-ml. bulb with a long stem, was transferred to the gas chromatography column by evaporation and suction as described,[9] the bulb containing the ether solution being immersed for 5 min. in a water-bath at 60° followed by 15 min. in an oil bath at 100°.

This method for transferring the acids on to the chromatography column is time-consuming and involves the removal of the column from the heating jacket. The method described by McInnes[13] whereby the ethereal solution is introduced *in situ* into a small receptacle at the end of the column appeared to offer certain advantages. In practice, however, it was found impossible to obtain sharp separations by this method, while any advantage gained by not having to remove the column from the apparatus was lost through having to allow the heating jacket and column to cool before introduction of the sample. Attempts to inject the ethereal solution directly into the end of the column was only successful when small volumes (about 50 μl.) were involved. As suggested by McInnes[13] losses of formic acid may occur when mixtures containing acids as high in the homologous series as dodecanoic acid are drawn on to the column by the method of James & Martin. Because of the high temperature and long suction time which is required to ensure complete transference of the less volatile higher members, it is possible for the formic acid to pass completely through the column. Under the conditions of application used in the present work, no formic acid was lost, provided that too great a suction was not applied to the column.

Gas chromatography

The apparatus used was that described by James & Martin,[9] fitted with the automatic titration unit; 0·02N-NaOH was used in the titration burette and the indicator was an 0·01% solution of phenol red in water. The chart was calibrated by introducing known aliquots of standard acid into the titration cell and measuring the resulting step height.

The herring extracts contained considerable amounts of acetic acid, with lesser quantities of formic acid, propionic acid and sometimes n-butyric and isovaleric acid and the following conditions were found suitable for complete separation of the components of such a mixture:

The column was 4 ft. in length and of 4–5 mm. internal diameter. It was packed with a mixture containing the following proportions by weight; Celite 20, D.C. 550 silicone oil 8, stearic acid 1 and orthophosphoric acid 1. The Celite was size-graded, heated and acid-washed before use as described.[9] The column was operated at 100° and the flow rate of carrier gas (nitrogen) was approximately 37 ml./min. Under these conditions separations of mixtures of acids in the range formic to n-valeric were achieved in 80 min. as illustrated in Fig. 1 (*a*). No losses of the acids studied occurred during chromatography.

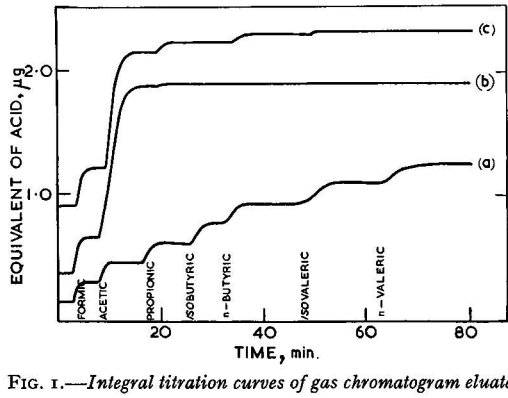

FIG. 1.—*Integral titration curves of gas chromatogram eluates*
(a) synthetic mixture, (b) from fresh herring, (c) from herring held for 90 h. (See text for conditions of running)

Paper chromatography of the volatile acids

Confirmatory evidence regarding the identity of the volatile acids was obtained by paper chromatography. Samples containing sufficient of the acids in a form suitable for application to the paper were obtained in the following manner. Minced herring flesh (600 g.) obtained from beheaded and eviscerated ('nobbed') fish was divided into three 200-g. portions and each portion was extracted with 2 × 400 ml. of 70% ethanol. The bulked extracts were neutralised with N-NaOH and concentrated by distillation at reduced pressure, whereby most of the alcohol was removed. This solution was acidified with N-H_2SO_4 and steam distilled, the distillation being continued until 400 ml. of distillate was collected. The distillate was neutralised with N-NaOH, concentrated to 10 ml. by distillation at reduced pressure and extracted with one 10-ml. portion of ether. This ether solution had the advantage of being capable of application to a paper at a rapid rate, without forming an overlarge spot.

Chromatography was one-dimensional, descending, on Whatman No. 1 paper sheets with a running distance of 18 in. from the starting line. Various solvents and sprays have been developed for use with volatile acids and these have been reviewed by Osteux *et al.*,[14] who developed solvents capable of separating all the lower acids, including formic acid and acetic acid, which were previously inseparable. By buffering their indicator spray they increased the stability and contrast of the acids spots.

In the present work, the solvent found to be most useful consisted of the following one-phase mixture, butanol : cyclohexane : propylene glycol : ammonia (conc. aq.) : morpholine : water, in the following volume ratio, 30 : 30 : 10 : 0·07 : 0·5 : 3·7.[14] In order to avoid loss of the more volatile acids on application to the paper, approximately 10 μl. of a 10% solution of morpholine in water was applied to each site before applying the ethereal or aqueous solution of the acids. Spots containing 200–300 μl. of the ethereal solutions from fish extracts were applied to the paper and run alongside 10 μl. spots of 0·1N solutions of known acids. The chromatograms were allowed to run overnight, when the solvent front travelled about 16 in. They were subsequently removed, partially dried in a current of cold air and sprayed on both sides with the buffered cresol red indicator described by Osteux *et al.*[14] The moist papers were hung in a drying cabinet at 60° for 20 min., when the acids were revealed in good contrast as yellow spots on a purple background. The spots were fully stable for at least 24 h., a considerable improvement on previous methods of spraying.

Although capable of separating formic and acetic acid, the above solvent will not distinguish between the isomers of butyric acid and those of valeric acid. These can be separated by extended running, using benzyl alcohol saturated with 1·5N-aq. NH_3 as solvent.[14] The bottom edges of the papers were serrated and the chromatograms run for 6 days. At the end of this period the papers were removed and sprayed directly with the buffered cresol red spray. Because of the non-volatile nature of the solvent, the papers could not be dried and remained translucent after spraying. The spots were marked immediately after spraying, as they spread rapidly on the wet paper.

Results

Post-mortem changes

Changes occurring during post-mortem spoilage were followed in whole herring held at 10–13° (ambient temperature) in an aluminium box with a loose lid. The fish were immature herring taken from the Moray Firth in October, 1957, with an average length of 21–22 cm. Samples of six fish were taken at regular intervals over a period of 4 days and stored at −30° pending analysis.

Fresh herring frozen alive in solid CO_2 contained about 10 mg. of acetic acid, 1 mg. of formic acid, 0·4 mg. of propionic acid and occasionally a trace (less than 0·03 mg.) of n-butyric acid per 100 g. of nobbed fish. Fig. 1 (*b*) and 1(*c*) illustrate the type of gas chromatogram obtained. The identity of these acids was substantiated by adding quantities of individual acids to a mixture prepared from fish flesh, and noting the expected increase in the corresponding zone on the gas chromatogram. Paper chromatography also provided further evidence in support of the identity of the acids, but n-butyric acid could not be detected on paper chromato-

grams of fresh fish although readily detected in stale fish. Its occasional appearance on gas chromatograms was quite definite, however.

Changes occurring in the content of the four major acids during the period studied are shown in Figs. 2 and 3, from which it will be observed that the behaviour of all the acids was similar. Little change occurred during the first 36 h., after which all acids began to rise rapidly. Other workers[1, 5] have noted the same behaviour when following the total volatile acid fraction in herring and sardines. During later stages of spoilage (in the sample taken after 72 h. and later samples) isovaleric acid began to appear on the gas chromatograms in very small amounts and its identity was confirmed by paper chromatography. The quantity present was too small for accurate measurement, but was not greater than 0·1 mg./100 g. in the oldest sample.

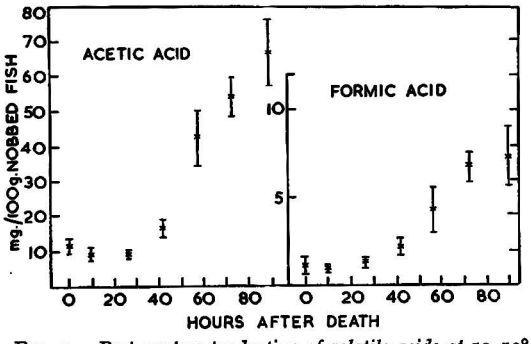

FIG. 2.—*Post-mortem production of volatile acids at* 10–13°
(Mean deviation is shown)

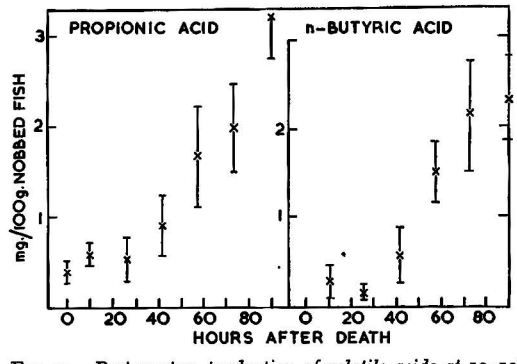

FIG. 3.—*Post-mortem production of volatile acids at* 10–13°
(Mean deviation is shown)

Experiments were also carried out to study the production of volatile acids in Scottish summer herring preserved in ice. Whole herring were held in cold storage for periods of up to 21 days in contact with three types of ice (*a*) normal ice, (*b*) ice containing 5 p.p.m. of oxytetracycline and (*c*) ice containing 5 p.p.m. of chlortetracycline. Fish were withdrawn at intervals over the period and determinations of volatile acid made on the beheaded and eviscerated fish. Three fish from each type of ice were analysed at each stage. Fig. 4 compares the production of total volatile acids in fish held in the three types of ice. (This figure was obtained from the titration of the steam distillate, as described previously.) Figs. 5 and 6 demonstrate the production of formic acid and acetic acid. It is apparent from these figures that the presence of antibiotics had no significant effect on the rate of production of volatile acids. The same was true for propionic acid and n-butyric acid, the former increasing from 0·3 mg. to 0·7 mg./100 g.

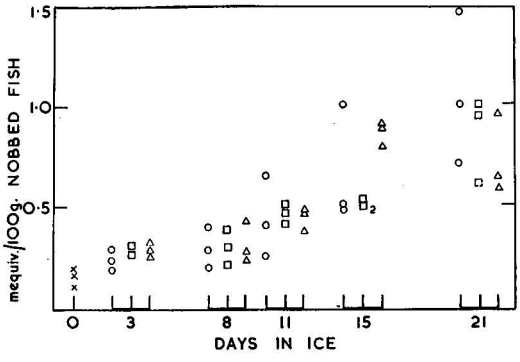

FIG. 4.—*Post-mortem production of total volatile acids in herring held in different ices*

○ normal ice
□ ice containing 5 p.p.m. oxytetracycline
△ ice containing 5 p.p.m. chlortetracycline

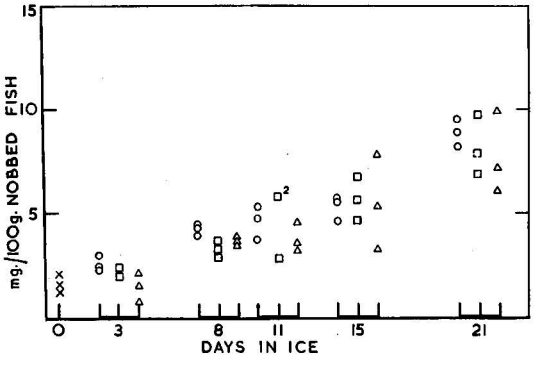

FIG. 5.—*Post-mortem production of formic acid in herring held in different ices*

○ normal ice
□ ice containing 5 p.p.m. oxytetracycline
△ ice containing 5 p.p.m. chlortetracycline

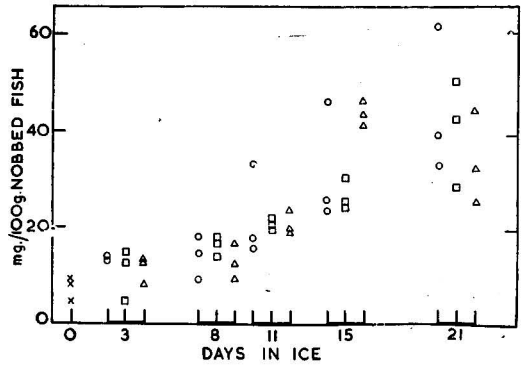

FIG. 6.—*Post-mortem production of acetic acid in herring held in different ices*

○ normal ice
□ ice containing 5 p.p.m. oxytetracycline
△ ice containing 5 p.p.m. chlortetracycline

and the latter from nil to 0·3 mg./100 g. in all cases. Mr. N. de Silva (personal communication) discovered that in herring held under conditions identical with the above, organoleptic tests showed a markedly beneficial effect of the antibiotics in retarding the rate of spoilage. This could suggest that the volatile acid value may indicate the post-mortem age of a fish rather than its condition. The apparent lack of effect of the antibiotics also suggests that either these acids may not be products of bacterial spoilage or that the bacterial species producing them are resistant to the effect of the antibiotic. Another possible answer is that bacteria present in the gut of the whole fish invade the flesh, remaining out of contact with the antibiotic, which does not penetrate below the skin.

Although a considerable increase in the content of these acids occurs during the initial stages of fish spoilage, it is unlikely that those produced in the flesh have any effect on the odour of the fish. It has been shown that the pH of herring flesh, which is 6·4–6·8 when fresh, increases

during spoilage after a slight initial decrease.[6, 15] Thus the acids would be present largely as their salts and would be non-volatile. Asakawa[16] noted that the lower volatile acids were present mainly in the salt form during spoilage of minced blue-fin tuna flesh.

Effect of cooking on the volatile fatty acid content of herring flesh

Fresh summer herring were beheaded and eviscerated and packed in 14-oz. lacquered tinplate oval fish cans, exhausted for 15 min. at 208·5° F and processed for periods of up to 5 h. at 240° F 10 lb. steam pressure. After being cooled under running water each can was opened, the contents thoroughly mixed and a sample taken for analysis. The results obtained are shown in Table, I from which it will be seen that no regular variation occurred in any of the acids studied. No butyric acid was detected in any of the fish.

Table I

Effect of heat processing at 240° F on the volatile fatty acids of herring
(each figure represents the mean for three fish)

Time of cooking, h.	Formic acid	Acetic acid mg./100 g. of flesh	Propionic acid
0	2·1	16·8	0·4
1	1·9	17·7	0·5
2	1·6	11·4	0·6
3	1·7	16·3	0·4
4	2·7	19·9	0·6
5	2·2	17·9	0·5

As mentioned previously, the non-volatile fatty acid value has been used for estimating the quality of fish after canning, the method depending on the value being unchanged during heat processing. The above results confirm that this is the case and show that none of these acids interacts to any appreciable extent with other flesh components during heat processing.

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DETECTION AND ESTIMATION OF FUNGAL AMYLASES IN FLOUR

By R. A. KNIGHT

As the methods normally used in the examination of flour do not reveal the presence of fungal amylases, a technique has been developed which may be employed for their detection and semi-quantitative estimation. This is based on the decrease in α-amylase activity which occurs when a flour extract containing calcium ions is heated at 68° for 30 min. Confirmation of the presence of fungal amylase and of the amount which has been added to flour can be obtained by using the relationship between cereal α-amylase activity and Hagberg number.

Introduction

' Diastatic activity ' is an important property of flour and can be considered to be dependent on two main factors :

(*a*) the susceptibility of the starch in the flour to attack by amylolytic enzymes which may be naturally present in the flour or added.

(*b*) the amylase content of the flour.

Amylases are generally classified into two main types, α- and β-amylase. The saccharogenic or sugar-producing enzyme (β-amylase) is rarely present in insufficient quantities in flour.[1] α-Amylase, the starch-liquefying and dextrinogenic enzyme, is however present in very small amounts in flour milled from sound wheat but increases with the degree of sprouting.[2] Flours deficient in diastatic activity may be supplemented by the miller or by the baker and the traditional diastatic supplement is malt flour.

In recent years, however, amylases derived from fungal sources have been increasingly used to improve the diastatic performance of flour. Such products are prepared in the form of highly active powders which may be diluted by the manufacturers to produce materials of uniform strength which have certain advantages.[3] Purified fungal amylases have been accepted in the U.S.A. as ingredients of bread and rolls.[4]

Properties of fungal amylases

Fungal amylase preparations, for example those derived from the mould *Aspergillus oryzae*, contain an amylase which is predominantly dextrinogenic in character.[5] Like the cereal α-amylases, this enzyme is unable to attack α-1,6-glucosidic linkages[6] and has a similar pH zone of optimum activity.[7]

In bread it has been found[8] that the amount of soluble dextrins is not increased by the addition of fungal amylases, because of their low thermal stability ; the residual maltose in bread crumb increases to a much less extent with increasing quantities of fungal amylase than with comparable amounts of cereal and bacterial α-amylases. Miller *et al.*,[9] however, using a rather more drastic extraction procedure, had earlier claimed that fungal amylase increases the quantity of soluble dextrins in bread crumb, although not to the same extent as malt.

Fungal α-amylases are inactivated at a lower temperature than cereal α-amylases[8–10] and the enzymes from both sources are protected to some extent from heat-inactivation by calcium ions.[11–14] There is a marked difference in the ratio of α-amylase to β-amylase between malt and fungal enzymes, it being reported[15] that this ratio is 2–3 : 1 for malt amylase and 7–9 : 1 for fungal amylase. Fungal α-amylase action is markedly higher in the presence of sodium chloride than in its absence.[16]

Experimental

Flour testing methods in relation to fungal amylases

Although an enzyme similar in several respects to that of malt α-amylase is present in fungal amylases, the methods which are normally applied in flour testing do not reveal with any certainty the presence of these materials in flour.

The Hagberg number[17] of flour shows a good correlation[18] with its α-amylase activity as

determined by the method of Jongh,[19] but additions of fungal amylase to flour do not significantly increase its Hagberg number. This is because the heat-treatment which is applied to the flour/water suspension during the test inactivates fungal α-amylase at an early stage and before the enzyme has had an opportunity to cause any marked degradation of the starch. Additions of malt to flour, however, result in a significant increase in its Hagberg number. The difference in response obtained when malt and fungal amylase are added to flour is illustrated in Table I.

Table I

Effect of additions of malt and fungal amylases on Hagberg number

Flour	Fungal amylase addition (oz./sack)	Hagberg number	Malt flour addition (oz./sack)	Hagberg number
A	0	15	0	15
	1·0	15	16	31
B	0	16	0	16
	1·0	16	16	31
	16	17	—	—
C	0	12	0	12
	0·5	12	16	38
	1·0	12	32	54
D	0	11	0	11
	16	11	16	23
	32	11	32	36

(In A and B, Preparation X was used; in C and D, Preparations Y and Z, respectively)

When used in conjunction with a determination of α-amylase activity, however, the Hagberg number can provide very useful information concerning the presence of fungal amylase in flour. Since the Hagberg number is not increased by fungal amylase additions, it would be expected that supplementation with fungal amylase would be revealed by a deviation from the expected α-amylase/Hagberg number relationship. This deviation is shown in Fig. 1 where those flours containing fungal amylase are clearly separated from unsupplemented samples.

In the *Amylograph* test as normally applied to flour, fungal amylases are not detected, as they are inactivated at an earlier stage in the heating process than cereal α-amylase and before marked changes in viscosity of the gelatinising starch occur. For example, comparable additions of α-amylase, added in the form of malt and fungal amylase, decreased the maximum viscosity of a flour/water paste by 400 and 0 units respectively.

Gas production in dough is increased by fungal amylases to an extent comparable to that of malt. This effect is illustrated by Table II which refers to the gas production of two flours containing supplements of malt and fungal amylase of equivalent α-amylase content.

Although *the maltose figure*[20] of flour is closely related to the degree of starch damage produced during milling,[21, 22] it would be expected that it would also be influenced by the quantity of α-amylase available to attack the damaged starch. Thus, the addition of malt to flour will increase its maltose figure by virtue of the introduction of α-amylase. This increase in maltose figure by malt is well known and has been employed to evaluate malt products for use in bread making.[23] Fungal

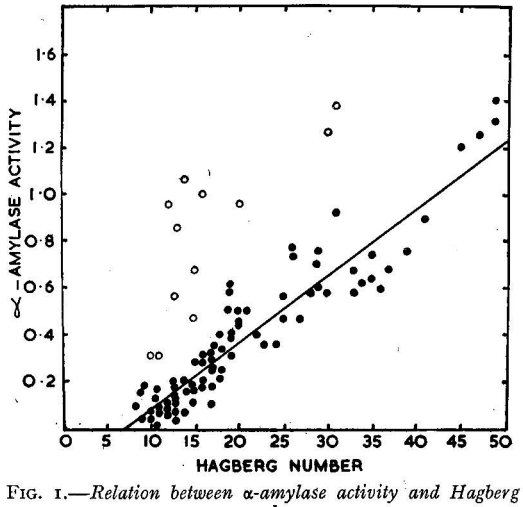

FIG. 1.—*Relation between α-amylase activity and Hagberg number*

● unsupplemented flours
○ flours supplemented with fungal amylase

Table II

Gas production of flours supplemented with malt and fungal amylases

(volume of gas, ml.)

	Flour A			Flour B		
	Control	Control + malt	Control + fungal amylase	Control	Control + malt	Control + fungal amylase
1st hour	29	25	30	30	29	30
2nd ,,	60	57	53	59	59	58
3rd ,,	58	58	57	61	59	59
4th ,,	54	51	51	55	55	54
5th ,,	36	52	51	26	55	56
6th ,,	17	47	49	16	32	32
Total	254	290	291	247	289	289

Table III

Maltose figures for flours with equivalent amounts of α-amylase from malt and fungal amylase

Addition to flour	Maltose figure (Blish–Sandstedt[20])
None	2·49
Malt extract	3·54
Malt extract heated at 68° for 30 min.	3·47
Fungal amylase extract	2·62

amylase does not, however, bring about a corresponding increase in maltose figure. This is illustrated by the results given in Table III where the additions of malt and fungal amylase are equivalent to 9 oz./sack (0·2%) and 0·9 oz./sack (0·02%) respectively, approximately equivalent additions of α-amylase.

It will be seen that the unheated malt extract increased the maltose figure by 1·05 units, whereas the fungal amylase extract produced an increase of only 0·13 units. Since these additions contained similar amounts of α-amylase, there would appear to be a fundamental difference in the mode of action of the enzymes from these sources. Heat treatment of the malt extract under the conditions used in this experiment (68° for 30 min. in the presence of calcium ions) will have inactivated the β-amylase present[12] and destroyed only a small proportion of the α-amylase activity.[24] The results obtained show that the β-amylase present in the malt extract had little or no effect on the maltose figure.

The loss of activity due to heat-treatment of the malt extract was 6·7% which is in good agreement with the loss of α-amylase activity expected. This confirms the view that α-amylases from malt and fungal sources differ in their action on starch in flour. As gas production is increased to a similar extent with equivalent α-amylase supplementations from these two sources, it would appear that fungal amylases may produce non-reducing dextrins which are assimilable by yeast. This problem is being investigated.

Since by the normal methods of flour testing the results of an examination of flours containing fungal amylase might indicate that wasteful and potentially harmful additions of malt flour would be needed, it was considered important that a test should be available which could be employed to detect the presence of fungal amylase in flour. Such a test might also be useful in the case of flours having a low Hagberg number and adequate gassing power where a diastatic supplement might be beneficial if fungal amylase were absent. The work described below resulted in the development of a method which enables commercial additions of fungal amylases to be detected and semi-quantitatively determined.

Basis of the method adopted for determination of fungal amylase

The method is based on one outstanding difference between cereal and fungal α-amylases, that is, the comparative stability of the former when heated in solution.[8–10] While calcium ions cause instability of β-amylase[12] and increase the stability of both cereal and fungal α-amylases,[11–14] the presence of a calcium salt in a flour extract would be expected to reduce the loss of cereal α-amylase to a minimum and this was considered to be desirable.

Since malt extracts, at their natural pH, only lose up to about 10% of their α-amylase activity when heated at 70° for 15 min. in the presence of calcium ions,[24] and fungal α-amylase

would be largely destroyed under these conditions, it appeared that the maximum differential between the two enzymes was likely to occur between 65° and 70°. Although the destruction of α-amylase by heat is dependent to some extent on the pH of the solution,[12] in the experiments described below no adjustment of the pH of the extracts was made. The pH of the various extracts was generally in the range 6·0–6·2.

Development of method

Experiments were carried out in which extracts of a flour having negligible α-amylase activity and of the same flour containing malt or fungal amylase were heated at 65°, 68° and 70° for various times and the residual α-amylase activity compared with that of the original extract. α-Amylase activity was determined by the method of Jongh[19] with minor modifications. The extracting solution contained 2 g. of calcium acetate[24] and 1 g. of sodium chloride per litre and β-amylase was added to the heated extract to overcome the effect of the loss of this enzyme during the heating period. It was found by experiment that an addition of 5 mg. of pure β-amylase in a total volume of 12·5 ml. of final reaction mixture was adequate for this purpose.

The results obtained in this series of experiments are given in Fig. 2, from which it will be seen that several different combinations of time and temperature could be employed in order to obtain a satisfactory differential between cereal and fungal α-amylase. The heating conditions finally chosen were 68° for 30 min., a combination which gives a high degree of contrast between the two enzymes in a reasonable heating time (no account having been taken in this work of the time, about 2 min., required for the extracts to reach a temperature of 68°). In addition, rather less precipitation of protein occurred at this temperature than at 70°, and the losses of cereal α-amylase were smaller.

It should be noted that these results were obtained when cereal and fungal amylases were heated in the presence of flour extract since these are the conditions that would occur in practice. In the absence of flour extract, dilute solutions of malt readily lose their activity on heating at 68° even in the presence of calcium ions, although fungal α-amylase does not appear to be markedly more unstable in the absence of flour extract than in its presence. In experiments with malt flours, losses of α-amylase activity amounting to over 70% were obtained when dilute extracts (0·025% w/v) were heated at 68° for 30 min. However, losses of less than 10% occurred when more concentrated extracts (0·5% w/v) were heated under the same conditions, these losses being comparable with those obtained by heating dilute malt extracts in the presence of flour extract. This illustrates the pronounced protecting effect of flour extract on cereal α-amylase, and confirms the finding of Kneen *et al.*[12] that the instability of enzyme solutions appears to be dependent on the concentration of accompanying substances in the extract rather than the amylase concentration.

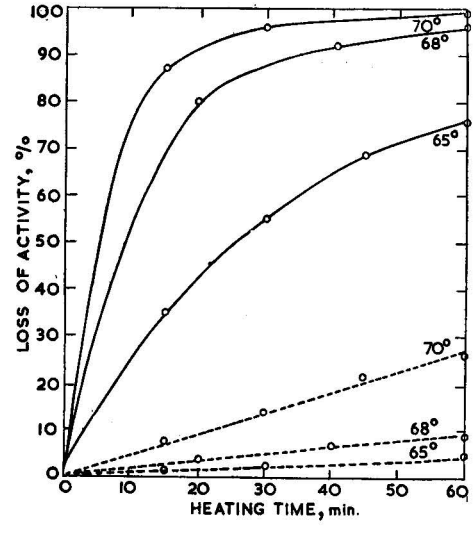

FIG. 2.—*Destruction of cereal and fungal α-amylases under different conditions of heating*

——— fungal amylase extract
- - - - - - - - malted wheat flour extract

When the above procedure was used to determine the loss of activity of four fungal amylases obtained from different suppliers it was found that, although there was a wide variation in α-amylase activity, the percentage losses obtained were similar (see Table IV).

Table IV

Fungal amylase	α-Amylase activity[19]	Loss of activity when heated at 68° for 30 min. in presence of flour extract, %
A	3100	86
B	1350	78
C	800	80
D	22,500	86

It would thus appear that commercial fungal amylase preparations are likely to behave similarly when heated under the above conditions and that a loss of α-amylase activity of a sample of unknown origin may be assumed to be about 83% (±5%) with reasonable confidence.

It would be expected that extracts of different samples of flour containing no fungal amylase would show some variation in loss of activity when heated at 68° for 30 min. This follows from various studies of the inactivation of cereal α-amylase,[12, 24, 25] and from the experimental errors involved in the measurement of the low α-amylase activities of normal flour samples. For example, Preece[24] found that the inactivation of α-amylase in extracts of malt heated at 70° for 15 min. ranged from 0·8 to 8·6%, average 4·3%.

In the present work, a series of 25 commercially available flours gave losses of α-amylase activity ranging from 1·8 to 16·1% with an average loss of 8·5%, when their extracts were heated under the above conditions. For the purpose of the detection of fungal amylase in flour a loss of 10% of the cereal α-amylase may be assumed without serious error.

Detection of additions of fungal amylase

Since the conditions of heating described above result in a wide difference between the extent of destruction of cereal and fungal amylases, the detection of normal levels of fungal amylase in flour is a comparatively simple matter. The quantities of fungal amylase that are usually employed contribute about 0·3–0·6 units[19] of α-amylase activity. Fungal amylase is, therefore, unlikely to have been added to a flour which has an α-amylase activity of less than 0·3.

The destruction of α-amylase that takes place when an extract of flour is heated at 68° for 30 min. will obviously depend on the quantity of fungal amylase which has been added to the flour and also on the α-amylase activity of the unsupplemented flour. Table V shows the losses of α-amylase that would occur when these factors vary between normal limits in the case of a typical fungal amylase preparation, assuming the losses of cereal and fungal α-amylases are 10% and 83%, respectively.

It can be seen that, except in those instances where very small additions of fungal amylase have been made or where the unsupplemented flour has a high α-amylase activity, the destruction of α-amylase is likely to vary from about 36 to 83%. This has been confirmed by an examination of a number of flours known to contain additions of fungal amylase. Eleven such flours, when examined by the method given below, gave losses of α-amylase activity ranging from 34 to 80%, the average loss being 57%.

Table V

Loss of α-amylase on heating flour containing added fungal amylase

α-Amylase activity of unsupplemented flour	Fungal amylase (oz./sack) added	Total α-amylase activity of flour	Loss of α-amylase after heating flour extract at 68° for 30 min., %
0	0·5	0·34	83
0	1·0	0·68	83
0·2	0·5	0·54	56
0·2	1·0	0·88	66
0·4	0·5	0·74	44
0·4	1·0	1·08	56
0·6	0·5	0·94	36
0·6	1·0	1·28	49

Details of method

In experimental work on flour containing small quantities of additive, e.g., 0·5–1 oz. of fungal amylase per sack of 280 lb. flour (0·011–0·022%), special care is necessary in sampling in order to eliminate errors due to uneven distribution.

A flask containing a weighed quantity of flour (5–10 g. is normally suitable) and about 3 g. of purified sand is brought to 30° in a water-bath, 100 ml. of extracting solution (2 g. of anhydrous calcium acetate and 1 g. of sodium chloride made up to 1 litre with distilled water), previously brought to 30°, are added and the flask well shaken. The mixture is incubated at 30° for 60 min. with shaking every 15 min. and then centrifuged and the supernatant filtered through a Whatman No. 4 filter paper, the first runnings being discarded.

About 20 ml. of filtrate in a stoppered tube are immersed in a water-bath maintained at 68° with the level of liquid in the tube below that of the water in the bath and the tube is agitated frequently for 2–3 min. to bring the extract rapidly to 68°. Exactly 30 min. after commencement of heating, the tube is removed from the water-bath and cooled immediately to room temperature under running water.

The α-amylase activity of the unheated and the heated extracts is then determined by the method of Jongh[19] using a reaction mixture composed of 8 ml. of extract, 2 ml. of β-amylase* solution (2·5 mg./ml.) and 2·5 ml. of 2·5% erythrodextrin substrate.

For qualitative purposes, a visual comparison may be made of the colour produced by withdrawing 1 ml. of reaction mixture after, say, 60 or 120 min., and adding to 5 ml. of 0·01N-iodine solution. After dilution with water to 100 ml. it will readily be apparent by visual inspection whether the loss in α-amylase activity is sufficient to indicate the presence of commercial quantities of fungal amylase, since the small loss of cereal α-amylase which occurs under the above conditions of heating is barely detectable by eye.

Application to flour samples

Estimation of fungal amylase

Owing to the wide variation in the activities of different samples of fungal amylases (Table IV), unless the particular preparation which has been added to a sample of flour is known, the quantity cannot be accurately determined. What can be measured, however, is the added α-amylase activity. On the assumption that the destruction of cereal and fungal α-amylases, when the above method is employed, is 10% and 83%, respectively, the approximate quantity of fungal amylase in a flour sample can be estimated by calculation.

Thus,

$$F = \frac{a(L - 10)}{73} \quad \text{and} \quad C = \frac{a(83 - L)}{73} \qquad . \quad . \quad . \quad (1)$$

where a is the total α-amylase activity of the sample, F is the activity due to fungal α-amylase, C is the activity due to cereal α-amylase and L is the observed % loss of activity when the flour extract is heated at 68° for 30 min.

This estimate may be confirmed by using the relationship between α-amylase activity and Hagberg number (see Fig. 1). The regression equation for this relationship is

$$y = -0{\cdot}196 + 0{\cdot}028x \qquad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where y is the cereal α-amylase activity determined by the method of Jongh[19] and x is the Hagberg number.

From the Hagberg number, the approximate cereal α-amylase activity of a flour can thus be obtained, and fungal α-amylase estimated by difference. In addition, the loss of α-amylase activity which occurs when the proposed method of heat-treatment is carried out should be in reasonable agreement with that calculated by assuming that 10% of the cereal and 83% of the fungal α-amylase, obtained from equation (2), has been destroyed.

Ten commercial flours known to contain fungal amylase were examined in this manner and the results obtained are given in Table VI.

* A suitable grade of β-amylase may be obtained from Wallerstein Laboratories, 180 Madison Avenue, New York, 16

Table VI

Results for commercial flours supplemented with fungal amylase

α-Amylase activity of flour	Hagberg number	Cereal α-amylase activity		% Loss of α-amylase activity on heating		Fungal α-amylase activity	
		From Hagberg number	Calc. from equation (1)	Found	Calc.	From Hagberg number	Calc. from equation (1)
0·32	10	0·08	0·01	80	65	0·24	0·31
0·47	15	0·23	0·20	52	47	0·24	0·27
0·57	13	0·17	0·20	57	61	0·40	0·37
0·68	15	0·23	0·26	55	58	0·45	0·42
0·90	29	0·62	0·53	41	33	0·28	0·38
0·96	12	0·14	0·25	64	72	0·82	0·71
1·01	16	0·26	0·43	52	64	0·75	0·58
1·07	14	0·20	0·41	55	69	0·87	0·66
1·27	30	0·65	0·85	34	46	0·62	0·42
1·38	31	0·68	0·64	49	47	0·70	0·74

In most cases the agreement between the results obtained by the two methods is reasonable, having regard to the sampling and experimental errors associated with this type of investigation.

Further experiments using flours containing known amounts of fungal amylase gave recoveries of 80–123% with an average value of 101%.

Conclusions

It has been shown that fungal amylases, in amounts normally used in commercial practice, may be detected and determined semi-quantitatively in flour by two different methods. The results obtained by the two methods are in reasonable agreement.

Acknowledgments

The author wishes to thank Dr. G. A. H. Elton, Director of Research, for helpful criticism. Acknowledgments are also due to Miss R. Bennett, in whose Department the Hagberg numbers of the various flour samples were determined, and Mrs. S. V. Stump for assistance in the experimental work.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JANUARY, 1959

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

INDEX OF AUTHORS' NAMES

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JANUARY, 1960

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Infra-red aerial photography in agriculture. S. Charter (*J. agric. Fd Chem.*, 1959, **7**, 536—539).—Stereoscopic photography at 700—1000 mμ (i.r.), from heights up to 20,000 ft., can give valuable information about the health of crops, probable yields, effects of fertilisers and pesticides, types of soil, water distribution, extent of weed and insect infestations, progress of irrigation and drainage schemes and of test plot studies, etc. Expert examination of photographs, a set of reference photographs and checking of results on the ground, are essential parts of the method. M. D. ANDERSON.

Characterisation of the Sebree–Chilcott soil series association (slick spots) in Idaho. F. M. Sandoval, jun., M. A. Fosberg and G. C. Lewis (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 317—321).—Slick spots (unproductive areas) in this Sierozem zone developed over calcareous materials were characterised by a very thin A_2 horizon overlying thicker B_2 layers with high exchangeable Na and sol. salts. A. H. CORNFIELD.

Characterisation and genetic study of a Dennis and a Parsons soil. C. D. Fanning and F. Bray (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 321—324).—Characteristics of these associated Prairie and Planosol soils are presented. A. H. CORNFIELD.

Central Russian turf podsols and grey forest soils compared with "sols lessivés" of W. Europe. P. Kundler (*Z. PflErnähr. Düng.*, 1959, **86**, 16—36).—These three soil types are similar with regard to general profile structure, distribution of humus and clay adsorption properties and structure. Textural differences in all three types are explained by silicate clay displacements. M. LONG.

Occurrence of soil "tumours" north-east of the Guánica Lagoon, Lajas Valley, Puerto Rico. G. Acevedo, M. A. Lugo-López and J. Ortiz-Vélez (*J. Agric. Puerto Rico*, 1959, **43**, 103—115).—Characteristics of soil tumours (mounds) oozing soil–water suspensions on an old alluvium are described. The tumours arise from water bursting through the soil, carrying with it large quantities of soil particles, due to the high upward pressure gradient induced during high rainfall or irrigation of adjoining upper lands. A. H. CORNFIELD.

Relations between physical characteristics of soils. I. Characteristics of the material quality. J. Járay (*Acta tech. hung.*, 1959, **24**, 285—328).—A very detailed discussion of the above characteristics is presented. The material quality of the soil is influenced by the surface of the aggregated particles, by the max. size of the particles and by the chemical properties; mathematical data are given. E. M. J.

Effect of vertical mulching and subsoiling on soil physical properties. J. F. Parr (*Agron. J.*, 1959, **51**, 412—414).—Vertical mulching (org. residues incorporated in the subsoil channel) of a silt loam resulted in higher soil bulk density, moisture content, and aggregation 10—14 months after treatment than did subsoiling alone. A. H. CORNFIELD.

Effect of synthetic polyelectrolytes on soil aggregation. M. A. A. Salam and A. G. Pollard (*Sci. J. roy. Coll. Sci. Lond.*, 1959, **27**, 28—35).—In four different soil types Krilium delayed the germination and emergence of rye-grass seedlings to extents which increased with the dosage. Small applications of Krilium to sandy, chalk and loam soils diminished and larger dosages increased the water-retaining capacities. In a heavy clay retention increased progressively with dosage. Increased aggregation produced by Krilium at all dosages tested was more apparent in the proportion of larger-sized aggregates (4—2 mm.). A. G. POLLARD.

Influence of lucerne, bromegrass and maize on soil aggregation and crop yield. S. A. Barber (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 258—259).—The aggregation index of a silty clay loam increased to a greater extent under bromegrass than under lucerne over 4 years. A mixed bromegrass–lucerne sward gave intermediate values. Continuous growth of maize produced the lowest aggregation index with little difference between years. There was poor correlation between maize yields and aggregation index. A. H. CORNFIELD.

Effects of drying on the mechanical strength of Lloyd clay. W. R. Gill (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 255—257).—The tensile strength of compressed moist blocks of Lloyd clay increased rapidly during the early period of ageing, but only slowly with further ageing. Increasing tensile strength was related to decreasing moisture content and the rate of increase varied with the container in which the block was ageing. A. H. CORNFIELD.

One-dimensional compression of partially saturated soil. Y. Yoshimi (*Dissert. Abstr.*, 1959, **19**, 2563).—The ratio of initial to total compression increased with increasing stiffness of soil structure, the initial air content and the pure water tension. The amount of consolidation is a function of time geometry, stresses and soil properties. Equations expressing rheological properties of partially saturated soil are discussed. O. M. WHITTON.

Pasture soil compaction by animal traffic. C. B. Tanner and C. P. Mamaril (*Agron. J.*, 1959, **51**, 329—331).—Animal traffic on 20 fine-textured pasture soils resulted in increased compaction and reduced air permeability in comparison with adjacent hay fields. The only soil which showed no effect from traffic was a silt loam, which had poor aggregate structure even without animal traffic. A. H. CORNFIELD.

Demonstration of clay displacement in soil profiles. H. P. Blume, E. Schlicting and H. S. Altemüller (*Z. PflErnähr. Düng.*, 1959, **85**, 227—244).—The tendency for clay to accumulate in some subsoils is due to displacement processes. M. LONG.

Moisture characteristics of Iowa soils. R. H. Shaw, D. R. Nielsen and J. R. Runkles (*Iowa agric. Exp. Sta.*, 1959, Res. Bull. 465, 411—420).—A till soil showed considerable variation in moisture content from one maize hill to another. This variation increased with depth. Moisture retained at 15 atm. tension (wilting point) varied with samples taken a few ft. apart, with a max. variation of up to 11% depending on soil type. Wilting point moisture % was significantly correlated with clay content. Laboratory methods for determining field capacity are unsatisfactory since they cannot evaluate field conditions which influence water movement. This applies particularly to poorly-drained soils. A. H. CORNFIELD.

Soil-moisture relationships of Rhodesian soils. E. R. Tillett and D. H. Saunder (*Rhod. agric. J.*, 1959, **56**, 61—64).—Apparent density, mechanical analysis, and moisture content at varying moisture tensions of 22 representative surface soils of Rhodesia were determined. The % of (clay + silt) in the soils was very highly correlated with moisture retained at both 0·33 atm. tension (field capacity) and 14 atm. tension (approx. wilting point). A. H. CORNFIELD.

Theoretical aspects of flow above the water-table in the drainage of shallow homogeneous soils. H. Bouwer (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 260—263).—Numerical expressions of the drainage flow above and below the water-table are obtained with an electrical resistance network for different hypothetical conductivity–tension relationships. Height of the capillary fringe has a considerable effect on drainage flow. The contribution from the zone above the fringe is negligible. Hysteresis causes a rising water-table to be lower over the drains than a falling water-table when the water-tables are at the same height midway between the drains. A. H. CORNFIELD.

Controlling soil moisture in pot tests. S. J. Bourget, B. J. Finn and K. F. Nielsen (*Agron. J.*, 1959, **51**, 429—431).—The method is based on embedding in the soil a porous cup which is attached by tubing, through a hole in the bottom of the pot, to a pan of water at a given distance below the pot, depending on the moisture tension required. The technique rapidly drains off excess water, applied to the soil surface, to any given moisture tension up to 100 cm. water. A. H. CORNFIELD.

Temperature and heat balance of soil. K. J. Kristensen (*Oikos*, 1959, **10**, 103—120).—Soil temp. at 2·5—150 cm. depth were measured for 3 years under bare soil and at 2·5—700 cm. depth under short grass at Copenhagen. At 50 cm. there was almost no diurnal fluctuation in temp. Seasonal changes in air and soil surface layer temp. are reflected at 100 cm. depth after a lag of about 18 days. Accumulation of heat by soil under short grass between April 1 and August 31 was about 3000 cal. per sq. cm., equal to 4—5% of the total radiation received. L. G. G. WARNE.

Multiple thermocouples for automatically averaging soil temperature at several sites. W. C. Burrows (*Agron. J.*, 1959, **51**, 370—371).—The equipment is described. A. H. CORNFIELD.

Physico-chemical properties of the Pinchen clay and the function of its free iron oxide and organic matter. H. Kun-Huang and C. Tsen-Tuo (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 270—273).—The sp. surface and standard free energy of adsorption of water vapour were both decreased by removal of org. matter from the clay. Removal of free Fe oxides decreased " available " water and sp. surface, but increased the free energy of adsorption. The binding forces of cations at exchange sites were little affected by the presence of org. matter or Fe oxides. A. H. CORNFIELD.

Movement of ions in soil columns (preliminary communication). H. Linser, H. H. Mayr and H. Unzeitig (*Z. PflErnähr. Düng.*, 1959, **86**, 57—65).—The values for the movement of NH_4^+ and NO_3^- in various soils correlate with the T value of the particular soil. M. LONG.

Cation removal using ion-exchange resins in the photometric determination of molybdenum with dithiol. K. Scharrer and W. Höfner (*Z. PflErnähr. Düng.*, 1959, **86**, 49—56).—Dowex-50 is satisfactory for the removal of cations in the determination of small amounts of Mo in plant matter, feeds, fertilisers and soils. M. LONG.

Effect of potassium and calcium activities in clay suspensions and solutions on plant uptake. R. J. Bartlett and E. O. McLean (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 285—289).—Uptake of K by soya-bean and barley from solution cultures containing clay in suspension was higher than from solutions of equal or higher K activity, whilst the reverse held for uptake of Ca. Bonding in the exchangeable form did not regulate K uptake, but may have regulated Ca uptake. A. H. CORNFIELD.

Ion movement in Wyming bentonite during electro-osmosis. H. S. Jacobs and M. M. Mortland (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 273—276).—The rate of removal of cations from bentonite–sand (1 : 20) systems during electro-osmosis decreased in the order Na, K, Mg, Ca. Electro-osmotic water flow per mequiv. cation removed decreased in the order Ca, K, Na. The rate of Na and K removal was proportional to the amount of these cations remaining in the system. Ca removal rate remained constant until about 50% of the Ca was left, and then decreased. The presence of a complementary cation increased the rate of removal of a given cation per unit of electricity used. Transference numbers during the early stages were 1·02, 0·96 and 0·61 for Na, K and Ca respectively. A. H. CORNFIELD.

Sodium adsorption ratio and residual sodium carbonate concepts of irrigation waters as they affect exchangeable sodium of soil under semi-arid conditions. L. O. Fine, E. J. Williamson, F. Wiersma and C. R. Umback (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 263—266).—In greenhouse tests with cropped soils using waters of high " residual sodium carbonate " (excess of $CO_3^{2-} + HCO_3^-$ over $Ca^{2+} + Mg^{2+}$) obtained by increasing HCO_3^- content, the % of exchangeable Na in the soil was greater than that expected from the Na-adsorption-ratio (SAR) values of the waters used. In field trials over 5 years with 15 in. annual rainfall the highest exchangeable Na was 2% above that expected from SAR values. This discrepancy between greenhouse results is probably due to return of Ca to the soil surface by lucerne and/or slow weathering of non-carbonate minerals. A. H. CORNFIELD.

Estimation of available soil potassium from plant analysis. M. W. Phillips and S. A. Barber (*Agron. J.*, 1959, **51**, 403—406).—In greenhouse pot tests with millet the total uptake of K was affected less than was % of K in the tissue by varying rates of N and P. Dry wt. yields of millet were positively correlated with total uptake of K and negatively correlated with % K in the tissue. In the field trials with maize receiving varying rates of N and K the total uptake of K by the plant was more sensitive to changes in available K than was % of K in the sixth leaf. A. H. CORNFIELD.

Comparison of several methods for evaluating the potassium status of Mississippi soils. L. E. Nelson (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 313—316).—Total K uptake by sunflowers in pot tests with 17 soils was significantly correlated with K extracted by six different chemical methods. Particularly high correlations were obtained with K extracted by N-NH_4OAc (pH 7·0), 0·5N-NH_4Cl–0·25N-HCl and 0·5N-HCl. In another experiment with 29 surface soils and 22 subsoils there were very high correlations between K uptake by sunflowers and exchangeable K both before and after cropping. A. H. CORNFIELD.

Plant analysis for determination of potassium requirements of arable soils. J. Köhnleer and N. Knauer (*Z. PflErnähr. Düng.*, 1959, **86**, 36—49).—Plant analysis provides the most satisfactory answer to the problem of K fertilising of cereals. The samples must include the whole plant and must be taken at a definite stage of plant development. M. LONG.

Simple flame photometer ; application to determination of potassium in soil and plant extracts. A. H. Cornfield (*Sci. J. roy. Coll. Sci. Lond.*, 1959, **27**, 36—40).—The construction and operation of the apparatus is described together with its use in determining exchangeable K in soils (N-NH_4OAc extract) and available K (1% citric or Morgan extracts). A. G. POLLARD.

Determination of aluminium ion activity in soil extracts. W. L. Lindsay, M. Peech and J. S. Clark (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 266—269).—A method is presented for calculating Al^{3+} activity from measurements of the concn. of Al, pH and ionic strength of the soil extract. The validity of the method was established by verifying the existence of a theoretical equilibrium relationship between the Al^{3+} and H^+ activities in soil extracts. The value (pH—$\frac{1}{3}$ pAl) for $CaCl_2$ extracts of three acid soils was fairly constant over the range 0·001—0·100 M-$CaCl_2$. A. H. CORNFIELD.

Aluminium in soils. III. Comparison of extraction methods for soils and clays. E. O. McLean, M. R. Heddleson and G. J. Post (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 289—293).—N-NH_4OAc (pH 4·8), N-NaCl and N-$BaCl_2$ removed comparable amounts of Al from a number of soils. N-NH_4OAc (pH 7·0) and Mehlich's triethanolamine–$BaCl_2$ reagent removed relatively little Al. Extractable Al decreased with increasing soil pH. N-NH_4OAc (pH 4·8) is probably the best reagent for determining extractable Al, particularly for soils with pH >5·5. The Al extracted was largely exchangeable. Extraction of Al from native and Al-treated clays is also reported. A. H. CORNFIELD.

Two methods of measuring available soil copper and the effects of soil pH and extractable aluminium on copper uptake by plants. R. L. Blevins and H. F. Massey (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 296—298).—The uptake of Cu by millet in pot tests with 34 soils was significantly correlated with both Versenate-extractable Cu (*Analyt. Chem.*, 1953, **25**, 655) and dithizone-extractable Cu (*Soil Sci.*, 1952, **73**, 341), but neither method was considered sufficiently precise for indicating the Cu status of soils. There was a significant negative correlation between Cu uptake by the plants and 0·1N-$CaCl_2$-extractable soil Al. Cu uptake was poorly correlated with soil pH. In short-term nutrient culture tests uptake of Cu by wheat plants decreased with increasing Al concn. in the culture. A. H. CORNFIELD.

Chemistry of soil copper. Lun-Shin Wei (*Dissert. Abstr.*, 1959, **19**, 2712—2713).—Cu added to soil was adsorbed first by org. matter, following Freundlich's adsorption equation, until the base-exchange capacity was saturated, and then by clay minerals, following Langmuir's adsorption equation. Cu was held more tightly by the org. matter than by the clay minerals, against both hydrolysis and acidification. Cu added to a Ca-saturated silt loam was irreversibly exchanged with Ca. In soils of normal pH range Cu is probably present mostly as a complex with org. matter, and as a (HO-Cu)–clay–H complex, with only a trace in exchangeable form. Extraction of Cu from soil by Versenate at pH near 4 was essentially an exchange reaction between Cu-Versenate and Cu-soil complexes ; for complete extraction of Cu from soil, Versenate should be used at higher pH. M. D. ANDERSON.

Release of iron oxide in red-brown soil formation from the weathering of limestone. I. Effect of carbonic acid. D. H. Khan (*J. Sci. Fd Agric.*, 1959, **10**, 483—486).—In carbonic acid leaching under laboratory conditions simulating leaching with rainwater of limestone rocks, there is progressive release of Fe (Si, Al, Ti, etc.) and partial removal of the alkaline earth carbonates. A reddish-brown to greyish-brown residue is obtained. Leaching with NH_4 acetate buffer removed the bulk of the carbonates and the residue obtained was similar to that in carbonic acid leaching. (12 references.) E. M. J.

Complexometric determination of extractable iron in soils. P. Fontana and G. Rossi (*Ann. chim., Roma*, 1959, **49**, 310—315).—An EDTA complexometric method is described. C. A. FINCH.

Uptake of strontium by pasture plants and its possible significance in relation to fall-out of strontium-90. R. S. Russell and R. J. Garner (*Nature, Lond.*, 1959, **183**, 1806—1807).—The authors question the conclusion of Vose and Koontz (*ibid.*, 1447) that, to ensure low concn. (*C*) of ^{90}Sr in milk, dairy cows should be restricted to an all-grass diet. During absorption of ^{90}Sr from uniformly contaminated soil the balance between grass and clover is not normally an important factor in determining *C*, but under other conditions the presence of clovers (deep root with low absorption) can minimise *C*. There is no evidence that aerial entrapment of ^{90}Sr by clovers exceeds that by grasses. W. J. BAKER.

Beneficiation of soils contaminated with strontium-90 ; beneficial effects of potassium. W. F. Libby (*Science*, 1958, **128**, 1134—1135).—Radishes were grown in soil treated with ^{90}Sr as nitrate with additions of K_2SO_4 or KNO_3 or only $^{90}SrSO_4$. The addition of sulphate was not very effective in reducing uptake of ^{90}Sr, but the addition of K was much more effective. 30 lb. of K^+ per acre reduced ^{90}Sr uptake by 40%. T. G. MORRIS.

The strontium-90 content of samples of pasturage in the [German] Federal Republic and its dependence on the strontium-90 content of the relative soils. A. Sittkus and E. Welte (*Naturwissenschaften*, 1959, **46**, 399—400).—The mean ^{90}Sr content of 22 samples of soil was 5·1 mc./km.^{2} at 10 cm. depth; that of pasturage was 49·0 $\mu\mu$c./100 g. There is a positive correlation between the radioactive contents of soil and grass, expressible by the regression curve $y = 0{\cdot}173\,x + 0{\cdot}70$ in which x is HCl-sol. ^{90}Sr in the soil, and y that in the plant. Scattering is due to atm. ^{90}Sr. W. H. KEMP.

Uptake of radio-strontium from the soil in pot trials. F. Scheffer and F. Ludwieg (*Z. PflErnähr. Düng.*, 1959, **85**, 244—249).—The uptake of ^{90}Sr by plants depends on the exchangeable Ca content of the soil and on the nature of the plant. In general high Ca levels are associated with low intake of ^{90}Sr. M. LONG.

Spectrographic determination of microelements in soils. P. Mirone and G. Rossi (*Ann. Chim., Roma*, 1959, **49**, 306—309).—The method is based on extraction with HOAc and concentration by pptn. with org. reagents. The resulting ppt. is used in the cavity of an electrode, using Ge and Pd as internal standards. The Ag, Co, Mn, Mo, Ni, Pb, Sn, Ti and Zn contents of eight alluvial soils of Piacentino are reported. C. A. FINCH.

Nitrogen movement and transformations in soils as evaluated by a lysimeter study utilising isotopic nitrogen and a field study. L. D. Owens (*Dissert. Abstr.*, 1959, **19**, 2709).—After application of $(NH_4)_2SO_4$ labelled with ^{15}N to soils (120 lb. of N per acre), losses of N by leaching were directly proportional to amount of moisture applied to the soil surface, and/or moving through the soil profile, between application and sowing of crop; uptake of N by the crop was inversely proportional to the leaching loss. The amount of fertiliser-N remaining in the soil at the end of the experiment was not affected by amount of moisture, nor was loss of N by denitrification (33% of the N applied). When N was applied in different forms, losses (determined from uptake of N by the crop) were greatest with NO_3^-, least with NH_4 salts, and intermediate with urea, differences increasing with increased moisture and increased rate of application. M. D. ANDERSON.

Non-symbiotic nitrogen fixation in a soil of the Nigerian rain-forest zone. A. W. Moore and J. N. Abaelu (*Nature, Lond.*, 1959, **84**, 75).—Twenty-week pot tests with a latosol (0—20 cm. layer from cropped area) show that increases in N up to 140 lb./acre can occur in bare soil and soil containing glucose or on which *Centrosema* is grown. The increase is small with added fertiliser (P + Mo). This non-symbiotic fixation is unimportant agriculturally because of almost continuous (in time) cover of indigenous growing plants. W. J. BAKER.

Nitrogen release from vegetable crop residues during incubation and their chemical composition. W. M. Iritani (*Dissert. Abstr.*, 1959, **19**, 2423—2424).—The N content of fresh vegetable residues was the main factor influencing the accumulation of mineral N during decomposition of the residues in soil; water-sol. N was twice as effective as the insol. fraction in releasing N. NH_3 accumulation was quite high and variable. A min. N content in the residues of 1·66—1·89% was necessary before any accumulation of mineral N occurred in excess of that in the soil alone. Neither total nor lignaceous C affected the N release, and the C/N ratio was a poor criterion for predicting N release. Dry matter production in tomato plants was highly correlated with the available N in the soil. Amounts of N obtained from maize residues were small. CO_2 release from the residues correlated better with the N release than did the C content of the residues. S. C. JOLLY.

Mineralisation of nitrogen and sulphur in sulphur-deficient soils. J. G. White (*N.Z. J. agric. Res.*, 1959, **2**, 255—258).—Incubation of S-deficient soils after liming those of low pH, showed that lime increased mineral N and SO_4^{2-}-S formed over a given period, compared with unlimed soil. The N : S ratio in neutral soils was ~8 : 1; mineral N and SO_4^{2-}-S accumulated in the ratio of ~10 : 1, in acid soils. After incubation the ratio varied between 20 : 1 and 40 : 1. Mineralisation of S may follow a pattern similar to that of N. (15 references.) E. M. J.

Sulphur uptake and residual studies in Northern Idaho using radio-sulphur. J. V. Jordan and G. O. Baker (*Idaho agric. Exp. Sta.*, 1959, Res. Bull. 42, 11 pp.).—In greenhouse tests with silt loam soils treated with various sources of labelled S (30 lb. of S per acre) the availability of the applied S to beans decreased in the order $CaSO_4$, $Fe_2(SO_4)_3$, $(NH_4)_2SO_4$, pea green manure, S, in the first crop. In the second crop the pea green manure furnished the largest amount of S. In field trials recovery of applied S (30 lb. per acre) over 3 years by lucerne was twice as great from $CaSO_4$ as from S. The S materials increased the protein % in lucerne and peas but had no consistent effect on P %. A. H. CORNFIELD.

Application of the turbidimetric method to the determination of small amounts of sulphate in soils. H. O. Jarrett (*Sci. J. roy. Coll. Sci. Lond.*, 1959, **27**, 41—45).—The modified $BaSO_4$ method described involves pptn. with saturated aq $BaCl_2$ in presence of 0·05—0·08N-HCl and use of gum acacia to stabilise the suspension. Turbidity is measured by absorptiometer. Limiting concn. of NO_3^-, PO_4^{3-}, Al^{3+}, Ca^{2+} and Fe^{3+} causing interference are determined. A. G. POLLARD.

Adsorbing power of various constituents of soil in regard to phosphoric ions and examination of soil reserves. R. Blanchet (*C. R. Acad. Agric. Fr.*, 1959, **45**, 247—252).—The adsorption of PO_4^{3-} by metallic hydroxides, especially $Al(OH)_3$, is considerably greater than by clays or limestones; the various adsorbants are in equilibrium and also with the soil solution. The activity of the adsorbed PO_4^{3-} is the more feeble as the adsorbent power rises. Three characteristics, equilibrium concn., isotopic P content diluted in 15 min., and the isotopic P content diluted in 3 days evaluate the availability of the reserves and the adsorbent power of the soil. (14 references.) M. C. M.

Reactions of metaphosphates in soils. J. Donoso-Torres (*Dissert. Abstr.*, 1959, **19**, 2732).—The hydrolysis of Na or Ca metaphosphate in acidified solution was an apparent first-order reaction. Na, NH_4 and Ca metaphosphates and KH_2PO_4, were mixed with two types of soil, which were maintained at 25% moisture and room temp., and orthophosphate was extracted by 0·1N-NH_4Cl and 0·5N-NH_4F after 1 hour, 6 weeks and 15 weeks. Considerable hydrolysis of metaphosphate (15—50%) had occurred even at 1 hour. After 15 weeks, amounts of condensed phosphate were greater with meta- than with ortho-phosphate in one soil, but similar in the other. Phosphates placed in soils in a vertical well penetrated to radial distances varying with soil and type of phosphate. Mechanism of transport was apparently by absorption of water vapour and subsequent movement of salt solution by capillary conduction. M. D. ANDERSON.

Effect of phosphate source, lime and time of phosphate application on absorption of applied phosphorus by plants. N. L. Chin, H. E. Ray, A. C. Caldwell and A. Hustrulid (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 299—302).—Wheat yields in pot tests with a silt loam (pH 6·9) were somewhat higher where P (100 lb. of P_2O_5 per acre) was applied as $Ca(H_2PO_4)_2$ (**I**) than as $CaHPO_4$ (**II**). Lucerne yields were similar with both sources of P. Utilisation of applied P was better from **I** than from **II**. Liming had no effect on wheat yields but increased lucerne yields. Liming decreased the utilisation of applied P. Time of application of superphosphate before planting (0—40 days) had no consistent effect on yields of oats or utilisation of applied P on several soils, although % P in the plant was usually higher when P was applied before planting. A. H. CORNFIELD.

Soil organic matter. I. Electrophoretic separation of acid-resistant components. H. H. Johnston (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 293—295).—A dark- and a light-coloured component were separated by paper electrophoresis from the acid-insol. NaOH-sol. fraction of three soils. Infra-red absorption spectra of the fractions showed them to have different structures, but both contained OH- and CO-groups. The similarity in N content and infra-red spectra between the dark-coloured components of the three soils indicates a similarity in constitution of the soil humic acids. A. H. CORNFIELD.

Extraction and determination of free amino-acids in soils. E. A. Paul (*Dissert. Abstr.*, 1959, **19**, 2419).—Extraction of soil with 0·5N-NH_4 acetate (pH 6·8) removed 20—25 free amino-acids in amounts of 0·05—0·5 μg. per g. of soil. Treatment of the soil with 1% of glucose and 0·3% KNO_3 to increase the microbial population, caused the production of 40 different amino-compounds including cysteic acid, aspartic acid, methionine sulphoxide, hydroxyproline, serine, threonine, proline, glutamic acid (**I**), glycine, alanine (**II**), valine, cystine, methionine, isoleucine (**III**), leucine (**IV**), β-alanine, tyrosine (**V**), β-aminobutyric acid (**VI**), lysine, histidine and arginine. Phosphoserine, taurine, cystathionine, diaminopimelic acid, ornithine and ethanolamine were tentatively identified, and eight other unknowns detected. The concn. of most of the amino-acids ranged from 1 to 5 μg. per g., but **I**, **II**, **III**, **IV**, **V** and **VI** were often present at concn. of 10—37 μg. per g. Concn. of amino-acids in treated soils were max. after 3 days' incubation, when the concn. were ~40 times their initial values, and then declined gradually. At 14 days the concn. were ~4 times their initial values. **I** was exceptional, its concn. increasing for 5 days, and being still 3·98 μg. per g. (i.e., 20 times its initial value) after 14 days. S. C. JOLLY.

Reasons for the improvement of soil structure of soils caused by liming. K. Hartge (*Z. PflErnähr. Düng.*, 1959, **85**, 214—227).—Comparison of the angles of contact between water and the film of org. matter covering soil particles and those between centrifuged soil solutions and glass and also of surface tensions of soil solutions before and after liming, indicate that an increase in org. matter at the

interface is an important factor in the improvement of structure caused by liming. M. LONG.

Influence of organic matter on the uptake of phosphorus from calcareous soil by barley. S. Y. Metwally and A. G. Pollard (*Sci. J. roy. Coll. Sci. Lond.*, 1959, **27**, 46—51).—In pot cultures, application of green manure (lupin) two months prior to the sowing of barley and application of P fertiliser increased the growth rate and P uptake of the seedlings. The direct nutritional effect of the green manure was small; its action depended on its capacity for restricting or retarding the reversion of available P added to soil. Under these conditions the nutrient efficiency of phosphates tested was in the order $Na_2P_4O_7$ and $NaPO_3$ > superphosphate and Mg phosphate > rock phosphate, the last-named having a slight negative action. A. G. POLLARD.

Effect of siliceous component of decomposing rice hulls on the solubility of phosphate. S. M. Bromfield (*Aust. J. agric. Res.*, 1959, **10**, 353—363).—The effect of decomposing rice hulls on the dissolution of sparingly sol. phosphates either directly, or by reduction of the phosphate-fixing power of Fe and Al oxides was studied. In culture tests rice hulls decomposed slowly and increased the solubility of Fe and $AlPO_4$ owing to greater hydrolysis as the pH of the cultures increased to 8, and, in the case of $AlPO_4$, to an interaction with silica. $AlPO_4$ was dissolved in presence of rice hulls over a pH range 5—8 with min. solubility near pH 7. Rice hulls also decreased the amounts of sol. PO_4^{3-} adsorbed by Al_2O_3 and Fe_2O_3 in this range. (27 references.) R. H. HURST.

Effect of liming and organic matter content on the availability of native and applied manganese. C. Sanchez and E. J. Kamprath (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 302—304).—Most of the sol. Mn^{2+} added to soil was converted to non-exchangeable form after 3—6 weeks' incubation and this conversion was accelerated by liming the soil. Air-drying soil prior to extraction with N-NH_4OAc (pH 7·0) resulted in higher exchangeable Mn values than did the use of moist soil, especially in a limed soil of high org. matter content. Addition of 2—10% of peat prior to incubation of unlimed soil increased the exchangeable Mn after 3 weeks, but decreased it in limed soil. A. H. CORNFIELD.

Synthetic action of enzymes in soils. G. Hoffmann (*Z. PflErnähr Düng.*, 1959, **85**, 193—201).—Paper chromatography shows that new compounds appear as a result of enzymic activity, and that some of these may be more polymerised than their precursors. Cell-free enzymes are equally active. Activity in soil may take the forms of a hydrolytic or synthetic metabolism. M. LONG.

Boron in agricultural land. C. H. Henkens and J. J. Lehr (*Landbouwvoorlichting*, 1959, **16**, 339—344).—Investigations were made on the relation between "heart-rot" in beet and the B content of the soil. As a general indication, when the B no. is <0·30, the disease can occur; B no. is defined as B content of the soil in mg./kg., obtained by boiling water extraction. J. M. HUBBARD.

Norite as clarifying agent in soil and crop research. J. C. van Schouwenburg (*Chem. Weekbl.*, 1959, **55**, 438—440).—Extracts of soil and crop samples may be clarified speedily by shaking with Norite. Measurements of ion concn. in standard solutions (varying the media), before and after treatment with a series of grades of Norite, indicate that it should be possible to find a grade which will enable accurate analysis to be made (particularly in acid medium) of given elements. J. M. HUBBARD.

Fertiliser and plant nutrient consumption 1957—8. W. Scholl, M. M. Davis, E. I. Fox and A. W. Woodard (*Farm Chem.*, 1959, **122**, No. 10, 66—74).—A digest of official statistics from the U.S. Dep. of Agric. A. G. POLLARD.

Fertilising practice. III. J. Prummel (*Versl. Landbouwk. Onderz.*, 1959, No. 65. 3, 32 pp.).—A survey of fertiliser practice on arable land, reclaimed peat soil and on sandy soil was carried out in the province of Groningen. Details of manuring and soil fertility from 30 farms are discussed. (21 references.) J. M. HUBBARD.

Fertiliser use in tropical agriculture. R. A. Webb (*Outlook on Agric.*, 1959, **2**, 103—113).—The subtractive technique of Webb described previously (cf. J.S.F.A. Abstr., 1958, ii, 214) is discussed. Taken together with the method of systematic variations due to Homès (which is based on the grouping of major elements into two similar groups, anions and cations; for any given total dressing there exists a best proportion for the ratio of N, S and P, and another for K, Ca and Mg; there is also a best ratio of total anions to total cations; the combination of these three proportions in one fertiliser gives the best mixture to be applied) offers hope of a more satisfactory evaluation of fertiliser requirements in tropical agriculture. (12 references.) R. H. HURST.

Nutrient balance and fertiliser practice. W. H. Allaway (*J. agric. Fd Chem.*, 1959, **7**, 470—473).—A critical review. M. D. ANDERSON.

Losses of anhydrous ammonia during application. J. H. Baker, M. Peech and R. B. Musgrave (*Agron. J.*, 1959, **51**, 361—362).—When anyd. NH_3 was applied to a number of soils at 81—261 lb. per acre at 4—8 in. depth the losses of NH_3 from the soil following application ranged from nil to 0·6% of that applied. A. H. CORNFIELD.

Availability of nitrogen in ammoniated bark used as soil amendment. T. R. Aspitarte (*Dissert. Abstr.*, 1959, **19**, 3084—3086).—Douglas-fir bark, freed from waxes by benzene, from tannins by hot water, and from cork by mechanical treatment, was ammoniated to different levels and incorporated with soil. The bark was fairly resistant to microbial attack, and was decomposed only 3—10% after 2 months at 28°. With increase of NH_3 content, there was increased output of CO_2 during decomposition of the bark. Addition of ammoniated or non-ammoniated bark to soil infested with *Phytophthora fragariae* decreased the incidence of red-stele disease in strawberries grown on the soil. Bark had a good effect on the state of aggregation of clay-loam soils. M. D. ANDERSON.

Influence of amino-acids on sugarcane and sugar recovery. I. Qualitative estimation of amino-acids in oil-seed cakes. S. C. Sen and Rajendra Prasad (*J. Instn Chem. India*, 1959, **31**, 19—21).—In an attempt to find a suitable cake for manuring sugarcane, paper-chromatographic identifications of the amino-acids (no. present indicated in parentheses) in the following cakes were made: mahua (17), castor (17), sesame (16), groundnut (14), rai (14), linseed (13), and mustardseed (12). O. M. WHITTON.

Calcium phosphate fertilisers. III. Effect of surface area on the availability coefficients of the dicalcium phosphates. IV. Relation between solubility in soils and availability coefficients of dicalcium and fused tricalcium phosphates. D. R. Bouldin and E. C. Sample (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 276—281, 281—285).—III. The availability coeff. of different granule sizes of $CaHPO_4$ (**I**) and $CaHPO_4.2H_2O$ (**II**) were well correlated with geometric surface area. Per unit of surface area the availability coeff. of **II** was 2·3 times that of **I**.

IV. Availability coeff. of **I**, **II** and fused $Ca_3(PO_4)_2$ (**III**) increased with decreasing granule size in greenhouse pot tests with oats. Per unit of surface area availability coeff. increased in the order **III**, **I**, **II** and were well correlated with concn. of P in solution after 14 days' reaction between the phosphates and soil. A. H. CORNFIELD.

Effect of granule size on the efficiency of granulated superphosphate used as pasture topdressing. F. B. Muller (*N.Z. J. agric. Res.*, 1959, **2**, 234—241).—Granulated superphosphate of particle size $\frac{1}{32}$—$\frac{1}{4}$ in. gave yields as good as or better than ordinary superphosphate (**I**). A powdered form under dry and the granular product under wet conditions gave better results than did **I**. When the granules were all >$\frac{1}{8}$ in. in diameter, yields were less when marginal amounts of phosphates were used. A granulated product ($\frac{1}{32}$—$\frac{3}{16}$ in. with >$\frac{1}{2}$ of the material between $\frac{1}{16}$ and $\frac{1}{8}$ in.) should be at least as effective as **I** when used under moist conditions. E. M. J.

Residual influence of phosphate fertiliser applied to a calcareous soil over a 6-year period. J. V. Mannering, G. O. Baker and M. LeBaron (*Idaho agric. Exp. Sta.*, 1959, Res. Bull. 41, 11 pp.).—The application of treble superphosphate (60—480 lb. of P_2O_5 per acre) in 1951 to a calcareous silt loam gave increased yields of barley straw and lucerne in that year, of field beans in 1955 and of sugar beet in 1956. Lucerne yields in 1952—4 were not increased, but % of P in the tissue was increased. Soil P extracted by both H_2CO_3 and 0·5M-$NaHCO_3$ reflected differences in rates of applied P even 6 years after application. A. H. CORNFIELD.

Sweet clover as a green manure. C. J. Willard and E. E. Barnes (*Ohio agric. Exp. Sta.*, 1959, Res. Bull. 839, 32 pp.).—Tests on many soil types showed that a two-year rotation of maize-small grains followed by a sweet clover catch crop maintained productivity nearly as well as did a 3—4-year rotation with one year of mixed hay including red clover and lucerne. The ploughed-in sweet clover produced as high yields of maize as did 6 tons of manure or 100 lb. of inorg. N per acre. A. H. CORNFIELD.

Use of spent mushroom compost. H. van Haut (*Z. PflErnähr. Düng.*, 1959, **85**, 201—214).—Whilst spent compost is excellent, if used after recomposting or if ploughed into soil some while before planting, mushroom mycelium present in the spent compost may have an adverse effect on crop yields. Adding lime, N or P also increase the rate of decomposition of the mycelium. The metabolic products of nematodes and bacteria in the compost may contribute to the adverse effect on yields. M. LONG.

Whey as a source of plant nutrients and its effect on soil. W. J. Sharratt, A. E. Peterson and H. E. Calbert (*J. Dairy Sci.*, 1959, **42**, 1126—1131).—By careful application, whey can improve soil structure and provide plant nutrients, but more complete utilisation of whey solids is accomplished by animal feeding. Application of whey to well-limed and near-neutral soil had no adverse effect on pH, but with soil of pH 5·0—5·5, acidity was increased sufficiently to affect plant growth. Lucerne tolerated, but did not benefit much from, a limited amount of whey. Grasses were more tolerant, and whey application increased the growth of bluegrass, especially during the second growing season, probably due to slow breakdown of N compounds in the whey. Moderate amounts of whey benefited soil aggregation. S. C. JOLLY.

Granular [fertiliser] products. Fisons Ltd. (Inventor: R. F. Knight) (B.P. 800,208, 1.4.55).—A mixture of $CaSO_4$ (e.g., gypsum, obtained by interaction of phosphate rock with H_2SO_4 in the production of phosphate fertiliser) (10—50 wt.-%) and NH_4 and/or alkali metal compounds (e.g., NH_4 sulphate, nitrate, or sulphate-nitrate, or KCl) is subjected to granulation, to give a free-flowing product. If desired, the second ingredient(s) may be used in solution or part solution form. F. R. BASFORD.

Fertilisers. Lummus Co. (B.P. 800,417, 16.3.56. U.S., 29.3.55).—Ca, K or Na metaphosphate is heated in presence of water and basic fertiliser material, e.g., limestone, dolomite, NH_3 or phosphatic rock (> 1 pt. per pt. of metaphosphate) at 120—175°/15—136 lb. per sq. in., to give a mixed fertiliser (containing mainly orthophosphate). F. R. BASFORD.

Granular mixed fertiliser. Imperial Chemical Industries Ltd. (Inventor: B. J. H. Mattinson) (B.P. 800,438, 23.1.56).—Granular mixed fertiliser containing NH_4Cl (or constituents which may result in its formation) is protected against caking by compounding (e.g., spraying the granular product or admixing during manufacture) with 0·001—0·2 wt.-% of a water-sol. salt of Cd or Mn^{2+}, the Na_2 salt of ββ′-disulphodinaphthylmethane, and/or a water-sol. polyphosphate. F. R. BASFORD.

Complete fertilisers. Wintershall A.-G. (Inventors: H. Keitel and W. Jahn-Held) (B.P. 799,428, 23.2.55).—As complete fertiliser there is claimed a composition of total nutrient >55%, containing 2—10% of MgO in assimilable form, e.g., as calcined or hydrated NH_4MgPO_4 and/or $(NH_4)_2Mg(SO_4)_2$, and characterised by $N : P_2O_5 : K_2O : MgO$ ratio 1 : 0·8—1·5 : 1·5—2·5 : 0·1—1·5. The composition also contains components with water-sol. P_2O_5 as well as those sol. in citric acid or citrates. A typical formulation is: KNO_3 (69), $(NH_4)_2HPO_4$ (21·9), $NH_4MgPO_4.H_2O$ (8·2) and micronutrients (0·9 pt.). F. R. BASFORD.

Fertilisers. Fisons Ltd. (Inventors: G. G. Brown and T. P. Dee) (B.P. 799,950, 31.12.54).—In the production of a phosphatic fertiliser, a phosphate rock (e.g., calcium phosphate type) is treated with a mixture of an ammonium salt [e.g., $(NH_4)_2SO_4$], HNO_3 and H_3PO_4 and the product obtained is treated with NH_3. I. JONES.

Fertilisers. Fisons Ltd. (Inventor: C. G. Bacon) (B.P. 798,690, 17.2.55).—Aq. $(NH_4)_2SO_4$ is added to conc. HNO_3 and the resulting solution is treated with NH_3 at high temp. (in a packed tower), to give molten NH_4 sulphate nitrate. The cooled product is subsequently granulated, prilled, crushed or milled, to give a crystalline mixed fertiliser. F. R. BASFORD.

Plant Physiology, Nutrition and Biochemistry

Effect of seed irradiation on germination and seedling growth of certain deciduous trees. M. B. Heaslip (*Ecology*, 1959, **40**, 383—387).—Seed of 18 species was irradiated with ^{60}Co γ-rays. Half of the species showed less than 30% survival after doses of 10,000 r but *Liquidamber styracifolia* and *Fraxinus americana* had a few survivors after 100,000 r. Sensitivity of the seed to irradiation increased as soon as seed dormancy was broken. L. G. G. WARNE.

Effectiveness of various solutions for simulating drought conditions as measured by germination and seedling growth. S. C. Wiggans and F. P. Gardner (*Agron. J.*, 1959, **51**, 315—318).—When radish and sorghum seed were placed in contact with solutions of various chemicals with concn. equivalent to 5 atm. osmotic pressure polyvinylpyrrolidone and NaCl almost completely inhibited, whilst sucrose, glucose and D-mannitol had little effect on, germination and a radicle growth. Concn. of the equivalent of 10 atm. or more of all chemicals greatly reduced or completely inhibited germination. The presence of 200 p.p.m. of three antibiotics and Dithane D-14 alone or in combination with a 2·5 atm. concn. of glucose had little effect on germination or radicle growth. A. H. CORNFIELD.

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Inhibition of germination of seeds by salicylhydroxamic acid (T2) and 5-bromosalicylhydroxamic acid (T40). H. Halweg and P. Krakówka (*Bull. Acad. polon. Sci. chim.*, 1959, **7**, 143—146).—The effects of T2 and T40 on the germination of vegetable, flower and cereal seeds were studied. Both acids inhibited the germination and growth of most of the tested plants. The min. inhibitory concn. of T2 and T40 was 15—62·5 μg. per 1 ml. of medium. Apart from these results, the compounds had a stimulating effect on the germination of peas and tomatoes. (11 references.) (In English.) R. J. MAGEE.

Relation between photosynthesis and photoperiodism in plants. G. A. Odumanova (*Dokl. Akad. Nauk SSSR*, 1959, **124**, 711—714).—Photoperiodic reactions of the plants in the absence of photosynthesis were investigated. Plants of *Brassica crenata* were cultivated in 14—15-hr.-day cycles, and *Perilla ocymoides* in the continuous lighting of luminescent tubes. Both plants produced abundant vegetative growth but were unable to start flowering. Leaves in CO_2-free atm. were unable to utilise even the optimal lighting conditions and the plant was unable to start reproduction process. A simultaneous photosynthesis with the photoperiodic impulse was imperative for normal growth of both short-day and long-day plants. A. GROCHOWSKI.

Effect of intensity and spectral composition of radiation on metabolism and harvest. N. P. Voskresenskaya and G. S. Grishina (*Dokl. Akad. Nauk SSSR*, 1959, **124**, 469—472).—Bean plants were grown in red light (520—710 mμ, max. at 620—680 mμ) at intensities 23—50 × 10^3 ergs/cm./sec. or in blue light (400—660 with max. at 460—540 mμ) with intensities 30—73 × 10^3 ergs/cm./sec. with 6 hr. of darkness. Metabolic changes and the wet and dry wt. differed with the light regime. Seed yields were much greater and their N content higher in blue than in red light. Blue-light plants showed the higher cytochromoxidase activity and earlier ripening. (11 references.) A. GROCHOWSKI.

Participation of alanine in biosynthetic processes in plants. E. A. Shilov and A. A. Yasnikov (*Dokl. Akad. Nauk SSSR*, 1959, **124**, 459—461).—The specific radioactivity of the rubber and carotene in the kok-sagyz plants and of the fatty acids in linseed on introduction of labelled alanine or acetic acid was investigated over 5—8 day periods and the relative effect of alanine was compared with those of the acetic acid and sucrose. Alanine donated two-carbon molecules of the acetic acid and the acetaldehydeimine both of which formed further links in the chain of the biosynthetic reactions. (11 references.) A. GROCHOWSKI.

Cation exchange between fragments of plant tissues and mineral solutions. F. Van Hoeck (*Ann. physiol. veg. Univ. Bruxelles*, 1958, **3**, 1—44).—Discs of plant material were immersed in various nutrient salt solutions and cation exchanges are examined. Toxic effects, due to imbalance of nutrients or to the presence of abnormal cations, are associated with endosmosis of K. Interrelationships between K, Ca, Mg and Zn are examined. A. G. POLLARD.

Relations between composition of the nutrient medium and that of the contents of the plant cell (*Gossypium hirsutum*, L.). G. H. J. van Schoor (*Ann. physiol. veg. Univ. Bruxelles*, 1958, **3**, 47—180). A comprehensive study. Special attention is given to the rôle of anions in the uptake of N, S, P, K, Ca and Mg by plants and in the subsequent distribution of these elements in the tissues. A. G. POLLARD.

Effect of cobalt on the elongation, respiration and deposition of cell material in the cell wall of *Avena* coleoptiles. M. Busse (*Planta*, 1959, **53**, 25—44).—Treatment of coleoptile cylinders with Co salts increased cell elongation caused by indolylacetic acid and restricted O_2 consumption and the deposition of cell-wall material. A. G. POLLARD.

Effect of radioactive phosphorus on rye plants. M. Aguado Marin (*Bol. Inst. nac. Invest. agron. Madr.*, 1959, **19**, 89—118).—Treatment of young rye plants with nutrient solutions containing ^{32}P produced a reduction in rate of root growth, chromosome breakage, anomalies in meiosis, partial sterility and hereditable branching of the ears. (26 photographs.) (33 references.) E. C. APLING.

Enhancement by inositol of the nodulation of isolated bean roots. N. Raggio, M. Raggio and R. H. Burris (*Science*, 1959, **129**, 211—212).—Isolated bean roots were grown in agar medium containing mixtures of different vitamins and amino-acids. It was found that *meso*inositol markedly increased the no. of roots nodulating and also the no. of nodules per root. T. G. MORRIS.

Salt resistance of protoplasm as a test for the salt tolerance of agricultural plants. G. I. Repp, D. R. McAllister and H. H. Wiebe (*Agron. J.*, 1959, **51**, 511—514).—Protoplasmic salt tolerance, determined by placing stem sections in NaCl of varying concn. followed by transferring them to slightly hypertonic glucose and then noting extent of plasmolysis, of a no. of species of plants correlated well with salt tolerance in the field. Increases in leaf succulence of

plants grown on salty soil agreed well with protoplasmic salt tolerance and yield. A. H. CORNFIELD.

Micro-determination of the lower reducing and non-reducing sugars in biological materials. W. Putzeys, J. Casier and R. Leyten (*Agricultura*, 1958, **6**, 239—257).—The sugars are separated chromatographically on paper. A modified Somogyi procedure is applied to the paper strips cut from the chromatogram. A. G. POLLARD.

Rapid and accurate automatic titration method for determination of calcium and magnesium in plant material with EDTA titrant. H. V. Malmstadt and T. P. Hadjiioannou (*J. agric. Fd Chem.*, 1959, **7**, 418—420).—In the method described, phosphate is removed from plant material, after wet digestion, by pptn. with $Zr(NO_3)_4$. Ca and Mg are extracted by CCl_4 as the diethyldithiocarbamate complexes. The Sargent Spectro-Electro automatic titrator is used, with standard disodium (ethylenedinitrilo)tetra-acetate, to determine Ca + Mg (indicator Eriochrome Black T; colour change wine-red to blue), and Ca (indicator Calcon, at pH 13; colour change pink to blue). Triethanolamine may be added before titration to mask traces of other metals remaining in solution, but in most cases is not necessary. (11 references.) M. D. ANDERSON.

Dynamics of indol-3-ylacetic acid in ripening and germinating maize seeds. V. V. Polevoĭ (*Dokl. Akad. Nauk SSSR*, 1959, **124**, 695—698).—Paper chromatography and colorimetry were used for determining the free and bound indol-3-ylacetic (**I**) contents in the maize seeds over the last month before ripeness and in the first hr. and days of germination. In dry maize seeds soaked for 2 hr. in water at 36° the **I** content in the endosperm was reduced by 40%, the water contained no **I**, and only a part of it was traced in the seed germ. Some **I** was utilised for biological processes. (11 references.) A. GROCHOWSKI.

Influence of heteroauxin on the cation content of tomato leaf ash. H. R. Bode (*Planta*, 1959, **53**, 212—218).—In young tomato plants the K content of leaf ash was highest in the youngest and least in the oldest leaves. The Ca contents were in the reverse order. Spraying the plants with indolylacetic acid was followed by an increased water intake by leaves with a much smaller proportional increase in dry matter. The K intake also increased markedly in the oldest and diminished in the youngest leaves. A. G. POLLARD.

Growth effects produced by 2,4-D [2,4-dichlorophenoxyacetic acid] in flowers and fruits of *Datura stramonium* L. and *D. tatula*. L. C. Wassberg (*Dissert. Abstr.*, 1959, **19**, 3105).—Effects of sub-lethal concn. of 2,4-D on *Datura* spp. are described. Responses of epidermal and vascular tissues, on flowering organs and on the thorns of the capsule walls are discussed. The existence of an extractable thorn-producing substance is suggested. M. D. ANDERSON.

Effect of seed treatment with streptomycin on Golden Acre cabbage seedlings. H. I. Borders (*Plant Dis. Reptr*, 1959, **43**, 549—551).—Cabbage seed soaked for 30 min. in 25—400 p.p.m. solution of streptomycin developed seedlings with colours ranging from deep purple (high concn.) to yellow-purple and died after attaining a height of about 0·5 in. Seed treated with 5—15 p.p.m. solution of streptomycin produced purple-yellow to yellow-green seedlings, but these eventually became green and grew normally. Seed treated with solutions containing 50—1000 p.p.m. of Aureomycin, 50—400 p.p.m. of Terramycin, and Arasan, Delsan and Dynactol produced normal seedlings which grew normally subsequently. A. H. CORNFIELD.

Inhibition of growth of excised tomato roots by 2-diethylaminoethanol. W. G. Boll (*Science*, 1959, **129**, 330—331).—2-Diethylaminoethanol (DEAE) at a concn. of 40 μM caused 50% inhibition in the growth of the main axis of excised tomato roots. At low concn. laterals were inhibited, but at higher concn. growth of the main axis was reduced but that of the laterals was relatively increased due to removal of the factors responsible for apical dominance which is shown in excised tomato roots. The inhibitory action of DEAE was unaffected by ethanolamine, but choline and 2-dimethylaminoethanol both relieved it to some extent. T. G. MORRIS.

Growth of excised grass embryos *in vitro*. S. Narayanaswami (*Bull. Torrey bot. Club*, 1959, **86**, 248—258).—Embryos of *Pennisetum typhoideum*, *Hordeum vulgare*, *Avena sterilis* and *Triticum vulgare* were used. Low concn. of maleic hydrazide stimulated coleoptile growth (cell extension phase only) but inhibited meristematic activity of the shoot apices. L. G. G. WARNE.

Mechanism of action of maleic hydrazide in tobacco and other plants. J. E. Baker (*Dissert. Abstr.*, 1959, **19**, 2726).—Respiration of tobacco seedlings and axillary-bud tissue was inhibited by 0·009—0·027M-maleic hydrazide, the effect being enhanced by lowering the pH of the medium to 4·0. Maleic hydrazide *in vitro* inhibited diaphorase, but no other of a large no. of enzymes studied (dehydrogenases, kinases, oxidases, nucleosidases, enzymes involved in oxidative and photosynthetic phosphorylation, etc.). Maleic hydrazide became bound to proteins and ribonucleic acid *in vitro*, the effect being more marked with the diethanolamine than with the K salt. M. D. ANDERSON.

Interaction of gibberellic acid and indolylacetic acid in *Impatiens*. J. Weijer (*Science*, 1959, **129**, 896—897).—Gibberellic acid (10^{-4} g. per l.) applied as a spray (5 ml. per alternate day) on highly inbred "double" (p_tp_t) lines of *Impatiens balsamina* L. caused full penetrance of doubling; controls gave only 67% of double flowering. Flowering was 3 weeks earlier than the controls and the plants were 2·5 times higher. Indolyacetic acid (IAA) caused slight stunting and delayed the flowering for 2 weeks. IAA and gibberellic acid together caused flowering 3 weeks early and the plants grew only to the normal size with full doubling. T. G. MORRIS.

Influence of gibberellic acid on endogenous growth substances of the Alaska pea. I. D. J. Phillips, A. J. Vlitos and H. Cutler (*Contr. Boyce Thompson Inst.*, 1959, **20**, 111—120).—In the first few days pea seedlings grown in red light contained less endogenous growth substance(s) than did those grown in the dark; after 8—11 days the amounts were similar, and after 21 days, the dark-grown plant contained the smaller amounts. Paper chromatography of pea extracts revealed indol-3-ylacetic acid, and a second growth substance occurred only in 7-day seedlings grown in darkness, and 11-day seedlings grown in red light. Pea seedlings grown in the dark or in red light, and sprayed with gibberellic acid (10 p.p.m.), showed respective increases of 100 and 180% in content of extractable growth substances 24 hours later. Growth-active zones were found at R_F 0·6—0·9 and 0·0—0·3, as well as at the positions previously observed. Material from zones R_F 0·4—0·7 and 0·7—1·0, of chromatograms of extracts of treated pea seedlings had both auxin and gibberellin-like activities. M. D. ANDERSON.

Determination of gibberellins by derivative labelling with diazomethane-^{14}C and by isotopic dilution analysis with tritium-labelled gibberellins. W. E. Baumgartner, L. S. Lazer, A. M. Dalziel, E. V. Cardinal and E. L. Varner (*J. agric. Fd Chem.*, 1959, **7**, 422—425).—Gibberellic acid and gibberellin A are determined in fermentation broths by reaction with diazomethane-^{14}C to yield the methyl-^{14}C esters, chromatographic separation on paper, examination with a Forro radiochromatogram scanner, and planimetric measurement of areas under the respective peaks. Sensitivity and accuracy can be increased at the expense of speed by extraction and liquid scintillation counting instead of scanning. The separation of gibberellins from process liquors is followed by adding gibberellic acid labelled with tritium and assaying successive samples by liquid scintillation counting, with a single isolation to determine the activity of the final product. The two methods are combined for determination of gibberellins in plant tissues by double isotopic dilution analysis. M. D. ANDERSON.

Infra-red determination of gibberellins. W. H. Washburn, F. A. Scheske and J. R. Schenck (*J. agric. Fd Chem.*, 1959, **7**, 420—422).—Gibberellic acid and gibberellin A, present together, are respectively determined by measurement of the absorbances at 12·86 and 10·85 mμ of a 15% solution in pyridine. Agreement with fluorometric and radio-isotopic methods was good. M. D. ANDERSON.

Paper chromatographic examination of plant-growth and -inhibiting substances in oat seedlings and maize scutellum. H. Bohling (*Planta*, 1959, **53**, 69—108).—From extracts of cylinders of oat coleoptiles three growth-substances [largely indolylacetic acid (**I**)] were separated chromatographically. Inhibitory substances were also detected in tips of roots and of coleoptiles. Extracts of maize scutellum yielded both growth-promoting (**I**) and -inhibiting substances. In the extracts **I** was destroyed by 30% H_2O_2; other active substances were more resistant. A. G. POLLARD.

Crops and Cropping

Factors affecting varietal differences in protein content of wheat. J. Seth (*Dissert. Abstr.*, 1959, **19**, 2710—2711).—In the field, high- and low-protein varieties of wheat did not differ in rate of absorption of N and amount of root growth. During grain formation, roots of high-protein varieties contained less N than those of low-protein varieties. There was no difference between the protein contents of the vegetative parts of plants grown in solution culture in the greenhouse. The protein content of the kernels was increased by foliar applications of N as NO_3^-, NH_4^+ or urea, and more markedly by soil applications, but varietal differences in protein content were maintained. A higher proportion of total N was present in the non-protein form in low-protein than in high-protein varieties. M. D. ANDERSON.

Effects of heat and loss of moisture on dormancy of wheat: interactions with "Mergamma D". P. D. Hewett (*Nature, Lond.*, 1959, **183**, 1600).—Dormancy of Cappelle Desprez Wheat by moisture

content 17·5%, or Peko wheat treated with Hg–BHC (Mergamma *D*) seed dressing, unlike dormancy in barley, is not overcome by heating at 38° either free or in moisture-proof polythene bags; with loss of moisture, dormancy may increase. Peko seedlings from samples dried at 38° show signs of stunting. Refrigeration leads to max. germination in both wheats, but increase in seed dressing gives increased stunting. Stunting of dressed wheat is reduced by heating with or without drying. Dressed barley responds similarly.
O. M. WHITTON.

Yield and composition response of wheat to soluble phosphate drilled in the row. J. P. Vavra and R. H. Bray (*Agron. J.*, 1959, **51**, 326—328).—When drilled in the row at sowing time water-sol. P (15 lb. P_2O_5 per acre) increased grain yields but had no effect on % P in the grain on a silt loam. Higher rates of P_2O_5 (30—60 lb. per acre) had little further effect in increasing grain yields. Application of NH_4NO_3 (15—60 lb. per acre) had no effect on yields or % P or % N in the grain and the responses to P were similar at all levels of N. A yield response equation is presented. Drilled P was utilised more efficiently than was broadcast P. A. H. CORNFIELD.

Relationships between climate and yields of maize and wheat in Spink County, South Dakota, and yields of milo and cotton at Big Spring, Texas. W. C. Moldenhauer and F. C. Westin (*Agron. J.*, 1959, **51**, 373—376).—Over 26 years in Spink County (northern Great Plains) crop yields were more closely associated with summer temp. than with summer rainfall. Spring wheat yields showed the highest correlation (negative) with June average max. temp., whilst maize yields showed the highest correlation (negative) with July average max. temp. At Big Spring (southern Great Plain) over 39 years milo yields were most highly correlated with pre-seasonal (Sept.—Apr.) rainfall. Yields were more highly correlated (negatively) with mean temp. during June, July and Aug. than with seasonal rainfall (May—Aug.). Cotton yields were most highly correlated (negatively) with Aug. average mean temp.
A. H. CORNFIELD.

Maize yields as influenced by nitrogen level and drought intensity. W. L. Parks and J. L. Knetsch (*Agron. J.*, 1959, **51**, 363—364).—An equation is presented relating maize yields with level of applied N and index of drought for 3 years of different rainfall conditions. The relationship accounted for 97% of the variability between treatment means. Such equations may be used for estimating probable yields at given levels of applied N or for determining optimum rates of N. A. H. CORNFIELD.

Nitrogen response of maize under variable conditions of drainage. J. Shalhevet (*Dissert. Abstr.*, 1959, **19**, 3080).—In poorly drained soil, anaerobic denitrifying micro-organisms cause rapid disappearance of N, and crops show symptoms of N deficiency. Applications of N increased the yield of maize grown on a continuously saturated soil, but yields were not as large as on adequately drained soil. O_2 ceased to be the limiting factor, and N deficiency became important, at a water-table 6—14 in. deep. Yields of grain were correlated with the oxidation-reduction potentials of soil below the water-table. M. D. ANDERSON.

Effects of αβ-dichloroisobutyrate sprays in preventing pollen shedding in maize. J. W. Cameron and F. M. Eaton (*Agron. J.*, 1959, **51**, 428—429).—Spraying the mid-season inbred Purdue 39A with 0·5% Na αβ-dichloroisobutyrate (**I**) 37 days after planting prevented pollen shedding from 80% of the main stalk tiller tassels. Spraying with a 1% concn. of **I** prevented pollen shedding but injured the ears. Spraying 44—51 days after planting did not prevent pollen shedding and caused high ear injury. Spraying other inbred varieties 37—51 days after planting had no effect on pollen shedding or caused ear injury where pollen shedding was reduced.
A. H. CORNFIELD.

Nutrition of rice plant (*Oryza sativa*, L.). V. Effects of ammonium and nitrate nitrogen on growth, yield and nitrogen uptake. A. Tanaka, S. Patnaik and C. T. Abichandani (*Proc. Indian Acad. Sci.*, 1959, **49B**, 386—396).—Rice was grown in solution culture with NH_4^+ and NO_3^- as N supplies. At moderate (20 p.p.m.) N levels, both are equally good as regards N uptake and grain yield, but NH_4^+ gives better straw yields. At high (100 p.p.m.) levels, NO_3^- is superior because NH_4^+ gives restricted root growth and high sol./protein-N ratios; NO_3^- promotes uptakes of K, Na, Ca, Mg, Mn and PO_4^{3-}. Better growth and crop yield are obtained with NH_4^+ given up to ear-initiation and NO_3^- during the elongation and reproductive stages. (16 references.) C. H. G.

Leaf composition of lowland rice and sugarcane as an indicator of their performance. G. B. Hong and J. van Schuylenburgh (*Neth. J. agric. Sci.*, 1959, **7**, 110—117).—The N/P ratio in the leaves of waterlogged rice decreased considerably, whilst the N/K ratio decreased slightly, from transplanting to maturity. Yields of grain and straw were highly correlated with leaf N/K ratio. In sugarcane the leaf N/P and N/K ratios remained fairly constant over the growing season. Sugar (% in the cane) increased with leaf N/K ratio up to ~1 and then decreased with further increase in this ratio. Yields of cane and sugar increased with the N/PO_4 ratio in leaves up to ~5 and then decreased with further increase in this ratio.
A. H. CORNFIELD.

Early and late season cultivation of rice. I. Physical and chemical properties of non-glutinous rices and their starches. H. Suzuki, S. Chikubu and T. Tani (*J. agric. chem. Soc. Japan*, 1959, **33**, 275—280).—Non-glutinous rice (varieties ordinarily grown in northern Japan) were cultivated as early (sown March, harvested Aug.) or late (sown July, harvested Nov.) crops in southern Japan. Differences in properties of the rice and starch between three varieties examined were greater than those between the two cultivation methods. The early-grown rice (polished) was difficult, while the late-grown rice was easy, to disintegrate in 1·7% aq. KOH. Water-uptake ratio and increase in vol. on cooking were similar for the early- and late-grown rices. Early-grown rice gave weaker iodine colour, showed lower pH and lower solid content in the residual cooking water. Granule sizes of the starches were similar. In the Brabender amylogram and alkali viscogram the difference due to growing methods was evident but the difference between varieties was not. The late-grown rice showed much higher viscoelasticity than did the early type. The concn. of KOH at which gelatinisation began was 0·23N for the late-grown and 0·35N for the early-grown rice starch. S. KAWAMURA.

Changes in sugar content during germination of rice seeds. Application of ion-exchange resin chromatography. T. Fukui and Z. Nikuni (*J. agric. chem. Soc. Japan*, 1959, **33**, 72—78).—Sugar contents of the endosperm, shoot and root of germinating rice seeds were determined chromatographically. The chief sugar of the embryo and endosperm of non-germinating rice seeds was sucrose, with small amounts of raffinose, glucose and fructose. Raffinose disappeared completely from the shoot and root of germinating seeds, while the amounts of sucrose, glucose and fructose increased. The ratios of the three sugars was constant during germination. The amount of glucose in the endosperm of germinating seeds increased markedly to a max. on the 8th day of germination, as did maltose and sucrose. Maltose was found only in germinating endosperms. An unidentified oligosaccharide composed of glucose and fructose was present in the later stages of germination. S. KAWAMURA.

Source and time of application of nitrogen for rye forage. H. D. Morris and J. E. Jackson (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 305—307).—Yields of rye forage on a sandy loam (pH 6) over 3 years were similar where N (120 lb. per acre) was applied as NH_4NO_3, $NaNO_3$, urea, or $(NH_4)_2SO_4$, but were lower where N was applied as $CaCN_2$. Applying all the N at planting (Oct.—early Nov.) gave lower yields of forage than did split application (half at planting and half top-dressed in Feb.). A. H. CORNFIELD.

Storage-rot susceptibility of potato tubers exposed to ionising radiation. W. J. Hooker and D. T. Duncan (*Amer. Potato J.*, 1959, **36**, 162—172).—Incidence of ring rot, *Fusarium* tuber rot, and bacterial soft rot developing during storage of tubers increased with the level of ionising radiation (5—500 kilorep) given to the tubers prior to storage. A. H. CORNFIELD.

Effects of seed-cutting dates on yields of potatoes. R. V. Akeley (*Amer. Potato J.*, 1959, **36**, 147—153).—Stands and yields of tubers of eight varieties of potato at five locations were not affected by date of cutting of the seed prior to sowing (up to 243 days) except with Sebago at one location, where freshly-cut seed produced the lowest yields. A. H. CORNFIELD.

Feeding of nitrogen to potatoes by means of farmyard manure and artificial fertilisers. II. J. Kortleven and H. Pijl (*Versl. Landbouwk. Onderz.*, 1959, No. 65. 1, 82 pp.).—A detailed report is given of trials to compare differences in N, moisture and starch contents in a crop developing under the influence of Nitro-chalk and that of farmyard manure. Generally the action per kg. of N in farmyard manure is the weaker; farmyard manure prolongs the life of the crop more, and this is reflected in the yield (higher % and greater wt. of big tubers). Nitro-chalk gives rapid starting development, which falls off later, especially with smaller dressings. (13 references.) J. M. HUBBARD.

Varietal response to potato seed-piece decay. K. Knutson, R. F. Line and C. J. Eide (*Plant Dis. Reptr*, 1959, **43**, 546—548).—Although potato varieties differed in susceptibility to seed-piece decay (due to *Erwinia* and *Fusarium* spp.), as indicated by % stand, the differences were not consistent over 2 years. Yields from infected seed-pieces were usually lower than from healthy seed-pieces.
A. H. CORNFIELD.

Phytic acid treatments for pre-peeled potatoes. B. L. Amla and F. J. Francis (*Amer. Potato J.*, 1959, **36**, 204—211).—Peeled potatoes dipped for 1 min. in 0·7% phytic acid or 0·5% Ca phytate + 1000 p.p.m. SO_2 and packed in polyethylene bags were not discoloured

after 16 days' storage at 4·4—5·6°. Packing in Cryovac bags following a 1-min. dip in 0·35% phytic acid or 0·25% Ca phytate + 200 p.p.m. SO_2 was equally effective. Potatoes treated with Ca phytate were slightly firmer than those treated with phytic acid. More alcohol was formed during storage in Cryovac than in polyethylene bags. A. H. CORNFIELD.

Acceleration of water uptake and germination of sugar-beet seed-balls by surface coating of hydrophilic colloids. S. T. Dexter and T. Miyamoto (*Agron. J.*, 1959, **51,** 388—389).—Coating the surface of sugar-beet seed with hydrophilic colloids (a quick dip in 0·75% agar, 0·75—1·5% gelatin, or 0·25—1·0% " algin," followed by air-drying) increased the rate of water uptake when the seed were sown in sand and accelerated rate of emergence when sown in the field. None of the treatments was injurious to the seed.
A. H. CORNFIELD.

Effect of lime, soil type and soil temperature on phosphorus nutrition of turnips grown on phosphorus-deficient soils. T. J. Army and E. V. Miller (*Agron. J.*, 1959, **51,** 376—378).—In pot tests with a fine sandy loam of low P status turnips showed poor growth and P uptake where no $CaCO_3$ was applied (soil pH 4·6), whilst both growth and P uptake increased considerably with application of $CaCO_3$ to give pH 6—7. Growth and P uptake on a silty clay loam (pH 5·4) were approx. doubled by application of $CaCO_3$ to pH 7—7·5. Increasing soil temp. (15·5—26·7°) increased both growth and P uptake, but had a much greater effect on the fine sandy loam than on the silty clay loam at all pH levels. Liming increased the dil. acid-sol. P in both soils at the end of the test, whilst increasing temp. increased dil. acid-sol. P only at the highest level of liming.
A. H. CORNFIELD.

Grain yields, evapotranspiration and water-use efficiency of grain sorghum under different cultural practices. P. L. Brown and W. D. Shrader (*Agron. J.*, 1959, **51,** 339—343).—Sorghum grain yields over 2 years averaged 11·4, 23·6 and 40·6 bushels per acre where the soil prior to planting had been wetted to field capacity to depths of 3, 5 and 7 ft. respectively. Evapotranspiration averaged 11·6, 14·8 and 17·0 in. of water, and stored soil moisture supplied an average of 49%, 60% and 65% of this evapotranspiration. Water-use efficiency for grain production increased with the depth to which initial soil moisture had been adjusted. Optimum plant population, as reflected in grain yields, increased with initial soil moisture depth, but optimum row spacing was similar for all depths. Both factors varied with season. A. H. CORNFIELD.

Fatty acids of sorghum leaf and stem. M. C. Burnett and R. L. Lohmar (*J. agric. Fd Chem.*, 1959, **7,** 436—437).—The lipids of sorghum leaf and stem were solvent-partitioned into three fractions, the fatty acid composition of which was determined by gas chromatography, and compared with that found for the original extract by alkali isomerisation. Gas chromatography gave the higher values for saturated acids, and lower for oleic acid, and probably gives a better picture of the composition. Fatty acid composition of leaf and stem differed markedly from that of grain, the chief acid being linolenic, which is absent from grain. Saturated acids (mainly palmitic) were more prominent in the leaf and stem, and linoleic and oleic acids in the grain. (16 references.)
M. D. ANDERSON.

Fertility requirements of ladino clover–orchardgrass pastures. W. H. Mitchell and L. J. Cotnoir, jun. (*Delaware agric. Exp. Sta.*, 1959, Tech. Bull. 325, 24 pp.).—Nitrogen fertilisation of ladino clover–orchardgrass pastures increased dry matter production but decreased the % of clover in the pasture. Application of P + K without N gave the best seasonal distribution of growth and highest levels of clover. Split application of N gave better seasonal distribution of forage than did a single application. Dressings of mixed trace elements increased yields at one of two locations.
A. H. CORNFIELD.

Influence of rates of nitrogen on coastal Bermudagrass. J. L. Stephens and W. H. Marchant (*Georgia agric. Exp. Sta.*, 1959, Circ. 13, 14 pp.).—Forage yields of coastal Bermudagrass and live-wt. gains in cattle increased with rates of annual application of N (50—200 lb. per acre). Annual interplanting with crimson clover or Borre lupin was somewhat more effective than the 50-lb. rate, but less effective than the 100-lb. rate, of N in increasing animal gains.
A. H. CORNFIELD.

Legume for native flood meadows. II. Phosphorus fertiliser requirements for maintaining stands of white-tip clover, *Trifolium variegatum.* C. S. Cooper and A. S. Hunter (*Agron. J.*, 1959, **51,** 350—352).—Annual application of 40 lb. of P_2O_5 per acre was sufficient for max. yields and P % of hay on a mixed clover–rush–sedge flood meadow over 3 years. Crude protein % in the hay was increased by P applications only in the second year. P fertilisation increased the proportion of clover in the hay. A. H. CORNFIELD.

Lucerne irrigation for maximum seed production. S. A. Taylor, J. L. Haddock and M. W. Pedersen (*Agron. J.*, 1959, **51,** 357—360).—Max. seed yields from lucerne were obtained with soil moisture in the range of suction equivalent to 2—8 bars (10^6 dynes per cm.^{2}). Highest yields were obtained by keeping the soil at low moisture suction until blossoming and then withholding irrigation water. Reduced yields occurred if plots were irrigated during blossoming.
A. H. CORNFIELD.

Production and botanical composition of legume–grass combinations and influence of legume on associated grasses. G. P. Tewari (*Dissert Abstr.*, 1959, **19,** 2426).—From field studies with vernal lucerne (*Medicago sativa*, var. Vernal), ladino clover (*Trifolium repens*), meadow fescue (*Festuca elatior*), smooth bromegrass (*Bromus inermis*, var. Lincoln), timothy (*Phleum pratense*) and alsike clover (*Trifolium hybridium*), the growing of legumes (lucerne, ladino and alsike clovers) in single, double or triple alternate rows between grass (brome, timothy) rows is not a practical means of controlling the composition of legume–grass pastures. S. C. JOLLY.

Metabolic changes induced in lucerne during cold hardening and freezing. C. R. Swanson and M. W. Adams (*Agron. J.*, 1959, **51,** 397—400).—Respiration during hardening of Arizona Chilean and Teton lucernes was determined daily in plants subjected to four combinations of day length and temp. Respiration increased in plants exposed to alternating temp. regardless of day length and was greater for the hardy Teton than for the non-hardy Arizona Chilean. Respiratory activity after freezing and thawing was related to variety and both temp. and day length during hardening.
A. H. CORNFIELD.

Chemical constituents of turtle grass, *Thalassia testudinum.* P. R. Burkholder, L. M. Burkholder and J. A. Rivero (*Bull. Torrey bot. Club*, 1959, **86,** 88—93).—This grass which grows in shallow tropical waters gave yields in Puerto Rico of 33 tons per acre, with 13% protein, 16% fibre and 36% other carbohydrates (on dry matter basis).
L. G. G. WARNE.

Method of increasing the phosphorus content of forages. R. Ferrando, S. Metivier and R. Gervy (*Nature, Lond.*, 1959, **184,** 76—77).—A second application on grassland, in late winter, of water sol P, especially if associated with N, raises the P content of forages. A preferred dressing is 20% Nitro-chalk and 18% Ca superphosphate (180 lb. of each/acre). Ca : P is 2·5 in comparison with 3·3 for the usual autumn dressing (P and PK) only.
W. J. BAKER.

Problems and practice in fruit tree manuring. G. Scheys (*Agricultura*, 1958, **6,** 271—303).—A review. The rôle of N, P, K, Mg, Ca, S, Si, Al and trace elements in the nutrition of fruit trees is discussed and practical aspects of mineral and org. manuring are considered. A. G. POLLARD.

Magnesium deficiency in apples and oranges. N. J. Halse (*J. Agric. W. Aust.*, 1959, **8,** 417—419).—Magnesium deficiency symptoms in leaves of apple and orange are described and illustrated by colour prints. Control of the deficiency by soil treatment and plant spraying is described. A. H. CORNFIELD.

Chemical control of plant populations in strawberries. C. W. Hitz (*Delaware agric. Exp. Sta.*, 1959, Tech. Bull. 324, 38 pp.).—Application of maleic hydrazide (1000 p.p.m., three sprayings from Aug. 1 to Sept. 15) was compared with hand-pruning (from Aug. 1 onwards) for the control of plant density in strawberry beds. Hand-pruning produced the best results with respect to plant and fruit size and yields. Spray treatments were less effective but gave better results than those of control plots. A. H. CORNFIELD.

Influence of exchangeable potassium and sodium in the soil on growth and chemical composition of Lovell peach seedlings and other crops. J. P. Martin, W. W. Jones and J. O. Ervin (*Agron. J.*, 1959, **51,** 418—421).—Growth of peach seedlings and five other species of crops was studied in base-saturated sandy loam and loam soils having K saturation 2—36% and Na saturation 1—27%. All species tolerated up to 22% K saturation in soil. With 36% K saturation moderate growth reductions and/or leaf injury occurred in peach seedlings, onion, lettuce, radish and carrot. At 13% Na saturation growth of peach seedlings was reduced slightly and at 27% Na saturation was reduced severely. The effect of increasing Na was similar at all levels of K saturation. The 27% Na saturation level reduced growth of radish, carrot and lettuce at all K levels, but reduced lucerne growth only at K saturation levels >8%. Growth of vetch was not affected even by 27% Na saturation at any K level. Reduced growth of peach seedlings was associated with high leaf K % (4% on dry matter basis) and with more than trace amounts of leaf Na. A. H. CORNFIELD.

Leaf diagnosis. I. Relation between abnormal characters in oranges and content of trace elements in leaves. C. Botella Soto, J. Royo Iranzo and E. Primo Yufera (*Bol. Inst. nac. Invest. agron. Madr.*, 1959, **19,** 51—66).—Analyses for ash, Fe, Mn, Zn, B and Cu are reported for old and young leaves from normal and abnormal

trees in various orchards in Valencia. Colour photographs are reproduced of the abnormalities studied. (23 references.)
E. C. APLING.

Leaf analysis in relation to yield and quality of pineapples. K. Kanapathy (*Malayan agric. J.*, 1958, **41,** 18—26).—Yields and size of pineapple fruit were increased by application of K at two locations, max. benefit being obtained with 50—100 lb. of K_2O per acre. High yields and quality (high sugar content) of the fruit were associated with high K (%) and K/P ratio in the leaf tips.
A. H. CORNFIELD.

Relationship between growth and mineral nutrition in pineapples. P. Martin-Prével (*Fruits d'outre mer*, 1959, **14,** 101—122).—Plants were treated with (i) commercial fertiliser (N : P : K = 1 : 1 : 2) or (ii) another with N : P : K = 1 : 1 : 6. The importance of the correct N balance on the wt. of fruit obtained is established. Overdosage of K is only partially balanced by diminution of Ca and Mg and this leads to the formation of org. acids in the fruit. The interaction of K on N, N on K and P, and K and Ca and Mg and also variations in the amounts of these elements in relation to the age of the pineapple and to the climate are investigated. (12 references.) M. C. M.

Effect of nitrogen, phosphorus and potassium fertiliser on fruit yield and composition of tomato leaves. J. L. Malcolm (*J. agric. Fd Chem.*, 1959, **7,** 415—418).—In a fertiliser experiment with tomatoes, yields and N content of leaves, increased with applications of N up to 150 lb. per acre; larger amounts decreased yield, and only slightly increased leaf-N. Applications of K up to 300 lb. per acre increased yields and the K contents of leaves. Applications of P up to 300 lb. per acre increased yields when supplies of N and K were near optimum, and also increased the P content of leaves. Yields showed N–P and P–K interactions; leaf composition showed N–K interaction. The value of P and K was obscured when too much or too little N was supplied. (16 references.)
M. D. ANDERSON.

Growth and chemical composition of onion as influenced by nutrition. J. D. Downes (*Dissert Abstr.*, 1959, **19,** 3077).—Onions were grown on sandy soil with three levels each of N, P, K, S, Mg, Cu, Mn and Zn, and effects on the growth and composition of the leaves and bulbs were determined. N gave increased leaf and bulb growth when it was a limiting factor, but excess of N caused large, probably toxic increases in the Mn content of leaves and bulbs. High levels of P hastened bulb growth while decreasing that of leaves; the P content was much higher in bulbs than in leaves. K increased the yields of both leaves and bulbs, and counteracted the effects of high N, probably by depressing the absorption of Mn and increasing that of Fe. High K also reduced the Mg content, which was little affected by applications of Mg. Zn reduced growth; Cu had erratic effects.
M. D. ANDERSON.

Cotton fertiliser trials on Blackland soils. D. A. Hinkle and J. F. Jacks (*Ark. agric. Exp. Sta.*, 1959, Bull. 613, 10 pp.).—Cotton on this clay soil made no response to P fertilisers. Optimum yields were obtained by application of 120 lb. of N per acre. N applications also increased "first-picking" yields.
A. H. CORNFIELD.

Response of cotton and maize to deep placement of fertiliser and deep tillage. W. H. Patrick, jun., L. W. Sloane and S. A. Phillips (*Proc. Soil Sci. Soc. Amer.*, 1959, **23,** 307—310).—Root development in the subsoil and yields of maize and cotton on silt loam soils were usually increased by deep tillage, in comparison with shallow tillage, and deep, compared with shallow, placement of fertiliser (500—600 lb. 12–12–12, $N–P_2O_5–K_2O$). Yield responses were greatest on soils with compact subsoils or hardpans and in growing season with below-average rainfall.
A. H. CORNFIELD.

Response of cotton to fertilisers in Puerto Rico. G. Samuels, J. P. Rodriguez and P. Landrau, jun. (*J. Agric. Puerto Rico*, 1959, **43,** 89—102).—Leaf-blade N, P and K values were better than petiole tissue values for indicating the nutrient status of the plants with respect to responses to fertilisers. Leaf values 45 days after planting were better than those after 60 or 90 days. No responses were obtained where the leaf blade, sampled 45 days after planting, contained N >5·0, P >0·4 or K >3·2% (dry wt. basis).
A. H. CORNFIELD.

Effect of the reniform nematode on yield, plant characteristics and fibre properties of upland cotton. J. E. Jones, L. D. Newsom and E. L. Finley (*Agron. J.*, 1959, **51,** 353—356).—The reniform nematode delayed maturity and reduced the size of boll, yield of lint, and in some years, lint %, but had no effect on seed size or fibre length, strength or fineness. The nematode increased wilt development on wilt-susceptible, but not on wilt-resistant, varieties.
A. H. CORNFIELD.

Nitrogen sources for Connecticut tobacco. H. C. De Roo (*Conn. agric. Exp. Sta.*, 1959, Bull. 623, 12 pp.).—Nitrogen supplied as NH_3, NO_3^- or urea, when supplemented with urea–formaldehyde fertiliser (side-dressed), was as effective as was cottonseed meal in improving yields and quality of tobacco.
A. H. CORNFIELD.

Ozone in high concentration as cause of tobacco leaf injury. H. E. Heggestad and J. T. Middleton (*Science*, 1959, **129,** 208—209).—Leaf injury known as "weather fleck" has been shown to be due to high concn. of O_3 in the air. Correlations between O_3 concn. and appearance of fleck indicated that 0·2 p.p.m. of O_3 was the threshold value for susceptible varieties of tobacco.
T. G. MORRIS.

Ecological significance of red-light sensitivity in germination of tobacco seed. P. V. Wells (*Science*, 1959, **129,** 41—42).—The light transmission of quartz sand and clay suspensions increased with increasing wavelength of light within the range 400—900 mμ. Thus a seed sensitive to red light can germinate at greater depths than non-sensitive seeds with less risk of desiccation.
T. G. MORRIS.

Effects of previous crops on growth, yield and certain chemical constituents of two soya-bean varieties. P. K. Dutta (*Dissert. Abstr.*, 1959, **19,** 2707—2708).—On high-fertility soils, the yield of soya-beans, as measured by wt. of seed and hay, protein and oil contents of seed, dates of flowering, no. of pods, leaf area, plant height and root wt., was not affected by the nature of the previous crop (maize, oats, wheat, flax, soya-beans).
M. D. ANDERSON.

Optimum plot size for soya-bean yield trials. C. A. Brim and D. D. Mason (*Agron. J.*, 1959, **51,** 331—334).—Optimum plot size in relation to shape, location, soil variability and costs was studied.
A. H. CORNFIELD.

Response of two sugarcane varieties to fertilisers at Rio Piedras, 1954—7. P. Landrau, jun., G. Samuels and S. Alers-Alers (*J. Agric. Puerto Rico*, 1959, **43,** 73—87).—Yields of cane and sugar per acre were increased by application of N, but not by P or K. The treatments had no effect on % of sugar in the cane. Leaf N values were highly correlated, whilst leaf P and K values showed poor correlation, with yields.
A. H. CORNFIELD.

Swellings on shoots of cacao plants deficient in copper. R. G. Lockard, P. Vanathevan and S. Thamboo (*Nature, Lond.*, 1959, **184,** 75—76).—Although swollen-shoot disease of cacao in W. Africa is caused by a virus, the possibility of Cu deficiency being contributory cannot be excluded. Severe symptoms (mainly increased xylem thickness) occurred after ~270 days, in plants grown in sand-culture without Cu.
W. J. BAKER.

Pest Control

Oxygen deficiency as a cause of disease in plants. H. F. Bergman (*Bot. Rev.*, 1959, **25,** 418—485).—A review with an extensive bibliography.
L. G. G. WARNE.

Progress in liquid pesticide formulations. E. Selz and P. Lindner *J. agric. Fd Chem.*, 1959, **7,** 540—543).—New blended anionic–non-ionic emulsifiers permit the prep. in liquid form of many different types of pesticides and pesticide–fertiliser mixtures. Less emulsifier is required; emulsifier in DDT formulations can be reduced from 20 to 2%.
M. D. ANDERSON.

Physico-chemical studies on agricultural sprays. III. Variation of phytotoxicity with chemical structure of surface-active agents. C. G. L. Furmidge (*J. Sci. Fd Agric.*, 1959, **10,** 419—425; cf. J.S.F.A. Abstr., 1959, ii, 118).—The complexity of the phytotoxic behaviour of surface-active agents is described. The non-ionic materials appear to be the safest to use on apples and plums. Damage resulting from the ionic types varies with their chemical nature and with the type of plant. With materials of similar chemical structure the phytotoxicity is governed by the physical size of the ion or mol. of the surface-active agent.
E. M. J.

Toxicology of thiuram disulphide fungicides. F. Bär (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2,** 106).—Experiments on assessing the subacute and chronic toxicity to animals of fungicide residues in plants are described. (In German.)
J. L. PROSSER.

Effects of certain variables on study of pesticide residues. S. Dormal (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2,** 106—107).—Concn., formulation and mode of application of pesticide, nature of substrate and of vegetation, degradation on the plant surface, nature of plant surface in relation to penetration, and sterilising and bleaching effects, are discussed. (In French.)
J. L. PROSSER.

Organic sulphur compounds as insecticides. E. Profft (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2,** 111).—Certain *p*- and *o*-thiocresol ethers, and their chloro and aceto deriv., possess insecticidal properties. Other compounds studied include pyridylethyl deriv. of acetothiocresol ethers and of 2-thiophenothiols, and addition

products of 2-vinylthienyl ketones and certain primary and s-aliphatic and aromatic heterocyclic amines. (In German.)
J. L. PROSSER.

Fungicidal behaviour of 2-o-chlorophenylimino-3-o-chlorophenyl-4-thiazolidone, 3-o-chlorophenyl-2, 4-thiazolidone and their acetoxy mercury derivatives. P. N. Bhargava and I. D. Saxena (*Res. J. Hindi Sci. Acad.*, 1959, **2**, 89—92).—These preparations, together with a number of derivatives, have been synthesised and their fungicidal activity has been examined. (From English summary.)
C. V.

Effect of amount of water used in application on the fungicidal efficiency of zineb. B. Peturson and F. R. Forsyth (*Plant Dis. Reptr*, 1959, **43**, 556—557).—The best control of wheat rust was obtained when tank-mix zineb (nabam 1·5 qt. + $ZnSO_4$ 0·75 lb. per acre) was applied in 5—10 gal. of water per acre. No control was obtained when the material was applied in 50—100 gal. of water per acre. In another year zineb 65% (2 lb. per acre) gave the same degree of control whether applied in 10 or 100 gal. of water per acre.
A. H. CORNFIELD.

Deposit of mist-concentrate spray as influenced by droplet size, air velocity, temperature and humidity. W. W. Gunkel (*Dissert. Abstr.*, 1959, **19**, 257).—A specially designed rotating-disc spray apparatus was constructed to produce droplets of uniform size, 50—150 μ in diameter, with devices to measure and regulate the speed of the spinning top, and to maintain a constant regulated flow of pesticide solution to the sprayer. The effects of droplet size, air velocity, temp., R.H. and leaf position, on the uniformity and extent of the deposits, were analysed. The larger droplets were more effective at the lower temp.
M. D. ANDERSON.

Water-dispersible DDT formulations. S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas (*J. sci. industr. Res.*, 1959, **18A**, 178—182).—A paste formulation incorporating a non-volatile lipoid-dissolving oil and containing thin needle-shaped DDT particles is considered the best.
A. M. SPRATT.

Absorption of some chlorinated hydrocarbon insecticides from soils into various crops. E. P. Lichtenstein (*J. agric. Fd Chem.*, 1959, **7**, 430—433).—Crops grown on soils treated 1—3 years previously with lindane, DDT or aldrin contained insecticide residues to extents depending on crop, type of soil, and nature and concn. of insecticide. Carrots absorbed more insecticide than did any other crop. Absorption was greater from sandy soils than from those with higher content of org. matter. The amount of insecticide absorbed by a given crop from a given soil was not in direct proportion to the concn of insecticide, relatively less being absorbed at the higher concn. Crops grown on aldrin-treated soils contained both aldrin and dieldrin. (17 references.)
M. D. ANDERSON.

New organic fungicide: 1-chloro-2,4-dinitronaphthalene. A. Soenen and B. Grossman (*Agricultura*, 1958, **6**, 183—238).—A review of recent experimental work on the use of this fungicide.
A. G. POLLARD.

Fungicidal activity of derivatives of 2-nitropropane-1, 3-diol. Z. Eckstein, E. Grochowski and T. Urbański (*Bull. Acad. polon. Sci. chim.*, 1959, **7**, 289—294).—A no. of 2-aryl-2-nitropropane-1,3-diols were synthesised; their i.r. spectra and their varying fungicidal activity towards *Fusarium culmorum*, *Alternaria tenuis* and *Rhizoctonia solani* are reported and discussed in relation to mol. structure and substituent position. Contrary to expectation, 2-(2′-quinolyl)-2-nitropropane-1,3-diol is completely inactive. (In English).
W. J. BAKER.

Dalapon residue in birdsfoot trefoil. M. M. Schreiber (*J. agric. Fd Chem.*, 1959, **7**, 427—429).—Birdsfoot trefoil, treated with dalapon to control grass, always contained residues of dalapon in amounts varying with rate and time of application, weather, etc. Applications in spring up to 5·0 lb. per acre effectively controlled grass, and residues at harvest 100 days later did not exceed 25 p.p.m. Residues 22 days after applications of 10 and 15 lb. per acre were 1250 and 1750 p.p.m. Residues of dalapon in forage crops may pass into foods such as milk. (14 references.)
M. D. ANDERSON.

Plant disease control by 5-nitrofuran derivatives in relation to chemical structure. R. G. Owens (*Contr. Boyce Thompson Inst.*, 1959, **20**, 141—149).—The effect of substituents in the 2-position of 5-nitrofuran on its power to control early and late tomato blights and powdery mildew of beans, was tested under greenhouse conditions. ED_{95} values for the more active compounds ranged from about 50 p.p.m. for 3-(5-nitro-2-furyl)acrolein to 250 p.p.m. for 1-(5-nitro-2-furyl)-2-nitroethylene. The presence of an alkylene group at the 2-position increased activity, which also depended on the terminal group, in the order: CHO > NO_2 > CN > COOH. Esterification of a terminal COOH enhanced activity, in the order: Et > Ne > Pr > pentyl. Activity was reduced by terminal hydantoin or pyrrolidone rings or by combination of two nitrofuran moieties. (14 references.)
M. D. ANDERSON.

Influence of chemical structure of 5-nitrofuran derivatives on absorption by fungus spores and on inherent toxicity. R. G. Owens and H. M. Novotny (*Contr. Boyce Thompson Inst.*, 1959, **20**, 151—165).—The influence of substituents in the 2-position on the absorption of 24 derivatives of 5-nitrofuran by conidia of *Neurospora sitophila* was determined. Some of the compounds were taken up in large amounts within 1 min.; with others, uptake was not detectable for <10 min. Rate of uptake during continuous exposure of spores to different compounds was not necessarily in the same order as total uptake in repeated exposures; probably some compounds affect the permeability of the spores. Relatively large amounts of most of the compounds were adsorbed, but compounds inhibiting germination were 1-(5-nitro-2-furyl)-2-nitro-ethylene and -prop-1-ene, 3-(5-nitro-2-furyl)acrolein and four alkyl 5-nitro-2-furyl ketones. The data are discussed with regard to the influence of 2-substituents on the control of tomato foliage blights in the greenhouse, and to the interrelations of inherent toxicity, extent to which the compounds are absorbed and their solubility in lipids. (16 references.)
M. D. ANDERSON.

Properties and fungicidal activities of aryloxyacetohydroxamic acids. II. Influence of bromine as substituent. Z. Eckstein and E. Czerwińska (*Bull. Acad. polon. Sci. chim.*, 1959, **7**, 223—227).—A series of bromophenoxyacetohydroxamic acids was prepared and tested for fungicidal activity against *Fusarium culmorum*, *Alternaria tenuis* (**I**) and *Rhizoctonia solani*. One Br atom introduced in position 2, 3 or 4 gives compounds selectively active only against **I**. Two Br atoms in positions 2,4, 2,5 or 2,6 cause complete disappearance of fungicidal activity. (In English.)
R. J. MAGEE.

Tetrin, a new antifungal antibiotic. H. L. Pote (*Dissert. Abstr.*, 1959, **19**, 2778).—Tetrin, synthesised by a streptomycete, inhibits filamentous fungi and yeasts but not bacteria. It was not toxic to young plants when sprayed on leaves at a concn. of 1000 μg. per ml., and did not damage plant seeds. Spraying decreased leaf spot in soya-beans, but increased the incidence of asparagus rust (*Puccinia asparagi*). Tetrin is a tetraene, absorbing at 291, 305 and 319 mμ in aq. solution; it is stable in basic and neutral solution, but not in acid; sol. in water and lower alcohols; insol. in ether, acetone and hydrocarbons.
M. D. ANDERSON.

Formulation of malathion dusts and powder concentrates. J. F. Yost and L. B. Frederick (*Farm Chem.*, 1959, **122**, No. 10, 64—65).—Factors relating to the stability and efficiency of malathion formulations (pH, storage temp., carriers, moisture content, nature of containers) are noted and recent practices described.
A. G. POLLARD.

Influence of captan on higher plants. II. Effect on specific enzyme systems. W. M. Dugger, jun., T. E. Humphreys and B. Calhoun (*Amer. J. Bot.*, 1959, **46**, 151—156).—The inhibitory effects of captan and of its tetra- and hexa-hydro-analogues on certain hexokinases, decarboxylases and on ribose-5-phosphatase in plants are examined.
A. G. POLLARD.

Diffusion of mercurial [fungicide] in fruit coat of treated seed. O. Lindström (*J. agric. Fd Chem.*, 1959, **7**, 562—566).—The penetration of org. Hg fungicides into seeds is accompanied by decreases in the concn. of Hg. in the atm. round the seeds, and in the outer layer of the seeds. Determinations of these values on wheat grains showed a rapid diffusion of mercurial towards the interior of the seed. The data are used with a simple diffusion model to obtain a single penetration parameter. Rate of penetration is strongly influenced by moisture content, the diffusion parameter increasing 500-fold when the moisture content of the wheat is raised from 12 to 18%.
M. D. ANDERSON.

Effect on honey bees of Thiodan applied to broad beans in a cage. T. Palmer-Jones (*N.Z. J. agric. Res.*, 1959, **2**, 229—233).—The spray was applied 1 hr. before the bees could visit the beans, and for 3 hr. the bees were repelled, but no mortality occurred. Later they collected nectar extensively from the bean flowers. Thiodan can therefore be applied to beans in flower, when bees are not flying.
E. M. J.

Effects of field applications of newer insecticides on honey bees. F. R. Shaw (*J. econ. Ent.*, 1959, **52**, 549—550).—Sevin (1—2 lb./100 gal.) was highly toxic to caged bees for 96 hr. Am. Cyanamid 5223 (dodecylguanidine acetate) had little effect. C. M. HARDWICK.

Plant growth as affected by ethylene dibromide fumigation of soils at two moisture levels. A. L. Brown, J. J. Jurinak and P. E. Martin (*Proc. Soil Sci. Soc. Amer.*, 1959, **23**, 311—313).—Ethylene dibromide (**I**) was injected into three soils at two moisture levels (air-dried and field capacity) at rates of 0·01—5·0 ml. per kg. of soil. Growth of tomato seedlings planted two weeks after treatment was seriously reduced on all soils where more than 0·01 ml. had been applied and the soils stored air-dried before planting. Where the soils had been stored at field capacity there was little growth reduction with up to 1·0 ml. of **I** in a loam and silty clay, whilst growth

was severely reduced with more than 0·05 ml. of **I** in a peaty muck. Plant-Br (%) increased with rate of application of **I**.
A. H. CORNFIELD.

Analysis of insecticidal thiophosphoric acid esters. E. Sándi (*Z. anal. Chem.*, 1959, **167**, 241—253).—Four halogenated analogues of methyl parathion are submitted to chromatography on a polythene-packed column (cyclohexane as stationary phase) in a buffer pH 4·6. These esters are readily separated from hydrolysis products (the corresponding nitrophenols) and can then be determined polarographically at the dropping Hg electrode, the buffer solution being used as solvent. J. P. STERN.

Micro-determination of 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)-ethane (TDE) in spray residues. I. Rosenthal, C. F. Gordon and E. L. Stanley (*J. agric. Fd Chem.*, 1959, **7**, 486—488).—The insecticide TDE is determined in plant extracts (after partial purification by solvent partitioning into acetonitrile, treatment with absorbent, and removal of HCl) by reaction with Na ethylate in dimethylformamide to yield 1-chloro-2,2-bis-(*p*-chlorophenyl)ethylene, which on treatment with H_2SO_4 gives a coloured carbonium ion complex with max. absorption at 502 mμ. The method detects 1·0 μg.; recovery of TDE is 62—65%. M. D. ANDERSON.

Colorimetric determination of malathion residues in cottonseed. M. N. Norris and E. J. Kuchar (*J. agric. Fd Chem.*, 1959, **7**, 488—489).—The colorimetric method previously described (see Norris *et al.*, J.S.F.A. Abstr., 1958, ii, 87) for determining residues of malathion is adapted for use on cottonseed by Soxhlet extraction with hexane, extraction of residues into acetonitrile, treatment with acid-washed alumina to remove coloured material, and extraction into CCl_4, followed by the procedure already described.
M. D. ANDERSON.

Chemical control of cereal rusts. J. G. Dickson (*Bot. Rev.*, 1959, **25**, 486—513).—A review with an extensive bibliography.
L. G. G. WARNE.

Antibiotics as seed treatments for control of grain smuts. W. F. Crosier (*Plant Dis. Reptr*, 1959, **43**, 616—618).—Treatment of seed with 0·5—1·0% actidione reduced partially the % of smutted heads in oats and wheat, but was ineffective for barley or for reducing seed decay of flax or seedling blight of wheat. Actidione and three of its derivatives, agrimycin, and griseofulvin did not control head smut of bromegrass. Cycloheximide and its acetate, semicarbazone and oxime reduced the incidence of oat smuts and nearly eliminated stinking smut of wheat, whilst agrimycin and griseofulvin gave only slight control. A. H. CORNFIELD.

Aphid populations on oats grown in various nutrient solutions. B. F. Coon (*J. econ. Ent.*, 1959, **52**, 624—626).—More *Rhopalosiphum fitchii* were found on seedlings given available N in nutrient solutions. The presence of P and K was also of some importance.
C. M. HARDWICK.

Commercial application of insecticides for control of sap beetles on sweet maize. J. T. Whitlaw, W G. Phillips and L. P. Ditman (*J. econ. Ent.*, 1959, **52**, 640—642) —The greatest reduction in no. of *Carpophilus lugulris* and the lowest reinfestation rate followed spraying with malathion. Residues of diazinon and parathion were more persistent than those of malathion. Increased use of oil was associated with greater toxicity. Spraying is most effective when 50% of the plants are in silk. C. M. HARDWICK.

Control of northern maize leaf blight, *Helminthosporium turcicum*, Pass., in the Everglades. R. S. Cox and D. S. Harrison (*Fla agric. Exp. Sta.*, 1958, Bull. 596, 20 pp.).—The effectiveness of a number of fungicides for controlling leaf blight decreased in the order mabeb, zineb + thiram, zineb, nabam + $ZnSO_4$ = Amobam = Dyrene.
A. H. CORNFIELD.

Maize seed treatments with fungicidal dusts. F. J. Nicholson (*Agric. Gaz. N.S.W.*, 1959, **70**, 37—38, 42).—Of materials tested, only "Tetroc" ("Coversan," 1 oz. per bushel of seed) consistently improved germination and yields. A. H. CORNFIELD.

Root and stalk rot in maize as affected by fertiliser and tillage treatment. D. T. Parker and W. C. Burrows (*Agron. J.*, 1959, **51**, 414—417).—High incidence of root and stalk rot of maize, due to *Fusarium moniliforme* and *Gibberella zeae*, occurred with high N applications. Where crop residues were left on the soil surface rot incidence was lower than where residues were ploughed under or removed. High applications of K had a significant, though slight, effect in reducing incidence of rot. Low rot incidence was associated with a low leaf N/K ratio. A. H. CORNFIELD.

Diseases of padi and their control. A. Johnston (*Malayan agric. J.*, 1958, **41**, 10—17).—A general account. A. H. CORNFIELD.

Field evaluation of Thiodan as an insecticide for potatoes. D. H. Moore (*J. econ. Ent.*, 1959, **52**, 564—567).—Thiodan spray was more effective than the usual parathion spray against *Epoasca fabae*, *Leptinotarsa decemlineata* and *Epitrix cucumeris* for three seasons on early and late crops. Thiodan mixed with fungicides resulted in more live foliage than when used alone. All treatments increased yields. C. M. HARDWICK.

Pentachloronitrobenzene and urea–formaldehyde for potato scab control. H. S. Potter, W. J. Hooker, W. Cargo and G. T. Stachwick (*Plant Dis. Reptr*, 1959, **43**, 633—637).—The application of pentachloronitrobenzene (50 lb.) or urea–HCHO (UF-85), 26% urea–59%, HCHO, 37 gal. per acre) reduced scab incidence in potatoes to about the same extent at a number of locations.
A. H. CORNFIELD.

Control of potato seed-piece decay with captan and streptomycin. D. F. Crossan (*Plant Dis. Reptr*, 1959, **43**, 543—545).—Potato seed-piece decay (*Erwinia carotovera*), resulting in decreased emergence, was reduced by treating the seed-piece with captan (2 lb. per 100 gal.) or streptomycin (100 p.p.m.) just prior to planting. Reduced emergence due to *Fusarium* infection was controlled by captan but not by streptomycin. A. H. CORNFIELD.

Control of turf grubs. J. B. Polivka (*Ohio agric. Exp. Sta.*, 1959, Res. Bul. 829, 30 pp.).—Annual applications of chlordane (5—10 lb.) or DDT (37·5 lb. per acre) controlled the grubs of the Japanese and June beetles and the northern masked chafer for 12 years.
A. H. CORNFIELD.

Fumigation of lucerne seed with methyl bromide for the control of the stem eelworm *Ditylenchus* (*Anguillulina*) *dipsaci*. A. B. P. Page, N. G. M. Hague, V. Jakabsons and R. E. Goldsmith (*J. Sci. Fd Agric.*, 1959, **10**, 461—467).—Nematodes removed from debris and treated on filter paper were all killed by fumigation at a concn.–time product of ≮850 mg. hr./l. The treatment had little effect on germination. In field trials six tons of lucerne seed fumigated at a concn.–time product of 1250 mg. hr./l. under a gas-proof sheet, showed no significant reduction in yield. Complete control may not have been obtained as eelworm symptoms appeared in fields sown.
E. M. J.

Control of *Hypera pastica* (Gyll.) on second-cutting lucerne in New York. C. S. Koehler, G. O. Poinar, jun., and G. G. Gyrisco (*J. econ. Ent.*, 1959, **52**, 590—592).—Heptachlor spray (4 oz./acre) controlled weevil larvae for 32 days and at 8 oz./acre, adults for 2 weeks. Chlordane (32 oz.) and lindane or endrin (4 oz.) were effective against the larvae for 14 days. Compounds were ineffective after one week.
C. M. HARDWICK.

Development of "ripe spot" on apples during storage. W. B. McGlasson and G. W. Botting (*J. Agric. S. Aust.*, 1959, **62**, 362—364).—Pre-harvest sprays of TMTD did not control development of ripe spot, due to *Gloeosporium album*, of Statesman apples during storage at 0°. Fruit picked on April 22 developed much less ripe spot during storage for 5—7 months than did fruit picked on May 13.
A. H. CORNFIELD.

McIntosh apple seedlings for the bioassay of fungicides for the control of apple scab. A. E. Rich and M. C. Richards (*Plant Dis. Reptr*, 1959, **43**, 540—542).—A technique for testing fungicides for the control of apple scab (*Venturia inaequalis*) using McIntosh apple seedlings with 3—4 leaves is described. Results obtained were closely correlated with field tests of fungicidal activity.
A. H. CORNFIELD.

Control of grey mould, *Botrytis cinerea*, Pers., on strawberries. P. M. Miller and E. M. Stoddard (*Plant Dis. Reptr*, 1959, **43**, 646—648).—Pre-harvest grey mould rot of strawberries was reduced to a greater extent by Thylate (3 lb. than by Phygon XL (0·37 lb.) or Orthocide (6 lb. per 100 gal.) with 3—5 sprayings given during May or May—June. Earlier applications gave poorer control. Thylate applied pre-harvest was also the most effective material for reducing the extent of grey mould 3—6 days after harvest.
A. H. CORNFIELD.

Control of peach twig borer on almonds and peaches in California. F. M. Summers, D. Donaldson and S. Togashi (*J. econ. Ent.*, 1959, **52**, 637—639).—Spraying of non-bearing trees with Thiodan, endrin, and Sevin reduced damage by *Anarsia lineatella* greatly. Guthion, parathion, Trithion and malathion were also promising. Thiodan, Trithion and Sevin were compared with DDT, Pb arsenate and parathion on fruiting trees. In no case were two applications adequate against a very heavy infestation. C. M. HARDWICK.

Control of shothole disease of chokecherry with dodecylguanidine acetate. R. E. Inman and J. L. Weihing (*Plant Dis. Reptr*, 1959, **43**, 536—539).—Among fungicides tested for control of shothole disease (due to *Coccomyces lutescens*) of chokecherry seedlings Cyprex (70% dodecylguanidine acetate, 1 lb. per 100 gal. applied 9 times at 2-weekly intervals commencing May 11) was the most effective.
A. H. CORNFIELD.

Diphenyl-induced variations in citrus blue mould. P. R. Harding, jun. (*Plant Dis. Reptr*, 1959, **43**, 649—653).—Properties of resistant and semi-resistant (to diphenyl) strains of citrus blue mould produced by continued exposure to diphenyl, are described.
A. H. CORNFIELD.

Control of pineapple gummosis in Puerto Rico. M. E. Pérez-Escolar (*J. Agric. Puerto Rico*, 1959, **43**, 116—127).—Of a number of materials tested toxaphene, chlordane and heptachlor effectively controlled gummosis when applied twice during the blossoming period and left residues within the fruit below the acceptable limits. A. H. CORNFIELD.

Effect of nitrogen, phosphorus and potassium levels on growth and virus content of excised tobacco-mosaic virus-infected tomato roots. A. Papasolomontos and R. E. Wilkinson (*Phytopathology*, 1959, **49**, 229).—The N and P requirements of virus-infected plants exceeded those of normal plants; the K requirement was similar in both. The concn. of virus particles in infected plants increased with the supply of N or P to the plants, K having little effect. A. G. POLLARD.

Systemic compounds for control of asparagus rust. H. H. Murakishi (*Plant Dis. Reptr*, 1959, **43**, 552—555).—Of seven materials tested actidione-S (semicarbazone analogue of cycloheximide) and D-113 (1,2-dichloro-1-methylsulphonylethylene) were the most promising for the control of asparagus rust, due to *Puccinia asparagi*. In field trials two applications of actidione-S (100 p.p.m.) were as effective in controlling rust as were 6 applications of Dithane Z-78 (2 lb. per 100 gal.). A. H. CORNFIELD.

Research on hop virus diseases at East Malling. J. T. Legg and P. J. Ormerod (*Brew. J., Lond.*, 1959, **95**, 247—250).—The evidence for transmission through the soil and for spread by a mobile insect agent is considered separately. The symptoms of split leaf blotch are described and reduction of yield (~50%) from affected plants of the variety Fuggle is discussed. E. M. J.

Methyl bromide for the control of root-knot nematode and *Fusarium* wilt. I. J. Thomason (*Plant Dis. Reptr*, 1959, **43**, 580—583).—Chisel application (8 in. depth) of methyl bromide (200—300 lb. per acre) 2 days prior to sowing beans controlled root galling, *Fusarium oxysporium*, and *Pythium* sp., but not *Rhizoctonia solani* or *Stemphyllium* sp. A. H. CORNFIELD.

Influence of nitrogen, phosphorus and potassium on the mortality of groundnut caused by root rot disease, *Sclerotium rolfsii*, Sacc. H. D. Dubey (*Agron. J.*, 1959, **51**, 369—370).—Mortality of groundnuts due to *Sclerotium* root-rot disease was unaffected by application of N (10 lb. per acre), increased by application of P (40—80 lb. of P_2O_5 per acre), and decreased by application of K (25 lb. of K_2O per acre). A. H. CORNFIELD.

Evaluation of six stickers in combination with Parzate for the control of coffee rust. R. B. Valdez, A. N. Pordesimo and F. T. Orillo (*Plant Dis. Reptr*, 1959, **43**, 562—564).—Addition of six different stickers to Parzate (Zn ethylenebis-dithiocarbamate) spray solutions had no significant effect on the extent of rust control. Parzate was as effective as was Bordeaux mixture for controlling rust. A. H. CORNFIELD.

Control of scab on pecan. J. R. Cole (*Plant Dis. Reptr*, 1959, **43**, 658).—Application of Bordeaux mixture 4–1–100 (two pre-pollination sprays) + Bordeaux mixture 6–2–100 (four cover sprays) gave the best control of pecan scab and the greatest increase in nut yields per tree. Puratised Agricultural spray, ziram and zineb, all with oil, also increased nut yields. All treatments increased nut size. A. H. CORNFIELD.

Control of oil palm seedling blight. A. Johnston (*Malayan agric. J.*, 1959, **42**, 14—20).—Leaf spot, due to *Curvularia*, of oil palm seedlings was controlled to a moderate extent by weekly spraying with 0·2% thiram or zineb from 6 to 10 months of age. 0·1% Cu oxychloride and 0·2% captan were relatively ineffective. Frond length was significantly increased by the zineb and captan treatments. A. H. CORNFIELD.

Toxicity of several insecticides to two species of beneficial insects on cotton. H. R. Burke (*J. econ. Ent.*, 1959, **52**, 616—618).—Contact and topical application showed dieldrin to be the least toxic to *Hippodamia convergens*. Endrin was much less toxic on contact than when applied topically. Toxaphene + DDT, dieldrin + DDT, heptachlor, DDT, parathion and methyl parathion gave similar results in both cases. When applied to cuttings the order of toxicity to *Orius insidiosis* was: toxaphene < DDT < dieldrin, heptachlor < aldrin, malathion and methyl parathion < parathion. C. M. HARDWICK.

Comparative spray coverage of cotton plants with a conventional and a high-clearance sprayer. R. E. Fye, A. R. Hopkins and W. W. McMillian (*J. econ. Ent.*, 1959, **52**, 592—596).—Both types of sprayer deposited most droplets where most *Anthonomus grandis* were found. The conventional sprayer gave better coverage of squares and the underside of leaves but this advantage was offset by greater damage to maturing terminals. C. M. HARDWICK.

Insect pests of ornamental plants. L. C. Kuitert (*Fla agric. Exp. Sta.*, 1958, Bull. 595, 51 pp.).—The pests and methods of controlling them are described. A. H. CORNFIELD.

Effect of fungicide-asphalt mixtures on growth of *Ceratocystis fimbriata*, *F. plantani*, *in vitro*. C. May and J. G. Palmer (*Plant Dis. Reptr*, 1959, **43**, 565—566).—Asphalt paint containing 0·5% of dichlone, ferbam, Phaltan or thiram prevented growth of *C. fimbriata* (which causes a fatal disease on the London plane tree) in *in vitro* tests. The asphalt paint by itself did not prevent growth of the fungus. A. H. CORNFIELD.

Dorsal contact toxicity of six insecticides to wintering larvae of European pine shoot moth. D. L. Haynes (*J. econ. Ent.*, 1959, **52**, 588—590).—No significant mortality was obtained by applying DDT, BHC or chlordane to the dorsal surface of *Rhyacionia buoliana*. Good field control suggests that DDT is a stomach poison. Dimethoate, malathion and Thimet gave high mortalities. C. M. HARDWICK.

Movement in soil of herbicides of the substituted urea type [diuron]. P. Subra and J. Guillemot (*Fruits d'outre-mer*, 1959, **14**, 123—124).—A method for investigating the movement consists of spraying pots of soil (3 types) with 80% diuron (1·6 kg./hr.), spraying with different vol. of water to represent 1—12 in. rainfall, and then planting *Vigna onguiculata* seeds in the pot; the main root of the plant stops growing when it has reached the level to which the herbicide has been washed down. Preliminary results indicate that the herbicide is washed down by the first few in. of water to a depth (2·5—3 cm., dependent on soil permeability) where it is stabilised; further "rainfall" moves it only slowly. This is particularly marked in permeable soil (sandy loam). C. H. G.

Preparation of "double phenoxy" compounds and preliminary evaluation of their herbicidal activity on mesquite seedlings. C. F. Krewson and R. Behrens (*J. agric. Fd Chem.*, 1959, **7**, 551—553).—Cl-substituted phenoxyalkyl esters of Cl-substituted phenoxyalkanecarboxylic acids ("double phenoxy" compounds) were synthesised, and tested for herbicidal activity on greenhouse-grown mesquite seedlings. Of 14 esters, six compared favourably in activity with the 2,4,5-trichlorophenoxyacetic acid and 2-(2,4,5-trichlorophenoxy)propionic acid used as standards. M. D. ANDERSON.

Comparative study on effect of α-naphthylacetic acid (NAA) and of 2,4-dichlorophenoxyacetic acid (2,4-D) and of their nitriles (NAN and 2,4-DN) on root growth. L. Ferenczy, G. Matolcsy and B. Matkovics (*Acta biol.*, 1958, **4**, 1—7).—2,4-DN was somewhat less active than the corresponding acid, 2,4-D, in inhibiting the root growth of seedlings of 8 spp. of dicotyledonous food plants. NAN was considerably less active than NAA. (29 references.) M. D. ANDERSON.

Attacking weeds with Simazin. T. Vossen (*Landbouwvoorlichting*, 1959, **16**, 270—276).—A report is given of field tests in 1958, using Simazin to control weeds in maize, potatoes, asparagus and other crops. Good results were obtained, without crop damage, for maize and old asparagus; Simazin is not suitable for potato crops. J. M. HUBBARD.

Absorption and translocation of dalapon in northern nutgrass (*Cyperus esculentus*, L). W. J. Saidak (*Dissert. Abstr.*, 1959, **19**, 2419—2420).—Using ^{14}C-labelled dalapon (2,2-dichloropropionic acid) it was shown that basipetal translocation was similar in mature leaves of nutgrass and quackgrass and was inhibited by concn. producing rapid necrosis. Most of the dalapon given to soil or to solution cultures was rapidly transported to the leaves by nutgrass plants, but high concn. inhibited this movement; little herbicide was found in the roots or tubers. Little translocation occurred when a leaf was dipped into a solution of dalapon. The erratic field control of nutgrass by dalapon is not necessarily due to differences in translocation to the tubers of the herbicide applied to the soil or leaves. S. C. JOLLY.

Comparative herbicidal activities of carbamates and *N*-substituted derivatives. D. Stefanye and H. R. DeRose (*J. agric. Fd Chem.*, 1959, **7**, 425—427).—Comparative tests of the toxicity to oats of *iso*propyl carbamates and their *N*-substituted derivatives are reported. The presence of a H atom on the N is not necessary to produce strong phytotoxic activity. A halogen in the *meta* position on the phenyl group increases activity. The carbamates are more toxic than the corresponding imidodicarboxylates. M. D. ANDERSON.

Herbicidal agents as possible aids for roguing diseased seed-potato plants. G. L. Barnes (*Amer. Potato J.*, 1959, **36**, 212—218).—In greenhouse and field trials diesel oil + 0·4% 2,4,5-T (triethanolamine salt), diesel oil + 30% *iso*propyl *N*-(3-chlorophenyl)carbamate, and diesel oil + 1% Dow General weed killer (55% 4,6-dinitro-*o*-s-butylphenol) were satisfactory for roguing diseased potato plants. Other materials had a slow killing action and failed to prevent regrowth from seed-pieces. A. H. CORNFIELD.

Effect of 2,4-D on growth, seed production and seed viability of rose clover, *Trifolium hirtum* All. W. A. Williams and O. A. Leonard

(*Agron. J.*, 1959, **51**, 383—387).—Application of 2,4-D amine and ester formulations (0·75—3·0 lb. acid-equiv. per acre) during the rosette and early bud stages had no effect on the germination of seed of rose clover. Seed and forage yields were reduced by applications at these stages, the ester form being the more toxic in this respect. Neither form caused damage when applied to ripe seed, whilst both forms drastically reduced seed production and germination when applied during the bloom stage. A. H. CORNFIELD.

Weed control in peas. G. A. Pearce (*J. Agric. W. Aust.*, 1959, **8**, 21—23).—Early-germinating weeds were controlled by spraying 2—3 days before pea emergence with MCPA or 2,4-D amine (2,4-D acid-equiv., 4 oz.) + DNBP, 0·5 gal. per acre. The only post-emergence treatment which did not damage the peas was 16% DNBP (1 gal. in 50 gal. of water per acre). A. H. CORNFIELD.

Chemical control of algae and other nuisance growths on greenhouse benches, pots and potting soil. O. D. Morgan (*Plant Dis. Reptr*, 1959, **43**, 660—663).—Application of Chemical 5400 [αα-trithiobis-(*N*-dimethylthioformamide)] at 800—1200 p.p.m. to greenhouse benches and pots prevented growth of algae for ~3 months. The treatment controlled algal growth on sand for ~5 months. Control of moss by the chemical was not as effective as was control of algae. A. H. CORNFIELD.

Controlling weeds by pasture renovation. J. M. O'Neil (*J. Agric. S. Aust.*, 1959, **62**, 444—450, 456).—A general account. A. H. CORNFIELD.

Control of the spear thistle, *Cirsium lanceolatum*, (L.), Scop. G. R. W. Meadly (*J. Agric. W. Aust.*, 1959, **8**, 33—35).—The weed is controlled by application of 2,4-D ester (1 lb. acid-equiv. per acre). A. H. CORNFIELD

Phosphoric esters. Olin Mathieson Chemical Corp. (B.P. 797,392, 17.7.56. U.S., 20.7.55).—Compounds $CO_2R{\cdot}CA : CB{\cdot}O{\cdot}PX(OR)_2$ (R is alkyl of 1—5 C ; X is O or S ; A and B together are trimethylene or tetramethylene), useful as insecticides, are obtained by interaction of the corresponding dialkyl halogenophosphate with an alkali metal salt of the enol form of the corresponding carbocyclic ketoester in an inert solvent. Thus, $(OEt)_2POCl$ is added dropwise at 70° to a suspension of the Na salt of ethoxycarbonylcyclopentanone in benzene, then after 3 hr. at the boil water is added and *1-ethoxycarbonylcyclopent 1-en-2yl diethyl phosphate* is isolated. F. R. BASFORD.

Insecticidal composition. S. C. Johnson & Son Inc. (B.P. 799,905, 14.2.56. U.S., 18.2. and 7.3.55).—An aerosol composition for use against house and garden insects, consists of <5 wt.-% of a toxic agent selected from DDT, methoxychlor, Strobane, and chlordane ; 0·5—5 wt.-% of pyrethrins ; a synergist for the latter, e.g., piperonyl butoxide (~5 pt. per pt. of pyrethrins) ; and volatile liquid carrier, b.p. 85—200°F (aliphatic hydrocarbon of <8 C, alkanol, ketone, ether or ester). F. R. BASFORD.

Trichloromethylthiosulphonate compounds. American Cyanamid Co. (B.P. 797,389, 21.6.56. U.S., 27.6.55).—Compounds $R(SO_2{\cdot}S{\cdot}CCl_3)_n$ (R is alkyl, aralkyl, alkenyl or alicyclyl ; *n* is 1—2), useful as fungicides, are obtained by treating $R(SO_2Cl)_n$ with alkali metal sulphite in aq. medium at 0—50° while keeping neutral with NaOH, then diluting with water and adding $ClS{\cdot}CCl_3$. Thus, a mixture of $MeSO_2Cl$ and aq. Na_2SO_3 is stirred at 5—10° while keeping neutral with 25% aq. NaOH, then 1 vol. of water is added. $ClS{\cdot}CCl_3$ is now charged during 1 hr., and after stirring during a further 1 hr. the pptd. *trichloromethyl methanethiosulphonate*, m.p. 54—54·5°, is filtered off. F. R. BASFORD.

Preparation and application of iminyl phosphates. California Spray-Chemical Corp. (B.P. 798,703, 24.11.55. U.S., 24.11.54).—Compounds useful, e.g., as miticides, comprise *O*-organo iminyl phosphates or thionophosphates. As an example of one method of prep., $(OEt)_2POCl$ is added slowly at room temp. to a mixture of benzene and the Na salt of acetoxime, and after subsequently heating at 75° during 2 hr. pptd. NaCl is separated off. The filtered liquor is distilled (molecular still), to give *Et₂ prop-2-ylideneiminyl phosphate*, b.p. 78—81°/9 × 10^{-3} mm. This (30) may be compounded with xylene (60) and commercial non-ionic, alkylphenol–polyethylene glycol ether emulsifying agent (10%), and an aq. spray prepared by diluting the resulting concentrate with 1000 pt. of water applied to Ford-Hook lima bean plants infested with *Tetranychus bimaculatus* Harvey, results in 100% kill thereof within 24 hr. F. R. BASFORD.

Fungicides. Farbenfabriken Bayer A.-G. (B.P. 799,320, 20.5.57. Ger., 18.5.56).—Tetramethylthiuram disulphide is compounded (4) with S (3—4·5 pt.), to provide a fungicidal composition highly active against *Venturia inaequalis* and *Phytophthora infestans*. F. R. BASFORD.

Horticultural pest control agents. M. Lumb and C. L. Duddington (B.P. 800,736, 27.1., 5.31., and 30.10.56).—A composition for use in control of eelworm infestation in soil comprises propagules (spores or hyphae capable of further growth) of a predacious fungus, viz., Moniliaceae, and a solid org. carrier decomposable in soil. Among the fungi which may be used the following are specifically mentioned : *Dactylaria thaumasia*, Dreschler, *D. candida*, (Nees) Sacc., *D. doedycoides*, Dreschler, *D. ellipsospora*, (Preuss) Grove, *Arthrobotrys conoides*, Dreschler, *A. robusta*, Duddington, and *Trichothecium flagrans*, Duddington. A suitable medium for propagating the fungus comprises glucose 0·25, peptone 0·25, glycine 0·5 NaCl 0·5, maize steep liquor 0·5, maize meal 0·1 and water to 100 wt.-%. F. R. BASFORD.

Sulphur-containing benzoxazole derivatives and compositions containing them. Boots Pure Drug Co. Ltd. (Inventors : H. A. Stevenson, D. Greenwood and J. E. Cranham) (B.P. 800,713, 27.4.55).—Compounds $R{\cdot}[CH_2]_m{\cdot}S{\cdot}[CH_2]_n{\cdot}R'$ (R is benzoxazol-2-yl ; R' is Ph optionally substituted by halogen or NO_2 ; *m* and *n* are 0—1 but when *m* is 0 then *n* is 1) are claimed, also compositions (dusts, suspensions or emulsions) containing them. The products are useful in the control of eggs and active stages of mites, especially *Tetranychus telarius*, L., *Metatetranychus ulmi*, Koch., and *M. bioculatus*, Wood-Mason. As an example of one method of prep., a mixture of *p*-bromobenzyl bromide, 2-mercaptobenzoxazole, K_2CO_3 and benzene is boiled during 2 hr., then solvent is removed and water is added, with pptn. of 2-(*p-bromobenzylthio)benzoxazole*, m.p. 70—72°. F. R. BASFORD.

Mercaptomethyl dialkyl dithiophosphates and insecticidal compositions [containing them]. Stauffer Chemical Co. (Inventor : L. W. Fancher) (B.P. 800,416, 15.3.56).—Compounds $OR(OR'){\cdot}PS{\cdot}S{\cdot}CH_2{\cdot}S{\cdot}C_6H_4Cl$-*p*, useful as insecticides and acaricides, are obtained by interaction of $OR(OR'){\cdot}PS{\cdot}SX{\cdot}$ with *p*-$ClC_6H_4{\cdot}S{\cdot}CH_2Cl$ in an org. solvent (X is alkali metal ; R and R' are alkyl of 1—5). Thus, a mixture of *p*-$ClC_6H_4{\cdot}S{\cdot}CH_2Cl$, Pr^iOH and $(OPr^i)_2PS_2K$ is boiled during 2 hr., then freed from most of the solvent, to leave a residue from which *OO-di-isopropyl* S-(p-*chlorophenylthiomethyl*) *phosphorothiolothionate* is isolated. F. R. BASFORD.

Insecticides. J. N. Escobar (B.P. 800,411, 4.8.55. Sp., 4.4.55).—The *OO*-dimethyl phosphorothiolothionate of diethyl mercaptosuccinate is compounded with a terpene hydrocarbon and the mixture is then treated with Cl_2 or NaClO to oxidise the phosphate and thereby provide a stable insecticidal composition free from unpleasant odour. If desired, pyrethrin or other insecticide may be incorporated. F. R. BASFORD.

Pest control composition. Fahlberg-List G.m.b.H. (B.P. 800,568, 12.6.57. Ger., 13.6.56).—The fungicidal activity of Cu-containing compositions is synergised by addition of a compound of Ag, Cu, Co, Mo or Sn, e.g., a prep. (**I**) containing 35% of Cu oxychloride and 3% of Co dichromate. For control of a hectare of land infested with *Phytospora*, 900 g. of Cu oxychloride added in the form of **I** was required, but 2500 g. of the Cu compound in absence of the Co. F. R. BASFORD.

Insecticidal compositions. State of Israel and M. Neeman (B.P. 800,490, 4.2.57. Israel, 12.2. and 3.6.56).—The compositions contain DDT and, as synergist, one or more compounds of the formula *p*-$ClC_6H_4{\cdot}SO_2{\cdot}NR_2$ where R is an *n*-alkyl radical having 2—7 C-atoms (e.g., *NN*-diethyl-*p*-chlorobenzenesulphonamide). I. JONES.

Production of salts of aryloxyethanolsulphuric acid half esters. Henkel & Cie G.m.b.H. (B.P. 798,476, 13.6.55. Ger., 14.6.54).—Water-sol. salts, viz., $OR{\cdot}[CH_2]_2{\cdot}O{\cdot}SO_3M$ (R is aryl ; M is cation), useful as selective weed-killers, are readily obtained by interaction of ROH with $X{\cdot}[CH_2]_2{\cdot}O{\cdot}SO_3M$ (X is halogen) at 50—150° in presence of acid-binding agent. Thus, a mixture of cryst. $Cl{\cdot}[CH_2]_2{\cdot}O{\cdot}SO_3Na$, PhOH and 8% aq. NaOH is boiled during 8 hr., then neutralised with a small amount of H_2SO_4. The solution is evaporated to dryness, the residue is extracted with EtOH, and the extract is freed from solvent, to give Na phenoxyethyl sulphate. F. R. BASFORD.

Animal Husbandry

Chromic oxide as an indicator for determining digestibility of beef cattle rations. N. W. Bradley (*Dissert. Abstr.*, 1959, **19**, 2704).—The rate of excretion of Cr_2O_3 by steers on a pelleted ration was found to be too variable to allow determinations of digestibility to be made on samples of faeces taken at random. Sampling plan should be determined for the exact conditions of each experiment. The patterns of excretion were similar after administration of Cr_2O_3 in capsules or mixed with the feed, but were more variable

with capsules. Variability was not lessened by giving food containing Cr_2O_3 frequently throughout the day at the max. rate of intake. M. D. ANDERSON.

Use of radioactive chromium oxide in digestibility determinations. E. A. Kane, W. C. Jacobson and P. M. Damewood, jun. (*J. Dairy Sci.*, 1959, **42**, 1359—1366).—Use of $^{51}Cr_2O_3$ as an indicator in these determinations economises time and labour without sacrifice of precision. Disposal of radioactive excreta, etc., presents a difficulty. A. G. POLLARD.

Composition of normal-acid fibre. D. M. Walker (*J. Sci. Fd Agric.*, 1959, **10**, 415—418).—Normal-acid fibre (NAF) extracted from coarse fodders and sheep faeces was analysed for lignin, true cellulose, cellulosan, protein and ash contents. Digestion of the original material with N-H_2SO_4 for 1 hr. causes loss of cellulosan and partial removal of protein, but most of the lignin and pure cellulose is retained in the NAF. (11 references.) E. M. J.

Biological value of proteins. A. de Vuyst, M. Vanbelle, R. Arnoud, W. Vervack and A. Moreels (*Agricultura*, 1958, **6**, 455—510, 531—583).—A critical review of numerous methods, *in vivo* and *in vitro*, for assessing the biological value of proteins. A. G. POLLARD.

Influence of increasing nitrogen level on essential amino-acid content and on biological value of the proteins of potatoes (B.L. Oser's E.A.S. index). Preliminary communication. I. W. Schuphan (*Z. PflErnähr. Düng.*, 1959, **86**, 1—14).—The biological value of potato protein is appreciably affected by N fertilisers. With low levels of P and K, the optimal N application was 40/60 kg./ha. and with high P and K levels up to 200 kg./ha. was needed. Of the amino-acid components of the proteins the proportions of leucine, isoleucine and arginine were most affected and lysine and phenylalanine only to a smaller extent by the level of N manuring Histidine showed only slight effects. M. LONG.

Rapid method for determining the optimal biological value of potato proteins in nitrogen fertiliser trials. Preliminary communication. II. W. Schuphan (*Z. PflErnähr. Düng.*, 1959, **86**, 14—16).—Protein quality is assessed as the E/R ratio, where both E the total yield, and R the crude protein (total N × 6·25) content are calculated on a dry matter basis. M. LONG.

Seed protein sources; amino-acid composition and total protein content of various plant seeds. C. R. Smith, jun., M. C. Shekleton, I. A. Wolf and Q. Jones (*Econ. Bot.*, 1959, **13**, 132—150).—The amounts of the several amino-acids found in the seeds of nearly 100 species of plants are listed. Many of the seeds are known to have high food values. (72 references.) L. G. G. WARNE.

Toxicity of amine-extracted soya-bean meal. J. Greenberg, D. J. Taylor, H. W. Bond and J. F. Sherman (*J. agric. Fd Chem.*, 1959, **7**, 573—576).—Soya-bean meal extracted with trichloroethylene (TCE) is toxic for many animals, the toxic substance formed being perhaps *S*-(dichlorovinyl)-L-cysteine. Commercial TCE contains an inhibitor, e.g., an org. amine, to prevent the corrosive action of degradation products. Soya-bean meal extracted with org. amines (primary, secondary or tertiary) was highly toxic to chicks when fed at a level of 20—40% in an otherwise complete diet. Chicks fed soya-bean meal extracted with acetone, ethanol or re-distilled TCE, survived and appeared normal. The material extracted by the amines was apparently not toxic, except in the case of butylamine. Triethylamine-extracted soya-bean meal inhibited the growth of rats. Glucose was toxic after extraction with triethylamine. Single amino-acids refluxed for 24 hr. with triethylamine were recovered unchanged except in the case of L-lysine, which showed evidence of change in composition. (15 references.) M. D. ANDERSON.

The respiration drift of harvested pasture plants during drying. W. L. Greenhill (*J. Sci. Fd Agric.*, 1959, **10**, 495—501).—The change in respiration rate of pasture plants was determined after they had been harvested and were being dried. Respiration continues throughout most of the drying period, at a progressively slower rate, until the moisture content is ~35% (dry wt. basis) when it ceases. Appreciable losses of dry matter occur, caused by continued respiration after harvesting, during haymaking, especially in warm humid weather. E. M. J.

Effect on silage of chopping and bruising the forage. C. H. Gordon, H. G. Wiseman, J. C. Derbyshire, W. C. Jacobson and D. T. Black (*J. Dairy Sci.*, 1959, **42**, 1394—1395).—Pre-ensiling mechanical treatment of forage markedly affected the final quality of lucerne silage. The value of bruising (finely chopped followed by bruising in a Silorator) for improving chemical quality was more pronounced in silage from afternoon-harvested forage, since this forage produced the lowest quality silage when coarse chopping was the only treatment. S. C. JOLLY.

Methods of ensilage of green forages. M. Vanbelle (*Agricultura*, 1958, **6**, 645—694).—A critical review of methods for making silage. Forage is best cut in the afternoon and (except for young grass) not allowed to wilt before filling in the silo. The benefit of chopping, crushing and laceration of fodders lies in the provision of a more uniform material for filling the silo with consequent better quality in the final product. Spraying the green material with aq. H_3PO_4 during crushing followed by covering the compacted mass with plastic sheeting has given promising results. A. G. POLLARD.

Chemical changes in lespedeza associated induced polyploidy. C. H. Hanson, W. A. Cope and R. M. Brinkley (*Amer. J. Bot.*, 1959, **46**, 36—39).—Among several species and crosses of lespedeza examined, colchicine-induced polyploids generally had lower crude-protein and Ca contents and higher K and (notably) tannin contents than the original species. The bearing of the data on the problem of digestibility of lespedeza forage for livestock is indicated. A. G. POLLARD.

Dried activated sewage sludge as a nitrogen source for ruminants. L. R. Hackler (*Dissert. Abstr.*, 1959, **19**, 2700—2701).—When dried activated sewage sludge was fed to steers and lambs instead of soya-bean oil meal, to provide up to 35% of the N of the diet, the steers gained less wt., and the lambs ate only half as much food and lost wt.; both developed diarrhoea. Metabolism experiments with lambs showed that some of the N of the sludge was utilised, but the apparent digestibility of the N of the diet was less than in diets with soya-bean oil meal or urea. M. D. ANDERSON.

Cocoa pods as fodder. C. Visser (*Landbouwdocumentatie*, 1959, **15**, 835—837).—A literature review, covering food value, influence on milk production and quality, and on health of cattle. In general, milk yield is reduced, but its fat content increased. (38 references.) J. M. HUBBARD.

Effect of feeding cod-liver oil and unsaturated fatty acids on rumen volatile fatty acids and on milk fat content. J. C. Shaw and W. L. Easor (*J. Dairy Sci.*, 1959, **42**, 1238—1240).—Addition of cod-liver oil, oleic acid or linoleic acid (I) to normal cow rations decreased the fat content of the milk, and lowered the molar concn. of acetic acid and increased that of propionic acid and total volatile fatty acids in the rumen fluid. I was the most active in this respect. Inconsistent data sometimes obtained by feeding the supplements to cows already receiving diets which decrease milk fat contents are discussed. A. G. POLLARD.

Pelleted lucerne hay for milk production. M. Ronning, J. H. Meyer and G. T. Clark (*J. Dairy Sci.*, 1959, **42**, 1373—1376).—When no concentrates were fed, lactating cows on pelleted lucerne hay ate more dry matter and produced more milk than did animals on chopped hay. Supplementation of chopped hay with ~12% of concentrates increased dry-matter intake and milk production to levels comparable with those with the pelleted ration, but supplementation of the pelleted hay had no significant effect. Butterfat % was not affected by either pelleted ration. S. C. JOLLY.

Effect of *Cercospora* disease on forage quality of lucerne. R. D. Brigham (*Agron. J.*, 1959, **51**, 365).—Lucerne leaves infected with *Cercospora medicaginis* were lower in % of crude protein and ether extract and higher in % of ash and N-free extract than were healthy leaves. A. H. CORNFIELD.

Comparison of methods for estimating the feed used for growth and maintenance of beef calves. W. W. Green, W. J. Corbett and J. Burie (*J. Anim. Sci.*, 1959, **18**, 548—554).—Mathematical relations between energy consumed by calves (total digestible nutrients or net energy), body wt. and gain in body wt. are presented. A. G. POLLARD.

Comparative energy requirements of sheep and cattle for maintenance and gain. W. N. Garrett, J. H. Meyer and G. P. Lofgreen (*J. Anim. Sci.*, 1959, **18**, 528—547).—Mathematical relationships are established between body wt. and the dietary requirement in terms of total digestible nutrient (TDN), digestible energy (DE), metabolisable energy (ME) and net energy, for the maintenance of energy equilibrium. TDN, DE and ME are equally serviceable in calculating energy requirements. Energy retention is determined by a modified comparative slaughter procedure, use being made of known relationships between the major chemical components of the body. A. G. POLLARD.

Responses of young calves to a diet containing salts of volatile fatty acids. W. G. Martin, H. A. Ramsey, G. Matrone and G. H. Wise (*J. Dairy Sci.*, 1959, **42**, 1377—1386).—The comparative effects of a casein–fat–glucose–starch–hay ration, a similar ration in which salts of acetic, propionic and butyric acids replaced the starch and hay and a whole milk ration on the volatile fatty acid (VFA), ketone and glucose contents of blood and urine are recorded. The data indicated that 3-week calves can absorb and metabolise VFA. A. G. POLLARD.

Strontium and calcium uptake and excretion in lactating cows. R. G. Cragle and B. J. Dermott (*J. Dairy Sci.*, 1959, **42**, 1367—1372).

—Following a single oral dosage of 1·7 mc. of ^{89}Sr, 0·55 and 1·8% of the dose was recovered in milk and urine respectively in 9 days. Corresponding data for ^{45}Ca were 3·77 and 0·82%. Excretion rates for ^{89}Sr in milk, urine and faeces may be represented as an exponential functon of time. A. G. POLLARD.

Effect of gestation on milk and butterfat production in dairy cattle. W. M. Etgen (*Dissert. Abstr.*, 1959, **19**, 3071).—Calculations from 1508 production records from two herds of cows showed that milk and butterfat production during a lactation increased with decreasing no. of days of gestation during the lactation. The increase became larger with increasing age of the cow. M. D. ANDERSON.

Effects of varying amounts of chlortetracycline in the rations of lactating dairy cattle. A. L. Shor, W. P. Johnson and A. Abbey (*J. Dairy Sci.*, 1959, **42**, 1203—1208).—Small amounts of chlortetracycline (**I**) appeared in milk from cows receiving 0·5 or 1·0 mg. of **I** per lb. of body wt. daily for 2 weeks, but this disappeared within 48 hr. of stopping administration of **I**; no **I** appeared in milk from cows receiving 0·1 mg. per lb. of body wt. daily. Initially milk production was occasionally reduced and appetite depressed slightly with the higher levels of **I** but these returned to normal within a few days. S. C. JOLLY.

Stilboestrol feeding and growth-hormone stimulation in immature ruminants. A. W. Struempler and W. Burroughs (*J. Anim. Sci.*, 1959, **18**, 427—436).—Experimental evidence presented supports the view that the growth-stimulating effect of stilboestrol on immature cattle and sheep operates largely by stimulating growth-hormone production. A. G. POLLARD.

Effect on reproduction of feeding diethylstilboestrol to dairy cattle. C. B. Browning, G. B. Marion, F. C. Fountaine and H. T. Gier (*J. Dairy Sci.*, 1959, **42**, 1351—1358).—Supplementary feeding of diethylstilboestrol at rates of 5—15 mg. per 1000 lb. live-wt. daily, had no ill effects on the oestrous cycle, conception or gestation. With 20 mg. levels some cows aborted. A. G. POLLARD.

Comparison between the carcass composition of Romney and Cheviot mature ewes. A. H. Kirton, R. A. Barton and E. Cresswell (*N.Z. J. agric. Res.*, 1959, **2**, 252—254).—The Cheviot carcasses contained significantly more protein and water and less fatty tissue in the shoulder region than did Romney carcasses. E. M. J.

Copper deficiency in sheep and "steely" wool. B. C. Jefferies (*J. Agric. S. Aust.*, 1959, **62**, 268—271).—A general account dealing with symptoms and methods of treatment. A. H. CORNFIELD.

Magnesium requirements of pigs. R. H. Mayo (*Dissert. Abstr.*, 1959, **19**, 3072—3073).—Mg is required by young pigs weaned at 3 or 9 weeks of age. Mg deficiency was indicated by lowered serum-Mg, and muscular weakness and twitching, followed by tetany and death. The amount of dietary Mg required to prevent the symptom (417 p.p.m.) was more than the amount necessary for normal growth (241 p.p.m.). Food efficiency was improved by addition of Mg up to 417 p.p.m. M. D. ANDERSON.

Phosphorus requirement, and comparative value of six phosphorus supplements, for growing pigs fed semi-purified diets. C. E. Jordan (*Dissert. Abstr.*, 1959, **19**, 2421—2422).—Based on feed consumption, feed efficiency and serum inorg.-P levels, an adequate P intake (as dil. H_3PO_4) for pigs fed a semi-purified diet from 6 weeks of age was 0·40%, i.e., 4·4 g. per day (0·30% was borderline). The P in H_3PO_4 was more readily available to swine than that in customary supplements. P was equally available from U.S. Pharm. dicalcium and commercial monocalcium phosphate, and rather less so from steamed bone meal. Curaçao phosphate and soft phosphate with colloidal clay were significantly poorer sources of P, not because of the high level of F (373 p.p.m. in soft phosphate), but because much of this F is more readily absorbed than is that in, for example, CaF_2. S. C. JOLLY.

Effect of feeding different combinations of soft phosphate with colloidal clay and dicalcium phosphate, with and without added fluorine for growing-finishing swine. S. M. Aldinger, V. C. Speer, G. C. Ashton, V. W. Hays and D. V. Catron (*J. Anim. Sci.*, 1959, **18**, 555—560).—Varying proportions of "soft phosphate with colloidal clay" (**I**) (0—40%) and of $CaHPO_4$ (0·75—1·7%) were added to a pig ration. With increase in the proportion of **I** there was a linear decline in rate of gain in body wt. and in feed efficiency. The undesirable action of **I** could not be attributed entirely to its F^- content. A. G. POLLARD.

Effectiveness of three different methods of iron administration to young pigs. M. E. Rydberg, H. L. Self, T. Kowalczyk and R. H. Grummer (*J. Anim. Sci.*, 1959, **18**, 410—414).—Pigs 5—15 days of age receiving injections of a Fe–dextran prep. showed greater increases in blood haemoglobin than did those treated orally with reduced-Fe pills or with solutions of Fe^{III} citrate. A. G. POLLARD.

Effect of pre-partum intramuscular iron treatment of dams on litter-haemoglobin levels. M. E. Rydberg, H. L. Self, T. Kowalczyk and R. H. Grummer (*J. Anim. Sci.*, 1959, **18**, 415—419).—Sows given intramuscular injections of an Fe–dextran prep. for 2 weeks before farrowing produced pigs having higher blood-haemoglobin levels during the critical post-partum period than did those given the injections 4 weeks earlier and those untreated. A. G. POLLARD.

Dermal and oral treatments of cattle with phosphorus-32-labelled Co-Ral. J. N. Kaplanis, D. E. Hopkins and G. H. Treiber (*J. agric. Fd Chem.*, 1959, **7**, 483—486).—When Co-Ral [*O*-(3-chloro-4-methylumbelliferone) *OO*-diethyl phosphorothioate] labelled with ^{32}P was given by mouth to steers, 35—38% of the dose (10 or 20 mg per kg.) was excreted in the urine and 35% in the faeces within 7 days. After applications of Co-Ral to the skin, 1—3% of the dose was excreted in the urine. The compound given orally was ineffective against stable flies and screwworm larvae, but it was highly effective by contact, and remained unchanged on the hair for several weeks after application. M. D. ANDERSON.

Feed for ruminant animals. Iowa State College Research Foundation (Inventor: W. Burroughs) (B.P. 799,095, 1.12.54).—Feedstuff for immature beef cattle is compounded with stilboestrol (0·03—2·7 mg. per lb.), to provide a growth-promoting prep. F. R. BASFORD.

Treatment of dressed poultry with antibiotic compositions to prolong the shelf life thereof. American Cyanamid Co. (B.P. 799,240, 2.7.56. U.S., 5 1. and 28.3.56.)—A composition, for use in preserving the shelf life of dressed poultry, consists of a water-sol. antibiotic, viz., chlortetracycline, tetracycline, oxytetracycline, or an acid salt or a metallic salt thereof (3—30 p.p.m.), a solid, non-toxic water-sol. acid (citric, gluconic, tartaric, malic, ascorbic or itaconic acid), a water-sol. diluent, and optionally a surface-active agent. F. R. BASFORD.

Treatment for residual liquors obtained by the distillation of alcohol from mash. R. A. G. Young (B.P. 800,935, 11.1.55).—The residual liquor (containing up to 15 wt.-% of solids comprising constituents of the original malt, etc.) is admixed (before or after reducing the water content thereof) with dry powder (≮20% of water), e.g., food substance or artificial fertiliser, then the mixture is finally dried, to give a product suitable for use, e.g., as animal feedstuff or fertiliser. F. R. BASFORD.

Veterinary compositions. Biorex Laboratories Ltd. (Inventors: S. Gottfried and L. Baxendale) (B.P. 799,415, 15.3.56).—A veterinary composition (emulsion, lotion, powder or pessary base), for topical or intramammary application, etc., in the treatment of inflammatory diseases in cattle, comprises glycyrrhetinic acid (or a salt or an ester thereof) dispersed in a pharmaceutical carrier (cocoa butter). F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Analytical-bromatological study of by-products of rice produced in the irrigable zone of the Badajoz plan. J. Remón (*An. Bromatologia*, 1959, **11**, 189—196).—Figures for fat, N-free extractive, fibre, protein, moisture and calorific value are reported for by-products of rice hulling. E. C. APLING

Rate of diffusion of oxygen through grain. S. W. Bailey (*J. Sci. Fd Agric.*, 1959, **10**, 501—506).—The rate at which O_2 can diffuse through a mass of grain (wheat, maize, barley or oats) was studied. In wheat the mean rate at 23° is 0·067 mg./sec. and a temp. dependence was observed. In maize, barley and oats the mean rates at room temp. are 0·0558, 0·0642 and 0·0721 respectively. The vol. of the intergranular air was measured in the four types of grain using a manometric method on a 500-ml. sample. E. M. J.

Distribution of sulphur among various components of wheat kernel, using sulphur-35. G. Seidman (*Dissert. Abstr.*, 1959, **19**, 3100—3101).—The kernels of wheat grown hydroponically on media containing ^{35}S showed most ^{35}S in the germ. Starch had some radioactivity, mainly in the dark (tailings) fraction. The water-sol. protein fraction contained less N than the water-insol. protein (gluten) fraction, but had about the same degree of activity, apparently because of the presence of sulphate. The activities of water-sol. proteins and dialysates from bran were lower than those of corresponding fractions from flour. Gliadin had more activity than glutenin. There was considerable activity in the fats, not related to protein content, but perhaps from sulphate or isothiocyanate. M. D. ANDERSON.

Proximate analysis of wheat flour carbohydrates. IV. Analysis of wholemeal flour and some of its fractions. J. R. Fraser and D. C. Holmes (*J. Sci. Fd Agric.*, 1959, **10**, 506—512; cf. J.S.F.A. Abstr., 1958, i, 151).—A bulked sample of wholemeal flours (632), three fractions sieved from the bulked wholemeal and three commercial fractions of endosperm, germ and bran were examined by methods previously recommended for determination of hemicellulose, cellulose, sugar and starch contents. Some changes in experimental detail were made, e.g., with those fractions containing more bran than a normal flour, and factor modifications are described, but with these, the methods previously detailed will give good assessment of the carbohydrate classes which form the "carbohydrate by difference" of all the fractions of the wheat grain. (13 references.) E. M. J.

Relation between baking value and surface tension of water extracts of wheat flour. L. Lásztity and E. Péterffy (*Élelmiszerkém. Tanszék. Közlem.*, 1959, **1**, 24).—The surface tension of water extracts of wheat flour and its relation to the baking value were studied. No significant correlation was found. The mean surface tension values are higher at low baking qualities but the individual values of a grade vary between too wide limits. E. M. J.

Polysaccharide constituents in starch. S. Augustat (*Ernährungsforschung*, 1959, **4**, 356—375).—A review of the literature on the constituents of starch is presented commencing with the year 1819. The "multiple" theory according to which most starches consist of two polysaccharide constituents, the linear chain of amylose and the branched chain of amylopectin, which has been adopted by most research workers is compared with the "unit" or "single constituent" theory according to which starch is considered to be a polysaccharide which resolves into two constituents during the processes of separation. Hypotheses are mentioned in which starch is thought to be a giant molecule, a uniform polysaccharide or has a completely different basis structure. There are relatively few experimental data. (84 references.) I. DICKINSON.

Shrinkage stresses in drying of gel-like and pasty materials. P. Görling and H. Beuschel (*Chem.-Ing.-Tech.*, 1959, **31**, 393—398).—Relationship between moisture distribution, shrinkage, and shrinkage stress is discussed. Experiments with macaroni dough are described, measuring the stress as a function of elongation, and the tensile strength and elasticity modulus as a function of the liquid content, under different drying conditions. A diagram is proposed allowing evaluation of tensile stress in relationship to liquid content and elastic deformation below the moisture limit for plastic deformation. H. FRIEDMANN.

Effect of harvesting time on stability (shelf-life) of baked cereal products such as bread rolls. D. Karp (*Grasas y Aceites*, 1959, **10**, 80—83).—The effect of harvesting time and of storage of grain, particularly rye, on its properties, and the stability of baked products made therefrom have been examined. (10 references.) L. A. O'NEILL.

Transformation of trehalose in baker's yeast. II. Synthesis of trehalose monophosphate and trehalose in various fermentation systems from brewer's and baker's yeast. M. Elander (*Ark. Kem.*, 1959, **13**, 457—474).—Brewer's yeast converted 5% of glucose into trehalose (**I**) and 18·7% into trehalose monophosphoric acid (**II**). Baker's yeast converted 30% of glucose into **I**. Although considerable amounts of **II** were formed initially, only small quantities were detected at the end of fermentation. The production of **I** and **II** by autolysis of baker's yeast was also investigated. (34 references.) (In German.) A. G. COOPER.

Determination of water in yeast cells (*Saccharomyces cerevisiae*). S. Rašajski and J. Veličković (*Bull. Soc. chim. Belgrade*, 1957, **22**, 515—525).—The methods of Montgomery and White (*J. Inst. Brew.*, 1945, **51**, 279) and Conway and Downey (*Biochem. J.*, 1950, **47**, 347) were used for samples of pressed yeast. Max. value (~21%) for intercellular water is obtained when measurements are made in 1—2% peptone solution. In solutions of polymers (e.g., polyvinyl alcohol, inulin) values are only 12—16% and are independent of polymer concn. (0·8—4·2%). High values (18—19%) are also attainable in 1—2% dialysed peptone solutions. Values obtained in solutions containing equal concn. (1·8%) of peptone and polyvinyl pyrrolidone are related linearly to the proportion of peptone or polymer in the solution. (13 references.) (In German.) W. J. BAKER.

Sugars and confectionery

Reduction of bisulphite to elemental sulphur by reducing sugars. D. L. Ingles (*Chem. & Ind.*, 1959, 1045—1046).—Examples are reported of oxidation of reducing sugars (1 mol.) by $NaHSO_3$ (4 mol.) in a 20 wt.-% aq. syrup at 50—100°, with simultaneous reduction of HSO_3^- to S. Reactions, also applicable to ascorbic acid and the Amadori product 1-deoxy-1-glycinofructose, are discussed in respect of foods containing SO_2 or HSO_3^-. (12 references.) W. J. BAKER.

Determination of sugars on paper chromatograms. C. M. Wilson (*Analyt. Chem.*, 1959, **31**, 1199—1201).—The sugars present in plant-cell-wall hydrolysates are determined by descending paper chromatography, development (thrice) with aq. BuOH–pyridine, immersion of the paper in a solution of aniline hydrogen phthalate, elution of the coloured spots with ethanolic HCl, and determination of the extinction at 390 mμ (for hexoses and rhamnose) and at 360 mμ (for pentoses). Results on 100 μg. of a sugar are reproducible to within 2%. J. P. STERN.

Determination of sugars using paper chromatography. P. Albers and R. Freiskorn (*Liebigs Ann.*, 1959, **622**, 150—159).—Chromatograms developed with aniline phthalate are measured photometrically, using non-transparent paper and non-monochromatic light. Error is <1%. C. A. FINCH.

Improved method for preparing pectate gels. A. M. Paton (*Nature, Lond.*, 1959, **183**, 1812—1813).—Addition of 0·1 wt.-% EDTA (Na_2 salt) to the pectin solution prevents formation of the weak gel during cooling and storage and ensures a firm gel within 12—24 hr. when the solution is poured over the Ca agar. W. J. BAKER.

Enzymic breakdown of pectins. J. Major (*Élelmiszerkém. Tanszék. Közlem.*, 1959, **1**, 25—29).—Attempts were made to obtain a pure conc. proteolytic enzyme prep. suitable for industrial purposes. Suitably pretreated colonies of moulds on bran were extracted with various salt solutions; 82% of the polygalacturonase and nearly all the polymethylesterase were eluted. Purifying and concentrating the enzymes needs further study. E. M. J.

Baker's yeast. Distillers Co. Ltd. (Inventors: M. A. Pyke, L. I. K. Ebbutt, R. M. Mackenzie and J. Cunningham) (B.P. 800,030, 17.11.54).—In the production of baker's yeast by aerobic growth in presence of continuously or intermittently added molasses (excepting processes in which beet molasses is added to yeast grown on beet molasses in the preceding stage), a product of enhanced maltase activity is obtained by maintaining an excess of fermentable sugar over that necessary for max. yield, thereby restricting the yield to at least 10% below the attainable max. F. R. BASFORD.

Panning of dough pieces. Baker Perkins Ltd. (Inventor: A. R. Palmer) (B.P. 800,422, 21.10.54).—An apparatus for use in the production of tinned bread is figured and claimed. F. R. BASFORD.

Fermentation and Alcoholic Beverages

Recovery of yeast from wine-making processes. M. Flanzy and P. André (*Chim. et Industr.*, 1959, **81**, 902—904).—In the alcoholic fermentation of grapes ~1 g. of yeast is produced for each l. of wine obtained. Fractional centrifuging has proved a promising method for separating the yeast from the tartrates, cellulose residues, etc., that are also found in the lees. J. M. JACOBS.

Potassium and sodium determination in wine. Comparison of flame photometric with gravimetric methods. W. Diemair and C. Gundermann (*Z. LebensmittUntersuch.*, 1959, **109**, 469—474).—Analytical data on 25 wines from <10 different countries are presented for both methods. The time-saving flame photometric method offers advantages, giving good reproducible values in serial work. E. M. J.

Reduction of aldehydes during alcoholic fermentation. Application to processing of heads. J. F. Guymon and M. S. Jaber (*J. agric. Fd Chem.*, 1959, **7**, 576—578).—The low-boiling (heads) fraction separated during the distillation of brandy contains aldehydes, which are objectionable impurities in alcoholic beverages. Yeasts are able to reduce aldehydes, and heads collected during distillation may be recycled in alcoholic fermentations to produce extra alcohol. The ability of various strains of yeast to reduce acetal, and to ferment in presence of acetal, varied considerably, and the same yeast was not necessarily active in both respects. Thus *Brettanomyces bruxellensis* reduced the largest amount of added acetal, but failed to ferment in presence of 15 mg. of acetal per l., which only delayed fermentation by other strains. Attention to the strain of yeast used for fermenting wines to be distilled should diminish the amount of aldehyde in the brandy distillate. (12 references.) M. D. ANDERSON.

A method of steeping [of barley] with aeration. D. Mauclaire (*Brasserie*, 1959, **14**, 134—136).—To hasten germination the barley is brought into contact with O_2 as soon as possible. In a series of vats it is immersed for 9 hr. (in the first for 6 hr.) and subjected to

aeration in the second and third for 45 hr., a slightly lower than usual temp. being maintained to get the best malt. E. M. J.

Washing of yeast. J. Apai and O. Janotkova (*Brauwissenschaft*, 1959, **12**, 142).—By the addition of chlortetracycline (20 μg./l. of washwater) in washing yeast cells, infection flora of beer is removed and fermenting power of yeast is stimulated. Flavour is not affected. E. M. J.

Fruits, Vegetables, etc.

Occurrence and significance of phenolic constituents of fruits. K. Herrmann (*Mitt. Lebensm. Hyg., Bern*, 1959, **50**, 121—136).—In this review of the occurrence of hydroxycinnamic acids, hydroxycoumarins, catechins, *leuco*anthocyanins, anthocyanins and flavones in 16 common fruits and in wine, the effect of these substances on the taste and usefulness of the fruits as well as the medicinal and phytopathological significance are considered. (123 references.) E. M. J.

Quantitative determination of arsenic residues in plant materials. H. Frehse and H. Tietz (*J. agric. Fd Chem.*, 1959, **7**, 553—558).—As in plant material is determined by wet-ashing, adding KBr, and distilling in a special apparatus with a trap in which $AsBr_3$ collects. As is measured as the molybdenum blue complex, preferably at 840 mμ in a 5-cm. cell. From 1 to 60 μg. of As per sample can be determined by direct reading from a standard curve. The method applied to apples 4 to 14 weeks after the last of 10 applications of a spray containing As, showed residues of up to 0·25 p.p.m. of As. (21 references.) M. D. ANDERSON.

Determination of Dyrene in apples by the Zincke reaction. D. L. Barry and D. J. Lisk (*J. agric. Fd Chem.*, 1959, **7**, 560—561).—Dyrene in apple tissue is determined by blending the sample with acetone, extracting Dyrene with $CHCl_3$, evaporating solvent, taking up the residue in benzene, chromatographing on alumina to remove interfering substances, and employing the Zincke reaction with pyridine and alkali to produce colour measured at 440 mμ. About 0·05 p.p.m. of Dyrene in 100 g. of apple can be detected; recoveries of added Dyrene averaged 97 ± 10%. Apples treated with Dyrene to control scab contained residues of about 0·1 p.p.m. M. D. ANDERSON.

Diphenylamine residues on apples. H. E. Harvey and P. J. Clark (*N.Z. J. Sci.*, **2**, 266–272).—The effect on residues of four different methods of applying diphenylamine was studied. The amount present depended on the treatment but in no instance did it exceed 8 p.p.m. and of this <90% was in the outer 2 to 4 mm. of the fruit. E. G. BRICKELL.

Chemical peeling of freestone peaches. J. A. Kitson and F. E. Atkinson (*Chem. Can.*, 1959, **11**, No. 7, 34—36).—Apparatus is figured and procedure described. O. M. WHITTON.

Aromas of fruit and their recovery. P. Dupaigne (*Fruits d'outre mer*, 1959, **14**, 127—141).—A review. (193 references.) M. C. M.

Occurrence, measurement and control of bitterness in carrots. P. M. Bessey (*Dissert. Abstr.*, 1959, **19**, 3076).—Bitterness developing in stored carrots appeared to be associated with early maturity and a warm growing season. Its appearance was hastened by ethylene or some other emanation from fruit. No relation could be found between bitterness and supply of Cu or Mn to the carrots, or concn. of O_2 or CO_2 in the storage atm. Bitterness was accompanied by increase in the fluorescence of light petroleum extracts of the carrots, and it can be rapidly assessed by spectrophotometric measurements at 240, 275 and 290 μm. M. D. ANDERSON.

Ascorbic acid contents of potato tubers harvested on plots of land given various fertilising treatments. C. Scholler, J. Causeret, G. Drouineau and L. Soubiès (*C. R. Acad. Agric. Fr.*, 1959, **45**, 533—537).—Although there were considerable variations in the yields of potatoes obtained from the different plots, no significant variation in the ascorbic acid content of the tubers was observed in relation to the nature of the fertiliser employed. (13 references.) J. M. JACOBS.

Insecticide residues in samples of peeled, canned tomatoes. M. E. Alessandrini, M. Doretti and G. F. Lanforti (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 110).—Very low or no toxic hazard exists with satisfactory control procedures using either chlorinated hydrocarbons or org. phosphates. (In English.) J. L. PROSSER.

Effect of varying chemical compositions brought about by processing methods on serum viscosity and water retention of tomato puree. G. L. Peters (*Dissert. Abstr.*, 1959, **19**, 3098—3099).—Rutgers tomatoes contain pectin of low methoxyl content, which shows increased η with increasing additions of Ca. The hot-break process gave tomato purees with higher pectin content, relative η, and water retention values, than purees obtained by the cold-break process. Addition of 0·25% of Calgon (Na hexametaphosphate) decreased the serum η and degree of water retention of tomato purees in the absence of added Ca, but gave increased η and water retention in presence of added Ca. The interrelations of pectin content, η and water retention, are considered. M. D. ANDERSON.

Colorimetric determination of Dyrene [2,4-dichloro-6-(o-chloroanilino)-s-triazine] residues in plant material. W. R. Meagher, C. A. Anderson, C. E. Gonter, S. B. Smith and D. MacDougall (*J. agric. Fd Chem.*, 1959, **7**, 558—560).—Resides of the fungicide Dyrene are determined by blending the sample with isopropyl alcohol, extracting the Dyrene into benzene, evaporating solvent, hydrolysing with acid to give *o*-chloroaniline, reducing with Zn to diminish extraneous colour, adding Celite and filtering, diazotising and coupling with *N*-1-naphthylethylenediamine to produce a magenta colour measured at 540 mμ. The limit of sensitivity of the method is 1·0 p.p.m., which is satisfactory in view of the low toxicity of Dyrene. Mean recoveries of Dyrene added to celery, onions, potatoes and tomatoes were 84 to 103%. M. D. ANDERSON.

Non-alcoholic beverages

Determination of orange juice in beverages. R. Intonti, F. Cotta-Ramusino and A. Stacchini (*Chim. e Industr.*, 1959, **41**, 603—608).—The following analyses are proposed for orange juices and beverages prepared therefrom, detailed procedures being given: Formol no.—this is based on the alkali required to neutralise the carboxyl groups of the amino-acids after addition of formaldehyde, and is determined electrometrically; ninhydrin no.—this is a measure of the amino-acid content, dependent on the reaction of the amino groups with ninhydrin and is determined spectrophotometrically; proline content—this is also determined spectrophotometrically after reaction with ninhydrin; arginine content—this is based on the colour reaction with 1-naphthol and bromosuccinimide and determined spectrophotometrically. L. A. O'NEILL.

Grapefruit skin oil and grapefruit-basis materials. E. Benk (*Riechstoffe u. Aromen*, 1959, **9**, 227—228).—The oil obtained by pressure of the skins or by solvent extraction, is used in the form of a basis material or essence which contains the oil mixed with 3—4% of thick grapefruit syrup. Details are given of the constitution and physical properties of the oils. The prepared beverages have a natural turbidity owing to presence of insol. constituents of the oil, but contain no flesh or peel particles. H. L. WHITEHEAD.

Tea, coffee, cocoa

Trace constituents of black tea. M. Myers, E. A. H. Roberts and D. W. Rustidge (*Chem. & Ind.*, 1959, 950—951).—Uncharacterised fluorescent spots were found in paper chromatograms of black tea extracts. Some of these were tentatively identified as 5,7-dihydroxycoumarin, (−)-epiafzelechin, phloroglucinol and pyrogallol whilst others may be coumarin derivatives. C. A. SLATER.

Chlorophyll content of tea. F. T. Wolf (*Bull. Torrey Bot. Club*, 1959, **86**, 184—189).—Average total chlorophyll content of green teas was 1·39 mg. per g. and for Oolong and black teas 0·82 and 1·01 mg., respectively. Chlorophyll *a* averaged 81% of the total in green, 78% in Oolong and 71% in black tea. L. G. G. WARNE.

Determination of small amounts of adulterants of the cocoa-fat group in chocolate and other confectionaries by paper chromatography. E. Pietschmann (*Fette Seif. Anstrichm.*, 1959, **61**, 682—686).—Fat characteristics which are typical in fat adulterants are described, together with a paper-chromatographic separation for typical adulterant fats. The isolated fat, after conversion into its hydroxamic acid deriv., is applied to strongly acetylated paper and developed with a 1 : 8 : 8 Et acetate/tetrahydrofuran/water mixture; zones are located on the dried strip by spraying with $FeCl_3$ reagent. Some R_F values are given. G. R. WHALLEY.

Fat adulteration in cacao products. XI. Common methods used for investigation of cacao butter and chocolate adulterants. A. Purr (*Fette Seif. Anstrichm.*, 1959, **61**, 675—682).—A preliminary scheme of selecting working material is given, together with five published methods for analytical detection of adulterant fats in cacao butter (by butyric acid no., iso-oleic acid value, solubility in acetone at −25°, dielectric constant and n). Selection of these methods is indicated for the detection of coconut fats, hydrogenated fats, vegetable tallows and other fats that have been hardened by physical means. (41 references.) G. R. WHALLEY.

Citrus fruit beverages and concentrates. Northern Dairy Engineers Ltd. and J. W. Shearman (B.P. 799,258, 26.2.57).—A method of preparing a citrus fruit flavoured liquor or beverage

comprises working, cutting or slicing the fruit, then centrifuging it while maintaining thereover a flow of water or aq. fluid (to absorb aromatic oils and extracted juice). Apparatus is figured and claimed. F. R. BASFORD.

Milk, Dairy Products, Eggs

Influence of vacuum treatment upon milk loss. A. C. Smith and L. R. Glazier (*J. Dairy Sci.*, 1959, **42**, 1234—1235).—Equations are presented expressing the increasing % milk loss with increasing degrees of flash-cooling in the various vac. chambers of the Vacu-Therm (a non-steam-injection vac. equipment), when used to remove off-flavours from liquid dairy products. S. C. JOLLY.

Recovery of added monoglycerides from milk. R. G. Jensen and G. W. Gander (*J. Dairy Sci.*, 1959, **42**, 1235—1236).—Monostearin and monobutyrin added to milk at levels of 0·1 and 2·0 mmol. per 100 g. of fat were recovered almost quant. by the silica-gel method (Jensen and Morgan, *ibid.*, 1959, **42**, 232). Purification methods for the two glycerides are outlined. S. C. JOLLY.

Effect of some preservatives on Babcock tests and fat hydrolysis in single milk samples. L. J. Manus and H. A. Bendixen (*J. Dairy Sci.*, 1959, **42**, 1236—1238).—The decreases in fat %, determined by the Babcock method, in milk samples preserved with $K_2Cr_2O_7$, K_2CrO_4, Milkeep (Na_2HPO_4 and tetrahydro-3,5-dimethyl-1,3,5-2H-thiadiazine-2-thione) or Mojonnier test fluid were small and non-significantly different during 7- or 14-days storage at 35° to 38°; with $HgCl_2$ as preservative, the decrease was >3 times that in chromate-preserved milk and approx. twice that in milk preserved with Milkeep or Mojonnier test fluid. The decrease in fat % was generally paralleled by increases in free fatty acid. S. C. JOLLY.

Rôle of xanthine oxidase in the development of spontaneously oxidised flavour in milk. L. W. Aurand and A. E. Woods (*J. Dairy Sci.*, 1959, **42**, 1111—1118).—Only a single enzyme system is involved in the development of spontaneously oxidised flavour in milk. A high xanthine oxidase activity is necessary for its development, but it can be controlled by the addition of xanthine oxidase inhibitors such as the pteridine derivatives, folic acid and 6-PA (2-amino-6-formyl-4-hydroxypteridine). S. C. JOLLY.

Whey preservation by hydrogen peroxide. L. Jasewicz and N. Porges (*J. Dairy Sci.*, 1959, **42**, 1119—1125).—Cheese whey was preserved for >10 days by the addition of 0·02% of H_2O_2 soon after separation. This concn. of H_2O_2 killed within 1 hr. 97% of the bacteria in whey grossly contaminated with $2·8 \times 10^7$ micro-organisms per ml., but was relatively ineffective against greater no. *Saccharomyces fragilis* was grown successfully in preserved whey in which the excess of H_2O_2 was destroyed by catalase. S. C. JOLLY.

Chemical stability of indicators and substrate indicators used for the detection of phosphatase activity. R. W. Henningson and F. V. Kosikowski (*J. Dairy Sci.*, 1959, **42**, 1294—1303).—Based on chemical stability, solubility, and rate and intensity of colour development, 2,6,-*N*-trichloro-*p*-quinoneimine is more suitable than is 2,6-dibromo-*N*-chloro-*p*-quinoneimine as an indicator in phosphatase testing. The stability of phenolphthalein phosphate and *p*-nitrophenyl phosphate, as dry powders and in solution, is also reported. S. C. JOLLY.

Separation of strontium-90 from calcium in milk. N. A. Myers (*Nature, Lond.*, 1959, **183**, 1807—1808).—The separation is effected, for 1 ml. of milk, on a 16·5-cm. column of Zeo-Karb 225 (50—100 mesh, Na form) by elution with ~30 ml. of 1% EDTA (Na_2 salt) at 22° and pH 8 (flow-rate 0·17 ml./cm.²/min.). Fraction from 8 to 20 ml. contains Ca, that from 20 to 25 ml. the ^{90}Sr; recovery is >90% for both. Milk-fat does not interfere and casein retention is nil. W. J. BAKER.

Removal of radioactive iodine and strontium from milk by ion exchange. P. Cosslett and R. E. Watts (*A.E.R.E.*, 1959, R 2881, 10 pp.).—From 96 to 98% of ^{131}I in contaminated milk can be removed by passing the milk through a short column of strong-base anion-exchange resin (Deacidite FF, Cl form). Up to 98% of ^{90}Sr can be removed similarly by a strong acid cation-exchanger (Zeocarb 225, Ca form). Milk is still palatable and each column will handle ~500 times its own vol., but is non-regenerable. ^{90}Y is not removed. W. J. BAKER.

Determination of radionuclides in milk ash. G. K. Murthy, L. P. Jarnagin and A. S. Goldin (*J. Dairy Sci.*, 1959, **42**, 1276—1287).—A modified method, suitable for the routine determination of radionuclides [total radio-Sr, ^{90}Sr (^{90}Y), ^{140}Ba and ^{137}Cs] in milk ash, is described. Using 1 l. of milk recoveries were as follows: Sr, 82 ± 2%; ^{90}Y (^{90}Sr), 77 ± 4%, ^{140}Ba, 85 ± 4%, and ^{137}Cs, 96 ± 3%. The overall recovery of ^{90}Sr, taking into account the recovery of Sr, was 64 ± 5%. These values were sufficiently precise for most purposes. S. C. JOLLY.

Rapid ashing of milk for radionuclide analysis. G. K. Murthy and J. E. Campbell (*J. Dairy Sci.*, 1959, **42**, 1288—1293).—An accurate method, requiring little attention from the operator, is described for the rapid routine ashing of 1-l. samples of milk for radionuclide analysis. Milk is added continuously at the rate of ~5 to 8 ml. per min. to a rotating crucible heated by a blast burner and finally ashed in a muffle furnace. One operator can ash 6—8 samples daily. S. C. JOLLY.

Isolation of immune globulins from milk and colostrum with rivanol. A. J. Kenyon, R. K. Anderson and R. Jenness (*J. Dairy Sci.*, 1959, **42**, 1233—1234).—The immune globulins (protein fraction with electrophoretic mobility of $-1·8$ to $-2·2$ cm.² volt^{-1} sec.$^{-1}$ at pH 8·6, ionic strength = 0·1) in colostrum, acid whey from skim milk, and crude lactoglobulin were separated semi-quant. from the other proteins by pptg. these other proteins with rivanol (6,9-diamino-2-ethoxyacridine lactate), centrifuging, and removing the rivanol from the supernatant liquid by adsorption on activated C. This procedure is simpler and quicker than the classical fractionation with $(NH_4)_2SO_4$, but recoveries need improving and checking before it can be used for the determination of immune globulins in milk. S. C. JOLLY.

Fractionation of milk proteins on anion-exchange cellulose. M. Yaguchi, W. G. Jennings and N. P. Tarassuk (*J. Dairy Sci.*, 1959, **42**, 1395—1396).—The proteins and enzymes in milk can be isolated, purified and characterised by applying dialysed skim milk to a column of anion-exchange cellulose (DEAF-SF; diethylaminoethanol on Solfa-Floc cellulose lattice) and eluting with phosphate buffers of varying pH and molarities (Sober and Peterson, *J. Amer. chem. Soc.*, 1954, **76**, 1711). S. C. JOLLY.

Direct analysis of lactose in milk and serum. J. R. Marier and M. Boulet (*J. Dairy Sci.*, 1959, **42**, 1390—1391).—Contrary to the findings of Barnett and Tawab (*J. Sci. Fd Agric.*, 1957, **8**, 437), both the amount of phenol added and the concn. of H_2SO_4 affect the determination of lactose in milk and serum by the colorimetric phenol–H_2SO_4 method of Dubois *et al.* (*Analyt. Chem.*, 1956, **28**, 350). Max. colour development occurred when the phenol concn. was between 5 and 12 mg. per ml. and H_2SO_4 concn. between 70 and 75% in the final solution. S. C. JOLLY.

Photometric milk fat determination. G. Haugaard and J. D. Pettinati (*J. Dairy Sci.*, 1959, **42**, 1255—1275).—A photometric method is described by which both fat content and average particle size are determined simultaneously. Interfering turbidity due to casein micelles is eliminated by use of a Ca chelating agent, and coherent scattering is eliminated by a five-fold dilution of the sample. Fat contents agree well with those determined by Babcock method. Applicability of the method to other dairy products is discussed. S. C. JOLLY.

Butterfat analysis. M. G. E.-D. Ibrahim, F. El-Said and A.-M. Ismail (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., 111—112).—Data are quoted on the linoleate content of pure and adulterated Egyptian butterfat obtained by thiocyanometry. A Pb salt–ether method for separating saturated from unsaturated acids is described. (In English.) J. L. PROSSER.

Distribution of polarographically active cystine in various fractions of cheese. A. Świątek and S. Poznański (*Roczn. Technol. Chem. Żywności*, 1959, **4**, 5—27).—Cystine contents of cheeses were studied directly on the samples and on aq., aq. NH_3, and other extracts of 20 Edam, Cheddar, Gruyère, Roquefort and Emmenthaler cheeses. Although the cystine contents were low, approx. 0·5 mmol. per 100 g. cheese, the use of 0·0025 mol. $CoCl_2$, N-aq.NH_3, and 0·1N-NH_4Cl electrolyte gave good polarograms. Repeatable results were obtained within 3% at the cystine concn. of 1×10^{-5} mmol. in 10 ml electrolyte. No cystine was found in the residue N and amino-N extracts of cheeses and this absence was confirmed by colorimetric and chromatographic analyses. In the aq. and aq. NH_3 extracts the amount of polarographically active S compounds identified as cystine varied from 0·44 to 1·38 mmol. per 16 g. total N. The cystine contents of the cheese proteins also varied considerably and were lower in the sol. (0·25—1·46 mmol.) and higher in the insol. proteins (0·48—2·26 mmol.). Generally the cystine contents of the sol. proteins increased with the ripening of the cheese. (26 references.) A. GROCHOWSKI.

Simplified determination of ash in cheese. E. Pijanowski (*Roczn. Technol. Chem. Żywności*, 1959, **4**, 47—59).—Complicated removal of the alkali salts in the standard cheese ash determination procedure was eliminated and direct incineration, wt. of the white ash and a Volhard's chloride estimation were found adequate when the total chloride and the casein contents are known from the general

analysis. The ash % is calculated from the formula $(100a - 0{\cdot}585n)/m - k/70 + s$, where a is wt. of ash in g., n ml. 0·1N-$AgNO_3$ for chlorides in the ash, m wt. of cheese sample in g., k % casein, s % NaCl in cheese. Laboratory tests confirmed accuracy of the method within 1%. The incineration over 2—3 hr. was recommended, but not the use of NH_4NO_3 as a bleaching agent. A. GROCHOWSKI.

Ultrasonic-energy attenuation in Cheddar cheese and effects of ultra-sound on the ripening process. A. E. Federer (*Dissert. Abstr.*, 1959, **19**, 3274).—The intensity of ultra-sound decreased by 50% in passing through 2·5 in. of close-textured Cheddar cheese. Sound transmission increased with firmness of cheese. The waves did not travel in a straight beam, but were scattered and reflected. A standing-wave pattern was found within the cheese at 400 kc but not at 1200 kc. The most favourable effects on the ripening of 1 lb. samples of cheese were obtained by treatment at 1200 kc between 2 Ba titanate transducer elements in direct contact with the cheese, operating for 90 sec. at 300 W. in series circuit, or 180 sec. at 250 W. in parallel circuit. M. D. ANDERSON.

Accelerating the ageing process in Cheddar cheese. T. R. Freeman (*Kentucky agric. Exp. Sta.*, 1959, Bull. 666, 16 pp.).—Rate of development of the flavour of Cheddar cheese was increased by raising the ripening temp. normally used and by substituting a starter prepared from a combination of *Streptococcus lactis* and *S. faecalis* for the conventional *S. lactis* starter. A. H. CORNFIELD.

Effect of addition of molasses to fodder [for milking cows] on quality of cheeses (Gruyère and Carré de l'Est). F. Favin, R. Ferrando, C. Moquot and G. Thieulin (*C. R. Acad. Agric. Fr.*, 1959, **45**, 232—237).—Addition of molasses to the ration of milking cows had no action on milk production and no appreciable influence on composition. The effect on quality of cheese was less than that of variation in the cheesemaker's technique and environment. M. C. M.

Effect of sugars on physical and chemical properties of egg albumin. E. L. Baker (*Dissert. Abstr.*, 1959, **19**, 3108).—The presence of 1·0M-glucose, galactose, fructose or sucrose prevents the appearance of about 30% of the SH groups usually formed in egg albumin on heating at 50° and pH 1·0, but does not affect the SH groups formed on denaturation by guanidine hydrochloride or Na dodecyl sulphate. Electrophoretic measurements of the amounts of native and denatured egg albumin present during heating at 50° and pH 3·0 showed that as much denaturation occurred in 24 hours in presence of glucose or mannitol as in 20 min. in their absence. M. D. ANDERSON.

Detection of *Salmonella* in egg products and other foods. T. Emmenegger (*Mitt. Lebensm. Hyg., Bern*, 1959, **50**, 145—158).—*Salmonella* (seven species) were found in 12% of egg products examined. The medium of Osborne and Stoke (nutrient broth containing Na selenite and Brilliant Green) was suitable for culture. This and the growth of pure cultures of the various micro-organisms by the Blue Agar plate method are described. (36 references.) E. M. J.

Production of Cheddar-type cheeses. Commonwealth Scientific & Industrial Research Organization (of Australia) (B.P. 799,121, 22.3.57. Aust., 26.3.56).—A machine for fusing cheese-curd is figured and claimed. F. R. BASFORD.

Edible Oils and Fats

Influence of quality of rape seed on stability of the oil. A. Rutkowski and Z. Makus (*Fette Seif. Anstrichm.*, 1959, **61**, 532—535).—The change in composition of the fatty acids which occurs at the end of the ripening period does not seriously interfere with the stability of the oil. Different oil storage conditions affect the oil stability, and the most stable oil is obtained from ripe seeds. The free-fatty-acid content and peroxide value of oils from damaged seeds are higher than those of oil from undamaged seeds. (13 references.) G. R. WHALLEY.

Photodecolorisation and rancidity of vegetable oils. II. Photosensitised decolorisation of groundnut oil. T. V. Subba Rao and G. Gopala Rao (*Grasas y Aceites*, 1959, **10**, 92—96).—Decolorisation of groundnut oil on exposure to sunlight is accelerated by presence of ZnO, TiO_2 or Mn ore. Addition of water enhances the effect. Only minor changes occur in the constants of the oil, other than a decrease in acid value resulting from the addition of ZnO. L. A. O'NEILL.

Rancidity of olive oil. VIII. Watts–Major test. R. Gutiérrez González-Quijano and J. M. R. de la Borbolla y Alcalá (*Grasas y Aceites*, 1959, **10**, 61—66).—Conditions for the Watts–Major test (colour development on treatment with $CCl_3{\cdot}CO_2H$ in AcOH solution, and phloroglucinol) have been studied, and extension of the reaction time for definitely oxidised oils to 35 min. is proposed. Relationships between the behaviour of a range of virgin and refined olive oils in the test, and peroxide value and stability have been examined. In general, oils giving a high value in test had low stability but the stability of oils giving a low value could not be predicted. With an oil stored in a closed container, the test value and peroxide value increased as the flavour deteriorated up to a certain point, when the test value fell. In an open container the test and peroxide values increased rapidly but the flavour remained acceptable. (10 references.) L. A. O'NEILL.

Detection of additions of soya oil to olive oil. A. Goded y Mur, R. Giménez Ruesga and C. de Francisco Galdeano (*An. Bromatologia*, 1959, **11**, 261—264).—The relationships of I val., peroxide value, Tortelli no., n and density are presented graphically to assist in the detection of adulteration, and the further examination of doubtful samples is briefly discussed. E. C. APLING.

Determination of impurities and " oxidised acids " in sulphur olive oil and its industrial evaluation. J. Gracián and M. Ventura (*Grasas y Aceites*, 1959, **10**, 67—75).—The effect of different solvents for determining the content of impurities in sulphur olive oil, the effect of removing the impurities from the oil prior to determination of " oxidised acids " (**I**) (acids insol. in light petroleum) and the change in impurity and **I** content of oils on keeping have been studied. An oil might be assessed on the basis of its total fatty-acid content instead of on the amounts of water, impurities and **I** present. L. A. O'NEILL.

Iodine value of unsaponifiable matter in differentiation of olive from other oils. L. Frontero (*Olii min.*, 1959, **36**, 154—156).—The I val. of the unsaponifiable matter (**I**) of olive and other oils differed considerably according to the procedure used for isolating **I**. Using the same method of isolation expressed olive oils and " rectified A " oils could be differentiated by this property from " rectified B " oils, esterified oils, other vegetable oils and esterfied animal fatty acids. L. A. O'NEILL.

Differentiation of olive oils classified as " rectified B." V. Sossi (*Olii min.*, 1959, **36**, 214—216).—The chemical constants, flavour and behaviour in various reactions of 10 oils classed as " rectified B " are tabulated. Oils prepared by esterification of the distilled fatty acids obtained from neutralisation pastes of olive or sulphur olive oils had similar characteristics to the classical " rectified B " obtained by neutralisation of extracted olive oil. Oils from other sources showed clear distinctions. L. A. O'NEILL.

Analysis of olive oil: colour reactions. S. Anselmi (*Olii min.*, 1959, **36**, 210—213).—Methods for detecting adulteration in olive oil, particularly colour reactions, are critically reviewed, and conditions for the Fitelson test for teaseed oil in olive oil studied. L. A. O'NEILL.

Insecticide residues in olive oils. M. E. Alessandrini, G. F. Lanforti and A. Sampaolo (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 110—111).—Very low or no toxicity is present in oils from olives treated with various insecticides, under controlled conditions. (In French.) J. L PROSSER.

Basic substances as synergists for fat antioxidants. H. S. Olcott and E. J. Kuta (*Nature, Lond.*, 1959, **183**, 1812).—Oil-sol. basic compounds [octadecylamine (**I**), proline (**II**), tri-isooctylamine] provide great protection against oxidation of, e.g., menhaden oil and pure trilinolein containing antioxidants [6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (EMQ), 2-t-butyl-4-methoxyphenol (BHA)]. They are not synergistic in the absence of the antioxidant, or if the latter is 2,6-di-t-butyl-4-methoxyphenol (BHT). Synergistic activity varies, e.g., **II** is highly effective in vegetable oils, whilst **I** is antagonistic to α-tocopherol but synergistic to γ-tocopherol. Induction periods (wt.-gain) at 50° for 200-mg. substrate plus antioxidant 0·5 and basic additive 2·5 μmoles are listed. W. J. BAKER.

Isolation, examination and determination of polyethylene glycol (Carbowax) surface-active agents added to edible fats. S. Anselmi, L. Boniforti and R. Monacelli (*Chim. e Industr.*, 1959, **41**, 421—424).—The fat is saponified, acidified, the fatty acids removed by extraction and the aq. phase containing the polyethylene glycol, glycerol, etc., examined. Polyethylene glycol is identified with an I/KI solution. The polyethylene glycol content may be determined by pptn. of the aq. solution with I/KI, dissolving the pptd. complex in KI and acetone and determination of combined I with aq. $Na_2S_2O_3$. The mol. wt. of the polyethylene glycol present may be determined i.r.-spectroscopically (OH absorption in the 2—3μ region). L. A. O'NEILL.

Formation of carbonyl compounds during the autoxidation of olefinic fats. K. Täufel and R. Zimmermann (*Ernährungsforschung*, 1959, **4**, 325—336).—Carbonyl compounds (21) which have been isolated from autoxidised fats are listed. The detection and identification of these compounds is particularly valuable in hydrogenated

fats where " reversion " has taken place. Carbonyl compounds are formed through a secondary reaction from the hydroperoxides of fatty acids: this formation and the behaviour is discussed in the light of recent literature. Half of the carbonyl compounds have probably a chain length of 4—10 C and it is thought that there are some between 2 and 18 C. As carbonyl compounds can be detected by taste and smell in a dilution of $1 : 10^6$ to $1 : 10^7$, very sensitive qual. and quant. analytical methods are important. 2,4-Dinitrophenylhydrazine has proved to be a valuable reagent for the identification and isolation of such compounds. 2-Thiobarbituric acid is a sensitive reagent for the detection of the oxidation of unsaturated fatty acids. (58 references.) J. DICKINSON.

Use of vitamin-A acid to vitaminise fat-containing foods. W. Wodsak (*Fette Seif. Anstrichm.*, 1959, **61**, 672—675).—Experiments are described to investigate the stability of vitamin-A acid (**I**) in animal and vegetable oils, fat–water emulsions and in skimmed milk powder. After storage for 6 months the % of **I** in groundnut oil, cottonseed oil, sunflowerseed oil and tallow are 92·5, 92·5, 95 and 84·5 respectively. G. R. WHALLEY.

Direct spectrophotometric determination of butylated hydroxyanisole in lard and hardened lard. P. V. Hansen, F. L. Kauffmann and L. H. Wiedermann (*J. Amer. Oil Chem. Soc.*, 1959, **36**, 193—195).—A method for determining 0—10 p.p.m. of butylated hydroxyanisole (BHA) in lard and hardened lard (I val. $\leq$10) utilises the strong absorption of BHA at 290 mμ. The lard is extracted with MeOH–$CHCl_3$. Butylated hydroxytoluene and tocopherols interfere with the method; in their absence it is more reliable than colorimetric procedures. (13 references.) G. R. WHALLEY.

Measurement of hardness of margarine and fats with cone penetrometers. A. J. Haighton (*J. Amer. Oil Chem. Soc.*, 1959, **36**, 345—348).—An equation is derived for standardisation of yield values measured with various penetrometers. Factors affecting the value are stated and conditions for the determination laid down.
C. A. BLAU.

Production of meat and fat products through centrifugal rendering. F. P. Downing (*J. Amer. Oil Chem. Soc.*, 1959, **36**, 319—321).—An account of the development of a low-temp. centrifugal rendering process of separating fat tissues on a manufacturing scale.
C. A. BLAU.

Improved lard shortening. T. Hedley & Co. Ltd. (B.P. 799,264, 8.7.55. U.S., 15.7.54).—The glyceride fat mixture consists of $\leq$90% by wt. of triglycerides of fatty acids of 16 and 18 C-atoms, the balance being monoglycerides and diglycerides. The mixture is derived principally from lard and contains a " Solids Content Index " of 12—20 at 92°F, 40—75% by wt. of the combined fatty acids of the trisaturates of the mixture is palmitic, the balance being predominantly stearic. The portion of the fat which melts at 40—103°F is <20% by wt. of the whole as approximated by " Solids Content Index " determinations at these temp., the fat having a polyunsaturates number <16. The products have wider plastic range of temp. than usual products. I. JONES.

Improving the taste and flavour of margarine and other fat-containing foods and edible products. Margarinbolaget Aktiebolag (B.P. 799,676, 30.11.55). Swe., 2.12.54).—Margarine and other fat-containing foods and edible products are given the taste and flavour of butter by adding to the product 1—50 g. per ton of at least one aliphatic lactone having 16—22 C-atoms (e.g., stearolactone).
I. JONES.

Meat and Poultry

Quantitative and qualitative determination of connective tissue content of meat and meat products. F. Lörincz and I. Szeredy (*J. Sci. Fd Agric.*, 1959, **10**, 468—472).—Total N is determined, then the N extracted with 0·05N-alkali at room temp. in 24 hr. is determined (Kjeldahl method) and the difference gives the connective tissue N. Animals of higher grades have less connective tissue than those in lower grades, and young may have more than older animals. The chewing (cutting) resistance of meat depends on the quality of connective tissue. Histological study indicates that the tissue fibres of older animals are thicker, more twisted, uneven in size and more closely woven together. (16 references.) E. M. J.

Fish

Pyruvic acid in the skeletal muscle of fresh and chill-stored, trawled codling (*Gadus callarias*). N. R. Jones (*J. Sci. Fd Agric.*, 1959, **10**, 472—474).—The levels of pyruvic acid in the muscle of trawled codling at death varied, with a mean level of 0·67 mg./100 g. During storage, mean values fell to 0·36 mg./100 g. after 8 days, then rose rapidly to 0·85 mg./100 g. at 12 days and more slowly to 0·92 mg./100 g. after 18 days. The values then fell to 0·47 mg./100 g. after 21 days. The possible causes of these changes are discussed. (11 references.) E. M. J.

Factors affecting the nitrate content of treated fish fillets. C. H. Castell and M. F. Greenough (*Fish. Res. Bd Can.*, 1959, **16**, 539—552).—Owing to variations in procedures in application of $NaNO_2$ there is a wide range in the average nitrite concn. (20—>200 p.p.m.) of nitrite-treated cod and haddock fillets from different fresh fish processing plants. The standard deviations in the nitrite values for individual fillets in similar groups may range from 3 to 40. Subsequent to the submission for publication of this paper the provision of Food and Drug Regulation for use of $NaNO_2$ in fresh and preserved fish in an amount $\geq$200 p.p.m. of the finished product was rescinded, 16 July, 1959, thus prohibiting the use of $NaNO_2$ in these items. E. M. J.

Sodium phosphate crystals on salt fish. W. J. Dyer, D. I. Fraser and J. R. Dingle (*Stud. Fish. Res. Bd Can.*, 1958, No. 523, 4 pp.; cf. J.S.F.A. Abstr., 1959, i, 53). E. M. J.

Influence of antibiotics added to ice on the digestibility of fish. A. Pujol and G. Varela (*An. Bromatologia*, 1959, **11**, 245—259).—Using cats as experimental animals, no effect on digestibility of fish protein was observed after storage in ice treated with penicillin, streptomycin, Aureomycin, chloromycetin or Biostat HC. (11 references.) E. C. APLING.

Dicarbonyl compounds as components of fish odour. G. F. Mangan, jun. (*Comm. Fish. Rev.*, 1959, **21**, 21—22).—Fresh skinless fillets of haddock, and similar fillets after 3 months at −10°, were distilled under high vac. at room temp.; the distillates were collected in receivers immersed in liquid N, and treated to obtain the 2,4-dinitrophenyl hydrazine derivatives. These were obtained in larger amounts from the frozen than from the fresh fillets. Solubilities and i.r. data indicated that the derivatives were formed mainly from aliphatic dicarbonyl and α-hydroxy compounds, containing four or fewer C atoms. These may be important components of odour and flavour. M. D. ANDERSON.

Acid-soluble nucleotides of salmon liver. H. Tsuyuki, V. M. Chang and D. R. Idler (*Stud. Fish. Res. Bd Can.*, 1958, No. 522, 8 pp.).—The acid-sol. nucleotide pattern of salmon liver is presented and compared with results obtained by other workers. Separated by anion-exchange chromatography at low temp., the relatively labile uridine-5′-diphosphate nucleotides of acetylglucosamine, galactose and glucuronic acid were obtained. The occurrence of these compounds and absence of uridine diphosphate glucose is discussed in relation to the rôle of inositol as a carbohydrate storage product. A new peptide-containing nucleotide succinoadenosine-5′-phosphosulphate (peptide) was found in the fraction which immediately follows adenosine-5-diphosphate. The parent base succinoadenine was also isolated. The pattern is simpler than that reported for rat liver and wheat. (41 references.) E. M. J.

Volumetric determination of oxidative stability of salted herring. R. Marcuse and K. Knutsen (*Grasas y Aceites*, 1959, **10**, 97—101).—The stability towards oxidation of salted herring (fillets) is estimated by volumetric measurement of the uptake of O_2 on storage of the products in closed jars. Evolution of CO_2 accompanies uptake of O_2, and the CO_2 may be absorbed with aq. NaOH distributed on a filter paper cylinder, but in comparative tests, e.g., of different antioxidants, this precaution is omitted. L. A. O'NEILL.

Rapid method of total lipid extraction and purification. E. G. Bligh and W. J. Dyer (*Canad. J. Biochem. Physiol.*, 1959, **37**, 911—917).—The method described, developed for use on frozen fish muscle, involves homogenising wet tissue with a mixture of $CHCl_3$ and methanol in proportions giving a miscible system with the water in the tissue; on dilution with $CHCl_3$ and water, and filtering the homogenate separates into methanol and chloroform layers, the latter containing all the lipids. For quant. extraction, the residual tissue is extracted again with $CHCl_3$ and filtered, and the filtrate is added to the previous filtrate; the methanol layer is removed, $CHCl_3$ is evaporated, and lipid is determined by weighing. The required proportions of $CHCl_3$, methanol and water, before and after dilution, are respectively $1 : 2 : 0{\cdot}8$ and $2 : 2 : 1{\cdot}8$.
M. D. ANDERSON.

Improved apparatus for separating steam-volatile compounds: use in determination of hexamethylenetetramine. N. Antonacopoulos (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 109—110).—The improved apparatus permits of more rapid separation of formaldehyde in the standard chromotropic acid method for hexamethylenetetramine in fish products. Distillation is from an alkaline mixture. (In German.) J. L. PROSSER.

Packaging of freshly cut red meats. Goodyear Tire & Rubber Co. (B.P. 799,478, 28.1.57. U.S., 16.7.56).—A packaging material for freshly cut meat (to maintain the red colour thereof) comprises a rubber hydrochloride film containing 25—40 wt.-% of ester plasticiser at least 10 wt.-% of which is a permanently fluid dialkyl adipate. A preferred plasticiser component consists of di-2-ethylhexyl adipate (15) and 2-ethylhexyl diphenyl phosphate (20), or di-2-ethylhexyl adipate (10) and dibutyl phthalate (25 pt. per 100 pt. of rubber hydrochloride). F. R. BASFORD.

Spices, Flavours, etc.

Colouring matters

Detection and isolation of synthetic colours from Cayenne pepper (*Capsicum annum*). M. Mingot Lorenzo (*Bol. Inst. nac. Invest. agron. Madr.*, 1959, **19**, 67—87).—Artificial colours are readily detected by paper chromatography or column chromatography on alumina using light petroleum containing 1% AcOH and CCl_4 as developing solvents respectively. Diagrams and colour photographs are reproduced illustrating the separations obtained with pure Cayenne pepper and a variety of adulterated samples. E. C. APLING.

Paper-chromatography of synthetic food dyes. H. Thaler (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 104—105).—Sharp separations among coal-tar dyes of similar structure can be effected by paper chromatography of a solution buffered to a standard pH, the preferred solution being ammoniacal t-Na citrate. (In German.) J. L. PROSSER.

Separation and identification of food colours. A. C. Cooke (*N.Z. J. Sci.*, 1959, **2**, 260—265).—The 17 water-sol. coal-tar permitted food colours were separated and identified by a paper-chromatographic method. R_F values are tabulated for 41 food colours, including those authorised in England, and details are given for the identification of the spots. E. G. BRICKELL.

Artificial colouring materials excluded from use in foodstuffs. I. C. Calzolari and E. Cerma (*Chim. e Industr.*, 1959, **41**, 425—430).—Methods are being examined for the determination of 17 colouring materials, recently banned in Italy from use in foodstuffs. The initial report deals with the polarographic determination in pure form of: Naphthol Yellow, Aniline Yellow, Chrysoidine, Orange I, Acid Bordeaux, Ponceau 2R, Biebrich Scarlet, Eosine and Phloxine. L. A. O'NEILL.

Preservatives

Suitability of chemical food preservatives. K. Raible (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 108).—Methods for establishing this, and for analysis of preservatives, are discussed. (In German.) J. L. PROSSER.

Properties of *p*-hydroxybenzoic acid esters towards higher animals. T. Sabalitschka (*Riechstoffe u. Aromen*, 1959, **9**, 224—226).—Details are given of the min. lethal and harmful dosages of Me, Et and Pr *p*-hydroxybenzoates and their Na deriv. as single or repeated dosages over long time to rats, mice, dogs, rabbits, etc. All these compounds are safe for use in human foods in preserving amounts: the Na deriv. are more toxic than the free esters. H. L. WHITEHEAD.

Antimicrobial activity of esters of *p*-hydroxybenzoic acid. K. Raible (*Fette Seif. Anstrichm.*, 1959, **61**, 667—669).—Concn. of 4—250 mg./100 ml. of Me, Et, Pr, Bu and benzyl esters of *p*-hydroxybenzoic acid in a nutrient broth at pH 5·5 have been utilised to assess their antimicrobial properties against *Lactobacillus arabinosus, Streptococcus faecalis, Escherichia coli, Micrococcus pyogenes* var. *aureus, Bac. subtilis* and *Saccharomyces* spp. Activity against these organisms increases with increasing chain length of the alkyl group. Lactobacilli are almost immune to the Me, Et and Pr esters. The application of butyl *p*-hydroxybenzoate as a foodstuffs preservative is discussed. (10 references.) G. R. WHALLEY.

Determination of antimicrobial activity of preservative combinations. H.-J. Rehm (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 109).—Antimicrobial activity of boric acid + other ingredients against *Escherichia coli, Aspergillus niger* and a wine lees stock has been studied, using isobol diagrams. It is suggested that compounds with the same antimicrobial-action mechanism have an additive effect in admixture, but mutually destructive results if the mechanisms are different. (In German.). J. L. PROSSER.

Inhibitory effect of combined chemical preservatives. H. G. Osman and A. el-Mariah (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 108).—Effectiveness of several org. acids, Na_2SO_3 and $NaHSO_3$, separately and in various mixtures, for inhibiting growth of *Saccharomyces cerevisiae* at pH 4 was investigated. Combinations of salicylic acid with any other component were more effective than either used alone. (In English.) J. L. PROSSER.

Antibiotics as meat, fish and poultry preservatives. H. Becker (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 105).—Use of antibiotics, especially Aureomycin, is discussed; experiments on the Acronize process are described. (In German.) J. L. PROSSER.

Comparative effectiveness of tetracycline antibiotics for fish preservation. B. A. Southcott, E. G. Baker, J. W. Boyd and H. L. A. Tarr (*Stud. Fish. Res. Bd Can.*, 1958, No. 514; cf. J.S.F.A. Abstr., 1958, ii, 82). E. M. J.

Preservation of fresh fish. H. L. A. Tarr (*Stud. Fish. Res. Bd Can.*, 1958, No. 524, 13 pp. Reprint *Archiv Fischereiwissenschaft*, 1957, **8**, 9—21).—Spoilage of fresh fish and its control are reviewed. Rate of bacterial spoilage is about twice as rapid at 2·5° as at −1°. The use of specially prepared ice, refrigerated sea water, and the effect of this on the stored fish, and the use of u.v. and γ-radiations, chemical preservation, $NaNO_2$, and antibiotics, especially Aureomycin (chlortetracycline, CTC), Terramycin (oxytetracycline, OTC), are discussed, CTC giving best results. CTC (0·15—1 mg./kg.) inhibits bacterial damage. On fishing vessels the immersion of the fish for 2 min. in CTC solution (5—25 mg./kg.) produces better quality fish fillets stored at 6° without affecting the taste of the subsequently cooked fish. (77 references.) E. M. J.

Use of antibiotics for control of spoilage in East Coast fisheries. I. Use of antibiotics on gutted cod and haddock aboard trawlers. II. Effect of antibiotics on keeping time of fillets. III. Factors affecting the amount of antibiotics taken up by fillets. C. H. Castell and M. F. Greenough (*Stud. Fish. Res. Bd Can.*, 1958, No. 551A).—I. Ice containing chlortetracycline (CTC) or oxytetracycline (OTC) (5 p.p.m.) added one or two days to the keeping time of well iced cod and haddock, and results were better when the fish were iced in boxes instead of the pens. Dipping the fish for 1 min. in solutions containing from 4 to 80 p.p.m. increased the keeping time up to 5 days longer.

II. CTC and OTC are the most efficient of the preservatives for increasing the keeping time of fillets. A concn. of 10 p.p.m. of the dips is recommended. The extension of the keeping time of stored fillets is dependent on the quality of the fish at the time it is dipped.

III. Factors influencing the amount of antibiotic taken up by fish muscle from a dipping solution are: concn. of antibiotic, length of time the fish are immersed, temp. of the dip, size of the fillet and the physical condition of the fillet (whether soft or flaked apart). Under usual plant conditions fillets pick up amounts of antibiotic in the range of 1/10 to 1/50 of that of the dipping solution. E. M. J.

Packaging

Prepackaging studies on fresh produce: *Capsicum grossum* Sendt. and *C. acuminatum* Fingh. B. Anandaswamy, H. B. N. Murthy and N. V. R. Iyengar (*J. sci. industr. Res.*, 1959, **18A**, 274—278).—A study has been made of the prepackaging of *Capsicum grossum* (sweet pepper) and *C. acuminatum* (green chillies) in bags of polyethylene, plain transparent cellulose and moisture-proof, heat-sealable transparent cellulose (M.S.T.). The shelf life of sweet pepper is almost doubled by packing in 150 gauge polyethylene film with adequate vents, also in M.S.T. bags at 100°F and 90% R.H., 76—88°F and 65—75 % R.H. and 47—50°F and 80—90% R.H. Green chillies only benefit from prepackaging in polyethylene bags at 76—80°F and 65—75% R.H., or at low temp. in all three materials. The ascorbic acid content of both products is unaffected by prepackaging. A. M. SPRATT.

[A] **Storage of preserved spinach in glass containers.** [B] **Storage of preserved carrots in glass containers.** F. Gstirner and S. N. I. Saad (*Z. LebensmittUntersuch.*, 1959, **109**, 483—487, **110**, 9—14). [A] The effects of temp. (4, 15 and 37°), of light, u.v. light, of darkness, of atm. pressure, of vac., and 60 mm. Hg., on the colour change of chlorophyll were observed during 45 days. In light the chlorophyll was bleached by atm. O_2; in vac. and higher temp. browning occurred. Stored under the most favourable conditions, viz., in vac. at 4° and protected from light, during longer storage (24 weeks) gradual decomposition of β-carotene, ascorbic acid and chlorophyll takes place, with no visual colour change.

[B] Effects of similar physical conditions and also the effect of adding ascorbic acid were studied on carrot brei. Colour and β-carotene content were diminished by oxidation with atm. O_2, but preserved in glass containers under vac. or protected from air, the brei showed only very slow, gradual change of colour and decrease of β-carotene content, undetected over a long period. This oxidative process was accelerated by light and heat. Least change occurred when the carrot was stored in vac., in darkness, at 4°. When pieces of carrot were used and ascorbic acid (100 mg./100g.) was added, in vac. at 4°, without light, storage life was ⁄36 weeks, with no change. Under similar conditions but at 15° and in light,

at 36 weeks loss of carotene was ~15% and with addition of ascorbic acid loss of carotene was ~30%. E. M. J.

Storage of packed foods. III. Improvement of the shelf life of margarine by antioxidants. E. Szilas and G. Palik (*Élelmiszerkém. Tanszék. Közlem.*, 1959, **1**, 16—20).—By use of propyl gallate or α-tocopherol acetate as antioxidants in stored margarine, best results were obtained when propyl gallate was mixed into the margarine, and α-tocopherol acetate was impregnated into the packaging materials. This latter process is less economical than direct use. Best storage life depends on the choice of suitable packaging materials, effective antioxidants and favourable storage conditions. E. M. J.

Should we pre-wrap fresh fillets in consumer packages? C. H. Castell (*Stud. Fish. Res. Bd Can.*, 1958, No. 532, 4 pp.).—Airtight wrapping retards the start of spoilage in frozen fish and, if spoilage has commenced, condition is not worsened thereby, but airtight wrapping does not retard spoilage in unfrozen fish and if spoilage has commenced offensive gases accumulate and may speed up the spoilage process. A wrapped fillet is protected from breakage and from contact with human hands. E. M. J.

Keeping time of wrapped fillets treated with an antibiotic. C. H. Castell (*Stud. Fish. Res. Bd Can.*, 1958, No. 540, 2 pp.).—If fresh untreated fillets are wrapped, they will probably remain in a very good state of preservation for 4 or 5 days, but if they have long-distance distribution the "shelf life" is short. Fillets that had been dipped in chlortetracycline (10 p.p.m.) then wrapped were acceptable up to 9 days and had a correspondingly longer "shelf life". E. M. J.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Nutritive value of bread protein as influenced by the level of protein intake, the level of supplementation with L-lysine and L-threonine and the addition of egg and milk proteins. J. B. Hutchinson, T. Moran and J. Pace (*Brit. J. Nutr.*, 1959, **13**, 151—163. Reprint).—Measurements were made of the growth of weanling rats, fed diets with different levels of bread protein (viz., 10·8, 15·0, 18·8 and 24·6%) and with different levels of supplementation of amino-acids, and of food consumption. Growth increased moderately as protein level was raised, but with a diet containing 24·6% of protein supplemented by 0·4% of L-lysine, growth rate was increased by ~88%. The following effects on growth rate were noted: at protein level of 13% + 0·5% of L-lysine, response was no greater than with addition of 0·25%, but if L-threonine (0·1—0·2%) was added with 0·5% of L-lysine, response was greater; at protein levels of 7—8% + 0·25% of L-lysine, the addition of 0·1% of L-threonine caused improvement; with bread protein 13% + L-lysine 0·5% + L-threonine 0·2%, performance was about the same as with casein, bread–casein, and bread–milk-powder mixtures at the same protein level. Growth rate on the supplemented bread was ~85% of that on whole-egg protein, and the protein efficiency ratio was ~80%. The influence of the levels of protein and of supplementary amino-acids on results observed is stressed. (21 references.) E. M. J.

Interrelations of linoleic acid with medium- and long-chain saturated triglycerides. H. Kaunitz, C. A. Slanetz, R. E. Johnson and V. K. Babayan (*J. Amer. Oil Chem. Soc.*, 1959, **36**, 322—325).—A study of the effect of linoleic acid on wt. increase, survival and reproduction, is made on rats fed a diet incorporating medium- (6—12 C) (**I**) and long-chain (14—18 C) (**II**) triglycerides. With 2% linoleic acid (**III**), rats fed **I** or **II** grew better than those fed a low-fat diet. Without **III**, those fed **I** grew better, those fed **II** less, than those fed low-fat diet. Rats fed **I** survived (2 years) better than controls. Weaning wt. with 2% **III** are equally high for **I** and **II** diets but lower for low-fat diet. (26 references.) C. A. Blau.

Combination of copper with amino-acids, peptides and proteins. J. F. Scaife (*Canad. J. Biochem, Physiol.*, 1959, **37**, 1033—1048).—Solutions containing an amino-acid, peptide or protein, were equilibrated with the sparingly sol. Cu salt malachite [$CuCO_3$,$Cu(OH)_2$], and determinations were made of the amount of Cu complexed to each substance, and of the free Cu in solution in equilibrium with each complex. The simple amino-acids formed complexes of the type CuR_2, appreciably dissociated in dilute solution; the extent of complexing is influenced by the nature of R. Histidine, tryptophan and other compounds containing more than one donor N atom, form more than one type of complex with Cu, the participation of the other N atoms in complexing being related to their basicity. Glycylglycine binds about twice as much Cu as glycine; the other glycine peptides, from tri- to penta-glycine, show progressively decreasing ability to bind Cu. Bovine plasma albumin and pepsin bound respectively 5 and 4·5 atoms of Cu per mol., the ratio remaining constant over a 20-fold range of protein concn.; the atoms of Cu were not all bound with the same affinity. Lysozyme bound very little Cu. (45 references.) M. D. Anderson.

Analysis of mixtures of amino-acids by gas-phase chromatography. C. G. Youngs (*Analyt. Chem.*, 1959, **31**, 1019—1021).—The amino-acids are converted to their *N*-acetyl butyl esters which are fractionated on a firebrick column coated with a hydrogenated vegetable oil. Max. deviation is 10%. G. P. Cook.

Determination of amino-acids by high-temperature paper chromatography. J. B. Himes and L. D. Metcalfe (*Analyt. Chem.*, 1959, **31**, 1192—1194).—Amino-acids in mixtures or protein hydrolysates can be resolved in a horizontal paper-chromatographic system at 60° with COEtMe–$EtCO_2H$–water (15 : 5 : 6). The spots are developed with ninhydrin and the intensities determined on a densitometer. (19 references.) K. A. Proctor.

Two-way separation of amino-acids and other ninhydrin-reacting substances by high-voltage electrophoresis followed by paper chromatography. M. L. Efron (*Biochem. J.*, 1959, **72**, 691—694).—The method is more rapid than two-way paper-chromatography. The first run is replaced by high-voltage electrophoresis. Desalting is unnecessary, because it occurs automatically during the electrophoresis. Movement of 43 substances is charted. J. N. Ashley.

Amino-acid analysis by horizontal paper chromatography. J. Chartier, F. W. van Klaveren and G. Vaillancourt (*Stud. Fish. Res. Bd Can.*, 1958, No. 531, 10 pp.).—The advantages of circular paper chromatography including the K.C.T. technique in which a paper with five machine-made slits is used, for the quant. determination of amino-acids in protein hydrolysates are discussed. With the system *n*-butanol–2-butanone–water–aq. NH_3 (5 : 3 : 1 : 1) six groups are obtained. Two groups are separated into their individual components with the system lutidine–water, three others with t. butanol–formic acid–water (75 : 0·8 : 24·2). Threonine appears as a single band. Length of the run is increased and separation is improved by dividing the paper into triangular segments and by putting the starting point near the periphery. (24 references.) E. M. J.

Purification of plant amino-acids for paper chromatography. J. F. Thompson, C. J. Morris and R. K. Gering (*Analyt. Chem.*, 1959, **31**, 1028—1031).—Basic amino-acids are retained on the NH_4 form of Dowex 50 resin, and other amino-acids are held on the H form. All the acids are eluted with ammonia and the hydrolysis of labile substances is avoided by carrying out the procedures at <6°. Recoveries are generally >97%. (22 references.) G. P. Cook.

Determination of amino-acids from plants by paper chromatography. J. F. Thompson and C. J. Morris (*Analyt. Chem.*, 1959, **31**, 1031—1037).—Neutral and acidic amino-acids are separated by two-directional chromatography, and leucine, isoleucine, phenylalanine and basic amino-acids by one-directional chromatography. All the spots are developed with ninhydrin either on the paper or after elution and are determined absorptiometrically at 570 mμ. The coeff. of variation is <5% in the concn. range 10—80 μg. G. P. Cook.

Microdetermination of ornithine. I. Reifer and L. Buraczewska (*Acta biochim. polon.*, 1959, **6**, 219—226).—Ornithine alone, or mixed with other amino-acids, is separated by paper chromatography in PhMe-Bu^nOH–aq. HCl solvent. The chromatogram is developed with ninhydrin at 75°. The ornithine spots are dissolved in HOAc + H_3PO_4, ninhydrin is again added, and after $CHCl_3$ extraction the extinction coeff. is compared with a blank. 2 mg.-% in plant material can be detected. B. Lake.

Catalysis of ascorbic acid oxidation by copper and its complexes with amino-acids, peptides and proteins. J. F. Scaife (*Canad. J. Biochem. Physiol.*, 1959, **37**, 1049—1067).—The rate of oxidation of ascorbic acid, catalysed by Cu and Cu complexes, was measured manometrically and chemically. With complexes of Cu with amino-acids, the more firmly bound Cu atoms were the more active catalysts. Cu complexed with glycine peptides had about half the mean catalytic activity of Cu complexed with amino-acids, and that of Cu complexed with proteins was lower still. Free Cu had 10 times the activity of Cu complexed with glycine, but in presence of increasing concn. of NaCl the activity of free Cu decreased, till at 0·178M-NaCl the activity of complexed Cu was twice that of free Cu. The small amount of Cu complexed with lysozyme had no catalytic activity, and inhibited the catalytic activity of free Cu and Cu-glycine complex. (46 references.) M. D. Anderson.

Unclassified

Food additives: toxicological problems. A. C. Frazer (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 96).—Techniques for evaluating possible harmful effects of a food additive are reviewed. (In English, French and German.) J. L. Prosser.

Analysis of food additives. O. Högl (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 98).—A review. (In English, French and German.) J. L. PROSSER.

Cumulative toxicity in relation to food additives and pesticides. R. Fabre and R. Truhaut (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 103).—Fat-sol. toxins, substances which react with body-tissue constituents, carcinogens, and materials acting indirectly by destruction of essential food principles, or formation of toxins by reaction with food constituents, are discussed. (In French.) J. L. PROSSER.

Mutagenic action of food additives. H. Lück (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 104).—Mutagenic action of various dyestuffs and other chemicals on cultures of *Escherichia coli* was undetectable at pH >7·5. Hexamethylenetetramine is active only at 0·1M and pH <7·5, as are also sorbic, benzoic, salicylic and mono-bromoacetic acids. Formic, acetic and propionic acids at pH 5·2 show no great decrease in bacterial count or increase in the extent of mutation. (In German.) J. L. PROSSER.

Presumptive detection of antibiotics in foods, using a simple micro-biological test. L. P. van der Mijll Dekker, D. A. A. Mossel, A. S. de Bruin and A. Manten (*J. Sci. Fd Agric.*, 1959, **10**, 475—478).—The food sample under test is mixed with double-strength agar containing triphenyltetrazolium chloride as indicator of bacterial growth. The culture medium is streak-inoculated with, e.g., *Staphylococcus aureus* or *Streptococcus cremoris* as test organisms and incubated. A blank test is prepared with a mixture of agar and an identical food sample known to be free from added antibiotics. To confirm the presence of an active concn. of an antibiotic, the procedure is repeated using a series of test strains; e.g., *Sarcina lutea, Bacillus cereus, Escherichia coli*, etc., so that the inhibition pattern is obtained and the antibiotic may be characterised. (28 references.) E. M. J.

Detection of plant thickening-materials and of polyphosphates, especially in mayonnaise. A. Blumenthal (*Mitt. Lebensm. Hyg., Bern*, 1959, **50**, 137—144).—Details are given for the separation of the thickening material in the sample and the identification by colour test using anthrone reagent. This is followed by more exact identification and detection of polyphosphates by paper chromatographic methods. The sensitivity of the procedure is tested by preparing, e.g., mayonnaise with known quantities of ingredients and different thickening materials. E. M. J.

Objective judgement of rancidity of soup preparations. B. A. J. Sedláček (*Z. LebensmittUntersuch.*, 1959, **109**, 480—483).—A quick method for fat extraction of soup prep. for reliable determination of peroxide and acid value of extracted fat is proposed. The soup powder + sand is ground with CCl_4 and filtered. Of this extract, 10 ml. are heated in a weighed dish, and the wt. of the residual fat is determined; 10 ml. are used to determine the peroxide value by the iodometric method; and a further 20 ml. are used for the acid value. A mixture of ethanol and ether (1 : 1) (25 ml.) is added and the solution is titrated with alcoholic 0·1N-KOH using phenolphthalein. Oxidation changes are also measured directly using ~5 g. of fat and determining the red colour obtained with 2-thiobarbituric acid at 530 mμ. E. M. J.

Comprehensive survey of catechins and catechin-tannins and their significance in foods. K. Herrmann (*Z. LebensmittUntersuch.*, 1959, **109**, 487—507).—This review covers the following: chemistry of catechins, occurrence in and extraction from plants, qual. detection and quant. determination, catechin-tannins, their significance as technical tannins, significance of catechins and catechin-tannins in pharmacology and medicine and in foods. In this last section, the browning of fruits, catechins in sweet musts, in wine prep., in tea and cacao are dealt with (in considerable detail) and also in cola, areca and pasta guarana. (164 references.) E. M. J.

Food products. Genatosan Ltd. (Inventors: F. A. Todd and N. J.van Abbe)(B.P. 800,530, 3.2.55).—Water-sol. protein or proteinaceous material (especially milk protein) is compounded with 0·1—1 wt.-% of a pure, water-insol. monoglyceride or sorbitan ester of a fatty acid, e.g., glyceryl mono-oleate or sorbitan monopalmitate, to provide a water-dispersible food product. The preferred protein in casein or a complex thereof with Na glycerophosphate. F. R. BASFORD.

Extruded protein products. Unilever Ltd. (Inventor: E. O. G. Batchelor-Williams) (B.P. 799,353, 13.10.55).—A product formed by extrusion and hardening of a protein (vegetable globulin or groundnut protein) is rendered transparent by heating in a water-insol. org. liquid b.p. ⩽115°, e.g., oil, fat or wax (groundnut oil), at ⩽100°/<1 atm., then subjecting to aq. treatment (immersion in aq. electrolyte) to rehydrate the fibre to equilibrium. The products are suitable as sausage casings. F. R. BASFORD.

Food products derived from cotton seed. Sudan Gezira Board (Inventor: D. Hepburn) (B.P. 800,533, 24.6.55).—Cotton seed kernel is puffed, then extracted with a solvent in which toxic pigment (but not cottonseed oil) is soluble, e.g., low-mol. alkanol ethanol, to leave an oil-containing kernel, suitable for use as a food product similar to puffed wheat. F. R. BASFORD.

Dried storage of micro-organisms. Commonwealth Scientific & Industrial Research Organisation (of Australia) (B.P. 799,644, 30.12.55. Aust., 19.1.55).—Viability of a dried micro-organism (cellular organism, virus, virus-like organism, etc.) during storage is maintained by compounding with a substance which inhibits or prevents damage to micro-organisms due to reaction between cellular protein and available carbonyl compounds present, e.g., non-reducing sugar or polyol, inositol, amino-acid or a carbonyl reagent (hydroxylamine, phenylhydrazine, semicarbazide, pyridinium-acetohydrazide chloride, trimethylammonium-aceto-hydrazide chloride or SO_2). F. R. BASFORD.

3.—SANITATION

Fumigation of maize in a large shell-concrete building. W. M. Graham (*J. Sci. Fd Agric.*, 1959, **10**, 478—483).—The maize in the sealed store of vol. 1,130,000 cu. ft. was fumigated with MeBr during five days. No fans were used to distribute the gas, but the heavily infested stacks were heating, thereby aiding distribution. The dead insects swept from the floor weighed 235 lb. The fumigation was more efficient and economical than that by sheet fumigation and 1386 lb. of MeBr had been used as against the expected 1400 lb. Extensive sampling of the maize and fabric failed to uncover any live pests. E. M. J.

Control of insects with pyrethrum sprays in wheat stored in ships' holds. G. L. Phillips (*J. econ. Ent.*, 1959, **52**, 557—559).—Spraying the ships' fabric and the grain surface with pyrethrum–piperonyl butoxide five times, during April–Sept., kept the no. of moths, weavils, beetles and psocids very low. One application of methoxy-chlor to the holds killed adult *Plodia interpunctella* immediately. The effect on the larvae could not be gauged as numbers were too low. C. M. HARDWICK.

Comparison of mortality and paralysis in the assay of pyrethrum preparations. J. M. G. Gradidge (*Sci. J. roy. Coll. Sci. Lond.*, 1959, **27**, 10—12).—Using grain weevils as test insects assays based on mortality indicate higher potencies of pyrethrum extracts (alone or synergised with piperonyl butoxide or sulphoxide) than do those based on paralysis. A. G. POLLARD.

Barthrin isomers and their toxicity to house flies in space sprays. W. A. Gersdorff, S. K. Freeman and P. G. Piquett (*J. agric. Fd Chem.*, 1959, **7**, 548—550).—Barthrin, the 6-chloropiperonyl ester of DL-*cis-trans*-chrysanthemumic acid, was found to be 32% as toxic as allethrin to house flies at the 50% mortality level, and the values for the D-*trans*, DL-*trans* and DL-*cis* isomers of barthrin were respectively 62, 38 and 29%. The ratio of the toxicities of *trans* and *cis* isomers was 2·18 for allethrin and 1·29 for barthrin. Knockdown activity of barthrin and its isomers fell with concn. almost as rapidly as lethality, whereas allethrin retains knockdown activity at concn. of low lethality. Synergism by piperonyl butoxide was as marked with barthrin and its isomers as with allethrin. (18 references.) M. D. ANDERSON.

Mode of action of synergised Bayer 21/199 and its corresponding phosphate in the house fly. R. E. Monroe and W. E. Robbins (*J. econ. Ent.*, 1959, **52**, 643—647).—*In vivo*, Bayer 21/199 and its phosphate were isotoxic to resistant house flies, but *in vitro*, the phosphate inhibited 435 times more cholinesterase. The addition of piperonyl butoxide did not affect *in vitro* results but increased the toxicity of both compounds 2·8-fold *in vivo*. This suggests that 21/199 is normally converted to its phosphate. (21 references.) C. M. HARDWICK.

Fluorinated analogues of DDT as toxicants and DDT synergists. M. S. Blum, J. J. Pratt, jun. and J. Bornstein (*J. econ. Ent.*, 1959, **52**, 626—628).—Of the 2,2-difluoro-1,1-di-*p*-halophenyl derivatives of ethane, ethanol and ethyl acetate, the *p*-bromo- and *p*-chloro-compounds were more toxic than the *p*-fluoro-compounds to susceptible *Musca domestica* and showed greater synergism with DDT. The ethane, ethanol and ethyl acetate derivatives decreased in toxicity as listed. C. M. HARDWICK.

***N*-Ethoxy-*N*-ethyl-*m*-toluamide insecticide.** R. T. Major and H.-J. Hess (*J. Amer. pharm. Ass., sci. Edn*, 1959, **48**, 485).—*N*-Ethoxy-*N*-ethyl-*m*-toluamide (**I**), an analogue of the insect repellant *NN*-

diethyl-*m*-toluamide, was prepared by reacting *m*-toluoyl chloride with *N*-ethoxyethylamine. I showed little systemic or contact toxicity in mice and rabbits, was toxic to *Aedes aegypti* and repellant to *Stomoxys calcitians*, but not repellant to *Musca domestica*.
A. G. COOPER.

Titrimetric determination of free carbonic acid in water. O. Thomann and A. Scherrer (*Mitt. Lebensm. Hyg., Bern*, 1959, **50**, 186—187).—The prep. is described and the use is recommended of a mixed indicator consisting of Thymol Blue and α-naphtholphthalein, claimed to give a better end-point than phenolphthalein gives in determining the H_2CO_3 in water. E. M. J.

Colorimetric analysis of water by membrane filtration. E. Novel and P. Burkard (*Mitt. Lebensm. Hyg., Bern*, 1959, **50**, 188—206).—The technique and various aspects of membrane filtration problems are surveyed. Various sterilisation processes are discussed. Using *Escherichia coli* as the test organism, growth on media containing Endoagar, gelose, gelose with lactose (with or without Bromocresol Purple), triphenyltetrazolium chloride, or Tergitol gave similar counts; growth on MacConkey medium and Eosin-methylene blue, and deoxycholate-containing media gave smaller counts. (28 references.) E. M. J.

Annihilating sea lampreys. Anon. (*J. agric. Fd Chem.*, 1959, **7**, 529—530).—Sea lampreys, preying on fish in the American Great Lakes, have been effectively exterminated in tributary streams in the larval stage by 3-trifluoromethyl-4-nitrophenol, which is effective at 0·5 to 4 p.p.m., and toxic to other fish only at 10 to 12 p.p.m. Compounds previously used were 3-bromo-4-nitrophenol and 3,4,6-trichloro-2-nitrophenol. M. D. ANDERSON.

Influence of basic fuchsin and sodium sulphite on EHC Endo membrane filter medium. R. E. Noble and M. Reitman (*J. Amer. Wat. Wks Ass.*, 1959, **51**, 614—622).—Basic fuchsin and Na_2SO_3 in the EHC Endo membrane prevented development of *Escherichia coli* in the suspension used for this study: low estimates were obtained in bacteriological analyses, of the no. of coliform organisms that could develop on this medium. O. M. WHITTON.

Wastes from fermentation industries; application of fermentation methods to wastes other than sewage. E. H. Lundin (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 82).—Problems in utilisation of wastes such as garbage are reviewed and the application of fermentation methods for disposal is considered. (In English.)
J. L. PROSSER.

Effectiveness of halogens or halogen compounds in detoxifying *Clostridium botulinum* toxins. A. R. Brazis, A. R. Bryant, J. E. Leslie, R. L. Woodward and P. W. Kabler (*J. Amer. Wat. Wks Ass.*, 1959, **51**, 902—912).—Free Cl_2 inactivates some *C. botulinum* in all detectable concn.; pH 7—10 does not affect rate (*R*) of inactivation but *R* is trebled on raising temp. from 5° to 25°. Over 0·5 mg./l. free Cl_2 is required to deal with Types B, C and E toxins. ClO_2 may be superior to Cl_2; chloramines are inferior. Effectiveness of I_2 is greater at pH 8·1 than at 6·25. (12 references.)
O. M. WHITTON.

Experimental spray irrigation of paperboard mill wastes. H. C. Koch and D. E. Bloodgood (*Sewage industr. Wastes*, 1959, **31**, 827—835).—The applicability of spray irrigation to disposal of paperboard mill wastes has been evaluated. Max. application rates have been established; no adverse effect on soil, or on maize or lucerne planted in it, has been observed. O. M. WHITTON.

Spray irrigation of dairy wastes. G. W. Lawton, G. Breska, L. E. Engelbert, G. A. Rohlich and N. Porges (*Sewage industr. Wastes*, 1959, **31**, 923—933).—Factors affecting spray-irrigation disposal of dairy wastes were studied. The method appears satisfactory under selected conditions, particularly of the irrigated area and of the volumetric loading and cation loading of the system. (11 references.) O. M. WHITTON.

Biochemical sewage purification on aerobic thermophilic basis. W. Husmann and F. Malz (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 91).—The course of reaction in thermophilic (55°) and mesophilic (20°) purification of sewage is discussed. The efficiency of the higher-temp. process is the smaller. (In German.)
J. L. PROSSER.

Metabolic energy balances in total-oxidation activated-sludge system. R. R. Kountz and C. Forney, jun. (*Sewage industr. Wastes*, 1959, **31**, 819—826).—Metabolic energy balances in a multiple-unit total-oxidation system were studied using dry skim milk as a source of org. matter. Total endogenous oxidation is not possible within reasonable time and size of treatment system. Of the influent ultimate O_2 demand ~60% appears as new activated sludge in a continuous-influent system. The actual endogenous loss per day of the total wt. of activated sludge in the system is ~2% and the accumulation of non-oxidisable sludge ~0·6%. (10 references.)
O. M. WHITTON.

Germicidal preparations. West Laboratories Inc. (Inventors: M. G. Sutton and M. M. Reynolds) (B.P. 799,721, 16.7.56).—A germicidal powder, concentrate or solution contains as active ingredient a loosely formed complex of iodine (≯28%) and an oxyethylated polyoxypropylene glycol, viz., $OH\cdot([CH_2]_2\cdot O)_x\cdot(C_3H_6O)_y\cdot([CH_2]_2\cdot O)_{x'}\cdot H$ of B.P. 731,603 (y is ≮15 and $[CH_2]_2O_{x'}$ comprises 20—90% of the total wt. of the compound). A typical concentrate suitable for use in cleaning sanitary equipment, comprises Pluronic *L*-62(44), 30—35% aq. HCl(2), iodine (17) and isopropyl alcohol (15 pt.). F. R. BASFORD.

***O*-(3,4-Dichlorophenyl) *NN'*-dialkyl phosphorodiamidothioates** Dow Chemical Co. (B.P. 800,220, 5.3.57. U.S., 5.3.56).—Compounds $3,4,1\text{-}Cl_2C_6H_3O\cdot PS(NHR)_2$ are claimed (R is Me or Et); they are active against mites, flies, roaches, etc., and are suitably compounded (in oil, aq. emulsion or dust) for use as insecticides. As an example of prep., 25% aq. NH_2Me is added during 1 hr. at 5—25° to a solution of $3,4,1\text{-}Cl_2C_6H_3O\cdot PSCl_2$ in benzene, then the mixture is washed with water and freed from solvent to leave O-3,4-*dichlorophenyl phosphoro-di-*(N-*methylamido*)*thioate*, m.p. 60–61°.
F. R. BASFORD.

1,3-Diazacycloalkane compounds. Monsanto Canada Ltd. (B.P., 797,714, 23.7.56. Can., 9.11.55).—The prep. of 1-dialkylaminoalkyl-2-thiono-1,3-diazacycloalkanes (-cyclohexanes or -cyclopentanes), useful as rodenticides, is described. One example given is the prep. of 1-2-(*dimethylaminoethyl*)-2-*thionotetrahydroglyoxaline*, m.p. 94—95°. J.A.C. ABSTR.

Clarifying water. Dow Chemical Co. (B.P. 799,621, 15.6.56. U.S., 18.7.55).—Turbidity in water is reduced by dispersing in the water a slurry of bentonite (10—500 p.p.m.), adding to the dispersion an acrylamide polymer (0·05—2 p.p.m.) agitating the mixture gently to form a readily settleable floc and then filtering. I. JONES.

4.—APPARATUS AND UNCLASSIFIED

Safety of pesticide chemicals: review of modern concepts and practices. L. W. Hazleton (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 99). (In English, French and German.)
J. L. PROSSER.

Determination of pesticide chemicals in foods and feeds: review of modern concepts, problems and methods. F. A. Gunther (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 101). (In English, French and German.) J. L. PROSSER.

Determination of aldrin, dieldrin and endrin residues. J. G. Reynolds (*XVII Int. Congr. pure appl. Chem.*, 1959, Abstr., **2**, 107).—Recovery and assay techniques are described. (In English.)
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Zinc-65 in foods and people. R. W. Perkins and J. M. Nielsen (*Science*, 1959, **129**, 94—95).—The ^{65}Zn content of farm produce grown under irrigation 30 miles downstream from an effluent containing trace amounts of the isotope is reported. In grass there was 440 times the amount in the water, but no other enrichment factor exceeded 31 (milk and beef). ^{65}Zn was detected in individuals who ate the produce, but in no case did the amount exceed 0·01% of the total permissible value for ^{65}Zn. T. G. MORRIS.

Spectrographic determination of strontium in ash of bone, milk and vegetation. M. S. W. Webb (*A.E.R.E.*, 1959, AM15, 8 pp.).—Bone ash, or CaSr oxalate fraction pptd. from ash of milk or vegetation, is diluted with C and $CuSO_4$ and pelleted. Pellets are burnt to completion in C cups at 7 amp. d.c., and spectra are evaluated by non-recording microphotometry (calibration with Fe-line intensity). Concn. of Sr are read from standard curves for (i) $SrCO_3 + Ca_3(PO_4)_2$ or (ii) CaC_2O_4,H_2O. From 50—350 p.p.m. of Sr can be determined with a coeff. of variation of ~3% at ~100 p.p.m.
W. J. BAKER.

Dried bovine plasma. I. Storage of spray-dried plasma and freeze-concentration of liquid plasma. J. Brooks and P. W. Ratcliff (*J. Sci. Fd Agric.*, 1959, **10**, 486—494).—Spray-dried bovine plasma, stored in air at 25°, rapidly acquired a fishy flavour; the absorption of O_2 and the development of fishiness were accompanied by an O_2-linked interaction between the disappearance of glucose and the development of fluorescence. Frozen, conc. plasma does not become fishy, but in respect of its functional properties as a possible albumin substitute, it is not equal to dried egg albumin. The prep. and properties of a freeze-concentrated plasma are described. (41 references.) E. M. J.

Potatoes a la Portugaise

Across the Tagus, not far from Lisbon, in the southernmost section of Estremadura, lies a rich agricultural area enjoying one of the finest climates in Europe. It is here, around the pretty white villages, that early potatoes are grown for the nearby Lisbon market. There was great concern when, mysteriously, the crop began to decline. Each year, in spite of intensive husbandry, the soil yielded less. The authorities were called in and, in a survey, discovered that the losses were due to the fact that nearly a third of the area was under attack by the potato root nematode (*Heterodera rostochiensis*).

Immediately, trials were undertaken with the highly effective Shell soil fumigant, D-D, which was injected into the soil four weeks before planting. A wise move indeed for, on lifting the potatoes in the following summer, it was found that the yield had increased from an original 6,000 kgs. to 22,000 kgs. per tare—an increase of *over six tons per acre*!

Neighbouring farmers were so impressed by these trials, which had been carried out under official auspices, that they began to apply the D-D treatment of their own accord. Today, many of them regularly fumigate their land, before planting, with D-D, especially where potatoes are grown year after year in the same soil. With D-D, the farmers are getting 'second helpings' . . . of potatoes *à la portugaise*.

D-D

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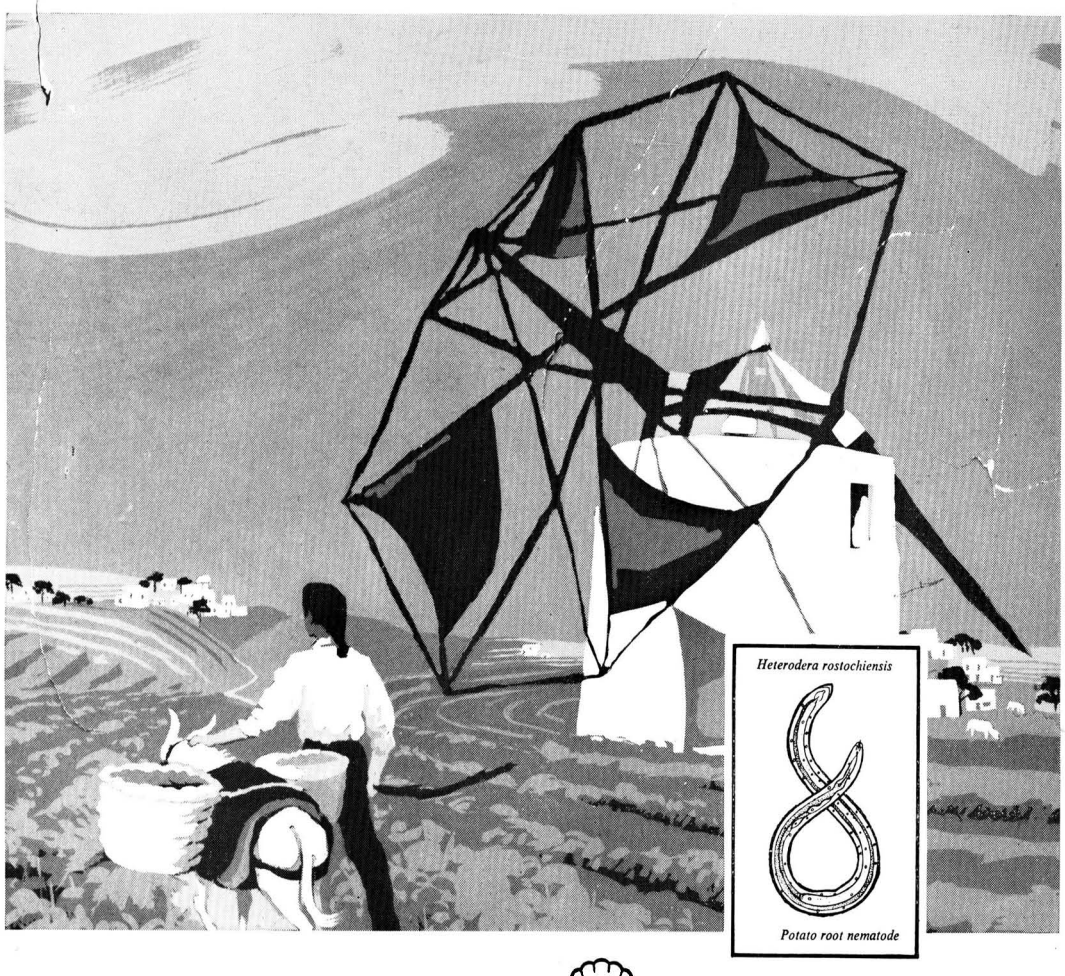

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